THE EFFECT OF COPPER IN VITILIGO[1]

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Recently many studies have been made to show that copper plays an important rôle in pigmentation. The initial observation dealing with the action of copper in this respect was an incidental finding made by Keil and Nelson (1) in 1931. While experimenting with rats in the study of nutritional or milk anemia, which is essentially a copper deficiency disease, these observers noted that the color of dark-haired animals changed to a silvery gray after the rats had been maintained on a milk diet for a number of weeks.

In the same year, Cuningham (2) made three important observations relevant to this problem. First, he noted that the skin of black-coated animals was apt to contain more copper than that of white-coated ones; second, that the copper content of the skin was concentrated mainly in the epidermis; and thirdly, "in vitro" experiments demonstrated the fact that copper accelerated the oxidation of dopa by skin extracts containing dopa-oxidase. Nothing further was added to our knowledge concerning the action of copper in pigmentation until Gorter (3), in 1935, conclusively demonstrated the fact that copper-free diets resulted in depigmentation of the hair of rats, rabbits, and cats and that this depigmentation disappeared following the administration of copper. On the other hand, no effect on melanogenesis was obtained if other minerals or vitamins were added to the diet. The young rat was the animal most susceptible in demonstrating this action. In the same year Sarata (4) made the first definite attempt to correlate the copper content of the skin with its degree

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of pigmentation. A study, carried out in mottled dogs and cats, showed that the copper content of pigmented hairs was much higher than that of the nonpigmented hairs of the same animal. Moreover, generally speaking, copper was found to be present in greater amounts in skin covered by dark hairs than in the skin underlying the colorless hairs. A similar study attempting to relate iron and manganese with pigmentation was entirely negative, but potassium was found in greater amounts in dark hairs than in colorless ones. On the other hand, contrary reports on a similar study were made by Saccardi and Guiliani (5) who found, in 1935, that the copper content of fur and feathers of various animals was greater in the case of the white than in the colored-coated animals. So far as I have been able to discover the observations of these latter writers have never been confirmed. In 1937 Yosikawa (6) and Naraska (7) supplemented Sarata's previous study. The former writer, Yosikawa, compared the copper content of grey and normally pigmented human hairs from aged individuals. Both types of hairs were taken from the same person. In all cases the copper content of grey hair was lower than that of colored hair. Naraska reported on the relationship of copper to pigmentation in the Mongolian spot. This study was made, he stated, because in this structure the pigmentation is of mesodermal origin as compared to the epidermal pigments in general skin and hair. Again, it was found that copper was more highly concentrated in the Mongolian spot than in the surrounding spot-free regions.

Thus, it seems that, according to most authors, epidermal and dermal pigmentation usually is accompanied by a quantitative increase of copper in the structure involved and that a diet specifically deficient in copper is apt to result in a failure of melanogenesis. It can hardly be denied, in the face of these observations, that copper plays an important rôle in the formation of pigment.

Just how copper operates in its melanogenetic action has been the subject of still further study. Clues to this action are given by a knowledge of the way copper acts in many metabolic processes. Copper is found throughout the animal and vegetable

kingdom (8) and almost wherever its type of action has been sufficiently well elucidated, it appears that copper is involved in metabolic activity in the manner of a catalyst. Its best known effect in this respect is its function in the conversion of iron into hemoglobin. In the formation of some other pigments copper plays a similar rôle. For instance, copper is needed for the generation of certain cytochromes found in yeast as well as heart and liver tissue. It has a catalytic effect on the oxidation of cysteine and ascorbic acid and it plays an important part in the regulation of many enzyme systems.

Inasmuch as dopa is a propigment, which, in the presence of an enzyme, dopa-oxidase, is converted into pigment, it can be readily appreciated that copper might materially influence this reaction. That such is the case was originally demonstrated in 1931 by Cuningham (2) who, as was mentioned above, was the first to report on the accelerating effect of copper in the dopa reaction when carried out with solution of dioxyphenylalanine (dopa) in the presence of dopa-oxidase. Sarata (4) while confirming Cuningham's experiments, was able to show that pigment could be precipitated from solutions of dopa when catalysed by copper even though dopa-oxidase, or any other enzyme, was absent from the solution. He, furthermore, noted that this catalytic action of copper was most marked at a certain optimum concentration, and that above or below this optimum the action of copper was much less pronounced. As a result of these studies, Sarata concluded that copper acts as a catalyst in the physiology of pigment formation; that it is taken up by young melanogenetic cells, where it remains even after pigment has been formed and with which it is eventually cast off in the stratum corneum, hairs, or feathers.

