THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Examination

This is to certify that we the undersigned, as a committee of the Graduate School, have given Paul McCullough Gilmer final oral examination for the degree of Master of Arts.

We recommend that the degree of Master of Arts.

be conferred upon the candidate.

Wm A. Riley
Chairman

A T Gertmenian

Royal N. Chapman

Date_____

10-20 5M

THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Paul McCullough Gilmer for the degree of Master of Arts. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Arts.

Wm A. Riley
Chairman

Royal N. Chapman

A. T. Edwards

Date _____

10-20 5M

STUDIES ON CERTAIN POISONOUS LEPIDOPTEROUS LARVAE AND PUPAE.

A THESIS

SUBMITTED TO THE GRADUATE FACULTY

OF THE

UNIVERSITY OF MINNESOTA

BY

PAUL M. GILMER

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE

DEGREE OF

MASTER OF ARTS

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INTRODUCTORY:

The larvae of certain of the Lepidoptera have long been famous, either for their grotesque appearance or for their supposed poisonous properties. The whole group has been of interest to the ordinary observer from the fact that many of the larvae are characterized by various so-called protective structures, giving them a horrifying appearance. These consist of spines, horns, peculiar knobs and the like; often bright colored but usually of a harmless nature; which have called forth such local names as, 'Hickory Horn Devil, Stinger Worms, Horn Worms', and the like.

The harmless nature of these organs has led to the statement being loosely made that poisonous properties among larvae of the Lepidoptera are rare. The author feels sure that this is the belief among most amateurs. The poisonous properties of the Io caterpiller (Automeris io) is known to the veriest tyro, but here in general their knowlege of the subject ceases. There are, however, among the Bombycids and kindred forms a number of types more or less notorious among collectors. Such include the brown-tail (Euproctis chrysorrhea), the flannel moths (Megalopygidae), the saddle-back (Sibine stimulea), and others.

In all of these the poisonous property is due to hairs or spines, generally the former, many of which are known to be in connection with glandular cells which undoubtedly secrete an active poison. Such is certainly the case with the io and brown-tail. With many others the urticating properties are supposed to be due merely to the mechanical effect of the hairs themselves.

In the latter part of the nineteenth century, beginning about 1870, entomologists in general began to take considerable interest

in such forms. There are numerous notes, especially in English journals, dealing with cases of infection with poisonous hairs, generally of Euproctis (Liparis) chrysorrhea, or E. auriflua. Some little work was done upon these to determine the cause of the trouble, and it was generally conceded that the small hairs were the mischief makers. Very little actual work was done previous to the work of Goossens[1] upon Cnethocampa processionea, the pine processionary of Europe.

This caterpillar had borne a deservedly bad reputation since ancient times, Pliny[2] making mention of its stinging properties. Goossens came to the belief that the poison was a secretion of the tubercles found on the hinder segments, which he believed the caterpillars smeared over the hairs. Anderson[3] believed the same to be true of Euproctis chrysorrhea and E. auriflua, and with this explaination most workers seems to have been satisfied.

It was the age of the collector and the student of life history, of the taxonomist rather than the histologist. To know and to avoid nettling species contented the average investigator. But little interest was shown in the question until the last decade of the century when Fabre[4] undertook to determine the source of the poison in Cnethocampa processionea. Beille[5] in 1895 finally settled the question in the case of the processionary, demonstrating the poisoning apparatus to be glands connected with poison hairs.

No other work was done until E.E. Tyzzer[6] began his investigation into the poison and poisoning apparatus of the brown-tail (Euproctis chrysorrhea), the same species that was the cause of the initial work done in the seventies and eighties in England. His work was further investigated by Miss Cornelia Kephart[7] in 1914 and the complete apparatus demonstrated. The previous year

the related form, Porthesia similis, had been investigated by Eltringham[8] in England with a very similar result. Altho Eltringham's interpretation of his findings differs much from Miss Kephart's there can be little question but that the structures are essentially similar.

Since the work of Miss Kephart there has been very little done with these poisonous forms. Stray notes to the effect that this or that species is urticating have appeared, but little has been done in the line of either further histological or chemical research towards the determination of the actual mechanism and cause of the urtication.

THEORIES AS TO THE CAUSE OF THE URTICATION:

There have been essentially two conflicting theories proposed to account for the urtication caused by these larval hairs.

The first of these, held formerly by the majority of scientist investigators, and still upheld by many, places the urtication as the result of the mechanical irritation caused by the entrance of the hairs under the skin. There is no question but that very similar lesions, at least in external appearance, may be made by such inert agencies as glass wool. This theory attributes the somewhat greater violence of the reaction to the fact that practically all these hairs are barbed, thereby producing greater traumatism. Variation in susceptibility is explained by the fact that skins vary in thickness, and individuals vary in reaction towards inert substances. There is little question that in some cases this theory is correct.

The second theory, first advanced by Goossens, Anderson, and some of the early investigators, and subsequently supported by most of the investigations undertaken, places the responsibility for the

irritation upon unknown chemical substances, either within the cavity of the hair or smeared on the outside. There is here, of course, no necessity for explaining the actual urtication, once the general thesis is granted, while individual variations in the violence of the reaction is explained upon the basis of variation in susceptibility.

Here again there is no question of the accuracy of the theory in many cases. The io moth, with its stinging spines, the maia (Hemileuca maia), and since the work of Beille and Kephart, the pine processionary and brown-tail may at once be classed in this group. Whether the reaction of a large number of other types is to be included in this class is a question yet remaining to be solved.

URTICATING PROPERTIES OF THE WHITE MARKED TUSSOCK MOTH:

While collecting cocoons and egg masses of the white marked tussock moth (Hemerocampa leucostigma) in Minneapolis and St. Paul for the use of the department, Mr. Arthur Hertig of the University of Minnesota was attacked by a very painful and diffuse rash, covering practically the whole body. For some days the connection between the cocoons and the rash was unsuspected, until Dr. H.H. Knight of the department of Entomology, upon investigating found that the trouble came from the cocoons, or rather from the hairs of the larvae entangled in the silk.

Some further experiments were made by Miss F. Defiel of the department, as the question somewhat paralleled that upon which she was at that time working. Her work confirmed Dr. Knight's suspicion.

NATURE OF THE PROBLEM:

Due to the fact that larval material was unavailable, the question was, in the beginning, of necessity attacked by the author

rather from the chemical than from the morphological standpoint. The problem offered two separate lines of investigation; first as to the actual cause of the urtication, whether it was a mechanical effect or an actual poisoning by some chemical agent; second, if the latter were the case, what the nature of the poison might be.

A search of the literature brought but a meager result. Howard[9] in 1895, in the report of the Bureau of Entomology, while commenting on the outbreak of H. leucostigma in Washington, wrote the following concerning the urticating properties of the larval hairs:

> "The barbed hairs just mentioned may occasionally produce considerable irritation of the skin of people upon whom the caterpillars may have crawled or dropped from trees. ******it is the shorter hairs from the sides which probably cause the irritation. They are very small and fall out readily, and when the caterpillar crawls over the skin of an individual who is warm and perspiring, the very sharp barbed hairs produce an irritation which in some individuals has been the cause of much discomfort, creating more or less irritation and swelling."

Göldi[10], in 1913, in his small booklet on pathological forms of insects, has referred to the tussock moth as primarily the poisonous moth of the United States. It is quite evident from his figures that his source of information must have been Howard's article above. Riley and Johannsen[11], in their Medical Entomology, in 1915 mention the above reference to Göldi as being 'a curious misunderstanding' as the hairs furnish only a local irritation.

These references seem to close the case against H. leucostigma, so far as literature on its urticating properties is concerned. Tyzzer seems to have used it as a 'check' against E. chrysorrhea in his experiments, and mentions that no irritation other than that

found on introducing any foreign substance of like nature under the skin, was noted. In the whole of the references there is nothing to show that the irritation is remarkable, or referable to aught but local reaction against a foreign material.

