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EXPERIMENTAL MEDICINE

ON THE PIGMENT OF THE NEGRO'S SKIN AND HAIR.

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PLATE XIV.

1. INTRODUCTION.

THE pigments of the animal body constitute a numerous and widely distributed class of substances. Almost all of the tissues and fluids contain more or less pigment either in solution or, what is more frequently the case, in the form of minute granules of various shapes.

Some of the pigments that are of great physiological importance—hæmoglobin and the biliary pigments, for example—have been the subject of careful investigation and are fairly well understood with respect to their physical and chemical properties, their origin and relationships, and the physiological functions which they subserve. There are many more pigments of the human body whose chemical properties are somewhat known, but whose physiological relationships are very obscure. As belonging to this class may be named the pigments found in melanotic tumours, those of the retinal and choroid coats of the eye and of the hair, and the colouring matters of serum, of the urine and of fats.

Another large class of coloured substances is still less understood. Of these substances we know at present little more than that they exist in this or that tissue of the body; not one of them has been the subject of extended chemical research. Instances of these are the dark pigments * normally present in the heart, liver, kidney, thyroid gland, suprarenal body and testicles, on the peritoneal surface of the intestines, in the pia mater and in nerve cells.

Prominent among these little-known pigments is that found in the epidermis of the dark-skinned races. This pigment is of interest not alone because it is the distinguishing characteristic of the great majority of the human race and because for them it serves a physiological purpose, but also because of its very probable relationship to the pigment more sparingly deposited in the skin of the so-called white races and to the dark pigments known as melanins, examples of which are found in the hair and in certain malignant tumours.

Should we reach a thorough understanding of the pigment of the negro's skin, and should this pigment prove to be identical with that in the skin of the white races, this knowledge might throw great light on many anomalous cases of pigmentation; for example, the bronzed skin of Addison's disease, the brownish patches appearing on the face and other parts of the body during pregnancy, the brown or black patches sometimes covering large areas of the body and known as

* Compare Maass, Zur Kenntniss d. körnigen Pigmentes im menschl. Körper. *Arch. f. mikr. Anat.*, Bd. xxxiv, p. 452.

nævi spili, nævi verrucosi, and nævi lenticulares, and the lesser pigmentations called freckles.

Perhaps also closely allied in chemical composition to these pigments is that which is developed in the course of various chronic skin diseases, such as psoriasis, lichen ruber, and xeroderma pigmentosum; also that due to chemical irritation, as after the application of mustard or cantharidal plasters or that which follows the repeated inroads of clothes lice and other parasites.

We shall show in the following paper that it is possible to isolate the pigment of the negro's skin, and we believe that the same or a similar method will be found applicable to many of the instances of pigmentation above enumerated, more particularly to extensive discolorations of the skin in white persons, as in Addison's disease.

2. Earlier Work on the Pigment of the Negro's Skin.

The only work of a chemical nature known to us on the pigment of the negro's skin is found in a brief but suggestive paper communicated in 1876 to the Chemical News by Prof. J. W. Mallet,* on behalf of his pupil Dr. F. P. Floyd. This investigator confined himself to a study of the pigment in its crude state—that is, the pigmentary granules plus the cell structure enveloping them. His material was obtained by cleansing with water scrapings of the skin and treating them with alcohol and ether to remove the fatty matters. Since no attempt was made to isolate the true colouring matter, or even the pigmentary granules themselves, the results arrived at are of very restricted value. Two instances will suffice to show how inapplicable to the isolated pigment is a statement which may be perfectly true of the pigment while still contained in the cells of the epidermis.† Floyd says that the "colouring matter" is insoluble in dilute alkalies, but we found the purified pigment to be soluble in the most dilute alkalies or alkaline carbonates or even in distilled water, provided only that the pigment used has been recently precipitated.

* On the Chemical Character of the Pigment of the Negro's Skin. *Chemical News*, 1876, vol. xxxiv, p. 179.

† The term pigment-granules in Floyd's paper can only be construed to mean the pigment-bearing cells of the rete mucosum.

Again, the main point in Floyd's paper and the one for which he has been widely quoted is his conclusion regarding the iron content of the pigments. This point, however, can not be settled by an analysis of strips of cuticle. On incinerating strips of dried cuticle taken from both black and white cadavers, he found that the ash from the former weighed approximately twice as much as that obtained from an equal quantity of the latter, and that it contained approximately twice as much iron. From this he concluded that in all probability the pigment of the negro's skin is a chemical modification of the red colouring matter of the blood. But it must be remembered that ferruginous, colourless compounds of a proteid character are present in every cell, and on that account no conclusion as to the iron contents of the pure pigmentary substance can be reached by Floyd's method. We shall show later that in the process of purifying the pigment, the amount of iron steadily decreases with the removal of the adherent inorganic compounds till only the slightest traces of this element are left—so small an amount, indeed, that we have concluded that the pure pigment contains no iron as a constituent of its molecule.

3. Method of obtaining and cleansing the Epidermis.

The method of obtaining the cuticle in which the pigment is lodged varies according to the state of the cadaver. When it has lain for some time in contact with aqueous fluids, the epidermis may easily be peeled off in large patches from the underlying corium and, what is a further advantage, many of the hairs remain embedded in the corium. Or the entire skin is first removed and then cut into pieces of manageable size and scrubbed with a stiff brush under a forcible jet of water, after which each piece is held for a few minutes under boiling water and then immediately subjected to a stream of cold water, a process which it may be necessary to repeat once or twice. The consequent expansion and shrivelling results in a separation between the rete mucosum and the underlying corium, and when the pieces are stretched out and fastened on a board the entire epidermis can be removed with ease, much as a piece of wet paper

is lifted from a table on which it has been flattened out. The corium, it may be added, appears perfectly white after the epidermis has been removed.

When these methods of peeling off the epidermis are not possible, the following method may be used. Large pieces of skin obtained from the dissecting room are allowed to soak for three or four days in a five- to seven-per-cent solution of potassium hydrate, after which the epidermis is removed by scraping with a nickel spatula. This method is inferior to those before mentioned on account of the greater difficulty in removing the hairs.

When a sufficient quantity of epidermis has been collected, it is treated first with water, then with alcohol and ether in large extractors especially constructed for the purpose. After ten days to two weeks of this treatment the shrivelled and dried epidermis has become grayish white on its upper surface, the whole appearance reminding one of paper made by wasps, and on its lower side black or brown according to the amount of pigment contained in it. The hairs are now carefully removed, a tedious process requiring many days of labour.

When no more hairs can be detected, the microscope reveals that the small white specks with which the lower surface is studded consist of dried hair follicles in each of which is embedded a minute hair. These hair follicles may also be removed with the help of broad nickel forceps, but this process requires so much time that it was only used in preparing the epidermis for the quantitative estimation of the pigmentary granules.

4. Action of Reagents on the Cleansed Epidermis.

The epidermis cleansed as described was subjected to the action of various chemical reagents as follows:

Alkalies.—Dilute solutions of the free alkalies (five to fifteen per cent) allowed to act on the epidermis at room temperature for some days will disintegrate it, and maceration at water-bath temperature brings about a very complete disintegration in a few hours. This is, however, no true solution of the pigment, for although a

large part of the turbid fluid will pass through filter paper with large pores, it clears up, if allowed to stand for a day or two, a closely packed precipitate being found at the bottom, while the fluid above is of a faint straw-colour or ruby-red, according to the strength of the alkali used (five to ten per cent) and the length of time it has been applied (two to six hours). This black sediment falls out more quickly if the fats have been thoroughly removed from the epidermis before maceration.

Even prolonged boiling with ten- to twenty-five-per-cent solutions of the alkalies extracts but very little of the pigment. Whatever the strength of the alkaline solutions used, the supernatant fluid, on being filtered and neutralized with an acid, throws down a precipitate that is found to contain a great deal of proteid matter which is presumably an alkali-albuminate with the admixture of a little pigment.

If the black precipitate that settles out from the turbid fluid as above described is collected on a flat filter and washed with a very weak solution of acetic acid, it will be found to consist of minute black or brownish granules which resemble the pigmentary granules seen in the lower cells of the cuticle in a microscopic section of the skin. Although the granules are thus resistant toward even strong solutions of the free alkalies, they dissolve readily in weak solutions of the alkalies under a steam pressure of 150° to 160° C. Thus it was found that when scraps of cleansed epidermis are heated in a sealed tube with a five-per-cent solution of potassium hydrate for a few hours at the above temperature, a clear black fluid is obtained which passes through filter paper with great ease and can be diluted with water to any extent without causing a precipitate. The addition of an acid to this solution throws down a black or brown flocculent precipitate which is found to possess the properties of a weak acid; it forms very soluble amorphous salts with free alkalies or their carbonates.

