Vitiligo is one of the common dermatoses, the percentage of which to all ambulatory patients in dermatological clinic is 1.9 in Kanazawa and 1.3 in Sendai. The etiology and pathogenesis, however, have not been thoroughly understood. Amongst various hypotheses, the theory of nervous disturbance is most widely accepted. It is well known that in some cases the site of eruption shows the tendency to follow the distribution of cutaneous nerve, and the observations of Sasamoto¹ and Takikawa² seem to support this view. Moreover the so-called pigment lability of the individuals induced by dysharmony of autonomic nervous system and disturbance of endocrine function is admitted to play a rôle in the etiology. The author has already presented several observations suggestive of the relationship between pigment production and nervous system. In the following I will first discuss the etiology of leucoderma centrifugum acquisitum Sutton, in which the participation of nervous system is most probable. Such a study, I believe, will throw some light on the dark etiology of vitiligo, too.

1. Sutton’s disease,³ the identity of which with vitiligo is still under discussion, is not extremely rare. In the recent 10 years we observed 9 cases including 2 atypical ones (Table XIII). The numbers of vitiligo during this period was 264. In most cases of Sutton’s disease, pigmented nevus in form of lentigo is situated at the center of the lesion. In some, however, hairy (Birger, Stokes, Krantz), papillary or usual pigmented nevi (Fuse, Arakawa) are noted, and rarely fibroma (Leszczynski, Mgebow and Pridsky, Richter), angioma (Rabut), verruca senilis (Petges, Reito, Szenthiralyi), psoriasis, sarcoid (Stokes), lichen planus (Robinson), scar tissue

<table>
<thead>
<tr>
<th>Case number</th>
<th>Age in years</th>
<th>Sex</th>
<th>Location</th>
<th>The lesion in the center of the patch</th>
<th>Concomitant vitiligo</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19</td>
<td>F</td>
<td>Right forehead and right mandibula</td>
<td>Lentigo</td>
<td>(−)</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>M</td>
<td>Left nape</td>
<td></td>
<td>(+)</td>
</tr>
<tr>
<td>3</td>
<td>31</td>
<td>M</td>
<td>Left neck</td>
<td></td>
<td>(+)</td>
</tr>
<tr>
<td>4</td>
<td>38</td>
<td>M</td>
<td>Back (two patches)</td>
<td></td>
<td>(+)</td>
</tr>
<tr>
<td>5</td>
<td>23</td>
<td>M</td>
<td>Right back</td>
<td></td>
<td>(+)</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>M</td>
<td>Right back</td>
<td></td>
<td>(+)</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>F</td>
<td>Chest</td>
<td></td>
<td>(+)</td>
</tr>
<tr>
<td>8</td>
<td>42</td>
<td>M</td>
<td>Abdomen</td>
<td>Papillomatous</td>
<td>(+)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>pigmented nevus</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>52</td>
<td>F</td>
<td>Left chest and right back</td>
<td>Keloid</td>
<td>(+)</td>
</tr>
</tbody>
</table>

* By Minor Itô.
Vitiligo

(Netherton and Curtis) or erythema (Mgebrow and Pridsky) are reported in place of the mole. The latter condition is beyond the category of vitiligo périnaevique.

In discussing the etiology of Sutton’s leucoderma, it must first be made clear whether the lentigo appears first or the leucoderma. It is difficult to solve this problem decisively because such trifling lesion as lentigo is likely to be overlooked and thus the statement of the patient cannot be accepted as accurate. Sutton, Weber, Richter, Shengsum, Hebijima stated that vitiligo is the primary eruption, while Stokes, Póor, Rostenberg, Gougerot, Sasamoto, Tosima, Kodama, Higuchi-Boku considered the depigmentation as secondary. Leszczynski, Reito, Mori hold eclectic opinion that leucoderma and mole are co-ordinated in clinical occurrence. Sutton in the histological study looked upon the hyperpigmentation of epidermis and the endothelial cellular infiltration in the corium in the central part as secondary changes following vitiligo. His theory was accepted by Schelmire and Bunch. Petges, Stokes, Casseberry found that what Sutton called endothel cell is no other than nevus cell, and Toyama, Goldhardt, Póor, Mgebrow-Pridsky accepted that the central lesion is true pigmented nevus. These findings lead to the conclusion that pigmented nevus is the primary eruption and thus the conception of vitiligo périnaevique is established. The author also noticed nevus cell in the histological study (Plate XIII, Figure 54) and is convinced of the initiality of nevus lesion.

The relationship between leucoderma Sutton and vitiligo is much discussed. Some authorities tried to take leucoderma Sutton as a distinct disease from vitiligo by following observation: the existence of cases in which vitiligo is not co-existent, incidence of spontaneous recovery, successful treatment with ultraviolet irradiation, differences in histological findings. These, however, cannot be definitive characteristics. For instance, most cases are combined with vitiligo (in my experience 8 out of 9), in some cases halo is noticed, extension over 10 cm in diameter is observed. Successful treatment with ultraviolet irradiation or spontaneous recovery can occur in vitiligo. And histological findings differ by cases. In my comparative study, transitory picture is obtained. These findings, in common with the results of dopa reaction, show that these two dermatoses are most likely to be identical.

The author holds to this conception and further tries to investigate the etiology of vitiligo from this standpoint. In my previous report I accepted Masson’s neurogenic theory of nevus cells and at the same time assumed that melanin production is influenced by the chemical transmission at the termination of vegetative nerve fibrils. Consequently the congenital abnormality in the distribution of termination of nerve fibrils at the site of nevus lesion is surmised. And the depigmentation of leucoderma Sutton may be caused by the abnormality of vegetative nervous system accompanying central nevus lesion in the sense of trophoneurotic disturbance. Judging from abovementioned considerations it may not be too eccentric to surmise the etiology of vitiligo and leucoderma Sutton in the following manner: in vitiligo the disturbance is found in the neighbourhood of spinal ganglion and hence the symmetrical or unilateral systematized arrangement while in Sutton’s disease the nervous disturbance limited in the area of nevus results in the circumscribed vitiligo périnaevique with only little tendency to extension.

2. Following cases of my recent observation are in favor of neurogenic theory.
   a) 55 years old woman with vitiligo of the back for three months duration. On
the lumbar region and in the middle line several dark brown islets are observed at regular intervals as if the area above the spinal ganglion are left intact (Plate XIII, Figure 52).

b) 50 years old man. Depigmented spots which developed recently appeared symmetrically on both sides of the spine (Plate XIII, Figure 53).

c) 3 cases of vitiligo in which the lesions are arranged symmetrically beginning from both sides of underlip to the sides of neck just like the predilection of bilateral herpes zoster of this area.

3. It is not rare to find vitiligious lesion with normal colored mamilla in the center. I have observed 8 such cases. As mamilla is physiologically rich in melanin and, moreover, is possessed of special nerve distribution, we can assume that these phenomena are closely related to Sutton's leucoderma. The absence of muscular reflex in vitiligious mamilla (Levy-Frankel and Juster)\(^7\) is in favor of this conception.

4. The rôle of vasomotor nerve in the etiology is suggested by following observations. In the histological study of vitiligo as well as leucoderma Sutton, dilatation of blood vessels and perivascular cellular infiltration are often noticed in the papillary, subpapillary layer in the neighbouring zone of depigmented area (Plate XIII, Figure 55). This, in accord with occasionally perceptible erythema on the perivitiligious area, and the érythème prévitiligineux of Milian, Maslot and Horowitz\(^6\) suggests the abnormality of vasomotor nerve. The abnormal cutaneous reagibility against various agents (carbon dioxide snow, canthalis plaster, epinephrine, morphine, caffeine, tuberculine, diphtheria toxin, myoarsphenamine, phenolphthaleine) found by Takikawa may support this theory.

5. In cases of vitiligo and Sutton's leucoderma, disturbance of sensibility is usually lacking. But in rare instances itching or hypesthesia is complained (Königstein, Thibierge, Sasamoto). In such cases it may be assumed that the disturbance of vegetative nervous system, which I already pointed out, may have extended to sensible nerve fiber which runs usually accompanied with vegetative nerve fibrils. Further evidence suggestive of nervous disturbance is the occurrence of vitiligo in tabes or following injuries of brain or peripheral nerve, and the fact that nerve leprosy is frequently accompanied with depigmentation.

Leloir and Chabrier, Dejerne,\(^8\) Marc\(^10\) payed attention to nerve atrophy in form of myelin fragmentation, increased Schwannian nuclei in the histological study. Darier, however, was sceptical about these findings. The author stained sections obtained from vitiligo and leucoderma Sutton with Seto's modificat of Bielschowsky's silver impregnation method. In thick nerve bundles (sensible nerve), hypertrophy and irregularity in thickness of axis cylinder and increase of Schwannian nuclei are observed. However, neuritis degenerativa atrophica is not ascertainable. Terminal reticulum of vegetative nerve fibrils, clearly stained in each section, is apparently normal. Naturally it is impossible to draw certain conclusion as to the functional state of nervous systme solely from the results of silver impregnation method which does not always give constant results.

6. We Itô and Watanabe\(^11\) examined local blood of vitiligious area. The differential count and oxydase reaction are almost the same as control peripheral blood. Peroxydase reaction, however, is markedly weaker. And, moreover, the results of oxidoreduction staining of Unna suggest specific local characteristics
especially in reducing activity of the site. This may have certain relationship with
the increased content of sugar and iodic acid reducing substance of canthalis blister
fluid at the vitiliginous area (Takikawa12)).