EXPERIMENTAL EVIDENCE:

The problem was first attacked from the standpoint of the actual cause of the urtication. Due to the complete absence of larval material all work was, of necessity, done with the hairs found in the old cocoons, collected the previous fall (1921) from trees in St. Paul and Minneapolis. An endeavor to separate the hairs from the cocoons speedilly showed itself to be impracticable. However, upon teasing the silk apart and examining the hairs microscopically, there were found to be three types recognizable; long plume-like black hairs with 'tufted' tips, coming from the front and caudal tufts of the larva; long, clear or whitish hairs, coarsely barbed, coming largely from the sides of the caterpillar; and lastly, shorter barbed hairs, resembling the last in general appearance, but with finer barbing, and about one third as long, coming largely from the white dorsal 'tufts' or tussocks.

POINT OF ENTRANCE:

A cocoon was rubbed vigorously on the skin of the forearm, which was immediately examined under a binocular dissecting microscope. The hairs were found abundantly scattered over the skin, but with very few penetrating. These last were without exception the shorter barbed hairs from the tufts, and the point of entrance was invariably the hair follicle. As the stinging began to manifest itself these penetrating hairs were gently moved with a dissecting needle. They were very firmly fixed, and the slight movement caused by the needle was at once manifested in a sharp increase in the

prickling sensation in the neighborhood of the hair. Any attempt to remove the hairs by means of forceps was invariably followed by a breaking off of the brittle hair, leaving a portion in the skin. In no case were hairs of the first type noted as penetrating the skin. Due to the resemblance of the last two types, it is not certain that some of the second may not have entered. However, no such long hairs were noted, and the broken parts of such as were removed by forceps proved to be the third type only. It is reasonably certain, therefore, that the irritation is primarily due to this last type.

Attempts were made to force the hairs thru the skin other than in the hair follicles without success. The hairs always broke before penetrating. Experiments made on the palms of the hands failed to produce infection, as did attempts to penetrate the backs of the last joints of the fingers, where no hair is present. Care was taken to prevent infection in the tender parts under the nail and nail root.

These experiments seem to show definately that the source of the urtication is the small hairs of the type found in the dorsal tussocks, and the point of entrance is the hair follicle.

NATURE OF THE URTICATION:

Upon vigorously rubbing in the hairs there is a latent period, varying from only a few seconds to, in some cases*, over twenty-four hours, before any reaction is noticeable. In the case of the author with untreated cocoons, this averaged close to thirty seconds. No irritation whatever was felt at the time of inoculation. The reaction began as a slight stinging, which rapidly increased to a sharp pain resembling a pin prick, or rather a series of them, as

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* In one case a latent period of over ten days was noted.

each hair sets up its own local irritation. In about a minute and a half a slight redness is noticeable, which rapidly spreads to somewhat beyond the area of the inoculation. Often a few moments before this redness appears there is a dry, crinkled appearance of the skin, resembling a slight sear from hot metal just before the blister appears. This appearance is not always noticeable as it is not very strongly marked.

At about two minutes the skin begins to show small papillae, rising above the general level, each having as its center an infected hair follicle. These rapidly increase, forming an indurated and granular appearing wheal at about the third minute. This wheal rapidly becomes white in color, with the reddened surrounding area showing in sharp contrast. Usually the white stage is reached within the fourth minute. This stage lasts a varying length of time, the swelling increasing, and the wheal becoming rounded and losing its granular appearance. The maximum is reached in about ten minutes in case there is no rubbing of the injured part; much quicker if it is rubbed. The wheal is now large, again red, rising smoothly from the surface, and is itself smooth. It is usually surrounded by small elevated papules, where isolated hairs have entered at a distance too great from the central wheal to be included. From these it is fairly evident that the central wheal is formed by the combined irritation of the whole infected mass; that is, it is a combination of a large number of such small papules. This of course explains the granulated appearance of the early stages.

The length of time this large wheal remains is largely a matter of degree of infection and individual susceptibility. In the author's case three hours usually saw the wheal going down and by twenty-four it was completely gone, the trace of the infection

being merely a large number of tiny pimple-like elevations.

At any time, from the time the wheal begins to go down until it has completely disappeared, and often as late even as two to three days after the original infection, the swelling may be renewed in almost as great a degree as originally by vigorously rubbing or 'scratching the affected part.

The author has noted in a number of instances that a subsequent infection near the source of an old one will cause the reappearance of the small pimple-like prominences that characterize the day old infections.

There have been a number of times that infections were much more persistant than usual, lasting on the forearm from two to three days. These infections were always accompanied by soreness to pressure, resembling a bruise, and were marked by more than ordinary swelling. No reason for this can be given other than that the particular cocoon was more virulent, as the proceedure was always the same.

There is some evidence that continued irritation of the infected spot will cause a particularly persistant and painful injury. In the case of one person, the rubbing of woolen underwear on the infected spot produced invariably a more than usual persistance. In the author's experience, infections of the arms were not especially troublesome, as the sleeveless underwear worn brought the infected spot in contact with the smooth cotton fabric of the shirt sleeve. However, an accidental infection on the point of the hip bone, where it was constantly chafed by the clothing and the belt, produced a very painful reaction, lasting over a week, and becoming so tender and sore as to interfere with sleep at night. In this instance the original swelling went down, leaving angry red papules,

the area of inflammation spread somewhat and the edges of the reddened area seemed particularly sensitive. The pain was of a sharp lancinating character, noticeable especially at night.

It might be noted here that this same character of pain was complained of by especially sensitive subjects.

The auther has found no remedy that will relieve the urtication.. Dr. Knight has reported verbally to the author that ordinary purslane (Portulaca oleracea) when rubbed at once on the infected spot will give considerable relief. This statement has not been verified because of inability to get purslane. However other fleshy leaved plants do not afford this relief.

SOLUBILITY EXPERIMENTS:

In attacking the problem of the poisonous nature of the hairs, the question of the solubility of any poisonous material, if present, offered the most obvious course.

The literature of work on similar hairs offered little that seemed favorable. Fabre and Goossens[12] had claimed the solution of a vesicating substance by extraction of the cast larval skins of C. processionea in ether and alcohol. Tyzzer also reported that the brown-tail hairs lost their urticating properties when extracted with water at 60° C.

Experiments were made with cocoons immersed and soaked for two weeks in the following media:

Ethyl alcohol	70%
Hydrochloric acid	2% --0.2%
Sodium chloride	5%
Acetone	
Chloroform	
Magnesium sulfate	5%
Potassium hydroxide	5% --0.2%
Benzene (C_6H_6)	pure
Water	room temperature -- 60°C (48 hrs)

The nature of the examination was two-fold: first to determine if the extract contained any urticating or vesicating agent; second to see if the hairs retained their urticating properties. The results follow:

EXPERIMENT I.

Solubility in 70% Ethyl alcohol.

Five cocoons were immersed and soaked for two weeks in about 30 c.c. of 70% ethyl alcohol. At the end of this time they were filtered off thru ordinary filter paper, washed four times in 70% alcohol, the filtrate being saved and the washings discarded. This gave a filtrate presumably containing any dissolved poison, and the hairs left on the paper free from any of the filtrate. Half of the filtrate was evaporated to a thick syrupy constancy, the other half used as obtained. Evaporation was done over a steam radiator, and took about three hours.

Test 1.

A bit of filter paper moistened in the plain filtrate was bound on the finger with sheet rubber, and covered with ordinary bandage. It was left over night. Result: negative for either urtication or vesication.

Test 2.

A bit of filter paper moistened in the evaporated filtrate was similarly bound on the finger and left over night. Result: negative for both urtication and vesication.

Test 3.

The hairs left on the filter paper from the original filtration were dried over the radiator until they were again completely dry. They were rubbed into the arm. Result: positive, with an apparent increase in virulence.

Test 4.

