FROM FILE #9

THE CUTANEOUS RESPONSE TO INDUSTRIAL AGENTS

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In the year 1899 Carl Herxheimer first described acneform eruptions in persons exposed to chlorinated compounds. He used the term "chloracne" for this problem. It was initially believed that chlorine itself was responsible for the eruption. However, several year later, it became obvious that chlorinated hydrocarbons were the provoking agents. Since that time industrial acne has become a rather common skin disease which may result from exposure to petroleum, cutting oils, coal tar fractions and chlorinated hydrocarbons of known chemical structure, such as chlorinated naphthalenes, diphenyls and diphenyloxides. In addition to pilosebaceous changes some of the chlorinated hydrocarbons are notoriously hepatotoxic and neurotoxic.

The activities of substances known to be acnegenic for man have been studied in a large variety of experimental animals, as well as in human subjects. The animals have included rabbits, mice, rats, dogs, cats and guinea pigs. Adams and his group, in 1941, described the reaction of rabbit skin to acnegens and claimed that the relative acnegenicity of a compound could be determined on the basis of the epithelial hyperplasia with its resultant thickening of the skin and follicle enlargement. Braun reported the production of follicular hyperkeratosis in the dog and rabbit following the application of a chlorinated naphthalene. We have not been able to produce pilosebaceous changes equivalent or comparable to human chloracne in the animals mentioned by local application, inhalation or ingestion of a known acnegen. The notable exception, however, is the skin of the

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external ear canal of the rabbit, which has been shown by Kerbrick[1,2] to respond rather vigorously to acnegenic stimulation.

Acne lesions may be induced in human skin by the repeated local application of such acnegenic substances as penta- and hexachloronaphthalene. Since such substances are also hepatotoxic, the experiments must of necessity be carried out with great care.

Objective

The purpose of this investigation was to determine the acne-inducing properties, in human skin, of three samples of sodium 2,4,5-trichlorophenate as compared with Halowax-1014, a known acnegen.

Materials and Method

A. The materials employed in this experiment were as follows:

Sample No. 3 was prepared from the plant Sodium Trichlorophenate; pH 10.0, and contained 22.4 percent T.C.P.*

Sample No. 4 was prepared by distilling trichlorophenol and then taking it up in caustic to form the phenate; pH 9.9, and contained 33.5 percent T.C.P.

Sample No. 5 was Sodium Trichlorophenate, prepared according to Diamond Alkali Company procedure in which dilution and filtration were employed; pH 9.9, and contained 31.0 percent T.C.P.

Halowax-1014 - a mixture of hexa and pentachloronaphthalene - was used as the positive control substance.

B. 1. Subjects: 12 human volunteers were employed for this investigation. They were white males of ages ranging from 47 to 78.

2. Application of materials: Each material was diluted with Plastibase (an oleaginous, non-water absorbing base containing 95 percent Liquid Petrolatum and 5 percent Polyethylene), in

* The material was obtained from a process exposure to which resulted in over 200 cases of chloracne and systemic effects.

concentrations which were demonstrated to be relatively non-irritating to rabbit skin. The concentrations of the test materials were increased in accordance with the skin response during the test as indicated below. A mixture of each material in Plastibase was applied once daily on flexural surface of forearm over a skin area measuring approximately 70 square centimeters. After application, the forearm was bandaged and retained for 24 hours until the next application.

Duration of Exposure, Concentration and Total Dose in Each Subject

Sample Number	Subject	Duration of Exposure (days)	Concentrations	Total Dose (gms)
3	J.P.	43	2.5% 53 gms + 3.5% 28 gms	2.2
	J.A.	43	2.5% 44 gms + 3.5% 14 gms	1.6
	C.P.	43	2.5% 44 gms + 3.5% 21 gms	1.8
4	J.C.	48	5.0% 4 gms + 7.0% 40 gms	3.0
	K.M.	28	5.0% 31 gms + 7.0% 10 gms	2.2
	R.T.	21	5.0% 35 gms	1.8
	E.J.	21	5.0% 31 gms	1.6
5	E.W.	43	2.5% 36.8 gms + 3.5% 11 gms	1.4
	K.S.	43	2.5% 38 gms + 3.5% 19 gms	1.6
	J.R.	21	2.5% 6 gms + 3.5% 19 gms	0.7
Halowax-1014	C.F.	43	20.0% 50 gms	10.0
	H.H.	48	20.0% 59 gms	11.8

3. Cutaneous biopsies: Biopsies were taken from the test and control sites in each subject prior to the initial application of the materials and at regular intervals during the duration of the exposure.

