STUDIES ON LIPOCHROMES *

IV. THE NATURE OF THE PIGMENTS IN CERTAIN ORGANS

CHARLES L. CONNOR

(From the Department of Pathology, Harvard Medical School, Boston, Mass.)

In previous papers the works of Willstätter and Stoll,[1] Van den Bergh and Snapper,[2] Palmer and his associates,[3] and others, have been referred to, and the methods, in part, by which they have established the identity of the lipochrome pigments with the carotin and xanthopyll of plants have been mentioned. I have been able to demonstrate the presence of carotin by chemical means in the liver, spleen, adrenal glands, corpus luteum, skin and fat, using a method which seems to be satisfactory for its quantitative estimation, at least for purposes of comparison.[4] But many tissues which are commonly said to contain lipochrome are not amenable to chemical examination because of their close association with other carotin-containing tissues, or because of the small amount present in the body. It was therefore necessary to resort to histologic methods to demonstrate the presence or absence of the pigment in such tissues. By the application of methods previously described,[5] and by chemical examinations, I have been able to confirm the presence of lipochrome in the tissues just mentioned but could not demonstrate it satisfactorily in the heart, ganglion cells, seminal vesicles, or in any other tissues which are commonly said to contain this substance. The pigment present in these last organs, as is well known, is a yellow to brown granular substance which is frequently tinged with fat stains, and therefore has been called lipochrome in this country, and lipofuscin in Germany. These two names are used to designate the substance in most English and American literature, but they actually represent different pigments. Borst originated the term "Lipofuscin" (Hueck [6]) because he thought it was derived from some sort of lipoid. Sehrt [7] named it "fat-binding wear-and-tear pigment" (*fetthaltige Abnutzungspigment*), and Lubarsch [8] used this term for over twenty years. From time to time during this period the question has been taken up in Lubarsch's laboratory and varying results

* Received for publication May 10, 1928.

reported by Brahn and Schmidtmann,[9] Salkowski,[10] and Staemmler.[11] Salkowski, and Brahn and Schmidtmann devoted their time to chemical examinations, comparing the substance to melanin. They found that it agreed well so far as elementary analysis is concerned, with the latter pigment, both containing carbon, hydrogen, nitrogen and oxygen in about the same proportions. The pigment which they analyzed contained no iron. Staemmler, using a combined iron cyanide and silver nitrate method found that all these pigments (of heart muscle, seminal vesicle, even adrenal cortex) could be blackened and he concluded that they were melanin or forms of melanin. Block,[12] however, insists that melanin is formed only in ectodermal or mesodermal melanoblasts, the first found in the epidermis, retina, scattered cells in mucous membranes and the nervous system, the second (mesoderma) in the choroid of the eye, and rarely in the corium. Melanin is present also in phagocytic cells (melanophores) in the corium but is not produced by these cells. Masson [13] agrees essentially with this, differing only in the name of the cells which form the pigment. Melanin is found only (Masson) in cells of the nervous system, and, while these are widely scattered, they can be differentiated from others by their morphologic and staining characteristics. Wells [14] says that melanin is probably not found even in pathologic conditions in cells which normally do not produce this pigment.

There are therefore, as regards melanin, two schools of thought: one that melanins form a group of pigments, all closely related, some formed in cells, others as the result of degenerative processes in tissues (Lubarsch), and the other that melanin is a specific substance elaborated by specific cells which, in man, are mostly of ectodermal origin, and in lower animals are of mesodermal and ectodermal origin (Bloch, Masson, and others).

Hemofuscin is a term used by von Recklinghausen [15] to name the yellow pigment found associated with hemosiderin in hemochromatosis. This is a yellow-brown pigment occurring in cells (endothelial or epithelial) or in the muscle and connective tissue cells of the liver, pancreas, intestinal wall and other organs in great abundance in this disease. Von Recklinghausen described brown atrophy of the heart and liver as localized hemochromatosis because of the presence of this pigment. Lubarsch and his school never recognized the name, but from the time of Sehrt's work in 1904 until recently, they have

considered it a fat-containing "wear-and-tear" pigment and adopted Borst's term, lipofuscin, for it. Recently, as has been mentioned, Lubarsch and his co-workers have tried to prove it to be melanin. Hueck identified hemofuscin with lipofuscin in 1912 and considered it to come from a lipoid, probably fatty acid. It was not until Mallory became interested in hemochromatosis that the name hemofuscin was restored.