Treatment

No definitive therapeutic measure is available as yet, and only various endocrine
preparations and ultraviolet irradiation either by itself or in combination with photo-
catalytic substances are tried at present. We have been studying the pigment
problem partly for the purpose of obtaining definitive treatment of vitiligo. In
animal experiment (Itō & Nakajo13)), it is possible to produce black pigment in melanin
free goldfish which has same chemical property as natural melanin by injection of p-
benzoquinone. This substance, however, has no therapeutic effect in human viti-
ligo. And the same for tyrosine, tryptophane and adrenalin. Itō and Watanabe14)
further examined the effect of several endocrine preparations and biologic active sub-
stances on melanin free goldfish. Among substances examined, only hematopor-
phyrine and natural anterior pituitary hormone can produce black pigment which
is maintained for relatively long period. In clinical application, both substances are
injected intracutaneously in the vitiliginous area with or without following ultraviolet
irradiation. Each injection is given at several days' interval when the local inflam-
matory changes have subsided. No side reactions are noticed except for slight por-
phyrinuria in cases of long continued porphyrine therapy. Usually after 3–4 months
treatment, tiny pigment flecks are newly formed which by enlargement and con-
fluence substitute the entire lesion. At the same time, hyperpigmented halo be-
comes more intensely pigmented. Histological examination of newly formed
pigment spots gave following results. Pigment granules are contained in the basal
layer and the dopa reaction is turned to positive. This newly formed pigment is
breached by hydrogen peroxide procedure and is identified with natural melanin.
This procedure, though rather time-consuming and expensive is superior to other
type of treatment in therapeutic results. Then, how is the mechanism of pigment
reproduction by these preparation is explained? Dietel, Zondek and many other
authorities observed that hormones of intermediary and posterior pituitary influence
color change in animals and matrimony lobe in fishes. Consequently pituitary
preparations are often applied in the treatment of vitiligo (Fournier, Cerenino, Ha-
yashi, Namiki). And Bloch15) observed hormone of ovarian follicle which is secreted
under the influence of anterior hypophysis, produced increased pigmentation on
the nipple of guinea-pig. In our experience neither the preparation of posterior
pituitary nor urinary gonadotropic hormone of anterior hypophysis are effective in
producing pigment in goldfish. On the contrary, the extract of anterior hypophysis
showed some effect. This is of interest in view of the fact that only this preparation
showed positive melanophore reaction. So it may be that some or other hormone
contained in the extract of anterior hypophysis may act as hormozyme (Euler) in
melanogenesis, or may activate dopa oxydase, in the sense of neurohormone of
Parker and Vilter.16)

Hematoporphyrine is a well known photocatalyser. In our experiment with
goldfish, melanin production was noticed solely on the back where the effect of ultra-
violet ray was strongest. Therapeutic result in vitiligo was most markable in area
Studies on Melanin

treated in combination with ultraviolet irradiation. Thus the effect of hematoporphyrine is attributed to photodynamic action on the contrary to that of anterior pituitary hormone.

SUMMARY

In the comment on the pathogenesis of vitiligo, nervous disturbance theory was accepted as most probable especially through the study of leucoderma Sutton which the author looks upon as a variety of vitiligo.

Several cases of vitiligo are presented in which the participation of nervous system was surmised.

No definitive therapeutic measure is available as yet. However, intracutaneous injection of anterior pituitary preparation and combined hematoporphyrine ultraviolet irradiation method gave superior result to other treatment.

References

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8) Milian, Maslot and Horowitz, ibid., 1930, 37, 479.
9) Habermann, Jadassohn's Handb., IV/2, 1933, 896.
11) Itô and Watanabe, this Supplement, 9.
13) Itô and Nakajo, this Supplement, 92.
14) Itô and Watanabe, this Supplement, 97.
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XIV. Dyschromatosis Symmetrica on the Extremities*

The clinical manifestations of pigmentary disturbance are far more complicated in our race than in the White and the Negro races. So we often observe such diseases as are not reported in foreign literatures. Dyschromatosis symmetrica hereditaria is a specimen in point. This disease was reported independently by Komaya,1) Matsumoto2) and by Toyama3) under three different designations. The characteristic cutaneous change consists of small pigment flecks resembling freckles mingled with reticulated depigmentation disseminated on the face and at the ends of extremities (Plate XIV, Figure 56). It is differentiated from freckles because of the co-existence of depigmentation and from xeroderma pigmentosum because of the lack of erythema and atrophy. However, the independence of this disease is not well established. For instance, the photosensitivity in xeroderma pigmentosum varies in a wide range according to cases and the clinical manifestation of hyper- and depigmentation differs according to the physiological color tone of the skin in individual race. So the lack of apparent photosensitivity and co-existence of hyper- and depigmentation cannot be always interpreted as pathognomonic feature of dyschromatosis symmetrica hereditaria. 4) have described previously that this disease belongs to same category to that of freckles and xeroderma pigmentosum from the genetical point of view.

Pigmentary disturbances similar to dyschromatosis symmetrica hereditaria are noticed under several conditions.

1. Safu in Caroline islands is one of them (Plate XIV, Figure 57). Although the high incidence of heredity is noticed by Matsunaga,5) it is difficult to exclude all postnatal factors such as framboesia and dermatophytosis (Melung of Ziemann for instance). Further dyschromatosis-like eruption following undoubtedly acquired disease is not so rarely observed in these islands.

2. Dyschromatosis symmetrica following pernio. It is not unusual to find depigmentation at the site of previous pernio eruption. However, the present author noticed that in small number of cases who suffer from pernio, symmetrical dyschromatosis-like lesion occurs chiefly on the dorsal surface of finger joints independently of pernio-scars. I have observed 13 such cases in the recent 10 years (Plate XIV, Figure 58). This, I assume, is resulted by the peripheral circulatory disturbance through coldness or by the recurrent inflammation due to coldness. According to Kuré and Yamagata,6) pernio is caused by trophic disturbance in hypotonic stage of parasympathicus. They thus recommended application of pilocarpine-lanoline ointment. Tomita7) in my clinic measured temperature of skin surface and tried functional test of arterioles. His findings show that pernio is caused by disturbance of vasomotor nerve especially in the arteriole portion on the ground of vegetative nervous system. The author8) obtained excellent therapeutic results in the topical application of camphor-lecithine-petrolatum ointment. This may

* By Minor Itô.
be explained by the assumption that lecithine which penetrated the skin is converted into choline or acetylcholine and thus exerts stimulation to parasymathicus. These findings disclose the labile autonomic nervous system of pernio patients, and thus I think that the pigmentary disturbance predisposes to these patients.

3. Dyschromatosis symmetrica following erythromalgie.9) In a 42 years old farmer who had suffered from erythromelalgia caused by mushroom poisoning Clitocybe acromelalga appeared a half year later irregular depigmented patches mingled with reticulated pigment spots on the back of both hands. The etiology of erythromelalgia is not entirely cleared. In Japan most of cases are caused by Clitocybe intoxication. And it is generally admitted that vagotonic individuals are predisposed.

COMMENT

Pernio and erythromelalgia are somewhat related in the etiology because both lesions may be interpreted as manifestations of vasomotor or trophoneurotic disturbance occurring at the ends of extremities. And it is interesting to find that in both conditions pigmentary disturbance is occasionally resulted. Müller10) observed similar depigmentation on the back of hands in a case of amyotrophic latalsclerosis. And I11) observed a woman, aged 61, of dyschromatosis symmetrica hereditaria in which the consanguinity of parents is stated. Since childhood she suffered from pernio and asthma, and after climacterium acroparesthesia appeared. Such cases are in favor of my theory because it is proper to assume that all these complaints developed on the ground of instable constitution of vegetative nervous system.

SUMMARY

In the comment of the etiology of dyschromatosis symmetrica hereditaria, Safu and dyschromatosis symmetrica following pernio or erythromelalgia, the role played by the constitutional lability of autonomic nervous system cannot be over-looked.

References

1) Komaya, Arch. f. Dermat., 1924, 147, 390.
6) Yamagata, Grenzgebiet, 1932, 6, 1533.
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XV. Riehl's Melanosis*

Owing to the abnormal conditions of life under the influence of World War II, it is quite naturally expected that unusual dermatoses are to be seen. What I call Riehl's melanosis in this article is a specimen in point. Towards the end of the war and after war period, it is not seldom to meet with cases showing slaty brown bluish pigmentation of the face quite distinct from chloasma either with or without follicular eruptions. Such incident is noticed not only in our clinic but by many other dermatologists in Japan. In the literature such cases are reported as Riehl's melanosis\(^1\) or melanodermitis toxica.\(^2\) But the identity of these two dermatoses is still under discussion. In this article similar cases are summarized under the title of Riehl's melanosis because this is the most popular designation. However, I do not agree with German authorities who define malanosis Riehl so narrowly.

**Clinical symptoms:** As it is too time consuming to make description of individual case, only the characteristic points are stated.