In 1934, Schroeder, and Grueneberg and Schade (quoted by Cornbleet (9)) demonstrated that vitamin "C" inhibits the dopa reaction.

Cornbleet (9) found that pigmentary precipitates can occur in a solution of dopa under the action of ultraviolet light alone, but he was able to accelerate this reaction considerably by adding copper to the solution. On the other hand, the addition of vita-

min C tended to slow down the reaction or, in other words, to neutralize the catalytic effect of the copper. Cornbleet concludes that the presence of these two substances, having antagonistic actions, so far as the oxidation rate of dopa is concerned, makes pigment formation susceptible to ready physiologic control.

Vitiligo, apparently, is a fertile field for the investigation of problems dealing with defects in pigment formation. No one, thus far, has proved, at least to the satisfaction of most dermatologists, the etiology of this condition. This subject has recently been reviewed by Becker (10), so that none but the most pertinent facts on this problem will be repeated here. Vitiligo, as a rule, presents histologically a normal epidermis and corium except for the complete absence of all signs of pigment. Of great importance is the fact that the dopa reaction is negative. This indicates, of course, that dopa-oxidase is not present in the tissue of the vitiliginous patch (11). Bruno Bloch concluded that the failure of pigmentation resulted from a "cellular exhaustion" of certain of the melanogenetic functions of the basal cells of the epidermis.

Masson (12) believes, in opposition to Bloch, that not all basal cells but only certain of the cells of the basal layer of the epiderm are capable of melanogenesis. These elements he calls dendritic melanoblasts and it is only these particular cells which are dopa-positive when they are functionally normal. In vitiligo Masson considers that he has been able to demonstrate certain "cellules claires" in the basal layer of vitiliginous patches, which he believes to be cells analogous to the dendritic melanoblast but which have lost the power of forming pigment and have become dopa-negative. Becker (10) in the article cited above, studied several cases of vitiligo histologically and was able to confirm Masson's findings of these peculiar cells in the basal layer of some vitiliginous patches.

Apparently, the epidermal cells in vitiligo, except for the failure of pigmentogenesis, are functionally quite intact. Although from time to time evidence has been presented to indicate that these cells react differently to various insults (see Pillsbury and Kulchar (13)) than do their neighboring normally pigmented cells,

still this does not apply to ordinary phenomena which are easily and regularly elicited.

Thus it seems that the outstanding immediate defect in vitiligo is a failure of pigmentogenesis, either of all the basal cells as Bloch believes, or of certain dendritic melanoblastic elements according to the ideas of Masson.

EXPERIMENTAL PROCEDURE

Inasmuch as copper has been shown to have an activating or catalytic action in cutaneous melanogenesis, an attempt was made to induce pigmentation in vitiligo, using this agent by intracutaneous injection and by inunction in the form of an ointment.

The subjects were five patients with classic vitiligo. The solution used for injection consisted of 0.1 per cent copper sulphate solution, of which 0.1 cc. was injected intracutaneously. The injections were repeated at the same sites at weekly intervals for three injections. Final readings were made one week after the last injection and were estimated in degrees of pigmentation as plus one to plus four. The injection sites chosen were as follows:

1. The center of a vitiliginous patch
2. The periphery of a vitiliginous patch
3. A normally pigmented portion of the skin in the neighborhood of the vitiliginous patch.

In four of the five cases studied, the above tests were duplicated, usually on a symmetrically opposite side of the body. One set of test sites was then painted with oil of Bergamot in 10 per cent alcoholic solution twice a day by the patient, while the duplicate test sites on the opposite side were not treated by oil of Bergamot.

An identical series of experiments was repeated, this time using an ointment containing 2 per cent copper sulphate[2] in place of the copper solution. This was rubbed on vitiliginous and normally pigmented areas on symmetrically opposite sides of the body by being vigorously applied by the patient 10 minutes twice daily over a period varying from 15 to 42 days.