Mallory, Parker, and Nye [16] have shown that this pigment is constantly associated with hemosiderin in hemochromatosis in a large number of organs, and describe it as forming granules of light yellow pigment which do not give the iron reaction, but stain with Nile blue, fuchsin, and safranin. It is soluble in alkalis and bleaches with hydrogen peroxide. Mallory [17] has further found it in normal organs, in the liver, pancreas, heart and kidneys. It was found in adults above the age of 45 and was always associated with hemosiderin. He found it associated with hemoglobin and hemosiderin in the wall of a chocolate cyst of the ovary and has concluded that hemofuscin is an intermediate product between the two.

Having ruled out the possibility that this "wear-and-tear" pigment is a lipochrome, after the development of methods by which the latter pigment could be rather definitely identified, it seemed necessary to apply these and other known methods to an examination of this mysterious pigment and to correlate, if possible, the results of other workers. Therefore a review of the properties by which all the known pigments are usually differentiated was made with the following result:

1. *Melanin:* Blackened with silver nitrate; precursor positive to dopa; does not stain with fat stains; insoluble; bleaches with oxidizing agents.

2. *Derivatives of Hemoglobin:*

   (*a*) *Hemosiderin:* Gives the iron reaction (Prussian blue with potassium ferrocyanide and acid); not bleached.

   (*b*) *Hematoidin:* Crystalline character; negative reaction to stains; no iron; soluble.

   (*c*) *Hemofuscin* (Mallory): Stains with basic fuchsin; no iron; bleached with hydrogen peroxide.

3. *Lipochromes:* Stain with fat stains; soluble in fat solvents; are easily bleached.

4. *Lipofuscin* (Borst): Stains irregularly with fat stains; insoluble; negative to silver nitrate; bleached with difficulty.

It was obvious from the start that these properties were by no means specific. For instance, practically all pigments can be bleached, and, while most pigments may be tinged with fat stains, I have previously found that lipochrome, in particular, does not take these stains. Consequently, a revision of the methods used had to be made and a technique developed, the details of which are as follows:

Technical Procedure

1. *Unstained Sections:* Formalin-fixed, cut on the freezing microtome.

2. *Fat Stains:* The staining of formalin-fixed frozen sections with acetone-alcohol solution of Scharlach R, and aqueous solution of Nile blue sulfate.

3. *The Dopa Reaction:* This is said by Bloch to be specific for the precursor of melanin in cells, revealing it as brown or black pigment in those cells which have a potential melanin-producing property. It does not affect, or only slightly darkens, fully formed melanin. Briefly, the technique is as follows: A 1: 1000 solution of *3–4* dioxyphenylalanine in triple distilled water which has been freshly boiled and which has a pH of 7.3 to 7.4, is made immediately before using. A buffer solution (an appropriate mixture of primary and secondary sodium phosphate) is added in sufficient quantity to keep the pH within the limits mentioned (about 2 cc. per 100 cc.). Fresh unfixed tissues cut on the freezing microtome are placed in this solution for from 16 to 24 hours at room temperature, or for 4 to 6 hours at 37° C. In using the method, sections of skin (I used mostly black guinea pig skin) are placed in the solution with the sections to be studied and examined from time to time for results. All tissues should be removed before greatly discolored and before a precipitate forms. The sections are washed in distilled water, allowed to dry in the air on a slide, cleared with xylol and mounted in balsam. Some sections were counterstained with methyl green-pyronin, as recommended by Bloch.

4. *Silver Nitrate Reaction:* Paraffin sections of formalin-fixed tissues were made at first, using Wright's rapid technique (Mallory and Wright, Ed. 8, p. 447), but considerable precipitate was always

present. Bielchowsky's method gave cleaner tissues, but also usually left some precipitate. Levaditi's method with blocks of tissue gave the most uniform results. These were made in the usual way and the first fifty or so sections discarded. Sections from the deeper layers contained little or no precipitate.

5. *Bleaching Agents:* Ferric chloride, peroxide of hydrogen, or direct sunlight were used to cause bleaching, and the reaction is recorded as positive if fading of the pigments could be caused by any process short of their actual disintegration by chemicals. Ferric chloride was used in saturated solution in 50 per cent alcohol and applied to the section on slides while under occasional observation. Hydrogen peroxide was used in 3 per cent solution into which the sections were placed for from 24 to 90 hours, sometimes changing the solution several times. Blocks of tissue were dried in sunlight before or after treating with dehydrating agents and gross observation made. (This could be applied to adrenal cortex, skin, corpus luteum, seminal vesicles and testicle.)