The number of the patient is 32. All but one are female cases. The age of patients is most frequently between 20 and 39 years, while according to foreign literatures middle aged women are often affected. Itching, erythema and swelling of the face are complained as prodroms. In some cases fits of erythema, swelling and desquamation are noticed and the pigmentation grew severer after each fit. Similar observation is also made by Joulia and others.\(^3\)

As regarding to the localization of the eruption, temporal, preauricular and mental regions never fail to show pigmentation. Other parts of the face, retroauricular portion and the sides of the neck may be involved, and in a few number of cases cubital fossae are also affected. The skin color of the affected area varies from slaty bluish brown to dark brown (Plate XIV, Figure 59). The skin surface in some is coarse and rough while in other is rather smooth and looks like powdered. Typical lichen planus-like eruption is found by none. By first glance the pigmentation forms rather diffuse patches, but on closer observation is found in tiny patches arranged reticularly corresponding to the superficial network of blood vessels. In long standing cases slight atrophy is noticed. In one case tinea amiantacea-like eruption is observed on the scalp. The pigmentation lose its intensity at retroauricular region and diminish into tiny patches and finally extinguish on the borderline to the breast. In a few number of cases pigment patches on the buccal mucous membrane are noticed, but yellow discoloration of sclera is not seen.

**Histopathology and histochemistry:** In six cases biopsy specimens were excised.

Specimen No. 1. 31 years old female patient complaining pigmentation on the face for two months. Epidermis is thinner than usual with shorter processes. Basal layer contains only small amount of pigment. In the papillary and in subpapillary layer, especially near hair follicles are pigment cells situated close to blood vessels.

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* By Susumu Iijima.
Studies on Melanin

Melanin granules are seen also in reticulum cells. No inflammatory changes throughout dermal layer.

Specimen No. 2. Obtained from a 30 years old woman. Duration of pigmentation more than two years. Thin epidermis. No hyperkeratosis. Several intrapapillary vacuoles are found. Perivascular infiltrates in the upper dermal layer consist of small lymphocytes and histiocytic cells. Pigment cells are increased in number. Melanin granules are phagocytosed in the reticulum cells, too. Elastic fiber is lessened in the infiltrated parts.

Specimen No. 3. Section obtained from a 37 years old female patient with pigmentation on the face for three years duration. Epidermis is slightly hyperkeratotic. Rete thickened. Basal layer is poor in pigment. In the corium, perivascular edema and infiltrate of round and histiocytic cells are noticed. These reach to the middle layer of corium. Pigment cells are unevenly distributed in the papillary and subpapillary layers.

Specimen No. 4. 29 years old female patient complaining intensive dark brown pigmentation with slight grade of erythema for two months. Epidermal cells are somewhat disordered with interepidermal vacuole formation near follicular openings. The pigment content of the basal layer is smaller than normal. Hair follicles are strongly dilated and filled with horny masses. In the corium moderate perivascular and perifollicular infiltrates consisting of lymphocytic and histiocytic cells are seen in the papillary layer, where numerous pigment cells are found especially in the vascular surroundings.

Specimen No. 5. 28 years old woman. Duration of pigmentary disturbance for four months. Rete cells are vacuolated at several places and loosely connected. Borderline to the corium not distinct. Dilation of follicular openings is remarkable. Here and there intrapapillary cell infiltration is noticed. Basal layer contains subnormal amount of melanin. Numerous pigment cells are situated in the papillary layer with inflammatory cells (Plate XV, Figure 60). These reach to the deeper layer of the corium along blood vessels.

Specimen No. 6. This was excised from a 54 years old woman. The cutaneous eruption consists of follicular keratotic papules and perifollicular pigmentation. Epidermis is nearly normal. Follicular openings are strongly dilated. Pigment content of the basal layer is rather increased. In the corium are hyperemia in the upper layer and small round cell infiltration in the surrounding of blood vessels seen, where melanin containing cells are noticed. Inflammatory changes are most remarkable in the neighbouring zone of hair follicles.

Brief summary of the histological findings is as follows. The epidermis shows various grade of inflammatory changes and these coincide with the clinical symptoms but not with the duration of the disease. The melanin content of the basal layer is uniformly decreased. In the cutis are perivascular lymphocytic and histiocytic infiltrations noticed. Pigment cells vary in number in cases, and their form coincides with usual chromatophores. Remarkable is the fact that in most of the cases histological changes are most prominent in the neighbourhood of hair follicles. The increase of melanin containing cells in the upper dermal layer reminds one of incontinentia pigmenti, and recently Doornink1) used the designation incontinentia pigmenti in the sense of a pathological feature and not a disease.
In the specimen No. 3. I stained the section with dopa reagent and got unexpectedly positive results. The technic employed is the modification of Fujiwara, and I found that after 90 minutes at 37°C, small pigment cells in the upper dermal layer undoubtedly darkened (Plate XV, Figure 61). As the free melanin granules in that layer are not stained black, it is obvious that it is not due to the adsorption of oxidated dopa. The dopa reaction of Bloch has been estimated to be an indicator of the melanogenetic activity of cells and in all reported cases of Riehl's melanosis the dopa reaction is always negative. So in order to confirm these results I stained the sections with peroxidase reagent and with permanganate-methylgreen method of Unna. The results are quite corresponding. Namely, with Oka-no's method peroxidase positive granules are indicated in the protoplasm, and with Unna's method the site of pigmentation stained with methylgreen as oxygen area (Plate IV, Figure 17). So it is found out that the pigmented area has high oxidizing potency. Therefore these results show that at least in some cases the dermal pigment cells in Riehl's melanosis act as melanoblast. Recently Capelli presented similar theory in the problem of melanin production.

Etiology and Pathogenesis: According to the conception of Vienna dermatologists all pigmentary disturbances, in which external irritants are suspected to play a rôle, are excluded from the category of Melanosis Riehl. Such theory is not accepted now because Arzt himself observed 3 male cases with apparently external causations, and Cartaud's case recovered by stopping the use of cosmetics. However, it is impossible to attribute such cases merely to external irritants. With regards to the etiology I will take up photosensitization through coarse cosmetics as external factor and hypovitaminosis as internal and as supplementary factor disturbance of endocrine and autonomic nervous system. Most of our cases are women of indoor activities having no occupational contact with tar, pitch or lubricating oil. So it is naturally suspected that crude cosmetics might be the external irritant. Such findings were reported also by Kitamura. In the analysis of the cosmetics used by patients I found in three of them admixture of lower hydrocarbons and moreover, all presented the flavor of bergamot oil. These two substances I will take up as external irritants. It is well admitted that the application of tar or oil on the cutaneous surface accelerates pigment production. If so, the occurrence of melanosis must be more frequent in our country. Therefore, the participation of another factor is readily surmised.

In the animal experiment of Itō and Nakajo, the injection of ascorbic acid and riboflavin can inhibit hyperpigmentation following bergamot oil application and ultraviolet ray irradiation. And this action is explained by the inhibition of the penetration of bergamot oil by these vitamins. If so, in hypovitaminotic state the penetration of such substance is easier than normal. The vitamin C content of the skin was tested by the modification of Rotter's method. The discoloration time of indophenole dyes is longer in this disease than controls. So it is possible that the patient's skin is more susceptible to external irritants. The same can be said with riboflavin. Though the determination of riboflavin was not made, we found higher level of pyruvic acid in patient's blood serum, and consequently surmise the deficient state of vitamins of same group. On the other hand the decrease of ascorbic acid means the loss of inhibitory action to the process of melanin
production itself.

As other signs of vitamin deficiency are almost lacking in my cases, it is not true hypovitaminosis but a sort of paravitaminosis in the sense of Comel. The rôle of autonomic nervous system in melanogenesis is not touched here. I mention only the frequent positive Aschner's phenomenon and positive pharmacodynamic reaction to adrenalin and pilocarpine.

The participation of endocrine system especially of adrenals and ovaries in this disease was already mentioned by Rolba. The results of recent investigations are not uniform. Adrenal insufficiency is noticed by Spira, hypofunction of thyroid by Carteaud, while Sirres found no endocrine disturbances. So we must wait for more comprehensive data in the future, and the fluorine-poisoning theory of Spira, hypercupremia of Panlais require further confirmation. The author refers only to the facts that in some cases the pigment patches were noticed on the buccal mucous membrane and that in such cases blood pressure was low.

According to the dopa theory of Bloch the pathogenesis of this dermatose is explained by the increased pigment production in the epidermis and by the dropping of melanin granules from the epidermis to the cutis. While on the other hand Meirowsky supported by the animal experiment of Lipschütz maintains that the pigmented cell in this disease is not chromatophore but melanoblast. In my above mentioned histochemical study the enhanced oxidizing potency of the pigmented area was actually ascertained and in one case even the positive dopa reaction was noticed. Therefore it is probable that at least in some cases melanin production takes place in the corium, in the so-called chromatophores. Such theory is partly supported by the recent findings of Capelli who think it possible that melanin, under some pathological condition, can be produced in tissues and cells which contain no specific enzymes.

**Treatment**: Various therapeutic procedures are reported in this therapy-resistant dermatose. By the repeated injections of ascorbic acid, adrenal preparation and epinephrine combined with external application of weak alcoholic solution of resorcinol, we observed improvement of cutaneous disorders in several cases. Kitamura recommended the injection of small amount of oxophenarsine. Recently Kawamura reported rapid recovery by intramuscular injection of BAL. In France the use of H365 is recommended by Grupper and others. However, in judging the effect of some or other method we must always keep in mind that spontaneous recovery is always possible in this disease.

**SUMMARY**

This disease occurs by the external application of various irritative, photosensitizing substances in individuals in hypovitaminotic states of vitamin C and B complex. And endocrine dysfunction and dysharmony of vegetative nervous system play supplementary rôle.

In the histochemical study the possibility of dermal pigment production is surmised.

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XVI. Influence of Bergamot Oil applied together with Vitamin C on Melanin Production*

In 1937 Urbach and Kral\(^1\) reported an interesting observation that the melanin production is inhibited when bergamot oil, which is known as the substance increasing cutaneous pigment, is applied with vitamin C which inhibits the dopa reaction. The authors attempted the follow-up study on this mechanism in human and animal experiments.

