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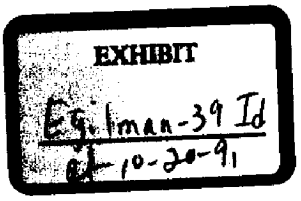

J. E.Braun, Superintendent
New York, N. Y.

July 8, 1955

H. O. Safety Engineering Dept.
Research Division

Diamond Alkali Company
Organics Chemical Division
Lister Ave.
Newark, N. J.

In accordance with your request for information concerning chlor acne, I am enclosing a number of articles I hope will be helpful. All articles and references in this department were photostated and photostats were taken from the latest medical books in the Medical Department, and Miss Steinberg reviewed indices of the Journal of the American Medical Association and articles appearing during the last five years in this Journal are also enclosed.

If there is anything further we can do, please let me know.

D. G. Vaughan, Secretary

By:

Chemical Engineer

FWSehl:
Encls:

"The Etiology of Acne with Special Reference to Acne of Occupational Origin".
The Journal of Industrial Hygiene & Toxicology - Sept. 1941.

"Skin Conditions Resulting from Exposure to Certain Chlorinated Hydrocarbons".
Reprint - The Journal of Industrial Hygiene, Vol. 20, No. 3, March 1938.

Page 112 - Diseases of the Skin - by G.C.Andrews, M.D.

Page 222 - Diseases of the Skin - by G.C.Andrews, M.D.

"Chlorinated Compounds-Precautions in Handling" -
Industrial Medicine - Jan. 1944

"Chlorinated Naphthalenes and Diphenyls" -
Industrial Medicine - Aug. 1943

"Chloracne from an Unusual Exposure to Arochlor" -
The Journal of the American Medical Assn. - April 24,1954.

Page 256 - Industrial Toxicology - by Fairhall.

"Hazards in Use of Chlorinated Naphthalene and Diphenyl" -
Reprint from The Industrial Bulletin, Industrial Commissioner of New York, Vol. 22; No. 2; Feb. 1943

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Page 91 -Abstracts - the Journal of Industrial Hygiene & Toxicology - April 1938.

"Precautions for Handling Chloro-Naphthalene, Chloro-Diphenyl, and Related Chlorinated Compounds" -
By: Halowax Products Division, 30 E. 42nd St., New York 17, N.Y.

DS 00022487

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ACNEFORM ERUPTIONS

With Remarks on Acne Vulgaris and Its Pathogenesis

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Part I

PILOSEBACEOUS IRRITANTS; OBSERVATIONS IN ACNES OF KNOWN EXTERNAL AND INTERNAL ORIGIN; AND PARTICULARLY IN ACNES DUE TO HALOGEN INGESTION; FOLLICULAR ACNEFORM RESPONSES TO PATCH TESTS

As the follicle is frequently the primary site of the pathologic process in acne of known external etiology, in acnes in which internal administration of bromides and iodides is the eliciting cause, in acneform eruptions due to the products of the tubercle bacilli, and in the ordinary form of comedo acne or *acne vulgaris sive juvenilis*, it seems permissible to discuss these conditions as a group. Moreover, the choice of this grouping is motivated by certain analogies which will be the object of further discussion. The grouping is, of course, more or less arbitrary, with no intention of implying that the pathogenetic process underlying these forms is necessarily the same.

Among the external causes of acne, chlorine gas, tars, oils, waxes, and greases are well-known offenders. As prototypes of acne of external origin, three recently observed cases may be cited.

Two young men and a young woman, all three without previous attacks of acne although in the acne ages (18, 23, and 27, respectively), all employed by the same firm and in the same kind of work, became suddenly afflicted with severe acne. These acne eruptions had begun within two months after the patients had commenced working at a job involving contact with hot oils and waxes, which not only touched the exposed parts of their skin, but also permeated their clothing. The patients stated that, of approximately 100 other employees at the same work, over 40 were similarly and abruptly affected with the same form of acne dermatosis. The three patients we examined presented a classic but very severe widespread comedo acne of the face, forehead, cheeks, chest, and back, with papules, pustules, and cystic lesions (see Fig. 1). Many lesions were also found in atypical localizations, on the arms, forearms, and thighs.

In spite of cessation of contact with the harmful agents (these patients did not return to work), and despite the most intensive treatment, the pathologic condition continued for an astonishingly long time (7 months, 1 year, and over 1 year, respectively).

At various sites and at varying intervals of time, patch tests performed on these patients, with the very waxes and greases with which they had come into contact, did not reproduce the acneform eruption, nor did they cause an eczematous response, nor in any way give evidence of a hypersensitivity of the skin to the materials applied.

These cases illustrate the clinical action of a substance with a capacity and a predilection for causing irritation of the pilosebaceous apparatus. Such a substance can produce the entire picture of a typical acne vulgaris eruption, including the comedo, papule, and pustule, the more cystic and deeper lesions, and the end result in characteristic pitted scarring.

These cases also demonstrate other important points: First, that the acne eruption may persist with surprising chronicity and refractoriness to therapy, even after the proven cessation of contact with the actual causative substance; and, second, that the application of a patch test, with

Read in part at the Annual Meeting of the Medical Society of the State of New York, Utica, May 16, 1934

the actual causative agent of the acne, need not necessarily be successful in the experimental reproduction of the dermatosis.

The second group of acneform eruptions consists of those not due to pilosebaceous irritation from without, but to the hematogenous distribution of the pilosebaceous irritant. Bromides and iodides are well known causes of such eruptions. That these halogens (as well as other substances distributed by the blood stream) can cause acneform eruptions seems to be dependent, first upon the fact that the rich circinoid arrangement of blood vessels and the hemodynamic phenomena around the follicles are particularly conducive to a concentration of circulating substances at these

Fig. 1.—Acne due to external contacts with grease and oil.

sites (Jadassohn [1]) second, that these halogens tend to be excreted through the sebaceous glands (R. O. Stein,[2] Kleeberg,[3] and others); and third, that these substances, for some as yet unknown reason, seem to possess a special capacity for irritating the pilosebaceous apparatus.

As evidence of the existence of this third factor, it may be stated that during the last two years we have tested all our cases of bromo- and iododermas by means of patch tests, with ointments containing 25 and 50 per cent potassium iodide and potassium bromide, respectively. In several cases, the patch test produced an eczematous response of diffuse and nonfollicular nature. (This, to a certain degree, confirms the findings of Bloch and Tenchio.[4]) On the other hand, particularly in *acneform* eruptions due to halogens, we observed not infrequently a response to the patch test, not of diffuse and eczematous character, but distinctly follicular and acneform in appearance, closely reproducing the follicular acneform eruption from which the patient was suffering (Figs. 2 and 3).

As far as we know, the observation of this *acneform* response to patch test in cases of acneform eruptions has not heretofore received attention. This form of reaction would seem to be of importance as an indication that a certain substance possesses a particular capacity for irritating the pilosebaceous apparatus of the

Fig. 2.—Acneform responses to patch tests with bromide and iodide on previously unaffected skin of chest of patient with acne vulgaris.

patient, or, stated conversely, as evidence of a hypersusceptibility of the patient's pilosebaceous apparatus to the particular substance applied.

Two further observations seem of interest in this connection. The first is that chronic iodide and bromide acnes can be clinically almost indistinguishable from acne vulgaris (Fig. 3). We have seen such cases due to the chronic ingestion of iodized salt or to the very occasional use of bromides. The second observation is that, even in classic cases of acne vulgaris, in which it was impossible to elicit any

history of halogen ingestion, and which were in no way to be suspected of a drug etiology, bromides and iodides occasionally elicited an acneform and follicular response when applied by means of patch test (Fig. 2).

The third group of follicular and acneform eruptions, namely, those due to the hematogenous distribution of the tubercle baccilus and/or its products, would seem to have many similarities as far as the mechanism of their genesis is concerned, with the hematogenous halogen acnes. For example, lichen scrofulosorum, better called tuberculosis lichenoides, which is probably one of the most common forms of tuberculid, is characterized by its predilection for follicular localization.

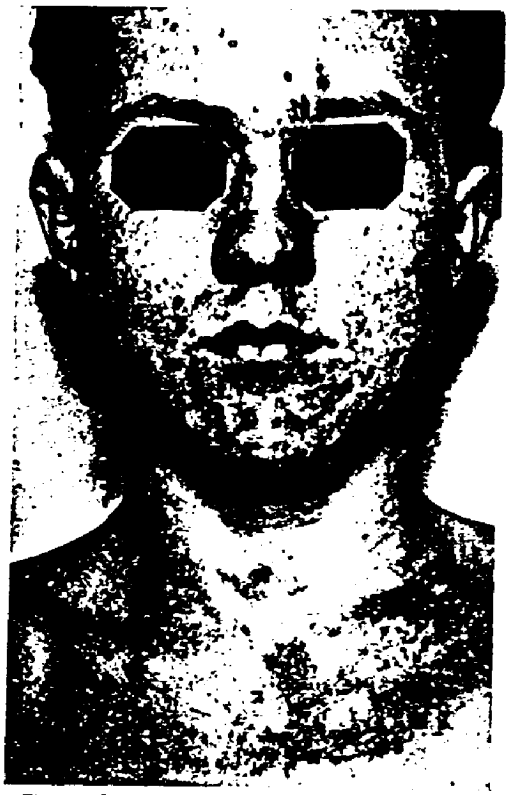

Fig. 3.—Iodide acne closely resembling acne vulgaris. (Note acneform response to iodide patch test on chest.)

Furthermore, most dermatologists now agree that the tubercle baccilus and/or its products can, in certain exceptional cases, produce eruptions so closely simulating acne vulgaris that it is in some cases impossible to differentiate clinically between them.

A third type of acneform eruption of tuberculous etiology is one which is almost indistinguishable from acne rosacea, namely, that known as the *rosacea-like tuberculid of Lewandowsky.** (For a complete report on this form of tuberculid, see MacKee and Sulzberger.[3])

The analogy between these tuberculous acnes and the halogen acnes is obvious. In the one case, the products of the tubercle bacillus, hematogenously distributed, selectively irritate the pilosebaceous apparatus. In the other case, the circulating halogen produces this effect.

At the Post Graduate Hospital, within the past four years, we have routinely tested a large number of patients (more than 100) with tuberculin patch tests. Many of the positive reactions to these

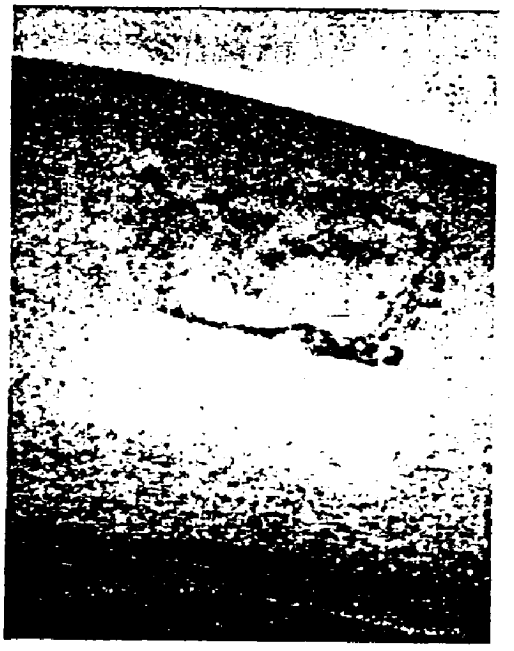

Fig. 4.—Acneform reaction to patch test with old tuberculin on previously unaffected skin of patient with acneform tuberculoderm. (Rosacea-like tuberculide.)

tests were of the follicular type. This form of reaction has been the rule, *particularly in the acneform tuberculids* and in lichen scrofulosorum (Fig. 5). The follicular response to tuberculin patch tests indicates that the statement made above, in regard to bromine and iodine, applies to tuberculin as well. For tuberculin would seem to be a substance possessing a particular capacity for irritating the pilosebaceous apparatus of certain individuals;

* The chronic use of iodides and bromides can produce not only acne vulgaris-like eruptions, but also clinical pictures resembling acne rosacea and rosacea-like tuberculid.

or, stated conversely, there is, in these individuals, a particular hypersusceptibility of the pilosebaceous apparatus to tuberculin.*

While the acneform eruptions discussed are not excessively rare, the fourth group, acne vulgaris, is of far greater frequency and, therefore, of far greater practical interest. Unfortunately, the present knowledge as to the pathogenesis of this condition is, to say the least, deplorably limited. The older theory of the bacterial etiology of acne vulgaris remains unproved and, to a great degree, discredited; the disease cannot be reproduced experimentally, in man or in animals, by means of the accused micro-organisms (Stein[3]). The newer theories dealing with the endocrine nature of the disease are vague and require crystallization and confirmation.

In view of this lack of knowledge, research into the cause of acne has lacked direction and guidance. It would, therefore, seem permissible and even desirable to suggest a working hypothesis which might prove of some value, if only in stimulating investigations along organized lines. From *many* hypotheses suggesting themselves, it seemed necessary to us to select *one* suitable for experimental investigation.

The hypothesis we have chosen includes the above outlined observations in acneform eruptions of known etiology, and it seeks to establish a common or related pathogenesis in acnes known to be of external causation, acnes known to be due to circulating hematogenous noxae, and acne juvenilis.

PART II

A WORKING HYPOTHESIS FOR THE STUDY OF ACNE VULGARIS

From the phylogenetic and ontogenetic points of view, the sebaceous glands and the hair follicles are to be considered invaginations of the surface epithelium. The cells of the hair bulb continue to undergo the same changes as those of the surface epithelium, namely, the changes of cornification; the sebaceous gland cells become differentiated in that they undergo not horny but fatty alteration, and these fatty cells form the secreta (holocrine secretion). It is obvious that an irritative stimulus with a capacity and predilection for affecting the pilosebaceous apparatus may produce hyperactivity: (*a*) of the sebaceous gland; (*b*) of the hair bulb; and (*c*) of the follicle wall and the epidermis at the points where the invagination takes place. It is, in particular, the hyperactivity of the epidermis of the follicle mouth, which seems to play a leading rôle in the formation of the primary lesion of acne vulgaris, namely, the comedo. For, as Kyrle,[4] Stein,[3] and others have shown, the first step in comedo formation is the epithelial irritation leading to *hyperkeratosis at the follicular mouth*, which gradually narrows the follicular orifice and finally forms a horny plug in the ostium. The hyperkeratosis and occlusion of the follicle, plus a hypersecretion of the simultaneously irritated gland, bring about the formation of the sebaceous and horny plug known as the comedone.

It is our opinion that variations in this mechanism may, in themselves, be sufficient to cause the entire picture of acne vulgaris and the accompanying (and frequently preceding) seborrhea. For one may have: (*a*) stimulation of seborrheic glands, with as yet unoccluded follicles, giving rise to seborrhea; (*b*) occluded follicles forming comedones (see Kyrle[4]); and (*c*) occluded follicles with hyperactive glands, in which the dammed-up secreta and any adjuvant substances coming from the circulation and excreted with the sebaceous material necessarily become concentrated.

This concentration of possible irritants and/or the mechanical irritation from pressure alone could, possibly, account completely for the subsequent lesions of papules, pustules, and abscess. It cannot be denied, however, that micro-organisms may play a rôle in the formation of the inflammatory and pyogenic development of the lesions. Such micro-organisms—as acne bacilli, staphylococci, micrococci, pityrosporons, bottle bacilli, demodex, etc.—not obligatory pathogens and normally found saprophytically on the skin and in the ostium of the follicle, become imprisoned within the follicle, under the horny plug. In this manner, these organisms may meet favorable conditions for growth and for the development of pathogenic activity in a follicle already damaged by stagnation and pressure. Or, the circulating substances may, in other ways, aid the pathogenic activity of bacteria (see Haxthausen, quoted by

* Similarly, trichophytin and oidiomycin patch tests are likely to produce follicular reactions.

Stein,[2]); or, on the other hand, the hypothetical substances may cause irritation only in follicles already abnormal, for instance, with anatomically deficient outlets. While these possibilities must naturally be considered, it does not seem to us that the action of the bacteria in acne can be primary; nor does our hypothesis necessitate the assumption of any bacterial activity whatsoever nor of any antecedent anatomic abnormalities. Both of these factors are unproved and would necessitate additional and complicating hypotheses.

If one sees as a primary cause of acne vulgaris a circulating substance or circulating substances with the capacity and predilection for stimulating, exciting, or irritating the pilosebaceous apparatus in certain predisposed individuals, it is obvious that close analogies exist between acne vulgaris and acneform eruptions due to the halogens and tuberculosis, and also those due to external pilosebaceous irritants.

It follows that one must look for the cause of acne vulgaris, either in the abnormal *quantitative or qualitative* presence of pilosebaceous stimulants or, in an abnormal susceptibility of the pilosebaceous apparatus to such stimulants, or in a combination of these factors.

In other words, to prove our hypothesis we must find either a substance or substances pathologically (quantitatively or qualitatively) present in acne vulgaris; or some substance or substances found and normally innocuous, but to which the pilosebaceous apparatus of acne patients can be shown to be hypersusceptible, or both.

Observations of the clinical course of acne vulgaris have naturally directed attention toward a certain group of possible causative agents. While acne vulgaris is practically unknown in infants and young children, it is an exceedingly common manifestation in the years immediately preceding sexual maturity and in early adolescence. A mild degree of acne is almost physiological in these years. Bruno Bloch[7, 8] examined 2,136 girls between the ages of six and eighteen years, and 2,055 boys between the ages of six and nineteen years. He found that comedones began to appear in these children between the ages of six and seven; and that, at the age of seventeen, 80 per cent of the girls had some degree of acne; and that, in boys, the condition developed earlier, so that, at *thirteen* years of age, 71 per cent of the boys were affected. He found, furthermore, a synchronous appearance of acne lesions and of pubic and axillary hair, and, in girls, of the first menstruation. These figures compose a statistical substantiation of the accepted clinical fact of the obvious connection between the awakening of the sexual endocrine activity and the appearance of acne vulgaris.

Bloch's findings showed not only that acne begins earlier in males, but that severe cases of acne were more than twice as common in boys as in girls; at nineteen years of age, there were 5 boys with severe acne to 2 girls with cases of equivalent severity. This finding fits in with our hypothesis. It would seem to indicate one of two things; either, that the male possesses intrinsically a follicular apparatus more sensitive to hormonal stimulation, and with a greater capacity for reacting to such stimuli; or

Fig. 5.—Exacerbation of acne vulgaris after 48 hours of ingestion of moderate doses of KI. The face was practically clear before this medication.

that more hormonal pilosebaceous stimulants (irritants) may be formed in the male than in the female. The formation of the beard and the heavier body hairs in the male would tend to substantiate this concept. True eunuchs, that is, those emasculated before puberty, generally have no beards or very sparse beards, and their growth of secondary sexual hair is likely to be of the female type. It has further been established that true eunuchs do not become bald. (In the 340 eunuchs examined, no sign of seborrheic alopecia could be found—Sabouraud.[9]) This observation seems to us to be of primary importance and can perhaps be explained in accordance with the theory that the male type of baldness is, in some way, the result of repeated overstimulation of the pilosebaceous apparatus of the scalp (see Hebra, Kaposi, Riehl, and others quoted by R. O. Stein,[2] page 62),

and that this overstimulation is, in turn, dependent upon male gonad activity.

There are yet other observations in acne vulgaris, which point to the circulating products of endocrine glands as being, in some manner closely connected with the development of the disease. There are numerous reports dealing with this subject. In this country, L. Hollander[10] and Schamberg,[11] among others, have called attention to observations suggesting such a connection. More recently, Kurzrock and Rosenthal[12] reported that the urinary excretion of estrin was subnormal in women suffering from acne. It is frequently observed that acnes exacerbate cyclically in connection with the menses; and that some cases are combined with dysmenorrhea. The acnes of older women (chin acnes, etc.) appear in a preclimateric manner, at the time when there is, according to endocrinologists, not infrequently a terminal endocrine dysfunction. Pregnancy often affects the pilosebaceous apparatus; and the influence of the pregnancy upon the acne dermotosis, as well as upon seborrheic conditions, may sometimes be apparent. Just as acne vulgaris does not occur before the beginning of sexual activity, so also the disease does not exist in the aged.

In view of all these considerations, it seems a logical conclusion that the products of the gonads and or of other endocrine glands in which there is increased or altered activity at the time of puberty, of pregnancy, and of menopause, either directly or indirectly contribute to the stimulation of the pilosebaceous apparatus: this stimulus physiologically produces the beard and the secondary sexual hair; and, in some cases pathologically, the formation of the comedo, and thus lays the foundation for acne. (Not only the sebaceous glands undergo such hormonal stimuli, but the embrologically related apocrine glands and the mammary glands of the female are synchronously and similarly stimulated.)

According to the concept outlined, acne vulgaris (and seborrhea and baldness, which are so frequently and familiarly associated with this dermatosis) would seem to be based upon an aberration in the normal and physiological process of hormonal pilosebaceous stimulation.

There are certain guides which can be used in the search for the hormone or hormones which may be actively engaged in the stimulation of the pilosebaceous apparatus. During the last months of intra-uterine life, there is a marked stimulation of the epithelium and particularly of the hair bulb and the sebaceous glands, which leads, as is well-known, to the formation of the vernix caseosa and the lanugo hair of the fetus. This hyperactivity ceases with birth, and both the vernix caseosa and the physiologic hyperkeratosis of the newborn rapidly disappear.

At first glance, it may appear to be no more than a chain of remarkable coincidences that the stimulation of the fetal pilosebaceous apparatus (vernix caseosa and lanugo hair) should occur at precisely the time when the maternal, and probably also the fetal, circulation contain the highest level of certain sexual hormones (*e.g.*, prolans, estrin); and that the hyperactivity of the pilosebaceous apparatus should cease abruptly when the infant is cut off from its supply of maternal hormones; and that there should be a renewed hyperactivity on the part of the pilosebaceous apparatus at precisely the time when the individual begins to produce his own sexual hormones. But this chain of events ceases to be merely an extraordinary coincidence, and becomes a basis for experimental investigation, when one hypothecates the direct or indirect causal relationship between the hormones and the stimulation of the pilosebaceous apparatus.

Part III

PRELIMINARY EXPERIMENTAL REPORT

Two possible modes of approach presented themselves for experimentation along the lines of our hypothesis. The first, to attempt to demonstrate, in cases of acne vulgaris, a pathologic quantitative or qualitative hormonal aberration, particularly in regard to the hormonal content of the blood stream, the skin, and the pilosebaceous apparatus. The second, to attempt to demonstrate a hypersusceptibility of the pilosebaceous apparatus of acne patients, directed toward normal hormones or their derivatives or constituents.

We have chosen to begin with the second of these approaches. It seemed possible that increased thyroid activity, frequently coincidental with increased gonad activity and leading to increase in circulating thyroxin (the iodine-containing hormone), might explain acne vulgaris as essentially of the same nature as a very chronic iodide

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acne. To investigate this possibility, patch tests with both thyroxin and iodide ointments were applied in the usual manner in 75 cases of acne vulgaris. None of these cases reacted to the thyroxin test, and only 4 reacted to the iodide. However, in these 4, the patch test produced typical and persistent lesions in the previously unaffected areas to which the iodide test had been applied (see Fig. 2). This number of positive reactors even to strong (50 per cent) potassium iodide tests is too small to be considered as strengthening the possibility that acne vulgaris is essentially a modified iodide acne (thyroxin-derivative acne).

Nevertheless, before coming to the conclusion that these results *entirely* contradict the possible rôle of iodine compounds in acne vulgaris, a continuation of experiments and modifications of technic seem indicated. This is particularly apparent when one remembers that many true iodide and bromide acnes do not necessarily give positive reactions to iodide patch tests; and that even in cases of acne due to external irritants, we were unable to elicit a positive reaction by means of the patch test application of the causative substances.

In a second series of experiments, we attempted to study the possible effect of iodides in acne vulgaris, not by external application of patch tests, but by ingestion and hematogenous distribution to the skin and pilosebaceous apparatus. In 20 cases of moderately severe acne vulgaris, in which there was no history of halogen ingestion, and in 21 control individuals (persons without acne), potassium iodide was given by mouth and in moderate doses (3 teaspoonfuls daily during two weeks, of the following: potassium iodide, 10.0 in aqua, qs. ad 120.0).

In 20, i.e., 100 per cent of the acne vulgaris cases, the ingestion of iodide as prescribed caused, after 2 to 3 days, distinct exacerbations of the dermatosis, with the formation of many new lesions, and flare-ups of existing ones. (See Fig. 5.) In some cases, only previously affected areas, *i.e.*, the typical acne sites, were affected; in others, a few acne lesions appeared in previously unaffected sites and in atypical localizations, for instance, on the forearms, buttocks, etc. (It seems noteworthy that these patients had all had negative patch tests to iodine in various forms. In some of them, such patch tests, repeated after the appearance of the exacerbation caused by KI ingestion, still failed to elicit a positive response.)

In only one of the 21 control cases did the administration of this amount of the iodide produce acne lesions; and in this one, only two small papules appeared on the forehead after two weeks of the iodide medication (the patient stated that he had had similar lesions in the past).

This experiment shows that patients with acne vulgaris differ from the norm in their reaction to iodides; and that their pilosebaceous apparatus is pathologically hypersusceptible to the direct or indirect effects of the drug. 1. *It is impossible to interpret this finding or, at present, to discuss the possible relationship of the demonstrable iodide hypersensitivity to the actual causative factors in acne vulgaris.* 2. *But this clinical fact remains: relatively small quantities of ingested iodides regularly produce new lesions and exacerbations of acne in acne patients.*

It was a logical consequence to the above results to have the third investigative measure consist in the attempt to treat acne vulgaris as though it were actually an iodide or bromide acne; and to see whether the results of this therapy would be sufficiently favorable to serve as a therapeutic test. The therapy consisted in the administration of sodium chloride by mouth. We prescribed this in a form which we have found very effective in the treatment of iododermas and bromodermas, and which we believe to be superior to other methods of sodium chloride administration in these dermatoses. The patient is instructed to take 16 gm. of sodium chloride daily, in addition to the normal intake; administered in the form of 1 gm. enteric-coated sodium chloride tablets, as prepared for us and now marketed by Eli Lilly Co. The results of this therapy were extremely gratifying in a few cases, but on the whole did not produce the rapid amelioration so frequently seen in true iodide and bromide acnes. *The number of cases reacting favorably is too small for us to consider these results as proof of the possibility that acne vulgaris is a modified iodide acne.*

It must be remembered, however, that acne may persist for a very long time, even after the cessation of the action of the eliciting agents. This is shown, for example, by the persistence of the cases of acne of external cause for long periods

after cessation of contact with the eliciting agent. (See Part I.)

The next series of experiments was performed to determine whether or not acne vulgaris patients possess a pilosebaceous hypersusceptibility to various hormones other than thyroxin, and to substances other than iodides.* For this purpose, we carried out patch tests in the usual manner, employing the following hormones and other substances on a series of 30 acne patients:

(1) Potassium iodide 5 per cent in aqua dest., later replaced by KI 25 per cent in aqua dest.

(2) Potassium iodide 5 per cent in lanolin, later replaced by KI 25 per cent in lanolin.

(3) Lanolin.

(4) Thyroxin, 1:1,000, in aqua dest.

(5) Parathormone.

(6) Acne vaccine (Parke Davis Co's combined).

(7) Antuitrin S.

(8) Follutein.

(9) Amniotin.

(10) Theelin.

(11) Antuitrin.

(12) Pituitrin.

(13) Glucose 10 per cent in aqua dest.

(14) Syrup. simplex.

(15) Lugol's solution, 50 per cent.

(16) Chocolate in lanolin.

(17) Chocolate in talc.

All of these tests were negative. It must again be stated that we do not consider the negative results of these relatively few experiments as conclusive. Continuation and modification of method is here necessary. It is to be pointed out, in particular, that our list of hormones is incomplete: for example, no experiments were undertaken with progestin and intermedin. Furthermore, the solutions employed may have been too dilute, or possibly even too concentrated. In these experiments we were limited to the employment of the commercially available concentrations. It would seem desirable to repeat these experiments, and especially with stronger solutions.

It should also be remembered that the action of hematogenously distributed hormones may differ radically from that of externally applied hormones. Such a difference in the action of hematogenously distributed and externally applied substances is the rule in many forms of drug eruptions, in which the external application or the injection into the skin frequently produces no reaction, while the ingestion even of small quantities of the same drug produces the dermatosis. This difference in reaction to externally applied and to ingested iodide has been demonstrated in the reported experiments in acne vulgaris. Such differences in reaction, though unexplained, are frequently encountered. The same difference of action of iodides may often be seen in *dermatitis herpetiformis*. In this disease the internal administration of iodides causes a high percentage of exacerbations of the dermatosis, while the patch test application elicits a decidedly lower percentage of positive reactions.

Unfortunately, and for obvious reasons, no attempt could, as yet, be made to study the possible effects of the internal administration of the hormones which were employed in patch-testing the acne patients.

In closing, we should like to point out that our hypothesis speaks not against, but rather in favor of the correctness of the clinical observation that certain cases of acne are harmed or even produced by drugs and foods, such as, for instance, iodides, chocolate, cheese, milk, etc. (See, for instance, Cleveland White.[3]) Perhaps substances other than hormones, for example, other metabolites, may play a rôle. For, if the follicular apparatus in an individual tends to become hypersensitive, such a hypersensitivity may be directed not to hormones, but to other substances; or, the existing hypersensitivity may become polyvalent and embrace in its scope other substances as well. Nor, as we have said above, do we wish to exclude absolutely the possibility that bacterial action enters into the pathogenesis of acne vulgaris; for the follicle and gland, first stimulated by hormonal or other influences, may then become more easily susceptible to bacterial noxae.

Furthermore, the beneficial effects of the best available modern acne treatment, namely, x-ray and desquamating procedures, obviously counteract the pathologic occurrences which we have outlined. The x-rays, by reducing the activity of the hyperstimulated gland and follicle orifice; and the desquamating measures, by removing the obstructive horny plug. These measures are still those of choice,

* Perhaps even more fully than thyroid hormone, theelin and the chemically related male sex hormone would fulfill many of the requirements of our hypothesis, *e.g.*, (*a*) present in both sexes; (*b*) increase at the end of pregnancy; (*c*) varying amounts secreted at different phases of the menstrual cycle; (*d*) absent in castrates; (*e*) promote cornification (vagina).

in acne therapy. Fortunately, excellent results may be obtained through their employment in the majority of cases of acne vulgaris. Where they fail, the sodium chloride treatment will occasionally bring success. Prolan injections will help in a certain number of acne patients. And other methods will help in yet other cases. However, a certain number still resist every known form of treatment.

It is to be hoped that a clearer understanding of the seemingly basic endocrinologic phenomena involved will lead, in the near future, to a more causal and effective therapy of acne vulgaris; and perhaps even to new and adequate methods for combating the closely related seborrheas and seborrheic alopecias.[14]

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DISCUSSION

Dr. Herman Sharlit: While we find it necessary to dissent from the validity of Dr. Sulzberger and his collaborators' procedures and the soundness of their hypothesis, we are indebted to them for a fairly complete selection of the clinical facts from which to discuss their hypothesis pro and con.

They point out that acne-like papules occur (1) from the exposure to external irritants (greases), (2) the ingestion of iodides and bromides, and (3) the toxins of tuberculosis; and while conceding that all these follicular papules are not identical with those of acne vulgaris, they all represent pathology about the same anatomical structures. And without expressly saying so, they are accepting a sameness about all these lesions in terms of the function of these structures: hence, their reference to pilosebaceous apparatus, a functioning unit, not an anatomical one. The pilosebaceous apparatus in man is a vestigial organ harking back to the day when we were completely covered with hair. We have several million such units now feebly functioning to cover us with hair. Briefly, it is a hair root and a hair channel lined by modified skin and lubricated by an oil from a parenchymatous secreting organ emptying its sebum into this channel. It functions in response to stimulation like other organs and, like them, is exposed to overstimulation, understimulation, and malstimulation (irritation). But before considering the effects of stimuli on the pilosebaceous apparatus, we must thoroughly appreciate the prime importance of anatomical fitness to function. The follicular canal must be smoothly lined and the oil effectively lubricating. Grease rubbed into the follicular openings may readily destroy the anatomical fitness to function and under stimulation evidence this disability by the evolution of an inflammatory reaction, the papule. Ingested iodine is stored in the skin and excreted in the hair; in excessive quantities these are tissue irritants causing inflammatory reactions wherever they lodge. Iodides and bromides can produce not only follicular papules but destructive lesions of large areas of skin. The circulating toxins of tuberculosis do not limit their irritating action to follicles necessarily; other and bigger lesions of skin have been attributed to this species of irritation. All these circumstances are but evidences of anatomical change due to local irritation, resulting in structural unfitness and emphasized and exaggerated in that unfitness in the presence of normal stimulation to the apparatus to function. None of this is evidence of any special irritability of the pilosebaceous apparatus for these substances, nor of unusual affinity of these substances for this apparatus.

It must be conceded that no known external irritant or circulating foreign substance is responsible for acne vulgaris and that the condition so uniformly appears synchronously with the maturity of sex function that it seems reasonable to suppose that the organism elaborates at about that time a substance or substances that operate, on the one hand, to influence the sex organs and, on the other, the pilosebaceous apparatus (a secondary sex factor). And with the throwing into the circulation of this pilosebaceous "stimulant" acne appears. To me this is reasonable physiology and in keeping with clinical facts. Dr. Sulzberger and his collaborators accept this statement of the situation but in terms of their hypothesis see in this elaborated stimulant a basis for the acne, to them this substance or substances probably elaborated by the glands of internal secretion have a perverted influence upon the pilosebaceous apparatus, to the extent that these substances are irritants and may produce acne after the fashion of iodides, tuberculosis toxins, and grease. We know of no substantiating evidence for this and cannot accept it. To us the explanation is as simple as when grease locally applied is the cause of acne,

namely, that the anatomically unfit follicular units (reflect on the comedone and its relationship to acne vulgaris), when put under the impetus to function, show their unfitness by a follicular papular reaction.

Our essayists undertook to test clinically their hypothesis by the use of the patch test. Of slight moment is the fact that these tests were all substantially negative. I am willing to assume that they were all positive, that they all produced follicular papules, and under these circumstances they would prove nothing with respect to their hypothesis concerning the pathogenesis of acne vulgaris, to wit, that the body elaborated stimuli to pilosebaceous apparatus circulating as specific irritants to set in motion the chain of events leading to the follicular papule. May I say in closing that the difference in the points of view of our essayists and ourselves may appear in the following analogy: A moving automobile is losing oil from its crank case and finally the motor breaks down. Our essayists say that the damage was done by the presence of gasoline in the gas tank, because if there were no gas in the tank the car could not move, and if the car were not moving the motor would not have broken. We say, in our simple way, perhaps, that the trouble resulted from the loss of the oil and that using the gasoline to drive the car was one way to show that the car was unfit for use.

DR. MARION B. SULZBERGER: I believe that Dr. Sharlit and I are in practically complete agreement, except perhaps on one point. I, of course, grant that the acne lesions appear in follicles which are in some way aberrant or altered at the moment at which the stimulant begins to act. However, it is a question in my mind as to whether this aberration is as simple as Dr. Sharlit imagines, and consists simply in a nevoid abnormality in the excretory passage. There are many facts which speak against this assumption (for it must be still regarded as a pure assumption, there being absolutely no proof of the preexistence of such an anatomic abnormality in the follicles of acne patients; clinical and histologic evidence both speak against this).

Furthermore, should there be a preexisting and predisposing anatomic or functional abnormality, the stimulus which produces the acne eruption in these follicles would still have to be considered part of the pathogenetic process; just as for instance, phenolphthalein ingestion and distribution must be considered as part of the pathogenetic mechanism in the production of a phenolphthalein dermatosis, which subsequently appears in apparently normal, but certainly in some way predisposed area of the skin. To use Dr. Sharlit's parable of the automobile, let us assume that an automobile is defective in some way, just as the follicle may be defective in some way. We have, in the case of the follicle, no way of discovering its defect and no way of repairing it. This may be the case in a defective automobile as well. The automobile is capable of functioning provided it is not overloaded or driven too quickly, just as the follicle is capable of functioning and does not become the seat of the pathologic process unless it and the sebaceous gland become stimulated beyond the point of tolerance (speed and overload).

The best way to keep the automobile in the story from breaking down is to prevent its being overloaded or driven at an excessive speed. Similarly we are trying to ascertain what hormones or substances bring about the overloading of the follicle and the excessive sebaceous secretion and thus perhaps learn to prevent or counteract this factor.

PRACTICE IN A PENITENTIARY

The problem of the prison doctor in Sing Sing, it appears, is to keep the well from pushing their way into the hospital, for anywhere from 100 to 150 show up each morning for sick call, out of an average of 2,600 convicts, lured by the prospect of better food, more comfort, and surcease from work. Dr. Charles C. Sweet tells about it in *Medical Economics*. As a matter of fact, the doctor assures us, prison life is good for the convict and thanks not only to the medical care that they receive but to the enforced regularity of their lives as regards exercise, eating, and hours of sleep, most prisoners improve remarkably in health within two or three months after coming to Sing Sing.

A picturesque figure is the hospital artist. This man, a negro and a lifer, says Dr. Sweet, is present at every operation. At a point where the pathological feature of the case is most clearly revealed, the operation is halted for a few seconds while he makes a pencil sketch and gets an accurate mental image of that field. Afterwards, in his office the final touches are put to the picture, and the completed pen drawing is filed with the patient's chart. These picture records, in color, are not only interesting but extremely valuable as part of the surgical records.

Whenever it is necessary to do a blood transfusion, donors are always chosen from among the prison population. The evening before the transfusion is to be made, an announcement goes out on the institution's radio that blood donors are going to be needed. The men get this message in their cells, and the next morning invariably a great number of volunteers show up at the hospital to have their blood typed. As many as three hundred men have appeared at one time in response to such a request, despite the fact that it is usually necessary to test four or five before finding a suitable donor. The one selected stays in the hospital that day and that night, and gets as his only special reward a chicken dinner, which he probably shares with somebody in the ward, perhaps the man to whom he has just given part of his own blood.

KAREN JORDAN

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Dermatitis from Synthetic Resins and Waxes*

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DERMATITIS in the manufacture of natural resins and from their use has been reported by numerous observers,[1] but dermatitis in the manufacture of synthetic resins and from their use has not been frequently reported [2] or studied.

The principal synthetic resins manufactured in the United States are:

1. Phenol-formaldehyde resins, sold under various trade names, such as Bakelite, Durite, Durez, Resinox, Indur, etc.
2. Urea-formaldehyde resins, sold under trade names such as Beetle, Plaskon, Unite
3. Coumaron resin, made from coal tar residue
4. Ester gums, which are natural resins, such as Rosin, Dammar and Copal, in which the acid of the resin has been combined and neutralized with glycerol
5. The glyptal resins, which are a combination of glycerol and an organic acid, such as phthalic and malleic acids
6. The vinyl resins, which are vinyl esters, ethers or halides combined with phenol, formaldehyde and hexamethylenetetramine
7. Furfural resins, obtained from combining furfural with phenol (furfural obtained from corn cobs by treating with H_2SO_4 and fractional distillation)
8. Chlorinated resins, obtained by chlorinating various oils and balsams
9. Chlorinated waxes, obtained by chlorinating mineral oils, paraffin, naphthalene, di-phenyl, etc.

These resins are used for many purposes:

For making plastics, such as telephone receivers, pipe stems, push buttons, ear p... cigar holders, bottle caps, buttons, ... artificial teeth plates, ornaments, bra... earrings.

For making varnishes, flexible co... floor finishes.

For making cements, wall boards, ... dishes, water containers, insulators, ... compounds, and numerous other objects ... for electrical insulations on condensers ... wires.

The most important of the re... from a standpoint of volume used ... skin hazards, are the phenol-form... hyde, the urea-formaldehyde and ... coumaron resins.

PHENOL-FORMALDEHYDE RESINS

These are made in two prin... varieties:

1. The cast resin, which is finished in ... factory
2. The molding resin, which is sold ... molding companies and completed in ... molding process

Phenol or cresol, formaldehyde ... ammonia are mixed in proper pro... tions in a kettle and heated a suffi... length of time and to the pro... temperature.[3] They combine and ... drawn out of the kettle in the form ... a syrup. This is run into pans ... allowed to cool, when it solid... This is known as "first stage r... or "alpha resin." The pans are ... heated, the resin re-melted and p... into suitable molds. These are p... in large, mineral oil filled carriers ...

* Read before the Industrial Hygiene Section of the American Public Health Association at the Sixty-fourth Annual Meeting in Milwaukee, Wis., October 8, 1935.

placed into *curing* ovens, where they are heated and go through the so-called beta and gamma stages into the completed cast resin.

After being taken out of the oven, the resin is taken out of the molds and oil, and washed with soap and water and dried, and it is then ready to be carved or lathed or bored into whatever object is desired.

During the course of these operations, formaldehyde is given off and the air of the room is strong with the odor of it, unless adequate forced ventilation is employed. Workers in the rooms who are sensitive to formaldehyde may develop dermatitis of the face, neck, and arms, as well as of the covered parts where there is friction, such as the belt line, the ankle at the shoe top, and the wrist at the cuff line.

The oil used in the curing process dissolves out of the resin some of the phenol and formaldehyde and, if used over and over again, it can contain considerable quantities of these substances. One sample which was analyzed after it came out of the ovens contained 2 per cent each of phenol and formaldehyde. The men handling this oil have their clothes splashed with it and at times suffer not only from oil acne and dermatitis of the legs, thighs, and other parts touched by the oil-soaked clothing, but also from dermatitis due to the irritating action of the phenol and the formaldehyde contained in the oil. The eruption in these cases is usually a diffuse erythematous base on which there are scattered the papules and pustules of an oil folliculitis.

The girls washing the oil off the resin, with soap and water, may also develop dermatitis of the hands and arms from the oil and also from the strong soap solution with which their hands are constantly wet. In many factories, the oil is treated with sodium hydrate after each baking in order to neutralize the phenol and formaldehyde it may contain, but if this is not carefully done, dermatitis may result from too much alkali.

The skin hazards to the users of these cast resins are practically nil, because when they are finished no phenol or formaldehyde are given off from them.

The molding resin is made up in numerous qualities, containing different proportions of phenol and formaldehyde and ammonia. It is carried to the alpha stage or beta stage, and then is ground, heated, and mixed with ground wood, zinc stearate, soap, dyes, and hexamethylenetetramine in a mix mill. From this mill it is discharged on to conveyors, where it is air cooled. While cooling, it forms lumps which are ground in mills to proper sizes fit for molding. The workers engaged in mixing, grinding, and packing the molding resin are all exposed to the dust of the irritating chemicals it contains, as well as to the formaldehyde which it gives off, the odor of which permeates the rooms. Dermatitis is a frequent occurrence in this occupation, especially if the rooms are not properly ventilated to draw off dust, and if the machines and jobs where there is dust are not properly protected by suction hoods.

In a factory employing about 400 workers, where no great care was taken to allay and prevent dust, 27 cases of dermatitis occurred in 8 months. Patch tests performed on 10 cases with various resins, hexa, 4 per cent solution of formaldehyde, and 2 per cent solution of phenol, showed that hypersensitivity to hexamethylenetetramine and to formaldehyde was the cause of 80 per cent of the occupational dermatitis in this plant. Hypersensitivity to phenol was also found, but in lesser degree than to formaldehyde and hexa.

The actual cause of dermatitis from exposure to hexa and formaldehyde is the same. They both finally decompose in the presence of warmth and moisture

into formic acid, which is the real skin irritant.

Dermatitis from the dust of the molding resins usually occurs at the points of friction with the clothing—the wrist, the belt line, the shoe tops and the collar line. It may, however, occur on the covered parts when the dust penetrates the clothing. The skin around the eyelids may also be affected. The eruption consists, usually, of scattered papules and vesicles on an erythematous base.

It gets well if the worker stays away from work, and returns again if he resumes work. In this factory, the dermatitis was more prevalent in the winter because the workers did not take showers in cold weather after their shift, whereas in the summer they were more likely to do so.

UREA-FORMALDEHYDE RESINS

These are made by mixing urea and formaldehyde in a kettle. No heat is required, the reaction generating its own heat. A syrupy, liquid results. This is mixed with bleached sulphite pulp, under heat and pressure, and dried in tray dryers. It is then mixed in a ball mill with pigments, zinc stearate, and a small amount of hexamethylenetetramine, about ½ per cent. It is then screened, and is ready for shipment. Minute amounts (fractions of 1 per cent) of other ingredients are added to different brands.

The manufacture of the urea-formaldehyde resins on a commercial scale is more recent than that of the phenol-formaldehyde resins, hence the two factories studied had more modern machinery and safety appliances than the phenol-formaldehyde resin factories. There were adequate ventilation facilities and good facilities for dust prevention. As a result, among 190 employees over a period of over 2 years, there had occurred only 4 cases of dermatitis. They were all due to hypersensitivity to formaldehyde. Two chemists in the experimental laboratory of these plants were hypersensitive to formaldehyde and suffered with dermatitis when exposed to it. One of them exposed his forearm in my presence to the mouth of an open bottle of formalin and almost immediately an erythema developed on the exposed skin.

In a molding plant in Ohio employing about 300 workers, where phenol-formaldehyde and urea-formaldehyde resins are used, 26 cases of dermatitis occurred in the first 10 months of 1934, and in a molding plant in Pennsylvania half of the workers developed dermatitis in the hot months of 1935.

The process of molding is practically the same for all the resins. The powder is placed in a "pill machine" and pressed into proper shapes for the molds. The pills are then placed into the molds where they are subjected to heat and pressure which shapes and hardens them. During the molding process gases are given off from the molds and the odor of formaldehyde is strong in the room and irritates the nose and throat and eyes of those unaccustomed to it.

There is an excess of powder in the molds which flows out during the molding process and is only partially "cured." This is called the "*flash*." When the molds are opened the flash is cleaned off the molds and filed off the molded object.

Patch tests with the resins with which they worked were performed in the winter on 2 active cases of dermatitis, and 10 workers who had recovered from dermatitis, gave only 2 positive reactions, 1 to the phenol-formaldehyde and 1 to the urea-formaldehyde resin. The examination and history of the 10 cases who had dermatitis but were free of it when the patch tests were made, showed that 2 had dermatophytosis and 1 probably had ptyriasis rosea. It

is probable that a considerable number of the 26 cases reported in 1934 were not of industrial origin.