4. Liver function tests: Thymol turbidity tests and prothrombin time tests were performed in each case prior to the initial application and in the 2nd, 4th and 6th week of the exposure. These were done to detect possible hepatic damage from the percutaneous absorption of the applied material.

Results

A. Clinical Observations

No. 3: During the first few days after the applications with this material were initiated, the skin at the site of exposure became red and pruritic in two of the three subjects. This reaction persisted for the entire period of the application and was intensified when the concentration was increased in each instance. In the other two subjects, microscopic comedones developed after 4 weeks and 6 weeks of the exposure (see Histological Findings). In one subject, J.A., clinical follicular enlargement appeared during the third week of application. The redness, pruritus and follicular reaction subsided gradually after the application of this material was discontinued.

No. 4: The cutaneous reaction to this material was clinically characterized by redness and itching. This was severe in two subjects and became more exaggerated when the concentration was increased. No folliculitis or follicular enlargement was noted.

No. 5: Erythema and pruritus characterized the reaction to this material. It was mild in one and moderate in two subjects. There was no clinically noticeable follicular change in any of the subjects.

Halowax-1014: The application of the control material was characterized by redness. In one case the reaction was noticeable in the first week, and in the second subject it appeared at the end of the second week. Folliculitis and enlargement became noticeable within 4 weeks after the application. As the application was continued, the erythema and folliculitis became progressively more severe. Following the discontinuation of the application, the erythema of the skin gradually subsided, but the folliculitis persisted and the comedones which were induced remained for several months.

B. Histological Findings

No. 3: In all of the three subjects prominent follicular dilatation and plugging with keratinous material were noted. This follicular reaction occurred during the second week of application in one subject (J.A.), after 4 weeks of application in a second subject (J.P.), and after 6 weeks in the third subject (C.P.). In some sections of these subjects marked dilatation of the follicle was noted with postule formation within the follicle. The follicular wall in these sections was thin, and keratinous material extended into the sebaceous duct with partial destruction of the follicle and sebaceous

acini. Perifollicular cellular infiltration, mild to intense, composed of lymphocytes and histiocytes, was noted in most of the sections. Eosinophilia was prominent in one case. A perivascular cellular infiltrate was noted in all three subjects, and was prominent in specimens taken from frankly irritated sites. The histological findings in these subjects were quite similar to those seen after the positive control, Halowax-1014, was applied. (The Na T.C.P. from this batch probably contained 2,3,7,8 tetrachloro dibenzo-p-dioxin.)

No. 4: The application of this material to four subjects produced mild, perivascular and perifollicular cellular reactions. Some follicular plugging was seen after 3 weeks of application of the 7.0 percent concentration of the material in one of the four subjects. This was not observed in the later sections of the same subject. The follicular plugging and cellular reaction appeared to be much less prominent than that which was produced by No. 3.

No. 5: The tissue reaction to this material was characterized chiefly by capillary dilatation and a lymphocytic perivascular and perifollicular reaction which became more prominent during the last 2 weeks of the application in two subjects. Follicular plugging was negligible.

Halowax-1014: In both subjects follicular dilatation, hyperkeratinization and plugging occurred as early as the second week of the application. This reaction was prominent and striking, and increased progressively during the exposure period. In one subject (H.H.) the follicular plugging was pronounced within 4 weeks. At this time a severe perifollicular eosinophilic and lymphocytic reaction, as well as

microscopic pustules in the follicle, were also noted. In this subject destruction of the lower part of the follicle was observed and after 4 weeks the sebaceous acini had disappeared. In the second subject the same type of intense follicular hyperkeratinization, dilatation and periadnexal reaction was observed. The perifollicular and perivascular infiltrate in this subject was composed chiefly of lymphocytes and histiocytes however. After 6 weeks of application characteristic comedo were observed.

C. Liver Function Tests

There appeared to be no abnormal results in the prothrombin time and thymol turbidity tests.