**Method:** The flexor surface of the forearm of an adult is rubbed with alcohol then the agents are rubbed for five minutes and are rubbed off with alcohol after half an hour. The irradiation is made using quartz mercury vapor lamp (the shortest wave length is 290 mp) with the uviol glass filter.

**Experiment with bergamot oil and analogous volatil oils or saturated and unsaturated fatty acids:** Of the agents examined including bergamot oil, volatile oleum lavandulae and terebinthine, saturated stearic and palmitic acid, unsaturated carboxylic and linolenic acid, none but bergamot oil increased pigmentation following ultraviolet irradiation.

**Experiment with several irritants:** As the result of applying 50% glycerin alcohol, 2% chrysarobin chloroform and coal tar and irradiating ultraviolet ray, all of them increased melanin production.

**Experiment with additional vitamin C:** The result which is showed in the Table XIV was obtained by intravenous injection of vitamin C (2 cc. contains 100 mg. ascorbic acid), applying each substance after half an hour and irradiating ultraviolet ray after 30 or 60 minutes. That is, only the result with bergamot oil was accordant with that of Urbach and in other applications the results were contrary (Table XIV).

<table>
<thead>
<tr>
<th>Table XIV</th>
</tr>
</thead>
</table>

Pigmentation after the Application of Pigment-increasing Substances and Ultraviolet Irradiation Following Vitamin C Injection

<table>
<thead>
<tr>
<th>Substances topically applied</th>
<th>Number of experimented persons</th>
<th>Skin reaction after irradiation esp. pigmentation</th>
<th>Relation with the experiment of Urbach</th>
</tr>
</thead>
<tbody>
<tr>
<td>80% bergamot oil alcohol</td>
<td>50</td>
<td>33</td>
<td>17</td>
</tr>
<tr>
<td>50% glycerin alcohol</td>
<td>12</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2% chrysarobin chloroform</td>
<td>10</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Coal tar</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\(C \equiv T\) Controlled area
\(T\) Tested (applied) area

Table XV showed the results of the experiments in which vitamin C was substituted for other vitamins, and the results of the injection experiments of vitamin B2 and vitamin B2 plus C were the same as those of vitamin C.

**Table XV**

*Pigmentation after the Application of 80% Bergamot oil Alcohol and Ultraviolet Irradiation with Another Vitamins or Hormones instead of Vitamin C*

<table>
<thead>
<tr>
<th>Substances subcutaneously injected</th>
<th>Number of tested persons</th>
<th>Skin reaction after irradiation esp. pigmentation</th>
<th>Relation with the experiment of Urbach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B complex</td>
<td>15</td>
<td>C&gt; T: 0  C= T: 0  C&lt; T: 15</td>
<td>Not coincides</td>
</tr>
<tr>
<td>Vitamin B2</td>
<td>12</td>
<td>C&gt; T: 10  C= T: 2  C&lt; T: 0</td>
<td>Coincides</td>
</tr>
<tr>
<td>Vitamin B2+C</td>
<td>6</td>
<td>C&gt; T: 6  C= T: 0  C&lt; T: 0</td>
<td>Coincides</td>
</tr>
<tr>
<td>0.1% adrenalin hydrochloride</td>
<td>15</td>
<td>C&gt; T: 12  C= T: 1  C&lt; T: 2</td>
<td>Coincides</td>
</tr>
<tr>
<td>Adrenocortical hormone</td>
<td>12</td>
<td>C&gt; T: 3  C= T: 2  C&lt; T: 7</td>
<td>Not coincides</td>
</tr>
<tr>
<td>Thyroid hormone</td>
<td>5</td>
<td>C&gt; T: 0  C= T: 0  C&lt; T: 5</td>
<td>Not coincides</td>
</tr>
</tbody>
</table>

*Experiment with hormones instead of vitamin C:* As showed in Table XV only in the experiment in which ultraviolet rays were irradiated five or ten minutes after the injection of adrenalin the pigment production was inhibited and this is identical to the experiment of Urbach.

In regard to the mechanism of the experiment, Urbach assumed that the skin comes to be photosensitive by repeated applications of bergamot oil resulting in increased pigmentation after ultraviolet irradiation, but that the decomposition product of vitamin C combines with bergamot oil and inhibits its action on the skin. However, our experimental results with vitamin B2 or adrenalin don’t support this theory. As the specific chemical affinity of vitamin C to bergamot oil can’t be admitted, the physical properties of this substance were examined in the following three experiments. 1) The refractory index of bergamot oil measured by Pulfrich’s refractometer is not significantly different from other plant oils (tsubaki, olive, peanut, sesame, hydnocarpus, bellandonnaseed, rice, soyabean, castor and linseed). 2) The layer of bergamot oil does not converge ultraviolet ray. 3) The ultraviolet absorption band of vitamin C together with bergamot oil is quite same to that of 80% bergamot oil-alcohol. Thus, the authors learned the mechanism of Urbach’s experiment was due neither to the mere chemical antagonism nor to the physical characteristics of bergamot oil, so we made biochemical observation by the following animal experiment.

*Animal experiment:* Clipping the hairs of costoventral region of albino rabbit as shortly as possible, making nearly the same experiment as those of human body.

*Results:* The state of dermatitis was seen after applying bergamot oil or coal tar, and it became still more remarkable by additional ultraviolet irradiation. The inhibition of the inflammation through vitamin C or B2 injection, was recognized neither macroscopically nor histologically; but the material being the albino rabbit, melanin could not, of course, be observed. When the penetration grade of topically applied
Studies on Melanin

oil was tested by Sudan III in case of bergamot oil application, many lipoid granules were recognized at hair-follicle, its surroundings and all over the corium, (Plate XV, Figure 63) and the accumulation of lipoid was increased by the ultraviolet irradiation. But when the ultraviolet irradiation was performed together with vitamin C injection, that is, in case of the experiment same as one of Urbach, lipoid granules gathered at the surface of stratum corneum in thick layer, its penetration into epidermis was almost suppressed. In corium it was limited only around hair-follicle (Plate XV, Figure 64), and had not a bit of difference from nontreated control specimens (Plate XV, Figure 62). Nearly same results were obtained in the experiments in which oleum lavandulae, terebinthine and coal tar were used. By the way the results were the same when vitamin B2 was used instead of vitamin C.

Histochemical examination of oxygen and reduction area by Unna’s method:2) The piece of skin used in the experiment on rabbit was examined and very interesting results were obtained; the reduction area was found at stratum corneum, hair-follicle and non-striated muscular fibers, and the oxygen area scattered in epidermis and cutis, besides around hair-follicle in the skin of non-treated normal rabbit (Plate XVI, Figure 65). When ultraviolet ray was irradiated, the oxygen area did not vary but reduction area around hair-follicle became more or less considerable. Besides there were no significant differences from the observation of non-treated rabbit to which the ultraviolet ray was irradiated together with vitamin C injection. But the specimen applying bergamot oil no notable alteration was found in the reduction area in comparison with the skin of non-treated rabbit. However peculiar alteration was found in oxyge area, i.e. it became remarkable in the lower part of epidermis and in the upper part of cutis (Plate XVI, Figure 66). The degree was still higher in the ultraviolet irradiated specimen. According to Urbach in the case to which vitamin C was injected, bergamot oil was applied, and ultraviolet ray was irradiated oxygen area became indistinct and on the contrary reduction area became distinct around hair-follicle and all over the epidermis and cutis (Plate XVI, Figure 67). The same alteration was proved when vitamin C was substituted for vitamin B2.

In the case to which only coal tar was applied, no alteration was found in reduction area but oxygen area became considerable and its degree became higher by ultraviolet irradiation. This result is contrary to that of bergamot oil application.

COMMENT

From the above animal experiments the authors obtained two important findings. The first is the phenomenon that in the specimen stained by Sudan III, it was admitted, each application penetrated deeply into skin when it was used either alone or together with ultraviolet irradiation, but the penetrability of these oils into epidermis was inhibited remarkably by the vitamin C or B2 injection. We have seen no report that the vitamin C or B2 has the activity to decrease the permeability of the skin. But it is noticed that these vitamins contain negative group in their chemical constitution, therefore this evidence is compared with the fact that the substances which contained negative group have the inclination to diminish the
permeability of cell. That in the experiment of adrenalin injection the results were accordant with those of Urbach only when the interval of injection and ultraviolet irradiation was short, may be due to the immediate disappearance of the activity of adrenalin in short time.

The second is that in the experiment of vitamin C injection the considerable differences between bergamot oil and coal tar, which have the activity to increase the melanin production, were found in the investigation of oxygen area and reduction area by Unna's method. The chemical natures of both being considered that the bergamot oil is apt to be oxidized in air, thus at the surface of epidermis bergamot oil which has checked to penetrate into epidermis by vitamin C is oxidized, and acts reductively, so melanin production is inhibited, while coal tar is hard to change even when it is set in air, so it would display different results from bergamot oil.

**SUMMARY**

The authors obtained quite similar results to those of Urbach's experiments, in which the inhibition of melanin production was recognized after the application of bergamot oil and ultraviolet irradiation following vitamin C injection. Furthermore the injection of vitamin B_2_ or adrenalin instead of vitamin C showed almost similar results.

From the histochemical observations of rabbit by Sudan III and by Unna's oxidoreduction method, we confirmed that vitamin C or B_2_ injection inhibits the penetration of topically applied bergamot oil. Thus it is guessed that bergamot oil is oxidized in air and supresses the melanin production as a reducing agent.