The same plant was visited in August, 1935. Twelve cases of dermatitis had occurred since the first visit 9 months before. This time 9 workers who had dermatitis during this period were patched with suspected samples of urea-formaldehyde and phenol-formaldehyde resins, as well as with hexamethylenetetramine. Four showed no reactions after 24 hours to any of the 7 patches applied. Five showed positive reactions to one or more of the resins, and of these 5, 3 showed positive reactions to hexamethylenetetramine.

Two of the 4 cases who showed no reaction had eruptions at the time the patches were applied. One was a case of dermatophytosis and the other an eczema which dated back to childhood. The weather was cold (less than 70°) during the period the patches were on, and this may have a bearing on the reactions, because, of the 26 cases reported in 1934, 18 had occurred during periods of hot weather in May, June, July, and August.

There can be little doubt that a considerable percentage of the dermatitis in this plant was of occupational origin.

That hexamethylenetetramine is a major causative factor, is shown by the fact that most of the cases occurred in workers handling the phenol-formaldehyde resin, which contains more of it than does the urea-formaldehyde resin.

Hexa, which was extensively used in the rubber industry as an accelerator and caused dermatitis, has now been almost entirely displaced by other accelerators. It is necessary in certain phenol-formaldehyde molding resins in order to furnish the necessary amount of formaldehyde and ammonia required to go through the gamma stage—or to completion.

In the urea-formaldehyde resin, hexa acts as a stabilizer to prevent the resin from hardening before it is molded.

The ventilating and dust prevention conditions in the molding plants were poor. There were no suction hoods over the molding machines. The suction hood over the "pill machine" was out of order. There was only window ventilation in the workrooms, and wash-ups or showers after work were not compulsory. With proper dust allaying facilities, such as forced ventilation, suction hoods, wet sweeping of floors after each shift, compulsory showers, protective clothing and ointments, the incidence of dermatitis in this plant could be decreased.

Dermatitis may occur in users of molded phenol-formaldehyde and urea-formaldehyde resin wares if the hexa is not all combined. Theoretically, it is all combined in the completed resin, but practically (as in some imperfectly cured pieces or pieces in which there was too much hexa in the molding compound to be completely taken up), there may be a sufficient amount remaining to cause dermatitis in hypersensitive people. The same thing may be true of the phenol, if not completely combined.

Dermatitis has been reported as due to contact with finished resin products, such as dermatitis of the ear from telephone receivers, and of the hands from varnishes.

In order to show that a dermatitis may have been caused by contact with a resin, it must be established that:

1. The dermatitis followed contact with the resin
2. That portion of the skin which came in contact with the resin was first affected
3. A powdered portion of the suspected resin placed on the clear skin near the eruption in the form of a patch test, gives a positive reaction if left on 24–96 hours

To determine the actual chemical in the resin which caused the dermatitis,

the nature and chemical composition of the resin must be determined, and patch tests performed on the patient with each of the chemicals composing the resin in order to determine to which of them the patient is hypersensitive.

The patches of the chemicals should be in such dilutions as will not cause reaction on the normal skin if allowed to remain on for 24 hours.

A 2 per cent aqueous solution of phenol can remain on the normal skin in the form of a patch test, for 24 hours without causing a reaction.

A 4 per cent solution of formaldehyde can remain on the normal skin, in the form of a patch test, for 24 hours without causing a reaction.

Powdered hexamethylenetetramine may remain on the normal skin, in the form of a patch test, for 24 hours without causing a reaction.

Powdered coumaron resin may remain on the normal skin, in the form of a patch test, for 24 hours without causing a reaction.

Coumaron resin is used in varnishes, adhesives, rubber, paint, chewing gum, lacquers, paper and fabric sizing, printing inks, and waterproofing.

It is made from the crude coal tar distillate, which comes off between 150° and 200° C.

This distillate is re-distilled to remove impurities such as benzol, toluol, zylol, naphthalene and tar acids—and a sharply fractionated naphtha is obtained. The naphtha is dried in lead-lined or stone-ware receptacles by treatment with H_2SO_4, in the proportion of 3 to 5 parts per 1,000. After standing a while, the acid is drawn off and fresh H_2SO_4 is added slowly and agitated until 3 to 5 per cent of acid is added. The kettle is kept cold during the reaction with brine-cooled refrigeration.

After the reaction takes place, the mixture is allowed to stand until the tar and sludge settle. They are then removed and the remaining oil is pumped to a neutralizing tank and treated with caustic soda to destroy the remaining acid. The oil is then washed with water to remove the soluble matter, and it is allowed again to settle and the water is removed.

The oil is now distilled and the solvent naphtha is distilled off and the naphthalene is removed by live steam. After this, there remains a heavy oil boiling at 320°–330° C., which is removed from the resin.

COUMARON RESINS

There are no skin hazards, outside of acid and alkali burns, in these operations, because they are totally enclosed. No skin lesions were found in a large plant and the medical records for a number of years showed no cases of dermatitis.

Dermatitis has occurred from coumaron resin in varnish which was used on heddle frames in a cotton mill. The skin on the forearms of the weavers was struck continuously by the moving heddle frames and some developed dermatitis. Patch tests showed that they were sensitive to chlorinated ceresin and coumaron resin in the varnish. The varnish grades of coumaron resin, especially the darker resins, may contain sulphuric acids resulting from the sulphuric acid treatments, if they are not carefully prepared, and it is these acids that cause dermatitis.

SYNTHETIC WAXES

The principal synthetic waxes manufactured in this country are chloro naphthalenes and the chloro diphenyls. They are both used for practically the same purposes—as electric insulators on condensers, as insulators on electric wires, in paints, varnishes, and lacquers, and as oil in transformers.

The chlorinated naphthalenes are made by passing chlorine through

naphthalene and replacing of the hydrogen atoms with chlorine. One or more of the hydrogen atoms can be replaced, forming mono, di, tri, up to per chloro naphthalene, in which 8 of the hydrogen atoms are replaced with chlorine, the formula being $C_{10}Cl_8$. The more chlorine, the more solid is the material.

In making chlorinated diphenyls, it is first necessary to manufacture diphenyl, $C_{12}H_{10}$. This is manufactured by passing benzol, C_6H_6, through molten lead, at a temperature of about 800° F., where 2 molecules of benzol combine to form 1 molecule of diphenyl, hydrogen being set free.

Diphenyl is a solid, crystalline-like substance. This is melted in closed cylinders and chlorine is bubbled through, replacing the hydrogen. One or more of the hydrogen atoms can be replaced, forming mono chloro diphenyl, di chloro diphenyl, up to deca chloro diphenyl, $C_{12}Cl_{10}$. Hydrochloric acid is a by-product of this reaction. The chloro diphenyls are liquid or semi-solid, up to the hexa chloro diphenyl.

Nonochloro diphenyl, $C_{12}HCl_9$, sold under the trade name of Arachlor, is used as an insulator for automobile electric wires and in condensers, and also as a de-lusterer of rayon.

In the manufacture of the chloro diphenyls, the workers are exposed to a benzol hazard when the diphenyl is made, as well as to a hazard from the inhalation of the fumes of diphenyl.

The workers engaged in chlorinating the diphenyl, especially that part of the operation where the crude Arachlor is being re-distilled to remove impurities, are affected with an acne-like condition of the skin. This also occurs in workers exposed to the fumes of the chloro naphthalenes, or Halowax. The fumes of these compounds cause acne on the face and neck and may penetrate the clothes and cause acne-like lesions to develop on the covered parts, the shoulders, and the belt-line, and even on the penis. The lesions on the skin resemble acne. They begin as small, pale, elevated papules, many having no openings in them. They develop into hard cyst-like elevations under the skin, some of which go on to suppuration, forming boils. Some of the lesions also occur at the mouth of the follicles and resemble the comedones and pustules of acne vulgaris.

In addition to these skin lesions, symptoms of systemic poisoning have occurred among workers inhaling these fumes. Those working with the chloro diphenyls have complained of digestive disturbances, burning of the eyes, impotence and hematuria. The latter symptom developed among a number of men making amino diphenyl, which is used in the manufacture of a rubber antioxidant. Cases of death from yellow atrophy of the liver have been reported among workers exposed to the fumes of the chloro naphthalenes.

Patch tests performed with Halowax and with the chloro diphenyls have yielded negative results. The skin lesions probably result from the mechanical plugging up of the follicles of the skin with the waxes as the fumes solidify on the skin.* The chlorine present in the waxes may have an irritating effect on the plugged follicles and cause suppuration.

PREVENTION

1. The protection of the workers from the irritating chemicals that compose the resins and waxes from the resins and waxes themselves. To do this, the process should be totally enclosed. If this is not possible, hoods with suction exhaust should be so placed over open processes that dust and fumes are pulled away from the worker and out of the room.

*I have recently seen the wife and child of a worker who had developed comedones and pustules from contact with his work clothes which were saturated with halowax and which he was accustomed to wear at home.

L. S.

2. The workrooms themselves should be ventilated by intake and exhaust fans to remove dust and fumes.

3. The floors, walls, and ceilings should be washed down at frequent intervals to keep them free of dust.

4. Two lockers should be furnished to each worker. One for his street clothes and one for his work clothes. The lockers for street clothes and work clothes should be in separate rooms, with the shower baths between the locker rooms. The worker coming to work enters the locker room for the street clothes, takes them off, and puts them in the locker and goes into the locker room where his clothes are kept and dons them. From this room he goes to the workrooms through a connecting door. At the end of his shift, he goes through this door to the work clothes locker room, takes off his work clothes and leaves them on the floor or bench to be washed and then goes to the shower baths and bathes and dries. Then he goes to the street clothes locker room, puts on his clothes and goes out of the door leading to the street. It has been estimated at one plant where such a system was instituted that 6 cents a day per worker will take care of furnishing clean work clothes each day.

5. New workers who are hypersensitive, but have only mild eruptions, should be given protective ointments and clothing and kept at work for about 3 or 4 weeks with the hope that they will develop an immunity or become "hardened." If this does not occur, they should be taken off the job.

6. New applicants for jobs should be carefully examined for skin diseases and those found to have them should not be employed.

7. There should be periodic medical examination of workers to detect cases of dermatitis and workers in chlorinated naphthalenes and diphenyls should be periodically examined for symptoms of systemic poisoning.

8. Laws should be passed making it compulsory for factories where there are skin hazards to adopt these measures.

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A.I.C.P. Guide for Public Health Nurses

A *GUIDE for Public Health Nurses* published by the New York City Association for Improving the Condition of the Poor, is a unique publication. A systematic description of cardiac conditions, for the benefit of public health nurses and a guide to the care which they can give patients has recently been prepared by the Bureau of Educational Nursing and has been subjected to the criticism of outstanding cardiologists.

Similar guides covering syphilis, child development, and tuberculosis have recently been revised and, although intended for the nursing staff of the A.I.C.P., they will readily be of interest to a wider circle.

LIVER LESIONS CAUSED BY CHLORINATED NAPHTHALENE [1]

By

FREDERICK B. FLINN AND NORMAN E. JARVIK

(Received for publication July 21, 1937)

In this country nine cases of yellow atrophy of the liver have occurred in shops in which chlorinated naphthalenes are being used to impregnate wire and other electrical equipment. The total number of employees exposed in the three plants was approximately 250. The fact that three of these cases have occurred, in each of three different plants, all widely separated and under different management but using the same type of material, indicates the possibility of a common etiological factor. The average time of exposure varied from 4 to 6 months.

Yellow atrophy of the liver is a rare disease. Osler [2] reported three cases among 28,000 patients examined. The records of 5,000 autopsies at the Presbyterian Hospital in New York, show three acute cases and fifteen sub-acute cases. Up to 1913, Umber [2], in Germany, had not seen a single case among 84 autopsies performed. In 1920, he reported two cases in 200 autopsies, and in 1921, eleven in 149 autopsies. Seyfarth [3], also of Germany, had had only one case up to 1915; but between 1915 and 1921 he found twenty-nine cases of yellow atrophy. The increase in the number in Germany appeared during the World War, at which time the use of chlorinated naphthalenes also increased. The majority of the cases, however, occurred in women during the middle or latter half of pregnancy, and no record of exposure to chlorinated naphthalenes in these cases has been reported.

We have been unable to find reports in the literature of any case of yellow atrophy occurring among the workers exposed to chlorinated naphthalenes either in this country or Europe. The hazard commonly recognized has been a cutaneous one, the lesion being in the

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We want to thank Dr. William C. Von Glahn for his assistance in studying the pathology of the animals autopsied.

form of an acne comedon. One hundred and two cases of dermatitis were found among 124 workers in Pennsylvania and a few mild cases in another plant. These cutaneous lesions have been reported by Wauer (4), Koelsch (5) and Teleky (6) under the name of "Perna Disease," which had resulted from exposure to a perchlornaphthalene. Perna wax is, in general, a mixture of chlorination products of naphthalene. It is yellow to dark brown in color, soluble in fats, contains 44 to 45 per cent of chlorine and sublimes at 130° C. Teleky showed that the cause of cutaneous injuries was definitely due to the easily volatile chlorine compounds contained in "Perna" to the extent of only 5 per cent. Lehmann (7) experimented with animals, and found that this chemical produced lesions of the liver which resembled acute yellow atrophy. Observers in France and Germany agree that in spite of the claim of individual susceptibility, 50 per cent of the workers showed cutaneous lesions.

The failure to find reports of any cases of atrophy among industrial workers exposed to chlorinated naphthalene may be explained by the history of similar industrial diseases. The medical profession at the beginning did not recognize the connection of the pathologic condition with an industrial exposure. Frequently, correct diagnoses are not made when the first cases make their appearance. In this instance the first cases in each locality were diagnosed as catarrhal jaundice in spite of the fact that catarrhal jaundice is rarely fatal. Post mortem examination which revealed the correct nature of the disease was not made until the second or third case appeared.

In the factory to which we were called to determine the etiological agent we found three types of chlorinated naphthalenes in use. They are sold under the name of halowaxes and, in this paper, will be designated as compounds *A*, *B*, and *C*. Compound *C* was introduced into the factory at the beginning of the 6-month period during which three cases occurred.

Compound *A* is a mixture of tri- and tetrachlornaphthalene. Compound *B* is a mixture of tetra- and pentachlornaphthalene and may contain some trichlornaphthalene. Compound *C* is a mixture of penta- and hexa-chlornaphthalene which is plasticized with a relatively small percentage of asphalt.

A boils between 600 and 650° F.; *B* boils between 615 and 655° F.; *C* boils between 680 and 730° F.

We determined the chlorine content and the melting point of each of these materials. The sublimate given off by *B* at 192° C. and that given off by *C* at 172° C. were collected and analyzed.

Compound	Chlorine percentage	Melting point
A	47.9	89 – 90° C.
B	49.5	117.5–122° C.
C	47.9	123 –132° C.
B sublimate	35.3	87 – 89° C.
C sublimate	53.1	117 –123° C.

While the circumstantial evidence pointed to the chlorinated naphthalenes as the etiological agent, we included in our study other materials that were being used in the plant during the period in which the disease developed. These substances were found not to produce the liver lesions. We therefore concentrated on the naphthalenes and used them in amounts that would be certain to produce results if they were at all toxic. In the *preliminary part of the study* we were not interested in determining the *minimum toxic dose*.

The first symptoms noted by the affected employee were a "run-down" condition and jaundice. As the jaundice increased there appeared an increase in general malaise, anorexia, attacks of dizziness, loss of weight and a tendency to vomit. Severe abdominal pains across the upper quadrant were noticed. The blood picture was normal except for a low blood sugar which in several cases was around 65 mgm. per 100 cc.

A typical gross autopsy finding showed an extensive necrosis, fibrosis and regeneration of the liver; acute enterocolitis with edema, fibrosis of the pancreas, acute pancreatitis, jaundice, ascites, edema of lower extremities, and subpleural hemorrhages.

EXPERIMENTAL

It has been our experience that the rabbit is the best laboratory animal for the study of liver lesions. Rabbits weighing approximately 2 kilos were used in the study. We also included a few rats and guinea pigs in these experiments, which brought the total number of animals used up to two hundred.

Dose. Fortunately for our investigation the State Division of Occupational Hygiene of Massachusetts had made a test of the air in the plant we were investigating when the last case appeared. Their findings are as follows: Rolling Department, 1 to 2 mgm. of wax per cubic meter of air; Soldering Department, 1 to 2 mgm. of wax per cubic meter of air; Impregnating Department, 11 to 20 mgm. of wax per cubic meter of air.

Our observations in this plant and laboratory experiments made

cells remain they often have compact, blue-staining areas in the cytoplasm. In this section, as in rabbit no. 533, there are wide spaces bordered by liver cells and at times containing large plugs of bile. It can be clearly seen that these spaces are distended bile canaliculi. Calcification is present in many of the areas of necrosis, stroma as well as liver cells. Large foreign body giant cells are found about the calcium. Some of the liver cells contain large droplets of fat, but total amount of fat is not great. Many liver cells contain bile. Cirrhosis negative: no proliferation of bile ducts.

Iron stain: Heart: No pigment. Lung: No pigment. Spleen: Small amount of blue pigment. Liver: Pigment red. Kidney: Very small amount of fine, red-staining pigment in epithelial cells of convoluted tubules.

For the purpose of determining the effects of smaller amounts of chlorinated naphthalenes we fed guinea pigs 5 mgm. per kilo of compound *B* for from 2 to 5 months. The pigs showed no clinical symptoms before death. The autopsies presented very much the same type of lesions as in the rabbits which had been receiving the large dose by injection. The liver surfaces were pebbled, and yellow areas beneath the capsule and deeper into the organ and areas of necrosis involving entire lobules were common.

A group of rabbits was injected with 2 mgm. of compound *B* per kilo of body weight. After a period of time sores began to appear in the skin. Histological section of these sores showed them to be areas of multiple, small necroses. As they only occurred in the chlornaphthalene animals we feel that this condition is due to the exposure.

The rabbits were killed after 7 months and autopsied. The picture of the liver showed the same type of necroses and yellow areas.

Our findings indicate that this small exposure does affect the liver of the rabbit although in these cases it will take a longer period of time to produce the lesions obtained by shorter exposure to the larger amount.

Discussion

From the facts presented one arrives at the conclusion that certain of the commercial, chlorinated naphthalenes are toxic if they are absorbed by the body. This conclusion is warranted by the following facts: (1) there was 100 per cent fatality among the animals receiving injections of compounds *B* and *C* and sublimate *C;* (2) the livers of the animals receiving the injections showed the early stages of acute atrophy of the liver; (3) in spite of the rarity of this disease there occurred three human cases of yellow atrophy of the liver in each of three widely separated plants all using the same type of material.

Whether the toxic material is the chlorinated naphthalene itself, or some impurity contained in the commercial product, is the subject of further study. However, one may take into consideration the following facts. Several addition compounds of naphthalene are known. They are probably the intermediate product of naphthalene, such as penta- and hexachlornaphthalene. One would expect the chlorine addition products of naphthalene to be relatively unstable. As a matter of fact, the simplest one, naphthalene dichloride, slowly evolves HCl at temperatures as low as 40° to 50° C., giving the relatively stable monochlornaphthalene, a true chlorine substitution product. The higher chlorine addition products, and the compounds containing both the substituted and added chlorine which may be present as impurities, are more stable than naphthalene dichloride but, under certain conditions, they will give up HCl to form true chlorine substitution products.

This is only a working hypothesis. However, the observation can be explained by assuming the presence of a trace of these relatively unstable addition products, which may be absorbed by the skin and there give off HCl but which, upon suitable heating, would be partly or completely destroyed so that the vapors would be relatively less toxic. This may be the explanation in the case of compound *B*. In the case of compound *C* one must conclude either that the toxic impurities, if any are present, are not destroyed by simple heating or that this particular polychlornaphthalene is toxic *per se*. It is recognized that batches of chemically pure naphthalene will chlorinate in different ways at times.

Summary

1. A 100 per cent fatality occurred when rabbits were injected subcutaneously with chlorinated naphthalenes *B* and *C* and the fumes of *C*. Guinea pigs fed by mouth gave the same result.

2. The liver lesions are those found in the early stages of yellow atrophy of the liver. After a longer exposure, small doses will cause lesions similar to those produced by the larger doses.

3. The cases of yellow atrophy of the liver among the employees using these chlorinated naphthalenes are probably due to an exposure to some chlorinated naphthalene.

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THE PROBLEM OF POSSIBLE SYSTEMIC EFFECTS FROM CERTAIN CHLORINATED HYDROCARBONS*

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THE use of chlorinated naphthalenes and compounds of allied pharmacological possibilities is extremely wide, and with the steady growth of the use of electricity is certain to expand much farther. For years it has been known that many of these compounds cause a troublesome acne, and there is a large literature upon this phase of the subject. Our investigations have not been concerned with chloracne but with the possibility of systemic effects following ingestion or inhalation of such products. In the spring of 1936, the Halowax Corporation, a division of the Bakelite Corporation, called our attention to three fatal cases of jaundice in workmen using chlorinated naphthalenes and chlorinated diphenyl, and requested that the subject be investigated as rapidly and thoroughly as possible.† In brief these cases were as follows:

Patient 1. Male, age 21. The previous medical history of this man was in no way significant except for the fact that he had an attack of jaundice about 6 weeks prior to his fatal illness. Late in December, 1936, he became badly constipated and had much abdominal pain and distention. When admitted to the hospital he was slightly jaundiced and was evidently very ill. He was somewhat anemic and his skin, particularly upon the arms, face, chest and back, showed many pustules. He died after a brief period in the hospital, and at autopsy was found to

* Received for publication June 30, 1937.

† The Halowax Company makes many products besides chlorinated naphthalenes, and it has come to our knowledge that all of these products are indiscriminately called "halowaxes" by purchasers and users, and are lumped together as possible causers of acne and even of systemic disease. Since "halowax" is merely a trade designation, care should be taken to describe compounds by their chemical names and thus avoid condemnations which are both troublesome and misleading.

have a cirrhosis of the liver with acute yellow atrophy superimposed upon it. This man had been exposed to low concentrations of vapors arising from a mixture of tetra and pentachlornaphthalenes, together with approximately 10 per cent of a refined chlorinated diphenyl. While both he and others engaged in the same work had chloracne, there were no other disturbances of health in fellow workmen, nor was there any precipitating cause for the acute yellow atrophy such as treatment with arsphenamine or exposure to dangerous concentrations of carbon tetrachloride.

Patient 2. This was a young man who died in February, 1936, after an acute illness characterized by jaundice. He had been exposed to fumes arising from a mixture of penta and hexachlornaphthalenes. There is no record of chloracne. The patient worked with a large number of other people of whom but one (Patient 3), a close friend, had significant illness.

Patient 3. Another young man employed with Patient 2. He became jaundiced in March, 1936, and died after an illness of 2 weeks. A careful autopsy resulted in a diagnosis of acute yellow atrophy of the liver. Here again no history could be obtained as to a precipitating cause, and there was no record of preceding attacks of jaundice.

In addition to these three very recent fatalities, we have learned of four other possible cases, none of them fatal. All of these have had jaundice and the entire group consists of isolated individuals who have been picked out of large groups having the same exposure. In but one instance, Patient 1, is there record of antecedent disturbance of health, and the general health of fellow workers has been good.

Such cases have not been reported in the medical literature and only occasionally can one find reference to systemic effects of any sort. For example, Courtois-Suffit (1934) reports on work done by Touraine and his associates (1934) who examined 60 workers who had been exposed to trichlornaphthalene. Of these 13 were found to have mild digestive complaints, anorexia, nausea and vertigo, but Courtois-Suffit remarks finally, "Absorption is certainly possible and we have for proof of it some of the digestive and general complaints which have been due to it. But they appear to be of little consequence considering the mildness of the digestive troubles and the absence of respiratory phenomena."

In Touraine's cases the exposure was to a trichlornaphthalene, whereas the American cases of acute yellow atrophy were exposed to compounds of higher chlorination. Our own experiments indicate that trichlornaphthalenes require enormous dosage, far beyond anything encountered in industry, in order to produce liver damage. Teleky (1927) reported a number of cases of chloracne in persons exposed to chlorinated naphthalenes with a chlorine content ranging from 14 to 53 per cent. He found that the lower the chlorine content the less the acne. Mittelstädt (1935) examined a number of cases of chloracne due to trichlornaphthalene and reported a number of vague general complaints but nothing in the nature of serious disease. Regarding his animal experimentation, Lehmann (1919) reported that animals fed chlorinated naphthalenes refused to eat after a time and that, whether poisoned by inhalation or by feeding, at death showed "peculiar" lesions in the liver. Flinn and Jarvik (1936) gave subcutaneous injections of enormous doses of chlorinated naphthalenes dissolved in paraffin oil to rabbits. The compounds used were as follows:

1. A mixture of tri and tetrachlornaphthalene.
2. A mixture of tetra and pentachlornaphthalene.
3. A mixture of penta and hexachlornaphthalene.

In addition, sublimates from (2) and (3) were collected in oil and injected subcutaneously.

None of the animals receiving (1) or the sublimate from (2) died, and even after 2 months were quite normal when autopsied. The first death in the animals receiving (3) occurred on the 12th day and the last died on the 26th day. Those receiving the sublimate from (3) were even more severely affected. Autopsy in these animals revealed striking changes in the liver, not, as described, entirely characteristic of acute yellow atrophy but sufficiently suggestive to cause the authors to conclude that "certain chlorinated naphthalenes or impurities contained in them are capable of producing yellow atrophy of the liver in the rabbit."

At the beginning of their paper, Flinn and Jarvik (1936) mention the fact that there have been three cases of acute yellow atrophy of the liver in men working with chlorinated naphthalenes but give no details in regard to them. These cases are undoubtedly the same as those described in the beginning of this paper.

One may summarize the meagre literature upon systemic effects from these substances as follows:

1. With the exception of the mention of acute yellow atrophy by Flinn and Jarvik (1936) there are no reports or even suggestions of serious effects upon human beings.

2. There is evidence (Teleky, 1927) that the degree of chlorination is significant in relation to the production of acne. In the work of Flinn and Jarvik (1936) the compounds producing serious liver injury were the most highly chlorinated of those tested, though the chlorine contents as given by analysis vary surprisingly little.

3. There are no published figures upon the amounts of various chlorinated naphthalenes in the air which will produce injury of any sort, and while the work of Lehmann (1919) and of Flinn and Jarvik (1936) point to the liver as a possible site of injury this indication rests upon such extreme dosage as to fail to apply directly to human exposure.

Experimental Work

In appraising the possible toxicity of any substance met in industry it is first necessary to determine the principal route of absorption. In the case of the compounds under consideration there can be no doubt that inhalation is their chief means of entering the body. They are used hot in a great variety of operations and volatilize in varied degree. They are often applied in solution in such volatile solvents as carbon tetrachloride and toluene. The amounts reaching the air under such circumstances are hardly detectable. It will however be shown that carbon tetrachloride adds to the toxicity of the chlorinated naphthalenes and allied compounds, and if there is possibility of inhaling these compounds in other parts of the factory then inhalation of carbon tetrachloride adds a decided hazard. Under such circumstances solvents such as toluene should be used.

Observation in a number of plants causes us to feel that even though

their initial loss in weight and were gaining. The liver lesions were similar to those found in rats fed penta and hexachlornaphthalenes plus 10 per cent chlorinated diphenyl but not so marked.

Summary of gross feeding experiments.—Of the various materials fed rats in large doses trichlornaphthalene plus traces of tetrachlornaphthalene was quite innocuous. Tetra and pentachlornaphthalene showed definite liver damage. Penta and hexachlornaphthalenes caused a similar grade of injury. The addition of chlorinated diphenyl to penta and hexachlornaphthalenes increased the toxicity. Chlorinated diphenyl alone produced liver lesions but in the dosage used was less effective than when mixed with highly chlorinated naphthalenes. In no case did the compounds used produce acute yellow atrophy but the lesions observed indicate this might be possible if one found a dosage which could act for the proper period of time.

Feeding Precise Doses by Stomach Tube

The compounds employed were suspended in gum acacia. In figuring the dosage the total amount a man of 50 kg. would inhale in an 8-hour day assuming an air concentration of 20 mgm. per cu. m. was first calculated and reduced to milligrams per kilogram. The rats and rabbits received this dose each day. The compounds used were those employed in the gross feeding experiments and the results were essentially similar though the lesions were less severe.

Subcutaneous Injections

The same gum acacia suspensions were injected subcutaneously into rats and rabbits, the dosage being calculated on the basis of 4 mgm. per cu. m. of air. Again similar results were obtained. In all such experiments there must of necessity be differences in the degree of effect but invariably the liver was the sole organ affected and the lesions were those already described many times.

Discussion

These experiments leave no doubt as to the possibility of systemic effects from the chlorinated naphthalenes and chlorinated diphenyl. As in the case of the effects upon the skin, the degree of chlorination seems to determine the systemic toxicity, and it is a striking thing that when trichlornaphthalene is reached systemic effects are never marked and are produced with the greatest difficulty. It is most remarkable, too, that all the compounds tested attack the liver and the liver alone. During the past few months we have determined the organically combined chloride in the livers of animals very severely poisoned by penta and hexachlornaphthalenes but have found no increase over normal figures, though the livers, as determined histologically, were very severely affected. At the present time we are conducting inhalation experiments on a chlorinated diphenyl containing 55 per cent of chlorine instead of 64 per cent as in the case of the experiments reported in this paper and on a compound with a chlorine content between tri and tetrachlornaphthalene. We are also determining the degree to which the diet may increase or decrease toxicity, this being suggested by similar work upon carbon tetrachloride.

In the basis of these experiments

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MORPHOLOGICAL CHANGES IN THE LIVERS OF RATS RESULTING FROM EXPOSURE TO CERTAIN CHLORINATED HYDROCARBONS*

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CHLORINATED hydrocarbons, particularly chlorinated naphthalenes and chlorinated diphenyl, have been used extensively in certain industries. Their use in the manufacture and preparation of many types of electrical equipment is constantly increasing. Although it is known that some of these compounds cause acne, only recently has the possibility of more serious systemic effects been recognized.

During the spring of 1936 we were informed of the occurrence of three fatal cases of jaundice in workmen using chlorinated naphthalenes and chlorinated diphenyl. At the request of the manufacturers of these compounds, we undertook an investigation to determine what systemic effects, if any, would result from the administration of a number of these compounds to experimental animals.

The known findings in the three fatal cases of jaundice, together with a review of the pertinent literature have already been reported (1).

The present paper describes the pathological changes observed in rats that had been exposed to various chlorinated naphthalene compounds and to chlorinated diphenyl.

MATERIALS AND METHODS

A detailed description of the apparatus and technic employed by the authors in this investigation has been published (1). White rats, maintained on a diet of Purina Dog Chow, supplemented by lettuce, eggs, milk, and cod liver oil, were used throughout the experiments.

* Received for publication September 5, 1937.

This is the second of three papers read at a symposium on chlorinated hydrocarbons given at the Harvard School of Public Health, Boston, June 30, 1937. The third paper will appear shortly.

The chlorinated hydrocarbons tested were the following:

Compound A. A mixture of tri- and tetrachlornaphthalenes.* Chlorine content 49.4 per cent.

Compound B. A mixture of tetra- and pentachlornaphthalenes. Chlorine content 56.4 per cent.

Compound C. A mixture of tetra- and pentachlornaphthalenes plus chlorinated diphenyl. Chlorine content 43.5 per cent.†

Compound D. A mixture of penta- and hexachlornaphthalenes. Chlorine content 62.6 per cent.

Compound E. A mixture of penta- and hexachlornaphthalenes. Chlorine content 62.6 per cent.

Compound F. A mixture of 90 per cent penta- and hexachlornaphthalenes, plus 10 per cent chlorinated diphenyl. Chlorine content 63 per cent.

Compound G. Chlorinated diphenyl. Chlorine content 65.0 per cent.

These preparations were selected because of their relative importance in industry. It should be noted that they also represent a wide range of chlorination.

All of the above materials were administered orally in varying doses. Compounds A, D, F, and G were selected for the inhalation experiments.

*This compound contained only small amounts of tetrachlornaphthalenes. In this paper it will therefore be referred to as trichlornaphthalenes.

†This compound consisted of a mixture of compounds B and G but in addition contained two plasticizers which have been considered to be inert. Without these materials the chlorine content of this compound would be between that of compounds B and D.

Inhalation Experiments

We were most interested in the results of the inhalation experiments because they more nearly simulate the type of exposure to which the workmen are subjected (1). The inhalation experiments were carried out in air-tight wooden compartments through which air, containing the volatilized compound being tested, was driven by electrical blowers. With this apparatus we were able to test simultaneously the effect of four compounds. Eighty rats were exposed to each compound. The methods for determining the daily air concentration (mgms. per cu. m.) of the chlorinated hydrocarbon being used, as well as the rate of airflow have been described (1).

Compound A (trichlornaphthalenes) was administered by inhalation to two groups of animals. In the first of these experiments, the rats were exposed to low air concentrations (average 1.31 mgms. per cu. m.) 16 hours daily for 134 days. In the second experiment the concentration was increased to an average of 10.97 mgms. per cu. m. This 16 hour daily exposure was continued for 102 days.

Compound D (hexa- and pentachlornaphthalenes) was administered to three groups of rats. In the first experiment the exposure consisted of 16 hours daily for 134 days, with an average air concentration of 1.16 mgms. per cu. m. In a second experiment the average air concentration was 1.44 mgms. per cu. m. This was maintained 8 hours daily for 143 days. In the final experiment, the average air concentration was increased to 8.88 mgms. per cu. m. and exposure, 16 hours daily, was continued over a period of 52 days.

Compound F (hexa- and pentachlornaphthalenes 90 per cent plus chlorinated diphenyl 10 per cent) was administered in low concentrations to two groups of rats. In the first experiment an average air concentration of 1.37 mgms. per cu. m. was maintained 16 hours daily for 134 days. In the second experiment, an average air concentration of 1.66 mgms. per cu. m. was inhaled 8 hours daily for 143 days.

Compound G (chlorinated diphenyl) was administered to two groups of animals in low concentrations. An average concentration of 0.57 mgms. per cu. m. was employed 16 hours daily for 134 days in the first experiment. In the second experiment (employing an average air concentration of 0.93 mgms. per cu. m.) the animals were exposed 8 hours daily for 143 days.

Feeding experiments

All compounds tested were employed in the feeding experiments. A weighed amount of finely ground material was mixed with rat food. This mixture was placed daily in one food container to supply the rats in a given cage. Thus each animal had an equal opportunity to ingest the compound supplied. As the number of rats per cage was reduced, the dosage supplied was proportionately decreased. Most compounds were supplied in large and small amounts.

Supplementary experiments were conducted on a smaller number of rats by feeding several of these compounds in small known amounts by stomach tube or by injecting them subcutaneously as suspensions in gum acacia.

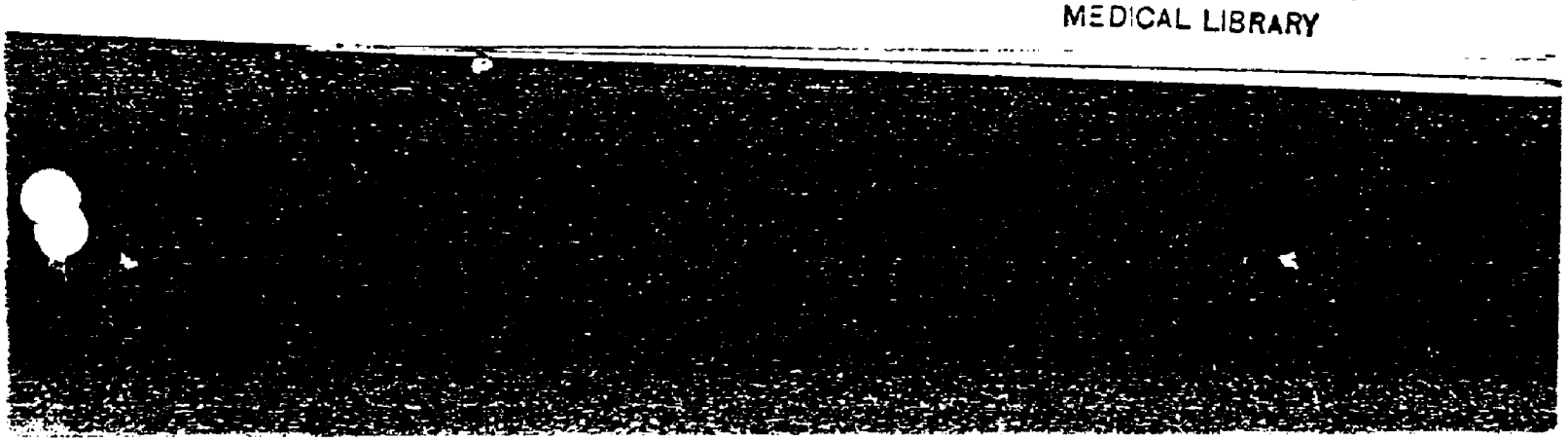
Carbon tetrachloride and alcohol administration to experimental animals

Seemingly the incidence of acute yellow atrophy in workers exposed to chlorinated naphthalenes and chlorinated diphenyl is very low. This single fact is of some importance in that it suggests that certain individuals may be more susceptible to the compounds or that in these instances the liver damage may have been intensified by some other agent. With this in mind, groups of animals that had been exposed for varying periods of time to certain chlorinated naphthalene compounds and chlorinated diphenyl were subsequently given a sublethal dose* of carbon tetrachloride and ethyl alcohol by stomach tube (0.75 cc. of each per kgm.). This dosage did not result in a single death among the 16 control animals and the degree of liver damage produced was quite constant and never very great. Administration of carbon tetrachloride and alcohol to rats exposed to the more highly chlorinated naphthalene compounds and chlorinated diphenyl caused extensive liver damage and proved widely fatal. Therefore, the use of carbon tetrachloride and alcohol has been employed regularly on representative groups of rats from each inhalation experiment.

Examination of tissues

Animals were sacrificed for pathological examination after varying periods of exposure. Complete autopsies were performed. Liver weights were recorded. In representative animals

* One cubic centimeter of carbon tetrachloride plus 1.0 cc. ethyl alcohol per kilogram when administered orally to normal white rats causes a 14 per cent mortality (2).



from each experiment, all organs excepting the central nervous system were examined microscopically. The livers of all animals were studied microscopically. Tissues were fixed in Zenker's fluid, 10 per cent formaldehyde solution, and absolute alcohol. The routine paraffin sections were stained with eosin methylene blue. Other stains employed were hematoxylin and eosin, phosphotungstic acid hematoxylin (Mallory), Foot's modification of Bielschowsky's stain (reticulum), Best's carmine stain, and Iron reaction with ferrocyanide of potassium (Mallory). In addition, frozen sections from certain liver specimens were stained with scarlet red, Lugol's solution, and methyl violet.

Results

Compound A. Trichlornaphthalenes

(a) *Exposure by inhalation to low concentrations (average 1.31 mgms. per cu. m.) 16 hours daily.*—The 80 rats subjected to these conditions showed no ill effects. Autopsies were performed on groups of 3 to 8 animals after 37, 72, 105 and 134 days' exposure. There were no significant abnormalities in liver weights.* Macroscopically the majority of the livers appeared normal although an occasional one was paler than normal. Rarely slight mottling was observed. Microscopically, the liver cells often appeared slightly enlarged, more granular than normal, and occasionally they were vacuolated. These slight abnormalities and the presence of a rare mitotic figure suggested that very slight injury to the liver cells had occurred. There was no demonstrable increase in the above changes after the first exposure period (37 days). Occasional livers of rats exposed for the total period showed

* Calculations pertaining to the weights of livers in these and subsequent animals are based on data in H. H. Donaldson's book "The Rat," 2nd ed. The Wistar Institute, Philadelphia, 1924 (p. 211).

PLATE I

FIGS. 1 AND 2. Camera lucida drawings showing the portal areas of the livers of two rats that were fed large doses of a mixture of tetra- and pentachlornaphthalenes and chlorinated diphenyl. The changes illustrated in figure 1 occurred within 48 days after exposure was begun. One should note the marked accumulations of hyaline globules in the cell cytoplasm, and the marked swelling of liver cells. Increased numbers of mitotic figures were also present. Similar changes are apparent in figure 2 which was made from the liver of a rat exposed 125 days. Degeneration in the central portions of the liver lobules is also present.

FIGS. 3 AND 4. These drawings illustrate the marked swelling and fatty vacuolization of liver cells observed in all rats that were fed small doses of tetra- and pentachlornaphthalenes. The changes shown in figure 3 were present after 26 days' exposure, those in figure 4 after 48 days' exposure. Note the mitotic figures in figure 3. The compound responsible for the changes contained no chlorinated diphenyl. Otherwise it was similar to the compound responsible for the lesions illustrated in figures 1 and 2.

FIGS. 5 AND 6. Camera lucida drawings of the portal areas of the livers of two rats fed moderate sized daily doses of a mixture of penta- and hexachlornaphthalenes for 14 and 29 days respectively. In the first of these figures one notes swelling of liver cells with narrowing and distortion of the sinusoids. The liver cells show an increased granularity of the cytoplasm with a massing of basophilic granules near the nuclei. There is also an excess of fat in small and medium sized vacuoles. This was more prominent in the central portions of the liver lobules. An increased spacing between liver cells and between liver cells and sinusoidal endothelium is present. After 29 days' exposure (fig. 6) the above changes are greatly increased. Numerous large intercellular spaces containing serous precipitate, strands of fibrin and leucocytes had developed between liver cells. These spaces were bounded by distorted liver cells. See also plate III, fig. 1.

Camera lucida drawings X 205

no definite microscopic change. (See fig. 3, plate II.) All other organs were normal.*

Further evidence that the liver tissue injury had been very slight was obtained from the carbon tetrachloride and alcohol test. Of 10 rats exposed for 144 days in the above manner and then fed 0.75 cc. per kgm. each of carbon tetrachloride and ethyl alcohol by stomach tube, none died. At autopsy and on microscopic examination there was little or no evidence of liver injury greater than that produced by the same dose of carbon tetrachloride and alcohol in normal control rats. (See plate IV, figs. 1 and 2, and plate V, fig. 1.)

The livers of 8 animals exposed to trichlornaphthalenes for 105 days and autopsied after a 2 month recovery period showed no definite pathological change. This is further evidence that this compound is only slightly toxic. These findings represent a marked contrast to the persistent hepatic lesions resulting from exposure to the compounds of higher chlorination.

(b) *Exposure by inhalation to high concentration (10.97 mgms. per cu. m.) 16 hours daily.*—In this experiment, the concentration of trichlornaphthalenes was approximately eight times that previously employed. Exposure was continued for 102 days. The 50 animals subjected to this concentration appeared normal. Groups of 3 to 17 rats were sacrificed after exposure periods of 31, 48, 59, 72 and 102 days. Most of the livers were pale yellow in color and slightly mottled. There was, however, no significant variation in liver weight. Microscopic examination revealed swollen liver cells with slightly increased granularity and vacuolization of the cytoplasm. Occasional degenerating and regenerating cells were observed. These changes, which were slightly more marked in the central portion of the liver lobules, were present after the first month of exposure. They

* In none of the experiments to follow were significant changes found in any organ other than the liver.

PLATE II

FIGS. 1, 2, AND 3. Liver changes resulting from the administration of trichlornaphthalenes are illustrated in these camera lucida drawings. In figures 1 and 2 are shown the most marked changes observed after prolonged (100 and 136 days respectively) feeding in daily doses of 3 gm. per 10 rats. The changes observed consisted of slight to moderate swelling of liver cells, increased granularity of the cytoplasm and finally, after prolonged exposure, fatty vacuolization of the majority of cells and complete fatty degeneration of occasional cells. Figure 3 is a drawing from a section of the liver of a rat exposed by inhalation to low concentrations of trichlornaphthalenes for 106 days. No striking or constant changes were observed in rats so treated. Occasional cells are slightly swollen and show increased granularity of the cytoplasm. A rare mitotic figure was observed in occasional sections.

FIGS. 4, 5, AND 6. Microscopic changes observed following the administration of a mixture of penta- and hexachlornaphthalenes are illustrated in these three drawings. Figure 4 shows the changes observed after feeding this preparation in 3 gm. daily doses per 10 rats, for a period of 30 days. The liver cells are markedly swollen and vacuolated. There is also necrosis and degeneration of scattered cells. Large intercellular spaces are observed. (See plate VI, figs. 1 and 2). Less marked but similar changes are observed in the livers of rats fed smaller doses of this preparation (see fig. 5). (26 days' exposure.) Figure 6 illustrates the liver cell changes that occurred after inhalation of low concentrations of penta- and hexachlornaphthalenes for 75 days. Note the swelling of the liver cells, the increased vacuolization and presence of large numbers of hyaline globules within cells. Mitotic figures were frequently observed.

Camera lucida drawings × 395.

were somewhat increased with longer exposure. After 102 days the liver cells were more markedly swollen and contained large and small fat vacuoles. Small hyaline droplets in the cell cytoplasm were occasionally observed. Mitotic figures, although never numerous, were increased in number. No structural changes had occurred in the liver lobules.

Carbon tetrachloride and ethyl alcohol were administered by stomach tube to 9 rats kept under these conditions. At autopsy their livers were slightly to moderately enlarged. They were yellow in color and the majority of them were mottled. The microscopic findings were variable. In one animal the observed necrosis was no greater than that seen after the administration of a similar dose of carbon tetrachloride and alcohol to normal control rats. The livers of 4 animals showed increased fatty degeneration in the central portions of the lobules with small necrotic foci. In the 4 remaining animals extensive central necrosis had occurred. In the most marked of these (fig. 2, plate V), the central one-half or two-thirds of each liver lobule was necrotic and in occasional areas two or more adjacent lobules had undergone degeneration. Hemorrhage and extensive leucocytic infiltration had occurred in these necrotic areas. The less damaged liver cells at the periphery of the lobules showed large numbers of mitotic figures, indicating accelerated regenerative activity.

(c) *Exposure by feeding.*—Nine of the 10 rats fed trichlornaphthalenes in doses of 3 gm. per day per 10 rats were sacrificed at varying periods of time from 9 to 136 days. The tenth rat died of a respiratory infection after 182 days' exposure. There was no significant abnormality in the weights of the livers of these animals. After 2 months' exposure, microscopic examination of the livers showed slight swelling of liver cells. This was accompanied by increased vacuolization of the cytoplasm due to the accumulation of abnormally large

PLATE III

FIGS. 1, 2, AND 3. Liver changes resulting from the administration, by feeding and inhalation, of a mixture of penta- and hexachlornaphthalenes (90%) and chlorinated diphenyl (10%) are illustrated in these drawings. Figure 1 is a drawing made from a section stained with P.T.A.H. In addition to other changes it demonstrates the presence of large and small intercellular spaces, interpreted as dilated bile capillaries. These spaces contained serous precipitate, fibrin, and occasional leucocytes. Note the sharply outlined boundaries of the spaces and the manner in which these spaces ramify between cells. This rat was exposed by the feeding of large daily doses for 12 days. Similar but less marked changes resulted from the feeding of small doses over a period of 28 days (see fig. 2). After 75 days' exposure by inhalation to low concentrations of this preparation, marked liver changes, consisting of cellular swelling, vacuolization, and hyalinization were apparent (fig. 3).