Glass wool was soaked in both the above filtrates, cut

into fine bits and rubbed vigorously into the skin. There was a reaction resembling at first that of the urtication, but unaccompanied by the typical sting and itch. The wheal formed went down in about half an hour, and except for a soreness to the touch, very distinct from anything found with the hairs, there was no further reaction. A check with plain glass wool gave exactly similar results.

Test 5. The extract evaporated from the filtrate was evaporated to dryness with glass wool in it. The wool was then cut and rubbed in. Reaction was exactly similar to Test 4.

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It is very evident from this set of experiments that ethyl alcohol is not a solvent for whatever chemical it might be that causes the urtication. The results were exactly parallel to those generally obtained with other urticating species. There is nothing in this set of experiments, other than a possible increased virulence of the dried hairs to warrant any theory other than the mechanical one.

EXPERIMENT II.

Solubility in HCl (2% - 0.2%).

The proceedure was the same as in Experiment I. Five cocoons were soaked for two weeks in each of the above percentages of hydrochloric acid. The hairs were filtered off, the filtrate saved and the hairs washed with distilled water until they were neutral to litmus on contact. The filtrate was divided and half evaporated as before. The hairs were dried over the radiator for four hours.

This same proceedure was used thruout except in the case of the KOH solutions. The same five tests were tried as in Experiment I. The results were as follows:

Test 1. The results were negative.(Plain filtrate)

Test 2. The results were negative.(Evaporated filtrate.)

Test 3. The result was positive. The hairs had a much increased virulence, causing a swelling as large as a small hen's egg, which remained sore to pressure for over forty-eight hours. There was in this case an itching sensation, causing a desire to rub the spot for a number of days. The reaction with HCl was the most intense experienced. (Dried hairs)

Test 4. Result similar to Experiment 1. Inference negative.

Test 5. Result negative, similar to Test 4.

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From this set of experiments there is some slight evidence of the presence of a true poison, whose virulence is increased by digestion in HCl. Since all free HCl was washed out, its action is precluded. A check experiment with glass wool soaked in the acid of the same concentrations gave nothing but the typical glass wool reaction. It may be noted that the difference between the acid treated and plain hairs is quite marked, and is characteristic. The poison is insoluble in HCl of the above concentrations. No difference in the reaction due to concentration was noted.

EXPERIMENT III.

Solubility in NaCl.

In this experiment the same proceedure was used, and the same five tests were made. The results were typically as above, except that there was no especial increase in virulance noticed in the dried hairs. There was no indication of solution of the poisonous material.

EXPERIMENT IV.

Solubility in Acetone.

Proceedure was as usual. Results were exactly comparable to Experiment III. There was no evidance of solution or alteration.

EXPERIMENT V.

Solution in Water.

The proceedure was varied, in that the extraction was for forty-eight hours at 60°C. for the second experiment. The first solution was prepared as usual. The results are taken up separately for reasons that will be given in the discussion.

By Usual Method: Tests 1 and 2 with this solution gave negative results; Tests 4 and 5, where the filtrate was rubbed in with glass wool gave a slight irritation, causing soreness and redness for two days. A number of small pustules containing typical yellow pus of streptococcus infection came up. This preparation produced results about as expected, as the odor showed putrifaction of the egg masses to be well under way. The fine glass wool very evidently caused here a number of tiny foci of infection with pyemic bacteria. Since the author is particularly resistant to such infections the application of argerol for one night checked the trouble. The author in no way attributes the inflammation to the solution of the poison from the hairs, as it was in every respect typically pyemic.

The dried hairs produced the usual reaction, in the author's opinion slightly less than usual, tho this is open to question.

Extraction at 60°C.: The results obtained here were negative for solution or alteration. Tyzzer obtained with the brown-tail a loss of toxic power in such extractions. So far as could be told there was no alteration in this respect in hairs so extracted in the case of H. leucostigma.

It is apparent from these results that there is little or no alteration or solution in water. The slight loss in toxicity beleived to have been noted in the long extraction, the author beleives to be due to the destruction of the poison by the bacteria of decay. Even here however, the loss is not so pronounced as to warrant a flat statement that it actually occurs.

EXPERIMENT VI.

Solubility in Chloroform.

Proceedure was as usual. Results were negative for solution. The two solutions when used produced a slight burning sensation in Tests 1 and 2, (bound on under rubber) with a slight redness the next day but no pain. Checks with chloroform produced identical results, which are evidently due to the chemical. There was no evidence of alteration in the hairs which reacted as tho untreated.

EXPERIMENT VII.

Solubility in $MgSO_4$.

Proceedure was as usual. Results were exactly similar to those with NaCl. There was no evidence of solution or alteration. Both these protein solvents failed to produce any solution.

EXPERIMENT VIII.

Solubility in Ethyl Ether.

Proceedure was as usual. The results were similar to those with ethyl alcohol; no solution, but a slight increase in virulence in the dried hairs. This is in no way comparable with that obtained with HCl, and is of such a slight degree as to leave a slight question as to its actual existance.

EXPERIMENT IX.

Solution in Benzene.

The proceedure was as usual. Results were completely negative so far as solution or alteration was concerned. The dried hairs were typical in reaction.

EXPERIMENT X.

Solution in KOH 5% - 0.2%.

Two solutions were used here, and the method of proceedure was as usual in each case.

Tests 1 and 2 were omitted because of the caustic action of the reagent itself. All others were performed. The results were, in the case of the higher concentration, invariably NEGATIVE, even in the case of the dried hairs. These hairs were examined under a microscope, and showed the contents to have been completely dissolved, leaving the hair clear but apparently unchanged. Examination showed that they entered as usual thru the hair follicle.

The lower concentration was used to obviate the possibility that the prolonged soaking in the caustic had altered the structure of the chitin so as to destroy its stiffness.

Upon trying the lower concentration hairs on a very sensitive subject reaction was obtained 24 hours after inoculation, with no signs of injury previously. The reaction was much milder than usual, consisting of a slight swelling and itching, but with none of the 'half raw' appearance that usually characterized infections on this subject. A similar reaction in other cases, as in treatment with heat will be noted under that head.

When allowed to stand for a week, the toxic properties of the low concentration material had returned almost to the full.

It is very apparent that some profound alteration in the toxic substance had taken place. The experiments with glass wool gave negative results showing that it was not solution but actual decomposition of some sort that had taken place.

To the author's mind these and the heat experiments that follow point conclusively to a definate chemical agent producing the

lesions following inoculations with the hairs. This completed the solubility experiments. The material is very insoluble, not responding to any ordinary solvent.

DIGESTION EXPERIMENTS:

From the work of McCaffrey[13] on the range caterpillar, Hemileuca olivia, where he calls attention to the increased susceptibility of workers from year to year, it was suspected that possibly the poisonous material might be some type of toxic protein, and the increase in susceptibility be the result of an anaphylactic reaction. It was therefore resolved to investigate the reaction of the hairs towards various protein digestants, in order to determine if there might be either solution or alteration.

The following general experiments were performed:

EXPERIMENT I:
Digestion in Pepsin-HCl.

Artificial gastric juice was prepared, using commercial powdered pepsin in quantity sufficient to cover the point of a small knife, and fifty c.c. of 0.2% HCl. Cocoons were immersed in this liquid and left for two weeks. The hairs were then filtered off and the filtrate saved. The hairs remaining on the filter paper were thoroly washed until neither the washings nor the hairs themselves gave any reaction to litmus. The hairs were dried over the radiator.

Test 1. The filtrate was evaporated to dryness over the radiator with glass wool. The wool was cut fine and rubbed in. The reaction was negative so far as urtication was concerned.

Test 2. The dried hairs were rubbed into the arm. Severe reaction took place. A check with hairs soaked in HCl gave an equal reaction. Evidently the increase was that noted where HCl was used.

Check:
Glass wool when placed in Pepsin-HCl and evaporated to dryness gave a negative reaction.

EXPERIMENT II.
Digestion in Artificial Pancreatic Juice.