Acids.—With the exception of nitric acid, dilute solutions (five to fifteen per cent) of the mineral acids allowed to act at room temperature do not dissolve out the pigment. Concentrated sulphuric

acid requires weeks to disintegrate the dried epidermis. For the first ten days the only effect is to swell the pieces of epidermis and bring out all their grooves and ridges. Even after two months thin filmy pieces are seen swimming in a dark fluid out of which settles no precipitate.

Concentrated hydrochloric acid is more effective; under its action in the course of two weeks all of the pigmentary granules have settled to the bottom, leaving the supernatant fluid but slightly coloured.

Concentrated nitric acid and fuming nitric acid are most effective solvents of the entire epidermis, the pigmentary granules included. After some hours a dark-brown fluid results which, on being freely diluted with water, throws down a dark-coloured flocculent precipitate. This precipitate, washed with alcohol and ether, is found to dissolve with ease in very dilute alkalies, yielding an intensely black solution. But if the nitric-acid solution has been allowed to stand for some weeks, dilution with water no longer throws down the precipitate and the solution has in the meantime become almost decolourized. Boiling the epidermis for a short time with much nitric acid of specific gravity 1.19 yields a dark-brown solution which, on the further application of heat, turns yellow and becomes finally almost entirely colourless. On diluting this solution with water, no precipitate is thrown down.

Glacial acetic acid allowed to act for months produces no marked changes to the naked eye; under the microscope, however, it becomes evident that certain changes in the cells have taken place, for the pigmentary granules are seen with greater distinctness. The action of chlorine and other oxidizing agents on the epidermis is the same as their action on the isolated pigment, which will be described later.

5. Methods of isolating the Pigmentary Granules.

From the experiments described in the foregoing pages it will be seen that the pigmentary granules of the epidermis must be classed with the most resistant and unalterable elements of the body.

For the purpose of learning what substances enter into their composition, we proceeded to isolate them by the following methods: A given weight of the epidermis, varying from 10 to 160 grammes, cleansed as before described, even the dried hair follicles being for the most part removed, was submerged in not less than twenty times its weight of a five-per-cent solution of pure potassium hydrate contained in large nickel bowls placed on the water bath.

The water in the bath was kept actively boiling, and the pieces of epidermis were for the first half hour constantly stirred with a nickel spoon; under these conditions the disintegration of the epidermis proceeded at a rapid rate, at the end of half an hour only very small particles remaining undissolved.

From time to time water was added so that the concentration of the solution should not go above five or six per cent. If after this process has been carried on for six hours the nickel bowl be set aside for twenty-four hours, there will be found in it a supernatant fluid of light-reddish-brown colour and a dense, black powdery precipitate consisting of the pigmentary granules.

Microscopical examinations were frequently made in order to follow the gradual disintegration of the epidermis step by step until there remained only minute brownish particles, not to be clearly distinguished except with the help of an immersion lens.

In Plate XIV, Fig. 1, we have given a drawing of a single cell detached from its neighbours showing the appearance of the granules. Fig. 2 represents the state of the disintegration after the potassium hydrate has acted on the epidermis for about two hours and a half. It will be seen that all cell outlines have been destroyed, the granules adhere together, forming brown or black lumps in each of which the individual granules are easily detected, and now and then a small aggregation of granules has a contour suggestive of the original cell form. Fig. 3 represents the condition after six hours of maceration. Light pressure on the cover glass causes the granules to fall apart and to appear in the field as discrete but very minute light-brown particles. As to the shape of these granules, we have concluded that they are rather elongated than round; this, however, can only

be determined by examining them under an enlargement of a thousand diameters or more and under favourable circumstances. The traces of foreign matter that may still be detected among the granules after long maceration may be removed by washing with warm hydrochloric acid, alcohol, chloroform, and ether. Thus treated, dried, and powdered, the granules cohere into little black lumps, as shown in Fig. 4. Rubbing up some of this powder with dilute alkalies gives us the picture under the microscope of individual granules, as seen in Fig. 3.

A few yellowish ovoidal and globular particles seen here and there were at first assumed to consist of a substance related to keratohyalin, but their inability to take up methylene blue and their disappearance in hot ether and hot alcohol show that they consist of fat. Small irregular black lumps and long black rods that were now and then found among the granules were shown by check experiments with pure keratin to consist of nickel sulphide. This is formed by the sulphur which is split off from the keratin acting on the nickel of the bowl and forming a thin coating of nickel sulphide, which breaks up into singular and varied forms.

Another and very simple method of isolating the pigmentary granules consists in the use of concentrated hydrochloric acid, which, as already described, entirely dissolves the epidermis excepting only the granules, which fall to the bottom as a black sediment. Treatment with so powerful an agent may remove from the granules much of their inorganic matter, but when it is desired to isolate the pigment itself rather than the pigmentary granules, this method promises to be of service because it enables us to isolate with ease any desired quantity of the granules, no matter how large.

The black granules, prepared by either of the above methods, washed and dried, become a snuff-coloured powder, not to be distinguished in appearance from that yielded by the pigment itself when it has been isolated from the granules.

But, as we shall see later, these two powders differ markedly in their behaviour toward reagents.

6. Possible Contamination of the Pigmentary Granules with Bacteria and Cell Fragments.

It might be supposed that the black precipitate described by us as consisting of the ultimate pigmentary granules would be found to be much contaminated with bacteria. Such is not the case, however. The usual methods of staining have failed to indicate their presence in sections of the dried and cleansed epidermis, and the following experiments were made to determine whether bacteria can retain their structural integrity when macerated in a five- to six-per-cent alkaline solution at water-bath temperature. In the first experiment 5 grammes of dried tubercle bacilli (pure culture) were suspended in 75 cubic centimetres of a five-per-cent solution of potassium hydrate contained in a small flask which was attached to a reflux condenser, and the mixture was then heated for four hours on an actively-boiling water bath. At the expiration of this time no bacteria could be detected either on direct examination of the resulting turbid fluid or by staining some of the precipitate thrown down by alcohol.

Nothing remained but amorphous flakes and stringy masses. A like negative result was obtained when scrapings from culture tubes containing the staphylococcus pyogenes aureus were subjected to the treatment just described. Nencki and Schaffer * have also shown that certain putrefactive bacteria (names not given) are largely dissolved when treated on the water bath with only 0.5-per-cent potassium hydrate, an insoluble, structureless residue remaining. Contamination of the pigmentary granules with bacteria must therefore be entirely excluded.

Neither can there be an admixture of cell fragments, for in the granules that have been collected on flat filters, washed with hot two-per-cent potassium hydrate, water, hot five-per-cent hydrochloric acid, hot alcohol, chloroform, and ether, the microscope fails to detect any but the merest traces of matter, excepting only the nickel sulphide obtained from the bowls. This statement is also confirmed

* Review in *Jahresb. d. Thier-Chemie*, Bd. ix, p. 385.

by the fact that the iron reaction (HCl and K_4FeCy_6) can now no longer be obtained.

But the most conclusive proof that no foreign matter of organic nature lies between the granules is found in the fact that from a given weight of granules an amount of crude pigment may be obtained by the method soon to be described whose weight equals that of the granules minus their ash. In other words, the crude soluble pigment with properties so different from the granules contains everything that they contain, barring only their great excess of ash.

The force of the above statement will become more apparent when we note the method of disrupting the granules, presently to be described, for the reagents used for this purpose are the very ones that have already done their work in dissolving out foreign organic matter.

7. Proportionate Amount of Pigmentary Granules in the Dried Epidermis.

Ten grammes of cleansed epidermis from which even the hair follicles had been removed were macerated for six hours in a nickel bowl as just described.

The pigmentary granules were then collected in two portions on small flat filters, washed with a warm, dilute solution of potassium hydrate, then with alcohol containing a very little acetic acid until the potassium hydrate, as shown by the flame test, was entirely removed, then with hot alcohol, hot chloroform and ether, and dried at 110° C. to constancy of weight.

The amount of pigmentary granules on the one filter weighed 0.2120 gramme, on the other 0.1089 gramme. The total residue of pigmentary granules, therefore, equaled 0.3209 gramme. From this amount we must subtract 0.0121 gramme as consisting of sulphide of nickel, leaving 0.3088 gramme of pigmentary granules. The granules would, therefore, constitute 3.08 per cent of the epidermis when cleansed as described.

In a second experiment 9.77 grammes of carefully prepared epidermis from various parts of the body was treated in the same man-

ner, except that the process of maceration was carried on in a porcelain instead of a nickel bowl. In this case the pigmentary granules obtained weighed 0.3693 gramme.

Neglecting the trifling weight of contaminating material that was derived from the porcelain bowl, we find the proportion of granules somewhat higher—viz., 3.78 per cent. It is well known that the epidermis varies in thickness in different parts of the body, and the difference in the above results is therefore easily accounted for.