FIGS. 4, 5, AND 6. The microscopic changes resulting from exposure to chlorinated diphenyl are illustrated in these figures. Figure 4 was made from the liver of a rat fed large daily doses for only 6 days. Marked swelling of cells and rapid regenerative activity are apparent. Figure 5 illustrates the changes that were uniformly produced by the feeding of small doses of this preparation. Note the extremely numerous hyaline inclusions in liver cells. This rat was exposed for 29 days. Very similar changes were produced by the administration of this compound by inhalation methods (fig. 6). The rat from which this drawing was made had been exposed to low concentrations for a period of 107 days.

FIG. 1. Camera lucida drawing × 95.

FIGS. 2 TO 6. Camera lucida drawings × 395.

amounts of fat. These changes increased slightly with longer exposure. (See plate II, figs. 1 and 2.) There were no architectural changes in the liver.

Because the effects resulting from large doses of trichlornaphthalenes were slight, this compound was not fed in small amounts.

Compound B. Tetra- and pentachlornaphthalenes

Exposure by feeding.—This preparation was employed only in feeding experiments. The daily food ration for 10 rats contained 0.5 gm. of this compound. All animals fell ill and either died or were sacrificed by the sixty-third day.

The livers were pale yellow in color, friable, and occasionally showed mottling. There were no significant variations in weights. On microscopic examination the same changes were observed in all rats. These consisted of moderate to marked swelling and rounding of the liver cells. The sinusoids were markedly narrowed. The majority of the liver cells contained large numbers of small fat vacuoles. This finding was most marked and first observed in the central portions of the lobules. In the least altered cells there was prominent massing of basophilic granules near the nuclei. Occasional necrotic cells were observed. Mitotic figures were present in increased numbers, indicating accelerated regenerative activity. The microscopic changes resulting from the feeding of tetra- and pentachlornaphthalenes are illustrated in plate I, figures 3 and 4. They are those of diffuse fatty infiltration and fatty degeneration.

Compound C. Tetra- and Pentachlornaphthalenes plus Chlorinated Diphenyl

Exposure by feeding.—Feeding (3 gm. doses daily per 10 rats) was continued with but one 4 day interruption for 130 days. Ten rats were so treated. Seven rats were sacrificed at varying periods of 47 to 124 days. Three rats died after exposure periods of 122 to 130 days. In all animals the livers were enlarged (33 to 90 per cent). The average weight increase was 71 per cent. They were also friable, pale yellow in color, and somewhat mottled. On microscopic examination, constant changes were observed. Practically every liver cell was swollen and rounded. Their cytoplasm contained large numbers of hyaline bodies. These were circular or oval in shape and varied in size from about half the size of a red blood corpuscle to twice the size of the nucleus of a liver cell. (See figs. 1 and 2, plate I.) In numerous instances, many small hyaline bodies had fused, forming large circular masses as large or larger than a normal

PLATE IV

FIG. 1. Photomicrograph × 45 illustrating the extent of the liver damage produced in 48 hours by 0.75 cc. per kgm. each of carbon tetrachloride and ethyl alcohol when administered by stomach tube to a normal control rat. The degenerative changes were limited to the central portion of the lobules.

FIG. 2. Photomicrograph × 220 of a section of liver tissue from a rat exposed by inhalation methods to trichlornaphthalenes in low concentration for 150 days. Following this exposure 0.75 cc. per kgm. each of carbon tetrachloride and ethyl alcohol was administered by stomach tube. The animal survived and was sacrificed 17 days later. No significant liver changes are demonstrable.

liver cell. These bodies stained brilliantly with eosin dye. They were often laminated and occasionally contained small clear fat vacuoles in their central portions. Mitotic figures in liver cells were sufficiently numerous to indicate an increased rate of regeneration (fig. 1, plate I). In the livers of 2 rats exposed for 124 and 125 days respectively, there were, in addition to the above changes, large areas of complete liver cell degeneration (fig. 2, plate I). For the most part this was limited to the central half or third of the liver lobules. Occasionally, however, there was complete degeneration of all liver cells except for a narrow zone of cells around the portal areas. In such livers there was a heavy polymorphonuclear and mononuclear inflammatory cell infiltration in the necrotic areas where liver cells were being removed.

The most conspicuous feature of the microscopic changes in the livers of these rats was the presence of large numbers of circular hyaline droplets in the cytoplasm of the liver cells. This material did not stain in a manner characteristic of amyloid and its staining properties were not like those of the hyaline observed in alcoholic cirrhosis. In frozen sections stained with Scharlach R, the staining reaction of these bodies was inconstant. The larger vacuolated hyaline bodies contained small droplets of fat while the majority of the smaller droplets did not stain red. Although similar hyaline droplets were observed in livers of rats exposed to various chlorinated naphthalenes, this type of degeneration occurred much earlier and to a much more marked degree in those rats that were exposed to preparations containing chlorinated diphenyl (figs. 1 and 2, plate I) or to chlorinated diphenyl alone (figs. 4, 5, and 6, plate III).

Compound D. Penta- and hexachloronaphthalenes

(a) *Exposure by inhalation to low concentrations (average 1.16 mgms. per cu. m.) 16 hours daily.*—Eighty rats living under these conditions appeared normal throughout the exposure period (134 days). Representative animals were sacrificed in groups of 3 to 15 animals after varying exposure periods as described in the experiments employing trichlornaphthalenes. There were no significant alterations in liver weights. Macroscopically the majority of the livers were light yellow in color and slightly mottled after the initial exposure period of [illegible] days there was evidence of slight [illegible] to

PLATE V

FIG. 1. A photomicrograph × 120 showing small areas of necrosis in central areas of the liver lobules. This rat had been exposed by inhalation to low concentrations of trichlornaphthalenes for 134 days following which carbon tetrachloride and ethyl alcohol were administered by stomach tube in doses of 0.75 cc. each per kgm. The rat was sacrificed 5 days later. The extent of necrosis is similar to that resulting from carbon tetrachloride and alcohol alone.

FIG. 2. In this photomicrograph × 45 are shown the most marked liver changes that were observed following the administration of carbon tetrachloride and alcohol (0.75 cc. of each per kgm.) to rats which had been exposed by inhalation methods to high concentrations of trichlornaphthalenes. This rat had been exposed to trichlornaphthalenes for 1 month. Very extensive necrosis was present in the central areas of each liver lobule. In occasional areas the major portion of several adjacent lobules were necrotic. This rat was autopsied 48 hours after the administration of carbon tetrachloride.

liver cells which appeared swollen and more granular than normal. The cytoplasm of occasional cells contained small acidophilic hyaline droplets and there was a moderate excess of fat in the form of tiny vacuoles (fig. 6, plate II). All microscopic abnormalities increased slightly during the second period (37 to 72 days) but no significant advance was detected between the 72nd and 134th day of exposure.

The livers of rats exposed for 105 days and then removed from exposure for a period of 2 months still showed cellular changes similar to those observed at the beginning of the recovery period. In a few of the specimens they appeared slightly less marked, indicating that some repair had taken place. In no instance was there evidence of increased damage.

Carbon tetrachloride and ethyl alcohol administered to rats exposed in the above manner for 144 days proved highly fatal, 9 out of 10 rats dying within the first 6 days—6 within 72 hours. At autopsy the livers of these rats were yellow and mottled and they were increased in weight (16 to 44 per cent). Microscopic examination showed widespread central necrosis similar to that observed in early acute yellow atrophy of man (see figs. 1 and 2, plate VII). The central one-half or two-thirds of each liver lobule showed complete liver cell necrosis and degeneration. Diffuse extravasation of erythrocytes had occurred and numerous polymorphonuclear and mononuclear leucocytes had invaded the necrotic areas. In the narrow periportal zones where the liver cells were less markedly damaged, there were large numbers of mitotic figures (fig. 2, plate VII).

(b) *Exposure by inhalation to low concentrations (average 1.44 mgms. per cu. m.) 8 hours daily.*—Animals were subjected to these conditions for 143 days. Groups of animals sacrificed after 42, 77, 98, 119, and 143 days' exposure showed liver changes that were essentially like those seen in rats exposed 16 hours daily. The carbon tetrachloride and alcohol test was uniformly fatal and produced liver changes that were identical to those already described and illustrated (figs. 1 and 2, plate VII).

(c) *Exposure by inhalation to high concentrations (average 8.38 mgms. per cu. m.) 16 hours daily.*—Penta- and hexachlornaphthalenes when administered in this concentration proved highly toxic. Eighty animals were

PLATE VI

FIG. 1. Photomicrograph × 220 of the liver of a rat exposed to penta- and hexachlornaphthalenes by the feeding of large daily doses for 16 days. The liver cells are swollen and markedly vacuolated. There is distortion of the liver cell columns. In frozen section preparations stained with Scharlach R, there was evidence of marked fatty degeneration. In addition to these changes, numerous oval, round and irregular intercellular spaces are present throughout the liver lobules. Such spaces contain a granular serous precipitate, strands of fibrin and occasional leucocytes. These spaces, insofar as could be determined, were due to dilatation of the bile capillaries and canaliculi.

FIG. 2. This photomicrograph × 215 when compared with the above figure demonstrates the similarity of the liver changes produced by the same compound administered in a different manner. In this instance the rat inhaled high concentrations of penta- and hexachlornaphthalenes for 38 days.

used, all of which lost weight and appetite. Four died by the end of the first month, 55 within 52 days. Most of these were markedly jaundiced. The remainder were sacrificed for pathological examination or succumbed to the effects of carbon tetrachloride and alcohol. Only 8 animals survived the 52 day exposure. The majority of livers were moderately or markedly enlarged. They were all yellow and in addition the majority were markedly mottled. Occasionally they were granular and their cut surfaces showed minute spaces which became more prominent after fixation. After an 8 day exposure, the liver cells were markedly swollen and moderate fatty degeneration was present. This was most marked in the central areas although all portions of the liver lobules were affected. The extent of liver injury rapidly increased so that after 30 days' exposure, marked structural as well as cellular changes were apparent. The microscopic appearances were identical to those to be described in rats fed large doses of penta- and hexachlornaphthalenes (figs. 1 and 2, plate VI) and in rats fed similar doses of 90 per cent penta- and hexachlornaphthalenes plus 10 per cent chlorinated diphenyl (see fig. 1, plate III and figs. 1 and 2, plate VIII).

Rats removed from exposure between the third and fifth week continued to die, the longest survival time being 35 days. Microscopic examination of the livers of these animals revealed no evidence of recovery.

Administration of carbon tetrachloride and alcohol again proved fatal to animals exposed in the above manner. The livers of such animals showed the characteristic massive necrosis previously described and illustrated.

(d) *Exposure by feeding.*—When fed in doses of 3 gm. daily to a group of 10 rats, penta- and hexachlornaphthalenes produced marked cellular and structural changes in the liver in a short period of time. All rats lost weight and appeared ill from the beginning. The longest survival time was 33 days. Although the liver edges were blunt and rounded, there were no significant weight variations. The livers were friable, yellow and mottled. Microscopically they showed marked swelling and vacuolization of the cells. There was also complete degeneration of scattered cells. Occasional mitotic figures were observed (see fig. 4, plate II). Suitable stains revealed very marked fatty degeneration. Occasional cells contained oval shaped or circular acid-

PLATE VII

FIG. 1. A low power photomicrograph × 45 illustrating the marked degree of degeneration of liver tissue resulting from the administration of a small dose of carbon tetrachloride and ethyl alcohol (0.75 cc. of each per kgm.) to an animal that had been exposed previously by inhalation methods to low concentrations of penta- and hexachlornaphthalenes for 134 days. This animal died within 24 hours after the administration of carbon tetrachloride.

Similar degrees of necrosis resulted from the administration of carbon tetrachloride and alcohol to rats which had been exposed to a mixture of 90% penta- and hexachlornaphthalenes and 10% chlorinated diphenyl or to refined chlorinated diphenyl.

FIG. 2. A higher power photomicrograph × 225 of the liver illustrated in the above figure.

ophilic hyaline inclusions. In addition to these cytological changes there were numerous varying sized intercellular spaces throughout the livers (see figs. 1 and 2, plate VI and fig. 1, plate III). These spaces were usually circular or oval in shape, although many of them ramified between cells in the liver columns or between liver cells and the sinusoidal endothelium in such a way as to suggest that they represented greatly dilated bile capillaries or canaliculi (fig. 1, plate III). They contained serous precipitate, a few strands of fibrin, and occasionally a few leucocytes. Rarely one observed one or two degenerating liver cells within these spaces. In some of the more markedly damaged livers, red blood corpuscles were also present. The above microscopic changes appeared identical to those observed in the livers of rats subjected to high concentrations of this compound by inhalation methods (figs. 1 and 2, plate VI). They were also similar to the changes that resulted from the feeding of another penta- and hexachlornaphthalene compound (figs. 5 and 6, plate I) and from the feeding of a mixture of penta- and hexachlornaphthalene (90 per cent) and chlorinated diphenyl (10 per cent).

When this preparation of penta- and hexachlornaphthalenes was fed in smaller amounts (0.5 gm. every second day to a group of 4 rats) the above described liver changes occurred less rapidly and to a lesser degree (see fig. 5, plate II).

Compound E. Penta- and Hexachlornaphthalenes

Exposure by feeding.—Feeding in doses of 1 gm. daily for 10 rats was continued with but one 4 day interruption. All 10 rats had died or had been sacrificed by the fifty-fifth day. These livers were friable and yellowish, and exhibited moderate to marked mottling. One animal was jaundiced. Of the 5 animals that were sacrificed, three showed enlargement of the liver (30 to 40 per cent); the remaining livers were normal in size. On microscopic examination the livers of rats exposed for 2 weeks showed marked cellular changes that appeared to be degenerative in nature. Although the liver cell injury was somewhat greater in the central portions of the liver lobules, no portion of the liver was spared. The liver cells showed varying degrees of swelling, increased granularity and vacuolization of the cytoplasm. Early injury was indicated by a massing of basophilic granules and small rod-shaped structures near the cell nuclei. The peripheral portions of such cells were acidophilic and vacuolated. Small irregular spaces were present between

PLATE VIII

FIG. 1. A photomicrograph × 95 of the liver of a rat fed a mixture of 90% penta- and hexachlornaphthalenes and 10% chlorinated diphenyl, in 3 gm. doses per 10 rats for 12 days. This rat survived for 23 days after feeding was stopped. A comparison of the liver of this rat with those of rats sacrificed earlier indicated that the liver changes had progressed considerably after administration of the compound had been terminated. One should note the marked change in the liver architecture. The spaces like those previously illustrated (figs. 1 and 2, plate VI) are very large and the remaining liver tissue is compressed. The liver cells show marked fatty degeneration.

FIG. 2. A higher power photomicrograph × 235 from another area in the liver illustrated in the above figure.

50 per cent). Such livers were yellow and mottled. Again there was extensive and widespread liver necrosis on microscopic examination (see figs. 1 and 2, plate VII).

(b) *Exposure by feeding.*—When fed in large doses (3 gm. daily for 10 rats) this compound proved to be very toxic. All animals appeared ill and feeding of the compound was stopped after 12 days. Despite this the rats continued to die, the last dying on the 35th day. Subsequent microscopic examination revealed that the liver changes had continued to progress after feeding was stopped. Macroscopically, the majority of the livers were enlarged, the largest showing an increase in weight of 118 per cent; the average increase was approximately 40 per cent. All livers were yellow, friable, and many of them were markedly mottled. Subsequent microscopic examination indicated that much of the mottling was due to hemorrhage into spaces within the liver tissue. In addition, the external and cut surfaces of the livers appeared slightly pitted or granular. The microscopic changes observed in the livers of the animals autopsied after 10 to 14 days' exposure consisted of moderate to marked swelling of liver cells accompanied by marked fatty vacuolization. Occasional liver cells showed additional signs of injury such as nuclear degeneration and polymorphonuclear leucocytic invasion. Scattered circular or oval shaped intercellular spaces (fig. 1, plate III) like those previously described in experiments where penta- and hexachlornaphthalenes were employed (figs. 1 and 2, plate VI) were observed. In the less markedly damaged livers, such spaces contained serous precipitate, strands of fibrin, and small numbers of leucocytes. In rats surviving 18 to 35 days, these spaces were much larger and often had attained the size of a normal liver lobule (see figs. 1 and 2, plate VIII). In these more severely damaged specimens, extensive hemorrhage had occurred (fig. 2, plate VIII). In sections stained with P.T.A.H. these spaces were seen to be bounded by flattened liver cells which showed sharp cell borders. Liver cells between such spaces were distorted, swollen, and showed marked fatty degeneration. In many of these livers there was a slight increase in connective tissue between the remaining cords of liver cells and around recognizable portal areas. Proliferative changes were occasionally observed in the bile ducts. This was indicated by increased numbers of ducts in certain areas and by mitotic figures in the bile duct epithelial cells. No recognizable dilatation of medium sized or large bile ducts was present. In many sections the architecture of the liver was so completely altered that it was difficult to recognize the portal areas. In all sections there was evidence of fatty and hyaline degeneration of the remaining liver cells. Slight polymorphonuclear leucocytic infiltration was present. This was somewhat more marked in the periportal areas.

Feeding of this mixture of penta- and hexachlornaphthalenes and chlorinated diphenyl in smaller doses (0.5 gm. every second day for 4 rats) resulted in liver damage that developed less rapidly. The rats were sacrificed after exposure periods of

21, 34, 58, and 81 days. Their livers were yellow, increased in size and showed slight mottling. Microscopically, the chief abnormality observed was marked fatty vacuolization of liver cells. No intercellular spaces like those described above were present.

Compound G. Chlorinated Diphenyl

(a) *Exposure by inhalation.*—Exposure by inhalation methods to low concentrations of chlorinated diphenyl was carried out on two groups of animals, each comprised of 80 rats, in a manner identical to that described in the foregoing sections. In the first group a 16 hour daily exposure, with an average concentration of 0.57 mgms. per cu. m., was employed. The second group of animals was exposed 8 hours daily to an average concentration of 0.93 mgms. per cu. m. Neither group of rats appeared ill. Representative animals were sacrificed in groups of 3 to 21 rats, after varying periods of exposure. In the first experiment, the animals were sacrificed after 37, 72, 105, and 134 days of exposure, in the second experiment after 42, 77, 98, 119, and 143 days. The livers were pale or slightly yellow and somewhat mottled. There were no constant variations in the weights of the livers. The microscopic findings in the two groups indicated that the different exposures had resulted in the same degree of liver injury. The pigmentation of the liver and Kupffer cells was somewhat greater in animals exposed 8 hours daily. In both experimental groups the carbon tetrachloride and alcohol test was highly fatal and led to the same extensive central necrosis of liver tissue that was observed in rats exposed to the toxic chlorinated naphthalene compounds (figs. 1 and 2, plate VII). The microscopic changes resulting from exposure to low concentration of chlorinated diphenyl alone were similar to those resulting from inhalation of penta- and hexachlornaphthalenes, plus 10 per cent chlorinated diphenyl. The most conspicuous change was hyaline degeneration (fig. 6, plate III) although swelling of liver cells, increased prominence of cytoplasmic granules and increased vacuolization were present. Mitotic figures were present in increased numbers. No microscopic evidence of recovery was found in the livers of rats exposed for 105 days and then removed from exposure for a period of 2 months.

(b) *Exposure by feeding.*—When fed in large daily doses (3 gm. for groups of 10 rats), chlorinated diphenyl proved highly toxic. Feeding was discontinued after 6 days. Seven rats died within the first 8 days. The 3 remaining rats gained in weight after the feeding was stopped and were finally sacrificed. The last animal was sacrificed 63 days after feeding was begun. The majority of livers showed a moderate increase in weight. Microscopically there was evidence that liver injury had occurred within the first few days. The cells were swollen, at times sufficiently to obscure the sinusoids. The cytoplasm of the liver cells was acidophilic in staining quality and contained small vacuoles. Small eosin-stained hyaline granules and globules were present. Mitotic figures were numerous (see fig. 4, plate III). There was little inflammatory cell infiltration and no structural changes were observed.

When smaller doses (0.5 gm. every second day) of this compound were administered to 10 rats, the first death occurred after 9 days. Two additional deaths occurred after 12 days and two more by the end of the fifth week. The remaining animals were sacrificed between the 40th and the 190th day. The livers were enlarged, the average increase in liver weight being 33 per cent. They were pale and slightly mottled. The most outstanding microscopic abnormality was the presence of large numbers of varying sized acidophilic hyaline globules in the cytoplasm (see fig. 5, plate III). These hyaline globules, although larger and more numerous, appeared identical to those encountered in the livers of rats exposed to other chlorinated diphenyl preparations. The liver cells were markedly swollen and showed fatty vacuolization. There was also increased regenerative activity. No structural changes were observed in any of the livers.

Discussion

Published reports concerned with the possible systemic effects of chlorinated naphthalenes have been few in numbers. Lehmann (3) in 1919 reported that animals exposed to chlorinated naphthalenes by inhalation or feeding refused to eat and at death showed "peculiar" lesions in the liver. In 1936, Flinn and Jarvik (4) reported the results observed following the daily subcutaneous injections into 30 rabbits of enormous doses (30 mgm.) of certain chlorinated naphthalene compounds dissolved in paraffin oil. The rabbits receiving a mixture of tri- and tetrachlornaphthalenes lived and when sacrificed, after a 2 months' exposure, no pathological changes attributable to the compound injected were demonstrable. The mixtures of tetra- and pentachlornaphthalenes and of penta- and hexachlornaphthalenes proved highly fatal and degeneration of liver tissue was observed. From their findings Flinn and Jarvik concluded that certain chlorinated naphthalenes or impurities contained in them are capable of producing yellow atrophy of the liver in the rabbit. It is noteworthy, in the light of the present experiments, that the compound of lowest chlorination (a mixture of tri- and tetrachlornaphthalenes) had no apparent effect on the well being of the animals and did not produce demonstrable changes in the liver although injected daily for a period of 2 months.

The present experiments demonstrate that chlorinated naphthalene compounds and chlorinated diphenyl are capable of producing marked liver damage in the white rat without demonstrable microscopic changes appearing in the other organs. Furthermore, the characteristics of the liver lesions resulting from comparable amounts of any given compound are the same, regardless of the method of administration (inhalation, feeding, or subcutaneous injection).

An attempt has been made throughout this study to grade the toxicity of each of the several compounds tested in accordance with the severity of the liver damage produced. Although some of these compounds appeared to produce similar degrees of liver injury, the toxicity of each compound seemed directly related to its degree of chlorination. Thus, a mixture of trichlornaphthalenes (chlorine content 49.4

per cent) proved to be the least toxic of any of the compounds tested, whereas chlorinated diphenyl (chlorine content 65.0 per cent) was highly toxic even in very low concentrations.

Trichlornaphthalenes, when compared to other naphthalene compounds of higher degrees of chlorination, were relatively innocuous. Even after prolonged feeding of large amounts of this material, the rats showed no evidence of ill health. After 2 months' exposure, microscopic examination of the livers revealed only slight to moderate degrees of fatty infiltration and degeneration of liver cells (plate II, figs. 1 and 2). These changes were slight when compared to those resulting from short exposures to compounds of higher chlorination. Exposure by inhalation to low concentrations of trichlornaphthalenes produced liver cell alterations that were never more than minimal (see fig. 3, plate II). Such animals withstood additional liver injury from carbon tetrachloride and alcohol in a manner indistinguishable from that of the normal animal. Somewhat greater liver injury resulted from inhalation when the air concentration of this compound was increased more than eight times that used in the previous experiment but again the liver changes were slight compared to those produced by comparable exposure to the more highly chlorinated compounds.

The mixture of tetra- and pentachlornaphthalenes employed in these experiments appeared to be considerably more toxic than was the mixture of trichlornaphthalenes. Animals fed small doses of the former of these preparations fell ill and died or were sacrificed by the 63rd day. Their livers showed extensive fatty infiltration and fatty degeneration (figs. 3 and 4, plate I).

Feeding of tetra- and pentachlornaphthalenes in combination with chlorinated diphenyl resulted in pronounced liver changes. These livers had increased in weight (average 71 per cent). Microscopic examination revealed a peculiar type of hyaline degeneration involving practically every liver cell (see figs. 1 and 2, plate I). This type of cell degeneration was more marked and occurred earlier after exposure to preparations containing chlorinated diphenyl than to any other compounds tested. Furthermore, it was most marked in the livers of animals exposed to refined chlorinated diphenyl (figs. 4, 5, and 6, plate III).

Comparable exposure of rats to penta- and hexachlornaphthalenes (Compound D), penta- and hexachlornaphthalenes (Compound E), and penta- and hexachlornaphthalenes (90 per cent) plus chlorinated diphenyl (10 per cent) (Compound F), resulted in liver changes having few detectable differences. The morphological changes were slightly greater in the livers of rats exposed to the last named compound. In these instances the hyalin degeneration of the cell cytoplasm was more marked. Although rats inhaling low concentrations of compounds D and F showed no demonstrable signs of ill health, microscopic examination of their livers revealed marked liver cell injury (fig. 6, plate II, and fig. 3, plate III). These lesions were still demonstrable after a 2 months' recovery period. Further evidence that liver damage had resulted from inhalation in low concentrations of these compounds was obtained from the carbon tetra-

chloride and alcohol test. Such tests were almost uniformly fatal and resulted in widespread liver necrosis (figs. 1 and 2, plate VII). These three compounds proved to be very toxic and resulted in a most unusual type of liver change when administered in large amounts by feeding (see fig. 1, plate III, fig. 1, plate VI, and figs. 1 and 2, plate VIII). Identical changes were observed following inhalation in high concentrations of compound D (see fig. 2, plate VI). In such livers the most conspicuous feature was the presence of large intercellular spaces. Examination of routine sections, supplemented by specially stained preparations indicated that these spaces had resulted from dilatation of bile canaliculi. The reason for such dilatation was not apparent. There was no corresponding dilatation of the large and small bile ducts at the periphery of the liver lobules and no inspissated bile was ever observed. The organically combined chloride in the livers of these rats was no greater than that observed in the normal animal. Although serious liver damage results from exposure to high concentrations of these compounds, the resulting changes are most unusual and do not resemble those of acute yellow atrophy on microscopic examination.

Of the various chlorinated hydrocarbons tested, chlorinated diphenyl gave evidence of being the most toxic. When administered by inhalation in very low concentrations (average 0.57 to 0.93 mgms. per cu. m.) liver cell changes were very pronounced after the first exposure period. The most striking change was the hyalinization of the cell cytoplasm (see fig. 6, plate III). Such cellular alterations were essentially unchanged after a 2 month recovery period. In these animals small sublethal doses of carbon tetrachloride and alcohol uniformly produced extensive liver necrosis and was highly fatal to them (figs. 1 and 2, plate VII). Chlorinated diphenyl fed in small doses produced similar but more marked liver injury (see fig. 5, plate III). In large doses this compound was highly fatal. The liver changes in animals dying after short exposures were inconspicuous and consisted mainly of swelling of cells and active regeneration (see fig. 4, plate III). However, animals removed from exposure before being fatally poisoned, subsequently developed hyaline degeneration of liver cells similar to that produced by prolonged administration of small doses of this compound.

Thus the results of the present study, as well as certain field studies that have been made (1) suggest that the solution of the industrial hazard involved is dependent largely on a reduction of the air concentration of these compounds to a level that will not produce liver damage. The present experiments indicate that lower air concentrations must be obtained in the case of the more highly chlorinated naphthalene compounds and chlorinated diphenyl than for trichlornaphthalenes if a safe environment for workmen is to be assured. Because of the pronounced toxic effect of small doses of carbon tetrachloride on the livers of animals already injured by exposure to chlorinated naphthalenes and chlorinated diphenyl, its use as a solvent for these compounds would appear to be very hazardous.

Although this investigation v
designed to determine what syst

effects, if any, would be produced in experimental animals by a group of chlorinated hydrocarbons, the pathological changes produced have been of such a nature as to justify further comment. Exposure of rats to highly chlorinated naphthalene compounds and chlorinated diphenyl results in a type of liver injury differing considerably from the liver lesions resulting from carbon tetrachloride (5, 6) and chloroform poisoning (7). The changes resulting from exposure to the compounds employed in this study involve all portions of the liver lobule. This is true despite the fact that the initial morphological changes are more pronounced in the liver cells nearest the hepatic veins. The changes observed are degenerative in nature. The successive degenerative changes were cloudy swelling, fatty infiltration and fatty degeneration and finally, the complete disintegration of the cell. Degeneration characterized by the accumulations of large amounts of acidophilic hyaline material was also observed. This feature was much more marked in the livers of animals exposed to the compounds containing chlorinated diphenyl. It is of interest that widespread degenerative changes of all liver cells can exist for several months without producing any evidence of ill health in the animals or without causing important structural changes in their livers. Such findings suggest that the alterations take place slowly and that the scattered necrotic cells are efficiently removed and replaced by new cells which also become injured. There was no observable morphological evidence to indicate that the regenerating liver cells had acquired an increased resistance to the injurious effects of the compound being used (8). The fact that liver cell changes were still present after a 2 month period during which the animals were removed from exposure to the more highly chlorinated compounds is further evidence that this type of injury is persistent and only slowly recovered from.

The administration in high concentrations of two compounds of intermediate degrees of chlorination resulted in marked structural changes in addition to marked degenerative changes in liver cells. The most conspicuous change was the occurrence of progressively enlarging intercellular spaces. These were interpreted as markedly dilated bile canaliculi. We are unaware of such changes having been previously described.

From the above it is apparent that exposure to highly chlorinated naphthalenes and chlorinated diphenyl not only results in liver changes having marked differences from those caused by other well known toxic agents, but also that the characteristics of the liver changes produced can be markedly altered quantitatively and qualitatively by varying the degree of exposure. For these reasons one would seem justified in suggesting that detailed studies on animals in which the types of liver injury observed in these experiments had been produced might further our understanding of liver cell function.

Summary

1. Studies concerning the effect upon white rats of a group of chlorinated naphthalene compounds and chlorinated diphenyl are reported. This investigation was undertaken because of the recent occurrence of 3 fatal cases of jaundice in men work-

ing in industrial plants where these compounds were being used. The compounds tested were selected as representative of a certain range of chlorination and because of their relative industrial importance.

2. Four of the compounds tested were administered by inhalation. The air concentration and exposure periods were varied. All compounds were tested by feeding in large and small amounts. In addition three of them were tested on small numbers of animals by subcutaneous injections. The effects of any given compound did not appear to be influenced by the method of administration. However, marked quantitative and qualitative differences in the effects of each of several compounds resulted from varying the air concentrations in inhalation experiments and the dosage employed in feeding experiments.

3. Macroscopic and microscopic examination of the tissues of rats exposed to these compounds indicated that the injurious effects are manifested solely in the liver. Blood examination during life revealed no significant abnormalities.

4. The liver changes resulting from exposure to the chlorinated hydrocarbons employed have been described and illustrated. As indicated by their ability to injure liver tissue the toxicity of these compounds increases with increasing degrees of chlorination. Thus a mixture of trichlornaphthalenes is relatively innocuous in low concentrations and when compared with compounds of higher chlorination, its toxic properties even in higher concentrations are relatively slight. Chlorinated diphenyl appears to be the most injurious compound of all those tested.

5. Administration of small sublethal doses of carbon tetrachloride and ethyl alcohol to rats whose livers have already been injured by the compounds under consideration is highly fatal and produces massive necrosis of the liver.

6. The significance of this study in relation to the industrial problem involved is discussed.

7. It is suggested that the types of liver injury observed in this study might be employed in experiments designed to study liver cell function.

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little difficulty with some of the terms. n this chapter it would have been well to xplain the difference in general between cute and chronic effects, as with many ubstances there is little similarity and the hronic effect is usually the more serious.

If the subject of workmen's compensation as a place in a book on industrial health, ne development of occupational disease egislation should have been mentioned. The two general types of occupational disease acts (all-inclusive and specific) with heir advantages or disadvantages are worth a paragraph or two.

The chapter on "First-Aid Treatment of njuries" treats only the traumatic injuries. Burns are taken care of in another hapter, but under first-aid a few words vo ave been helpful describing what to lo cute cases of poisoning, cyanide, n. pills, etc., where the patient is still reatning but where prompt action is necesary.

It does combine in one compact volume nd in a readable style much information n industrial safety and health that otherwise would require reference to many ources. For the beginning students of industrial engineering, industrial safety and ealth, or personnel relations, it should rove a helpful book. The operating ngineer and manager will want more complete information on the particular exposures in his plant and the industrial hysician, if interested in prevention, will want more on the engineering methods of control.—*S. W. Gurney.*

INDUSTRIAL CANCER. By *E. W. Baader*, in "NEUE ERGEBNISSE AUF DEM GEBIETE DER KREBSERKRANKUNGEN," Adam and Auler, Leipzig, 19[redacted] (28).

A valuable study [redacted] the history of industrial cancer. [redacted] well known forms of industrial c[redacted] cluded: the lung and nasal canc[redacted] England in nickel workers, the l[redacted] of general workers in Japan, and the liver cancer c. fishers in the Curische Haff, caused by th[redacted] ch, *Opistorchis felineus*.—*L. Teleky.*

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THE SYSTEMIC EFFECTS RESULTING FROM EXPOSURE TO CERTAIN CHLORINATED HYDROCARBONS*

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CHLORINATED naphthalenes and diphenyls, because of their electrical, heat and moisture-resisting properties, and because they are non-inflammable, are used extensively for insulating wire and in the manufacture of electrical condensers. The chlorinated naphthalenes are naphthalenes in which one or more of the hydrogen atoms has been replaced by chlorine. There is, thus, a series of these substances beginning with monochlornaphthalene and going on to the octochlor derivative. In industry they usually occur in mixtures in which more than one chlorinated product is present. On the whole, the higher the chlorination, the more toxic this material becomes. In the manufacture of chlorinated diphenyls, C_6H_6 is converted into $C_{12}H_{10}$ which, in turn, is chlorinated to $C_{12}Cl_{10}$; the substitution products range from the monochlor to the decachlor diphenyl.

A rather characteristic acneform skin eruption resulting from exposure to these substances has been recognized for a great many years—indeed, ever since they began to be manufactured about 25 years ago. These skin eruptions came into some prominence in Germany during the war and have been attracting sporadic interest in this country ever since. An investigation of this condition, as it appeared among a group of young workers engaged in the manufacture of electrical condensers, was reported in a recent issue of this *Journal* (1).

Experience has shown that the medical practitioner is still somewhat unfamiliar with the skin eruptions, even

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The chlorinated naphthalenes are sometimes referred to as "Halowax" by purchasers and users. While the Halowax Corporation manufactures these substances, it also manufactures many others. Since the term "Halowax" is merely a trade name for substances manufactured by this Company, it should not be used indiscriminately. In all instances, substances should be designated by their chemical names only.

though they are by no means uncommon in the industries where the chlorinated naphthalenes and diphenyls are used. The reason for this is that any one physician is likely to see such cases only rarely, unless he happens to be practicing in the immediate vicinity of one of these factories where large numbers of workers are employed. That the relatively rare systemic effects resulting from such exposure almost invariably go unrecognized is not surprising under the circumstances. It is of the greatest importance however, that physicians become acquainted as promptly as possible with the clinical and pathological pictures presented by these patients, particularly as regards systemic effects, since failure to detect early clinical manifestations of toxicity, and to remove susceptible individuals promptly from further exposure, may, on occasion, result fatally.

Review of Literature on Systemic Effects*

Experimental studies.—The first mention in the literature of systemic effects from chlorinated naphthalenes is that of Lehmann in 1919 (2). He found that animals which were fed or which inhaled these substances lost appetite and at death showed "peculiar" lesions in the liver.

In 1936, Flinn and Jarvik (3) experimented on rabbits with three different compounds; (A) a mixture of tri- and tetrachlornaphthalene, (B) a mixture of tetra- and penta-chlornaphthalene and (C) a mixture of penta- and hexa-chlornaphthalene. They also used sublimates of (B) and (C) given off at 192°C and 172°C respectively. Large doses, approximately 15 mg. per kg were injected subcutaneously each day.

Animals receiving compound (A) and the sublimate of compound (B) showed no lesions attributable to these substances when killed at the end of 2 months. The 30 animals, however, which had received the higher chlorinated compounds (B) and (C) and the sublimate of (C), all died in from 12 to 26 days. Autopsies uniformly showed extensive damage.

The next important study to be published was that of Drinker, Warren and Bennett (4). These investigators administered chlorinated hydrocarbons by inhalation, subcutaneously and by mouth, to white rats. Mixtures of (a) tri- and tetrachloronaphthalene; (b) penta- and hexachloronaphthalene alone, and (c) with 10% refined chlorinated diphenyl; and (d) chlorinated diphenyl were used.

Briefly, these experiments showed, that the tri- and tetrachloronaphthalenes produced relatively unimportant pathological changes in the liver until extremely high concentrations were used. Animals exposed for 6 weeks to the higher chlorinations, however, in relatively low concentrations regularly showed minor degrees of liver damage even though, as a group, there were no clinical evidences of such toxicity while alive. Exposed to still higher concentrations, the rats lost weight and appetite, and began dying after 8 days' exposure—many with severe jaundice. Examination of the liver of these animals at autopsy revealed marked central fatty degeneration with necrosis of liver cells.

It is of considerable interest that

* A review of the literature on skin manifestations appeared in the article by Mayers and Silverberg mentioned above (1).

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the exposed rats, which gave no clinical evidences of disease, promptly died from acute yellow atrophy of the liver when given a very small dose of carbon tetrachloride—a dose well tolerated by control animals.

Two other points of interest were brought out by the study: (1) in the rats, which on autopsy showed even marked pathological lesions of the liver, no abnormalities were found in the other organs—a finding not uncommon in experiments on animals, but rather rare in humans who die from acute yellow atrophy of the liver; (2) even the less important pathological changes induced in the liver by these chlorinated hydrocarbons in the lower concentrations were found to be very persistent, being present even 2 months after cessation of exposure.

Clinical Reports.—Clinical reports of cases of systemic poisoning from the chlorinated naphthalenes are as yet rare in spite of the length of time that these substances have been in use. No doubt the infrequency of such reports has been, in part, due first, to the fact that cases of systemic poisoning are unusual occurrences—the element of individual susceptibility appearing to play an even more important rôle than usual in this situation—and second, to failure on the part of physicians to recognize cases of poisoning. Until recently there has been general lack of knowledge of the toxicological properties of these chlorinated hydrocarbons, and there is still relatively little information available regarding the clinical picture of industrial poisoning from them.

The danger that lies in such a lack of knowledge is exemplified in a situation that recently came to the attention of the Labor Department in New York State, in which a physician had been treating a severe case of jaundice in a young woman exposed to chlorinated naphthalenes. After a long and serious illness, when the girl was on her way to recovery, the physician expressed the opinion that within a few weeks she would be able to return to her former work. Whatever the cause of the jaundice in this case, there is good reason to believe, on the basis of the experimental work previously cited, that further exposure to such substances would have entailed a grave risk to the patient's life.

In 1934, Courtois-Suffit (5) reported on the work of Touraine who, with his associates, examined 60 workers exposed to trichlornaphthalene and found mild digestive disturbances and dizziness in 13, but nothing of a more serious nature.

In 1935, Schwartz suggested the possibility of systemic disease from exposure to these substances in a talk before the American Public Health Association (6).

In 1936, three fatal cases of jaundice in chlorinated naphthalene workers were recognized in this country. These were reported by Flinn (3) and Drinker (4) who summarized the cases briefly.

All three of the men were young and in none could any predisposing cause other than their industrial exposure be found to account for their illness. Two of the men who had worked side by side died within 2 months of each other. Both had been exposed to mixtures of penta- and hexachlornaphthalene, and one had been exposed to a mixture of tetra- and penta-chlornaphthalene with 10% chlorinated diphenyl. In both, the

diagnosis of acute yellow atrophy of the liver was made on autopsy. In the third case no autopsy was reported, but death occurred after an acute illness characterized by jaundice. In one case dermatitis characteristic of the effect of chlorinated naphthalenes had preceded the jaundice.

In addition to these fatal cases, Drinker (4) also mentions four cases of non-fatal jaundice among individuals with similar exposure. No details are given.

Case Histories

Because of the obvious need for more clinical data in regard to the effects of chlorinated naphthalene exposure, we are reporting 3 cases in persons who, after exposure in the course of their work to these known hepatoxic substances, died of acute yellow atrophy of the liver, and in whose cases no other etiological factors could be discovered even after very careful investigation. The first case was seen in consultation at the Lincoln Hospital in New York City by Adelaide Ross Smith. The second and third cases were seen in consultation at the New Haven Hospital by Dr. Leonard Greenburg, (now Executive Director of the Division of Industrial Hygiene of the New York State Department of Labor) when he was Commissioner of Health of New Haven, Conn. The plant conditions under which the patients had worked were carefully investigated.

*Case 1. H. F.**

A 17 year old girl was admitted to the Lincoln Hospital, New York City, on the service of Drs. Kenneth Taylor, Edwin Hauser and Scott Johnson on April 26, 1932 in a semi-comatose condition. She was intensely jaundiced on admission.

Her past medical history, obtained from members of the family, was entirely negative with the exception of a tonsillectomy in 1930. Until the onset of the present illness she had been unusually healthy and free from symptoms of any kind.

The occupational history was as follows: After being graduated from grammar school she attended high school for 1 year and then obtained a job, her only one, with a concern manufacturing electrical condensers for use in radios. She worked at this one place for 7 months and stopped working there 7 days before her admission to the hospital.

Her work consisted of soldering and labelling condensers. It is possible, from the information given, that she may also have assisted in the sealing operations, but this could not be definitely ascertained. In any event, in the soldering of the condensers she was exposed to the fumes of tri- and tetrachlornaphthalene with which the condensers were originally impregnated. At the same time she was exposed to fumes of the higher chlorinated naphthalenes from the sealing operations conducted in close proximity to the tables at which the soldering was done.

The present illness began about 5 months before admission to the hospital—or approximately 2 months after starting work in the plant—at which time she noticed several pigmented areas on her face. These continued to increase in severity and extent, and caused her to visit the outpatient department of the Lincoln Hospital. Here sugar was found in her urine and she was referred to the skin clinic where a diagnosis of acute catarrhal jaundice was made. Her rectal temperature, at that time, was 100°. A diffuse papulo-pustular eruption was also present on her face. On being questioned about it, the patient stated that it had been present to her knowledge for about 2 months.† From then on she suffered from

* Grateful acknowledgement is made of the courtesy extended by the staff of Lincoln Hospital and the Medical Examiner's Office in granting permission to use the records in this case.

† Investigation revealed the fact that some of her co-workers were also suffering from a similar acneform eruption, and one girl discontinued her work because of it. None of these other girls suffered any systemic disease.

impairment: 100% retention in 5 min., 85% retention in 30 min.

Blood chemistry: Non-protein nitrogen, 28 mg./100 cc.; Urea N, 11 mg./100 cc.; Serum total proteins, 6.15 mg./100 cc.; Serum total albumin, 2.66 mg./100 cc.; Serum total globulin, 3.49 mg./100 cc. A/G ratio, 0.76; Blood sugar, 68.0 mg./100 cc.; Blood calcium, 10.32 mg/100 cc.; Blood phosphorus, 4.12 mg./100 cc.; Serum fatty acids, 11.3 mg./100 cc.; Serum lipoid phosphorus, 7.5 mg./100 cc.; Serum total cholesterol, 10.5 mg./100 cc.; Serum free cholesterol, 63.0 mg./100.

Sugar tolerance test was within normal limits.

X-ray of abdomen showed hepatic flexure of colon unusually high.

Course. The patient was put on a high carbohydrate diet and his general condition improved but the jaundice persisted. He was discharged on May 16, 1934 with the diagnosis of toxic hepatitis.

He was admitted again on June 1, 1934 complaining of abdominal swelling of 3 days' duration, weakness, anorexia and edema of the legs which had developed soon after his discharge. The physical examination showed deep jaundice, systolic murmur, abdomen distended and tympanitic with dullness in the flanks, moderate pitting edema of the legs. Examinations of the urine, blood and stool showed no important variations from the original findings with the exception of a drop in white cell count to 6,400 with 74% polys., and a decrease in serum albumin to 1.88%. Serum CO_2 content was 54.54. Serum chloride was 98.0 mg./100 cc.

The patient's course after the second admission was rapidly downhill. The distention could not be controlled and he soon passed into complete coma. A convulsion occurred on June 8th and on June 10th he died.

*Anatomical diagnosis.** Extensive necrosis, fibrosis and regeneration of liver, acute entero-colitis with edema, fibrosis of pancreas, acute pancreatitis, jaundice, ascites, edema of lower extremities, focal pneumonia (bilateral), subpleural hemorrhages, cloudy swelling of kidneys.

Case 3—C. C.

A young man 22 years of age was admitted to the New Haven Hospital on February 28, 1935 with the complaints of jaundice, abdominal pain, nausea and vomiting of bloody material. The history given was as follows:

He had worked in the same wire coating plant as the previous patient (F. D.). The present illness had begun with jaundice 2 months previously with no other symptoms. This continued for about 1½ months. Two weeks before admission he became more jaundiced and concomitantly developed upper abdominal pain, malaise, nausea and finally vomiting—the vomitus becoming bloody in character. He was treated by his family doctor with no relief. His condition became worse and he finally became delirious and incoherent. Hospitalization was advised.

Summary of physical examination—The patient was comatose, irrational and vomiting bloody material. Positive findings of significance were as follows: Temperature not elevated. Blood pressure 102/68. Generalized jaundice, petechiae over the extensor surfaces of the arms; tenderness over the upper quadrants of the abdomen. No liver dullness percussable.

Course—His condition became rapidly worse, coma setting in soon after admission. The vomiting continued. The patient died about 24 hours after admission.

Anatomical diagnosis—Extensive necrosis and regeneration of liver; jaundice, acute lymphadenitis of portal nodes; ascites; perienteritis of jejunum; cloudy swelling of heart and kidneys; acute pulmonary congestion; healing exanthematous rash of fore-arms.