**References**

2) Unna, Arch. f. Mikroskop. Anat., 1911, 78, 1; Biochemie der Haut, Jena 1913; Histochemie der Haut, Wien 1928.
XVII. Relationship between Melanin Production and Dioxybenzenes

Oettel recognized in his experiment of hydroquinone intoxication that in chronic intoxication the black cat are decolorized and turned in gray-white and guessed it being due to the interruption in the process of oxido-reduction. Getting the suggestion from his experiment, we made the experiment on goldfish, using hydroquinone and its isomers, resorcinol and pyrocatechol.

Method: The black goldfishes (Kurodemekin—Plate XVI, Figure 68) used in this experiment are black since the brood and their body color does not vary for life and the black pigment is believed to be melanin. Goldfishes weighing 20-40 g. were fed in water temperature at 15°-20° C. 0.5% saline solutions of resorcinol, hydroquinone and pyrocatechol respectively were made and 0.05 cc. of each solution was injected subcutaneously every other day in the ventral region of Kurodemekin.

Results: The results are shown in Table XVI.

| TABLE XVI |
| Decoloration following Dioxybenzenes Injection on Black Goldfish |
| (The sign # shows the original black tone of Kurodemekin, #, ++, +, −, shows the degree of black tone in turn) |

<table>
<thead>
<tr>
<th>Number of fishes</th>
<th>Before injection</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5th inj.</td>
</tr>
<tr>
<td>0.9% NaCl</td>
<td>5</td>
<td>(###)</td>
</tr>
<tr>
<td>0.05 cc.</td>
<td></td>
<td>2 d*</td>
</tr>
<tr>
<td>0.5% resorcinol</td>
<td>5</td>
<td>(###)</td>
</tr>
<tr>
<td>0.05 cc.</td>
<td></td>
<td>1 d</td>
</tr>
<tr>
<td>0.5 hydroquinone</td>
<td>5</td>
<td>(###)</td>
</tr>
<tr>
<td>0.05 cc.</td>
<td></td>
<td>1 d</td>
</tr>
<tr>
<td>0.5% pyrocatechol</td>
<td>5</td>
<td>(###)</td>
</tr>
<tr>
<td>0.05 cc.</td>
<td></td>
<td>3 d</td>
</tr>
</tbody>
</table>

* Number of dead fishes during the experiment

In hydroquinone the fading began at the third injection, the red-yellow tone extended gradually from the injected part to costo-abdominal region, tail and head equally (Plate XVI, Figure 69), and after tenth injection the goldfish became indistinguishable from red one (Akademekin—Plate XVI, Figure 70). The margin of fins, bulbi and palpebra, however, remained black, and the decoloration of the

pigment was not recognized. The injections of resorcinol, pyrocatechol and saline (control) show no decoloration at all.

The goldfish decolorized by hydroquinone injection regenerated the black pigment slowly and diffusely from the margin of fins where the black pigment remained in about three weeks after the end of the injection, and became the original Kurodeme-kin in forty days (Table XVII).

<table>
<thead>
<tr>
<th>Number of fishes</th>
<th>After the last injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>18 days</td>
</tr>
<tr>
<td>2 (−)</td>
<td>1 (±)</td>
</tr>
</tbody>
</table>

**Influence of sunlight and temperature of water:** The black pigment of amphibia or fish, etc. is often influenced by sunlight or temperature. We performed the experiments, changing these conditions. In regard to the conditions of sunlight we made three gradations of intensity of illumination; the first was “dark,” in which case the aquarium of goldfish was covered with black cardboard, the second is “lighted” in case of which no direct rays were shone, and the last is “well lighted” in which the aquarium was set at the side of window towards the south. In consequence of the experiments of hydroquinone injection to five goldfishes in each conditions, the decoloration appeared in “well lighted”, in “lighted” and in “dark” in turn and the regeneration of the black pigment after the last injection was fastest in “well lighted” revived completely in month, and was latest in “dark.”

In respect of the water-temperature, making the experiments in greenhouse in winter under the following conditions; temperature below 15°C, 15°C–20°C, and above 20°C. We found both depigmentation and pigmentation was fastest in case of the optimal temperature (15°C–20°C) to goldfish.

**Experiment with vitamins, hormones, etc.:** vitamin C, B₂, B-complex, adrenocortical hormone, glutathione, albalite (bleaching agent), etc. were injected on black goldfish by the same method to that of hydroquinone but no depigmentation was observed. Merely the light brownish red color was observed after 20 minutes of 0.05 cc. adrenalin injection (0.1%) but it returned to black after several hours.

**Histological investigation of skin and scale of goldfish:** Large quantity of melanin were contained in the scales and epidermis of black goldfish, and yellow-scarlet lipochrome mixed irregularly with them. Melanin granules of scales and epidermis decreased by the hydroquinone injection, melanin disappeared completely in the specimen which was excised after seventh injection, and dopa reaction turned to negative. But the chromatophores in corium were kept normal throughout the experiment and were not influenced by hydroquinone. Microscopically the temporarily fading of melanin following the adrenalin injection was the contraction of melanin granule and the dopa reaction was always positive at the same degree.
Studies on Melanin

COMMENT

It seems reasonable to consider that melanin is decolorized by reducing potency of hydroquinone as Oettel guessed, but we can’t be convinced of it because the decoloration of melanin was not recognized in case of pyrocatechol which has stronger reducing potency. If the injection of hydroquinone induces regressive metamorphosis in epidermis, it is not explained why it acts only on epidermal melanoblast selectively, and causes no alteration in other tissues.

In order to make clear the character of hydroquinone, the alkalization potency of skin was examined by the litmus cutaneous test, according to Leszczynski and Falik’s method.

Method: The alkalization potency is indicated as follows; red litmus solution (pH 6.46) and blue litmus solution (pH 7.0) are injected intracutaneously at the same time, and the duration that it took till both red and blue wheals change into the equal dark blue is measured.

In this experiment albino rabbits were used instead of goldfish, and litmus cutaneous test was made after half an hour after making previous subcutaneous injection of the dosage of one hundred times as much than goldfish culculating from dosis letalis minima of dioxybenzenes to goldfish and rabbit. The results were shown in the Table XVIII.

<table>
<thead>
<tr>
<th>Table XVIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkalization Potency measured by Leszczynski-Falik’s Litmus Method</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal number</th>
<th>Before injection</th>
<th>After 1st inj.</th>
<th>After 3rd inj.</th>
<th>After the last injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>2% hydroquinone 1.25 cc.</td>
<td>20' 40''</td>
<td>36'00''</td>
<td>43'20''</td>
<td>38'00''</td>
</tr>
<tr>
<td>2% pyrocatechol 1.25 cc.</td>
<td>20 30</td>
<td>37 40</td>
<td>46 30</td>
<td>38 15</td>
</tr>
<tr>
<td>2% resorcinol 1.25 cc.</td>
<td>20 20</td>
<td>54 15</td>
<td>56 00</td>
<td>25 12</td>
</tr>
<tr>
<td>2% vitamin C 1.25 cc.</td>
<td>22 00</td>
<td>56 05</td>
<td>54 00</td>
<td>14 18</td>
</tr>
</tbody>
</table>

While the time of normal litmus test in rabbit skin was 19–22 minutes, it lengthened twice upwards at the first or third injection of hydroquinone and pyrocatechol. The lengthened values by hydroquinone showed for a week after the last injection, but by pyrocatechol returned next day to the normal value. It is learned from the results that resorcinol is stable, excludes out of body without alteration in vivo and pyrocatechol is labile, changes easily, and stands still for short time in vivo. Contrary to them, as the degree of stability of hydroquinone is moderate and it stays long in body, it is guessed that the reducing potency of it is displayed thoroughly,
and the alkalization potency of the injected body, it is appeared, is suppressed, consequently the melanin production which is the oxidation is diminished. While dopa reaction was weakened in the section fixed with acid formalin, but (on the contrary) it can be proved after several days in the sections fixed with neutral one, and this fact supports our inference. That is, it is guessed that the hydroquinone injection causes the disorder of alkalization potency of skin and lets the dopa-oxydase reaction weaken, and at last suppresses the melanin production.

**Summary**

Fading of melanin in the epidermis and scales was found by hydroquinone injection on black goldfish. Dopa reaction turned to negative after the decoloration had been completed, but the melanin in the chromatophore remained unchanged. Optimal temperature for this process was $15^\circ-20^\circ$ C., and ultraviolet ray accelerated both depigmentation and repigmentation.

Two isomers of hydroquinone, resorcinol and pyrocatechol, however, were proved ineffective in spite of the stronger reducing potency of the latter than hydroquinone.

The mechanism of the depigmentation by hydroquinone could not be explained by mere reduction of melanin, but rather by the special suppression against dopa oxydase through its alkalization potency.

The temporary fading by adrenalin injection was microscopically the contraction of melanin granules.

**References**

2) Leszczynski and Falik, Dermat. Wschr., 1936, 103, 965.
XVIII. Influence of Benzoquinone on Melanin Production*

The experiments of melanin production, using benzoquinone which is the catalyser in the metabolism of amino acid and is chemically contrary at all to hydroquinone which has the decolorizing action of melanin mentioned in the previous report were performed.

Method: The yellow-red and white goldfish (Wakin) were used. The condition of the feeding and the method of injection were the same as the previous report; 0.5% saline solution of p-benzoquinone was freshly made, and 0.05 cc. of it was injected subcutaneously every other day at the costoventral region of goldfish.