Interpretations

In comparing the relative effects on the human pilosebaceous unit of the test materials and Halowax, it must be understood that it was not possible to use concentrations equivalent to Halowax-1014 because the test materials were all primary irritants in concentrations lower than that of Halowax. Nevertheless, it is evident from the pathological observations that material No. 3, which was prepared from the plant Sodium Trichlorophenate, produced follicular hyperkeratinization, dilatation, severe changes in the follicle and sebaceous acini, as well as a perifollicular reaction, all of which were seen in the Halowax-1014 treated skin. That these changes were not as severe as Halowax-1014 in all instances may be attributed to the fact that it was necessary to employ much lower concentrations of material No. 3. Although 20 percent Halowax was employed in the control subjects, it was not possible to apply concentrations greater

than 3.5 percent of material No. 3.

Since the follicular alterations produced by material No. 4 were mild by comparison to No. 3 (even though higher concentrations of No. 4 were used), this does demonstrate that its effect on the pilosebaceous system of human skin is considerably less intense. The pilosebaceous effects of material No. 5 may be considered to be even less than either No. 4 or No. 3, since follicular hyperkeratinization plugging and sebaceous gland changes were negligible.

From these experimental observations in human skin we may conclude that the plant Na T.C.P. in the concentration employed in the experiment is definitely acnegenic. By comparison, the sample to Na T.C.P. prepared by the distillation method (No. 4) and the dilution method (No. 5) are much less acnegenic. The fact that No. 4 and No. 5 did not induce significant pilosebaceous plugging and dilatation does not exclude them as potential acnegens if concentration plays an important role in their acne-inducing action. It does, however, demonstrate that in equivalent concentrations the potential acnegenicity of Nos. 4 and 5 is considerably less than material No. 3. Recently it was determined by Kimmig and Schulz[3,4] that the acnegenic material in this reaction product is 2,3,7,8 tetrachlorodibenzodioxin.

Under the conditions of the investigation, the application of the test materials, as well as the control, appeared to produce no systemic effects. Two types of liver function tests performed periodically during the exposure demonstrated no abnormal changes.

The response of the follicle and sebaceous epithelium to the material contained in Na T.C.P. is quite fascinating. What we are observing in the sebaceous gland is not in the realm of cell death,

degeneration or true atrophy. We may speculate as to what does occur locally. The epithelium of the outer root sheat of the distal part of the follicle, infundibulum and sebaceous duct proliferate and produce keratin which is retained in the duct and infundibulum. This stimulation also affects the undifferentiated cells lining the sebaceous acini which usually give rise to lipid forming cells. Undifferentiated epithelial cells are formed and the sebaceous acini are transformed into small masses of undifferentiated keratinizing epithelial cells which eventually become part of the temporarily thick wall of the comedo. With further keratinization these cells do not continue to proliferate actively and the final structure is a keratin filled funnel or cyst lined with thin flat epithelium sans sebaceous cells.

The disappearance of sebaceous elements has been noted with specific polycyclic aromatic hydrocarbons which are carcinogenic. There is no evidence, however, to demonstrate that the acnegenic halogenated compounds are carcinogenic.

In conclusion let me review the evolution of the microscopic changes when the human pilosebaceous unit is stimulated by specific acnegenic substances. This is the sequence:

1. Follicular pore dilatation and hyperkeratinization. This may extend to sebaceous duct.
2. Modulation of follicular epithelium and sebaceous duct cells to produce a hyperplastic ~~relatively undifferentiated by~~ keratinizing epithelium.
3. Progressive disappearance of lipid forming cells and lower part of follicle. In some instances the hair follicle continues to grow actively and in a few instances sebaceous acini remain attached to the plugged follicle.

4. Periadnexal and perivascular infiltrate with lymphocytes, some plasma cells. In two instances, marked cosinophilic infiltrate noted.
5. Hyperplastic lining of comedo thins out to a single cell lined sac.
6. This persists or becomes a small cyst attached to the epidermis.

This study demonstrated that one sample from a reaction vessel producing sodium trichlorophenate induced chloracne in volunteer subjects when the diluted material (2.5%) was applied daily for as long as 43 days. No systemic reactions were observed. Two other samples of sodium trichlorophenate prepared by other methods failed to produce acneform lesions.

REFERENCES

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