DISCUSSION

These three cases show the occurrence of similar pathological changes in the liver in three young adults known to have been working with chlorinated naphthalenes and diphenyls—all were exposed directly or indirectly to the higher chlorinated hydrocarbons. In

* Autopsies on this and the following case were performed on the pathological service of the New Haven Hospital by Dr. H. M. Zimmerman. Grateful acknowledgement is made of his courtesy in permitting use of the records.

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Anatomical diagnosis—Extensive necrosis and regeneration of liver; jaundice, acute lymphadenitis of portal nodes; ascites; perienteritis of jejunum; cloudy swelling of heart and kidneys; acute pulmonary congestion; healing exanthematous rash of fore-arms.

DISC[redacted]

These three cas[redacted]he occurence of similar pa[redacted]hanges in the liver in three y[redacted]ts known to have been working with chlorinated naphthalenes and diphenyls—all were [illegible]d directly or indirectly to the [illegible]chlorinated hydrocarbons. In all three cases, a most careful investigation failed to reveal any other predisposing cause for the condition. In the first case in particular, that of a healthy young girl on her first job, the absence of any conditions predisposing to liver damage either preceding or following her exposure to the substances in question is especially clear.

It is of interest to note that two of the cases apparently had suffered from at least one previous attack of hepatitis followed by a certain degree of improvement before the onset of the fatal attack.

In view of the fact that Drinker, Warren and Bennett found no lesions in organs other than the liver in their experimental animals, it is noteworthy that such lesions were conspicuous in all three of the cases here reported. Such lesions, are, indeed, usual findings in cases of acute yellow atrophy of the liver in humans regardless of etiology. On the basis of present knowledge, it would be impossible to say whether they are the result of primary intoxication or are merely secondary to liver damage. In the light of the animal experiments, however, one would incline to the latter view and speculate as to the part played by length of exposure, in the final pathological picture.

It has been mentioned that in Drinker's (4) experiments, rats with no evidence of clinical disease while being exposed to the chlorinated naphthalenes, promptly died from acute yellow atrophy of the liver when given doses of carbon tetrachloride so small as to be harmless to control animals. This prompts speculation as to whether death from acute yellow atrophy of the liver in workers similarly exposed occurs only in those having some pre-existing substratum of liver damage—such as might follow an attack of catarrhal jaundice, for example—and that this might account, in part at least, for the fact that only a very occasional worker out of a large group will suffer from systemic effects of exposure to these substances. In the three cases reported however, no history was given suggesting that any hepatic disorder prior to exposure had occurred.

The presence of a papulo-pustular eruption in two cases is of interest. In one it antedated the systemic symptoms. This type of eruption is characteristic of the dermatitis caused by chlorinated naphthalenes, and until recently was the only disturbance attributed to them.

The presence of "aggregations of comedones" such as were found in Case I is also characteristic of the skin eruptions produced by these substances. The question as to whether or not the skin eruption in such a case is in any way connected with the onset of systemic effects cannot be answered, since thus far no correlation has been established between skin lesions and systemic disease (1).

It is interesting, therefore, that in Case I the skin eruption apparently antedated all evidences of systemic disease. Had the girl been promptly removed from further exposure, when it was first observed, it is possible that her life would have been saved; or had the physicians first called upon to treat the jaundice (of whatever origin) in her case, or in the second case here reported, recognized the danger of continuing exposure to the chlorinated naphthalenes, these deaths might possibly have been averted.

Recommendations for Medical Control

1. The authors wish to stress the need for the conscientious reporting by physicians of *all* illnesses occurring among workers exposed to the chlorinated naphthalenes and diphenyls, particularly cases which have been carefully worked up, so that the clinical disease entities resulting from such exposure can become further clarified and thus more readily recognized in the future. Fewer errors in the diagnosis and management of these cases would occur, and workers' lives could undoubtedly be saved in this way.

2. Persons suffering from the typical acneform eruptions should be removed from further exposure.

3. Persons who have, at any time in the past, had any liver disease—even a mild catarrhal jaundice—should not work with these substances; nor should workers with a history of typhoid fever, malaria, gall-stones or other diseases known to affect the liver adversely.

4. Persons receiving arsphenamine treatment for syphilis; or those who are taking drugs believed to be injurious to the liver in susceptible persons, should not be further exposed in their work to potential liver poisons.

5. Persons working with the chlorinated naphthalenes and diphenyls, if requiring a general anesthetic for an operation, should not be given chloroform or avertin, and vice versa. individuals who have recently received such anesthetics should not immediately thereafter go back to their former work or to work with other substances believed to be potentially toxic to the liver.

6. Pregnant women should not be exposed because the liver, in pregnancy, appears to be peculiarly susceptible to injury.

7. Experience seems to indicate that by proper attention to ventilation and medical supervision of workers the chlorinated naphthalenes and diphenyls can be used in industry with safety.

Summary

The systemic effects resulting from exposure to certain chlorinated naphthalenes are discussed, and the literature of the subject briefly summarized. Three liver deaths in workers handling these substances are presented in some detail, with autopsy findings. Recommendations for prevention are given.

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OBSERVATIONS ON THE FATE OF PENTACHLOROPHENOL IN THE ANIMAL ORGANISM*

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THE manufacture of pentachlorophenol and sodium pentachlorophenate, and the use of these materials for the preservation of wood and other products have created certain opportunities for human exposure to them and to solutions containing them. Absorption may occur as the result of the inhalation of dust in the manufacture or handling of pentachlorophenol or its sodium salt, while percutaneous absorption may result from contact with solutions containing pentachlorophenol or its sodium salt.

The acute and chronic effects of pentachlorophenol upon experimental animals have been described in previous communications (1, 2, 3, 4, 5) but further information was needed in order to pave the way for studies on human beings. First, it was necessary (A) to determine the largest amount of pentachlorophenol that could be ingested daily without causing retention in the blood. Then, because the rapid elimination of pentachlorophenol in the urine had suggested that determination of the urinary concentration might offer a means of estimating the extent of the absorption of the compound, it was desired to learn (B) the relationship between the concentrations in the blood and urine following its ingestion.

(A) Determination of the Maximum Daily Oral Dose of Pentachlorophenol that Will Not Cause Retention in the Blood of Rabbits

Previous observations (4) had shown that the daily feeding of 3 mg. of pentachlorophenol per kilogram of body weight to rabbits, over a period of 90 days, caused accumulation of the compound in the blood up to an approximate concentration of 0.6 mg. per 100 ml. In similar manner, each of 15 rabbits was given 90 doses (on successive days excepting Sundays) of the sodium salt in the form of a 0.25 per cent aqueous solution, each dose being equivalent to 1 mg. of pentachlorophenol per kilogram of body weight. None of the animals showed signs of poisoning. The analytical [illegible] for the blood, (drawn as in previous experiments

TABLE 1

PENTACHLOROPHENOL IN THE BLOOD OF RABBITS FOLLOWING REPEATED ORAL DOSES OF SODIUM PENTACHLOROPHENATE, EACH EQUIVALENT TO 1 MG. OF PENTACHLOROPHENOL PER KILOGRAM OF BODY WEIGHT

IDENTIFICATION NUMBER	NUMBER OF ORAL DOSES	PENTACHLOROPHENOL mg./100 ml.*
D 6865	2	0
D 6866	5	0
D 6867	7	0
D 6868	9	0
D 6869	11	0
D 6870	14	0
D 6871	16	0
D 6872	18	0
D 6873	20	0
D 6875	23	0
D 6876	26	0
D 6877	30	0.15
D 6864	33	0.15
D 6865	37	0.16
D 6867	40	0.10
D 6867	44	0.10
D 6868	48	0.10
D 6870	52	0.40
D 6872	56	0.20
D 6873	61	0.10
D 6874	68	0.20
D 6875	72	0.15
D 6876	75	0.15
D 6864	77	0.30
D 6865	81	0.15
D 6867	85	0.20
D 6870	90	0.30

* The volume of samples was 10 ml.

16 hours after administration), are summarized in Table 1. The chemical method of analysis em-

* Received for publication January 23, 1943.

(B) The Relationship Between Blood and Urinary Concentrations of Pentachlorophenol

Single oral doses of sodium pentachlorophenate equivalent to from 50 to 200 mg. of pentachlorophenol per kilogram of body weight were administered to rabbits. (The lethal oral dose is about 275 mg. per kilogram.) The animals were kept under restraint for 8 hours, during which time their urine was collected by catheterization and samples of blood were obtained by cardiac puncture. Eight hours after the administration of 50 mg., the blood level was 4 mg. per 100 ml., the urinary concentration 92 mg. per 100 ml.; at the corresponding time after the administration of 100 mg., the blood level was 10 mg. and the urinary concentration 344 mg. per 100 ml.; after administration of 200 mg. the respective results were 13 mg. and 372 mg. (Figures 1, 2, 3). Determinations made 24 hours later revealed very considerable decreases in concentration in both blood and urine.

Discussion and Conclusions

Sodium pentachlorophenate, fed to rabbits daily (excepting Sundays) until 90 doses, [illegible] equivalent to 1 mg. of pentachlorophenol per kilogram of body weight, had been given, caused only a doubtful retention of the compound in the blood. Analyses carried out during the course of the experiment gave results which were either negative or only slightly above the limits of the analytical error.

Comparison of the blood and urinary concentrations of pentachlorophenol during the first 8 hours following the ingestion of 50, 100 and 200 mg., respectively, per kilogram of body weight, demonstrates a sharp rise in urinary pentachlorophenol in response to a slight increase in the blood level. The constancy of the relationship between urinary and blood concentrations over several hours, and the very much higher concentration of the compound in the urine than in the blood, confirm earlier observations and suggest that urinary determinations may offer a sensitive means of estimating the extent of human absorption and, consequently, the severity of exposure to pentachlorophenol during its manufacture or handling.

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THE ETIOLOGY OF ACNE WITH SPECIAL REFERENCE TO ACNE OF OCCUPATIONAL ORIGIN*

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IN SPITE of an enormous literature and very considerable volume of work, precise etiological factors in relation to acne vulgaris remain unknown.

Acne vulgaris is a very widespread and common condition and many cases are available to every practitioner for study. The occurrence of acne in industry has also received considerable attention and a determination of the cause of this type might throw considerable light on the etiology of acne vulgaris. Acne has also been described as arising from the action of bromides and iodides taken internally but the lesions produced differ fundamentally from acne vulgaris in that the essential lesion, the comedone, is absent. Acne arising locally from oil and tar has also a widely different distribution. In the occupational acne to be described the fundamental lesion is present and the condition is strictly comparable with acne vulgaris. A brief review of factors associated with acne vulgaris upon which most observers are agreed is instructive.

It is agreed that the essential lesion is the comedone, which is caused by a hypersecretion of sebum and hyperkeratosis of the mouth of the lanugo follicle, the blackhead being formed by the oxidation of the keratinised cells. It can only occur where sebaceous glands are present and these are larger and more numerous in certain regions. The regions most commonly affected are the forehead, temples, cheeks and chin, especially on the sides. The chest, back and deltoid region may also be affected.

The reasons for localization are discussed more fully later.

The disease occurs most commonly at puberty, i.e. at ages 12–18, and remains until 20–30 years of age.

Bloch (1) has demonstrated the "statistical relation between acne and puberty" and considers "the same cause is responsible for both. Thus acne is a consequence of the physiological function of the sexual glands and variations in degree are due to the follicular apparatus of the individual differing in its sensitivity to the hormone."

There is a growing literature on the relation between acne vulgaris and hormone factors and Werner (2) states that an endocrine factor may initiate the skin disturbance, the infective process being secondary.

Lawrence (3) and Templeton and Truman (4) report beneficial results from hormone therapy, e.g. Anuitrin

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'S' in acne vulgaris and menstrual disorders. Wile (5) concludes that although there is a diminished secretion of estrogen in many cases of acne there is not sufficient proof of its significance to justify its general therapeutic use.

A survey by Hinrichsen and Ivy (6) of the incidence of acne includes groups of subjects living under different social conditions. They conclude that acne appears and reaches a more severe stage at an earlier age in girls than boys which is in harmony with the fact that sexual maturity occurs earlier in girls. For all grades of acne there was no significant sexual difference in its incidence. The frequent use of creams and lotions does not alter the incidence of acne in its broadest sense but may possibly decrease the incidence of severe involvement.

Acne then, results from a hypersecretion of sebum which occurs temporarily at puberty or more commonly in relation to seborrhea, a true hypersecretion of a more permanent character.

The sebaceous glands are not supplied with nerves and an increased secretion can therefore only occur under limited conditions:

(a) From an increase in size of the gland,
(b) From stimulation of the gland,
  (i) From congestion of the capillaries (heat, exercise, alcohol).
  (ii) From irritation by (a) excretion, (b) local irritation.

There is fairly general agreement upon other related factors. The sex distribution is equal. Heredity, inasmuch as seborrhea is often a familial factor, is a definite related condition to acne vulgaris and also occupational acne—the greasy skin favors the growth of organisms and causes dust to adhere. Local irritation from dirt, dust, oil, tar and turpentine is of considerable importance as a predisposing factor. Season and social position are not widely accepted as predisposing causes. The problem of diet is reviewed by Hellier (7) who concludes that excess of carbohydrate and fats increase the tendency to acne.

There is a considerable doubt as to the relation of bacteria to acne and several organisms have been stated to be a casual factor.

(1) The acne bacillus described by Unna in 1893 and cultivated by Sabouraud in 1897. It is invariably present in comedones showing no sign of inflammation and also in those which have broken down.
(2) The acne bacillus in combination with staphylococci.
(3) Staphylococci alone particularly staph. albus.

A harmless mite (*Demodex folliculorum*) is also present.

Goldsmith (8) reviews the relation of staphylococci and the microbacillus to acne and concludes there is no direct relation.

Acne in Industry

Brief Historical Review

The occurrence of acne in industry has been noted for many years and has usually been described under the name of "chlor acne." Very many substances have been blamed as the causative agent and it was soon recognized that the name "chlor acne" was a misnomer inasmuch as exposure to chlorine or to many compounds con-

taining chlorine, does not give rise to the disease.

Bettmann (9) reported cases in workmen engaged in unpacking a hydrochloric acid tower constructed with tarred plates. Numerous other chlorinated organic products have been blamed.

Herxheimer (10) observed and described the disease in workers engaged in the electrolytic manufacture of chlorine and stated it only appeared in association with some form of coal tar. Carbon electrodes were in use. He considered the cause might be chlorobenzene formed by the passage of chlorinated phenol into the blood. The disease is considered an ordinary tar or paraffin eczema by Barres and Courtois-Suffit (11).

Rambousek (12) blamed chlorinated tar products given off in the electrolytic manufacture of chlorine by action of this product on the carbon anode. The anodes were made from tar, bitumen and ground charcoal and the containing vessels painted with pitch varnish. The cases of acne disappeared on the substitution of magnetite for carbon as the anode. He also described an outbreak during work with a hot mixture of saltcake and tar.

Wauer (13) described "Pernakrankheit" resulting from exposure to higher chlorinated naphthalenes.

Koelsch and others (14) blame chloronaphthalenes as does Teleky (15) who further states the injuries produced are proportional to the chlorine content of the product and considerable improvement results if the chlorine content is decreased from 30% to 8%, the loosely bound chlorine being eliminated.

Nicolas and Lacassagne (16) report a case with widespread lesions distinguished from acne vulgaris by the localization of the eruption.

Flury and Zernik (17) describe acne-like inflammation of the sebaceous glands due to chloronaphthalenes and occurring on the uncovered skin of the face and arms and covered parts where rubbing takes place.

Schwartz (18) described chlor acne on exposed and covered parts of the body from the fumes of chlorinated naphthalenes. Patch tests were negative.

Jones and Alden (19) suggest as the ultimate cause styrene di-chloride and chloroethylbenzene.

Mayers and Silverberg (20) describe cases of skin disease resulting from exposure to chlorinated hydrocarbons. The most important skin lesions were of an acne-form nature. Chlorinated diphenyl is also very similar in action to chlorinated naphthalenes.

Acne in a Chemical Industry

During the past 6 years I have had the opportunity of studying occupational acne in a chemical industry. Every man has received a complete physical examination but females are excluded because of the small number engaged and the fact that no females have been exposed to agents known to cause acne. Table 1 indicates the number of employes engaged in the industry, by age groups, and in table 2 is shown the type of skin disease discovered.

The Incidence of Acne.—The diagnosis of acne has been established by the presence of comedones sufficiently obvious to warrant treatment. The

TABLE 1

EMPLOYES EXAMINED

AGE	FACTORY 1		FACTORY 2		TOTAL
	Workers	Staff	Workers	Staff	
15	6	—	12	—	18
15–20	115	3	128	1	247
20–25	168	15	132	13	331
25–30	174	43	111	8	336
30–35	211	32	121	18	382
35–40	171	31	127	24	353
40–45	149	26	122	21	318
45–50	138	29	114	21	302
50–55	144	21	99	12	276
55–60	121	8	64	7	200
60–65	142	4	55	2	203
Total	1,539	215	1,085	127	2,966
	1,754		1,212		

The incidence of susceptible (age 15–30) employes at Factory 1 ... 29.0%
The incidence of susceptible (age 15–30) employes at Factory 2 ... 32.0%

Thus there is approximately an equal percentage of susceptible employes and an equal incidence of acne in both works. It will be seen later that the cases of occupational acne are approximately equal at both works. Table 3 indicates the age distribution and severity of the cases of acne.

112 cases occurred between the ages of 15 and 30 = 66% of total cases
128 cases occurred between the ages of 15 and 35 = 70% of total cases

The Incidence of Occupational Acne.—The cases of occupational acne have occurred in the following occupations.

A. Workers engaged in a process using molten chloronaphthalenes.
B. Workers actually engaged in the manufacture of chloronaphthalenes.
C. Workers engaged in experimental processes with chlorinated naphthalenes and chlorinated diphenyl.

TABLE 2

EMPLOYES FOUND TO BE SUFFERING FROM SKIN DISEASE

WORKS	ACNE INCLUDING OCCUPATIONAL ACNE	INDUSTRIAL DERMATITIS EXCLUDING ACNE	ECZEMA	VARICOSE ECZEMA	PSORIASIS	IMPETIGO	SEBORRHEA	ACNE ROSACEA	FUNGUS INFECTIONS	SCABIES	FURUNCULOSIS	URTICARIA	ALOPECIA	ICHTHYOSIS	TOTAL
Factory 1	99	16	13	7	6	5	22	—	1	—	5	2	1	1	178
Factory 2	70	59	8	3	3	1	22	3	3	1	5	3	—	—	181
Total	169	75	21	10	9	6	44	3	4	1	10	5	1	1	359

classification adopted by Bloch has been roughly followed, i.e.

\+ = 5–20 comedones
++ = 20–50 comedones or papules and pustules.
+++ = 50 or more comedones or papules and pustules.

The total incidence of acne was 169 cases or ... 5.7%
Acne expressed as a percentage of total skin diseases ... 47.0%
The incidence of acne at Factory 1 5.6%
The incidence of acne at Factory 2 5.77%

D. Cases occurring at other or customers' factories.

The cases occurring in the first three groups are set out in table 4.

Description and Occurrence of Cases.—The cases I have had the opportunity of observing have arisen during the manufacture of chlorinated naphthalenes or from the use of these bodies to impregnate various articles.

TABLE 3

ANALYSIS OF CASES OF ACNE

AGE	FACTORY 1				FACTORY 2				TOTAL
	Slight +	Moderate ++	Severe +++	No.	Slight +	Moderate ++	Severe +++	No.	
15	1	1	—	2	—	—	—	—	2
15–20	14	14	2	30	3	5	—	8	38
20–25	17	8	2	27	2	13	2	17	44
25–30	4	9	1	14	3	10	3	16	30
30–35	2	3	—	5	1	9	1	11	16
35–40	5	2	—	7	—	1	1	2	9
40–45	1	2	1	4	—	4	1	5	9
45–50	—	4	1	5	—	5	—	5	10
50–55	—	3	—	3	—	2	—	2	5
55–60	—	2	—	2	—	2	—	2	4
60–65	—	—	—	—	—	2	—	2	2
Total				99				70	169

TABLE 4

INCIDENCE BY AGE GROUPS

GROUP	TOTAL	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60
A—Factory 2	4	—	—	1	1	—	1	1	—	—
B—Factory 2	13	—	1	5	1	3	3	—	—	—
C—Factory 1	20	8	7	3	1	—	—	—	—	1
Total	37	8	8	9	3	3	4	1	—	1

During the process of manufacture it is impossible to determine the precise stage of chlorination most likely to cause acne but cases 1–4 (table 5A) have occurred from exposure to a product approximating tetrachloronaphthalene.

It does not appear possible that any decomposition product of the bodies is responsible for the development of acne which is due to a specific effect of the chlorinated naphthalene. Acne has therefore, been produced under conditions of exposure to the fumes of heated chlorinated naphthalenes. (One case occurred from exposure to the fumes of chlorinated diphenyl.) No case of acne has arisen from handling the cold products within the works, although approximately 50 men have been exposed to this risk. Very many times this number have been at risk handling the products in the cold state outside the works, but no cases of acne have been reported. The conditions necessary for the production of

acne are that the chloronaphthalenes shall be present in a finely divided state. This may occur when the products are volatilized by heating or are present in a solution which is allowed to evaporate or as dust. These conditions have been widely recognized and may occur under varying conditions of manufacture and use of the products. It is instructive to note that employes exposed to constituent products do not contract acne. Thus no cases of acne have arisen during the manufacture and use of chlorine where I **have had the opportunity of examining repeatedly 150 men over a period of 6 years.** 4 workers exposed for varying periods from August 1936 to monochloronaphthalene have not contracted acne.

Tables 5A and 5B indicate the employes affected with occupational acne and points of interest and photographs are referred to subsequently.

Case No. 1, A. C., is described in detail as being the most severe and illustrating many typical features of the disease.

Case 1—A. C. Age 43 years. Pipefitter. Was medically examined on July 7, 1932 previous to starting work at Factory 2.

Medical and Family History. Nothing serious or relevant. *Conclusion:* Physique good. Fit for any employment. Note: Few acne spots were present at the time.

***History.* Commenced work in 1932 as a pipefitter.**

In January 1933 commenced work on a process using molten chloronaphthalenes in an open vessel. No particular precautions were taken with regard to ventilation as at the time it was not suspected that the manufacture and use of chloronaphthalenes required special precautionary measures.

The summer was very hot and he was perspiring freely from this and his other work. He wore a blue flannel shirt and noticed the dye ran, to which he attributed his skin lesions.

Condition on Dec. 20, 1933. Physically sound. Eyes dark brown. Hair dark brown and fairly thick. No anemia. Slight icterus of conjunctivae. Perspires normally. Slight seborrhea of scalp. Teeth: uppers, denture; lowers fair. Personal cleanliness good. Teetotal. Skin dark, fairly moist but not greasy.

The condition commenced in April 1933 as "pimples" on the lower part of both arms (flexor aspect) and between the legs.

Constitutional disturbance. Complains of feeling "below par." Becomes tired easily. Loss of rest due to irritation of the skin. *Itching very marked.* For the past 3 weeks has had pain after food with nausea.

Local disturbance (see fig. 1). There was no preliminary irritation, erythema or edema. No other members of his family or associates have been affected. The skin is normal between the lesions described and there is no alteration in the normal pigmentation of the skin. The scalp, forearms, axillas and hands are free from lesions. The *face shows a few* comedones only. The observations are of interest and are further discussed later in view of the nature of his work at which he wore a shirt with sleeves rolled above the elbows. Comedones are very numerous on the neck and extend slightly into the hair but infection is slight. Few comedones with slight infection are present behind the ears and under the chin. The comedones are numerous upon the chest and many infected follicles are present. The breasts are especially affected with large follicles. Secondary infection is prevalent upon the upper abdomen and comedones are numerous upon the back, especially in the lumbar region. Comedones are very numerous upon the arms and many are infected. The upper part of the thighs, the scrotum and the penis have many infected follicles.

***Progress.* Removed from work with chlorinated naphthalenes in January 1934. Improvement commenced on the face, back and arms. Lesions on the chest persisted** up to November 1935 when the infection subsided. Few blackheads remained. Several dull red pigmented scars remain to date. (See photograph.)

TABLE 5A

Cases of Acne

Group "A", Cases 1–4 inclusive. Workers engaged in the use of chloronaphthalenes.
Group "B", Cases 5–17 inclusive. Workers engaged in the manufacture of chloronaphthalenes.

Case	Age	Exposure: Degree	Exposure: Total period, months	Date of onset of acne	Latent period	Distribution of acne	Severity	Time taken to recover after removal from contact with causative agent
					mos.			
1. A. C.	43	Severe	12	Apl. 1933	4	Generalised	Severe	2 yrs.
2. T. J.	25	Severe	12	Mar. 1933	2	Generalised	Severe	4 yrs.
3. H. A.	34	Slight	3	May 1934	3	Face	Slight	2 wks.
4. W. H.	47	Slight	13	May 1936	13	Chest	Slight	
5. E. A. D.	42	Mod. severe	40	Dec. 1935	24	Face	Slight	Not removed
6. W. P.	39	Mod. severe	36	Mar. 1935	16	Face, neck. Cysts present	Mod. severe	8+ mos.
7. J. A. T.	38	Mod. severe	18	Sep. 1935	18	Face	Moderate	4½ mos.
8. T. C.	24	Mod. severe	5	Dec. 1935	5	Face	Moderate	1 yr. Progressive 6 mos. after removal
9. M. S.	30	Mod. severe	5½	Feb. 1936	4½	Face	Moderate	1 yr.
10. W. H.	28	Mod. severe	18	Sep. 1937	24	Face	Slight	Not removed
11. C. M.	26	Mod. severe	17	Dec. 1935	2½	Face	Severe	6+ mos.
12. W. W.	41	Mod. severe	4	July 1937	13	Face	Slight	4 mos.
13. R. T.	35	Mod. severe	3	Dec. 1937	2½	Face, forearms, neck	Severe	4+ mos. Progressive 3 mos. after removal
14. J. S.	26	Slight	Occasional	Jan. 1936	?	Face	Moderate	
15. A. C.	26	Moderate	5	Nov. 1937	1	Face	Slight	Not removed
16. J. McD.	25	Moderate	Occasional	Jan. 1936	?	Face	Slight	
17. J. T. L.	41	Slight	Occasional	Sep. 1935	?	Face	Slight	

Case 2—T. J. Age 25 years. Pipefitter. Was not medically examined until Dec. 28, 1933 when the acne was well established.

History. First employed at Factory 2 in 1923 as a laborer.

Family History. Nothing relevant.

Personal History. Nothing serious or relevant.

Present Condition. Physically sound. Physique fairly good. Eyes grey. Hair light brown, medium thick. No anemia. No jaundice. Teeth—Dentures (4 years). Perspires normally. No seborrhea. Personal cleanliness—fairly good. Teetotal. Skin—moist, dark, smooth, greasy. Had never had any skin trouble before January 1933 but had a few blackheads. He also wore a blue flannel shirt, and noticed the dye ran.

The acne commenced in March 1933, gradually at first. The irritation was marked especially when he became warm. Pimples appeared on the lower part of the back and stomach (i.e. top of the pants), and spread to the back, arms, face, back of head, legs and scrotum.

Constitutional Disturbance. None. General health good.

Local Disturbance. The eruption is not anything like so extensive as the previous case. It is to be noted he was not so exposed to fume. No members of his family or associates have become affected.

The skin is normal between the lesions described, and there is no alteration in pigmentation of the skin elsewhere. Practically the whole of the body is affected with comedones, but infected follicles are few. The scalp is free.

Face—Comedones very numerous. Infected follicles few. *Neck*—Comedones very numerous, especially behind ears. Some cysts are present. *Back*—Pustules old and new numerous. Comedones few. *Ankles*—Pustules numerous. *Buttocks*—Some infected follicles.

Progress. **Slow but steady progress was made after removal from the process. Many blackheads** remained on the face until March 1934 but few became infected. Numerous blackheads were present on the buttocks until August 1937.

I have had the opportunity of studying the mode of production of cases of acne arising from exposure to chlorinated naphthalenes in industries other than the actual manufacture of the product. They have indicated that exposure to vapor is a necessary factor and handling the cold product does not produce acne.

Cases of particular interest were observed in 1936 (D. Limited 21.12.36) when 2 boys were exposed to vapor and developed acne of the face and neck after a latent period of 5 months. Their hands and arms were mostly exposed but no lesions developed in these areas.

Predisposing Factors and Susceptibility.—In my opinion the majority of workers will become affected with acne if exposed for more than 2 to 3 weeks to concentrations of fumes which are considered more than slight. In most operations it is only possible to estimate the amount and duration of exposure. Out of 81 workers exposed to fumes 37 became affected with acne, i.e. 45%. The exposure was more than slight in only seven of the unaffected cases. Four of these employes were more than 40 years of age, the other three being 24, 25 and 35 respectively.

In cases described by Fulton and Matthews (21) 101 persons or 78% became affected. Jones and Alden (19) state that 23 people out of 24 were affected from the late summer of 1932 to October 1933. Flury and Zernik (17) state that susceptibility is very variable and estimate that 50% of workers suffer.

Table 7 indicates susceptibility in employes exposed to some risk of acne but not affected with the disease.

Certain other factors are related to susceptibility.

Age. The most important factor causing occupational acne is the degree and period of exposure and the age

TABLE 5B

CASES OF ACNE

Group "C", Cases 18 37 inclusive. Workers engaged in research on chloronaphthalenes.

CASE	AGE	EXPOSURE TO CHLORINATED NAPHTHALENES		EXPOSURE TO CHLORINATED DIPHENYL		DATE OF ONSET OF ACNE	LATENT PERIOD	DISTRIBUTION OF ACNE	CAUSE	SEVERITY	DATE OF REMOVAL FROM EXPOSURE	TIME FOR RECOVERY
		Degree	Period	Degree	Period		mos.					
18. C. I. S.	27	Occasional and slight (considerable before Dec. 1936)	3 yrs.	Occasional and slight	1½ yrs.	Aug. 1937	?	Face. None previously	Chlorinated naphthalenes	Slight	Not removed	6 mos.
19. W. T. T.	21	Slight	4 yrs.	Moderate	2½ yrs.	July 1937	?	Face. None Previously	?	Moderate	Not removed	9 mos.
20. R. V. F.	31	Moderate	4½ yrs.	Moderate	4 yrs.	Oct. 1937	7	Along and under mandible. None previously	Chlorinated naphthalenes	Moderate	Not removed	
21. J. E. T.	19	Moderate	16 mos.	Moderate	7 mos.	Nov. 1937	9	Face. None previously	"	Slight	Not removed	
22. A. H.	18	Moderate	20 mos.	Moderate	3 mos.	Oct. 1937	8	Face. None previously	"	Moderate	Feb. 1, 1938	3 mos.
23. J. A.	18	Slight	2½ yrs.	Slight	8 mos.	Nov. 1937	?	Face. None previously	"	Slight	Not removed	2 mos.
24. F. S.	17½	Moderate	9 mos.	Nil	—	Oct. 1937	7	Forehead and forearms. None previously	"	Severe	Nov. 16, 1937	6 mos. Worse immediately after removal
25. P. S. C.	26	Moderate	8 mos.	Slight	7 mos.	Mar. 1937	4	Forehead and around eyes. None previously. No secondary infection	"	Moderately severe	Mar. 24, 1937	8 mos. Became worse immediately after removal

25. P. S. C.	26	Moderate	8 mos.	Slig'	7 mos.	Mar. 1937	4	ously Forehead and around eyes. None previously. No secondary infection	"	Moderately severe	M [illegible] 1937	removal 8 mos. Became worse immediately after removal	
26. N. H. G. B.	27	Moderate	18 mos.	Nil		Jan. 1937	12	Elbows, face and forearms. None previously	"	Moderately severe	May 1937	12 mos.	
27. H. C.	19	Slight	2-3 mos.	Nil	—	Sep. 1937	?	Chin. None previously	?	Slight			
28. H. F.	21	Moderate	3 yrs.	Nil		Feb. 1935	2	Face	Chlorinated naphthalenes	Moderately severe	Not removed. Exposure lessened	3 yrs.	
29. R. J. H.	18	Moderate	1 yr.	Nil		Oct. 1937	8	Face. None previously	"	Slight	Not removed		
30. D. M.	18	Occasional	12 mos.	Nil	—	Sep. 1937	7	Face	"	Slight	Not removed	6 mos.	
31. N. B.	56	Slight	11 mos.	Slight	3½ mos.	Mar. 1938	3	Around eyes. None previously	"	Slight	Not removed	2 mos.	
32. T. A. F.	24	Slight	4 mos.	Nil		Nov. 1937	2½	Face. None previously	"	Slight	Dec. 31, 1937	1 mo.	
33. S. T. G.	23	Slight	4 mos.	Nil		Sep. 1937	5	Face and forearms. None previously	"	Slight	Not removed	3 mos.	
34. J. H.	21	Slight	6 mos.	Slight	3½ mos.	Apl. 1938	3	Face. Had seborrhoea previously	Chlorinated diphenyl	Slight	Not removed		
35. J. M.	22	Slight	2 mos.	Nil		Feb. 1938	1½	Face	Chlorinated naphthalenes	Slight	Not removed		
36. W. W.	23	Slight	5 mos.	Nil		Sep. 1937	7	Face and elbows. None previously	"	Moderate	Not removed	8 mos.	
37. F. G. H.	17	Slight		Nil	—	Nov. 1937		Face	"	Slight	Not removed		

factor is not so evident as in acne vulgaris.

70% of cases occurring were in the age group, 15 to 30 years, while only 54% of the people exposed fell in this age group. Generally speaking, the disease developed more easily and the latent period was shorter, while the mild cases took longer to subside, in younger employes.

Type of skin and presence of skin disease.— The choosing of workers for employment where there is a risk of acne obviously excludes those affected with acne or seborrhea. This factor therefore cannot be estimated. It is probable that they are more susceptible and in one case where the acne developed in a worker affected with seborrhea before the risk was realized,

FIG. 1. Case no. 1. A. C. Photograph May 13, 1934

the acne proved very intractable. I could find no relation between the color

general. It has been held that brunettes are less susceptible than blondes

FIG. 2. Case no. 1. A. C. Photograph November, 1937

FIG. 3. Case no. 2. T. J. Photograph May 13, 1934

FIG. 4. Case no. 6. W. P. Photograph December 10, 1937

of the skin, hair or eyes with susceptibility to acne or skin disease in

to skin disease generally (Klauder and Brown, 22).

FIG. 5. Case no. 11. C. M. Photograph December 10, 1937

the condition to arise and for aggravation to take place during the summer months. On theoretical grounds this is probable, as increased activity of the skin glands occurs, with less evaporation and consequently more secretion in the skin causing adherence and solution of the chloronaphthalenes.

Cleanliness. This is a factor of the very greatest importance. I am convinced that where the general level of cleanliness of the individual is low the acne develops more rapidly (see Case 13) and vice versa.

One man (Case 5) exposed more than any other employe to the fumes of chloronaphthalenes remains free from acne and the reason appears to be

FIG. 6. Case no. 13. R. T. Photograph April 19, 1938

Season. The seasonal influence appears to be of small importance. Allowing for the latent period there appears a slightly greater tendency for

personal cleanliness. This employe is an experienced process worker who is fully aware of the importance of cleanliness. The factor of cleanliness

operates generally in the prevention of industrial dermatitis.

Family History. I have been unable to trace any related factor under this heading. No case of any member of a worker's family being affected has come to my notice.

General Health. All workers were examined before engagement, during work with chloronaphthalenes and after the development of acne; a study of the results of examinations provided no relevant information. No general defect, type of development or physique predisposed to the development of acne. No disturbance of health occurred during or after the development of acne or during work with chloronaphthalenes. A study of sickness records has not indicated any related factor.

TABLE 6

EXPOSED EMPLOYES WHO HAVE NOT CONTRACTED ACNE. REPEATED EXAMINATIONS, 1932–1939

EMP. NUMBER	AGE	EXPOSURE	TIME	DEGREE OF EXPOSURE
4	37–56	Use of chloronaphthalene	1–6 wks.	3 slight, 1 moderate
14	20–55	Mfg. " "	Odd spells to 3 yrs.	11 " 3 "
26	19–47	Research in " and chlorinated diphenyl	1 mo.–5 yrs.	12 " 7 "
			2 days–6 mos.	Slight

TABLE 7

SUSCEPTIBILITY

	AGE PERIODS									
	15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60
Cases of acne	—	8	8	9	3	3	4	1	—	1
Number at risk, not affected	—	1	9	9	7	9	2	4	—	3
Number not affected where exposure is considered to be enough to cause acne	—	—	1	1	—	1	1	1	—	2

TABLE 8

SEASONAL INFLUENCE

MONTH	CASES
January	3
February	3
March	4
April	2
May	2
June	—
July	1
August	—
September	7
October	6
November	5
December	4

The Mechanism of Production of Acne

1. *General.* The precise mechanism of production of acne has remained undetermined and in the case of acne arising from chlorinated naphthalenes it is unknown whether the effect is a local one or arises from absorption of the product into the body. Dis-

cussing the matter in his book, Prosser White (23) supported by others, considers it a direct external effect, while other authorities report a dermatitis due to elimination by the skin glands. The relationship, if any, between acne due to iodides and bromides and that due to chlorinated naphthalenes and acne vulgaris has not been elucidated. Sulzberger, Rostenberg and Sher (24) note the similarity of acneform eruptions produced by external agents such as oil and tar, by internal agents such as iodides and bromides and acne vulgaris. To explain follicular eruptions caused by halogens the following reasons have been advanced:

1. The rich convolution of blood vessels around the follicles.
2. Their special capacity for irritating the pilo-sebaceous apparatus.
3. The excretion through the sebaceous glands.
4. The response to patch tests is follicular, i.e. the pilo-sebaceous apparatus is oversensitive.

Patch tests have generally been negative in cases of acne from chloronaphthalenes. Mayers and Silverberg (20) applied patch tests with ointments of potassium iodide and potassium bromide to cases of "chlor-acne" without finding evidence of hypersusceptibility.

Oppenheim (25) describes an occupational acne due to bromine occurring in men manufacturing gas bombs. The eruption was situated on the forehead and cheeks and slightly on the neck and chest. He did not decide whether the condition was due to absorption and excretion or to a local action.

In his report for 1936, H. M. Chief Inspector of Factories states that the weight of evidence seems to point to the skin affection being the result of external irritation, as opposed to internal absorption and excretion. In support of this he states that no free chlorine or decomposition product of the wax compound was found in the atmosphere of the "spinning" process held responsible for nearly all the skin cases in one firm. An important case has been described by Fulton and Matthews (21) of a skin eruption occurring in an infant aged $2\frac{1}{2}$ years. The eruption was of an acneform nature, mostly affecting the cheek and forehead and extensor surfaces of the arm and forearms. The father has a generalized chlor acne as a result of exposure to hexachloronaphthalene and chloro-diphenyl. The mother was affected on cheeks, buttocks and extensor thighs and a sister aged 11 months had comedones on both cheeks. The father returned home in his soiled working clothes and played with the child without changing. The father and son slept together, the father always sleeping in his underwear.

In considering this case it is instructive to read the remarks of Macleod (26) on the grouped comedones of infants and young children. The comedones tend to be grouped in clusters, are medium in size and widespread in distribution. He suggests they are due to local irritation from the skin of the mother or the wearing of dirty, greasy clothes. They have been described in several members of one family and a bacterial origin is suggested.

Acne occurs in paraffin workers from a direct effect on the skin. Through the courtesy of Dr. Scott of Broxburn,

West Lothian, I was able to see cases at the Scottish Shale Oil Industry and obtain his opinion as to the cause, which is purely mechanical. In type and distribution the lesions are quite distinct from "chlor-acne" and are prevented by the application of lanoline and the use of washing facilities.

There are many points of great interest in the determination of the method of production of acne of occupational origin.

1. The latent period which occurs between exposure to the products and development of acne.
2. The site and type of the eruption.
3. The changes produced in the skin.
4. The systemic effects of chloronaphthalenes.

The Latent Period. This is referred to by most writers on occupational acne. In my cases this period varied from 1 month to 2 years. The average period was 7 months. Fulton and Matthews (21) give as an average period, 24.3 months. Severe cases developed in 5 to 9 months but only one person who was in contact with vapors of the hot wax developed acne before 2 months. It would indicate that the lesions are not produced by a simple obstruction of the glands, but that some physiological change is produced in them, i.e. hypersecretion. This might be owing to external or internal action. Of equal importance is the observation that repeated crops of blackheads occur after removal from contact with chloronaphthalenes, even with adequate treatment. In the absence of gross infection this period, in moderate cases, is about 4 months. In severe cases with infection 2 or more years elapse before the eruption subsides.

The site of eruption and difference from acne vulgaris. At first sight it would be thought that if acne of occupational origin is produced by a local action it would occur on exposed parts of the skin, and possibly elsewhere if produced by internal absorption. It must be remembered, however, that the histological structure of sebaceous glands and hairs in various parts of the body determine the site of the acne lesions. The function of the sebum is to hinder rapid evaporation and conserve heat. The sebaceous glands are most densely situated where subcutaneous fat is minimal, e.g. on scalp, nose, lip, chin, the middle line of the front and back of the trunk, i.e. those areas which especially need to be protected against loss of heat. The scalp is almost never affected although it abounds in sebaceous glands and a reason for this has been advanced by Bregman (27). He states that the sebaceous glands in the parts of the skin where acne vulgaris usually occurs are covered with lanugo hairs and therefore lack strong vigorous arrectores pilorum. The expulsion of the contents of sebaceous glands, adequate under normal circumstances, easily becomes inadequate with hypersecretion of sebum as occurs at puberty. In my cases the distribution of the lesions has been seen earliest and most commonly upon the face. Commencing on the outer side and below the eye to the malar prominence, the comedones became widely distributed over the cheeks as is clearly shown in photographs of cases 6, 11 and 13. Numerous comedones are present behind the ears. Few are present on the forehead and the nose is characteristically spared, as

is the mid-line of the face generally. In severe cases the lesions are found on the neck, chest, upper abdomen, arms and extensor aspects of the forearms, particularly about the elbows (photograph case 13). The back, thighs and buttocks may also be affected. I have not seen the eruption on the legs. The most characteristic feature is the enormous number of very fine blackheads around the eye and this observation, with the absence of the very noticeable greasiness, distinguishes the eruption from acne vulgaris. Secondary infection is present to a far less degree than would be anticipated from the number of comedones. When it does occur it proves to be a very intractable condition resulting in the formation of cysts (case 6) and violet scars (case 1). 2 severe cases (numbers 1 and 2) showed widespread lesions with secondary infection. The distribution in these cases was very interesting. The forearms were very slightly affected and the face and neck affected to a lesser degree than the chest. The men were engaged in a process using molten chloronaphthalenes where ventilation was poor. They wore blue flannel shirts with short sleeves. The absence of the eruption on the forearms and the mild type on the face indicates to my mind that these areas were washed more frequently than other parts of the body and supports the view that the eruption is due to an external action on the skin. The back of the neck was affected with many comedones and two factors probably explain this distribution; firstly, the scant cleansing and secondly the rubbing of the shirt neck. Case 1 was not in the "acne age" and the face therefore less susceptible, whereas the body developed acne owing to the clothes rubbing the irritant well into the skin.

Precise Local Changes. The probable mechanism of production is that chlorinated naphthalenes are deposited on the skin and clothes in fine particles and being lipoid soluble, are dissolved in the sebum. The sebum is chiefly composed of the esters of the higher alcohols and fatty acids, a small quantity of fat as triglyceride and traces of cholesterol. The solubility of chlorinated naphthalenes in lanoline (taken as a substitute for sebum) is considerable and experiments prove that more than 1 part of chloronaphthalenes dissolves in 8 of lanoline. In the absence of cleanliness the chloronaphthalenes irritate the sebaceous glands, causing an excess of cell growth and secretion, followed by plugging of the gland and possible secondary infection. As previously stated, cleanliness is a most important factor.

Jones and Alden (19) have described the microscopical examination of the skin of the chest of a negro, aged 26 years, who had a typical acne arising from chlorinated diphenyl. The man commenced work in April 1930 and worked regularly until the end of 1933. He first noticed blackheads on the face, neck, arms and legs in May 1933. The skin was seborrhoic and nothing abnormal was detected on physical examination. Cystic dilation was noted in hair follicles and sebaceous glands with destruction of the hair, marked thinning and atrophy of the epithelium of the follicles and a heavy plug of keratinized material partly filled the cystic cavity. In some areas there was a superficial plug at the surface opening; others showed the

surface open and the plug deeply situated. No purulent exudate was present. There was a zone of moderately dense connective tissue surrounding the enlarged follicles with slight edema and infiltration by lymphocytes, but no leucocytes were noted. Slight edematous changes were noted in the occasional sebaceous glands present but they were strikingly few. The sweat glands were normal.

The changes produced in the skin may be marked in localized and varying parts of the body, the rest of the skin being normal, again indicating a local external action of the causative agent.

Inasmuch as animals do not suffer from acne, little aid can be obtained from experimental studies. Reference has been made to patch tests which are invariably negative and in view of the long latent period, such tests would not be expected to give positive results. Repeated patch tests might prove positive. I exposed the skin of a normal area of human back (17 times) to the vapor of highly chlorinated naphthalenes from Nov. 19, 1937 to Feb. 22, 1938. The skin was not cleansed after the applications but no acne was produced. The experiment was conducted as follows:

About 200 gm. of the material were placed in a liter flask which was closed with a rubber bung carrying two glass tubes (1) inlet, reaching almost to the surface of the chloronaphthalenes, (2) outlet, connected by rubber tubing to the inlet tube of the reservoir. The material in the flask was kept molten by a low flame. Air was pumped into this flask from an enema bulb at the rate of one squeeze per second, which swept the vapor into the reservoir. The reservoir consisted of the top half of a large flask, around the edge of which pressure tubing (slit lengthwise) was fitted to make a more even and resilient joint when applied to the body.

The neck of the reservoir was fitted with a bung carrying two glass tubes. (1) Inlet, which reached three-quarters of the depth of the vessel to ensure a direct impingement of the vapor on the skin, (2) Outlet, leading to a Dreschel bottle partly filled with water to act as a trap and a flow indicator. The heating of the flask was regulated to maintain a constant supply of vapor in the reservoir.

A series of investigations was conducted to determine any alterations in the bacterial flora of the skin during exposure to chlorinated naphthalenes as described in the preceding experiment. No change could be determined nor were any new features determined in the bacteriological examination of cases of established acne.

Since no specific reactions for chloronaphthalenes could be found, experiments to detect their excretion by the skin and urine were considered impracticable.