Artificial pancreatic juice was prepared with commercial dry pancreatin sufficient to cover the point of a small knife, and 50 c.c. of 0.2% $NaHCO_3$. Cocoons were placed in 50 c.c. of the solution and permitted to digest for two weeks. The hairs were filtered off, and the filtrate treated as in Experiment I. The hairs were washed carefully with distilled water until neutral to litmus and were then dried as usual over the radiator.

Test 1.
The evaporated filtrate was rubbed into the arm by means of the usual glass wool. There was no urticating reaction.

Test 2.
The hairs were rubbed into the arm. Reaction was normal or nearly so. There seemed to be a slight decrease in reaction, tho this was so small as to be uncertain. If present it is believed it is due to the alkaline medium, which from other experiments seems to possess the property of decreasing the virulence.

EXPERIMENT III.
Digestion in 2% HCl by Boiling.

Cocoons were immersed in about 300 c.c. of 2% HCl and boiled vigorously in an Erlenmeyer flask for five hours. The concentration of the acid was held about constant by adding every fifteen minutes a quantity of distilled water equal to that lost. At no time did the concentration more than double due to loss of water.

After five hours of boiling the mixture was allowed to settle and decanted thru a filter. The filtrate was saved for tests of solution, and the sediment thrown on the filter. This was washed

with distilled water, and finally with 0.2% KOH and then again with distilled water until a neutral point was reached. The alkali was used merely to neutralize the acid which washed out very slowly. Not enough was used to completely neutralize, as the final washings showed at first an acid reaction to litmus.

Test 1. The filtrate was evaporated with glass wool and tested in the usual manner with negative results.

Test 2. The hairs on the filter were dried as usual. Very complete digestion had taken place, and the silk was completely gone, the remains being hairs, pupal shells, and a brownish 'sludge' composed largely of debris from the pupal cases.

The mixture was rubbed vigorously into the arm. The results were negative. After standing a week they were again tried with similar results.

Both times examination was made under the dissecting microscope and the hairs were found imbedded in the normal manner in the hair follicles.

Microscopic examination of the filtered off material showed the hairs normal in barbing, but very clear and 'glassy' in appearance, with absolutely nothing in the hair cavity. To all appearances the chitin was unattacked.

There had evidently been a complete solution of all organic material except the chitin itself, and with this solution had gone the toxic agent. This in turn had been destroyed by the action of the acid. This was the only evidence obtained tending to show a protein nature to the irritant, unless possibly a slight anaphylactic reaction could be so classed.

HEMOLYSIS EXPERIMENTS:

Hairs were teased out under the disecting microscope on a slide. This was moistened with a drop of blood from a fresh prick in the finger. This was covered with a cover slip and placed at once under the high power of the microscope. There was no sign of lysis of the corpuscles, nor was the crenation noted by Tyzzer in the brown-tail evident. It is very evident that the poison has no such hemolytic power as that of Euproctis chrysorrhea.

OXIDATION EXPERIMENTS.

Attempts to oxidize the toxic agent were made. Three agents were used; $KMNO_4$, $K_2Cr_2O_7$, and H_2O_2. All agents were used in a very dilute condition, no attempt being made to ascertain the strengths of the first two, the last being ordinary commercial peroxide of hydrogen of the brand called 'Dioxygen.' The results were as follows:

EXPERIMENT I.

Strong $KMnO_4$.

A strong solution of $KMnO_4$ was made up, strong enough to showa purple color by reflected and a black by transmitted light. Cocoons were boiled with this. A heavy precipitate of MnO_2 was thrown down which was filtered with difficulty. When dried no results were obtained with the hairs. Examination under the microscope showed there were none in the follicles, and finally under the compound microscope showed that the action of the chemical had been so great as to very profoundly alter the structure of the hairs. These were black, brittle, and the characteristic barbs were lacking.

EXPERIMENT II.

Weak $KMnO_4$.

A very weak solution of $KMnO_4$ was made up, just sufficiently strong to give a light wine red with transmitted light. Cocoons were soaked for one week in this solution. The solution became a

clear brownish towards the last, showing a fair precipitate of MnO_2. The hairs were filtered off, washed and dried. No reaction was obtained from these hairs. Examination microscopically showed, however, typical penetration, and the hairs themselves were typical in form, stained slightly brownish, and contained no material in the hair cavity. They were apparently normal in elasticity, requiring a considerable distortion to break. These hairs were tested again after standing a week, with the same results.

A second test made with a still weaker solution gave in the author's case a similar result. With a sensitive subject, however, small papules appeared after a few hours, showing an incomplete loss of the toxic substance.

EXPERIMENT III.

Weak $K_2Cr_2O_7$.

A weak solution of $K_2Cr_2O_7$ was made by dissolving a very small crystal in distilled water, giving a weak yellow solution. To this was added three drops of concentrated H_2SO_4. To 75 c.c. of this solution was added five cocoons. These were allowed to stand for eight hours, when a small bit of cocoon was used for a test. The result was positive with the dried bit, and very little change had taken place. Sixty hours after immersion a second trial was made. This gave a positive reaction but much less pronounced than at first. A microscopic examination of the structure showed the hairs essentially unaltered, but solution of the contents to be taking place. After eighty hours the results were negative for the author but with a sensitive subject a reaction typical in type but much milder than usual resulted about eight hours after infection. After standing twelve days in this solution the results were completely negative.

Examination of the hairs showed them unaltered in structure but

completely empty of contents. They seemed to penetrate in a normal manner. In the sensitive subject these hairs gave a very slight tingling only, with very little redness but no swelling or papule formation. It is believed that this reaction was a local one due to mechanical irritation only as it was very short in duration, (½ hr.) and did not resemble a typical infection even in the early stages.

EXPERIMENT IV.

H_2O_2.

A commercial solution of 'Dioxygen' was used. There is no way of knowing the exact concentration, but it is probably about 4%. This experiment was a complete failure. The egg masses, silk, and old pupal remnants in the cocoons caused such a rapid loss of the oxidizing power of the peroxide that the contents of the hairs themselves were unaffected. It is doubtful if the hair cavities were even penetrated before the chemical power of the liquid was lost.

.....

The results of these oxidation tests were significant, especially when compared with the action of KOH and prolonged boiling with HCl. In all cases where the hairs were unaltered in essential structure, negative reaction was always accompanied by solution of the contents of the hair. The tests with bichromate showed this loss to be a progressive one, and accompanied by a gradual reduction of the urticating power, even going so far as to lose the ability to produce typical urtication for some hours.

This last may be due to solution of the material contained in the chitin itself, the time being necessary for further diffusion from the remnant remaining in the hair cavity.

The complete loss of urticating ability with the final solution of the hair contents affords practically incontrovertable evidence that the urtication is not due to mechanical injury but primarily to

some poisonous substance contained in the hair cavity.

HEAT EXPERIMENTS.

Still keeping in mind the idea that the urticating material might be of a protein nature, it was resolved to try the effect of heat upon the urticating properties of the hairs. It was applied in two ways; dry heat and moist. The latter was tried both with superheated steam, and by boiling in water in an open vessel.

EXPERIMENT I.

Dry Heat:

Test 1. Six cocoons were placed in a glass vessel and subjected to dry heat in an oven at 100°C. for fifteen minutes. The cocoons were removed and cooled. Upon testing the result was positive for urtication. The reaction time was shorter (ten seconds until the initial prickling was felt) and the reaction itself somewhat more severe. The course of the reaction was normal but accelerated.

Test 2. Six cocoons were placed in a glass dish and heated for forty-five minutes at 120°C. Upon cooling the raction appeared about normal, possibly a little less than normal, and the reaction time was about forty-five seconds to the initial prickling. It may be noted here that Tyzzer found for the brown-tail, that baking at 115°C. for one hour completely destroyed the toxic agent. The only effect of a heat five degrees greater for forty-five minutes was to slightly delay the time of reaction. It is probable that there was no change whatsoever in the material, except possibly such as took place at the heating to 100°C. (a possible initial break down of the toxo-protein complex) but that the slight delay was due to complete dessication of the contents of the hair, and the need for softening this material by the body fluids.