8. The Substratum of the Pigmentary Granules.

We believe that a perusal of the following pages will leave no doubt that the pigmentary granules which settle in the bowls when the epidermis is macerated in warm solutions of potassium hydrate are no other than the pigmentary granules that may be seen under the microscope in every cross-section of the negro's skin. We grant that we must remain in ignorance as to whether the long maceration necessary to free the granules from the cells in which they are lodged may not have extracted from the granules themselves some constituent of a proteid or keratinoid character. It would appear probable, however, that had this been the case, the granules would have fallen to pieces as do bacteria when their soluble proteids are removed from them by treatment with hot alkalies.

We must also concede it to be possible that the water, alcohol, and ether used have removed from the granules some constituent soluble in these agents, but this error, if it be such, is unavoidable.

We have already alluded to the fact that the granules become somewhat translucent and pale after long treatment with hot alkalies. This change becomes very apparent when microscopic sections of healthy skin taken from a living subject are thus treated on the warm stage and examined from time to time. This has been kindly done for us by Dr. T. C. Gilchrist. Dr. Gilchrist has watched under the microscope the sections during the different stages of their disintegration under the action of alkalies of varying strength, and has demonstrated that the granules remaining are those originally deposited in the epidermis.

Post * has also made this observation, for he says that the elementary granules are seen as pale, somewhat swollen rods when they have been decolourized † by concentrated potassium hydrate which has been allowed to act on them at room temperature for some weeks.

Are we to conclude from these observations that the pigmentary substance is lodged in a substratum of a different character, that there is a ground substance which constitutes, so to speak, the body of the granules and which is permeated with the pigment? In other words, are we to look upon these granules as a kind of trophoplast, like chlorophyll granules, and like them composed of a substratum of proteid matter which is stained with a pigment?

It appears that histologists who have given attention to the subject incline to this latter view. W. v. Nathusius, ‡ for instance, has recently shown in his paper on the fibrillary structure of the keratinoid cells of the fibrous coat of hairs that these cells may be so far disintegrated with warm ammonia as to leave undissolved their pigmentary granules, lying for the most part in rows as originally deposited in the cells, but deprived of some of their pigment. This investigator also furnishes drawings of the granules arranged in rows in the isolated fibrils, their natural pigment removed, and methyl violet or fuchsin substituted.

Still other evidence that there is present in these granules a non-pigmented substratum is presented by Reinke, who has recently sought to establish the histological similarity of these structures to the trophoplasts of the botanist. Reinke has found that the pigmentary granules of the peritoneal cells of the larva of the salamander may be bleached with hydrogen peroxide and then stained with safranin. He concludes as follows: "Inasmuch as the pigmentary granules can

* Virchow's *Archiv*, Bd. cxxxv, p. 493.

† Post evidently does not use "Entfärbung" in its literal sense. In our experience, potassium hydrate never entirely removes the pigment unless the granules have first been subjected to an acid.

‡ *Arch. f. mikr. Anat.*, Bd. xliii, p. 153. In the explanatory remarks devoted to the drawings v. Nathusius refers to detritus lying among the fibrils. We must therefore assume that the ammonia leaves much undissolved matter in addition to the fibrils and granules.

* *Arch. f. mikr. Anat.*, Bd. xliii, p. 393.

be freed of pigment and again coloured with a staining dye, it would appear to be a justifiable conclusion that the substratum of the granules is something different from the pigmentary substance."

We must say, however, that Reinke's argument does not seem to us conclusive. The substance constituting the pigment may have been left *in situ* after having been bleached by the hydrogen peroxide and the substance that took up the new dye may be no other than the original pigmentary substance itself. In a word, Reinke has not proved that the hydrogen peroxide removes anything from the granules.

No objection can be made, we think, to the following method of demonstrating that there is in these granules a substratum of a non-pigmentary character. Some of the granules isolated and purified as already described are macerated for a week or ten days at room temperature with a large excess of hydrochloric acid of five per cent. The flask containing the granules and the acid is shaken a few times a day, and every second day the acid is poured off and fresh acid added. At the end of the time the granules are washed free of acid with the help of a suction pump or a centrifugal machine, and are then treated on the water bath with dilute (two- to five-per-cent) potassium hydrate. An astonishing change has taken place; the granules now yield up their pigment in large amount to weak alkalies and a fluid of a deep-brown, almost black, colour may be filtered off in a short time.

When examined under the microscope, immediately after the coloured substance begins to leave them, the granules may still be detected as mere shades of their former selves.

Soon, however, only faintly coloured *débris* is left, as when bacteria are similarly treated. This *débris* must originate in the pigmentary granules, and it can represent nothing else than the substratum of Reinke and others.

We have attempted to stain the granules just as they were beginning to give up their black pigment to the potassium hydrate. This would have made the proof of a separate substratum absolutely complete, for we should have had on the one hand the clear, black

solution, and on the other the minute elements coloured with the artificial dye. But after being treated with potassium hydrate, the ground substance seems incapable of taking up the new dye. We tried, therefore, another method. After the treatment with cold hydrochloric acid of five per cent, a small quantity of the granules was placed in a glass tube with a mixture of one volume of alcohol and six volumes of concentrated ammonia. The tube was sealed and then kept in a water bath at 100° C. for six to ten hours. This process extracts from the granules a little of their pigment and they will then take up gentian violet with avidity, as seen in Plate XIV, Fig. 5.

The certain presence of a non-pigmented ground substance in the pigmentary granules has led us to inquire whether it would not be possible to isolate from white epidermis and white hair minute elements which would correspond in every particular, except colour, to the black granules of the negro's skin and hair. We therefore subjected to the treatment already described such structures as white horn, white hair of the dog and of the rabbit, merino wool, and scales of epidermis from a person afflicted with ichthyosis. We found that white horn dissolved in five- to ten-per-cent solutions of warm potassium hydrate to a perfectly clear fluid. The other keratinoid structures yielded varying small amounts of a highly resistant proteid. We have boiled this substance for four hours with a five-per-cent solution of hydrochloric acid with the result that only a part of it went into solution, and if the substance be again subjected to hot potassium hydrate of five per cent, only a little passes into solution. In short, even the alternate application for a long time of boiling alkali and acid does not entirely dissolve this resistant proteid.

We assume this body to have properties very like that of the ground substance of the granules, and if this assumption be correct, it explains why it is so difficult to remove from the pigment all traces of the contaminating ground substance. Of all the keratinoid structures just referred to, white hair from the dog is the most resistant to alkalies and appears to contain the largest proportion of the unknown proteid.

As the quantity of this proteid is in any case small, a large amount

of material is necessary, and most of our experiments were made on merino wool.

We shall soon publish the results of our observations on the chemical properties of this hitherto overlooked proteid. We may say that by our present method of macerating merino wool and the other keratinoid structures with potassium hydrate this resistant proteid is left behind, not as white granules, but as an amorphous compound. After isolating the proteid just referred to, we found that v. Nathusius* has shown that when white hair is treated with a boiling aqueous solution of methylgreen, minute, deeply coloured particles may be detected which correspond in size and in position to the "air spaces" of Kölliker in white hair and to the pigmentary granules in coloured hair. Von Nathusius therefore concludes that the same histological element is present in both white and black hair, differing in the latter case only in its pigment.

9. The Inorganic Constituents of the Pigmentary Granules.

Having seen that the pigmentary units may be isolated in any desired quantity, it becomes of interest to learn what inorganic constituents enter into their composition. This problem is, furthermore, one of physiological importance, as bearing on the broad question of the distribution and localization of the inorganic constituents of the body.

Numerous difficulties have been encountered in the analysis of the granules obtained from the epidermis. Their ash content is found to be too high, when they are not washed with hydrochloric acid, to remove the amorphous material which appears to be precipitated among them by the alkali used. This material promptly gives an iron reaction with HCl and K_4FeCy_6—a test which was applied in vain to the granules. This foreign material may readily be removed by washing the granules with hot hydrochloric acid of two to five per cent; but this process deprives the granules themselves of some of the inorganic matter properly belonging to them. We have incinerated the granules both before and after washing with hot hydro-

* *Loc. cit.*, p. 152.

chloric acid, and find that in either case an error is introduced into the analysis, the total ash being too large in the first and too small in the second. The true value lies somewhere between the two. In the case of the hair granules soon to be described, the ash content obtained by incineration without previous washing with hydrochloric acid gives, in our opinion, a correct value, because no foreign matter appears to be precipitated upon them.

Stating for the present the results of our analyses in round numbers, we find that the inorganic constituents of the pigmentary granules of the hair dried at 110° C. without previous washing with hydrochloric acid make up rather more than eleven per cent of their weight. Washed with hydrochloric acid of five per cent, their ash content still amounts to nearly seven per cent.

The pigmentary granules of the epidermis dried at 110° C. without previous washing with hydrochloric acid show an ash content of twenty-six per cent. After thorough washing with hot hydrochloric acid this percentage is reduced by one half.

The great difficulty of entirely removing all adherent foreign matter from the epidermis of a cadaver causes us to view with suspicion this very high ash content of the epidermal granules, and we can not at the present writing assert positively, as we can for the ash content of the hair, that no foreign inorganic matter was present. But, as has been described, the greatest pains was taken to cleanse the skin thoroughly, and the results we give are taken from several accordant analyses.