Results: The results obtained were shown in the Tables XIX and XX.

**Table XIX**

Black Pigment Production following p-Benzoinnone Injection on Yellow-Red Goldfish

<table>
<thead>
<tr>
<th>Number of fishes</th>
<th>Before injection</th>
<th>After</th>
<th>3rd inj.</th>
<th>5th inj.</th>
<th>8th inj.</th>
<th>10th inj.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9% NaCl</td>
<td>5 (-)</td>
<td>5 (-)</td>
<td>4 (-)</td>
<td>3 (-)</td>
<td>3 (-)</td>
<td></td>
</tr>
<tr>
<td>0.05 cc</td>
<td>(-)</td>
<td></td>
<td>1 d*</td>
<td>1 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5% p-</td>
<td>10 (-)</td>
<td>3 (+)</td>
<td>2 (+)</td>
<td>2 (+++)</td>
<td>6 (##)</td>
<td></td>
</tr>
<tr>
<td>benzoquinone</td>
<td></td>
<td>5 (+)</td>
<td>5 (+++)</td>
<td>4 (##)</td>
<td>1 (++)</td>
<td></td>
</tr>
<tr>
<td>0.05 cc.</td>
<td></td>
<td>2 d</td>
<td>1 d</td>
<td>1 (+)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Number of dead fishes during the experiment

**Table XX**

Fading of the Black Pigment after the Last Injection of p-Benzoinnone

<table>
<thead>
<tr>
<th>Number of fishes</th>
<th>After the end of injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 days</td>
</tr>
<tr>
<td>7 (##)</td>
<td>2 (###)</td>
</tr>
<tr>
<td>5 (###)</td>
<td>4 (##)</td>
</tr>
</tbody>
</table>

As the influence of p-benzoquinone injection to the yellow-red goldfish (Plate XVI, Figure 71), black-brown pigment production was seen at the injected part or the margin of fins after the third injection, the black tone strengthened and extended. As the number of injection goes on the godfish showed thorough the appearance of

Influence of Benzoquinone on Melanin Production

black one after the tenth injection (Plate XVII, Figure 72), i.e., after twenty days from the beginning of the experiment. The strength of the black tone could be recognized still for about one month after the end of injection (Plate XVII, Figure 73), but the fading of the black color was seen gradually in two or three months after the end of injection but it took considerable time to return to the original yellow-red one.

The results of the experiments made on yellow-red goldfish and white one with or without yellow-red spots were shown in the Table XXI.

**Table XXI**

<table>
<thead>
<tr>
<th>Black Pigment Production following p-Benzquinone Injection on White-Yellow-Red Goldfish and White Goldfish</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of fishes</strong></td>
</tr>
<tr>
<td>-----------------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Yellow-red goldfish</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>White goldfish with</td>
</tr>
<tr>
<td>yellow-red spots</td>
</tr>
<tr>
<td>White goldfish</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

The white goldfish was high in the rate of death, the black pigment production was very slow, and the injected part and the margin of fins became black at first after the fifteenth injection. In white goldfish with yellow-red spots (Plate XVII, Figure 74) the considerable black color tone appeared in the yellow-red spots and at the margin of fins after about 12th injection (Plate XVII, Figure 75).

Influence of sunlight and temperature of water: In consequence of the experiment in yellow-red goldfish under the same conditions to the previous report, the black color tone appeared earliest in "well lighted" and latest in case of "dark." In regard to the water temperature most considerable black tone appeared at the optimal temperature (15°–20° C). By the way, it is very interesting that two white goldfishes with yellow-red spots among those in which the black tone was produced by benzoquinone had faded once after the experiment, and remained as a white yellow-red one during the winter, but the said black spots as were seen in the previous experiment reappeared again unexpectedly at the middle of May next year, and this may be, it is guessed, because the metabolism of goldfish was promoted by the influence of sunlight and the temperature.

Experiments with additional vitamins and gluthatione: It must be noticed that in white yellow-red goldfish the black pigment production due to the previous injection of p-benzoquinone was remarkable in the region in which lipochrome situated. Lipochrome is a compound of intricate composition, and its principal component is carotinoid pigment. This reminds us of the relation between carotinoid and vitamin A, so we used this with p-benzoquinone. In the experiment, using vitamin C, E2 and gluthatione, etc. with p-benzoquinone, the black pigment appeared more
remarkable as shown in Table XXII, in case of that vitamin A, C, B₂ and glutathione were used accompany with p-benzoquinone than that only p-benzoquinone was injected.

**Table XXII**

Experiment adding Vitamins and Glutathione on Yellow-Red Goldfish

<table>
<thead>
<tr>
<th></th>
<th>Number of fishes</th>
<th>Before injection</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>5th inj.</td>
</tr>
<tr>
<td>1.0% p-quinone</td>
<td>5</td>
<td>(−)</td>
<td>2 (++ )</td>
</tr>
<tr>
<td>+ vitamin A</td>
<td></td>
<td></td>
<td>2 (± )</td>
</tr>
<tr>
<td>1.0% p-quinone</td>
<td>5</td>
<td>(−)</td>
<td>2 (++ )</td>
</tr>
<tr>
<td>+ vitamin B₂</td>
<td></td>
<td></td>
<td>2 (+)</td>
</tr>
<tr>
<td>1.0% p-quinone</td>
<td>5</td>
<td>(−)</td>
<td>2 (+)</td>
</tr>
<tr>
<td>+ vitamin C</td>
<td></td>
<td></td>
<td>1 (+)</td>
</tr>
<tr>
<td>1.0% p-quinone</td>
<td>5</td>
<td>(−)</td>
<td>2 (+)</td>
</tr>
<tr>
<td>+ glutathione</td>
<td></td>
<td></td>
<td>3 (+)</td>
</tr>
</tbody>
</table>

**Histological investigation (Plate XVIII, Figures 77–82):** There were no melanin in the scales and epidermis of yellow-red goldfish, there was only yellow-red lipochrome, and dopa reaction was negative. In case of white goldfish there was no pigment cells, but mere guanine crystal. Histological investigation of the scales and skin of the yellow-red goldfish which became visible black following the p-benzoquinone injection, many black-brown pigment granules were produced newly in the scales and epidermis, mixed with the ready-existing lipochrome which became almost invisible as the degree of black color tone became distinct and the same appearance as the microscopic observation of the scales and epidermis in the black goldfish (Kurodemekin) is observed. Dopa reaction turned obviously to positive and the black pigment faded after several hours' immersing in hydrogen peroxide.

**Chemical investigation of newly produced black pigment:** The nature of melanin being yet not made clear, its identification is very difficult, and there are only the following standard of it: dopa reaction, the silver reducing agency, the decoloration by hydrogen peroxide, and the hard solubility in most solvents, etc. but all these are not always precise. Therefore, it must be disputable to consider the black pigment produced by benzoquinone as melanin. We tried with many kind of solvents in order to examine by spectroscopical method, but could not succeed to dissolve it at all. Only light-brown extract was obtained by the method of Przibram¹ and Tutshku;² it is to immerse the tissue in the solution consists from 9 parts of 75% alcohol and 1 part of ether and set at the dark, cool place for ten weeks. Then the extract is treated with sulphuric and nitric acid till the lipochrome reaction turns negative. The black human hair, the skin-piece of nevus pigmentosus and the sepia of cuttlefish were compared with spectroscopically as the control by the same procedure. The continuous absorption band was shown, at the center of which was 4000 Å, and the special absorption spectrum was not seen both in the extract from scales or skin of goldfish and those of controlled materials. This result was the
Influence of Benzoquinone on Melanin Production

same as was reported by Dannee, and it is guessed that it is owing to the complex constitution of melanin. Thus I guessed newly produced black pigment is melanin from these chemical natures.

Experiments with tyrosine, dopa, pyrrol and adrenalin: We made the experiments in yellow-red goldfish, using tyrosine, dopa, pyrrol, tryptophan and adrenalin, those which are considered as melanogen; hydrogen peroxide as the oxidizing agent, copper or gold preparations as the catalyst, and pilocarpine as the contrast to adrenalin with the single injection or combined with the ultraviolet irradiation, but in any cases the black pigment was not produced.

COMMENT

In regard to pigment experiment by benzoquinone, only Mayer's experiment in vitro is cited but it is not useful for our purpose. The fact that black-brown color tone appeared first at the margin of fins distant from the injected part coincides with the fact that the margin of fins remained black even when the fading of melanin was noticed in the hydroquinone experiment of the previous chapter. Therefore it is clear that the black substance appeared by $p$-benzoquinone injection was not the deposit of oxidized benzoquinone. The periphery of fins is widest in the external surface of body and so the degree of absorption of oxygen from water is highest and oxydase is activated sufficiently. Moreover the fact that the red spots turned to black selectively in white yellow-red goldfish in our experiment can't be considered as the result of deposition of oxidized benzoquinone. In the spectroscopic test, $p$-benzoquinone which becomes black when left standing in air presented the obvious absorption band at 3600 $\AA$. This is quite different from the continuous absorption band of black-brown extracted solution obtained from the tissue after $p$-benzoquinone injection.

Thus from the abovementioned histological and chemical observation (p. 94) we guessed that the black pigment produced in the experiment of $p$-benzoquinone is melanin.