Test 3. Heating at 140°C. for one half hour gave results in every way comparable to those of the 120°C. test. The reaction time was the same, (45 seconds) and the results similar in every respect.

Test 4. Seven cocoons were heated for one hour at a temperature varying from 175°C. to 200°C., averaging most of the time 185°C. This heat was sufficient to produce browning and a slight crinkling of the silk of the cocoons, and was estimated to be close to that which would produce structural change in the chitinous hairs.

The cocoons were cooled and tried on the author. The result was negative, tho a slight prickling at the time of the inoculation showed that the hairs were penetrating. Microscopic examination showed typical entrance at the hair follicles.

A cocoon of this material was inoculated into a sensitive subject within ten minutes of leaving the oven. It gave at first no response, but twenty-four hours later a typical redness and small pimples appeared. This same material twenty-four hours later (48 after leaving the oven) gave a normal reaction with this subject.

A second set of cocoons, heated and then steamed in an autoclave at 100°C. (no pressure) for two minutes, when dried over the steam radiator to get rid of the surplus moisture gave a typical reaction.

Apparently here the delay was due to a complete dehydration, the ability of the hairs to react returning after rehydration had taken place. However as a check, a portion of the heated material was allowed to stand in a dessicator (H_2SO_4 type) for forty-eight hours. Much to our surprise the reaction had returned almost to normal, thus showing it was not of necessity due to dehydration but possibly to some type of reversible reaction in the material itself. Further experimentation with moist heat served to confirm this suspicion.

EXPERIMENT II.

Moist Heat at 100°C., by Boiling.

Cocoons were boiled for five minutes, first, and later for fifteen minutes in water in an ordinary Erlenmeyer flask of 500 c.c. capacity. The temperature reached in free boiling was a fraction of a degree above 100°C. The results were as follows:

Test 1.

Boiling for Five Minutes.

Six cocoons were boiled in water for five minutes. They were then filtered off and dried as usual over the steam radiator.

When rubbed into the arm the hairs produced the usual reaction, but at a much slower rate (initial prickling in one minute) and to a much less degree. They amount of swelling was barely sufficient to produce a typical consolidated wheal.

Test 2.

Six cocoons were boiled in water for fifteen minutes, filtered, dried as usual and rubbed into the arm. The result was practically negative, only a very slight and transitory redness was produced without the usual prickling. It is believed the redness was due to friction rather than to the action of the cocoon hairs.

This material was permitted to stand a week and used again. Much to the experimenter's surprise the virulence had returned in full force, a typical reaction was experienced in the usual time.

EXPERIMENT III.

Pressure Steam Heat.

Cocoons were placed in a glass container and submitted for varying lengths of time to steam heat under pressure in an ordinary steam autoclave. Pressures varied from nothing to ten pounds.

Test 1.

Steam Heat for Fifteen Minutes at 0 lbs. Pressure.

Six cocoons were heated in a steam autoclave for fifteen minutes

at ordinary pressure, the door of the autoclave being partially open, and the gauge registering 0 thruout. The cocoons were removed, cooled and used damp. A positive reaction was obtained, but much less in degree than ordinary, and very slow in developing, the first stinging appearing at the end of the second minute. The infection did not pass the preliminary red stage, no pustules appeared, and the reaction was over in half an hour.

It was thot possible that the fact that the cocoons were damp might have softened the hairs so that they did not penetrate as readily as formerly. Examination showed, however, that a very considerable number were in the hair follicles.

In all subsequent experiments the material was dried by heat at 110°C., in a drying oven for one minute to remove excess moisture.

Test 2. Heat for Fifteen Minutes at 5 lbs. Pressure.

Six cocoons were steamed in the autoclave for fifteen minutes at five pound pressure. They were removed, the excess water evaporated in the dry oven, and the dried cocoons were rubbed on the arm.

The reaction here was practically the same as for heat at no pressure; the reaction was about the same time in appearing (2½ minutes) and the nature of the reaction identical.

Test 3. Heat for Fifteen Minutes at 10 lbs. Pressure.

Six cocoons were heated in the autoclave at 10 lbs. pressure for fifteen minutes, dried in the oven for one minute, and rubbed into the arm. The results were as follows:

Three minutes after inoculation there was no sign of any reaction. The site of the inoculation was rubbed vigorously for two minutes. Redness developed, probably due to friction, and a slight prickling was noted. There was no papule formation, and it is doubt-

ful whether the prickling was not the result of friction. No further developments were noted.

Twenty-four hours later no reaction was noticed on inoculation, but at the en of forty-eight hours a slight reaction was given in the author's case, proceeding very slowly to the wheal stage. Test was made was made with a sensitive subject, with typical results.

It will be noticed that in all heat reactions the poisonous principle was altered gradually by the increase in temperature, but that complete destruction was not obtained. Upon standing under atmospheric conditions the nettling properties returned almost fully.

Moist heat was more effective than dry, and steam seemed more effective than hot water at the same temperature, since the result of equal exposure in time to free steam and boiling water give a difference. Steam heat at ten pounds pressure gave results very comparable with dry heat at 185°C., and in much less time. The actual heat applied at this steam pressure was in the neighborhood of 104°-105°C. The autoclave had no means of taking any accurate record.

It is probable that the steam was forced into the hair cavity forcing out the contained air by pressure, and thus subjecting all parts to the action of the heat. Apparently the presence of moisture greatly accelerated the alteration of the toxic substance. However, it should again be noted that exposure to atmospheric conditions for a day or two restored almost to the full the toxic effect.

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It is rather hard to get at just what has actually occurred. Since the actual composition of the toxic agent is in question, there is no way of attacking the problem from the purely chemical standpoint. However the author believes the evidence tends to support a theory of a protein (protoplasmic) molecule with a toxic group adsorbed upon it.

The resistance to dry heat and the relative susceptibility to moist is typical of many protein substances, which coagulate readily under steam pressure and resist many degrees higher dry heat.

The origin of the poisonous material, that is the fact that it is the product of living cells, and is apparently so closely linked with the protoplasm of the hair cavity as to be impossible of removal without the solution of that protoplasm, as was shown by attempts to dissolve it from the hairs; the fact that its destruction is coincident with the destruction of the hair protoplasm, all points to a very close linkage between the two.

It should again be noted that virulence may be increased by the action of acids, decreased by alkalis of great dilution to the point of temporary inhibition, and subjection to heat also alters it temporarily, but does not destroy the toxic agent.

Altho the evidence warrants no definate statement, the author believes the following hypothesis at least explains the facts observed, and that there is nothing inherent in the evidence against it.

....

It is evident that the toxic agent is closely associated with the protoplasmic contents of the hair. Histological evidence discussed later shows that it is apparently secreted by a special gland cell. Until we are able to isolate the poison in a more or less pure form we can only speculate as to its nature and as to the mechanism of its action physiologically.

As a basis for discussion the author presents the following hypothesis as a possible explaination of the facts.

The toxic agent is linked in either a physical or chemical bond with the protein molecule of the protoplasm. When introduced into the body the poison molecule is absorbed by some tissue element

which has a greater'affinity' (chemical or physical) for it than has the hair protoplasm. The absorption produces the typical swelling, irritation, etc., of the infection. The reaction is probably brought to a close locally by the walling off of the infected spot by leucocytes and the slow absorption by them of the poison agent. Until this final removal is complete, rubbing will break down the leucocyte dam, causing a return of the first symptoms. The time that may elapse in which return of reaction will take place varies with the degree of infection, and the individual idiosyncracies of the person.