Preceding application of the ointment, a 10 per cent alcoholic solution of oil of Bergamot was applied to the experimental sites on one side of the body.

Subsequently, the experiments described above were repeated on two of the patients. These experiments differed from the preceding ones only in the fact that different copper preparations were used. Instead of the 10.1 per cent copper sulphate solution, a colloidal copper preparation[3] (containing 0.32 mgm. of copper

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[2] The ointment was prepared by making a saturated solution of the copper sulphate in water and incorporating it in a lanolin base.

[3] The colloidal copper used in this experiment was supplied to us by the Mulford Colloid Laboratories, Philadelphia. It was described by them as a casein protective colloid with the copper present either in the form of the oxide or the hydroxide, and contains a total of 37.8 mgm. of solids per cubic centimeter and 0.32 mgm. of copper per cubic centimeter.

per cubic centimeter) was injected intracutaneously, and in place of the 2 per cent copper sulphate ointment a stronger ointment containing 10 per cent copper sulphate was employed.

It was planned to obtain biopsies of the normal and vitiliginous skin after they were treated by copper, but unfortunately, none of the patients consented to this procedure. Studies of the dopa reaction and staining for the presence of copper, therefore, could not be performed.

EXPERIMENTAL RESULTS

The results were constant. In no case did any degree of pigmentation appear in the vitiliginous areas. In some of the patients, a slight degree of pigmentation developed at the sites of injected copper in the normally pigmented areas, but this was attributed to the inflammatory effect of the copper rather than to any specific pigmentary action. In 2 patients ordinary catarrhal stock vaccine was injected as a control, which led to pigmentation of a greater degree than that of the copper-injected sites. Copper rubbed into the skin in the form of an ointment produced no pigmentation except in one instance when a mild grade of pigmentation appeared on a normal portion of the skin following the combined use of the 2 per cent copper ointment and oil of Bergamot. Here, however, the oil of Bergamot had resulted in a mild dermatitis and the patient had been exposed to sunlight, so that undoubtedly we were dealing in this instance with a case of Berlock dermatitis.

Ultraviolet light had no effect in inducing pigmentation with copper in two of the cases in which it was tried. Here, four successive doses of ultraviolet light were administered at weekly intervals in conjunction with the 10.1 per cent copper sulphate injections, the colloidal copper injections and copper inunctions (2 and 10 per cent) using the technic described above.

The results were entirely negative, except that the injected sites were a little more deeply pigmented in the normal skin exposed to ultraviolet light than on the normal skin sites which were protected from the radiation. The vitiliginous patches, however, showed no change.

The combined effect of ultraviolet light, oil of Bergamot and copper, when carried out with the above technic on one patient

TABLE 1

Degree of pigmentation developing at each injection site when read one week after the last injection

Degrees of pigmentation estimated as plus one to plus four

INITIALS	SEX	COLOR	COMPLEXION	NUMBER OF INJECTIONS EACH SITE	COPPER SULPHATE SOLUTION—0.1 PER CENT				COPPER (0.32 MG./CC.) AS PROTECTIVE COLLOID				CONTROL VACCINE
					No oil of Bergamot		Oil of Bergamot		No oil of Bergamot		Oil of Bergamot		
					Vitiligo	Normal	Vitiligo	Normal	Vitiligo	Normal	Vitiligo	Normal	
R. Y.	M	W	Brunette light skin	3	0	1	0	0	—	—	—	—	—
M. S.	F	C	Medium dark skin	3	0	1	0	1	0	1	0	1	2
L. J.	F	C	Medium dark skin	3	0	2	0	2	0	1	0	1	3
J. R.	M	W	Brunette dark skin	3	0	0	0	0	—	—	—	—	—
E. M.	F	C	Medium dark skin	3	0	1	—	—	—	—	—	—	—

TABLE 2

Degree of pigmentation developing after application of copper-containing ointment (readings made the day of the last application)