The acids present in the granules are silicic, sulphuric, and phosphoric; the bases * are calcium, magnesium, and iron, the order of statement representing the relative amounts. Treatment of the granules with hot hydrochloric acid of five per cent reduces the salts of sulphuric and phosphoric acids to a low figure, only about ten per cent of these constituents remaining, while the percentage of silicic acid is but little affected. Iron constitutes over four per cent of the

* A little aluminium oxide was also found in the ash from both kinds of granules. We must, however, reserve for a later paper the question whether a small amount of aluminium is contained in the human epidermis and hair.

ash of the epidermal granules when these have not been washed with hydrochloric acid, or about one per cent of the dry granules.

A trace of this element is retained by the granules with great tenacity in spite of repeated treatment with hydrochloric acid; but, as we shall see later, iron does not constitute an integral part of the molecule of the pigment.

That silicon is present in the pigmentary granules of both the skin and hair, we can have no doubt. As found by us in the granules of the hair, for example, no foreign source can be claimed for it when we state that the hair was cut for us from the heads of clean negroes in the hospital wards; that it was most carefully picked over and washed with hot water, alcohol, and ether; that it was macerated in nickel bowls, and the granules collected on flat filters placed on perforated porcelain plates in glass funnels, the sides of the filters projecting upward along the wall of the glass funnels, so that the granules themselves were hardly in contact with the glass, and the silicates taken from the glass by the alkaline fluid would all be carried into the filtrate. Besides, the amount of silicon found is too large to be derived from the glass funnel, even if no care had been taken. A conclusive proof that silicates are present in the granules of both the epidermis and the hair, and that they pass into solution along with the pigment and adhere to it with great tenacity, is seen in the following fact. It is possible, as we shall see, to prepare from the epidermis a specimen of soluble pigment that shall contain no less than twelve per cent of ash, including the silicates. Such a pigment will, nevertheless, remain in solution in alcohol of eighty to ninety per cent for an indefinite period, provided only that a freshly precipitated pigment be used. But silicates that have been dissolved out of utensils of glass and porcelain by alkalies, when added even in small amount to an alcoholic solution of the pigment of the strength referred to, fall out promptly, as we found by actual trial, and carry down with them considerable pigment.

The above furnishes one of several proofs of the close union that exists between the substratum of the pigment and the inorganic constituents of the pigmentary granules. Other instances of this condition

are seen in the firm union that exists between the proteids of the body and the mineral compounds accompanying them.* In the vegetable world we find the same close connection existing between cellulose and mineral compounds, like silicates of iron and calcium. Small quantities of inorganic material still adhere to cellulose, even after repeated solution in ammoniacal cupric oxide and repeated precipitations and washings with hydrochloric acid, thus furnishing a striking reminder of the readiness with which our pigment remains in solution in alcohol, notwithstanding the considerable quantity of adherent mineral matter. Lange † concludes, as the result of his experiments in this field, that the mineral constituents which can not be entirely removed from cellulose are distributed in the membranes of the cell as particles of an insoluble compound of the element in question; and when the membranes are dissolved, these mineral compounds are held in suspension in the solution, and filtering holds back only a portion. Some such hypothesis may also explain the " close union " referred to above as existing among the constituents of the pigmentary granules.

That silicon should be found in the pigmentary granules of both the epidermis and the hair need not occasion surprise. Silicon has long been known to be one of the constituents of hair; it has also been found in the epidermal scales from a person afflicted with ichthyosis,‡ and traces of it exist in the urine. It is found to a considerable extent in the ash of feathers, and Henneberg # has found it in the blood of chickens to the amount of 0.0139 per cent. It is very probable that silicon may be proved to be an indispensable constituent of the integumentary structures, more especially of their pigmentary granules.

As to the function of these inorganic constituents, we can only offer surmises. It may be that they serve the purpose of a mordant, enabling the substratum of the granule to hold and " fix " the solu-

* Cf. M. Nencki, Bemerk. üb. d. sogenannte Asche d. Eiweisskörper. *Arch. f. exp. Path. u. Pharmakol.*, Bd. xxxiv, p. 334.

† *Ber. d. deutsch. chem. Gesellsch.*, Bd. xi (1878), p. 826.

‡ Halliburton, Chemical Physiology and Pathology, p. 566.

Ann. d. Chem. u. Pharm., Bd. lxi (1847), S. 257.

ble pigment.* Closer study of this interesting question may perhaps furnish an insight into nature's method of dyeing. We have already said that it is only possible to dissolve the granules in weak alkalies at water-bath temperature when they have first been subjected to maceration with hydrochloric acid. Now, it can be easily shown that the acid removes only inorganic constituents from the isolated granules, and it would therefore seem that the presence of this inorganic matter is in some way essential to the retention of the pigment, since its removal renders the granules soluble in a medium which before failed to dissolve them.

The ash content of the granules remains high, even if large deductions are made, as in the case of those derived from the epidermis.

Our analyses are verified in this respect by one made many years ago by Scherer,† who includes in his analyses of proteids that of the pigment called by him the pigmentum nigrum oculi. To obtain it, the black pigment-bearing cells of the choroid coat of oxen's eyes were brushed off with camel's-hair brushes under water, allowed to settle, and were then strained through a filter of linen cloth, in order to remove any fragments of tissue; the inky-black filtered fluid was then evaporated, and the residue extracted with boiling alcohol and ether, and dried; 0.345 gramme of the dried substance gave 0.035 gramme of ash, or 10.1 per cent.‡ In Scherer's analysis the entire pigment-bearing cells of the choroid coat were incinerated; but had he isolated and analyzed the granules themselves, we have no doubt the ash content would have approximated that found by us in the hair. It seems highly probable that all pigmentary granules contain a great deal of inorganic matter.

* Such a theory must, however, take account of the fact that white feathers contain fully as much silicon as do those that are coloured, also that the feathers of birds living on grains contain more silicon than those living mainly on soft fruits. *Vide* Gorup Besanez, *Ann. d. Chem. u. Pharm.*, Bd. lxi (1847), p. 47.

† *Ann. d. Chem. u. Pharm.*, Bd. xl (1841), pp. 1-65.

‡ Scherer gives the ash as amounting to 9.8 per cent, but, according to his own figures, it is as above.

10. Methods of isolating and purifying the Pigment.

It is only necessary to apply chemical reagents in the proper sequence in order to dissolve out of the granules the pigment contained in them. Thus, if the granules that have settled out from the alkaline macerating fluid be washed free of alkali by decantation, and then digested at room temperature with five to ten per cent HCl for ten days, the acid being renewed every two days, and, after being washed free of acid, be now again subjected to the action of warm dilute alkalies, it will be seen that the alkaline solution becomes at once deeply coloured. After some hours of maceration on the water bath with five- to ten-per-cent solutions of potassium hydrate, only a small residue, consisting largely of inorganic matter, is left undissolved, and repeated digestion with ten-per-cent potassium hydrate reduces this undissolved residue to trifling proportions.

Instead of digesting the granules with hydrochloric acid at room temperature, the same result may be obtained by boiling them with the acid for four hours. This method was used by us only once for the special purpose of studying the effect on the iron content of the ash. At all other times only cold five-per-cent hydrochloric acid was used that we might run no risk of removing iron from the molecule of the pigment.

The black alkaline solution of the pigment is now diluted with an equal volume of water and filtered; it remains perfectly clear on standing, a minute sediment of finely divided sulphide of nickel being found at the bottom of the vessel. In our early work we precipitated the pigment out of its first alkaline solution by means of a large excess of a mixture of six volumes of alcohol and one of ether. After allowing the precipitate to settle for some days, the supernatant fluid was removed with the siphon, and the dark-brown precipitate was collected on flat filters, washed with warm alcohol, to which a little acetic acid had been added, redissolved with the help of a trace of potassium hydrate or of ammonia, and reprecipitated with alcohol and ether. This process was repeated seven or eight times. In the later stages ammonia was used to redissolve the pigment, and, after the solution had

been effected, the ammonia was driven away on the water bath before the solution was filtered. In such a case the pigment still remains in perfect solution after the removal of the ammonia. With the gradual removal of the potassium used to effect the first solution, it became, however, increasingly difficult to precipitate the pigment with alcohol. Finally, it remained in solution in a fluid containing not far from ninety per cent of alcohol, and, in order to precipitate the pigment, it was found necessary to add considerable acetic acid. This fact made it seem probable that the pigment would be found nearly pure, but incineration showed an ash content as high as 12.4 per cent. We were therefore obliged to abandon this method of isolating the pigment, and to reserve the precipitation with alcohol and ether for the later stages of the process, after the ash content had been reduced by alternate treatment with alkalies and acids. Hirschfeld,* toward the close of his valuable paper on the pigment of the choroid coat, regrets that he did not sooner observe that an excess of alcohol precipitates the pigment, as he might have used this method to isolate it.