The chemical process of melanin production is the difficult problem, which has not been made clear, but the hypothesis of Evans and Raper explains it as follows; tyrosine turns to dopa by tyrosinase, it is oxidized by dopa oxydase to dopa-quinone, and afterwards becomes black-brown melanin after oxidizing without requiring enzyme. In view of this hypothesis, the results of our experiment are explained as follows. No dopa reaction was found both in epidermis and scales of yellow-red and white goldfish but the injection of $p$-benzoquinone dopa reaction turned to positive and melanin production was observed. Thus $p$-benzoquinone is considered as a biocatalyser. This was confirmed by the observation that melanin production increased when such activator like vitamin C, B$_2$ or gluthatone was added. In regard to the relationship between melanin and lipochrome, there are the treatises of Kutschera-Aichbergen and Abeloos, etc. The close correlation between melanin production and carotinoid substance is surmised according to the following facts obtained from our experiment; the first is that the melanin is found mixed with lipochrome in black goldfish and the second is that vitamin A which was considered as a biocatalyser by Euler and Hellström (1928) presents the activator-action.
Summary

The black pigment was found after \( p \)-benzoquinone injection in yellow-red or white goldfish and dopa reaction turned to positive at the same time.

The pigmentation took place earlier in goldfish with pre-existent lipochrome granules than in white one.

Temperature and sunlight were found to influence the melanin production.

This pigment production was accelerated by injection of vitamin A, B\(_2\), C or gluthatione.

The black extract of the newly pigmented scales and skin of goldfish presented all chemical and physical characteristics of human melanin.

Thus it is guessed that \( p \)-benzoquinone participated in the melanin production as a kind of activator.

References

1) Przibram, Pfüger's Arch., 1913, 153, 385.
2) Tutschku, Biochem. Z., 1923, 135, 585.
XIX. Influence of Hormones and Others on Melanin Production*

In lower vertebrates their color change is due to the concentration or dispersion of melanin exists in pigment cells. These pigment cells concerned are under the control of the sympathicus, and so it is generally recognized that the endocrine influence which is closely connected with the sympathicus has a great importance. In higher vertebrates the direct relation between the pigment cell and the nervous system cannot be elucidated, but Jores observed that the human melanophore-diffusing hormone is related to the dark adaptation which causes the movement of retinal pigment. In addition to that, the correlation between the pigment of skin and the autonomic nerve or the endocrine status in the human body is able to know in Addison's disease, menstruation and gravidity, but the most part of this question cannot be elucidated as yet.

We have investigated in order to make up previous experiment and observed the color change of goldfish caused by various hormones and poisons of vegetative nervous system.

Experiment on Black Goldfish (Kurodemekin)

Materials and methods of this experiment are quite same as Itō and Nakajo’s. Results: It is generally recognized that the concentration of melanophore in fishes is provoked by feeding with epinephral substance or by injection of adrenalin, and Itō–Nakajo’s experiment confirmed these facts. We observed in the same way, the temporary decoloration due to the melanophore concentration which is provoked by injection of ephedrin or wake-amines (1-phenyl-2-methyl aminopropane hydrochloride), both of them have sympathicotropic action. On this experiment, the decoloration provoked by injection of ephedrin was less intense than that of adrenalin, but was much more durable; when provoked by wake-amines, the decoloration was slight and continued only short time. Althouth the melanophore concentrating action with ergotoxin is observed by Barbour and Spaeth on fishes, by Leszczynski on frog, but this action is very slight in our experiment. (Table XXIII)

It has been known that chinine or cocaine is capable of giving positive melanophore reaction, but when adrenalin was taken with them, the melanophore concentrating action of adrenalin was suppressed slightly.

It is confirmed by many investigators that the injection of insulin in large dose presents the increased adrenalin in blood as the results of hypoglycemia. Therefore we attempted the experiment by the injection of insulin in large dose but no decorolation in the skin of goldfish was observed. Injection of vitamin P had no influence upon this experiment.

* By Minor Itō and Masahiko Watanabe. Published in Tohoku Igaku Z., 1947, 36, 1 (Japanese).
Studies on Melanin

Table XXIII
Temporary Decoloration on Black Goldfish
(The sign ### shows the original black tone of goldfish, ++, +, and + show the degree of black tone in turn)

<table>
<thead>
<tr>
<th>Number of fishes</th>
<th>Before injection</th>
<th>After injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of fishes</td>
<td>10'</td>
</tr>
<tr>
<td>0.1% adrenalin 0.05 cc.</td>
<td>5</td>
<td>###</td>
</tr>
<tr>
<td>4% ephedrin 0.05 cc.</td>
<td>2</td>
<td>###</td>
</tr>
<tr>
<td>Wake-amine 0.05 cc.</td>
<td>1</td>
<td>###</td>
</tr>
<tr>
<td>0.1% ergotoxin 0.1 cc.</td>
<td>3</td>
<td>###</td>
</tr>
<tr>
<td>0.1% adrenalin 0.1% chinine 0.05 cc.</td>
<td>2</td>
<td>###</td>
</tr>
<tr>
<td>0.1% adrenalin 0.5% cocaine 0.05 cc.</td>
<td>2</td>
<td>###</td>
</tr>
</tbody>
</table>

Experiment on Yellow-Red Goldfish

Though it is generally recognized that the specific action of pituitary hormone disperses melanophore, yet chinine, cocaine, acetylcholine, caffeine, yohimbine, strychinine, acetic acid, etc. are also the substances which give the positive melanophore reaction although they have no specificity. In addition to that, Zondek and Krohn5) found in their experiment on Thoxinus laevis that the matrimony lobe is due to the dispersion of black and red pigment and is selectively formed by the administration of intermedin. In Komori's6) experiment on Acheilognathus tabira, the matrimony lobe was selectively formed by pituitary hormone, and it was also formed by yohimbine, morphine, ergotine, chloral hydrate, strychinine, ether, chloroform and atropine. Lipshutz, Bloch and Schrafl,7) Tsubota8) observed hypertrophy of mamma and hyperpigmentation of mammilla by the application of ovarial hormone in their experiment on guinea-pig.

As indicated on Table XXIV in the series of our experiments with hormones and poisons, we could observe the production of black pigment by injection of pituitary hormone of the anterior lobe, acetic acid and of hematoporphyrin. The gonadotrophic hormone extracted from urin could not produce the black pigment, but by applying pituitary hormone of the anterior lobe (prolan A), we have observed the positive result coincides with the process of the melanophore reaction, and from this fact, we suppose that this hormone plays probably the part of Euler's so-called hormozyme. It was described that the injection of diluted acetic acid—Thumberg called it a sort of metabolic substance—has a melanophore dispersing action. In our experiment also, we observed the production and the enlargement of black fleck. Hematoporphyrine is a substance with photodynamic action; applying it in our experiment at the same time with the ultraviolet irradiation, we observed the increased
Influence of Hormones and Others on Melanin Production

**TABLE XXIV**

Pigment Production with Hormones or Poisons on Yellow-Red Goldfish

(−, ±, +, ++...Grade of the pigmentation)

<table>
<thead>
<tr>
<th></th>
<th>Number of fishes</th>
<th>Before injection</th>
<th>After 5th inj.</th>
<th>10th inj.</th>
<th>15th inj.</th>
<th>20th inj.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pituitary hormone (anterior lobe)</td>
<td>3</td>
<td>−</td>
<td>1(+)</td>
<td>1(+*)</td>
<td>1(+)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1(±)</td>
<td>1(+)</td>
<td>1 d</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1(−)</td>
<td>1(+)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gonadotropic hormone from urine</td>
<td>6</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Pituitary hormone (posterior lobe)</td>
<td>4</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Thyroid hormone</td>
<td>2</td>
<td>−</td>
<td>1(−)</td>
<td>1 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone</td>
<td>2</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Luteosterone</td>
<td>2</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Folliculin</td>
<td>2</td>
<td>−</td>
<td>−</td>
<td>2(±)</td>
<td>2(±)</td>
<td>2(±)</td>
</tr>
<tr>
<td>0.8% acetic acid</td>
<td>2</td>
<td>−</td>
<td>−</td>
<td>2(+)</td>
<td>1(+)</td>
<td>1 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1(+)</td>
<td>1(+)</td>
<td>1 d</td>
<td></td>
</tr>
<tr>
<td>Yohimbine</td>
<td>2</td>
<td>−</td>
<td>−</td>
<td>1(−)</td>
<td>1(−)</td>
<td>1(−)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 d</td>
<td>1(−)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematoporphyrin</td>
<td>3</td>
<td>−</td>
<td>−</td>
<td>1(+)</td>
<td>1(+)</td>
<td>1(+)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2(−)</td>
<td>1(−)</td>
<td>1(−)</td>
<td>1(−)</td>
</tr>
<tr>
<td>Hematoporphyrin</td>
<td>2</td>
<td>−</td>
<td>1(+)</td>
<td>1(+)</td>
<td>1(+)</td>
<td></td>
</tr>
<tr>
<td>with ultraviolet ray</td>
<td></td>
<td></td>
<td>1(+)</td>
<td>1(+)</td>
<td>1 d</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1(−)</td>
<td>1(+)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>4</td>
<td>−</td>
<td>2(−)</td>
<td>2(−)</td>
<td>2 d</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 d</td>
<td>2(−)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Number of dead fishes during the experiment

melanin production in the region about the dorsal fin of goldfish (Plate XVII, Figure 76).

**SUMMARY**

Adrenalin, ephedrin, such wake-amines as 1-phenyl-2-methyl aminopropane hydrochloride could produce temporary decoloration in black gold-fish. Total and permanent fading of melanin, however, could not be achieved with these substances.

Pituitary hormone of the anterior lobe, hematoporphyrin and acetic acid produced black pigment in yellow-red goldfish. Other substances examined (thyroid hormone, pituitary hormone of the posterior lobe, acetylcholine, etc.) were found useless.