Systemic effects associated with occupational acne. A study of the systemic effects of exposure to chlorinated naphthalene sheds little light upon the production of acne. Unless the lesions are widespread people affected complain of little except the disfigurement and irritation. Vague symptoms such as lassitude, anorexia, malaise and depression may be complained of in serious cases. Nothing is to be detected on physical examination, although all employes exposed

received repeated complete physical examinations.

Case 1, the most serious I have seen, was investigated in hospital. He complained of malaise, anorexia and lassitude. His conjunctiva had a slight icteric tinge but nothing abnormal could be detected. Liver tests were not performed.

Investigation of the urine showed no albumin or sugar, mucus was excessive, a few epithelial cells and occasional leucocytes and red blood cells were present, no casts or renal cells were observed.

A fractional test meal showed a poor secretion of HCl. The total acidity curve was normal for the first half-hour and then dropped from 208.8 to 10% in one hour and a quarter.

No occult blood was present in feces.

Chloride estimation. Blood: Total Chlorides = 583 mg. NaCl in 100 cc. Urine: 9.23 gm. NaCl in a 24 hours' specimen. Both these were non-fasting specimens.

Fulton and Matthews (21) state that blood and urine examinations in their cases reveal nothing abnormal. 10 exposed cases and 2 controls showed the icterus index (as tested with the Klett Calorimeter) to be raised, but it returned to normal after 1 week.

The effect of chlorinated hydrocarbons on the liver has lately received considerable attention. In 1935 Doctor H. Taylor in a private communication indicated to me that exposure to chloronaphthalenes (in very high concentrations), caused severe damage to the liver, kidneys, and lungs of rats after a period of a few weeks. The chloronaphthalenes were heated and the atmosphere blown over the rats in the form of a mist.

Drinker, Warren and Bennett (28) state that chlorinated naphthalenes and chlorinated diphenyl attack the liver and the liver alone. The higher the chlorination the greater the injury. Compared with benzene and lead tetraethyl they are only slightly toxic and operations employing them can be easily safeguarded. The precise fate of chlorinated naphthalenes in the body is unknown. It has been suggested that since they are rapidly oxidized, the chlorine combining with sodium, the opportunity for it to produce acne-like eruptions such as occurs with bromine is impossible.

The systemic effects are discussed and the literature reviewed by Greenburg et al. (29). 3 liver deaths are noted in detail. **Recommendations are given for the prevention of such cases and it is advised that persons suffering from typical acneform eruptions should be excluded from further exposure.** Drinker (30) also discusses the systemic effects and states that neither a high calcium diet nor the administration of xanthine prevent liver injury. When a mixture of penta and hexachloronaphthalene is given to dogs by mouth the urinary chlorides rise, indicating that in some way the body cells detach chloride from this body. A high chlorine content in the naphthalene does not enhance toxicity.

Genner and With (31) at the Finsen Institute at Copenhagen have investigated the relationship of skin diseases such as lupus, psoriasis and eczema to disorders of metabolism traceable to the liver. It was noted that tarry applications to the skin caused transitory interference with the functions of the liver. In other respects the findings did not support

the theory that hepatic disorders are related to skin disease. Abnormal activity of the sebaceous glands is noted in association with puberty and endocrine disfunction, also as a result of the action of iodides, intestinal toxemia and avitaminosis. It is also enhanced in pulmonary tuberculosis and hyperthyroidism and over-action of the anterior pituitary and by cholesterol-like bodies containing the estrogenic principle (Desaux, 32). Digestive disturbances lower the resistance of the skin to infection as also do blood states such as anemia, and sedentary occupations cause a poor peripheral circulation. Sulzberger (33) has pointed out that many derivatives of tar are structurally like the estrogenic hormone. Lesions other than acne resulting from exposure to chlorinated naphthalenes have not been described in Great Britain. (J. C. Bridge, 1936 (Report, H.M. Senior Medical Inspector of Factories.)

Prevention

Successful prevention might possibly provide a clue to the precise method of production of acne. In all cases the acne arose after exposure to the causative bodies in finely divided condition, most commonly as fume. This produced acne by reaching susceptible areas of the skin either by direct local action or by absorption and subsequent excretion. As previously stated it is my opinion that local action is the mechanism of production. **The fundamental principle in prophylaxis, therefore, is to prevent the worker coming into contact with fumes or fine particles.** Normal methods of working and successful prevention of the condition unfortunately do not enable one to discriminate between the two possible methods of production. Reduction of external fumes is obtained by enclosure of the plant and adequate ventilation by exhaust above the level of the chloronaphthalenes. Ventilation of the building is difficult as the bodies have a high specific gravity and therefore, a high vapor density. The avoidance of over-heating is of great importance and a temperature of 10 to 15°C. above the melting point is all that is required for industrial purposes. Protection of the skin and adequate removal of the bodies should prevent the development of lesions. If, however, acne develops under these conditions stringently observed, it would indicate that the bodies were absorbed into the system and subsequently excreted. If skin protection was adequate it would indicate lung and/or bowel absorption. Personal protection places a considerable onus on the worker which has been proved time and time again to be unsuccessful in any form of safety, also the bodies have been proved capable of causing systemic effects. I tried various skin protectives yet acne still developed after reducing exposure to a minimum. Owing to the solubility of chlorinated naphthalenes in fats the greatest care is necessary in selecting a protective agent.

"Lemon Cream" and also a mixture of equal parts of lanolin and cotton seed oil have both been given an extensive trial.

Lemon Cream

	%
Ivory soap flakes	7.48
Chemically pure glycerine	26.40
Sodium silicate (P.84)	24.20
Tragacanth	0.20
Oil of lemons	0.16
Water	41.60

In cases where I have watched the cleansing of the skin I am not satisfied with the value of any protective tried, but co-operation is difficult to obtain as employes have a complete disregard for the early manifestations of acne.

The following protectives are recommended: A, by the Halowax Corporation; B, by Mayers and Silverberg (20).

A

Zn. oxide	½ oz.
Lot. calamine	½ oz.
Salicylic acid	20 gm.
Ad. Lan. Hyd.	4 fluid oz.

B

Pot. sulphette (50% filtered solution)	60 gm.
Zn. oxide	30 gm.
Precipitated sulphur	30 gm.
Glycerine B.P.	5 cc.
Soluble fluid rose	1 cc.

Closely connected with protection is the removal of the chloronaphthalenes from the skin and prevention from long and close contact. I am convinced this is the most important factor in prophylaxis after reducing fumes to a minimum. The provision of adequate washing facilities with hot water and shower baths should be under supervision by the foreman or other responsible person. It is suggested by Mayers and Silverberg (20) that owing to the fact that chlorinated naphthalenes are insoluble in hot water, cold cream should be smeared on the skin to remove the wax before washing. I have no experience of this method but have found the use of hot water and Neko soap (Parke, Davis & Co.) to be adequate.

General Preventative Measures

General preventative measures are advised by manufacturers of chloronaphthalenes:

1. Education of the workers as to the cause and method of production of acne.

2. The provision of a complete change of clothes for workers (overalls—light colored, highly starched, closely woven, full length sleeves and a proper neck; full length **underclothing laundered at least once a week**).

I tried the effects of hoods and Gamgee masks but the condition was aggravated by this procedure. I consider the cause to be excessive sweating and rubbing into the skin of the chloronaphthalenes which collected under the mask.

3. Adequate locker accommodation.

4. Avoid touching body and face with the hands; no rags for wiping nose and face. Wash before meals. No meals on the plant.

Other measures may also reduce the incidence of acne:

1. Medical selection of workers: (a) Avoid adolescents. (b) Avoid employes with oily skin and established acne or seborrhoea.

2. Medical inspection at frequent intervals: (a) Eliminate susceptibles. (b) Remove from contact all early cases.

3. No other work to be performed involving excessive perspiration.

Summary

Acne arises from exposure to the fumes and fine particles of chlorinated naphthalenes in a very high percentage of workers exposed.

The latent period and effect of cleanliness, the absence of systemic effects and the distribution of the eruption indicate that the occurrence of acne is due to a local effect on the skin.

Experience has indicated that provided simple precautionary measures are adopted, products known to give rise to acne may be manufactured and used with safety.

The information contained in this paper is published by permission of the Directors of Imperial Chemical Industries Limited, to whom the thanks of the author are due.

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dling this type of material in the San Diego Division of the Consolidated Vultee Aircraft Corporation there has not occurred a single case of serious intoxication from exposure to any toxic material throughout this large plant.

Chlorinated Naphthalenes and Diphenyls

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THE chlorinated naphthalenes comprise a group of chemical substances made by the addition of various amounts of chlorine to a naphthalene base. The amount of chlorine may vary from three to six or possibly more atoms providing compounds from trichlornaphthalene to hexachlornaphthalene.

In the same manner, the varying amounts of chlorine are added to a diphenyl base providing a group of compounds of varying chlorine content.

As a rule, the final substances, as used in industry, are mixtures of differing chlorinated naphthalenes. Earlier, these were, as a rule, possessed of a lower degree of chlorination and later on the compounds were usually of a higher degree of chlorination with varying amounts of chlorinated diphenyls added thereto.

These substances are not new. Perchlornaphthalene was used in Germany during the last war.

It is to be clearly understood that these groups of compounds are not manufactured by any one company but rather by approximately five in the United States, and they are sold under varying trade names by the manufacturers.

The chlorinated naphthalenes and diphenyls are valuable industrial products. Because of certain properties which they possess, we may briefly state these valuable properties as follows:

1. Resistance to water and alkali.
2. High insulating value. They possess high dielectric constant.
3. Thermo plasticity.
4. Quite stable chemically.
5. Flame resistant.

For these reasons, these substances possess much value industrially in the making of electric condensers, and in the insulation of wire and cable, etc.

Method of Use in Industry

THE chlorinated naphthalenes and diphenyls are used in industry by two methods:

1. A cold method in which the material is dissolved in a solvent usually a mixture of petroleum naphtha and tolu[redacted]
2. A [redacted]d wherein the material is rendered plastic b[redacted]

Toxic Ef[redacted]

IN THE [redacted]tates, the first three cases of acne produ[redacted]orinated naphthalenes were reported by Sulzberger[1] and his co-workers in 1934. Fulton and Matthews,[2] of the Pennsylvania Department of Labor, reported 101 cases of dermatitis in a wire insulating plant in 1936.

In 1936, Dr. Louis Schwartz,[3] of the United States Public Health Service, described cases of acne produced by chlordiphenyls, and in the same year, Jones and Alden[4] reported similar findings.

In 1938, Mayers and Silverberg described acneform skin eruptions in workers making electrical condensers.[5]

Time does not permit a discussion of the histology of these dermatological changes produced by this substance.

In general, it can be said with a fair measure of certainty that these changes are brought about in the skin, primarily by the contact of the skin with the vapors of these compounds, or by actual contact with them in the form of solid or particulate matter. We have found, for example, that workers exposed to fumes have more acne than those exposed to dust alone, and we have also found that workers exposed to the solid material or its particulate form have developed dermatitis. In this connection, the repo[redacted] Fulton and Matthews is very interesting. **They p[redacted] and describe a case of an infant, 2½ years of age developed the dermatitis as a result of contact wit[redacted] soiled working clothes of its father who was in[redacted] habit of playing with the child prior to changing clothing.**

More recently, Schwartz[6] has described cases of dermatitis among cable strippers employed at shipbuilding establishments and working with cables which had been impregnated with these compounds.

Systemic Effects

IN THE United States, systemic effects in the form of acute yellow atrophy of the liver were reported by Flinn and Jarvik[7] in 1936 among men working with tetra and pentachlornaphthalene. These findings were verified by animal experimentation. In 1937, Drinker, Warren and Bennett[8] reported such liver changes among animals exposed to the material by inhalation, and similar findings were obtained with the chlorinated diphenyl compounds. These latter authors also mention four non-fatal cases among humans and the three fatal cases previously cited by Flinn and Jarvik.

I should like to cite two or three cases which we have encountered in our own experience[9] and which may be of interest from the point of view of duration of exposure prior to this type of poisoning. A girl 17 years of age was engaged in soldering electrical condensers which were impregnated with tri- and tetrachlornaphthalene for a period of approximately seven months. The autopsy for this case showed mild to severe degeneration of the liver and presented a clear picture of what is generally known as acute yellow atrophy. It was apparent in this case that the latter organ displayed areas of healing and new areas of pathology.

In the second case, a man 24 years of age developed jaundice after five months of exposure in the coating of wires with wax containing the higher chlorinated naphthalenes. He was away from work for a period of time and four months later the jaundice became quite intense, with general malaise and loss of appetite. After two months more of work he was sent to the hospital, where he rather rapidly passed away. The autopsy in this case showed extensive areas of necrosis and fibrosis of the liver with regeneration.

In a third case, published by Drs. Mayers and Smith,[10] of the staff of the New York State Department of Labor, an 18 year old girl became ill after five months of exposure in the soldering of electrical condensers. The waxes in this case were composed principally of trichlornaphthalene. She was hospitalized and recovered after a long and slow period of convalescence.

Recent Industrial Experience

DURING the past year the Division of Industrial Hygiene of the New York State Department of Labor has conducted an investigation in two cable plants

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Round Table Discussion, Regulation in the Use of Toxic Substances. [redacted]

ation, a large number of cases of dermatitis und, and several deaths due to liver damage w rs in the industry.

ation of workers in the two factories r vely few cases of enlarged liver—five in alpation.

arison in these two plants showed a very much ncidence of acneform dermatitis in one of the s compared with the other; namely 21% as d with 60%, the former being in the cold establishment and the latter being in the es- nent employing the hot process only. The der- appeared to take place on the average in about ths in the hot process plant as compared with hs in the cold process plant.

ions

ESULT of our general re-study of this whole blem and our experience in two recent out- as well as several isolated experiences we desire several basic conclusions:

lorinated naphthalenes and diphenyls are in highly toxic compounds and must be used with care. **Industrial hygienists should make every see that such exposures are controlled, in so umanly possible.** In this effort, we do not be- safe to rely on limiting atmospheric concentra- t rather to depend on a maximum of mainte- d engineering control.

xic exposures may be more readily controlled e cold or solvent method of impregnating cable y than where the hot process is used.

nown cases of liver disease in our experi- lable history and experience generally o exposure to vapors or fumes from the hot There is no clear evidence in our experience, it is possible that skin absorption may pro- stemic poisoning. Physiologically we see no vhy this is not possible. However, most der- cases do not develop liver damage.

y, I should like to call attention to a group of endations which were agreed upon by all of the nvolved in the last outbreaks of poisoning as utmost importance in control of this hazard.

endations

s there is a very good reason for using the hot hod of impregnation all new installations se the cold or solvent method of impregnation orinated naphthalenes and diphenyls. Where method is now being used it should be changed cold, if possible, or surrounded with every rotective measure.

neral hygienic [redacted] **should be followed,** o case should t [redacted] lowed to supersede ing control of t [redacted] source of the ex- he operations in [redacted]

llowing hygienic [redacted] may be considered actice where thes [redacted] nds are handled. Two lockers for ea [redacted] exposed to chlorin- xes (one for working and one for street

rk clothes above the underwear should l laundered at least twice a week by the n

h rkers should change to clean underwear nd of each shift before getting into his street

upervised cleaning: (1) At noon the workers move outer clothing and scrub hands and face pervision. (2) At the end of the shift they e required to take a supervised shower before

(e) Protective skin creams or protective clothing should be provided by the management at the discretion of the foreman, nurse, medical, or plant superintendent.

(f) All departments handling chlorinated synthetic waxes should be thoroughly cleaned according to a prearranged schedule. This should include the removal of all deposits of waxy material from the machines, floors and surrounding objects. **Workers doing the cleaning should be provided with protective clothing and supplied air or organic vapor masks where exhaust ventilation is inadequate or not possible.**

3. The foremen of all departments where this material is handled should be apprised of the toxic nature of the material and instructed in safe handling procedures. These men should make it their duty to check up on the workers in their departments and instruct them in safe practice.

4. Pre-employment and periodic physical examinations should be made of all exposed workers. These should include the taking of a full clinical history, with special emphasis on gastro-intestinal disturbances and dermatitis. In addition, the skin should be carefully examined periodically and the more reliable liver function tests performed. Gastro-intestinal complaints developing in a worker at any time should be a signal for an immediate medical check-up. A history of liver disease, jaundice, or antisyphilitic treatment should automatically exclude a worker from jobs involving a possible toxic exposure. Pregnant women should not be employed where there is a possible exposure to the synthetic chlorinated waxes.

5. Engineering control of plant operations cannot be overemphasized but specific recommendations are not applicable to all cases. It would be wise for a plant using this class of materials to check their control measures with the state industrial hygiene agency, the insurance carrier and some competent consultant before occupational disease occurs.

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Abstract of Discussion

MANFRED BOWDITCH (Boston): A few issues ago an article appeared in the *Journal of the American Medical Association* dealing with that subject of cable wire treating. One of the two authors is here; he might say a word or two on that subject.

DR. IRVING R. TABERSHAW (Boston): Those were experiments to remove wax from the skin, and, as you know, it is practically impossible to get off. DR. GREENBURG mentioned scrubbing, but that doesn't do it. As a matter of fact, workers themselves have attempted everything,

Preventive Industrial Dentistry

DR. C. R. TAYLOR discussed "Preventive Industrial Dentistry."

Preventive industrial dentistry had its beginnings in the early 1900's, securing increased attention after World War I. But it was not until 1942, when a consultant was added to the U. S. Public Health Service staff, that considerable impetus was given to the movement. More recently a group of industrial dentists has been formed by the American Dental Association for the study and planning of services. The State of Michigan also has a dental consultant.

It is important at the present time to plan additional features, and also to plan permanent programs.

An example of what can be done was detailed with reference to the emergency dental clinic set up at Ypsilanti, Michigan, where a U. S. Public Health Service dental consultant was detailed. War workers are here given first attention, and others as opportunity permits, including extractions, fillings, x-ray films, and treatment of Vincent's angina and gingivitis.

As yet no data have been gathered on the saving of lost time, but this is now being obtained. The important thing about the whole set-up is the ability to cooperate with community dentists and to provide facilities for workers on an emergency basis.

The Pomona plan was referred to, where dentists set aside one day a week for workers, and also use evening hours for day workers.

Industrial physicians can participate to good advantage in this program, along with the employer, the employee, and dentists.

Examples of clinical dental problems were described and commented upon and, finally, a pre-payment plan for children with respect to controlling dental problems in the younger group was mentioned as a possibility.

C.C.A. Program

MR. HOWARD STRONG closed the sessions, speaking on "The Industrial Health Program of the Chamber of Commerce of the United States of America."

The general public health aspects of this program were outlined, being described by several publications of the Chamber which were distributed at this session.

It is the purpose of the Chamber of Commerce of the U.S.A. to stimulate local programs, secure local cooperation, not with respect to technical problems, but rather to help to spread and apply general information.

Use is made of an advisory council chosen from different communities, as previously announced. Cooperative relationships are carried out with official agencies, such as the American Medical Association, the American Public Health Association, the U. S. Public Health Service, and others.

It is believed that the Chamber of Commerce has a major job to do in educating the public, to help the public become more health-conscious, and to provide information for public release to the press, radio, printed bulletins, and other forms.

The second phase of this program is action as a result of information released. Health committees have been formed in city chambers of commerce and examples of the public health activities of these were summarized.

Booklets and bulletins have been prepared for distribution on various health problems.

It is proposed that an annual appraisal of community health programs be made by state health departments, with the advice of the U. S. Public Health Service.

Chlorinated Compounds

—Precautions in Handling—

DR. A. G. CRANCH, Director of Union Carbide & Carbon Company's Toxicological Laboratory, submits the following precautions for handling certain chloro-naphthalene, chloro-diphenyl, and related chlorinated compounds. These were developed, under his direction, by the Halowax Products Division, (30 East 42nd St., New York), and are presented here not only for their value in avoiding harmful effects from these chlorinated materials but also on account of the applicability of these precautions to operations where analogous materials are employed. The practice of the manufacturer in distributing this information to all of its customers, and to health agencies generally, is to be commended.

PRECAUTIONS for Handling Chloro-Naphthalene, Chloro-Diphenyl, and Related Chlorinated Compounds:

These compounds constitute a group of synthetic waxes made by the chlorination of a coal tar derivative. The compounds most commonly handled in industry are those containing chlorinated naphthalenes, chlorinated diphenyls, and chlorinated diphenyl oxide. These materials have certain characteristic toxic properties. Their toxic action is evidenced commonly in the development of a form of acne, which usually does not appear until after some weeks or months of continuous exposure to these materials. This exposure may be either to fumes from the hot material or from continued contact with the solid material. It can only follow repeated or continuous contact, and will not develop because of short and infrequent exposures. It closely resembles common acne, but differs in that it is usually encountered on the face — including forehead and temples — neck, of the arms, and sometimes around the waistline, rather than simply on the face and back, which are the more usual locations for common acne. Usually the acne from this industrial exposure is accompanied by a larger proportion of comedones (blackheads) than is the case with common acne; also, itching may be a prominent symptom.

In exceptional cases liver damage may occur through inhalation of the fumes. This is a rare form of poisoning, in contrast to the appearance of acne, and seems to have no relation to the extent or duration of the acne, which may have occurred, but is dependent on other factors. It does not result from casual exposure, but is a cumulative effect.

Experience has shown that, despite their toxic properties, these chlorinated materials may be safely used if proper control measures are observed. **Under good operating conditions the appearance of acne should be reduced to a minimum, and easily brought under control.** With these precautions, cases showing liver damage probably would never occur.

Preventive Measures

MECHANICAL: Wherever these materials are handled good housekeeping conditions must be maintained. There should be no careless scattering of material. All equipment and work places should be thoroughly cleaned preferably every day. Those engaged in this work should take the same personal precautions as are outlined for operators. **Equipment should be of an enclosed type, and fumes and vapors removed by exhaust ventilation.** This is especially important where the materials are handled in a hot condition. Where the so-called cold process is used, in which the chlorinated waxes are dissolved in a solvent, the precautions should be taken to control the vapor given off by the solvent itself at room temperature, and by the compound as well when the solvents are driven off later by heating.

Our engineering department is ready at any time to advise regarding proper design for ventilating equipment for proposed or installed processes. The efficiency of

sting installations can be checked by special equipment, which we will make available when required.

HYGIENIC: Care must be used in the selection of workers. Young people are more prone to develop acne. It would not be desirable to employ such individuals on this work unless their skin is clear, dry, and free from excessive waxy secretion. Those showing an oily skin with a tendency to the formation of blackheads or acne should not be employed. Those having a history of skin disease, liver disorders, or alcoholism should not be employed. Those recently exposed to chlorinated volatile solvents, or those under treatment requiring the use of the heavy metals, as in syphilis, should not be employed. **After employment, there should be close supervision of the workers to detect any evidence of acne in its earliest stages, or any evidence of digestive disorders, jaundice, or other symptoms suggesting liver involvement.** ***Employees showing such symptoms should apply at once for treatment by a qualified physician who is aware of the possibilities present in their industrial exposure.***

PERSONAL HYGIENE: (a) All unnecessary prolonged contact with these materials should be avoided. This as to vapors from the heated material, and exposure to the solid.

(b) There should be a complete change from street clothes to working clothes before going to work. Work clothes should be supplied including such items as starched and laundered cover-alls of light color and close weave; socks; caps; and underwear (preferably union suits with long sleeves and legs). Gloves and aprons should also be supplied where indicated. Sleeves should be buttoned at the wrist. Cover-alls should be buttoned at the neck. Fresh work clothing should be supplied for regular operators twice a week or oftener, daily in hot weather. It should not be taken home for laundering. Provision should be made by the employer for this. If exposure is occasional or intermittent, a different schedule might be warranted.

(c) Separate lockers should be provided for street clothes and work clothes, which should never be kept in close contact in the same locker.

(d) It is essential that face and hands be washed before eating, and that a shower be taken on quitting work. It is recommended that the daily shower should be supervised to make certain that washing is thoroughly carried out before resuming street clothes and leaving the work place.

(e) A suitable skin cleanser should be provided. Strong solvents or soaps containing mineral abrasives should be avoided. Powdered soap containing a corn meal abrasive is probably the most suitable soap, but there are a number of recently developed cleansers based on sulfonated vegetable oil, which may prove to be very satisfactory.

(f) Protective creams offer an additional protection when applied to the face and hands, or to other exposed skin surfaces. These should be applied before work and renewed at least once or oftener, if necessary, through the day. Contact of soiled hands with the face should be avoided. Soiled waste or rags should not be used on the face.

(g) Employees should be cautioned as to the effects of excessive use of alcohol, exposure to chlorinated hydrocarbon solvents, or continued exposure while under medication involving the use of the heavy metals, such as arsenic or bismuth. They should also report at once any evidence of dermatitis, or digestive or liver disturbance.

Light, Temperature, Humidity

—Effects in the Working Environment—

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THE EFFECTS of Temperature, Humidity, Air Movement and Radiant Heat.

A. Acute Effects of High and Low Temperatures: These topics will be discussed from the point of view of their relation to accidents, together with suggested methods of obtaining optimum conditions and of preventing their harmful effects. These physical factors are of importance to industrial workers because they control the amount of heat which can be lost by the body. If sufficient heat cannot be lost, the pulse rate, blood volume, body temperature, respiration and, at high temperatures, the metabolism increase. Because of the cutaneous dilatation, the diastolic blood pressure may fall, if not well compensated. When excessive sweating occurs, salt and water may be lost from the body in sufficient amounts to cause a fall in the concentration of sodium chloride and dehydration of the blood. All of these factors place a marked strain on the circulatory system, the heat regulating mechanism and other structures of the body, which, if extreme, may lead to heat cramps, stroke or exhaustion. Heat cramps are characterized by spasmodic cramps of the skeletal muscles. They may be cured by sodium chloride given either by mouth or intravenously. They are due to the loss of sodium chloride and water from the body through the sweat, and may be prevented by taking salt and water by mouth at intervals during the work shift. Heat exhaustion presents the symptoms of shock to a greater or less degree. It is presumably due to a failure of the cardiovascular system to compensate for the adjustments necessary in exposures to high temperatures. It is reported that the incidence of heat exhaustion in industrial workers has been greatly reduced since salt or salt and sugar tablets have been provided. Heat stroke (or sun-stroke) is characterized by a very high body temperature, often associated with delirium and convulsions. The reason why the heat-regulating mechanism fails in these cases is not understood. It should be noted that many industrial cases show symptoms common to more than one type of heat disease. This is especially true of heat cramp and exhaustion.

The chief effects of exposure to low environmental temperatures among industrial workers are local changes in the skin and subcutaneous tissue of the extremities leading to acute transient inflammatory reactions, frost-bite, chilblains, etc., which may, if extensive, lead to necrosis and gangrene of the tissues.

B. Effects of Temperature and Humidity on Resistance to Disease:

Exposure to high temperatures followed by chilling predisposes industrial workers to acute respiratory diseases such as pneumonia, but does not appear to affect the incidence of tuberculosis. Chronic rheumatism appears to be higher in some industries where sudden changes in temperature and excessive moisture are present. Exposure to high temperatures may also increase the rate of absorption of harmful chemical substances. There is at present no very sound evidence that either high or low humidity *per se* affects the resistance to disease.

C. Relation of Temperature to Work and Accidents:

The acute effects of high temperature are greatly increased when a man is doing heavy physical work, since exercise itself causes an increase in heat production, pulse rate, respiration and body temperature. At the same time, workmen are unable to do as much physical work at high temperatures. Even a slight rise in air temperature decreases the capacity for physical work, and at very high temperatures the ability to work is greatly curtailed. Low air temperatures sufficient to cause numbness also reduce ability to work. It is reported that the

Industrial Intoxication Due to Pentachlorophenol

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OF THE halogenated derivatives of benzol, pentachlorophenol is becoming increasingly important as an **insecticide and fungicide.** Along with its sodium and potassium salts it is being used extensively to impregnate wood, leather, paper, paints, and glues as a protective medium against the action of bacteria, moulds, algae, and insects. For this purpose pentachlorophenol possesses a number of advantages: it is a potent insecticide and fungicide, with an antiseptic action 50 times stronger than that of phenol (Ehrlich and Bechold), is inexpensive, fairly insoluble in water and has a low vapor pressure (Fairhall).

In the technical production of pentachlorophenol, chlorine and partially chlorinated benzol are vaporized and brought to reaction in a quartz tube. The reaction product is finely dispersed hexachlorobenzol, hydrogen chloride, and chlorine. Hexachlorobenzol is extracted and mixed with **sodium hydroxide**, with which it reacts at a temperature of 240° C to form the sodium salt of pentachlorophenol. Free pentachlorophenol is obtained by the reaction of the sodium salt with dilute hydrochloric acid. The final product is centrifugalized, dried, and filled into barrels. Fig. 1 is a schematic illustration of the production process.

Our attention was focused on the industrial hazards involved in the production method described by compensation claims of **10 to 17 workers** employed in the pentachlorophenol production department of a **west-German plant.***

To our knowledge, industrial intoxications of the type described in this paper have not been published previously, although a number of authors, notably Americans, have pointed out this industrial hazard, and performed animal experiments to study the toxicology of pentachlorophenol.

All of the workers gave a case history of **severe skin eruptions and furunculosis.** All but one still showed definite **signs of extensive acne, more than a year after discontinuation of production. Eight of 10 men examined gave a clear-cut history of neuralgic pain of the lower extremities** in the course of their skin disorders or (one case) shortly before. Four still complained of **bronchitis,** which had been quite severe in seven during pentachlorophenol production. In addition to these complaints, various other disorders were reported.

The department in question had been engaged in the production of pentachlorophenol from August, 1948, until February, 1949. In addition to this substance, there had also been some experimentation in the production of trichlorophenol. No further production took place since February, 1949, and with the exception of a certain amount of contact which some of the workers had with pentachlorophenol and associated products in the transfer of machinery and apparatus, none of the workers had contact with other noxious products later.

A distinction must be made between the symptoms due to **irritation of the upper respiratory**

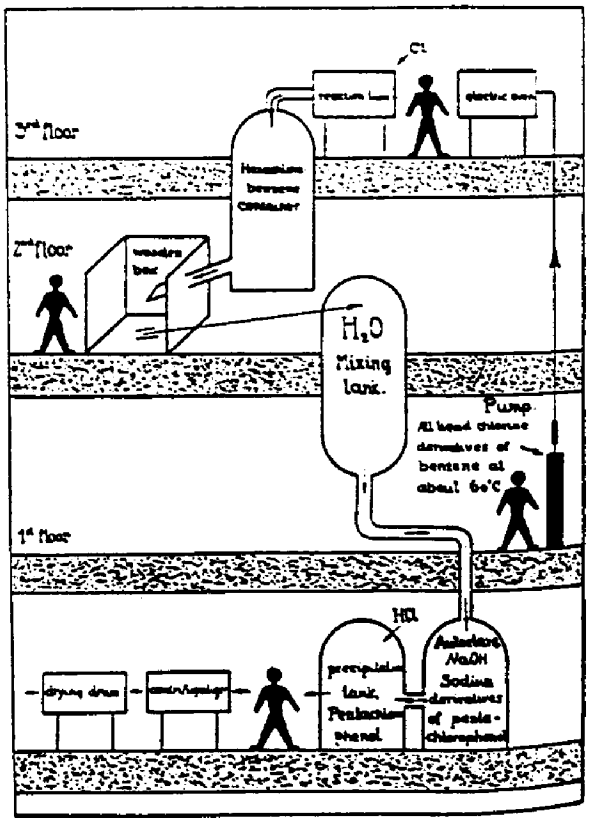

Fig. 1.
Schematic illustration of pentachlorophenol production process.

*Dr. med. O. BRINKMANN of the department of the State Industrial Physician (Staatlicher Gewerbearzt) of Westphalia-Lippe also examined the cases in question and arrived at similar results as the authors. The problem of industrial pentachlorophenol intoxication was discussed at the last meeting of German industrial physicians at Hamm/Westph. on October 25, 1950, by Brinkmann and one of us (Bauer).

Fig. 2.

Fig. 3

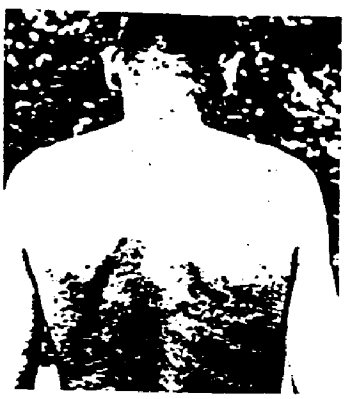

Fig. 4.

passages and the eyes, which occurred during exposure, and usually subsided in the course of a few hours, and the chronic disorders, most of which began or reached their greatest severity weeks, or even months, following exposure. All of the men complained of irritation of the eyes with pronounced lacrimation, irritation of the nasal mucosae, coughing, sneezing, and chest oppression during work. Chronic bronchitis developed in seven of the cases mentioned, and subsided in all but two cases within a few weeks following discontinuation of production.

The first signs of acne were reported by the foreman of the plant in December, 1948, almost five months after the beginning of pentachlorophenol production. No new cases occurred until February, 1949, when four other workers began to develop an acne. The other five workers, i.e., one-half of the men examined, did not notice any skin eruption while engaged in the production process. In these, first signs of a skin disorder appeared in March (one case), April (three cases), and May (one case) of 1949.

According to their own case histories, medical records, which were available in all cases, and findings at the time of examination by the authors (June, 1950), all workers presented essentially the same disorder: an acne disseminata punctata with varying degrees of secondary pustular infection, small and large furuncles, brown pigmentation, and some cicatrization. In all cases, the skin disorder was most severe during the spring and summer of 1949. More than a year later, however, four of the workers still had severe degrees of acne, and four others still showed definite signs of the disease. In one case, the remaining acne lesions were slight, and in only one of the men had the condition cleared up completely.

Eight of the 10 workers examined gave a case history of neuralgic pain of the lower extremities. In one case, this disorder began almost three months before the first signs of acne; in all others, it occurred simultaneously with the skin eruptions or weeks to months later. In all cases, the condition is referred to as neuritis or sciatica by the examining physician. In descriptions of the condition, weakness of the lower limbs, paresthesias, severe pain of the gluteal and femoral region and along the course of the sciatic nerve are mentioned. Unfortunately, no detailed analysis of sensibility and reflexes was made. Since the present examination revealed no definite signs of neuritis in a neurologic sense in any of the workers, we prefer to leave the question open as to whether neuritis or neuralgia is the more appropriate term to describe the painful condition experienced by the pentachlorophenol workers.

A number of other disorders were registered, of which heart complaints (palpitation, shortness of breath) in four cases, disturbances of libido (four cases) and, curiously, bursitis of the elbow (four cases) merit mention. For the last mentioned condition, work histories of the patients in question afforded no mechanical explanation.

No laboratory studies were conducted during exposure or at the period of maximum development of the disorders described. Present examinations gave no conspicuous results. A slight increase of the blood sedimentation rate in six of the 10 cases, with values ranging from 11 to 24 mm, according to the Westergren method, was associated with the acne condition still prevailing. Examination of the urine revealed traces of albumin in two cases, one of which was diagnosed as a chronic nephritis, possibly due to anti-syphilitic medication. There was a slight increase of the $CaCl_2$-coagulation threshold in four cases, positive thymol turbidity test in one of these and two additional ones. Moderate anemia, without conspicuous color index shifts, was found in two cases. The white blood count revealed moderate degrees of leucocytosis not exceeding 12,100 in four cases. Differential counts were inconspicuous. Summarily, it may be said that the laboratory results yielded no new aspects of essential importance.

In an evaluation of the disorders described, there is no question of the relation between the work performed and the irritation of the eyes and upper respiratory passages occurring during exposure. The subsequent occurrence of the same type of skin eruptions in all of the workers involved in the production of pentachlorophenol,

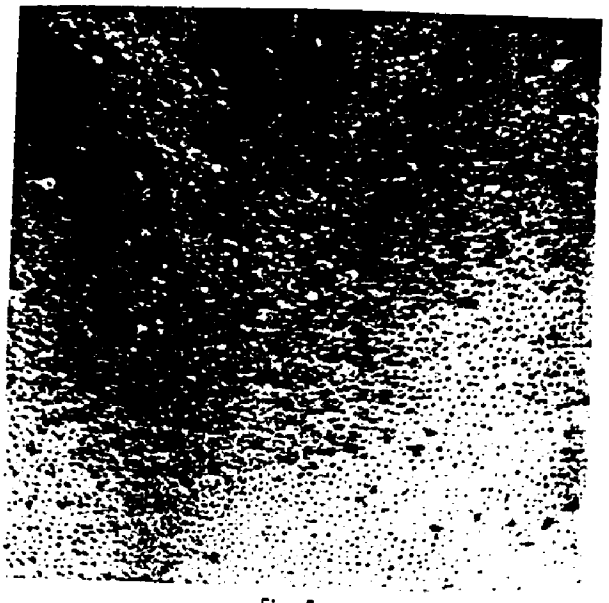

Fig. 5.
Skin eruptions in pentachlorophenol workers.

Fig. 6.
Bursitis of elbow in pentachlorophenol worker.

and the occurrence of neuralgic conditions of the lower extremities in eight of 10 workers points to a definite connection between the exposure to pentachlorophenol and or its intermediary products. Whether the other disorders mentioned are also attributable to a noxious action of chemical substances encountered during work may not be decided beyond a certain degree of probability on the basis of available data.

While it is impossible to rule out the action of other intermediary products (monochlorobenzol) completely, the conditions of work point strongly to the dominating role of pentachlorophenol and hexachlorobenzol in producing the disorders described. In some of the workers, technical experimentation involving **trichlorophenol** and tetrachlorobenzol may also have played a role; however, all of these men spent most of their time in the production of pentachlorophenol.

Figs. 2-6 are photographs of the skin disorders found in all, and Fig. 6 shows the bursitis which was present in four, of the pentachlorophenol workers.

Experimental Data

VON OETTINGEN has summarized the literature on the halogenated derivatives of phenol in Bulletin No. 190 of the National Institute of Health. Quoting the results of Deichmann, Machle, Kitzmiller and Thomas in rabbits, he states that pentachlorophenol is **readily absorbed through the skin and from the gastro-intestinal tract.** Absorption studies indicate that it is distributed throughout the entire organism, and that within the limits of the dosage employed by the authors quoted, 70% are excreted essentially within 24 hours with the urine, and 9% are metabolized. Other studies employing intraperitoneal injection in rats yielded higher percentages of metabolization. It is pointed out that, in contrast to phenol, pentachlorophenol is not conjugated with sulfuric or glucuronic acid.

The application of pentachlorophenol to the skin resulted in extensive local damage, the intensity of which was determined in part by the solvents used.

The **experimental** administration of pentachlorophenol produces central depression followed by an increase in respiratory rate and volume, increased pulse rate associated with a primary rise and subsequent fall of blood pressure, progressive **neuromuscular weakness**, and terminal rise of body temperature. The glycogen contents of the liver are depleted, suggesting that final convulsive seizures may be due to hypoglycemia. There is increased peristalsis and diuresis, later oliguria. In fatal poisoning, heart failure is the cause of death.

In chronic pentachlorophenol poisoning the animals became **listless**, defecated frequently, became **less active**, showed slight **motor weakness and**, prior to death, convulsive seizures.

Pathological examination revealed marked rigor mortis, **extensive damage to the cardiovascular system**, especially the small vessels, **swollen thymus, dilatation of the heart**, congestion of the lungs, trachea and bronchi, **enlarged hyperemic and sometimes edematous liver**, congested kidneys and hemorrhages, leucocytic infiltration, and albuminous fatty degeneration. The brain and spinal cord showed slight chromatolysis of nerve cells and diffuse areas of lymphocytic and mononuclear infiltration on histological examination (Kehoe, Deichmann, Gruebler, Kitzmiller, Thomas, Boyd, McGavack, Terranova, Piccione—quoted from von Oettingen).

Experiments on rabbits were conducted recently by the Gewerbehygienische Laboratorien of the Farbenfabriken Bayer, Elberfeld. Finely pulverized pentachlorophenol was applied to the

Fig. 7.
Left: Normal rabbit's ear. Right: Acne eruptions on rabbit's ear powdered 10 times with pentachlorophenol.

Fig. 8.
Skin of rabbit powdered with pentachlorophenol 11 days.

Fig. 9.
Skin of rabbit, dusted with pentachlorophenol. Histological section showing necrosis of subepithelial layers and massive leucocyte infiltration.

ears of the animals with a cotton swab daily for a period of 10 days. On the fourth to sixth day a slight erythema of the skin resulted; two to three days later, a pronounced swelling of the hair follicles was seen, which increased in intensity for some days following the discontinuation of pentachlorophenol application. Similar results were obtained with a 5% pentachlorophenol mixture with talcum (see Fig. 7).

Employing a similar technic on the shaved skin of rabbits, we could observe intense local irritation following the application of pulverized pentachlorophenol three to four days after exposure. The skin became red, and the veins dilated and prominent. Small excoriations developed in several places. In some areas, without signs of external injury of the skin, an infiltrated, brown-red, plateau-like area resembling tanned leather developed. Histological examination revealed extensive necrotic changes in the upper sub-epithelial layers. There was a massive accumulation of leucocytes immediately above the muscularis, suggestive of phlegmonosis. In other skin areas nodular infiltrations could be found. Our findings seem to coincide with the lesions described by Fairhall as the result of prolonged application of a 1% solution of pentachlorophenol to the skin (see Figs. 8-10).

Without a discussion of the extent to which secondary infection may have played a role in producing the lesions described, it seems essential that they could be produced by the gentle application of pentachlorophenol with a cotton swab, a procedure involving no mechanical injury of the skin.

Studies on the systemic effects of experimental pentachlorophenol intoxication produced in the manner described are still under way.

Comment

WHEREAS the animal experiments described are suggestive of a similarity in the mechanism of skin lesions produced experimentally to those occurring as the result of an occupational hazard in man, there is as yet no definite information available indicating that the systemic lesions found in animals parallel those found in man. The occupational cases presented in this study clearly indicate one thing, however, that **pronounced systemic lesions do occur as the result of exposure to pentachlorophenol and related compounds.** The clinical findings point to a striking similarity with the intoxications due to chlorinated naphthalenes (Perna-disease described by Teleky), in which typical skin changes designated as "**chloracne**" and identical with those found in our cases, **neuralgic disorders and damage to the liver** may occur. The fact that skin lesions are not confined to the exposed areas, strongly suggests that the extensive alterations of this organ cannot be explained by mere contact. More plausible is the assumption that they

Fig. 10.

Same specimen as Fig. 9. Nodular infiltration in skin area dusted with pentachlorophenol.

are a partial (to be sure, the most conspicuous) manifestation of a general intoxication involving the internal organs as well. Assuming this, and considering the similarity to intoxications due to chlorinated naphthalenes, it seems important to consider the possible toxic effects on the liver. We are reminded of the occurrence of a number of fatal cases of toxic jaundice in workers exposed to chlorinated naphthalenes (Schwarz, Good and Pensky, Flinn and Jarvik, McLetchie and Robertson—quoted from Hamilton and Hardy). Whether the occurrence of neuralgic conditions is dependent upon toxic alterations of nerve tissue is still open to investigation. Results of animal experiments cited above (chromatolysis of nerve cells, lymphocytic and mononuclear infiltration) are strongly suggestive of such changes. For practical purposes in dealing with workers exposed to halogenated benzol- and phenol-derivatives, it seems well to consider this possibility in view of the present tendency to attribute all conditions resembling sciatica to mechanical alterations of the vertebral column. It is recalled that sciatica was the initial finding in one of our cases later developing the typical picture with generalized acne.

It has already been stressed that, in addition to pentachlorophenol, a number of other chlorinated benzol derivatives may have played a part in producing the clinical findings. On the evidence available at present, it is hard to assess the exact role played by one or the other of the chlorinated benzols and phenols involved. Considering the amounts of potentially hazardous substances involved, the results of animal experiments, and the similarity of the disease to the condition produced by highly chlorinated naphthalenes, however, we are inclined to consider pentachlorophenol as the noxious substance primarily responsible.

Summary

TEN cases of industrial intoxications due to pentachlorophenol are reviewed. An outline of the production process, results of animal experiments, and the clinical symptomatology, which is characterized by irritation of the mucosae and upper respiratory tract, neuralgic pain, and generalized acne of many months duration, are introduced.

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Connecticut Committee

THIRTY members were assigned to the committee by the House of Delegates. At the first meeting held in the State society building on May 24, 1950, DR. R. J. HINCHEY was appointed secretary and eight members were elected to the executive board: DRS. BARTON, CARTER, CLARKE, GRAY, KILGUS, PHARRIS, VESTAL, and YEAGER. Reports were discussed concerning publications, small plant industrial medical coverage, industrial dentistry, standard practices in methods of air sampling, multiphasic screening examinations, proposed industrial heart clinic at Yale, policies concerning workmen's compensation legislation, various meetings of medical and related professional groups, manufacturer's association and labor organization liaison activities, "ultrasonic noise" developments, alcoholism in industry, and section on occupational health plans. The formation of county committees on industrial health in five counties was reported, and it was agreed that the major effort of the committee this year would be to implement the development of county committees.

—From "Report of the Committee on Industrial Health," by JOHN N. GALLIVAN, M.D., Chairman, in *Connecticut State Med. J.*, May, 1951.

...nts. Thyroid function may be depressed in a higher ...centage of cases if urethane therapy is maintained for ...longer period. This possibility is currently being in...tigated.

SUMMARY

Twenty patients were treated with rectal suppositories ...urethane. The therapeutic effectiveness of rectally administered urethane is equivalent to that of orally administered urethane. Urethane administered rectally is as effective as Fowler's solution in spacing irradiation therapy. Undesirable side-effects were minimal. It is concluded that rectal administration of urethane is preferable to oral or intravenous administration.

2065 Adelbert Rd. (6) (Dr. Weisberger).

CHLORACNE FROM AN UNUSUAL EXPOSURE TO AROCHLOR

J. Wister Meigs, M.D., Jack Jonathan Albom, M.D.
and
Bernard L. Kartin, M.D., New Haven, Conn.

The first outbreak of acne-like lesions due to high ...ling chlorinated compounds in industry in this coun... was noted by Schwartz [1] in 1936. Subsequently, dur... World War II, various reports of acne-like lesions in ...kers exposed to certain chlorinated naphthalenes and ...phenyls followed. Collier [2] reported 12 cases of chlor... of the face in workers exposed to the fumes or dust ... chlorinated naphthalene and one fatal case due to ... yellow atrophy of the liver. Fifty-five cases of acne... dermatitis were reported by Kelley [3] in 200 persons ...osed to chlorinated naphthalene (Halowax). Good ... Pensky [4] described 52 cases in electricians, who ...dled the cold-finished product for the most part. In ... of their cases was systemic involvement found. ...hwartz's report [5] concerned the involvement of elec...cians, who installed and stripped wires in ships during ... war. The chlorinated naphthalene, which was im...nated into the asbestos and wrapped around the wire ...nsulation, flaked off in the stripping process. In the ... described, two months elapsed before the appear... of the chloracne of the face. There was no systemic ...olvement. Peck,[6] Cranch,[7] and Greenburg [8] discuss ... chlorinated naphthalenes and chlorinated diphenyls, ... appearance of the dermatological lesions, their value ...ndustry, and precautions in handling these chemicals.