When the combined protein and toxic molecules are subjected to the action of heat of the proper temperature, or to that of dilute alkali, two reactions take place. First, the protein molecule is acted upon in the usual manner and altered. As a result the bond between the two is severed and the toxic molecule is freed. It is in turn acted upon by heat or chemical undergoing a slight change chemicslly that destroys or at least reduces to practical impotence, its toxic qualities. This reaction, however, is probably a mere rearrangement of the molecule without loss of any element, unless possibly a dehydration takes place. Upon standing this action is slowly reversed, and the original molecule reconstituted as it was. That a dehydration may take place is shown by the fact that subjection to moisture in the case of the heated material hastened the return reaction, but that it is not merely a physical reaction dependent upon the presence of free water is shown by the fact this this same heated and dry material nevertheless recovered its power even in a dessicator over sulfuric acid. The return to virulence is apparently not complete, there being sufficient loss to be noted, tho not very great in amount. This is due to two possibilities; the complete destruction of a portion of the toxic molecules, or possibly to the fact that in the case

of alteration as above postulated there arrives a stage of 'equilibrium' between altered and unaltered molecules, where no further return is possible. The author is rather inclined to this belief, tho the evidence might as easily be interpreted in the other manner.

As additional support to the above postulate, is the fact that dry heat at 100°C. for fifteen minutes in place of decreasing the reaction actually increased the speed of it. This was probably due to the fact that the first action had already set in, that is, the bond between the protein and toxic molecules was severed, and the alteration of the protein begun. The toxic molecule, however, was as yet unchanged and was present in the free state ready for immediate union with the appropriate tissue element.

A somewhat similar reaction probably takes place with the presence of free acid in the mixture. Again a breaking of the bond takes place, freeing the toxic molecule. The acid slowly reacts with the protein, so altering it as to make subsequent readsorption of the toxic molecule impossible, and the increase becomes permanent. It would seem probable, since the normal blood reaction is slightly alkaline and some solution must take place in the blood, that the toxic molecule is insoluble in acids, hence cannot be obtained from them. Since the attempt to neutralize this acid material and then extract with isotonic carbonate solution was not made, this is of course speculation.

It must be freely admitted that this hypothesis is based upon exceedingly slender evidence, and it is advanced merely as a working basis for the explanation of phenomena which seem unexplainable except upon some such working scheme.

CHART of REACTIONS. Type of Reaction.	No effect	Increase	Decrease	Note
Solubility				
Ethyl Alcohol	+	-	-	
Ethyl Ether	+	-	-	
Benzene	+	-	-	
Chloroform	+	-	-	
Acetone	+	-	-	
HCl	+	-	-	
KOH	+	-	-	
H_2O	+	-	-	
H_2O (60°C.)	+	-	-	
NaCl	+	-	-	
$MgSO_4$	+	-	-	
Chemical Action				
Acid (HCl 0.2% - 2%)	-	+	-	
Alkali (KOH 0.2%)	-	-	+	Temporary loss.
(KOH 2%)	-	-	+	Complete loss.
Oxidation				
$KMnO_4$	-	-	+	Complete loss
$K_2CR_2O_7$	-	-	+	Progressively lost to completion.
H_2O_2	+	-	-	Failure.
Heat				
Moist				
100°C. by boiling	-	-	+	Partial loss
100 C. steam	-	-	+	Slight loss
15 lbs. steam	-	-	+	Temporary loss
10 lbs. steam	-	-	+	Temporary loss
Dry				
100°C.	-	+	-	Shorten of time.
120°C.	-	-	+	Delay in time.
140°C.	-	-	+	Delay in time
185°C.	-	-	+	Temporary loss.

HISTOLOGICAL EVIDENCE.

With the completion of the chemical work it became necessary to support and supplement it with as complete an histological examination as possible. For this purpose endeavor was made to rear from egg masses sufficient larvae for a complete series. At the same time advantage was taken of the rearing to find if possible whether there was any difference in the poisoning in the various instars.

Larvae were hatched from eggs in the University insectary, and fed upon lettuce. For some reason, possibly due to the unnatural food used, but more likely to too great humidity, it was found impossible to grow them beyond the fourth instar.

Examination of the larvae had shown, however, that from the moment of hatching they were poisonous, becoming increasingly so with the development of the typical 'tussocks' in the third and fourth instars.

It was also found that the poisonous type of hairs were scattered over the body in company with the other hairs on the lateral and dorsal tubercles, particularly those of the first and last body segments. They were easily distinguished be being shorter and occuring usually in tufts of from six to a dozen hairs.

Examination was made of the various instars histologically, especially of the hairs and their appendages. Considerable difference was found between the various types of cells in connection with the hairs. These might be divided into two general types; the true trichogen cells, which secrete the chitin of the hair and its 'follicle', and true gland cells which communicate with the hairs themselves thru the small pore at the proximal end of the hair.

Of the trichogen cells there appeared to be two distinct types;

first the usual enlarged 'hypodermal cell' usually rather triangular in section, or probably actually pyramidal or conical, generally crowded away from the hypodermis to such an extent as to often appear as a second hypodermal layer where the hairs are close together. They communicate with the pore in the proximal end of the hair by a long narrow'neck' and may be readily identified by the fact that they contain the usual unbranched nucleus. (Figs. 4 and5) These cells are typical of the smaller types of hairs, and are found in the poison hairs.

The second type of trichogen is immense in size, being conspicuously the largest cells found in the larva. From the peculiar shape they might very properly be called 'trident' cells. This three pronged appearance is caused by the fact that the cell apparently secretes not only the trichogen itself, but also the cup-like 'follicle' or socket in which it is borne. In the larger hairs this socket is a large heavy ring, setting down somewhat below the level of the surrounding chitin, as a pipe might project slightly thru the sides of a cask. Apparently these trident cells are cup-like, surrounding and secreting this ring, while the usual filament projects from the center and up into the hair. This arrangement gives the typical three pronged appearance in section.

These trident cells are found secreting the hairs, and particularly the black 'plume' hairs on the first and last body segments. They are so large in size, and the fact that the nuclei appear branched led to the belief, at first, that they were probably the true poison secreting glands, or at least gland cells of some type. However the inability to demonstrate any other trichogen cells in connection with these large hairs, and the fact that they did undoubtedly fit socket-like about the hair follicle has confirmed the later

belief that they are but modified trichogen cells, and that their great size is probably due to the very large size of the hairs secreted by them.

These cells are concentrated particularly in the tubercles on the first and last body segments, tho they are found in smaller numbers and size associated with the long white hairs scattered over the lateral tubercles. They are shown in the figures on Plate 6.

THE POISON APPARATUS.

The poison hairs are found scattered in small tufts all over the body. There are usually from six to a dozen hairs in each tuft, tho they occasionally appear solitary. They are found largely on the lateral tubercles of each segment in rather few numbers, in fair quantity on the first thoracic segment, associated with the plume hairs in the dorsal tubercles, and rather more plentifully on the last abdominal segment with the similar dorsal plume tubercles. It was noted that when disturbed the larvae had a habit of elevating the last few segments and wagging them back and forth, a habit possibly associated with the presence of these hairs, and used as a means of protection.

By far the greater number, however, are found in all instars, except the first two, in the dorsal white tufts on the first four abdominal segments, which give this moth its name 'tussock moth'. Here the hairs occur in almost 'pure culture' and in great numbers. They are characteristically short, and attached very loosely, so that they drop out with but little difficulty. Each hair is attached to a trichogen cell and a gland cell, the latter crowding together below the hypodermis so closely as to give the appearance of a second or even a triple thickness of cells. Beside the neck of each such cell may be found the true trichogen cell, usually pushed down

below the hypodermal layer, and often times so crowded as to appear as a part of the gland, being forced into the gland cell itself. Examination under high power, however, in such cases readily shows the dividing line between the two cells.

Usually the gland cell communicates with the hair by means of a short thick neck, abutting up against the follicle chitin ring and completely filling it except for a thin fiber of trichogen protoplasm. However, where the crowding is very great, as in the center of a tussock, the gland cells will be found in many cases communicating by means of a rather elongated and slender neck. In such cases this thread of protoplasm may be seen entering the follicle ring of chitin in company with that from the trichogen.