Recently we have proceeded as follows: A large quantity of cleansed epidermis—say 100 grammes—is macerated in a number of large nickel bowls with twenty to thirty times its weight of a five-per-cent solution of potassium hydrate. After the maceration has continued for six hours, the black fluid is poured into a large glass jar, and six volumes of alcohol are added. In a day or two a dense black precipitate has settled out, while the keratoses and alkali-albuminates derived from the epidermis remain in solution. The black precipitate is next subjected to the action of cold dilute hydrochloric acid, as already described. The granules are then dissolved in potassium hydrate in nickel bowls on the water bath, the solution is diluted with water and filtered, and the pigment is precipitated out by the addition of acetic acid in excess. After the pigment has settled out, the red, supernatant fluid is decanted, and the pigment is collected on flat filters and washed free of acid. For this purpose water is at first used, but, when the pigment begins to dissolve in the wash water, absolute alcohol is substituted. The pigment is now again dissolved

* *Zeitschr. f. physiol. Chem.*, Bd. xiii, p. 420.

in dilute potassium hydrate (two to five per cent), heated in the water bath for some hours, and again filtered, when a small residue of insoluble flocks will be found on the filter; it is then again precipitated with an excess of acetic acid, allowed to stand as before, collected, and washed. It is now dissolved in ammonia, the ammonia driven off on the water bath, the solution filtered, and the pigment precipitated out with alcohol and ether, to which a very little acetic acid has been added. This process is repeated until it is found that the ash content has been reduced to one or two per cent. After this, the pigment is washed with hot alcohol, chloroform, and ether until these reagents remove nothing further from it. The ash content is thus reduced to a low figure, varying from 0.8 to 1.2 per cent in the instances where the pigment was purest.

11. The Pigmentary Granules and the Pigment of the Negro's Hair.

While the study of the pigment of the negro's skin has been the chief purpose of our investigation, we have also isolated the pigmentary granules and the soluble pigment of the negro's hair. From 600 to 1,000 grammes of negro's hair, cleansed with great care and treated with alcohol and ether in large extractors, were macerated in large copper kettles over a free flame with ten times their weight of a five-per-cent solution of potassium hydrate, care being taken not to allow the temperature to rise above 95° C., or the solution of potassium hydrate to go beyond a concentration of six per cent. The black, turbid fluid was then poured into glass jars containing a large excess of alcohol or a large quantity of dilute hydrochloric acid of such strength that, when the turbid fluid had been added, the whole should have a plainly acid reaction. If alcohol is used, the black precipitate is flocculent; if hydrochloric acid is used, the precipitate falls out in large lumps that have a sticky feel, because of an admixture with an alkali-albuminate of a glue-like character. In this latter mixture sulphuretted hydrogen is also given off in large quantities, which is not the case when the turbid alkaline fluid obtained from the skin is similarly treated. The main constituent of the precipitate in both

cases is the pigmentary unit of the hair, a large black, rod-like particle, very much resembling in form certain bacteria. Plate XIV, Fig. 6 shows these rods in a piece of crushed hair, which had been macerated in hot potassium hydrate for fifteen minutes—a length of time found sufficient to remove the scaly covering of the hair and soften its fibrous substance. Fig. 7 represents the pigmentary granules of the hair after treatment in a closed tube with alcohol and strong ammonia, and subsequent staining with gentian violet.

In order to isolate the pigment of these granules, the black precipitate thrown out by alcohol or hydrochloric acid is treated in the way already described as applicable to the pigment of the skin. The pigment finally obtained is not distinguishable in any way from that obtained from the epidermis. As with the epidermis, so also with the hair, maceration in concentrated hydrochloric acid in a closed bottle entirely dissolves all of it but the granules. Twenty cubic centimetres of hydrochloric acid thrown on a few curls of negro's hair entirely disintegrate them in the course of a week. At the end of this time the supernatant fluid has a reddish-violet colour, while a black precipitate, consisting of the typical granules, has settled at the bottom. On account of its convenience, we propose to use this method in future work on the pigment of the skin and hair.

To our great surprise, a given weight of hair contains a smaller proportion of pigmentary granules than does a like weight of epidermis. Thus, 10 grammes of negro's hair yielded only 0.1889 gramme of granules, or 1.9 per cent, as compared with 3.08 to 3.78 per cent of granules contained in the epidermis. This result was afterward abundantly verified in the isolation of pigment from large amounts of hair.

12. Alkali-albuminates and Peptone-like Bodies formed by the Action of Alkalies on the Epidermis and Hair.

When the alcoholic fluid which is decanted from the granules is freed of its alcohol by distillation, and the resulting alkaline fluid is made acid, an alkali-albuminate, already referred to as having a ropy, sticky character, is thrown out. When the macerating alkaline fluid

containing either hair or skin granules is put into weak hydrochloric acid, in order to precipitate the granules, this alkali-albuminate at first remains in solution, although the fluid has an acid reaction, and can be precipitated by the addition of more hydrochloric acid to the fluid filtered off from the granules. N. Sieber * has also called attention to this substance in her paper on the pigment of the choroid coat and of the hair. In the case of the epidermis, an alkali-albuminate is also formed, but its physical properties are entirely different. It falls out as a powdery sediment, and does not form a translucent glue-like mass on drying, as does the alkali-albuminate from the hair. Both of the alkali-albuminates, when collected on filters, washed, and dissolved in a little alkali, now fall out immediately on neutralization with a mineral acid, not requiring an excess of acid, as they do when still mixed with the alkali used for maceration and with the soluble products of the epidermis and hair. They also dissolve readily in a little hydrochloric acid of twenty-five per cent, and are completely precipitated from this solution by the addition of ammonium sulphate or sodium chloride.

We think it highly probable that these albuminates are derived, in large measure, from the keratins of the skin and hair by the action on them of the potassium hydrate; for Lindwall † has shown that when the keratin obtainable from the shell membrane of the hen's egg is treated with sodium hydrate, an alkali-albuminate and peptone-like bodies are formed. We may also add that the fluids from which the alkali-albuminates referred to have been separated, all promptly give the red biuret reaction at room temperature, and, as the fluid no longer contains a native proteid or an alkali- or acid-albuminate, it is safe to conclude that we have in solution some peptone- or albumose-like body in addition to the alkali-albuminate referred to.

* *Arch. f. exp. Path. u. Pharmakol.*, Bd. xx, p. 364.

† Beiträge zur Kenntniss des Keratins. Reviewed in *Jahresb. d. Thier-Chemie*, Bd. xi, p. 38.

13. Chemical Properties of the Isolated Pigments.

The isolated pigment of the negro's skin presents the appearance of a dark-brown or snuff-coloured powder. If a little of this powder is placed upon a strip of blue litmus paper and moistened with a drop of water, it gives a markedly acid reaction. If it be heated in a platinum crucible, a mixed odour of burnt horn and pyrrol is perceived and the pine-sliver reaction for pyrrol is obtained, with such promptness and intensity that we must assume this substance to be present in considerable quantity among the distillation products of the pigment. Toward the close of the incineration the odour of pyrrol disappears, and an overpowering odour of hydrocyanic acid is given off. Freshly precipitated and washed free of potassium, the powder is soluble in water and in a large excess of alcohol of ninety per cent. Allowed to stand over sulphuric acid for months, or dried to constancy of weight by heating at 120° C., the pigment changes from snuff-colour to a dense black, its particles shining like anthracite coal. It loses its free solubility in water, alcohol, and dilute alkalies, but long-continued digestion on the water bath with strong alkalies again dissolves it.

The pigment is entirely insoluble in the usual organic solvents, as ether, chloroform, amyl alcohol, acetone, glycerin, acetic ether, xylol, toluol, carbon disulphide, phenol, and pyridine. It is precipitated from its aqueous solutions by potassium ferrocyanide, copper sulphate, ammoniacal zinc chloride, silver nitrate, lead acetate, calcium acetate, barium hydrate, and calcium hydrate. The addition of sodium chloride, magnesium or ammonium sulphate, and of other neutral salts to an aqueous solution induces a complete precipitation of the pigment. Alkaline solutions examined with the spectroscope show no absorption bands.

The addition of an acid in excess to the alkaline solutions causes the pigment to fall out at once as a brownish-black flocculent precipitate. Concentrated hydrochloric acid does not dissolve the pigment, and appears to have no damaging effect on it, even long boiling with a twenty-five-per-cent solution leaving the pigment unchanged. Con-

centrated sulphuric acid only slowly dissolves a little of the pigment, and, when the solution of what little is taken up is dropped into ice water, the pigment falls out apparently unchanged.

Concentrated nitric acid easily dissolves the pigment, forming with it a dense black solution, from which it may be precipitated on dilution with water. It may be boiled with this acid or repeatedly evaporated with it without loss of colour. Glacial acetic acid does not dissolve the pigment.