From these facts it is clear that complete decoloration of melanin is difficult by injection of melanophore-dispersing substances, and a special bio-catalytic substance such as hydroquinone is required for this purpose.

**References**

1) Jores, Klin. Wschr., 1933, 12, 1599.
Studies on Melanin

2) Itô and Nakajo, this Supplement, 88, 92.
7) Bloch and Schrafl, Arch. f. Dermat., 1932, 162, 268.
XX. General Conclusion*

In summarizing our experiments mentioned in the foregoing chapters, the following conclusions can be reasonably reached.

The Participation of Nervous System in Melanin Production

By the results of Bielschowsky-Seto's silver impregnation method, it is found that nevus cell of pigmented nevus originated from neuroectodermal system which is homogenetical to Schwannian cells just as Masson stated. In an autopsy case of abortive Recklinghausen's disease without neurofibroma, preneurinomatous changes were noticed microscopically in the pigmented area and in solar plexus. Further circumscribed increase of Schwannian nuclei of larger nerve bundle is observed in the deeper dermal layer of pigmented area. These findings disclose the fact that there exists close relationship between Recklinghausen's fleck and neurofibroma, and are influential data of nerve cell theory stated by Verocay, Held, Kyrle, Masson, Cailliau, Jausion-Levy-Coblentz-Spillmann and recently by John and Ormea.1) The frequent occurrence of so-called neurofibromatous structure of nevus cell is reported by Becker2 who in the histological study of 741 cases of pigmented nevi found in 72.5% the structure of the third type of Miescher.3) Thus the nevus cell which may present polymorphism according to the local peculiarities, is uniformly of neuroectodermal origin. These facts give clue also to the polymorphic histological picture of melanocarcinoma.

On the contrary, the pigment cell of Mongolian spot, blue nevus and nevus fusco-caeruleus belong to mesenchymal melanoblast. In the latter two diseases which are properly interpreted as localized malformation, the histological structure is apparently more exaggerated than in physiological character i.e. Mongolian spot, and the affinity to nervous system is more conspicuous suggesting the coincidence with endo- or perineurium. This finding as well as the results of phylogenetical investigation of Mongolian spot, leads us to the conclusion that the pigment cell in these dermatoses belong to homogenetical melanoblasts in the sense of perineurale Pigmenthülle of Weidenreich. Though Mongolian spot disappears ontogenetically in a prescribed duration in which it becomes estranged itself from nervous system and on the contrary, blue nevus and nevus fusco-caeruleus are interpreted as the localized malformation close to nervous system and exist for life. This reminds one of the importance of correlation between melanin formation and nervous system. Thus we were able to verify both histologically and genetically that both ectodermal and mesodermal melanoblasts are closely related to nervous system. The conception of mesectoderm of Platt4) which was introduced into the genetical experiment on the mechanism of development of pigment cell and pigment flecks in amphibia by Harrison,5) Holtfreter, Du Shane,6) Twitty,7) Rosin, De Lanney and Tada is of interest in this connection.

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In many clinical cases of systematized nevi, incontinentia pigmenti, nevus ophthalmo-maxillaris Ota and Mongolian spot, the eruption are localized in such a manner as one can easily assume the coincidence with the distribution of cutaneous nerve. Such cases are reported in the corresponding chapters. In various pigment disorders, functional abnormalities of vegetative nervous system are disclosed by the results of histamine scratch test and diaphoretic test. In some cases of pigmented nevus and incontinentia pigmenti, the abnormality of trophic nerve distribution resulted in the asymmetry of the breast glands. In several cases of nevus spilus en nappe, even the sensible nerve was involved in the affection. These are homolateral nervous disturbances accompanying pigmentary disorders and are favorable to our theory.

In benign melanomas, I believe, it is possible to distinguish rather distinctly those originating from Schwannian cellular ectodermal melanoblasts and those from mesenchymal melanoblasts corresponding to endo- or perineurium. So it is theoretically not impossible to differentiate histologically melanosarcoma and melanocarcinoma. This differentiation, however, is not always possible in individual cases. We believe that this problem will be given a solution in further progress in the investigation.

The Role of Enzymes in Melanin Production

The conflict of the monism and dualism on this problem was already mentioned. We tried indophenolase reaction in the melanoblasts which were considered as dopa positive by many investigators such as cells of basal layer, pigmented nevus, Mongolian spot, blue nevus, nevus fusco-caeruleus and malignant melanoma or pigment cell of Riehl's melanosis and found numerous indophenolblue granules in their protoplasm. Their size and form coincide quite well with those of preexistent melanin granules. And they are more numerous in cells with little melanin content. While on the other hand positive peroxydase reaction is principally limited to nuclei, and the reaction is especially strongly positive in the nuclei of basal cells, nevus cells and dermal melanoblasts, where diffuse or granular staining of protoplasm is also noticed. Endothel cells of blood capillaries present strong positive reactions, and indophenolblue granules and peroxydase positive granules overflow to the surrounding tissue. And at the same time local blood leucocyte of pigmented area shows stronger peroxydase reaction than normal area. This shows that there occurs exchange of both enzymes between myeloic leucocytes and tissue cells. These biochemical reactions exhibit the passing phase of melanin production in the presence of two enzymes, oxydase and catalase in the protoplasm. In permanganate methylgreen staining of Unna the site of melanogenesis stains always with methylgreen as oxygen areas. And in vitiligo the papillary layer reduces permanganate as reduction areas. In melanoma amelanotic foci also reduce permanganate, like nevus cell nest without melanin. Thus the lack of melanogenesis in malignant melanoma is no less than a phase of extremely rapidly metastatic and infiltrative growth of tumor cells and cannot be estimated as a measure of malignancy. This finding coincides with the experimental results of Greenstein on the oxygen consumption of melanotic and amelanotic mouse melanoma suspension.

It is well known that malignant melanoma is rare in Negros and that it frequent-
General Conclusion

It occurs in area which is physiologically poor in pigment such as finger tip, sole and lip. So it may be properly assumed that the disturbance of biochemical condition resulting in abnormal melanin production in heterotopic area is a cause of malignancy like the incidence of malignant melanoma in white horse.

In our follow-up study of Urbach-Kral's experiment, the increase of oxygen area is noticed in area hyperpigmented by the application of bergamot oil. The injection of ascorbic acid and riboflavin prior to the application of bergamot oil and ultraviolet irradiation completely inhibits hyperpigmentation. On histochemical examination the increase of reduction area is noticed. By injection of these vitamins the cutaneous penetration of bergamot oil is reduced and the decomposition product of this substance reacts rather reductively on the skin resulting in the suppression of melanin production. In Riehl's melanosis the deficiency of vitamin C and B complex is surmized by the increased value of pyruvic acid and iodic acid reducing substance of blood serum. These vitamin deficiencies are important etiological factors in Riehl's melanosis. The abovementioned results of Unna's staining, indophenol oxydase reaction and peroxydase reaction point to the importance of local biochemical condition specially of redoxpotential and the action of biocatalyst in normal and pathological melanogenesis.

In the experiment with black goldfish, only one of the three isomers of di-oxybenzenes, namely hydroquinone can suppress melanin production. This action is attributed to the decreased alkalization potency of cutaneous tissue by hydroquinone. Recent observation of Dejust, Ropshow, Caillau on the dermoepidermal hydrogen ion concentration supplies further influential data to this theory.

On the other hand, by injection of p-benzoquinone to yellow-red goldfish, black pigment is formed and the dopa reaction of scale turned to positive. This newly formed pigment is identified as melanin. As all other oxidizing agents failed to give similar results, it is not a mere oxidation by p-quinone but a special catalytic action of this substance on melanogenesis.

That the enzyme tyrosinase is indispensable for melanogenesis is admitted by almost all investigators. This ferment is actually identified in the extract of human and animal melanosarcoma by Niklas. In normal human tissue, however, it has been impossible to obtain tyrosinase activity. This may be due to the presence of inhibitor substance like ascorbic acid and sulfhydryl compounds (Hoff). While on the other hand Bloch and Raper emphasized the participation of dopa oxydase. And in my experiment indophenol oxydase and peroxydase are observed in the histochemical study, so their participation in melanogenesis is readily surmised. Thus I believe the two enzymes theory is more probable. Then how is the action of p-quinone in the goldfish experiment explained? According to Mann and Keilin in the presence of small amount of o-quinone polyphenolase catalyzes not only polyphenol but also monophenol. p-Benzquinone may act in the same way as an activator and thus accelerate melanogenesis. Both indophenol oxydase and peroxydase has common substrate and their difference lies only in acceptor substance. So their activities are presented at various passing phases of biochemical condition of individual tissue cell. The manifestation of dopa oxydase activity represents a phase of this cellular activity and cannot always be estimated as the existence of specific enzyme. This pigment production in goldfish appears first and most
Studies on Melanin

prominently at the place where carotinoid pigment is preexistent. And the addition of vitamin A, B₂ and C to p-quinone accelerates melanin production. So the participation of cytochrom system cannot be overlooked.

From the above histological and histochemical researches, I have concluded that melanogenesis is a part of biological oxidizing processes which is carried on under the influence of nervous system. In this sense the chemical transmission theory of Loewi-Dale and/or neurohormonal theory of Vilter is introduced in the mechanism of melanogenesis.

References

1) John and Ormea, Arch. f. Dermat., 1951, 192, 478.
2) Becker, Arch. of Dermat., 1949, 60, 44.