The value of the chlorinated naphthalenes and di...enyls in industry is due primarily to their resistance to ... and alkali, high insulating value (high dielectric ...stant), thermoplasticity, chemical stability, and flame ...stance, as described by various writers.[9]

The characteristic lesions of chloracne are pinhead to ...-sized pale straw-colored cysts formed by the plug... of the orifices of the sebaceous glands, resulting in ...tion of the secretion and in the keratinization of the ... membrane. Comedones are present but are not a ...ing feature. In nearly every worker exposed suffi...ly to these chlorinated compounds for a few months ... lesions will develop. The exposure may be either to ... from the hot material or to the solid material on ...nued contact. Repeated or continuous contact is ...ial. Lesions have not been reported after short or ...quent exposures. Vesiculoerythematous eruptions, ...en in acute eczematous contact-type dermatitis and ... erythematous eruptions with pruritus, have also ... described.[9a]

This paper reports the development of lesions of chloracne in seven workers employed in a chemical plant concerned with organic chemical production.

HISTORY OF EXPOSURE

A chemical company had for some months been using molten salt at 350 F as a medium for supplying accurately controlled quantities of heat to a large jacketed reaction chamber. Because of the dangers of solidification of the salt in the return line, as well as the corrosion problem, it was decided to use a chlorinated diphenyl (Arochlor) as the heat exchange material. The same apparatus was used. This included an oil-fired furnace containing the heating coils, a steel pipe supply line to the reaction chamber, a return line to a large sump pump, and an outflow line from the sump to the furnace (see figure). The reservoir for the sump pump had a capacity of about 400 gal. (about 1,680 liters).

The chemical product, designated as organic acid A, was manufactured with the use of molten salt for heat exchange from January to December, 1949. At that time, chlorinated diphenyl was substituted as a heat exchange material. It was soon apparent that under certain conditions there was slight leakage of vapors from a number of places, particularly around the cover of the sump, and also from all gasketed connections in the system. Because of the known toxicity of these substances, the assistance

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Associate Professor of Occupational Medicine, Department of Public Health (Dr. Meigs), Assistant Clinical Professor of Dermatology, Department of Internal Medicine (Dr. Albom), and Assistant Clinical Professor of Medicine, Department of Internal Medicine (Dr. Kartin), Yale University School of Medicine.

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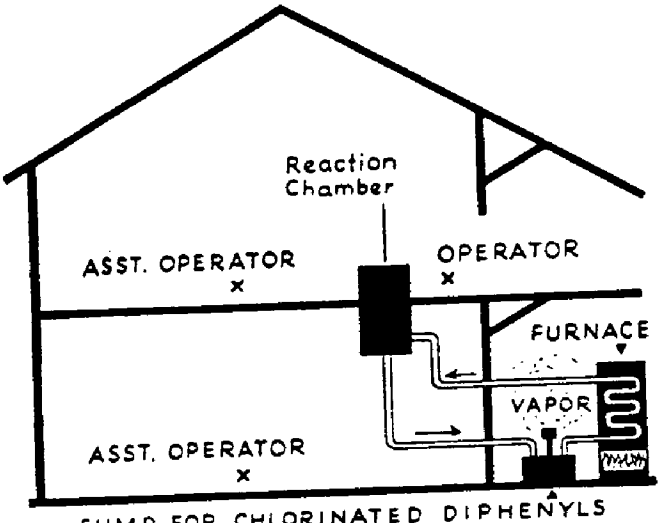
of the Bureau of Industrial Hygiene, Connecticut State Department of Health, was sought and received. A field study under conditions of obvious vapor leakage was said to have shown negligible air concentrations of the chlorinated diphenyls in the actual breathing zones of the workers. In this study, which was four months prior to the dermatological findings reported here, the air concentration of chlorinated diphenyls was reported by the bureau to be 0.1 mg. per cubic meter of air. The recommended maximum allowable concentration is 1.0 mg. per cubic meter. The figure shows that most of the leakage was at points outside the building but under a roof. No one worked regularly at the points of leakage. Nevertheless, repeated attempts were made to control vapor leakage, without complete success. This operation continued for 19 months without incident or recognition of skin or other manifestations of exposure to chlorinated diphenyls. Each employee had a complete physical examination by an internist prior to working in this environment.



Schematic drawing of the heat exchange system in which the chlorinated diphenyl was used and the points at which workers were exposed to fumes.

An operator making organic acid A ("operator," figure) was sent to one of us because of acute contact dermatitis of the face. In addition to the contact-type of dermatitis, there were noted pinhead-sized straw-colored cysts and comedones on both cheeks and the forehead. A diagnosis of chloracne was made, and the source of exposure was determined. Examination of other workers in this working environment uncovered six additional cases. In all the face was involved especially the cheeks circumorbitally, the forehead, ears, and in one case the mastoid region. **All employees were examined carefully by an internist. The seven employees in whom chloracne had developed had liver function tests performed.** Tests included direct and total bilirubin determinations and 24 and 48 hour cephalin flocculation, thymol turbidity, and alkaline phosphatase determinations. Six of the subjects had completely normal test results. One employee had borderline cephalin flocculation and thymol turbidity. Thirteen months later repeated liver function tests showed an unchanged cephalin flocculation and improved thymol turbidity. Results of complete blood cell counts and urinalysis were normal in all instances. All had normal blood pressures with no other clinical evidence of any chlorinated diphenyl toxicity.

After the recognition of these cases of chloracne, all but one of the gasketed joints in the heat exchange system, including the cover of the sump, were welded together. A hand hole 6 in. (15 cm.) in diameter was left with a gasketed cover so that the system could be drained or filled when necessary. **After that time no vapors were** visible, and the odor of chlorinated diphenyls was barely detectable in the immediate vicinity of the sump pump. **Continued careful observation of workers has revealed no new cases of chloracne.**

COMMENT

The unusual feature of this outbreak of dermatitis was the long period of exposure before any cases were recognized. The sudden recognition of seven cases after exposure up to 19 months was due to the especially careful examination of the skin of all exposed employees after discovery of the first case. Of 14 men exposed or potentially exposed to the vapors of chlorinated diphenyls, presented clinical evidence of chloracne. There was no a very good correlation between the apparent degree of exposure and the development of signs of disease. For example, a foreman, an assistant foreman, and a plant superintendent whose duties would appear to have exposed them only incidentally to the toxic agent had mild to moderate signs. The mean length of exposure of those in whom signs developed was 14.3 months and of those who did not show signs was 11.4 months, but there was considerable overlap, with chloracne developing in one worker after only 5 months in contrast to another who showed no signs even after 19 months' exposure to vapors. Since the manifestations were exclusively on exposed areas of the skin, it appears that the vapors were deposited directly on exposed skin and did not go through the clothing. The nature of each factor determining the appearance or nonappearance of lesions is not clear. Skin pigmentation may be a factor. Three of the workers were Negroes, and none of them had chloracne. No correlation with perspiration could be made, but all cases were discovered toward the end of the summer.

Prevention consisted of controlling the leakage of vapors. The fact that tests of the air, even in the presence of vapors, showed only negligible amounts of chlorinated hydrocarbons indicates that this type of intermittent but fairly long continued "mild" exposure is not innocuous. The low concentration of the chlorinated diphenyl in the air might account for the fact that lesions developed in only 50% of those involved.

SUMMARY

Seven cases of mild to moderate chloracne of the face and head occurred among 14 chemical operators exposed from 5 to 19 months intermittently to small concentrations of the vapors of a chlorinated diphenyl (Aroclor). Leakage of these vapors from a heat exchange system occurred chiefly outdoors, but chloracne was observed among men working inside the adjacent building. **In all cases the condition cleared up after treatment. Control of vapors by welding all joints in the heat exchange system prevented recurrences.**

310 Cedar St. (Dr. Meigs).

THE CUTANEOUS AND TOXICOLOGICAL ASPECTS OF PRODUCTS EVOLVED IN THE 2,4,5 TRICHLOROPHENOXYACETIC ACID SYNTHESIS

by

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Margaret Walker, B.S.
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July 1, 1954

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in the
Department of Preventive Medicine and Industrial Health
College of Medicine
UNIVERSITY OF CINCINNATI, CINCINNATI, OHIO

The Cutaneous and Toxicological Appraisal of Products Evolved in the 2,4,5 Trichlorophenoxyacetic Acid Synthesis

Submitted by the Monsanto Chemical Company, Nitro, W. Va.

I. General Objectives

A. To determine the potential acnegenicity and systemic effects of products evolved in the synthesis of 2,4,5 trichlorophenoxyacetic acid.

B. To further explore the pathogenesis of "occupational acne", and the syndrome which resulted from exposure to operations concerned with the production of 2,4,5-T, described in 3 previous reports from this Laboratory.

II. Experimental Background

A survey of the literature brought to light one paper which concerned itself with experimental work performed on rabbits. Adams et al.[1] discussed the reactions of rabbits to compounds reported to have caused acneform dermatitis in man. They concluded that their evidence "shows this acneform dermatitis to be the visible response of the skin to an irritant acting upon it from the exterior, and that this response took the form of epithelial hyperplasia, secondary inflammatory and degenerative changes, and finally, regenerative processes".

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The technique described by Adams and his co-workers was employed in this laboratory in connection with another investigation in which known acnegens were applied in the prescribed manner. Their results were

not reproduced. This method was therefore regarded as useless for the present study.

The observations of Parnell[2] on the postnatal development of the sebaceous glands of the rats appeared to provide a means of studying acnegens in this animal. Parnell described the following developmental changes in the skin of rats from birth to 31 days:

A.	Day 0 to day 17:	Sebaceous glands are added slowly and dermal fat increases.
B.	Day 18 to day 27:	Sebaceous cell enlarges and fills with sebum at the expense of dermal fat.
C.	Day 27 to day 28:	Sebum cells disappear in the secretory phase.
D.	Day 28 to day 31:	Stratum germinativum is activated, and sebaceous cells are replaced rapidly from the deeper layers of the cell wall.

Here then was a known pattern of development of sebaceous glands whereby the application of a suspected acnegenic material, in the proper time sequence, might alter phase "C" of the cycle and perhaps lead to changes in the sebaceous glands and ducts comparable to those observed in acne.

Several other reasons supported the selection of the rat as an experimental animal. First, the hair cycle and concomitant epidermal changes in the rat were well known[3]. Secondly, the sebaceous glands of the rat are well defined and easily recognisable histologically. Thirdly, there were available data on the effect of applying various chemical substances upon the epidermis of the rat[4].

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In the initial group of experiments one of the suspected acnegens, 2,4,5 trichloroanisole, and several control substances were applied for periods of varying duration and initiated during the first month of life (in consideration of Parnell's description of the development of sebaceous glands). When completely negative results were observed in these experiments, the application of the same substances in various phases of the hair cycle was decided upon. It has been reported by one investigator that carcinogens are more effective when applied upon the skin of the mouse in the anagen, or growth phase. Another investigator was of the opinion that a 0.6 per cent solution of methylcholanthrene in benzene produced the maximum of damage to the skin of mice when the hair was in the telogen, or resting phase. Hence in the second group of experiments the rats were subjected to test in various stages of the hair cycle.

III. Purpose of Present Experiments

A. To study the effects of repeated applications of the products submitted, and of known acnegens, on the developing sebaceous gland and hair follicle of young rats.

B. To study the effects of the products submitted, and of known acnegens when applied during specific stages of the hair cycle, e.g., anagen, telogen.

C. To study the effects of repeated applications of the products submitted, and of known acnegens, upon the skin of human volunteers, with particular reference to the pilosebaceous unit.

D. To obtain information regarding the effects of the percutaneous absorption of the substances submitted, when they are applied upon rat or human skin over extended periods of time.

IV. Materials and Methods

A. Experimental Animals:

The rats were bred in this laboratory from an albino strain obtained from Carworth Farms, Rockland, New York. The resultant litters averaged 10 - 12 animals. Weaning was carried out at the end of 3 weeks. Rats were not rebred for at least one month following weaning. Only male rats were used throughout the experimental work.

B. Chemicals Employed:

Materials from the 2,4,5-T process:

1. 2,4,5 Trichloroanisole
2. Pentachloroanisole
3. 2,4,5 Trichlorophenoxyacetic acid
4. "Stringer Cake" (crude 2,4,5 trichlorophenoxysodium acetate)

5. Acetone C.P., specific gravity 0.792.

Known acnegens employed as controls:

6. "Halowax 1001"
7. "Halowax 1014"

The test solutions used for application upon the skin were prepared as 5 per cent by weight in acetone solution except in the case of "Stringer Cake", which was dissolved in water.

TABLE V

The Effects of Repeated Applications of Pentachloroanisole, 2,4,5 Trichlorophenoxyacetic Acid and 2,4,5 Trichloroanisole to the Skin of Human Subjects

Summary of Experimental Conditions and Observations

Experiment #11

Subject Identification	Age (Years)	Present Status of Skin (Prior to application)	Previous Skin Trouble	Test Material	Number of Applications (Days)	Total Period of Applications (Days)	Quantity Applied (grams)	Control	Clinical Findings	Microscopic Findings
C.F. (female)	16	Normal	None	Pentachloroanisole (5 gms. in 100 gms. acetone)	53	59	1.06	Acetone	None	Normal
O.O. (female)	69	Normal	None	Pentachloroanisole (5 gms. in 100 gms. acetone)	53	59	1.06	Acetone	None	Normal
V.R. (female)	48	Normal	None	2,4,5 Trichlorophenoxyacetic Acid (5 gms. in 100 gms. acetone)	53	59	1.06	Acetone	None	Normal
C.H. (female)	60	Normal	None	2,4,5 Trichlorophenoxyacetic Acid (5 gms. in 100 gms. acetone)	53	59	1.06	Acetone	None	Normal
F.D. (male)	19	Normal	Severe acne vulgaris of face. Topical therapy.	2,4,5 Trichloroanisole (5 gms. in 100 gms. acetone)	69	98	1.38	Acetone	None	Normal
S.D. (female)	16	Normal	Acne vulgaris, old. Face scarred. Recurrent acne vulgaris, shoulders. Topical therapy.	2,4,5 Trichloroanisole (5 gms. in 100 gms. acetone)	67	98	1.38	Acetone	None	Normal

C. Techniques:

1. Animal Experiments

A skin test site, measuring approximately 1 cm in diameter and located with its anterior edge at the shoulder level on the dorsum of the animal, was used. Hair was removed from this site by means of an electric clipper, and this routine was repeated sufficiently often to keep the hair above the skin surface less than 2 mm in length during the experiment.

Animals were painted daily with a fully wetted camel's hair brush (No. 1-3536 type camel's hair brush - M. Grumbacher, N.Y.). Originally a daily application consisted of 3 separate paintings with a fully wetted brush. Later in the course of the experiments, 5 fully wetted applications of the brush were made each day. The total dose per day of each substance applied was calculated and recorded. Daily painting of the animals was continued for an appropriately planned length of time. (See tables for total dose applied in each experiment and duration of exposure.) Following this, the animals were anesthetized with Nembutal®, given intraperitoneally, and killed for examination.

In the investigations with trichloroanisole and the Halowaxes, treatment periods of varying duration were employed and initiated in different phases of the rat hair cycle.

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The animal experimentation was controlled by treating a litter mate with the vehicle used as a solvent for the chemical being appraised. Other controls were used in different phases of the total experimental process, and were as follows:

a. Untreated.

b. Rubbed - the skin of the animal was stroked 20 times with a 2" by 2" gauze pad.

c. Brushed - the skin of the animal was stroked or brushed 15 times with a dry camel's hair brush.

d. Petrolatum - the skin of the animal was rubbed with white petrolatum.

2. Human Experiments

A skin test site measuring approximately 2 inches in diameter and located over the center of the scapula was used. The skin area was painted daily with one of the several materials under test for varying periods of time as indicated in Tables IV and V. The test site was observed at frequent intervals, and at the cessation of the applications, biopsy of the skin was carried out, without anesthesia, using a rotary-driven electric punch. Excised tissues were fixed in a 10 per cent solution of formalin, sectioned and stained with Hematoxylin and Eosin. Serial sections of each biopsy were studied microscopically.

D. Summary of Experimental Procedures:

The details of each experiment are recorded in Tables I - VI and may be summarized as follows:

1. Experiments #1 - #6 (Table I, Sections A and B).

2,4,5 trichloroanisole and the control substances were applied repeatedly upon the skin of young rats. These applications were initiated at various ages during the first months of life and maintained for periods of varying duration.

2. Experiments #7 - #8 (Table II).

2,4,5 trichloroanisole and the control substances were applied repeatedly upon the skin of young rats during specific phases of the hair cycle. The first group of rats was treated during 3 successive anagen phases, and the second group during 3 successive telogen phases of the hair cycle.

3. Experiment #9 (Table III).

2,4,5 trichlorophenoxyacetic acid, pentachloroanisole and "Wringer Cake" were applied repeatedly upon the skin of rats on 60 days in an attempt to determine the effect of such prolonged contact with the skin of animals prior to applications of the same substances upon human skin.

In Experiments #1 - #9, inclusive, the animals were carefully observed for evidences of systemic intoxication.

4. Experiment #10 (Table IV). 9327337

"Halowax 1014", a known acnegen, was applied repeatedly upon

the apparently normal skin of 4 human subjects over a period of 27 days.

5. Experiment #11 (Table V).

Pentachloroanisole and 2,4,5-T were applied repeatedly upon the apparently normal skin of 2 groups of human subjects (2 in each group) over a period of 59 days. 2,4,5 trichloroanisole was applied upon the normal areas of the skin of 2 persons who had acne vulgaris over a period of 98 days.

V. Summary of Findings

A. Multiple daily applications of a 5 per cent solution of trichloroanisole upon the skin of young, male rats, over varying periods up to 21 days, produced neither gross or pathological changes in the skin nor evidence of systemic intoxication.

B. There were no differences in the effects induced by applications of trichloroanisole, regardless of whether such applications were initiated in the anagen phase, or in the telogen phase of the rat hair cycle; in either case, they were continued over 3 successive periods totaling 4 to 39 days.

C. Repeated applications of 5 per cent solutions of pentachloroanisole, 2,4,5 trichlorophenoxyacetic acid and "Ringer Cake", upon the skin of respective groups of young, male rats, over the period of 60 days failed to produce changes in the skin or other organs.

D. Application of 5 per cent solutions of "Halowax 1001" and "Halowax 1014" upon the skin of respective groups of male rats over periods up to 31 days failed to produce changes in the skin or other organs.

E. Applications of a 5 per cent solution of 2,4,5 trichloroanisole upon human skin, made over a 3-month period, produced no changes in the skin.

F. Application of 5 per cent solutions of pentachloroanisole and 2,4,5-T upon human skin over a period of 2 months produced no changes in the skin.

G. Application of a 5 per cent solution of "Halowax 1014" upon human skin over a 4-week period produced no changes in the skin.

VI. Interpretations and Considerations for Future Investigation

It is fairly evident from the results of these experiments that neither the cutaneous changes nor the systemic effects sustained by exposed men have been reproduced by the experimental use of 4 of the suspected materials or of the known acnegens such as "Halowax 1001" and "Halowax 1014", in the manner described. Quantities as great as 372 mgms of trichloroanisole and the Halowaxes upon the skin of rats were applied over a period of 36 days (31 applications). As much as 1.2 gms of 2,4,5-T and of pentachloroanisole were applied over the period of 60 days without any noticeable effect, and 1.5 gms of "Wringer Calx" were applied over a similar period with no change. In humans, contact with 0.46 gms of Halowax, divided into smaller doses applied on 23 days,

and with 1.06 gms of pentachloroanisole, divided into equal individual doses on 53 days, and with 1.06 gms of 2,4,5-T, in corresponding manner on 53 days, as well as 1.38 gms of 2,4,5 trichloroanisole on 98 days, produced no injurious effects.

It is apparent that the factors which brought about the cutaneous and systemic effects among the workmen in the plant were not duplicated in the animal or human experiments. Some pertinent questions to be raised in connection with our failure to induce clinical or pathological effects with either the 4 specific materials from the 2,4,5-T process or with the known acnegens, are these:

A. Is the rat an appropriate experimental animal for such an investigation?

B. Were the concentrations of the materials, applied upon the skin of the rat or of man, or perhaps absorbed into the tissues, from this source, adequate to stimulate the cutaneous effects?

C. In view of the further question which is implied in the latter part of Questions 2, above, should not other routes of absorption into the body be considered in the pathogenesis of "chloracne"?

By the implication of these questions we may be guided into the several experimental approaches to the problem which should be considered for the continuation of this investigative program.

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1. Although the skin of the rat appears to have remained unaltered through contact with the concentrations employed in the manner indicated, it may be possible to induce pilosebaceous changes in the rats with higher epicutaneous concentrations of acnegens and other types of exposure. Nevertheless, a variety of other animals should be employed for the epicutaneous appraisal of known acnegens and the 2,4,5-T materials. It would seem logical to employ animals subject to the spontaneous development, or the experimental induction, of follicular inflammation, such as the dog or cat. It would be well to consider also, specific sites in the skin of experimental animals, in which there is an abundance of large sebaceous glands. Such a site is the external auditory canal of the rabbit (not the skin of the auricle which was employed by Adams and his co-workers). The usefulness of this area for the investigation of sebaceous gland problems has been suggested by Montagna[5] and described recently by Rambrick and Blank[6].

2. Since the dosages of materials were applied uniformly throughout each experiment, the dose/weight relationship changed greatly in many instances, with the rapid growth of the young rats, which growth was not inhibited by the experimental procedures. For the 3-day old rats the dose calculated in gm/kilo was in some instances greater than 1.0, but with rapid increase in weight

the dose in gm/kilo decreased to as little as 0.1. It is also possible that 5 per cent concentrations were insufficient to produce pilosebaceous changes in animal or human skin. In future work it is suggested that much higher concentrations be used over larger areas of the skin of both experimental animals and human subjects.

3. In the course of the occupational exposure at the Nitro plant there were opportunities not only for cutaneous contact with the chemical materials but for inhalation and possible ingestion of these materials as well. Since in so many cases the cutaneous involvement was very wide-spread, endogenous distribution of the toxic material to the affected organs, including the skin, was a possibility. Respiratory absorption of the acnegens should be considered seriously in this connection. In future investigations animals should be subjected to respiratory exposures to the suspected acnegens, and perhaps to their ingestion. It is likely that cutaneous absorption of acnegens under experimental conditions may be limited. To enhance such absorption the addition of surfactants to the applied solutions m[illegible] tried.

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4. It is poss[illegible]cle that some or all of the 4 substances from the 2,4,5-T materials chosen for the initial investigation are not acnegenic. Nevertheless, since the methods of appraisal produced negative results with known acnegens, these 4, as well as the remainder of the materials submitted, should be investigated in future studies.

VII. Recommendations

The investigation which has been carried out thus far with Monsanto materials and known acnegens should be regarded as exploratory. This initial experience indicates clearly that the entire problem requires considerable experimental probing, and that investigative activity should be carried out simultaneously in several directions if progress is to be made.

That new cases arise currently in the 2,4,5-T synthesis, despite careful reorganization of the process and institution of rigid hygienic precautions, indicates that acnegens are still being evolved and that the potential hazard is still present.

It seems advisable, therefore, that a fundamental investigation of the pathogenesis of "chloracne" and a detailed appraisal of the materials which gave rise to the "Nitro Syndrome", be carried out. Methods of study must be developed before the major objectives can be approached. It will require the combined skill and experience of several scientific disciplines to devise adequate methods for identification and appraisal of the acnegens involved. Such an investigation requires long-term planning and organization, so that the problem may be approached from several directions simultaneously. It should be considered on a time basis of not less than 3 to 5 years.

The program for the next 2 years should include the following general experiments:

A. Determine the cutaneous and systemic effects of known acnegens and the materials from the 2,4,5-T process, when applied in high concentrations and over large areas of the skin of various experimental animals, including dogs, cats, rats, calves, etc.

B. Determine the cutaneous and systemic effects of known acnegens and chemical materials from the 2,4,5-T process, when administered repeatedly over an extended period by inhalation and by feeding. A variety of experimental animals should be used.

C. Utilise the sebaceous-rich skin of the external auditory canal of rabbits and determine the effect of acnegens on such areas.

D. Continue the human experiments, in which known acnegens and chemical materials from the 2,4,5-T process are to be applied repeatedly in high concentrations upon cutaneous areas commonly affected, such as malar eminences, preauricular areas, ala nasi, and large areas of the back. Surface active agents may be used to increase the percutaneous absorption of the acnegens and to enhance the pathological changes.

When a method has been developed that is capable of inducing pilosebaceous changes in the skin of animals comparable to those which occur in man, the cytologic and cytochemical appraisal of such changes which may be induced by individual acnegens should be carried out.

This might include the consideration of alterations in lipids, phosphatases, other esterases, glycogen, and keratin, as determined histochemically.

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July 1, 1952

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TABLE I

The Effects of Repeated Applications of 2,4,5 Trichloroanisole, "Halowax 1001", and Control Treatment to the Skin of Young Rats, Initiated During the First Month of Life

Summary of Experimental Conditions and Observations

(Section A) - Experiments #1 - #3a

Test Materials	Controls	Number of Animals (male rats)	Date of Birth	Age Start of Test (Days)	Age Completion of Test (Days)	Age at Necropsy (Days)	Number of Applications (Days)	Duration of Applications (Days)	Total Quantity Applied (Grams)	Clinical Findings	Microscopic Findings
	Untreated	1	8-11-53	-	-	1/2	-	-	-		
	Untreated	1	"	-	-	4	-	-	-		
	Untreated	1	"	-	-	7	-	-	-		
	Untreated	1	"	-	-	11	-	-	-		
	Acetone	1	"	3	10	11	7	8			
"Halowax 1001" (5% by wt. in acetone)		1	"	3	10	11	7	8	0.084	None	None
2,4,5 Trichloroanisole (5% by wt. in acetone)		1	"	3	10	11	7	8	0.084	None	None
	Untreated	1	"	-	-	32	-	-	-		
	Acetone	1	"	3	31	32	24	29			
"Halowax 1001"		1	"	3	31	32	24	29	0.288	None	None
2,4,5 Trichloroanisole		1	"	3	31	32	24	29	0.288	None	None

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TABLE I (2)

Section A - Experiments #1 - #3a (Continued)

Test Materials	Controls	Number of Animals (male-rats)	Date of Birth	Age Start of Test (Days)	Age Completion of Test (Days)	Age at Necropsy (Days)	Number of Applications (Days)	Duration of Applications (Days)	Total Quantity Applied (Grams)	Clinical Findings	Microscopic Findings
	Untreated	1	8-11-53	7	-	32		25	-		
	Acetone	1	"	7	31	32	21	25	-		
"Halowax 1001"		1	"	7	31	32	21	25	0,252	None	None
2,4,5 Trichloro-anisole		1	"	7	31	32	21	25	0,252	None	None
	Untreated	1	"	7	-	24	-	-	-		
	Acetone	1	"	7	23	24	15	17	-		
"Halowax 1001"		1	"	7	23	24	15	17	0,18	None	None
2,4,5 Trichloro-anisole		1	"	7	23	24	15	17	0,18	None	None

(Section B) - Experiments #4 - #6

	Untreated	1	8-11-53	21	-	21	-	-	-		
	Untreated	1	"	21	31	32	-	-	-		
	Acetone	1	"	21	31	32	9	11	-		
"Halowax 1001"		1	"	21	31	32	9	11	0,108	None	None
2,4,5 Trichloro-anisole		1	"	21	31	32	9	11	0,108	None	None
	Untreated	1	8-19-53	13	-	13	-	-			
	Untreated	1	"	13	-	17	-	-			
	Untreated	1	"	13	41	42	-	-			
	Acetone	1	"	13	41	42	23	28		None	None
"Halowax 1001"		1	"	13	41	42	23	28	0,276	None	None
2,4,5 Trichloro-anisole		1	"	13	41	42	23	28	0,276	None	None

TABLE I (3)

(Section B) – Experiments #4 – #6 (Continued)

Test Materials	Controls	Number of Animals (male rats)	Date of Birth	Age Start of Test (Days)	Age Completion of Test (Days)	Age at Necropsy (Days)	Number of Applications (Days)	Duration of Applications (Days)	Total Quantity Applied (Grams)	Clinical Findings	Microscopic Findings
	Untreated	1	6-24-53	25	–	85	–	–	–		
	Untreated	1	"	25	–	85	–	–	–		
	Untreated	1	"	25	–	85	–	–	–		
	Untreated	1	"	25	62	63	–	–	–		
	Acetone	1	"	25	62	63	31	36			
	Rubbed Control	1	"	25	62	63	31	36			
	Petrolatum Control	1	"	25	62	63	31	36			
"Ihlovax 100]"		1	"	25	62	63	31	36	0.372	None	None
"Ihlovax 101]"		1	"	25	62	63	31	36	0.372	None	None
2,4,5 Trichloro-anisole		1	"	25	62	63	31	36	0.372	None	None

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TABLE II

The Effects of Repeated Applications of 2,4,5 Trichloroanisole, "Halowax 1014" and Control Treatment, Administered During Three Successive Anagen and Three Successive Telogen Phases of the Rat Hair Cycle

Summary of Experimental Conditions and Observations

Experiments #7 - #8

Test Materials and Controls	Number of Animals	Date of Birth	Age Start of Test (Days)	Number of Applications (Days)	Age Completion of Test (Days)	Age Resumption of Test (Days)	Number of Applications (Days)	Age Completion of Test (Days)	Age Resumption of Test (Days)	Number of Applications (Days)	Age Completion of Test (Days)	Total Quantity Applied (Drops)	Age Necropsy (Days)	Clinical and Microscopic Findings
Rubbed or Brushed Control	4	10-24-51	6	12	19	30	16	48	65	11	78	-	115	
Acetone	6	"	6	12	19	30	16	48	65	11	78	-	115	
"Halowax 1014"	3	"	6	12	19	30	16	48	65	11	78	0.78	115	None
2,4,5 Trichloroanisole	4	"	6	12	19	30	16	48	65	11	78	0.78	115	None
Acetone	1	11-18-51	21	10	31	49	10	60	81	11	92	-	138	None
2,4,5 Trichloroanisole	3	"	21	10	31	49	10	60	81	11	92	0.62	138	None

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TABLE III

The Effects of Repeated Applications of 2,4,5 Trichlorophenoxyacetic Acid, Pentachloroanisole and "Wringer Cake" to the Skin of Rats for 60 Days

Summary of Experimental Conditions and Observations

Experiment #9

Test Materials and Controls	Number of Animals	Date of Birth	Age Start of Test (Days)	Number of Applications (Days)	Age Completion of Test (Days)	Total Quantity Applied (grams)	Age Necropsy (Days)	Clinical and Microscopic Findings
Acetone	2	1-1-54	41	60	110	-	111	None
2,4,5 Trichlorophenoxyacetic acid	3	"	41	60	110	1.20	111	None
Pentachloroanisole	3	"	41	60	110	1.20	111	None
"Wringer Cake" (5 gms dissolved in 100 gms distilled water)	3	"	41	60	110	1.50	111	None

TABLE IV

The Effects of Repeated Applications of "Haloxax 1014" to the Normal Skin of Human Subjects

Summary of Experimental Conditions and Observations

Experiment #10

Subject Identification	Age (Years)	Present Status of Skin (Site of Application)	Previous Skin Trouble	Test Material	Test Material	Number of Applications (Days)	Total Period of Applications (Days)	Quantity Applied (grms)	Clinical Findings	Microscopic Findings
M.W. (female)	66	Normal	None	"Haloxax 1014" (5 gms. in 100 gms. acetone)	"Haloxax 1014" (5 gms. in 100 gms. n. octane)	23	27	0.16	None	Normal
S.B. (female)	77	Normal	None	"Haloxax 1014" (5 gms. in 100 gms. acetone)	"Haloxax 1014" (5 gms. in 100 gms. n. octane)	23	27	0.16	None	Normal
N.J. (female)	65	Normal	None	"Haloxax 1014" (5 gms. in 100 gms. acetone)	"Haloxax 1014" (5 gms. in 100 gms. n. octane)	23	27	0.16	None	Normal
M.M. (female)	61	Normal	None	"Haloxax 1014" (5 gms. in 100 gms. acetone)	"Haloxax 1014" (5 gms. in 100 gms. n. octane)	23	27	0.16	None	Normal

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TABLE VI

Summary of All Experimental Data on Repeated Cutaneous Applications of 2,4,5 Trichlorocarbanilide, "Halowax 1001", and "Halowax 1014"

Material *	Number of Animal	Number of Applications	Duration of Applications	Clinical Findings	Microscopic Findings
2,4,5 Trichlorocarbanilide	18	7 - 39	8 - 72 *	None	None
"Halowax 1001"	7	7 - 31	8 - 36	None	None
"Halowax 1014"	4	31 - 39	36 - 72 *	None	None

* Animals were treated through only one phase of three consecutive hair cycles.

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5

CHLORINATED AROMATIC CYCLIC ETHERS AS THE CAUSE OF CHLORACNE

noted

By J. Kimmig and K.H.Schulz

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Naturwiss., 44, 337-338 (1957)

Personnel engaged at various stages in the usual industrial hydrolysis of tetrachlorobenzene to form trichlorophenol and in the further processing of the latter present dermatological symptoms of varying intensity. On the basis of anamneses and diagnoses, these symptoms are ascribable to chloracne. The clinical picture is characterized by comedones, pustules, and smaller sebaceous retention cysts affecting the face, neck, chest, back and the limbs. In some cases the hepatic tissues are also affected.

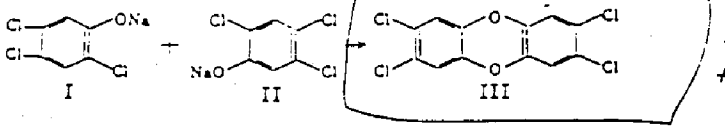
Animal experiments were carried out with a view to determining the etiology of this intoxication. Chloracne-inducing substances administered externally to rabbits' ears resulted in inflammation. The picture evolved in the course of 2 to 4 weeks was comparable to that due to chloracne in man. Furthermore, it was established that neither the starting material, 1,2,4,5-tetrachlorobenzene, nor the resulting trichlorophenol can be held responsible for the above dermatological complaints. Therefore the intoxicating factor had to be sought in the by-products, occasionally present as impurities in the trichlorophenol and its subsequent derivatives[1].

A number of compounds, that might possibly be formed under the manufacturing conditions was prepared by the manufacturers, taking into account the latest concepts concerning the reaction mechanism of the high-pressure phenol process[2]. We tested these compounds on rabbits' ears, and found the following derivatives to be very highly active: 1) trichlorodibenzofuran, 2) tetrachlorodibenzofuran, 3) 2,3,6,7-tetrachlorodibenzodioxin (2,3,6,7-tetrachlorodiphenylene dioxide).

The variously chlorinated diphenyl ether, together with the unsubstituted and monochlorinated dibenzofuran were completely inactive. The chloracne-inducing action appeared to depend on the number and position of the chlorine atoms in the benzene ring, and on the cyclic ether structure.

The highest activity was exhibited by 2,3,6,7-tetrachlorodibenzodioxin, which produced the characteristic symptoms in rabbits' ears, even when used in 0.01 to 0.002% solutions. When higher concentrations were used, the animals soon died of hepatic necrosis. The high general toxicity of this compound is shown in the case of peroral administration. Single oral doses of 0.05 to 0.1 mg/kg of body-weight resulted in death within 1 to 2 weeks. Autopsy revealed extensive necrosis and engorgement of the liver.

Whereas nothing definite is known as yet about the formation of chlorinated dibenzofurans in the high-pressure phenol process, it has been established that tetrachlorodibenzodioxin is formed by the condensation of two molecules of sodium trichlorophenoxide, accompanied by the elimination of two molecules of NaCl.



where I and II represent two molecules of sodium 2,4,5-trichlorophenoxide and III is 2,3,6,7-tetrachlorodibenzodioxin (2,3,6,7-tetrachlorodiphenylene dioxide).

Moreover, tetrachlorodibenzodioxin, identical to the synthetic model compound, was isolated from the by-products arising in the industrial high-pressure phenol process.

Another author[3] chlorinated dibenzodioxin for a different reason, and obtained a tetrachlorodibenzodioxin, in which the positions of the Cl atoms were unknown. In its chemical and toxicological properties, this compound differed only slightly from ours, synthesized, or isolated from

technical trichlorophenol. In a chemical assistant engaged in the laboratory preparation of this compound, contact with the latter led to severe chloracne. The compound, prepared by the above methods, resulted in similar symptoms in animals.

It should be added that neither pure 2,4,5-trichlorophenol, nor pure pentachlorophenol induced inflammation in rabbits' ears. It is therefore to be concluded that the much-discussed cases of chloracne following contact with pentachlorophenol are not to be attributed to this compound, but to toxic by-products related to those described in this article. Analogously to the above reaction, the heating of sodium pentachlorophenoxide should lead to chlorinated dibenzodioxins, primarily to octachlorodibenzodioxin.

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ENGLAND

CLINICAL AND EXPERIMENTAL INVESTIGATIONS CONCERNING THE ETIOLOGY OF CHLORACNE

by K.H. Schulz

Arch.klin. exp. Derm., 206 (1957)
589 - 596

A number of workers in a chemical factory at Hamburg developed certain dermatological symptoms between the middle of 1954 and spring 1955. On the basis of anamneses and diagnoses, these symptoms were attributed to chloracne. Altogether 31 people were affected, whose individual clinical pictures will not be discussed in the present article. The clinical picture corresponded to that frequently obtained in occupations necessitating contact with perchlorinated naphthalenes and diphenyls. The symptoms comprised numerous comedones, pustules, some furuncles and retention cysts, all affecting invariably the face, neck, and the nape (see Fig. 1), and in some cases also the back, chest, genitals, and the limbs.

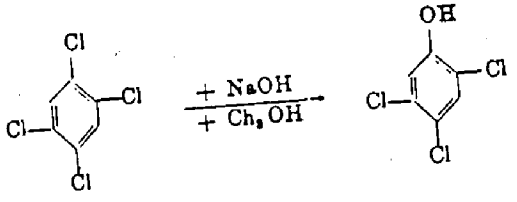
Fig. 1:

Chloracne arising from contact with technical trichlorophenol (chemical worker, aged 35)

In addition to dermatological symptoms, several patients presented chronic conjunctivitis and blepharitis. General subjective complaints, such as loss of appetite, debility, and nausea were often expressed. Fortunately, however, objectively detectable affections of the liver, kidneys, central nervous system, and the hemopoietic apparatus, were always absent.

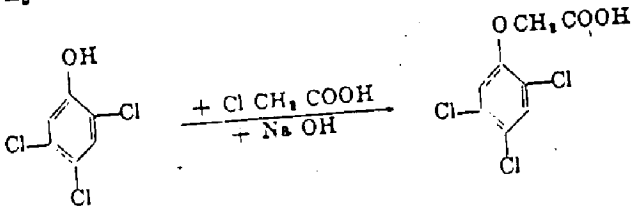
All the patients had worked in the 'Trichlorophenol Section' of the factory, manufacturing 2,4,5-trichlorophenoxyacetic acid, which is a widely used, highly active herbicide, prepared from α-hexachlorocyclohexane in a multistage process whose last two steps, being the most important ones in the present context, are as follows:

1. Preparation of trichlorophenol by the alkaline hydrolysis of tetrachlorobenzene, carried out at 170°C in the presence of sodium hydroxide and methanol:



Tetrachlorobenzene — 2,4,5-trichlorophenol

2. Preparation of the final product by the addition of chloroacetic acid and sodium hydroxide to the trichlorophenol:



2,4,5-trichlorophenol — 2,4,5-trichlorophenoxyacetic acid

It was soon found that only those workers were affected who were engaged in the preparation and the further processing of trichlorophenol.

It should be mentioned in this connection that cases of chloracne have been found among personnel engaged in the manufacture of trichlorophenol in chemical works not only in Hamburg, but also in Southern and in Western Germany, these cases being occasionally accompanied by severe afflictions of the liver.

Trichlorophenol itself was first assumed to be the original cause of intoxication. According to previous reports[1], contact with highly chlorinated phenols, e.g. pentachlorophenol, causes chloracne. This assumption, however, soon had to be abandoned, for the following two reasons:

1. Work with trichlorophenol had proceeded for two years without resulting in any harmful effect. The complaints first arose after a change in the preparation of trichlorophenol, whose details can no longer be neglected.

2. The results of the animal experiments clearly disprove the causative function of pure 2,4,5-trichlorophenol.

Animal experiments

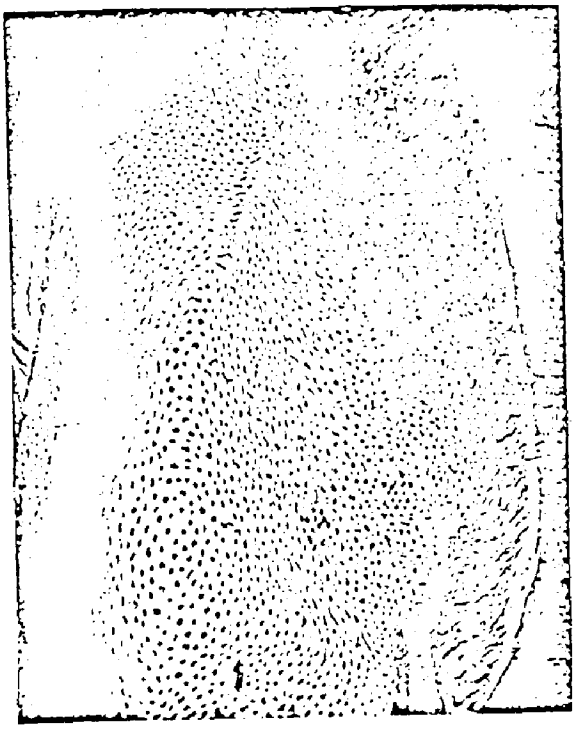
The animal experiments were carried out on rabbits' ears, on which Hofmann and Neumann[4], Braun[2], and Landes[6] and others used highly chlorinated naphthalenes and induced symptoms very similar to those of chloracne in human subjects. In each animal, the inside of one ear was painted daily with the substances to be tested for their chloracne-inducing activity, whilst the other ear, serving as a control, was treated with the solvent only. The duration of the experiments varied between 3 and 8 weeks.

The chemical plant mentioned above supplied the technical 2,4,5-trichlorophenol (95% pure), which was employed in 2% and 5% solutions in polyglycol and resulted in the following symptoms: inflammation of the ears, accompanied by the reddening of the surface and moderate swelling,

developed in about 5 to 7 days; a few days later the picture changed - the diffuse inflammation had subsided and new symptoms appeared, which closely resembled those of chloracne in man. Follicles, filled with hyperkeratotic material, projected out of the plane of the skin, so that the surface of the latter assumed a sandpapery appearance (see Fig. 2). In contrast, ears treated with the solvent alone developed no symptoms.

Fig. 2:

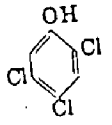
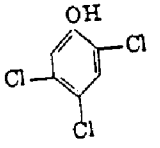
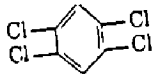
Rabbit's ear after a 3-week treatment with a 3% solution of technical trichlorophenol in polyglycol (follicular hyperkeratosis, follicular distension)



From the histological point of view, an acanthotic thickening of the epidermis was observed, together with edema and moderate cell infiltration in the cutis. The follicles were strong and their epithelia, distended almost infundibularly and filled with keratotic material, were extended.

Unlike technical trichlorophenol, chemically pure, twice-distilled trichlorophenol did not induce these symptoms even after an 8-week application. Experiments using the distillation residue gave once more the same positive results. The results are shown in Table 1 below.

Table 1. Results of animal experiments carried out with a view to determining the etiology of chloracne (by painting rabbits' ears)

Substance	Structural	Concentration %	Results	
			Ear	Hepatic toxicity
2,4,5-Trichlorophenol (technical grade)		5	++	slight
2,4,5-Trichlorophenol (chemically pure)		5-10	ϕ	ϕ
1,2,4,5-Tetrachlorobenzene		5-10	ϕ	ϕ
Distillation residue of trichlorophenol	-	1-5	++	slight

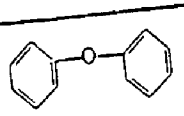
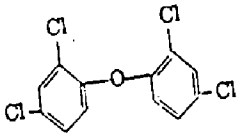
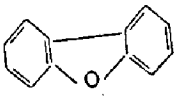
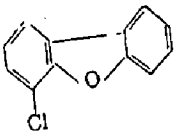
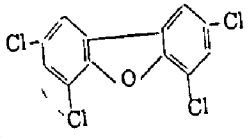
ϕ = None

It emerges from these results that the substance causing chloracne is not trichlorophenol itself, but arises in all probability as a by-product in the alkaline hydrolysis of tetrachlorobenzene into trichlorophenol.

Further investigations* aimed at gaining more information of the nature of the substances causing chloracne. Since, owing to extraneous circumstances, the residue could not be separated, synthetic compounds were used in these experiments. Naturally, such compounds were selected that were likely to be formed in the industrial production of trichlorophenol from tetrachlorobenzene. The results are listed in Table 2.

* Carried out in collaboration with Dr. Sorge, Head of the Trichlorophenol Section.

Table 2: Results of animal experiments carried out with a view to determining the etiology of chloracne (by painting rabbits' ears)

Substance	Structural formula	Concentration %	Results: Ear	Results: Hepatic toxicity	Remarks
Diphenyl ether		5-10	ϕ	ϕ	
Mono-, di-, tri-, and tetrachlorinated diphenyl ethers		5-10	ϕ	slight	
Diphenylene oxide (dibenzofuran)		5-10	ϕ	ϕ	
Monochlorinated dibenzofuran		5-10	ϕ	ϕ	
Tri- and tetra chlorinated dibenzofuran		0.1-5	(+)	very strong	Positions of the Cl atoms are uncertain

ϕ = none

Experiments with diphenyl ethers showed that neither the unsubstituted nor any of the mono-, tri-*, and tetrachlorinated ethers was capable of producing symptoms of chloracne in the rabbits' ears.