Very dilute solutions of the pigment lose their colour when acted upon for a few days by neutralized hydrogen peroxide, and dark-brown flocks settle out. Chlorine passed into an alkaline solution of the pigment immediately deprives it of its colour. When the pigment is repeatedly evaporated down in a porcelain bowl with nitro-hydrochloric acid, there remains a reddish-yellow resinous mass, insoluble in alkalies, water, alcohol, or ether, which dissolves readily in amyl alcohol. On evaporation, a reddish-brown resinous mass is obtained, the nature of which we have thus far been unable to determine.

The behaviour of the pigment toward reagents, as described, makes it evident that it is a very resistant substance. This view is still further strengthened by the following experiments: 2.6 grammes of hair pigment, with an ash content of 2.19 per cent, and a sulphur content of 1.7 per cent, was dissolved in 500 cubic centimetres of a five-per-cent solution of potassium hydrate, and kept for four and a half hours in an autoclave at a temperature of 185° C. At the end of this time the black solution was filtered, the pigment thrown out with acetic acid, collected, washed, and dried. On examination, it was still found to contain much sulphur. It was therefore again dissolved in 500 cubic centimetres of a five-per-cent solution of potassium hydrate, and kept for three hours at a temperature of 200° C. The solution not having changed in colour, it was brought up to a potassium hydrate content of thirty per cent, and was again kept for two hours at a temperature of 200° C. The black solution was then treated in the usual way to recover the pigment. As nearly as we could judge, fully one third of the pigment originally put into the

autoclave had disappeared, and the part left had more markedly acid properties than before. The recovered pigment was found to have a somewhat higher sulphur content. Thus, 0.3551 gramme gave 0.0496 gramme of barium sulphate, and therefore contained 1.9 per cent. of sulphur. Unfortunately, part of the solution from which the sulphuric acid was to be precipitated was spilled, and there has been no opportunity to repeat the experiment, so that we must place the sulphur content somewhat higher. We judge it to be in the neighbourhood of 2 per cent. We conclude that the proteid material of the ground substance had been decomposed by this treatment, for the odour of skatol became very apparent when steam was allowed to escape from the autoclave. The recovered pigment no longer emitted the odour of burnt feathers on being incinerated, but the presence of nitrogen was nevertheless conclusively shown by Lassaigne's method. The pyrrol reaction could no longer be obtained from the vapours emitted on dry distillation. This last fact is of importance. The presence of much pyrrol among the products of dry distillation of the melanins has led certain chemists to suspect that these substances are pyrrol derivatives, and we ourselves at first held this view. In two trials with a pigment obtained as above, and in a third trial with a pigment that had been heated to 260° C. with a saturated solution of barium hydrate, we failed to get the pine-sliver reaction for pyrrol, although a far smaller amount of the original pigment gave the reaction unmistakably. The vapours given off on burning some of the pigment purified in the manner just described with barium hydrate are difficult to characterize; but the odour of burnt feathers and of pyrrol is certainly not present.

In the experiment just referred to, in which barium hydrate was used to free the pigment from the ground substance, 1 gramme of pigment was kept at 260° C. with a saturated solution of this agent for four hours. After such treatment, this specimen of pigment, as we have already noted, failed to give the pyrrol reaction; and, strange to say, it was almost entirely precipitated from its solutions in nitric acid on the addition of an alkali, even when this solution no longer contained any barium.

We believe that the last-named method furnishes us with a means of entirely removing the ground substance from the pigment, and it must be left to later research to show with its help whether the substance thus obtained is a single chemical individual.

The substance obtained when the pigment is heated under pressure with potassium hydrate is perhaps closely related to the hippomelaninic acid obtained by Berdez and Nencki,* by fusing with potassium hydrate the melanin isolated from melanotic tumours of the horse.

It will be seen that we are only at the beginning of a true chemical knowledge of the melanins of the skin and hair. It would seem very probable that the melanins thus far described by investigators were all more or less contaminated with the proteid to which we have referred. But it is certainly a great advantage to see our way to an easy removal of all contaminating substances, and we propose, with the help of this method, to continue our investigations into the chemical nature of the melanins of the skin and hair.

14. Combustion Analyses.

Below are given the results of combustion analyses of pigment from the skin and hair prepared in various ways. We are fully conscious of the fact that these analyses do not assist us in establishing an empirical formula for our pigment, for the reason that the true pigment is still contaminated with the proteid-like substance that constitutes the groundwork of the pigmentary granules. Our object in making them was to learn whether different methods of preparing the pigment and prolonged treatment with strong alkalies really alter the so-called isolated pigment, when neither colour nor chemical behaviour have been in the slightest degree affected. Had spectrophotometric methods been applied, it is probable that they would have supplemented these analyses in a valuable way, or even have rendered them unnecessary.

* *Arch. f. exp. Path. u. Pharmakol.*, Bd. xx, p. 359.

Pigment of the epidermis, prepared by repeated precipitation with alcohol and ether and a little acetic acid out of solutions in dilute potassium hydrate, and finally out of solutions in ammonia, from which, however, the ammonia had been expelled before precipitation by the agents named. The special feature of this method is the mild treatment with potassium hydrate. The pigments of both the epidermis and hair are excessively hygroscopic, so that all weighings must be performed in well-closed capsules.

0.2972 gramme of pigment burned in oxygen current with lead chromate, finely powdered lead chromate being also strewn on the substance in the combustion boat, gave 0.5468 gramme of CO_2 and 0.1000 gramme of H_2O. The ash content of this specimen, as determined by previous analysis, was 3.17 per cent; calculating for ash-free substance, we find that the above specimen contained 51.83 per cent C and 3.86 per cent H.

0.3656 gramme of substance gave by fusion with KOH and KNO_3, after previous oxidation with concentrated nitric acid—the analysis being carried out in detail according to the directions of Hammarsten *—0.0928 gramme of $BaSO_4$ = 3.60 per cent S, calculated for ash-free substance.

0.2065 gramme of substance gave by the method of Dumas 24.7 cubic centimetres of nitrogen with the barometer at 759.2 millimetres and the temperature 28.5° C. N = 0.02802 gramme, or, calculated for ash-free substance, 14.01 per cent.

Hair pigment prepared in the same manner as skin pigment, except that it was subjected for a longer time to treatment with potassium hydrate. 0.1922 gramme gave 0.0073 gramme ash = 3.79 per cent.

0.2439 gramme of pigment gave 0.4536 gramme of CO_2 and 0.0746 gramme of H_2O, or C = 52.74 per cent and H = 3.53 per cent.

0.8114 gramme of pigment gave 0.1897 gramme of $BaSO_4$, the analysis being carried out as already described for the sulphur analysis of the skin pigment. Calculated for ash-free substance, S = 3.34 per cent.

0.3953 gramme of substance gave by the method of Dumas 34.8 cubic centimetres of nitrogen, with the barometer at 760.2 millimetres and the temperature 26.5° C. N = 0.03995 gramme; correcting for ash, we find N = 10.51 per cent.

The percentage composition calculated for the ash-free pigment is therefore as follows:

* *Zeitschr. f. physiol. Chem.*, Bd. ix, p. 289.

Pigment of the Epidermis.		Pigment of the Hair.	
C =	51·83	C =	52·74
H =	3·86	H =	3·53
N =	14·01	N =	10·51
S =	3·60	S =	3·34
O =	26·70	O =	29·88
	100·00		100·00

It will be seen from the above table that the pigment of the epidermis differs markedly in its nitrogen content from that of the hair. This difference is very likely due to the more prolonged treatment with potassium hydrate. We have found that the method of preparation has a great influence on the percentage composition of the pigments, and it seems impossible to prepare two specimens from the same original material so that they will give the same analytical results. Thus, a specimen of pigment from the epidermis, analyzed in the early stages of purification by the alcohol method, had the following composition: C = 52.17 per cent. H = 4.14 per cent. N = 12.8 per cent. S = 4.02 per cent. O = 26.87 per cent.

The pigment of both skin and hair was next treated on the water bath with a very strong solution of the potassium hydrate, and the effect noted.

A quantity of pigment whose ash content had already been reduced to a low figure was dissolved in a ten-per-cent solution of potassium hydrate, and this solution was reduced in a nickel bowl on the water bath to fifty or sixty per cent in strength. Water was then added and the evaporation repeated, and this treatment was kept up for six hours. The solution was then diluted and filtered, and the pigment precipitated with an excess of acetic acid, after which it was collected on flat filters and washed with alcohol until all traces of potassium and acetic acid had been removed. The pigment was now taken up in ammonia, the ammonia expelled on the water bath, and the black solution filtered and precipitated with a large excess of alcohol and ether and a small amount of acetic acid. It was then collected on flat filters, washed free of acetic acid with alcohol, and then treated with hot alcohol, chloroform, and ether. Thus prepared, the pigment of the epidermis has the following composition:

0.1333 gramme of pigment from the epidermis gave 0.0011 gramme of ash, consisting of minute dark specks = 0.82 per cent.