The gland cells are rather large cells, tho much smaller than the enormous trident cells of the plume hairs. The nucleus usually appears single and very large, tho in many the typical 'multinucleate appearance' caused by sectioning a branching nucleus shows that this rather typical gland habit is the actual condition here, but that the branches must be short and stubby rather than thin connecting threads with enlarged ends as in the trident cells.

There is little or no variation in structure due to the age of the larva. The true poison hairs of the first instar were typically like those of the fourth, where they occur. The difference was a matter of quantity and distribution rather than structure. In fact in the first instar the glands seemed proportionally larger, but this was due rather to the much small size of the larva as a whole than to an actual larger size in the gland cell itself.

It will be noted that the condition here is different from that found by Miss Aephart in the brown-tail, where tufts of hairs were supplied each from a single large gland cell, with a small thread

of protoplasm connecting hair and gland. Here, as is usual, each hair is armed separately; an independant unit.

SUMMARY

1. The larvae of the white marked tussock moth (Hemerocampa leucostigma) is a true venomous caterpillar; whose virulence ordinarily is not great, but varies with individual susceptibility, very few being totally immune.

2. This poisonous effect is due to the smaller white hairs scattered over the body, chiefly on the lateral tubercles and in the dorsal 'plume' tubercles, of the first thoracic and last abdominal segments in the first two instars, and found there, and particularly localized on the white dorsal 'tussocks' of the first four abdominal segments in the later instars.

3. These hairs retain their virulence after being shed, both in the cocoons and in the shed larval skins.

4. The poisonous material is particularly resistant to chemical agents, being insoluble in all ordinary solvents, and resisting the chemical action of those reagents. The virulence is increased by acids, and decreased to the point of temporary loss by alkalis of low concentration. It is resistant to both moist and dry heat, but loses its urticating power temporarily upon treatment with tens pounds pressure steam heat for fifteen minutes, or 185°C. dry heat. This urticating power is regained with but little loss after a few days time at room temperature.

5. The urticating material is apparently in a close relation to the protoplasm filling the hairs, since it cannot be destroyed by ordinary means without dissolving out the protoplasmic content of the hairs. This relation is probably only a physical one,

(adsorption on the protein molecule) as the poison is not destroyed by agents which unquestionably would alter the dried hair contents (KOH, 0.2% HCl, etc.).

6. The poison is the product of a special gland cell, communicating directly with the hair, and independant of the trichogen or hair forming cell.

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PLATE II.

Effect of Untreated Hairs on the Skin of the Fore-arm.

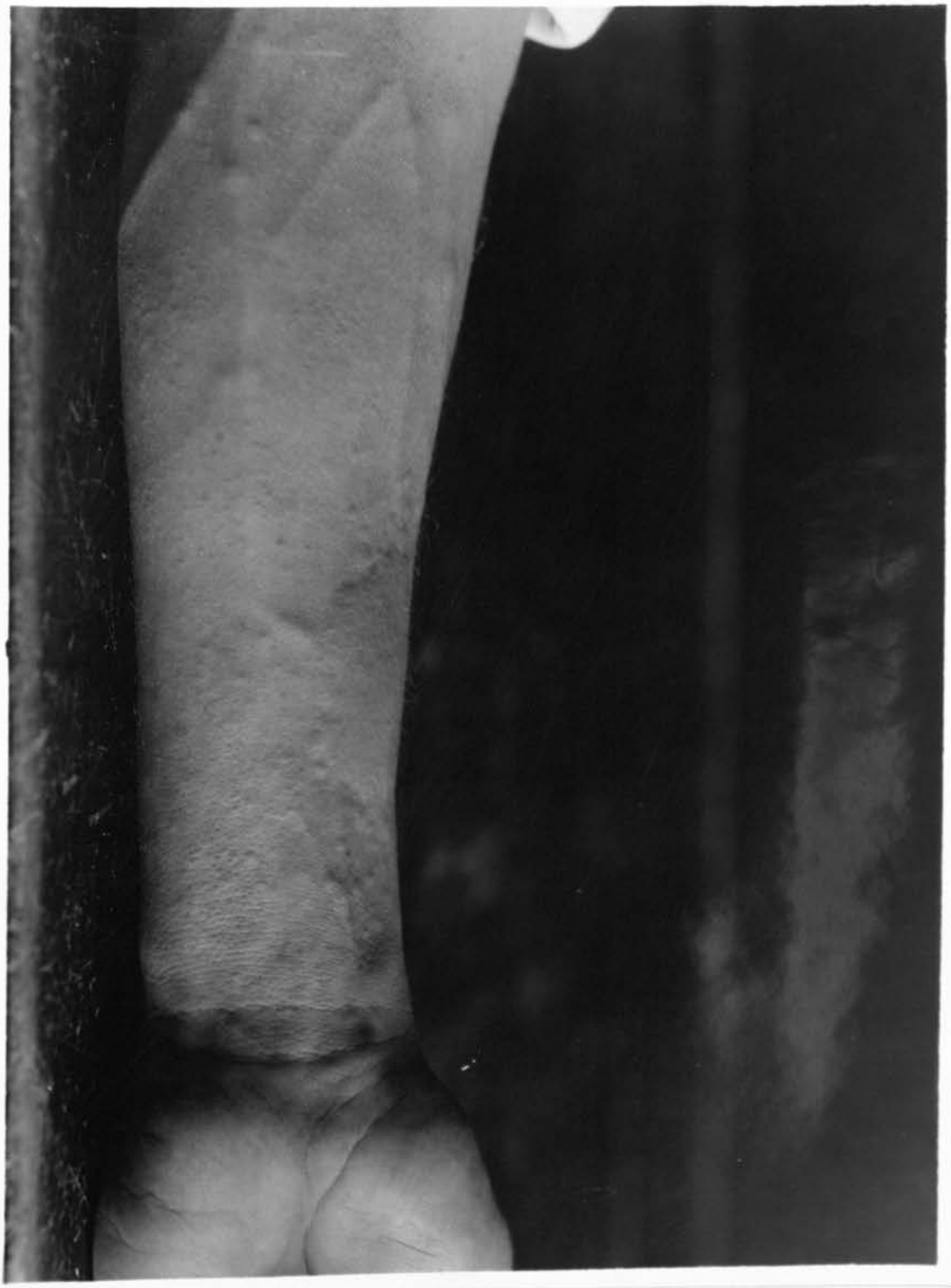
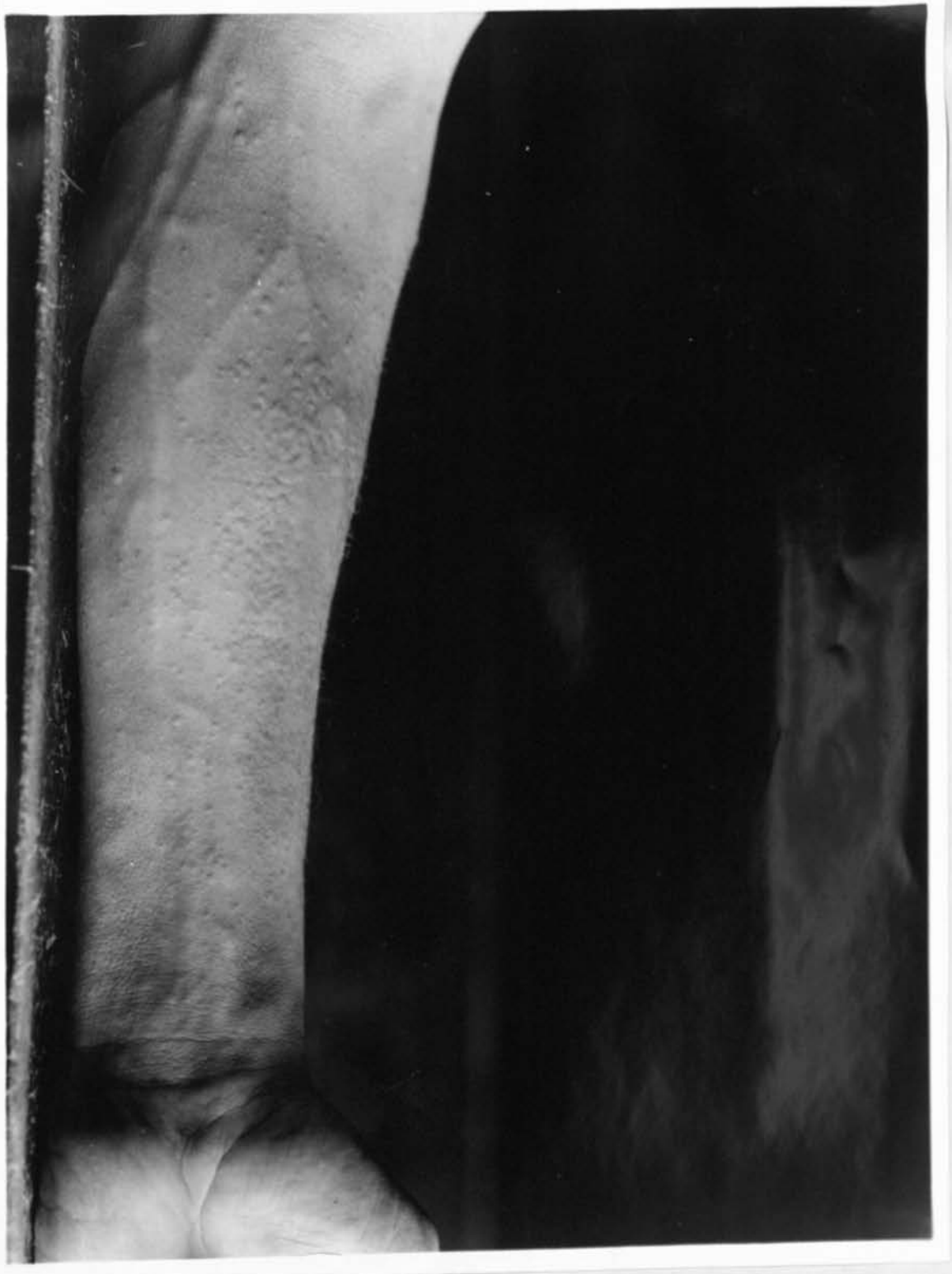