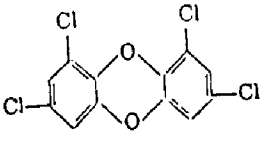
Since their formation under the relevant reaction conditions is possible, the chlorine derivatives of dibenzofuran (diphenylene oxide) were next tested. Dibenzofuran differs from diphenyl ether in the presence of a furan structure by ring closure taking place between two benzene rings. The unsubstituted and the monochlorinated compounds were found to be

* Translator's note: Sic. Dichlorodiphenyl ether is omitted.

inactive in the animal experiments. The tri- and the tetrachlorinated dibenzofurans, on the other hand, exhibited a strong hepatic toxicity. Daily painting of the ears (over an area having a diameter of about 3 cm) with 0.1 and 0.5% solutions led to death in 2 to 3 weeks. Autopsy revealed an appreciable engorgement of the liver and distended necrotic tissues, occasionally imbibed through hemorrhages. A single peroral application of a dose of 1 mg/kg led to severe hepatic conditions, which could be studied with the aid of the Hofmann-Oettel[5] modification of the bromosulfthalein reaction. The symptoms described above appeared also on the ears of the animals, but to a smaller extent, as in the experiments with the distillation residue. Therefore the question still remains whether tri- and tetrachlorinated dibenzofurans were the true cause of the cases of chloracne under consideration.

Although the question may be posed whether the highly chlorinated naphthalenes and diphenyls, whose chloracne-inducing activity has long been known, could be regarded as the cause in the present case as well, from a chemical viewpoint it is hardly probable that these compounds can be formed under the reaction conditions in question.

Clinical observation led a step nearer to elucidating the nature of the cause. A chemical laboratory assistant has recently developed severe chloracne. This patient had been engaged in the laboratory preparation of highly chlorinated diphenylene dioxides in a laboratory outside Hamburg, where, after about 10-14 days, he developed chloracne in conjunction with facial dermatitis. Since the patient worked with chemically pure compounds, it must be assumed that highly chlorinated diphenylene dioxides are capable of evoking symptoms of chloracne in man.



Tetrachlorodiphenylene dioxide
(positions of the Cl atoms are uncertain)

These compounds may have a causative function in the chloracne cases

found in the chemical plant as well, since, from a chemical viewpoint, chlorinated diphenylene dioxides may be formed in the hydrolysis of tetrachlorobenzene into trichlorophenol.

Animal experiments with chlorinated diphenylene dioxides are in progress, and the results obtained so far indicate that tetrachlorodiphenylene dioxide has a high hepatic toxicity in rabbits.

Although the proof is still incomplete and the distillation residue of the technical trichlorophenol has not been investigated, it is hoped that a contribution has been made to the etiology of chloracne, particularly with the aid of the highly chlorinated diphenylene dioxides. Further investigations are aimed at the final elucidation of this problem.

Discussion

A. Szakall (Hamburg): Comedones on the zygomatic arch of patients wearing spectacles are probably ascribable to the chafing of the surface cells of the horny layer, followed by cell proliferation with increased horny formation. The inner surface of the spectacle frame in contact with the skin becomes rough* in use, and gives rise to friction. Pinkus [J. invest. derm., 19, 431 (1952)] has shown that a weakening of the horny layer leads to cell proliferation, with increased horn formation. The removal of four surface cell-layers is sufficient to result in cell proliferation.

P. Keller (Aix-la-Chapelle): Is it possible that the formation of large comedones around the eyes in patients wearing spectacles is due to a chlorine content of the synthetic material of the frame ? These comedones were found in a tank officer around that eye only, which he used with the periscope, and the formation of the comedones persisted for years afterwards.

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* Translator's note: Sic.

O. Braun-Falco (Mainz) : With respect to Prof. Keller's remarks concerning the follicular 'spectacles hyperkeratosis', this is believed to have a physical cause (e.g. the pressure of the frame), and resembles in this connection the comedones appearing after x-ray contact irradiation.

References:

1. E.W. Baader and H.J. Bauer, Industrial Intoxication due to pentachlorophenol. Industr. Med. a. Surg. 20, 286-290 (1951).

2. W. Braun, Chlorakne, Monographien zur Zeitschrift Berufsdermatosen (Chloracne, Monographs of the Journal of Occupational Dermatosis) vol. 1. Aulendorf: printed by Editio Cantor.

3. H. Grimmer, Occupational acne caused by chlorinated aromatic hydrocarbons, Zbl. Arbeitsmed. u. Arbeitsschutz, 5, 76-83 (1955).

4. H. Th. Hofmann, and W. Newmann, Animal experimental investigations of the dermatological effect of chlorinated naphthalenes, Zbl. Arbeitsmed. u. Arbeitsschutz 2, 169-173 (1952).

5. H.Th. Hofmann, and H. Oettel, The use of the bromosulfthalein reaction as a simple micromethod in the study of the hepatic function,Arztl. Wschr. 1954, 965-967.

6. E. Landes, Discussion, Bemerk. Dermat. Wschr. 130, 1191 (1954).

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5

CHLORINATED AROMATIC CYCLIC ETHERS AS THE CAUSE OF CHLORACNE

noted
Milbury

By J. Kimmig and K.H.Schulz

University Clinic of Dermatology, Hamburg-Eppendorf

Naturwiss., 44, 337-338 (1957)

Personnel engaged at various stages in the usual industrial hydrolysis of tetrachlorobenzene to form trichlorophenol and in the further processing of the latter present dermatological symptoms of varying intensity. On the basis of anamneses and diagnoses, these symptoms are ascribable to chloracne. The clinical picture is characterized by comedones, pustules, and smaller sebaceous retention cysts affecting the face, neck, chest, back and the limbs. In some cases the hepatic tissues are also affected.

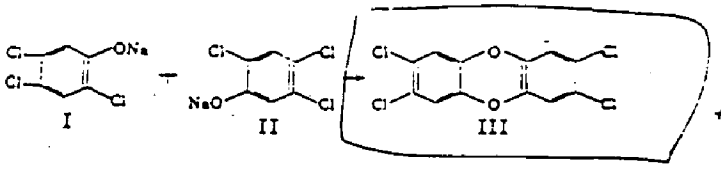
Animal experiments were carried out with a view to determining the etiology of this intoxication. Chloracne-inducing substances administered externally to rabbits' ears resulted in inflammation. The picture evolved in the course of 2 to 4 weeks was comparable to that due to chloracne in man. Furthermore, it was established that neither the starting material, 1,2,4,5-tetrachlorobenzene, nor the resulting trichlorophenol can be held responsible for the above dermatological complaints. Therefore the intoxicating factor had to be sought in the by-products, occasionally present as impurities in the trichlorophenol and its subsequent derivatives[1].

A number of compounds, that might possibly be formed under the manufacturing conditions was prepared by the manufacturers, taking into account the latest concepts concerning the reaction mechanism of the high-pressure phenol process[2]. We tested these compounds on rabbits' ears, and found the following derivatives to be very highly active: 1) trichlorodibenzofuran, 2) tetrachlorodibenzofuran, 3) 2,3,6,7-tetrachlorodibenzodioxin (2,3,6,7-tetrachlorodiphenylene dioxide).

The variously chlorinated diphenyl ether, together with the unsubstituted and monochlorinated dibenzofuran were completely inactive. The chloracne-inducing action appeared to depend on the number and position of the chlorine atoms in the benzene ring, and on the cyclic ether structure.

The highest activity was exhibited by 2,3,6,7-tetrachlorodibenzodioxin, which produced the characteristic symptoms in rabbits' ears, even when used in 0.01 to 0.002% solutions. When higher concentrations were used, the animals soon died of hepatic necrosis. The high general toxicity of this compound is shown in the case of peroral administration. Single oral doses of 0.05 to 0.1 mg/kg of body-weight resulted in death within 1 to 2 weeks. Autopsy revealed extensive necrosis and engorgement of the liver.

Whereas nothing definite is known as yet about the formation of chlorinated dibenzofurans in the high-pressure phenol process, it has been established that tetrachlorodibenzodioxin is formed by the condensation of two molecules of sodium trichlorophenoxide, accompanied by the elimination of two molecules of NaCl.



I II III

where I and II represent two molecules of sodium 2,4,5-trichlorophenoxide and III is 2,3,6,7-tetrachlorodibenzodioxin (2,3,6,7-tetrachlorodiphenylene dioxide).

Moreover, tetrachlorodibenzodioxin, identical to the synthetic model compound, was isolated from the by-products arising in the industrial high-pressure phenol process.

Another author[3] chlorinated dibenzodioxin for a different reason, and obtained a tetrachlorodibenzodioxin, in which the positions of the Cl atoms were unknown. In its chemical and toxicological properties, this compound differed only slightly from ours, synthesized, or isolated from

technical trichlorophenol. In a chemical assistant engaged in the laboratory preparation of this compound, contact with the latter led to severe chloracne. The compound, prepared by the above methods, resulted in similar symptoms in animals.

It should be added that neither pure 2,4,5-trichlorophenol, nor pure pentachlorophenol induced inflammation in rabbits' ears. It is therefore to be concluded that the much-discussed cases of chloracne following contact with pentachlorophenol are not to be attributed to this compound, but to toxic by-products related to those described in this article. Analogously to the above reaction, the heating of sodium pentachlorophenoxide should lead to chlorinated dibenzodioxins, primarily to octachlorodibenzodioxin.

References:

1. K.H. Schulz, Lecture delivered at the 23rd Conference of German Dermatologists, Vienna, May 23-27, 1956.

2. A. Luttringhaus and D. Ambros, Chem. Ber. 89, 472 (1956).

G. Wittig and L. Pohmer, Chem. Ber. 89, 1334 (1956).

3. H. Stockmann, Personal communication.

CLINICAL AND EXPERIMENTAL INVESTIGATIONS CONCERNING THE ETIOLOGY OF CHLORACNE

by K.H. Schulz

Arch.klin. exp. Derm., 206 (1957)
589 - 596

A number of workers in a chemical factory at Hamburg developed certain dermatological symptoms between the middle of 1954 and spring 1955. On the basis of anamneses and diagnoses, these symptoms were attributed to chloracne. Altogether 31 people were affected, whose individual clinical pictures will not be discussed in the present article. The clinical picture corresponded to that frequently obtained in occupations necessitating contact with perchlorinated naphthalenes and diphenyls. The symptoms comprised numerous comedones, pustules, some furuncles and retention cysts, all affecting invariably the face, neck, and the nape (see Fig. 1), and in some cases also the back, chest, genitals, and the limbs.

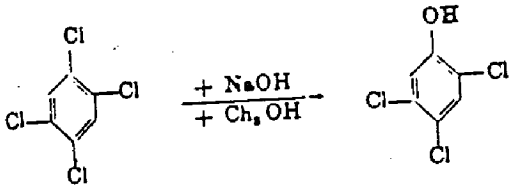
Fig. 1.

Chloracne arising from contact with technical trichlorophenol (chemical worker, aged 35)

In addition to dermatological symptoms, several patients presented chronic conjunctivitis and blepharitis. General subjective complaints, such as loss of appetite, debility, and nausea were often expressed. Fortunately, however, objectively detectable affections of the liver, kidneys, central nervous system, and the hemopoietic apparatus, were always absent.

All the patents had worked in the 'Trichlorophenol Section' of the factory, manufacturing 2,4,5-trichlorophenoxyacetic acid, which is a widely used, highly active herbicide, prepared from α-hexachlorocyclohexane in a multistage process whose last two steps, being the most important ones in the present context, are as follows:

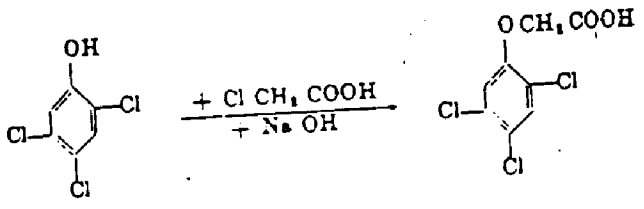
1. Preparation of trichlorophenol by the alkaline hydrolysis of tetrachlorobenzene, carried out at 170°C in the presence of sodium hydroxide and methanol:



Tetrachlorobenzene

2,4,5-trichlorophenol

2. Preparation of the final product by the addition of chloroacetic acid and sodium hydroxide to the trichlorophenol:



2,4,5-trichlorophenol

2,4,5-trichlorophenoxyacetic acid

It was soon found that only those workers were affected who were engaged in the preparation and the further processing of trichlorophenol.

It should be mentioned in this connection that cases of chloracne have been found among personnel engaged in the manufacture of trichlorophenol in chemical works not only in Hamburg, but also in Southern and in Western Germany, these cases being occasionally accompanied by severe afflictions of the liver.

Trichlorophenol itself was first assumed to be the original cause of intoxication. According to previous reports[1], contact with highly chlorinated phenols, e.g. pentachlorophenol, causes chloracne. This assumption, however, soon had to be abandoned, for the following two reasons:

1. Work with trichlorophenol had proceeded for two years without resulting in any harmful effect. The complaints first arose after a change in the preparation of trichlorophenol, whose details can no longer be neglected.

2. The results of the animal experiments clearly disprove the causative function of pure 2,4,5-trichlorophenol.

Animal experiments

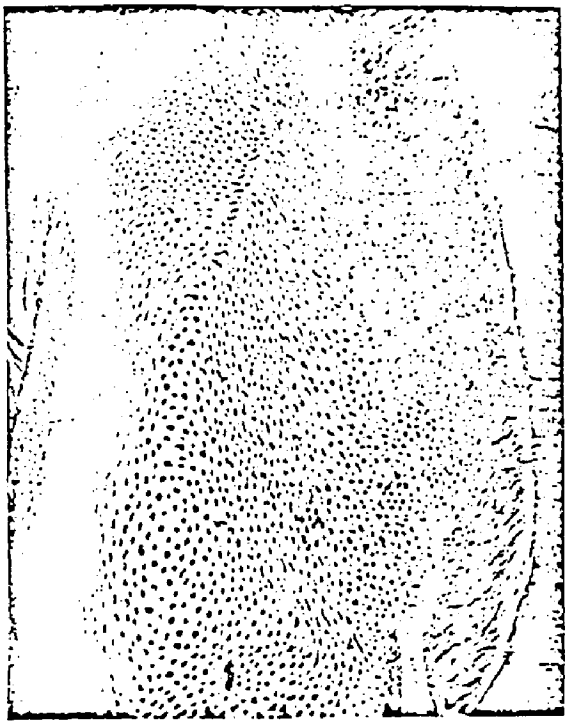
The animal experiments were carried out on rabbits' ears, on which Hofmann and Neumann[4], Braun[2], and Landes[6] and others used highly chlorinated naphthalenes and induced symptoms very similar to those of chloracne in human subjects. In each animal, the inside of one ear was painted daily with the substances to be tested for their chloracne-inducing activity, whilst the other ear, serving as a control, was treated with the solvent only. The duration of the experiments varied between 3 and 8 weeks.

The chemical plant mentioned above supplied the technical 2,4,5-trichlorophenol (95% pure), which was employed in 2% and 5% solutions in polyglycol and resulted in the following symptoms: inflammation of the ears, accompanied by the reddening of the surface and moderate swelling,

developed in about 5 to 7 days; a few days later the picture changed - the diffuse inflammation had subsided and new symptoms appeared, which closely resembled those of chloracne in man. Follicles, filled with hyperkeratotic material, projected out of the plane of the skin, so that the surface of the latter assumed a sandpapery appearance (see Fig. 2). In contrast, ears treated with the solvent alone developed no symptoms.

Fig. 2:

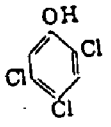
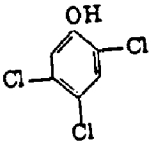
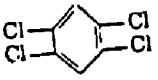
Rabbit's ear after a 3-week treatment with a 3% solution of technical trichlorophenol in polyglycol (follicular hyperkeratosis, follicular distension)



From the histological point of view, an acanthotic thickening of the epidermis was observed, together with edema and moderate cell infiltration in the cutis. The follicles were strong and their epithelia, distended almost infundibularly and filled with keratotic material, were extended.

Unlike technical trichlorophenol, chemically pure, twice-distilled trichlorophenol did not induce these symptoms even after an 8-week application. Experiments using the distillation residue gave once more the same positive results. The results are shown in Table 1 below.

Table 1. Results of animal experiments carried out with a view to determining the etiology of chloracne (by painting rabbits' ears)

Substance	Structural	Concentration %	Results	
			Ear	Hepatic toxicity
2,4,5-Trichlorophenol (technical grade)		5	++	slight
2,4,5-Trichlorophenol (chemically pure)		5-10	ϕ	ϕ
1,2,4,5-Tetrachlorobenzene		5-10	ϕ	ϕ
Distillation residue of trichlorophenol	-	1-5	++	slight

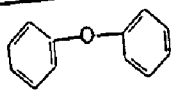
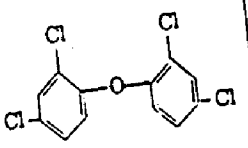
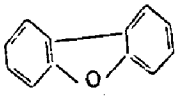
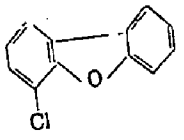
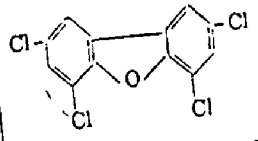
ϕ = None

It emerges from these results that the substance causing chloracne is not trichlorophenol itself, but arises in all probability as a by-product in the alkaline hydrolysis of tetrachlorobenzene into trichlorophenol.

Further investigations* aimed at gaining more information of the nature of the substances causing chloracne. Since, owing to extraneous circumstances, the residue could not be separated, synthetic compounds were used in these experiments. Naturally, such compounds were selected that were likely to be formed in the industrial production of trichlorophenol from tetrachlorobenzene. The results are listed in Table 2.

* Carried out in collaboration with Dr. Sorge, Head of the Trichlorophenol Section.

Table 2: Results of animal experiments carried out with a view to determining the etiology of chloracne (by painting rabbits' ears)

Substance	Structural formula	Concentration %	Results: Ear	Results: Hepatic toxicity	Remarks
Diphenyl ether		5-10	ϕ	ϕ	
Mono-, di-, tri-, and tetrachlorinated diphenyl ethers		5-10	ϕ	slight	
Diphenylene oxide (dibenzofuran)		5-10	ϕ	ϕ	
Monochlorinated dibenzofuran		5-10	ϕ	ϕ	
Tri- and tetra chlorinated dibenzofuran		0.1-5	(+)	very strong	Positions of the Cl atoms are uncertain

ϕ = none

Experiments with diphenyl ethers showed that neither the unsubstituted nor any of the mono-, tri-*, and tetrachlorinated ethers was capable of producing symptoms of chloracne in the rabbits' ears.

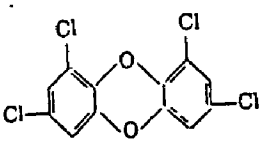
Since their formation under the relevant reaction conditions is possible, the chlorine derivatives of dibenzofuran (diphenylene oxide) were next tested. Dibenzofuran differs from diphenyl ether in the presence of a furan structure by ring closure taking place between two benzene rings. The unsubstituted and the monochlorinated compounds were found to be

* Translator's note: Sic. Dichlorodiphenyl ether is omitted.

inactive in the animal experiments. The tri- and the tetrachlorinated dibenzofurans, on the other hand, exhibited a strong hepatic toxicity. Daily painting of the ears (over an area having a diameter of about 3 cm) with 0.1 and 0.5% solutions led to death in 2 to 3 weeks. Autopsy revealed an appreciable engorgement of the liver and distended necrotic tissues, occasionally imbibed through hemorrhages. A single peroral application of a dose of 1 mg/kg led to severe hepatic conditions, which could be studied with the aid of the Hofmann-Oettel[5] modification of the bromosulfthalein reaction. The symptoms described above appeared also on the ears of the animals, but to a smaller extent, as in the experiments with the distillation residue. Therefore the question still remains whether tri- and tetrachlorinated dibenzofurans were the true cause of the cases of chloracne under consideration.

Although the question may be posed whether the highly chlorinated naphthalenes and diphenyls, whose chloracne-inducing activity has long been known, could be regarded as the cause in the present case as well, from a chemical viewpoint it is hardly probable that these compounds can be formed under the reaction conditions in question.

Clinical observation led a step nearer to elucidating the nature of the cause. A chemical laboratory assistant has recently developed severe chloracne. This patient had been engaged in the laboratory preparation of highly chlorinated diphenylene dioxides in a laboratory outside Hamburg, where, after about 10-14 days, he developed chloracne in conjunction with facial dermatitis. Since the patient worked with chemically pure compounds, it must be assumed that highly chlorinated diphenylene dioxides are capable of evoking symptoms of chloracne in man.



Tetrachlorodiphenylene dioxide
(positions of the Cl atoms are uncertain)

These compounds may have a causative function in the chloracne cases

found in the chemical plant as well, since, from a chemical viewpoint, chlorinated diphenylene dioxides may be formed in the hydrolysis of tetrachlorobenzene into trichlorophenol.

Animal experiments with chlorinated diphenylene dioxides are in progress, and the results obtained so far indicate that tetrachlorodiphenylene dioxide has a high hepatic toxicity in rabbits.

Although the proof is still incomplete and the distillation residue of the technical trichlorophenol has not been investigated, it is hoped that a contribution has been made to the etiology of chloracne, particularly with the aid of the highly chlorinated diphenylene dioxides. Further investigations are aimed at the final elucidation of this problem.

Discussion

A. Szakall (Hamburg): Comedones on the zygomatic arch of patients wearing spectacles are probably ascribable to the chafing of the surface cells of the horny layer, followed by cell proliferation with increased horny formation. The inner surface of the spectacle frame in contact with the skin becomes rough* in use, and gives rise to friction. Pinkus [J. invest. derm., 19, 431 (1952)] has shown that a weakening of the horny layer leads to cell proliferation, with increased horn formation. The removal of four surface cell-layers is sufficient to result in cell proliferation.

P. Keller (Aix-la-Chapelle): Is it possible that the formation of large comedones around the eyes in patients wearing spectacles is due to a chlorine content of the synthetic material of the frame? These comedones were found in a tank officer around that eye only, which he used with the periscope, and the formation of the comedones persisted for years afterwards.

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* Translator's note: Sic.

O. Braun-Falco (Mainz) : With respect to Prof. Keller's remarks concerning the follicular 'spectacles hyperkeratosis', this is believed to have a physical cause (e.g. the pressure of the frame), and resembles in this connection the comedones appearing after x-ray contact irradiation.

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6. E. Landes, Discussion, Bemerk. Dermat. Wschr. 130, 1191 (1954).

Translated

by

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28, ALEXANDRA ROAD, LONDON, S.W.19

ENGLAND

Diamond Alkali Company

INTER-OFFICE CORRESPONDENCE

DACO 1-18-1A

DATE 20 July 1962

TO Mr. E. G. Wolner - Cleveland

FROM Mr. R.A. Guidi - Newark

SUBJECT: [illegible]

REPLY REQUESTED BY (DATE)

I am attaching a copy of a letter addressed to Dr. Bleiberg from the Medical Director - USA Department of Health, Education, and Welfare. These two gentlemen are personal friends. Dr. Bleiberg had hoped that Mr. Birmingham might be more helpful. Unfortunately, the only suggestions made had been attempted, with negative results.

I have now arranged to have a Chemist from Aetna visit the plant July 25th, 26th, and 27th. These visits are for the purpose of taking air samples at various vents in hopes that we might more specifically locate the source of the Chloracne. The Aetna Chemist, Mr. Walter Hendershot made a preliminary investigation on July 3, 1962. After his visit, we discussed his observations at length. He reported general conditions were well above average and could offer no specific ideas at that time.

If, after Mr. Hendershot has completed his work, we feel additional assistance is required, I am contemplating contacting the New Jersey Industrial Division, Dept. of Health. Any additional ideas or suggestions will be greatly appreciated.

R.A. Guidi

RAG/mm

DS 00017408

OCCUPATIONAL CHLORACNE CAUSED BY AROMATIC CYCLIC ETHERS

by

J. Kimmig and K. H. Schulz

University Clinic of Dermatology, Hamburg-Eppendorf

Dermatologica, 115 (1957) 540 - 546

Thirty-one cases of chloracne were found among workers engaged in the production of 2,4,5-trichlorophenol and in the conversion of the latter into 2,4,5-trichlorophenoxyacetic acid and its esters. Animal experiments, carried out by painting rabbits' ears, have shown the cause of chloracne to be not trichlorophenol itself, but toxic by-products formed in the alkaline hydrolysis of 1,2,4,5-tetrachlorobenzene into 2,4,5-trichlorophenol. Compounds that may arise in this process were synthesized, and tri- and tetrachlorodibenzofuran and tetrachlorodibenzodioxin were found to be highly active. Furthermore, 2,3,6,7-tetrachlorodibenzodioxin was isolated as a by-product formed in the manufacture of trichlorophenol, and the possibility of its formation from sodium trichlorophenoxide was established.

Chlorinated compounds, used as industrial materials or formed as by-products, occupy an important position among the chemical and physical intoxicants capable of producing acne and folliculitis.

The first cases caused by these compounds were observed at the beginning of the century with the introduction of the electrolytic chlorine process. The view put forward, and later corrected, by Herxheimer, according to which the disease is caused by free chlorine, has led to the confusing but common designation "chloracne".

Since the introduction of chlorinated naphthalenes, used in various branches of industry on account of their useful properties (resistance to acids, non-inflammability, waterproofness, good insulation), contact with these compounds has often resulted in chloracne[2,3,8]. Both Wauer[9] and Teleky[8] have suggested the term "Pernakrankheit" ("perchloronaphthalene disease"), which again is unsatisfactory in many respects.

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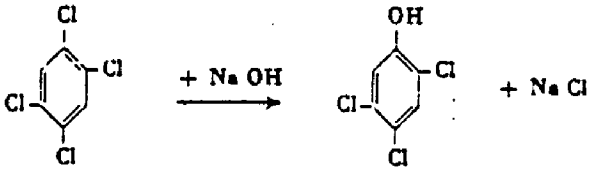
EXHIBIT "J"

aving been used with more than 100 substances in the past two years, the rabbit-ear test enables the chloracne-inducing activity of a substance to be ascertained with high probability.

The first results were reported by one of us[7] at the 23rd Congress of German Dermatologists in Vienna. It was first shown that, contrary to previous assumptions, 2,4,5-trichlorophenol was not to be considered as the chloracne-inducing factor. This emerged from the observation that the expected symptoms were not produced on the rabbits' ears with a 5% solution of pure 2,4,5-trichlorophenol in polyglycol, but they were produced by the technical product. Since the starting material, 1,2,4,5-tetrachlorobenzene, was inactive on the rabbits' ears, it was to be assumed that the intoxicant was among the by-products formed in the alkaline hydrolysis of 1,2,4,5-tetrachlorobenzene into 2,4,5-trichlorophenol.



1,2,4,5-tetrachlorobenzene

2,4,5-trichlorophenol

Since the distillation residue could not at first be separated, a number of such synthetic compounds were selected for testing, which were likely to be formed in the hydrolysis of tetrachlorobenzene at 180°C, these compounds being the chlorine derivatives of diphenyl ether and of dibenzofuran (diphenylene oxide).

Whilst the chlorine derivatives of diphenyl ether and unsubstituted and monochlorinated dibenzofuran were inactive in the animal experiments, trichloro- and tetrachlorodibenzofuran resulted in the expected symptoms, even when they were applied in 0.05% solutions.

In addition to the above symptoms, both compounds exhibited strong ~~hepatotoxic properties.~~ The oral administration of a single dose of ~~0.5 to 1 mg/kg led to~~ severe hepatic conditions in rabbits. However, chlorinated dibenzofurans could not be detected as a by-product in the production.

The clinical examination of a laboratory assistant who developed a severe case of chloracne after contact with tetrachlorodibenzodioxin indicated the possible causative function of chlorinated dibenzodioxins (diphenylene dioxides). Further experiments with rabbits' ears showed tetrachlorodibenzodioxins, especially 2,3,6,7-tetrachlorodibenzodioxin, to be very highly active. Three or four painting with 0.01 - 0.005% solutions, applied at intervals of 3 to 4 days, were found to be sufficient to produce severe inflammation and follicular hyperkeratosis. A single dose of 0.05 - 0.1 mg/kg (per os) resulted in severe hepatic conditions, and, in most cases, in death within 8 to 20 days. Autopsy revealed distended necrotic tissues and engorgement of the liver.

OCCUPATIONAL POISONING IN THE PRODUCTION OF CHLOROPHENOL COMPOUNDS

by H. [redacted] H.[redacted] and U. Spiegelberg, Hamburg

(From the Neurological Clinic (Director: Prof. Janzen), the Dermatological Clinic (Director: Prof. [redacted]) and the Clinic for Psychiatric and Nervous Diseases (Director: Prof. Bürger-Prinz), University Hospital Hamburg-Eppendorf)

Archiv für Geiverbepathologie und Geiverbehygiene, 18 (1961) 538-555

Introduction

In at least three factories in Western Germany groups of workers engaged in the production of chlorinated phenols have recently developed acne which took a protracted course and was followed by further complications.

Baader and Bauer and also Brinkmann (1950/51) reported cases among a group of [redacted] in a factory situated in Nordrhein-Westfalen. In this factory the workmen were engaged in the production of [redacted]. In addition to a comedo-acne associated with secondary pustular infection and furuncle formation of various degrees, most of the affected workers complained even in the early stages of their skin disease of [redacted], and indefinite [redacted]. Review of the documentation based on 17 cases* led to the following results: All 17 affected cases had acne; out of these 4 could be regarded as severe, 8 as moderately severe, and 5 as medium severe to light; almost all patients had [redacted] In 4 cases bursitis of the cubital joint could be established.

As far as other complications were concerned, [redacted] bronchitis, [redacted], and [redacted] fatal case; [redacted] patients developed [redacted] (in 7 cases intensive pain in the lower limbs, [redacted], in [redacted] without atrophy, and in 2 cases the [redacted]

*We wish to express our gratitude to the Berufsgenossenschaft der Chemischen Industrie for putting at our disposal their records and other documents, and for their understanding attitude during prolonged clinical observations.

Seven of the patients complained of psychological disorders such as continuous [redacted].

A greater number of [redacted] (about 60 cases, Prof. Berg) occurred [redacted] in the middle [redacted] among workers engaged over a long period (in most cases for several years) in the [redacted] (saponification of 1,2,4,5-tetrachlorobenzene to 2,4,5-trichlorophenol by treatment with methanolic sodium hydroxide). Similarly to a third group of cases in the Hamburg district, to be discussed in greater detail below, persons working with trichlorophenol frequently [redacted] some time [redacted]. In a discussion following a lecture by Spiegelberg at the Congress of Northwest German Neurologists and Psychiatrists, held in 1960 in Luneburg, in which the author described [redacted] after occupational poisoning, Janzarik [redacted].

The third group consisted of [redacted]. These had been working in the trichlorophenol department of the factory in which a herbicidal substance, [redacted] was produced from technical 2,4,5-trichlorophenol by heating in autoclaves with sodium hydroxide and monochloroacetic acid. After completion of the esterification, the final product was purified by repeated recrystallization. The task of the workers consisted first of all in the filling of the autoclave; for this purpose trichlorophenol had to be taken out of open barrels by means of shovels. During this procedure a fine dust arose and spread all over the premises.

Other procedures were connected with the filling and operation of centrifuges, and with the inlet and outlet tube systems. As those workers who had been in particularly close contact with trichlorophenol developed the most intensive skin lesions, it seemed quite plausible to regard the technical trichlorophenol as the causative agent. The correctness of this view will be discussed below in connection with the problem of etiology.

The present author's clinical observations

At the present time, [illegible] 9 of the 31 workers of the Hamburg Factory mentioned above are [illegible], prolonged [illegible] of the lower limbs, [illegible], but mainly for marked [illegible]. The following table shows the complaints and damage to health established in these patients. In all cases the skin lesions developed on the whole according to an identical pattern: first numerous comedos developed, initially on the face (particularly the cheeks above the zygomatic arc, the forehead, the temples, the chin and the ears), and later secondary infection led to the development of folliculitis, pustules, furuncles, and retention cysts. Still later, these lesions spread in most cases onto the lateral parts and the back of the neck, the upper half of the back, the chest, lower arms, the genital organs and the thighs. Numerous furuncles developed mainly on the back of the neck, and on the back. These efflorescences were usually extremely densely arranged, and hardly any follicle remained unaffected.

In some of the workers who had apparently suffered a more intensive exposure the described acneiform lesions were preceded by a dermatitis associated with erythema and edema which extended to the periorbital region, the cheeks, and the forehead. Almost at the same time, blepharoconjunctivitis developed in some of the patients; similarly to the skin lesions, this condition took a chronic course.

The summary of the findings set forth in the table shows that, in addition to the described changes, some patients had partly spots and partly larger areas of pigmentation on the face, which endowed the skin with a dirty gray-brown appearance.

The [illegible], taken as a whole, is [illegible] and later by some other authors [illegible] in persons exposed to chlorinated naphthalenes, diphenyls, and other aromatic compounds. (For more details see W.Braun

and also A. Risse-Cundermann (1959)). These forms of occupational poisoning are generally referred to by the term chloracne or "Perna-disease", although these terms do not express correctly the precise nature of the condition in question.

In our cases the dermatological manifestations took a particularly tenacious course. The therapeutic measures applied by us (evacuation of the comedos, and external keratolytic and antibacterial treatment combined in more severe cases with systemic administration of antibiotics were insufficient to prevent the formation of new comedos, retention cysts, and furuncles during the first one or two years, although the patients were no longer in contact with the toxic substance in question. At present, in most of the patients residual changes consist in densely arranged deep scars, which are particularly disfiguring when localized on the face (pseudoatrophodermia vermiculata).

All affected workers without exception complained of marked fatigue and weakness in the legs, frequently accompanied by pains particularly in the proximal leg muscles. These complaints were pronounced even in early stages of the disease, sometimes even preceding the development of more serious skin lesions. The records or spontaneous communications by the patients revealed the presence of paresthesias only in 2 out of 9 cases. We failed to demonstrate in any of the cases localized paresis or atrophy, weakening of the reflexes, or the absence of muscular extension reflexes which would have suggested toxic polyneuropathy. Two of the subjects investigated indicated loss of sensitivity in the lower limbs, combined with isolated epicritical disorders. Electromyographic examination revealed no reliable signs of neurogenic lesions, as one would have expected in the case of lesions of the peripheral nerves. The fact that experiments based on serial stimulation revealed the premature development of fatigue requires further confirmation both with regard to the method and the findings obtained. These findings show that the described neuromuscular disorders do not fit into the picture of a toxic polyneuritis or polyneuropathy.

The electroencephalogram showed in 6 cases an abnormal picture, which consisted of very labile frequencies, and of partly assymetric dysrhythmic complexes. One of the patients showed accentuated dysrhythmia and increased cerebral excitability after photic stimulation. The EEG changes obtained by us were not characteristic, and yielded no important new facts pointing to the correct diagnosis.

Some of the workers investigated by us complained of headache, attacks of vertigo, and a tendency to orthostatic collapse. 5 of the 9 patients investigated showed marked signs of excessive vegetative irritability such as sweating on hands and legs as well as in the axilla, increased dermographism, and a suggestion of a positive Chvostek sign. Blood pressures taken in Out-patient Departments were all found to be in the normal range. In 5 out of 9 patients the blood pressure was around the lower limit of the normal range. A tendency towards orthostatic collapse could not be observed either in out-patients nor in in-patients. In 2 cases there was a suspicion of myocardial damage.

Fig. 1 Fig. 2 Fig. 3

Fig. 1-3. Chloracne developing during exposure to technical trichlorophenol

4 out of 9 patients presented abdominal complaints such as a feeling of fullness and pressure in the stomach and the hepatic region, and complained of slight abdominal pains. Gastric secretion was disturbed in 4 cases, including 3 cases of hypoacidity and one case of hyperacidity. In one case gastritis could be established by radiological investigation.

In the course of observations on out-patients as well as on in-patients great

efforts were made to establish the presence of [redacted]. Lability tests led in all cases to negative results, but in 2 cases the bromophthalein test revealed a slight delay in the excretion of that substance. [redacted] led to [redacted]. In 2 cases the lesions consisted of mild perihepatic changes; in the third case the sections revealed the presence of a [redacted] with mild inflammatory and [redacted]; in this latter case the clinical and histological picture was consistent with the state after virus hepatitis. In the same case deposits of both an iron-containing and iron-free yellow-brown pigment were observed; this pigment, however, was not identical with the gray iron-free pigment found by Kalk and Wildhirt in cases of chlorophenol poisoning. The presence of red cells in the urine of one of the patients who showed no other signs of kidney lesions remained unexplained.

All cases suffering from chlorophenol poisoning underwent detailed psychiatric and psychopathological investigations, and the [redacted] found in these cases are of particular interest. In 6 cases the course of the disease could be followed up for about two years. It proved possible to take objective anamneses and also to carry out experimental psychological investigations**

With a great degree of conformity, all patients described a subjective syndrome of complaints which extended, in addition to symptoms suggestive of polyneuropathic lesions of the extremities, or cardiovascular and abdominal symptoms, to the psychological and intellectual sphere, but above all to the vitality and the emotional-impulsive behavior of the patients (Burger-Prinz). The patients particularly complained of [redacted], such as feeling of [redacted].

The basic mood of the patients was described as having deteriorated towards a feeling of [redacted] and towards [redacted]. Frequently the patients were of ill humour, with a component of [redacted]. Emotional changes proper were increased [redacted], a tendency to [redacted].

*Prof. Dr. HORNBOSTEL and Dr. SCHONFELDER, I.Med. Univ.-Klinik Hamburg-Eppendorf.
**In this context we express our gratitude to W. von Schubert for carrying out the tests in question (Rorschach-Psychogram and the Hamburg-Wechsler intelligence test for adults).

General lack of vigour, loss of verve and drive were symptoms observed in all cases examined by us. The subjects revealed loss of initiative, interest, loss of willpower, they tired easily and showed a diminished capacity both in the physical and the psychological and intellectual sphere.

This picture of the subjective and objective psychopathological anamnesis can be rounded off by a number of other symptoms recurring with greater or lesser regularity: disorders of the basic instincts could be observed in practically all cases. Thus all subjects complained of a considerable loss of sexual vigor and most of them also of loss of libido. Their appetite deteriorated and occasionally [illegible] weight occurred. In most patients [illegible] required concentration increased, in the sense of increased need for time combined with difficulties in getting [illegible].

Some patients complained of loss of mental capacity, aboveall loss of memory and the ability to learn. Most of the patients became intolerant to alcohol. Finally, there was a tendency to hyperesthesia with excessive sensitivity to noise and light. Almost all patients stated that the described psychopathological syndrome was subject to considerable variations.

Changes in the intensity of the symptoms in the sense of diurnal variations with a predominance in the early morning, mid-morning or in the evening were described by the patients; some were free from complaints for intervals of several days or weeks.

For the sake of completeness, two further rare phenomena will be described here. Two of the subjects stated that exposure to cold (cold showers or ablutions) led to a more or less prolonged relief from the general complaints and the polyneuropathic symptoms. One of the patients adopted peculiar eating habits which changed from time to time: e.g. for a while he lived exclusively on brown bread, then changed to milk-soups and in another period he consumed daily three litres of milk neglecting any other food.

In contrast to the polymorphous and polysymptomatic subjective picture, it does not take long to enumerate the objective psychopathological signs. In the course of exploratory talks, the majority of patients displayed a soft, depressed, dull mood, and it was hard if not impossible to cheer them up. They gave the impression

having [redacted], their psychomotor processes were lax and tired. The picture was that of a reduction in drive on the basis of an organic cerebral lesion, rather than that of inhibition. As regards the emotional behavior of the patients, they seemed to be at times less accessible and responsive, and on other occasions, on the contrary labile and easily upset. Two patients were found to be true hypochondriacs, and another one showed a slight but distinct [redacted].

The psychological tests are important, as they reveal more subtle defects in intellectual performance and psycho-organic emotional disturbances. The majority of patients who underwent the Hamburg-Wechsler intelligence test for adults showed a significant increase in the proportion of decomposition (Wechsler) which at least points to [redacted]. In the Rorschach psychogram coartation of the type of emotional response, signs of [redacted], lack of concentration, decreased speed, and stickiness of thought processes, as well as a tendency toward perseveration suggested organic changes in the brain.

Discussion

Observations on three independent groups of workers exposed to chlorophenol, with a [redacted], thus [redacted] the most important symptoms of which are as follows:

1. Initial dermatitis of the face and conjunctival irritation, followed by acne, which in some cases may also develop more gradually. The acne spreads mainly over the face, later extends to the back of the neck, the shoulders, and the upper parts of the trunk. In severe cases the whole body is covered with comedos, retention cysts, nodules, pustules, furuncles, and spotty pigmentation. In some cases the mucous membranes of the face and the upper respiratory tract show a higher degree of irritation, and are partly associated with prolonged blepharoconjunctivitis.

2. In some cases disorders of the internal organs, [redacted] which shows deposits of an iron-free gray pigment, give a characteristic picture in biopsy samples. In some cases [redacted] and in a few cases [redacted]

only on the rabbit ear but also on human skin, one of us (Schulz) carried out an experiment on himself: a circumscribed skin area on the lower arm was twice painted with a 0.01% solution. Two days later a mild dermatitis developed, followed after several days by follicular dermatosis and comedos which could be easily demonstrated histologically. This seems to constitute sufficient evidence for the etiological importance of the substance in question in the origin of the conditions described in this paper. It does not seem impossible that other chlorinated aromatic compounds of highly toxic properties may be formed, which had hitherto escaped identification and investigation by animal experiments.

The experimental and clinical observations constitute an impressive example of the fact that substances involved in large-scale chemical production processes in small quantities as side products may be of importance in the occupational pathology of the substance in question. This aspect should not be overlooked in examinations concerning the mass occurrence of occupational poisoning. If it can be shown that the contaminations of a main product are responsible for the occupational disease, a basic preliminary condition for successful prophylaxis of the disease will be fulfilled.

In our special case we have been able to change the production technology by a modification recommended by the plant chemical team. These changes made it possible to avoid the formation of the highly toxic multiply chlorinated dibenzodioxin and dibenzofuran. Ever since, the plant continued to produce 2,4,5-trichlorophenol and to transform it into the herbicidal substance 2,4,5-trichlorophenoxyacetic acid, but no symptoms of intoxication have been observed among the staff.

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I'm going to get these English references.

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Screening Tests for Urinary Uroporphyrin

Technical Considerations and Presentation of a New Method

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Elevation of urinary uroporphyrin occurs regularly in the porphyrias and is a relatively specific phenomenon. Urinary uroporphyrin must be purified and concentrated for qualitative demonstration at low but clinically significant levels. A new procedure based on the adsorption of uroporphyrin to talc, its insolubility in ether, and its brilliant red fluorescence under ultraviolet light is a rapid and highly sensitive screening test. Unlike other methods now in use, this test is significant when negative. Urine specimens from 12 patients with porphyria and 74 of 75 random hospital specimens of urine containing 50 to 100 mcg. per liter of added uroporphyrin gave positive tests while weak positive reactions were seen in only 6 of the same 75 specimens without added uroporphyrin.

THE PORPHYRIAS are inherited and acquired errors of metabolism characterized by overproduction of the porphyrins and their precursors, and capable of producing characteristic cutaneous and acute symptomatology. While the diagnosis can sometimes be made clinically, it must always be proved by laboratory means.

Ideally, quantitative urinary and fecal studies should be carried out whenever porphyria is suspected. Practically, the limitations of time, cost, and complexity of quantitative techniques, and the lack of necessary instrumentation often limit initial diagnostic efforts to the use of screening procedures for the qualitative demonstration of porphobilinogen and porphyrins in the urine.

Watson et al. have reviewed the subjects of qualitative testing for porphobilinogen.[1] This communication will survey the problems attendant to qualitative demonstration of urinary uroporphyrin, including some commonly used methods and their limitations, and will describe a new and more sensitive technique.

Some Characteristics of Porphyrins and Their Analytic Implications

All qualitative tests for porphyrins depend on demonstration of the characteristic red porphyrin fluorescence under ultraviolet light. This fluorescence is so intense that concentrations as low as 1 mcg. per liter in pure solution can be visually detected.

Porphyrin fluorescence is significantly affected by the wave length of the exciting ultraviolet light. Porphyrins will fluoresce when excited by light in the 3,600 to 3,700 A band (maximum 3,650 A), 4,000 to 4,200 A band (maximum 4,050 A), and in the bands near to their other absorption maxima, but the wave lengths closest to 4,050 A are the most efficient.[2]

Unfortunately, other fluorescent substances in urine[3, 4] can totally or partially mask the porphyrin fluorescence at low but clinically significant concentrations. Almost all urines when excited by 3,650 A light (which is obtained by means of the Wood's ultraviolet filter) display a blue-white fluorescence, apparently emitted by products of nicotinic acid metabolism.[4] This fluorescence, often intense under the 3,650 A light, is absent or minimal under the 4,050 A light. Likewise, the yellow fluorescence found in a majority of urines even after acidification is excited to a much greater degree by 3,650 A than by 4,050 A light.

Because we were unable to find an inexpensive, commercially available 4,050 A light source, we devised a simple adapter to take the place of the Wood's filter over the bulb of the readily available mercury vapor diagnostic lamp. The adapter is made from a coffee-can cover and supports a blue filter. This filter transmits ultraviolet light in the 4,000 to 4,400 A band with a peak transmission at

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4,100 A and a minimal transmission in the 3,600 to 3,700 A band.

We have also found it helpful to view the resulting fluorescence through a no. 1 yellow photographic filter. This eliminates incidental blue light reflected from the surfaces of the glassware and is especially helpful in detecting porphyrin fluorescence at lower concentrations.

Solubility and Significance of Urinary Porphyrins

Differential solubility of porphyrins in organic solvents, a function of the number of carboxyl groups on the aliphatic side chains, permits their definitive separation by extraction techniques. Separation of coproporphyrin from uroporphyrin forms the basis of qualitative and quantitative urinary porphyrin analysis (Table 1). (Protoporphyrin, normally present in the stool, almost never appears in the urine, even in persons who have porphyria.)