0.1139 gramme of another specimen of pigment from the epidermis that had not been allowed to stand so long while in a state of solution gave 0.00142 gramme of ash, consisting of minute black specks = 1.24 per cent.

0.1570 gramme of pigment from the epidermis gave 0.3044 gramme of CO_2 and 0.0723 gramme of H_2O. Correcting for an ash content of 1.24 per cent, we have C = 53.56 per cent and H = 5.11 per cent.

0.2357 gramme of pigment from the epidermis gave 30.0 cubic centimetres of nitrogen, with the barometer at 767.4 millimetres and a temperature of 18° C.; correcting for ash, N = 15.47 per cent.

0.4126 gramme of pigment from the epidermis gave 0.0750 gramme of $BaSO_4$; correcting for ash, S = 2.53 per cent.

0.4170 gramme of pigment from the epidermis gave 0.07006 gramme of $BaSO_4$. This particular analysis was made with a specimen containing 2.34 per cent of ash. Correcting for ash, we have S = 2.36 per cent.

Pigment of hair treated with strong potassium hydrate as previously described:

0.2598 gramme gave 0.0057 gramme of a reddish ash = 2.19 per cent.

0.1665 gramme gave 0.34075 gramme of CO_2 and 0.0799 gramme of H_2O. C = 57.06 per cent; H = 5.45 per cent.

0.1646 gramme gave by the method of Dumas 17.6 cubic centimetres of nitrogen at 20° C., with the barometer at 758 millimetres. N = 12.87 per cent.

0.7337 gramme gave 0.0924 gramme of $BaSO_4$. S = 1.77 per cent.

Placing the results in parallel columns—

Pigment of Epidermis.		Pigment of Hair.	
C =	53·56	C =	57·06
H =	5·11	H =	5·45
N =	15·47	N =	12·87
S =	2·53 : 2·36	S =	1·77
O =	23·33	O =	22·85
	100·00		100·00

Comparing the later analyses—that is, after the prolonged digestion with strong potassium hydrate—with those previously obtained with the help of less destructive methods, we see that the sulphur content has been considerably lowered for both pigments, and the carbon hydrogen and nitrogen content raised.

We are inclined to believe that this change in the sulphur con-

tent of the pigments is due to the fact that the contaminating substance derived from the pigmentary granules has been in part decomposed by the potassium hydrate. The fact that it is impossible to isolate two specimens of the pigments so that their percentage composition does not vary with even slight differences in the method of preparation, also points to the conclusion that we are not yet dealing with a single chemical individual, that our so-called isolated pigment is a mixture of at least two substances, one of which is responsible for this variability. It seems very improbable that this variability can be referred to the pigmentary compound, for this retains its colour and its general chemical character, however severe the treatment to which it is subjected.

15. The Iron Content of the Isolated Pigments.

The pigmentary granule, as we have seen, contains much iron, but, as the pigment isolated from these granules is purified, we find that the iron content steadily diminishes until but the merest trace remains, only to be accurately estimated by the spectrophotometric method.* We must therefore conclude that iron is not present as a constituent part of the molecule of the pigment; that it does not enter into its chemical structure, as does the iron of hæmatin, for example, but is present only as an impurity.

The present diversity of opinion as to the origin and iron content of the melanins, of which our pigment is one, is, we must believe, owing to the fact that a clear distinction has not always been made between the complex pigmentary granule and the pigment itself. The former contains iron; the latter does not.

In support of our view as to the low iron content of the pigmentary substance, we present, first, analyses of pigment with a relatively high ash content, and then analyses showing the ash content reduced to a low limit.

Thus, a specimen of pigment prepared by precipitating its first solution in potassium hydrate with strong acetic acid, and then wash-

* Cf. Mörner, *Zeitschr. f. physiol. Chem.*, Bd. xi, p. 87.

ing the collected pigment thoroughly with this acid, had an ash content of 3.3 per cent and an iron content of 0.14 per cent, estimated by titration with a weak solution of potassium permanganate. We always found this high percentage of iron when the pigment had not been repeatedly dissolved and precipitated.

Another specimen which had been prepared by the method of frequently repeated precipitation with alcohol gave the following results: 0.3863 gramme yielded 0.0134 gramme of ash, or 3.46 per cent. The amount of this ash taken up by dilute hydrochloric acid, after first rendering the silicic acid insoluble, was 0.0079 gramme. The amount of iron in this solution, estimated by the gravimetric method of Ludwig and Gottlieb,* was found to be 0.000385 gramme (0.00055 Fe_2O_3 obtained). The iron content of the pigment in this case was therefore 0.099 per cent. The residue of the ash insoluble in dilute hydrochloric acid consisted for the most part of silicon dioxide, and weighed 0.0055 gramme. In this instance we see that the iron has been much reduced, although the ash remains high, on account of the alcohol method being used.

A still lower iron content is found in specimens of pigment that have been prepared by the method of prolonged treatment with potassium hydrate, as already explained.

I. Thus 0.2253 gramme yielded 0.00529 gramme of a black, powdery ash. Of this ash 0.00359 gramme passed into very dilute hydrochloric acid, after having first rendered the silicic acid insoluble. The iron of this amount was found by titration with a weak solution of permanganate to be 0.00017 gramme, and constituted therefore only 0.076 per cent of the pigment. The part of the ash insoluble in dilute HCl consisted of silicon dioxide and aluminium oxide.

II. Pigment = 0.1333 gramme, ash obtained = 0.0011 gramme, or 0.82 per cent, and containing 0.00003 gramme of iron, as estimated by colour titration with KCNS. This specimen of pigment therefore contained only 0.02 per cent of iron.

III. Pigment = 0.1139 gramme; ash obtained = 0.00142 gramme, or 1.2 per cent, and consisting of minute black specks with a fused appearance. The iron of this ash, as determined by titration with a weak

* *Arch. f. exp. Path. u. Pharmakol.*, Bd. xxvi, p. 139.

solution of permanganate, amounted to 0.00016 gramme, or 0.14 per cent of the amount of pigment taken.

The ash of the hair pigment was also analyzed as above described, and with similar results. When purified until a low ash content was obtained—say 2.19 per cent—the iron was also found to amount to far less than 0.1 per cent. Thus, 0.4492 gramme of substance gave 0.0078 gramme of ash, the iron of which, as determined by titration with weak permanganate, amounted to 0.00026 gramme, or 0.057 per cent.

The iron determinations recorded in this paper, both of the granules and of the pigment, were made in three ways, and we have usually indicated in each case which method was employed. When only a mere trace of iron was thought to be present, the method of analysis was by colour titrations with potassium sulphocyanide; the second method, the one most frequently employed, was by titration with a solution of potassium permanganate, each cubic centimetre of which was capable of oxidizing 0.00043 gramme of iron; the third was by the gravimetric method of Ludwig and Gottlieb, which has been found trustworthy up to the limit of half a milligramme of iron, when only small filters and small quantities of reagents are used. Thus, one of us (Abel) recovered by this method half a milligramme of iron from a few cubic centimetres of a solution containing precisely that amount of iron which had been standardized by Dr. T. B. Aldrich. Miss Katharine Porter also recovered by this method 0.00239 gramme of Fe_2O_3 out of 0.00244 gramme taken. The Fe_2O_3 was prepared from pure ferric chloride.

16. The Origin of the Pigmentary Substance.

When a pigment is found to contain iron, this fact has been thought to furnish some evidence that it is a derivative of hæmoglobin; but if no iron is present, it may still claim that source, for the animal organism contains a number of pigmentary substances, such as hæmatoidin and bilirubin, that have no iron in their composition, and which, nevertheless, are derived from hæmoglobin. This class of iron-free pigments, it will be remembered, lack the element sulphur.

The coloured substances of the epidermis and hair agree with this class in containing no iron, but they differ in that they contain a considerable amount of sulphur, which we must consider to be present as a constituent part of their molecule. We are not at present acquainted with any coloured natural derivative of hæmoglobin that contains sulphur, nor do we know of any such produced by chemical means. The proteid moiety of hæmoglobin contains but little more than a half per cent of sulphur, while our pigment contains at least three times this amount.

The presence of sulphur in the pigment makes it certain that it is derived from a proteid, and we can as easily suppose some proteid of the blood to be the direct precursor of the pigment as that hæmoglobin should be decomposed in order that its proteid moiety may serve this purpose. We should have an equal right to assert that keratin is derived from hæmoglobin.