Degrees of pigmentation estimated as plus one to plus four

INITIALS	SEX	COLOR	COMPLEXION	2 PER CENT COPPER SULPHATE OINTMENT					10 PER CENT COPPER SULPHATE OINTMENT				
				Number of days ointment was used	No oil of Bergamot		Oil of Bergamot		Number of days ointment was used	No oil of Bergamot		Oil of Bergamot	
					Vitiligo	Normal	Vitiligo	Normal		Vitiligo	Normal	Vitiligo	Normal
R. Y.	M	W	Brunette light skin	28	0	0	0	2*	—	—	—	—	—
M. S.	F	C	Medium dark skin	28	0	0	0	0	21	0	0	0	0
L. J.	F	C	Medium dark skin	42	0	0	0	0	21	0	0	0	0
J. R.	M	W	Brunette dark skin	15	0	0	0	0	—	—	—	—	—
E. M.	F	C	Medium dark skin	28	0	0	—	—	—	—	—	—	—

* Result of Berlock dermatitis.

gave results identical to that of the ultraviolet experiments described above.

DISCUSSION

The failure of copper to induce pigmentation in the skin of vitiliginous patches is difficult to explain in the light of the modern investigative literature. No one, so far as I have been able to find, has analysed the copper content of vitiliginous skin, although analogous studies made on piebald animals would make one suspect that it should be lower than that of normal skin. Studies have been made by Cornbleet (9), however, on vitamin C, which tend to show that vitamin C is held in the skin by pigment and that, therefore, the ability of non-pigmented skin to retain vitamin C is quite limited. The third element in the mechanism of pigment formation is dopa-oxidase and this, as discussed above, has been found to be absent in the vitiliginous patch.

While it is true that the logical way for inducing pigmentation in vitiligo would be to employ a method which causes the production of dopa-oxidase in the involved cells, such an approach has, to my knowledge, never been possible. Therefore, the next best method of attacking the problem of failure of melanogenesis in vitiligo seemed to me to utilize the catalytic action of copper on dopa as described above. This seemed quite reasonable because "in vitro" experiments showed that copper was able to produce pigmentary precipitates from solutions of dopa free of enzymes, including the enzyme dopa-oxidase.

The failure of copper to induce pigmentation in vitiliginous patches can be explained on the basis of one of several possibilities, for example:

1. Although dopa, or a dopa-like substance, is present in vitiliginous epidermis, copper does not behave "in vivo" as it does "in vitro," and the intervention of dopa-oxidase is necessary to complete the reaction for pigment formation in vivo.

2. The promelanin found in vitiliginous skin has different properties from that of dopa itself, requiring dopa-oxidase for pigmentogenesis.

3. The analogue of the melanoblast (i.e. "cellules claires" of

Masson),—or the ordinary basal cell of the epiderm, if one subscribes to the views of the Bloch school,—has lost its ability to take up promelanin from the blood stream and, therefore, none is present to be acted upon by copper.

4. It is possible that normal pigment-forming cells elaborate their own propigment and that the corresponding cells in vitiligo have lost their ability to produce this substance for themselves. Some experimental evidence has been reported to support this last point of view; for Goldsmith (14) in his recent review of pigmentation, states that pigment can be produced in tissue cultures of pigmented cells; and also that excised skin, when warmed and exposed to ultraviolet light, undergoes increased pigmentary changes.

Exposure of the copper-treated vitiliginous patches to ultraviolet light, and their treatment with oil of Bergamot plus ultraviolet exposure caused no melanogenetic activity. This emphasizes the fact that a lack of available copper cannot of itself explain the failure of melanogenesis in vitiligo.

The pigmentation that occurred at the control sites of copper injection in normal skin must have been due to the irritating effect of the injected copper, rather than to any specific pigmentary action, since ordinary catarrhal vaccine, injected in corresponding sites, produced a greater degree of pigmentation than did copper itself.

CONCLUSION

1. Copper, by intracutaneous injection in the form of a 10.1 per cent copper sulphate solution or in the form of colloid suspension, or by inunction as a 2 and 10 per cent ointment, has no effect in inducing pigmentation in the non-pigmented patches of vitiligo.

2. The above result is not modified when the copper-treated patches are painted with 10 per cent alcoholic solution of oil of Bergamot, exposed to ultraviolet light, or subjected to the simultaneous use of both of these agents.

3. These results seem to indicate that the local diminution in the amount of copper is of itself not the actual or sole cause of the failure of pigmentogenesis in vitiligo.

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