Table 1.—Use of Organic Solvents to Separate Porphyrins*

Extraction by	Extractability of Uroporphyrin	Coproporphyrin	Protoporphyrin
n-Butanol	− or =	+	+
Ethyl acetate	=	−	+
Ethyl ether	−	+	+
Chloroform	−	=	+

− = well extracted; − = not extracted; = = poorly extracted, or only under special conditions.
* From Schwartz et al.[3]

Coproporphyrin is found in most urines in qualitatively demonstrable concentrations, the normal daily excretion being 100 to 300 mcg. per day.[3] These values are increased by modest consumption of alcohol in the absence of any demonstrable hepatic dysfunction. Elevation of urinary coproporphyrin to several milligrams per liter occurs in heavy metal poisoning and in a variety of hepatic, hematologic, and neoplastic disorders.[4]

Conversely, urinary uroporphyrin is not qualitatively demonstrable at the normal levels of 10 to 30 mcg. per day.[5] Although intermittent findings of urinary "noncoproporphyrin" from 50 to 500 mcg. per day have been reported as incidental findings in some severe systemic diseases[6] this sporadic elevation is distinctly uncommon and occurs far less often than the corresponding elevation of urinary coproporphyrin.[4] Considerable elevation of urinary uroporphyrin is demonstrable in the symptomatic phases of the porphyrias. Lower concentrations of urinary uroporphyrin, from 200 to 500 mcg. per liter, are highly suggestive of latent porphyria or of porphyria in remission.

Porphyrins can be quantitatively adsorbed to the surfaces of a variety of inert materials. Aluminum oxide, calcium phosphate, fuller's earth, kieselguhr, lead acetate, and magnesium oxide have all been used to concentrate porphyrins from large volumes of urine as a preliminary to chromatography, crystallization, quantitative determination, or qualitative demonstration.

Techniques for the Demonstration of Uroporphyrin in Urine

The casual demonstration of porphyrin fluorescence in a raw or acidified urine by placing the specimen under the Wood's filtered light or other filtered light has no real significance. This can often be demonstrated in normal urines which contain relatively small amounts of the nonspecific coproporphyrin. Conversely, when considerable blue-white or yellow masking fluorescence is present, a common occurrence under the Wood's filtered light, the lack of characteristic porphyrin fluorescence does not guarantee the absence of excesses of uroporphyrin.

In all of the commonly used qualitative and quantitative methods, uroporphyrin and coproporphyrin are separated by differential extractions. Ethyl acetate and ethyl ether are most often used. The former has the advantage of being nonexplosive, but the latter provides a more rapid and clean-cut separation of the coproporphyrin from the uroporphyrin.[2]

The persistance of detectable porphyrin fluorescence after extraction of coproporphyrin from the urine at pH 4 to 6 signifies uroporphyrinuria. However, a negative result does not exclude the possibility that abnormal uroporphyrin levels are present with the fluorescence masked and quenched by other substances. As much as 1,000 mcg. per liter of uroporphyrin can escape detection in this way. At higher levels, porphyrin fluorescence is always observed.

Ideally, a screening test should eliminate the coproporphyrin, concentrate the uroporphyrin, and minimize the interfering effects of other substances present in the raw urine. One widely used technique is based on adsorption of the uroporphyrin to a gelatinous calcium phosphate precipitate after prior extraction of the coproporphyrin.[8, 10] This method eliminates most of the pigments and yellow fluorescent substances, but carries along significant amounts of the blue-white fluorescing material. The degree of adsorption of the uroporphyrin to the precipitate is variable, and the final solution actually represents a 2:5 dilution of the original uroporphyrin. In our experience, this method has not been reliable at uroporphyrin concentrations of less than 500 mcg. per liter. Occasional negative tests are seen with as much as 1,000 mcg. per liter when the final solution is examined under the Wood's filtered light.

Another method is based on adsorption of uroporphyrin to insoluble lead salts. Here lead acetate is added to urine from which the coproporphyrin has been extracted.[11] This method not only eliminates most of the blue-white fluorescing substances, but also achieves a threefold concentration of the uroporphyrin in the test sample. However, the pigments and yellow fluorescing substances which re-

main are also concentrated in the final solution. This method is reliable at uroporphyrin concentrations of 200 mcg. per liter when the 4,050 A light is used. Negative tests at uroporphyrin levels of 500 mcg. per liter are not uncommon when the final solution is examined under the Wood's filtered light.

Talc Disk Method

With [12] has described the use of a small, tightly packed talc disk of 2 to 3 mm. thickness for the separation of porphyrins from urine. The disk is prepared by pouring a suspension of talc in water onto filter paper in a small Buchner funnel connected to a source of suction. This disk will adsorb relatively large quantities of porphyrins from urine passed through it.

Blue-white fluorescing substances pass through the disk, but pigments and yellow fluorescing substances are retained with the porphyrins. With [12] eluted the porphyrins (and other substances as well) when he used a 1:9 mixture of 1 N hydrochloric acid and acetone prior to chromatography or crystallization.

Table 2.—Comparison of Qualitative Uroporphyrin Tests

Concentration of Added Uroporphyrin	50-70 Mcg Liter		100 Mcg Liter		200 Mcg Liter	
Wavelength of Exciting UV Light	3,650 A.	4,050 A.	3,650 A.	4,050 A.	3,650 A.	4,050 A.
Calcium phosphate	1/3	0/3	1/17	11/17	2/5	4/5
Lead acetate	1/4	4/4	3/16	14/16	2/5	5/5
Disk method	3/4	4/4	15/16	16/16	5/5	5/5
	Positive/Total					

The talc disk method, modified as described below, is the basis of a simple and highly sensitive screening test for urinary uroporphyrin.

1. A 20 ml. portion of urine at pH 4.5 to 5 is heated at 100° C. (212° F.) for 10 minutes in the dark. This step converts nonfluorescent porphyrin precursors to uroporphyrin while destroying a certain amount of the coproporphyrin present. The pH adjustment is conveniently achieved by adding to the urine 5 ml. of acetate buffer at pH 4.8 (4 parts saturated sodium acetate, 3 parts water, 1 part glacial acetic acid).
2. The urine, cooled to room temperature, is passed through the talc disk with the aid of suction.
3. The disk is washed, in situ, with 30 ml. of an 80% acetone, 20% water mixture. (This removes almost all of the interfering substances from the talc without causing significant uroporphyrin loss.)
4. The porphyrins are eluted from the disk with 10 ml. of a 10% 1.5 N hydrochloric acid, 90% acetone mixture. (This solution contains all the uroporphyrin and some of the coproporphyrin present originally, but purified and concentrated 2 times. If, when examined under the 4,050 A light, it does not fluoresce, or only emits minimal fluorescence, then the test is negative. It will be necessary to proceed further with about half of the urines.)
5. The acid-acetone eluate is combined with 5 ml. of acetate buffer and extracted for 2 minutes with 100 ml., then 1 minute with 50 ml. of ethyl ether. (This extraction removes all of the remaining coproporphyrin. The acetone disappears into the ether phase leaving the uroporphyrin dissolved in about 5 ml. of an aqueous solution is first acidified with 0.5 ml. to 1 ml. stances, and concentrated 3 to 4 times from the original urine.)
6. The final aqueous solution is examined in a thin-walled glass tube under the filtered ultraviolet light. If the 4,050 A light is used, the solution is first acidified with 0.5 ml. to 1 ml. of concentrated hydrochloric acid. A definite red fluorescence under the 4,050 A light or unequivocal pink or magenta under the Wood's light is considered a positive test.

When a 20 ml. aliquot of urine is used, positive tests are routinely found at uroporphyrin concentrations of 100 mcg. per liter in the raw urine. The sensitivity of the test may be varied by increasing or decreasing the size of the urine sample. Exclusive of heating, the entire procedure takes around 10 minutes to perform.

Table 3.—Summary of Cases of Porphyria

Case	Type	Uroporphyrin	Porphobilinogen (Watson-Schwartz Test)
1	PCT	++++	Negative
2†	AIP	++++	Positive
3	AIP	++++	Positive
4*	AIP	++++	+
5†	AIP	++++	Positive
6	AIP	++++	Positive
7*	VP	++++	Negative
8*	VP	+++	Negative
9	VP	++++	Negative
10	VP	++	Negative
11†	VP	++++	Negative
12	VP	++++	Negative

PCT = porphyria cutanea tarda, sensu strictiori; AIP = acute intermittent porphyria (classical Swedish); VP = atypical sip (Negro patients).
* Acute attack less than one year ago.
† Acute attack years ago.

This method has been applied to 75 separate 24-hour urine specimens obtained at random from the hospital clinical laboratories. Pure uroporphyrin I was added to 20 ml. samples of urine at concentrations equivalent to 50 and 100 mcg. per liter. An equal portion of each urine without added uroporphyrin served as its own control. Of the test urines, 74 of the 75 gave distinctly positive results. The one false negative at the 100 mcg. per liter level became definitely positive at 200 mcg. per liter. Six of the 75 specimens without added uroporphyrin gave weakly positive results.

A comparison of the 3 methods was carried out in 25 urine specimens (Table 2). In each, there was no detectable endogenous uroporphyrin by the disk

method. The results show that the disk method is consistently positive at lower uroporphyrin levels than the other 2, even if the Wood's filtered light is used. However, each of the methods is more sensitive when the final solution is examined under the 4,050 A light.

Elevation of Uroporphyrin in the Various Porphyrias

Elevated uroporphyrin can be demonstrated in the urine of virtually all symptomatic cases and many latent cases as well.[13-18] In patients who have the Swedish form of acute intermittent porphyria, urinary uroporphyrin is normal or slightly elevated when the urine is fresh, but heating in the dark converts significant amounts of precursors (especially porphobilinogen and uroporphyrinogen) to uroporphyrin. Patients who have the South African form usually have some elevation of urinary uroporphyrin, and this is supplemented by conversion of precursors in the acute phase. Our own experience in cases of porphyria is summarized in Table 3.

Thus, the qualitative uroporphyrin test will be positive in any form of porphyria if the patient has symptoms. Although further studies are indicated for intermediate reactions, a 4-plus reaction may be considered diagnostic. Conversely, the diagnosis of porphyria may be confidently excluded in a symptomatic patient whose urinary uroporphyrin test is negative.

Summary

Elevation of urinary uroporphyrin occurs regularly in the porphyrias, and very uncommonly in other conditions. The presence of interfering substances in urine necessitates purification and concentration of uroporphyrin for its qualitative demonstration at lower, but clinically significant, levels. The modified talc disk method is a rapid and sensitive screening procedure for urinary uroporphyrin. The test is applicable to any type of porphyria, and a negative result has much significance in excluding the diagnosis of all types of porphyria. An inexpensive adapter can be made for the production of 4,000 to 4,400 A ultraviolet light. This light band is superior to that produced by the commonly used Wood's light in qualitative testing for porphyrias.

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A DEFINITION OF SCIENCE.—"Scientific work," she said one afternoon as we were walking over the hills, "has a value of its own, whether you're liking it or not. It's—there. It's permanent. It's work which is always going to last. It's real creation."—Snow, C. P.: *The Search*, New York City: New American Library of World Literature, Inc., 1960.

ACQUIRED PORPHYRIA

In this issue of THE JOURNAL (page 88) Cam and Nigogosyan report their clinical observations made on 348 patients with porphyria cutanea tarda. This large number of patients afflicted with a relatively rare disease was seen in the course of 4 years, in 3 southeastern provinces of Turkey, where Dr. Cam is regional dermatologist. He had been holding this governmental position for a number of years without having observed a single patient suspected of having porphyria, when first, in the summer of 1955, and then, in much larger numbers, during the following summer, patients visiting his clinic showed symptoms characteristic of porphyria cutanea tarda. He correctly suspected a toxic cause for this sudden appearence of a syndrome which usually is considered of hereditary nature. Through epidemiological inquiries, his suspicion centered on hexachlorobenzene, a chemical used since 1955 by the government to protect seed wheat from fungal contamination. The time of appearance of so many cases of manifest porphyria and their geographic location coincided to such an extent with the time of introduction of hexachlorobenzene as a fungicidal agent and its regional distribution, that a causal relationship was difficult to disclaim. Indeed, the porphyrinogenic nature of this halogenated benezene, which should not be confused with the insecticide, benzene hexachloride, has subsequently been demonstrated in animal experiments.

The concept that some forms of porphyria cutanea tarda may be acquired rather than inherited is not new, but the report from Turkey provides the first direct evidence, implicating a specific toxin to which the afflicted patients were exposed. Early investigators of porphyria and porphyrin metabolism, including Gunther and Stokvis, believed that in some instances human porphyria may be acquired rather than inherited. In noting the frequent association of this syndrome with chronic alcoholism, Waldenstrom, Brunsting, Tappeiner, and others have suspected chronic nutritional liver disease as a possible etriological factor for this disturbance in porphyrin metabolism. Reports from South Africa, concerning the occurrence of this syndrome in Bantus with nutritional deficiencies, have further strenghtened this concept. Moreover, the successful production of experimental forms of hepatic porphyria in animals, using a variety of compounds, including allylisopropylacetylcarbamide (Sedormid) and hexachlorobenzene, has demonstrated that profound disturbances in porphyrin metabolism may be acquired in the absence of genetic predisposition. The recent outbreak of porphyria in Turkey seems to represent the human counterpart to these animal experiments.

In spite of these observations, it should be remembered that, for the various syndromes of human porphyria, including porphyria cutanea tarda, evidence for genetic transmission of the metabolic defect has been demonstrated beyond reasonable doubt. Thus, a detailed search for a hereditary pattern, including determination of fecal and urinary porphyrins in family members, would appear essential before the possibility of an acquired disturbance is considered.

RUDI SCHMID, M.D.
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PURPOSE

The porphyrins have a pink-to-red fluorescence when viewed by ultraviolet light at about 4,000 A. Uroporphyrin can be separated from coproporphyrin because of different solubilities in acid solutions.

METHOD:

1. Transfer 25 ml. of urine to a separatory funnel and add 10 ml. glacial acetic acid.
2. Extract twice with 50 ml. portions of ether, collecting and combining the ether extracts. The aqueous residue is saved for a subsequent step (5).
3. Wash the combined extracts in a separatory funnel with 10 ml. of 5% HCl. Collect the HCl fraction and view by ultraviolet light.
4. A strong red fluorescence indicates the presence of large amounts of coproporphyrin. Confirm spectroscopically.
5. View by ultraviolet light the aqueous portion saved from the ether extract in Step 2. Red fluorescence indicates the presence of uroporphyrin. (Confirm spectroscopically) and by the Waldenstrom method, as follows. (Can be omitted)
6. Using dilute (0.3%) HCl, adjust the aqueous residue to a pH of 3.0 to 3.2.
7. Extract twice with 50 ml. portions of ethyl acetate. Save and combine the ethyl acetate extracts.
8. Extract the ethyl acetate extract 3 times with 2 ml. portions of 0.3% HCl. (3% by volume). Combine the acid extracts and view by ultraviolet light. Red fluorescence indicates uroporphyrin.

REMARKS:

1. Small amounts of coproporphyrin are present in normal urine.
2. Uroporphyrin is not normally present in the urine.
3. Ethyl acetate will not extract all the Waldenstrom uroporphyrin and little or none of the pure uroporphyrin type I found in congenital porporphyria. However, ethyl acetate extraction will concentrate the more commonly occurring Waldenstrom uroporphyrin sufficiently to detect its presence.

REAGENTS:

1. 5% HCl: Add 50 ml. of concentrated HCl slowly to 200 ml. of distilled water. Dilute to 370 ml.

REFERENCE:

DS 00024360

Wintrobe, John D., Laboratory Hematology, P. 360.

April 22, 1963

COPY

Dr. Bleiberg
22 Ball Street
Irvington, New Jersey

Dear Dr. Bleiberg:

I am returning herewith the article entitled, "New Method Bars Damage of Light to Human Skin".

Many thanks for having left it with me.

Very truly yours,

RAG/nc
Enclosure

R. A. Guidi
Plant Manager

DS 00024439

New Method Blocks Damage of Light To Human Skin

(Journal Tribune—World Wide Report)

MINNEAPOLIS — A new method of protecting the skin against cutaneous sensitivity to light has been developed by Drs. Kamori M. Fukuda and Walter J. Rumge, of the University of Minnesota. It has produced clinical results described as "very satisfactory."

The investigators found that a mixture of 0.015 per cent dihydroxyacetone or lawsone with 5 per cent dihydroxyacetone in a 50 per cent isopropanol-water solution, or in an ointment, applied to the skin will chemically react with the stratum corneum and produce a chemically altered keratin that filters out 95 per cent of the visible light, and 20 per cent of the infrared radiation reaching the skin.

According to the researchers, unlike presently available commercial products, their protective filter cannot be washed off since it is part of the stratum corneum. They said that approximately three to six applications of the topical mixture produce within a few hours "maximum protection for sunlight-sensitive patients."

Photosensitivity Effective

Once the protective skin filter is induced in the stratum corneum, it can be kept "at a maximally efficient level of protection by reapplying the topical mixture once or twice a week in the majority of patients with sunlight sensitivity."

The investigators reported: "During the past two years we have treated 75 patients with the following disorders: porphyria, polymorphic light eruption, porphyria cutanea tarda, erythropoietic hydroa aestivale, urticaria solaris, subacute lupus erythematosus, and vitiligo. All were protected from light.

"This topical mixture has not produced any contact dermatitis, nor have we observed any adverse side effects. Chromatographically purified quinones and dihydroxyacetone must be used, as impurities will cause contact dermatitis and instability of the topical preparation."

Patients, they added, have been satisfied with the cosmetic properties of the new preparation, since it can be applied in a water-alcohol solution or a nongreasy vanishing cream and does not wash off or stain clothing.

"If the preparation is used more than once a day," according to the report presented at a staff meeting of the University of Minnesota Hospitals, "it will impart a natural brown color to the skin: this color simulates a natural tan. If the topical preparation is used less than every other day, no change in skin color from the topical mixture can be perceived even though the patient obtains maximum protection. The color induced in the stratum corneum is primarily due to the quinone and not to the dihydroxyacetone. Individuals using this preparation can acquire a natural tan but at a slower rate than.

Special Article

Fluorescing Erythrocytes and Porphyrin Screening Tests on Urine, Stool, and Blood

Investigation of Photosensitivity

Derek J. Cripps, MD, MS, and Henry A. Peters, MD, Madison, Wis

Selected methods have been described for detecting an increase of porphyrins in the urine, stool, and blood. These methods are rapid and are particularly useful for use in outpatients or office practice to evaluate patients with sunlight sensitivity in whom porphyria is suspected. Also included are photomicrographs of fluorescing erythrocytes and a table giving examples of quantitative porphyrin values in stools, erythrocytes and urine that may be observed in different types of porphyria.

PORPHYRIA in man may occur from acquired or inborn derangements of porphyrin synthesis, and depending on the primary site of this synthesis, the porphyrias are usually grouped into erythropoietic or hepatic. Symptoms of photosensitivity in porphyria are associated with the presence of increased porphyrins (uroporphyrin, coproporphyrin, and protoporphyrin) in the plasma or erythrocytes. The principal wavelengths of light responsible for provoking photosensitivity are in the region of 400 mμ (violet light) and this action spectra [1-3] corresponds with the maximal absorption spectra of porphyrins when measured in vitro with a spectrophotometer.[4] Patients with porphyria may therefore give a history of photosensitivity occurring through window glass which transmits lights of wavelengths greater than 320 mμ but absorbs the sunburn wavelengths.

Patients with photosensitivity in whom porphyria is suspected can be routinely screened by the following simple and rapid tests on their urine, stool, and blood. These tests were selected as they can be conveniently performed in office practice or on outpatients. The fundamentals of these porphyrin screening tests have been described elsewhere for urine,[5] stool,[5,6] blood,[7] and for fluorescence microscopy observations of erythrocytes[8]; but, these tests have been modified and summarized to facilitate the interpretation of results.

The clinical features of the porphyrias are well documented[9,10] and need not be repeated in this article. Examples of quantitative porphyrins in the urine, stool, and blood that may be detected in the various types of porphyria have been summarized in Table 1, references for these examples are as follows.

In erythropoietic porphyria (a case reported by Varadi[11] in 1958, but the porphyrin determinations are recent), the uroporphyrin and coproporphyrin, which are mainly series[1] are increased in the urine, stool, plasma, and erythrocytes. In erythropoietic protoporphyria (12 cases,[12-15] two unreported), the urinary porphyrins are normal; fecal protoporphyrin is usually increased but may occasionally be normal,[16-18] so the diagnosis is made by the detection of increased erythrocyte protoporphyrin. There has been only one reported patient with erythropoietic coproporphyria.[19] In this patient, the urine and stool porphyrins were normal, but the erythrocyte coproporphyrin was increased. The number of patients (15 cases,[3,20,21] 10 unreported) with symptomatic porphyria or porphyria cutanea tarda, summarized in Table 1, is small, but the series includes several urine and stool porphy-

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rin (including uroporphyrin) determinations on each patient; however, the results are similar to a larger series of 66 cases reported by Eales.[22] In symptomatic porphyria, stool coproporphyrin usually exceeds the protoporphyrin, and urinary uroporphyrins are high. In acute intermittent porphyria (not associated with photosensitivity), the porphyrin precursors, porphobilinogen and Δ-aminolevulinic acid, are increased in the urine during an attack,[23] but may still be high, although decreasing, in remission[24-27]: fecal porphyrins are usually normal[28]; urinary coproporphyrin may be increased; but, urinary uroporphyrin is probably increased by in vitro conversion from porphobilinogen.

In the mixed or variegate porphyria[22] (hereditary), stool porphyrins (particularly protoporphyrin) are high, and urinary coproporphyrin may exceed the uroporphyrins; during an acute intermittent attack, increased porphobilinogen and Δ-aminolevulinic acid may be detected in the urine; in latent forms or asymptomatic relatives the stool porphyrins only may be increased.

There have been 30 reported cases of hereditary coproporphyria recently reviewed by Goldberg et al,[28] which is characterized by excessive coproporphyrin in the stools and urine. This condition may be asymptomatic, but certain drugs, such as barbituates, may produce acute intermittent attacks, and increased porphobilinogen and Δ-aminolevulinic acid in the urine. The range of stool coproporphyrin in 10 cases[28] has been appended to Table 1.

Materials and Methods

Rapid porphyrin screening tests on urine, stool, and blood

Materials and Apparatus

1. Amyl alcohol
2. Acetic acid, glacial
3. Diethyl ether: Anesthetic or analytic reagent grade.
4. Mixture of reagents, diethyl ether: glacial acetic acid, 5:1 (by volume).
5. Mixture of equal parts of reagents: amyl alcohol, acetic acid, and ether.
6. 1.5 normal hydrochloric acid (HCl). Mix approximately 120 ml of concentrated acid to 1 liter of distilled water.
7. 3 N HCl
8. 0.1 N HCl
9. Test tubes
10. Glass stirring rods
11. Disposable pipettes
12. A Wood's lamp

Urinary Porphyrins (For Uroporphyrin and Coproporphyrin).—*Method* 1.—Put 4 ml of urine into a test tube; add 10 drops of acetic acid and 1.0 ml of amyl alcohol. Shake gently. Examine under a Wood's lamp.

Result.—A positive test is indicated by a pink or red fluorescence in the upper amyl alcohol layer.

If an unexplained negative test is obtained on fresh urine, the presence of porphyrinogens can be confirmed by oxidation to form porphyrins with 0.05% iodine.

Method 2.—The sensitivity of the above test can be altered by transferring the upper amyl alcohol layer to a clean test tube with a disposable pipette and shaking with 10 ml of 1.5 N HCl (the fluorescence of the porphyrins is increased in HCl).

RESULT.—Negative or faintly pink fluorescence indicates a normal test, with a total urinary porphyrin below 250 μg/liter to correspond with the upper limit of normal porphyrins[29] suggested in Table 1. A stronger pink or red fluorescence indicates a positive test.

Interpretation.—The urinary porphyrin screening tests detect the presence of uroporphyrins or coproporphyrins or both which can be increased in the hepatic porphyrias and erythropoietic porphyria, but not in erythropoietic protoporphyria.

Increased urinary porphyrins may be found in several conditions not associated with photosensitivity, such as coproporphyrinuria of lead poisoning, liver disease, chemical intoxications, eg, carbon tetrachloride and infections, and some anemias.[6,29]

Stool Porphyrin Screening Tests.—*Method* 1.—A piece of fresh stool, the size of a small cherry stone (about 0.5 gm) is placed in a test tube and mixed into a paste with 0.5 ml of glacial acetic acid by stirring with a glass rod. Then about 4 ml of ether is added and the contents of the tube thoroughly mixed with the glass rod. Allow the liquid (acid ether) extract to clear, and then decant into a clean test tube. Add 2 ml of 1.5 N HCl. Shake thoroughly and when the phases are separated, examine under a Wood's light.

RESULT.—Normal stools yield a negative or faintly pink fluorescence in the lower acid layer. If coproporphyrin or protoporphyrin or both are present in excess, then the fluorescence in the lower acid layer is correspondingly in-

Table 1.—*Examples of Quantitative Porphyrin and Erythrocytes in the*

Type of Porphyria		Stools (μg/gm. dry weight) CP*	PP*	UR*
			Erythropoietic	
Erythropoietic porphyria (1 case, 5 determinations)	R*	20,790	310	+
	M*			
Erythropoietic protoporphyria (12 cases, 90 erythrocyte and 69 stool determinations)	R	2.1 to 132	21 to 1,642	N
	M	16.9	332	
Erythropoietic coproporphyria (1 case)		±	N	N
			Hepatic	
Symptomatic porphyria (15 cases, 89 urine and 59 stool determinations)	R	40 to 379	18 to 272	1.8 to 231
	M	158	104	66
Acute intermittent porphyria: during attack, 26 cases; during remission, 13 cases	R	±†	±	N
	R	12.7 to 70.4	10.5 to 115	
Porphyria variegata, cutaneous only (25 cases)	R	100 to 986	129 to 1,750	±
	M	355	638	
Porphyria variegata, during attack (20 cases)	R	105 to 1,834	70 to 1,943	±
	M	628	804	
Normal values, upper limit	R	27	75	...

*CP, coproporphyrin; PP, protoporphyrin; UR, uroporphyrin; PBG, porphobilinogen; ALA, aminolevulinic acid; M, mean; R, range; X, example; and N, normal.

†In hereditary coproporphyria (a variant of acute intermittent), the stool coproporphyrin is increased. In ten cases, it was 87 to 15,420μg/gm. dry weight.

‡Uroporphyrin can form in vitro from porphobilinogen (a pyrrole); hence, determination of fresh specimens are advised.

creased. Red fluorescence in the upper ether layer is due to chlorophyll derivatives and may be ignored.

Method 2.—The following method, described by Eales et al,[6] is similar, but has the advantage that a large number of fecal porphyrin screening tests (441) have been compared with the corresponding quantitative results.

A small button of stool (enough to cover a dime) is mixed into a paste with 3 ml of reagent (mixture No. 5 containing equal parts of amyl alcohol, acetic acid, and ether). The solvent layer is decanted into a clean test tube and 2 ml of 1.5 N HcCl is added, shaken thoroughly, and examined under a Wood's light.

RESULT.—The presence of porphyrin (coproporphyrin, protoporphyrin, or uroporphyrin) in the lower acid layer was graded as follows: 0 (normal), bluish-green fluorescence; ±, tinge of pink; 1+, definite pink; 2+, marked pink; and 3+, brilliant red.

Interpretation.—Increased stool porphyrins may be found in most forms of porphyria (Table 1). The ranges of normal stool porphyrins in nine series were reviewed by Eales,[29] but remain difficult to define precisely. The upper limit of normal quantitative stool porphyrins used in Table 1 is selected as a guide, and doubtful values have to be interpreted with other biochemical tests and clinical features.

One difficulty in comparing stool qualitative screening tests with quantitative results (Table 2) which are expressed in micrograms per gram dry weight (for uniformity) is that the amount of fresh stool used for a screening test varies because it is not accurately weighed and its water content is variable. The ratio of dry to fresh weight in our last 50 determinations was 1:4 (mean, SD ± 2.5).

Increased stool porphyrins noted in patients without photosensitivity may occur in asymptomatic porphyrias (related family or latent forms), gastrointestinal bleeding, meat eating, intoxication, such as lead poisoning, liver damage, and possibly due to alteration of intestinal bacterial flora from antibiotics.[8,20]

Blood Porphyrin Screening Test.—*Method.*—Five milliliters of the ether acetic acid mixture No. 4 is placed in a test tube. Four drops of blood (0.2 ml) are added and thoroughly mixed with a stirring rod. The supernatant is decanted in the second test tube, to which 1.0 ml of 3 N HCl is added and mixed well, and the test tube is viewed under ultraviolet light. The suggested quantity of blood and reagents are double that originally described,[7] but can again be proportionately increased until the investigator is accustomed to observing porphyrin fluorescence.

RESULT.—An intense red fluorescence in the lower acid layer indicates an excessive amount of protoporphyrin or coproporphyrin, or both. Porphyrins in normal blood are usually undetectable by this method. (If 0.1 N HCl is used rather than 3 N HCl, coproporphyrin only is extracted). The presence of increased erythrocyte porphyrins may also be confirmed by fluorescence microscopy examination.

Interpretation.—This test is designed to give a positive reaction if the blood porphyrin level

Values Observed in Stools, Urine, Different Types of Porphyria

Erythrocytes (μg/100 ml)			Urine (mg/day or liter)			
CP	PP	UR	CP	UR	PBG*	ALA*
			Erythropoietic			
1,250 to 2,370	135 to 480	1,570 to 4,360	27.57	54.14	N	N
1,705	336	2,650				
1.4 to 53.6	197 to 2,820	N	N	N	N	N
11.6	980					
408	128	128	N	N	N	N
			Hepatic			
N	N	N	0.102 to 1.27	0.22 to 10.9	N	N
			0.576	4.321		
N	N	N	±	±	4.0 to 162.4	4.0 to 174.9
			98 to 1,134		1.2 to 99.2	5.0 to 43.2
N	N	N	0.035 to 5.10	0.005 to 0.89	0.3 to 13.8	0.3 to 27.8
			0.732	0.207	2.0	4.9
N	N	N			10 to 142	7 to 103
			Wide scatter		45.4	33.8
2.5	52.0	...	0.283	0.040	1.9	5.9

is above 52 μg/100 cc, which is selected as the upper limit of normal[30] in Table 1. A positive test result would be found in erythropoietic porphyria (which would also have a positive urine and stool test); and in erythropoietic protoporphyria, but in this case the urinary porphyrins would be normal).

A false-positive test could be obtained if the plasma porphyrins were sufficiently high, which might rarely occur in cutaneous hepatic porphyria, and the test should then be repeated with 0.1 ml of packed red cells. Increased erythrocyte porphyrins may be detected in patients who are not affected with photosensitivity, for example, in lead poisoning, iron deficiency anemia,[31] sideroblastic anemia treated with pyridoxine,[32] or griseofulvin.[30]

Fluorescence Microscopy Screening Test.—This screening test may be used for detecting increased erythrocyte porphyrins, but is particularly useful for screening possible instances of erythropoietic protoporphyria in which the clinical features in remission could be slight.

Method.—A few drops of peripheral blood, diluted at the ratio of 1:5 approximately, are examined under a cover slip with an oil immersion objective using an ultraviolet fluorescence microscope. If photography for erythrocytes containing protoporphyrin (Fig 1) is required, an iodine tungsten light source[8] can be used, which will prevent the rapid fading of protoporphyrin fluorescence that would occur with a mercury vapor ultraviolet fluorescence microscope.

RESULT.—The number of fluorescing erythrocytes (fluorocytes) 1,000 erythrocytes counted on two occasions in five of the 12 patients (Table 1) with erythropoietic protoporphyria varied from 12% to 25% (Fig 1) and corresponds with counts made by previous observers.[8] There was some difference in the individual fluorocyte fluorescence, but the percentage of fluorocytes was approximately proportional to the increased erythrocyte protoporphyrin which varied from 467 to 2,820 μg/100 cc. Fluorocytes were rarely seen in normal individuals that we have screened, but may be observed in patients with conditions associated with increased erythrocyte porphyrin. For example, in a patient with iron deficiency anemia and an erythrocyte protoporphyrin level of 90 μg/100 cc, and fluorocyte count was 1%. However, in a patient with chronic lead poisoning and an erythrocyte protoporphyrin level of 626 μg/100 cc, the fluorocyte count was 100%, but as might be expected, individual cell fluorescence was much less than would be found in erythropoietic protoporphyria (with a similar quantity of erythrocyte porphyrin), but a fluorocyte count which was limited to 12% to 25%.

The number of fluorocytes in peripheral blood from two individuals with erythropoietic porphyria that we have studied varied from 5% to 6%. More than one half of these fluorocytes were nucleated (normoblasts) and the fluorescence was greater in the nuclei than the cytoplasm (incorrectly printed[8] but amended in an erratum) (*Arch Derm* 93:401 (April) 1966). The fluorescence in the nuclei may be predominantly uroporphyrin[33] (Fig 2).

COMMENT.—The sensitivity of these porphyrin screening tests depends not only on the efficiency of porphyrin extraction into hydrochloric acid, but the interpretation of the intensity of porphyrin fluorescence observed by the investigator with his Wood's lamp in the dark. For example, the erythrocyte porphyrin screening test[7] was based on a trial in which the

Table 2.—*Comparison of 441 Consecutive Fecal Porphyrin Screening Tests With Corresponding Quantitative Determinations**

Grade	No. of Specimens	Fecal Porphyrin†	No. of Samples		
			Variegate	Symptomatic	Nonporphyric
0	226	34 ± 31	1	11	113
±	61	93 ± 50	4	13	44
1+	65	203 ± 151	21	7	27
2+	46	652 ± 444	39	7	1
3+	43	1,237 ± 636	43	1	0

*From Eales et al.[6]
†Fecal porphyrin values are given in micrograms per gram, dry weight.

fluorescence of pure protoporphyrin in 1.5 N HCl was detectable with our Wood's lamp, using a concentration of 0.1 μg/ml, but not with 0.05 μg/ml. If the investigator wishes to determine the sensitivity of these tests himself, it is suggested that studies are made with a known concentration of coproporphyrin, which can be present in all the tested specimens. The concentration of coproporphyrin in 0.1 N or 1.5 N HCl is first determined in a spectrophotometer,[34,35] or against a standard in a fluorometer,[36] then, this coproporphyrin in hydrochloric acid is adjusted to pH 3.2 (red with Congo red) by adding solid sodium acetate, which is added to the ether extract of the specimen (eg, urine or stool) to be tested. If 1 ml of coproporphyrin in a concentration of 0.1 to 0.8 μg/ml is added to the ether extract from the erythrocyte screening test, there would be an approximate increase of 100μg to 800μg coproporphyrin/100 ml packed cells, and the intensity of porphyrin fluorescence can be observed and compared by the investigator.

Quantitative Determinations

The methods of determining quantitative porphyrins we used are as follows: urinary porphobilinogen and Δ-aminolevulinic acid as described by Mauzerall and Granick[37]; urinary porphyrins as described by Rimington in a Broadsheet[34] which is based on an earlier report[38]; fecal porphyrins as described by Rimington,[34,35] which has an advantage over the method of Schwartz et al[36] in that determinations are performed on random stool specimens and expressed as micrograms of porphyrin per gram dry weight of stool. This method is being used by an increasing number of investigators,[6] with the result that quantitative determinations can be more easily compared[20]; erythrocyte porphyrin determinations are based on a method described by Rimington et al.[30]

Quantitative Erythrocyte and Plasma Porphyrin Determinations

The method used by us is based on that described by Rimington.[30] The increasing interest in quantitative porphyrins has prompted us to describe the method in detail and to illustrate it with an example of the calculations used to determine erythrocyte porphyrins in a patient with erythropoietic porphyria (Fig 2).

Method.—A measured quantity of blood with added anticoagulant ethylenediaminetetracetic acid (EDTA) is used, usually 5 to 10 ml, if normal porphyrins are expected, but less than 2 ml can be used if the patients have increased erythrocyte porphyrins.

The packed red cell volume (V) and plasma volume (V) are calculated from the hematocrit. The blood sample is centrifuged at 3,000 rpm for ten minutes to separate the plasma, which is removed, and the remaining red cells are gently mixed with twice their volume of 0.9% sodium chloride and again centrifuged. This process is repeated, usually twice is sufficient. The plasma and sodium chloride washings for porphyrin determinations are added to 50 ml of an ethyl acetate: acetic acid (4:1 ratio) mixture, and allowed to stand overnight at 4 C in the dark. If hemolysis (from trauma) has occurred, the plasma and washings must be discarded. The remaining red cells are treated the same way. On the following day, both the erythrocyte and plasma extracts can be filtered through a sintered funnel under suction to allow separation from the protein residues. The red cell debris is continuously washed with the ethyl acetate and acetic acid mixture until the filtrate containing hematin and porphyrin becomes colorless. The filtrate is washed twice with saturated sodium acetate solution, which forms the lower

phase, which is then transferred to a second separating funnel. These sodium acetate washings are shaken with a little fresh ethyl acetate to reextract any remaining coproporphyrin and protoporphyrin, and are then added to the main bulk, which is washed once with 3% sodium acetate solution. The sodium acetate washings which contain the uroporphyrin fraction are combined. If the red cells should contain an excess of uroporphyrin, further extraction of uroporphyrin from the red cell debris is obtained by washing them with 10% ammonium hydroxide, which is then added to the sodium acetate washings. It was found necessary to perform this additional procedure for erythropoietic porphyria, but not in erythropoietic protoporphyria.

The main hematin filtrate is then repeatedly shaken with about one fifth its volume of 3 N HCl until all the porphyrins, coproporphyrin, and protoporphyrin are removed, which is confirmed by examining the successive acid extracts for fluorescence under a Wood's lamp. If the porphyrin fluorescense is strong, the acid volume (A) is measured and an aliquot (B) is then used. Solid sodium acetate is added to the 3 N HCl solution until a Congo red test paper no longer turns blue. This neutralized HCl solution (pH 3.2) is then shaken in the separating funnel with 50-ml portions of ether so that coproporphyrin and protoporphyrin are extracted into the upper ether phase. The ether extract is washed once with 3% sodium acetate and these washings are combined with the 3 N HCl which is once more shaken with ether to extract any remaining porphyrins. The two ether extracts are combined and washed with a little water. Coproporphyrin is extracted from the ether with successive 2- to 3-ml portions of 0.1 N HCl until the acid extract no longer shows any red fluorescence under a Wood's lamp. The combined acid extracts are mixed and the total volume (v) recorded. Protoporphyrin is then extracted in the same way, using 1.5 N NCl, and the total volume (v) is recorded.

Meanwhile, the sodium acetate and ammonium hydroxide washings containing uroporphyrine are brought to pH 3.0 to 3.2 with concentrated HCl, transferred to a separating funnel, and shaken with about 50 ml of ethyl acetate. The phases are allowed to separate, the uroporphyrin remaining in the upper ethyl acetate layer. The lower (aqueous) phase is run into a second separating funnel and shaken again with about 50 ml of ethyl acetate; after the phases have separated, this ethyl acetate extract is combined with the first one and washed with distilled water, which is discarded. Uroporphyrin is then extracted with 2- to 3-ml successive portions of 1.5 N HCl as described for coproporphyrin and protoporphyrin, and the volume of acid extract (v) is recorded.

The acid solutions are centrifuged and the concentration of porphyrin can be determined in a spectrophotometer[34,35] (Uvispeck or Beckman DU 2) which requires no standard, or measured in a fluorometer[36] against a standard solution. The following calculations are based on a determination in a spectrophotometer using the correction formulae and constants suggested by Rimington.[34,35] The constant (C) for protoporphyrin is 1.226; for uroporphyrin, 0.832, and for coproporphyrin, 0.73 (in 0.1 N HCl) or 0.837 (in 1.5 N HCl).

The optical densities of the acid solutions are measured in cells of 1-cm pathway at 380μ (D 380), 430μ (D 430), and the third measurement is the optical density obtained in the Soret region where the peak of maximum absorption is determined (D Max). The Soret maximum for coproporphyrin in 0.1 N HCl is usually at 399.5μ or in 1.5 N HCl at 401μ; for protoporphyrin in 1.5 N HCl, it is usually at 407μ; and for uroporphyrin in 1.5 N HCl, the peak is usually at 405μ.

Calculation.—The following formula was used:

$$[2\ D\ Max - (D\ 430 + D\ 380)] \times C \times \frac{v}{V} =$$

μg porphyrin/100 ml packed red cells

For example, the quantitative erythrocyte, porphyrins determined in the patient with erythropoietic porphyria (Fig 2) were calculated as follows:

The original blood sample was 2.08 ml, and the hematocrit, 24%; therefore, the volume of packed red cells (V) was 0.5 ml. The volume of acid extract (A) was 116 and the aliquot (B) of this was 50 ml. For uroporphyrin determinations, a 25-ml aliquot was taken from the total sodium acetate and ammonium hydroxide washings of 125 ml.

Coproporphyrin: $[2 \times 0.165 - (0.027 + 0.044)] \times 0.73 \times \frac{23.3}{0.5} \times \frac{116}{50} \times 100 = 2{,}360 \mu g/100cc$

Protoporphyrin: $[2 \times 0.032 - (0.009 + 0.003)] \times 1.226 \times \frac{10.1}{0.5} \times \frac{116}{50} \times 100 = 299 \mu g/100cc$

Uroporphyrin: $[2 \times 0.075 - (0.026 + 0.009)] \times 0.632 \times \frac{19.2}{0.5} \times \frac{125}{25} \times 100 = 1.840 \mu g/100cc$

This work was supported by USPHS grant AMO 9995 from the National Institute of Arthritis and Metabolic Disease. The Broadsheet in which Rimington[5] described urinary porphyrins was obtained from Dr. R. B. H. Tierney, Pathology Laboratory, Barnstable, Devon, England at 50 cents a copy.

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patients, with use of blood, throat washings or swabs and stools or rectal swabs in various tissue cultures failed to yield cytopathic hemadsorption or interfering agents.

Occupational Acne. Fabio Londoño[1] (Bogota, Colombia) reviewed the literature and reports **5 cases** of extensive follicular eruption in men engaged in spraying with **weed killers** (Killex and Exteron). The patients were aged 21-25 years. The spray contains the butyric ester of 2,4-dichlorophenoxyacetic acid, the methyl **ester of 2,4,5-trichlorophenoxyacetic** acid and solvent. The dermatosis has been called **chloracne** and follicular eleoconiosis.

The disease was clinically similar in all patients and was characterized by a profuse acneiform eruption, starting on the face and extending to the trunk and limbs, appearing 3-9 weeks after contact with the spray. The eruption included comedones, pustules, sebaceous cysts and furunculoid elements. The affected skin displayed a slate gray discoloration, more evident on the face. In all patients, the facial lesions were mainly comedones or small miliary cysts, whereas lesions on the trunk were mainly inflammatory (papules, pustules and furunculoid elements) and large cysts (Figs. 68 and 69). The dermatosis, although more marked on seborrheic areas, extended over nonseborrheic zones.

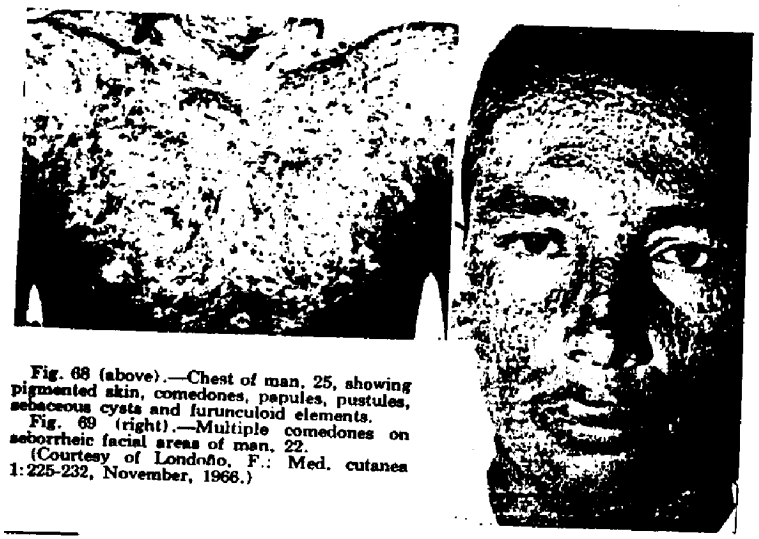

Fig. 68 (above).—Chest of man, 25, showing pigmented skin, comedones, papules, pustules, sebaceous cysts and furunculoid elements.
Fig. 69 (right).—Multiple comedones on seborrheic facial areas of man, 22.
(Courtesy of Londoño, F.: Med. cutanea 1:225-232, November, 1966.)

(1) Med. cutanea 1:225-232, November, 1966.

The disease was seen in tropical regions where spraying is usually performed and appeared twice, with a seasonal prevalence from November through January (1962-63 and 1963-64).

Bacteriologic examination showed a coagulase- and mannitol-negative staphylococcus.

Histologic study showed accumulation of fatty material in the pilosebaceous glands with or without inflammatory reaction around them. Sebaceous cysts surrounded by acute inflammatory exudate or by foreign-body giant cells and histiocytic granulomas were observed.

Treatment included elimination of contact with the spray and administration of antibiotics and estrogens. Dermabrasion was used on facial lesions, with satisfactory cosmetic results in all the patients. In 1 patient, ultraviolet therapy was used for the rest of the lesions.

These eruptions should be considered chloracne and not follicular eleoconiosis, an important differentiation from the etiologic and prophylactic points of view. The condition is definitely an occupational disease, and the worker thus affected should receive the benefits of compensation laws.

► [Chloracne, as illustrated by the author, can be very destructive and lead to disfiguring scars. It would be important to determine why certain individuals are susceptible while others are relatively little affected or completely immune. If screening methods were available (? patch tests with occlusion), those individuals susceptible could be excluded from this type of exposure. We are far behind in the field of occupational counseling.—Eds.]

Dogger Bank Itch is discussed by M. L. Newhouse[2] (London). Dogger Bank itch, an allergic dermatitis first described by Bonnevie (1948) is caused by the Alcyonidium hirsutum or A. gelatinosum, which is a seaweed-like colony of the phylum Bryozoa. It is most commonly found in the Dogger Bank area of the North Sea. In recent years many Lowestoft trawler fishermen have been affected by a severe dermatitis thought to be due to contact with the sea chervil, which has been identified as A. gelatinosum.

Crews of 55 trawlers were interviewed, and the hands, forearms, face and neck were inspected. Skin disease was common; the prevalence of all occupational skin diseases was 19.7% and that of nonoccupational skin diseases was 7.1%. Thirty-two (7.1%) of those interviewed were found to have Dogger Bank itch. Eighteen of these were patch tested with alcyonidium or serial dilutions of an homogenate of the colonies. Tests were

(2) Proc. Roy. Soc. Med. 59 (pt. 1):1119-1120, November, 1966.