17. Total Amount of Pigment and of Pigmentary Granules in the Skin of One Negro.

The entire skin, with the exception of that on the palms of the hands and soles of the feet, was removed from the cadaver of a tall, slender negro, and from this skin the epidermis was removed with the exception of a few small areas. After being cleansed with water, alcohol, and ether, the epidermis thus obtained was found to weigh 34.08 grammes. Making a liberal allowance for the thicker epidermis left on the feet and hands, and the small areas not removed from the knees and elbows, we may assume that the entire removable epidermis of the negro in question, when freed of hairs, fatty substances, water, etc., would have weighed 40 grammes. 9.9306 grammes of this epidermis yielded 0.2182 gramme of pigment, the pigment being gathered and weighed immediately after its first precipitation. Calculated on this basis, we find that the epidermis of this cadaver must have contained at least 0.8789 gramme of pigment. The individual in question was judged to be a full-blooded negro, but his colour was of the bronzed order rather than deep black. The epidermis was also thinner than usually seen, and the case may therefore be assumed to

contain somewhat less than the average amount of pigment. We therefore assume the average amount of the pigmentary substance, as at present isolated, to be in the neighbourhood of 1 gramme for an adult negro of average body surface. If we assume, also, that the pigmentary granules as lodged in the living cell contain sixty-five per cent of water and about five per cent of mineral constituents, there would be found in the skin of the average negro about 3.3 grammes of pigmentary granules.

18. Function of the Epidermal Pigment.

While we ourselves have done no work on the function of the pigment, the subject is one of such interest that we must be allowed a brief account of the investigations of others.

Hom * exposed his own hand alongside of that of a negro to the direct rays of the sun, with the result that his own hand became inflamed, while that of the negro remained unaffected, thus showing how great a protection is afforded by the pigment of the negro's skin. C. Eijkmann,† of Batavia, has recently published a most interesting experimental research on the regulation of body temperature among whites and Malays in Batavia. The relative ability of the two kinds of skin to radiate and conduct heat, and the extent to which sweating takes place in the two races, were carefully studied. By the following experiment the two kinds of skin were also compared as to their ability to absorb sunlight, in which respect they were found to differ markedly. Two thermometers exactly alike were treated as follows: The bulb of one was covered, first, with a piece of skin from a Malay, and outside this with a piece of skin from a white man. On the second thermometer the order of the skin wrappings was reversed, the white skin being put next the bulb and the brown skin outside. After a period of exposure in a moist chamber to the direct rays of the sun,

* Cited from Boubnoff, Ueber das Permeabilitätsverhältniss der Kleidungsstoffe zum chemisch wirkenden Sonnenstrahl. *Arch. f. Hyg.*, Bd. x, p. 363. This investigator's name is given as Horn by Eijkmann in Virchow's *Archiv*, Bd. cxl, p. 157.

† Virchow's *Archiv*, Bd. cxl, p. 125.

the first thermometer registered 47.5° C., the second 50.1° C. Eijkmann also says that the brown skin felt distinctly warmer to the hand than the white skin. Now Widmark * has shown that the ultra-violet rays of the spectrum are far more effective in causing that superficial inflammation known as sunburn (erythema solare) than are the rays of less rapid undulation. Indeed, sunburn and that more marked inflammation resulting from exposure to a very powerful electric arc light are both caused to so preponderating an extent by the ultra-violet rays that heat, the degree of illumination, and other factors that have hitherto been regarded as causative agents, may be entirely neglected.

Hammer † has furnished corroborative experiments by covering the skin with ointments and lotions containing sulphate of quinine, which has the power of changing violet rays and ultra-violet rays to less refrangible ones, and has thus succeeded in greatly lessening the inflammatory action of sunlight and of the arc lamp. Bouchard is also cited by Paul Bert ‡ as having demonstrated that violet rays are more effective in raising a blister than rays of less refrangibility. Hammer has also abstracted from the literature of dermatology a number of interesting cases of great sensitiveness of the skin to light, and in all of these cases it was shown that the chemically active rays alone were responsible for the inflamed condition of the skin.

It is a matter of common observation that the photograph of a negro is indistinct and unsatisfactory compared with that of a white man, the light reflected from the black skin having been largely deprived of its actinic quality. Boubnoff's # experiment with frog's skin placed over sensitive plates has also shown how effective are thoroughly pigmented areas in absorbing the chemically active rays of light. It may therefore be assumed that it is one function of the pigment to absorb the chemical energy of the sun's rays, and, although the pigmentary layer of the skin would thereby tend to take on a higher

* Cited at length in Hammer. Ueber d. Einfluss des Lichtes auf d. Haut. Stuttgart, 1891, pp. 29-33.

† *Op. cit.*, pp. 33-41.

‡ *Rev. scientifique*, 1878, No. 42, p. 987.

Loc. cit., p. 362.

temperature, yet the inflammation of the more sensitive vascular corium underneath is in reality prevented.

19. Conclusions.

The pigmentary granules of the negro's skin and hair can be freed in several ways from the cells in which they are lodged and collected in any desired amount.

As thus obtained, these granules are found to be insoluble in dilute alkalies, dilute hydrochloric acid (hot or cold), alcohol, or other organic solvents when applied in the order named. If, after they have been subjected to the action of dilute hydrochloric acid, they are again treated with dilute alkalies, they are found to give up their pigment, and, on the continued application of heat, the granules dissolve entirely in the alkaline solution, leaving only an insignificant residue.

The pigmentary granules are composed of a colourless ground substance or substratum, a pigment, and much inorganic matter. Their inorganic constituents, as thus far determined, are calcium, magnesium, iron, and silicic, phosphoric, and sulphuric acids; and these constituents possibly play an important part in the deposition and fixation of the colouring matter in the granules.

The pigment isolated from the granules, and sufficiently freed from adherent inorganic matter, contains only the merest trace of iron—so little, in fact, that we must think of it when entirely pure as free of iron. Heating the isolated pigment with barium hydrate at a temperature of 260° C. entirely frees it from the closely adherent ground substance, and it is then found that the vapours of pyrrol are no longer emitted when it is subjected to dry distillation, and the odour of burnt feathers is no longer discerned, although nitrogen is still present.

We can not conclude as the result of our work that the pigment is a derivative of hæmoglobin; it seems to us more probable that it is ultimately derived from the proteids of the parenchymatous juices.

The total quantity of soluble pigment in the skin of a negro of average size is found to weigh about 1 gramme; the weight of the pigmentary granules is about 3.3 grammes, if we are right in our assumption

that they contain sixty-five per cent of water and five per cent of mineral constituents in their natural state in the epidermis.

The pigments of the epidermis and hair of the negro are very likely identical. In the present state of our knowledge we can only say that it seems highly probable that the pigment of the negro's hair is not different from the dark pigment found in the hair of the white races, and we may infer that the pigment of the black skin differs only in amount and not in kind from that deposited in the skin of the white man.

Description of Plate XIV.

Fig. 1.—A single cell from the lowest stratum of the rete mucosum, showing the pigmentary granules.

Fig. 2.—The epidermis after maceration for two hours and a half with a five-per-cent solution of potassium hydrate.

Fig. 3.—The same after maceration for six hours. The pigmentary granules here seen have been spread out in a thin layer by slight pressure on the cover glass.

Fig. 4.—The dried pigmentary granules in the form of the snuff-coloured powder described in the text, mounted in water.

Fig. 5.—The pigmentary granules of the epidermis stained with Ehrlich's gentian-violet solution after having been boiled in a sealed tube with a mixture of concentrated ammonia and alcohol.

Fig. 6.—A piece of hair macerated for fifteen minutes in five-per-cent potassium hydrate and then crushed and mounted. It shows the large ovoidal pigmentary granules.

Fig. 7.—The isolated pigmentary granules of the hair stained with Ehrlich's gentian-violet solution, after having been partially decolourized by means of concentrated ammonia and alcohol.

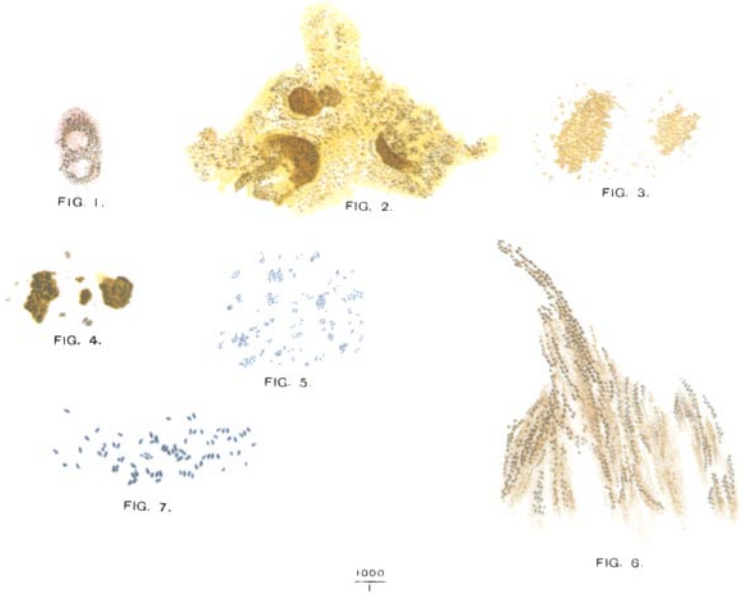

FIG. 1.

FIG. 2.

FIG. 3.

FIG. 4.

FIG. 5.

FIG. 6.

FIG. 7.

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