PLATE II.

Effect of Comminuted Glass Wool on Skin of the Fore-arm.



12-21-8M

PLATE III.

Effect of Hairs Treated with 0.2% HCl on Skin of the Fore-arm.

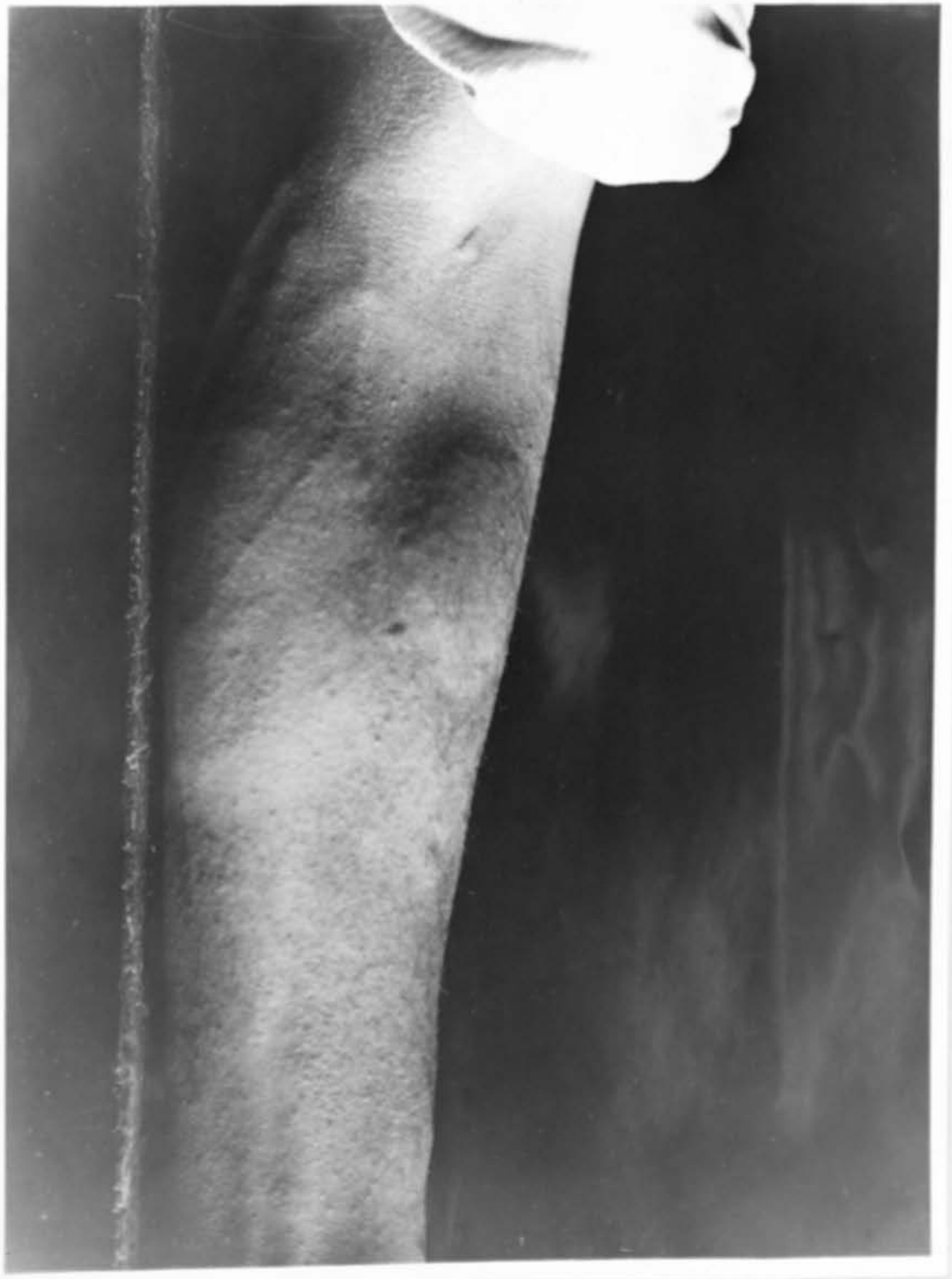
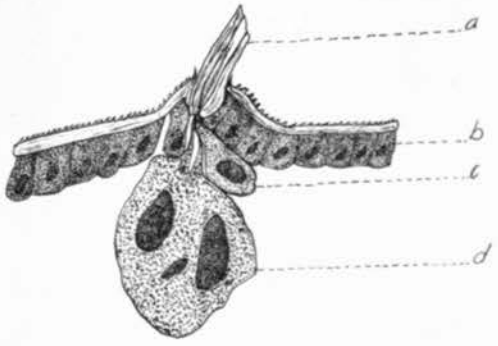


PLATE IV.

Poison Gland Cell, Hair, and Trichogen Cell of H. Leucostigma.

Plate 4

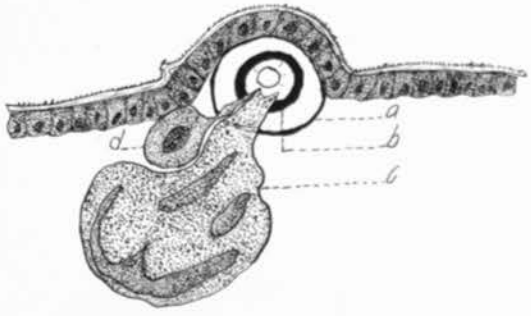


a - hair
b - hypodermis
c - trichogen cell
d - poison gland cell

PLATE V.

Poison Gland Cell, Hair and Trichogen Cell of H. leucostigma.

Plate 5



a follicle ring
b hair
c poison gland cell
d trichogen cell

PLATE VI.

Trichogen (Trident)Cells of'Plume'Hairs of H. leucostigma.