6. *Fat Solvents:* Acetone and chloroform were used, the chloroform after dehydration with acetone. The usual process of making paraffin sections was not relied upon to dissolve out fat and lipochrome, as frequently some of the less soluble lipoids, such as cholesterol, are left in the tissues and give false reactions with fat and basic fuchsin stains. Chloroform alone, without previous dehydration of the tissues, will not extract all the lipochrome.

7. *Alcoholic Potassium Hydroxide:* Used according to a method previously reported: 10 per cent potassium hydroxide in 70 per cent alcohol plus an equal amount of 4 per cent formaldehyde solution. The tissues are placed in this solution for from 40 minutes to 2 hours, dried on a slide, and observed directly or after mounting in glycerine. This solution will dissolve the tissue if prolonged, and one must vary the time according to the tissue under investigation. The pigment, if lipochrome, collects in small masses, or precipitates as crystals which become visible in the microscope. Melanin and hemoglobinogenous pigments are not affected.

8. *Basic Fuchsin:* This is said by Mallory to stain hemofuscin. It also stains other substances, such as lipoids and the cytoplasmic granules of tissue mast cells. It does not stain melanin. The stain was used 0.5 per cent in 50 per cent alcoholic solution upon tissues after thorough extraction with fat solvents, and most sections were

differentiated in 95 per cent alcohol until practically colorless. The pigment which stained by this method could not be decolorized by alcohol.

9. *Iron Reaction:* Mallory's method with ammonium sulphydrate followed by potassium ferricyanide and acetic acid was used. The sections were counterstained with basic fuchsin, and differentiated in 95 per cent alcohol.

10. *Chemical Examination for Lipochrome:* Organs were dissolved and saponified in alcoholic potassium hydroxide, dehydrated, and extracted with petroleum ether after a method previously described. In some cases the resultant pigment was identified by spectroscopic examination.

Tissues Examined

The source of the tissue, and the pertinent histories are as follows:

I. *Heart Muscle:*

1. From a six weeks' old infant, dying following an operation for pyloric stenosis; very little pigment present.
2. From a 44 year old woman with nephritis and a large heart; abundant pigment present.
3. From a man of 79, death from peritonitis following a ruptured duodenal ulcer. Weight of heart 280 gm. A fair sample of brown atrophy.
4. From a man of 81 dying of arteriosclerosis and bronchopneumonia. Heart normal in weight; abundant pigment.

II. *Liver:* From the four cases named above, and several from guinea pigs. The latter reacted essentially the same as the human livers.

III. *Intestine:* From the men of 79 and 81 years.

IV. *Spleen:* From the four cases mentioned above and from a case of lobar pneumonia in a young man.

V. *Seminal Vesicle and Prostate:* From the old men, and a man of 21 with pneumonia.

VI. *Adrenal Glands:* Eight from various sources, all essentially normal.

VII. *Corpus Luteum:* From surgical specimens at the Peter Bent Brigham Hospital.

VIII. *Skin:* From several of the adults mentioned above, from a 3 months' old infant and from a black guinea pig. All normal tissues.

IX. *Testicle:* From two old men.

X. *Carotin Lesion:* A granulomatous lesion which followed the injection of pure carotin into the peritoneal cavity of a guinea pig.

XI. *Old Hemorrhage:* A section of ovary containing a hemorrhagic cyst, the wall of which was formed by granulation tissue and endothelial cells. A great deal of pigment was present in cells and interstitial tissues.

Results

Heart: In unstained sections the characteristic pigment is noted in granular yellow to brown masses at each end of the nucleus, or irregularly scattered throughout the muscle. It becomes rusty-red with Scharlach R., green with Nile blue sulfate. The color is deepened by silver nitrate and after prolonged treatment some of the dense masses appear almost black. It can be bleached after extraction with chloroform and treatment with hydrogen peroxide; is harder to bleach with ferric chloride alone. It is negative to fat solvents, alcoholic potassium hydroxide and dopa. No lipochrome could be extracted. Some of the pigment gives the iron reaction; most of it stains intensely red with basic fuchsin, but some is only tinged with the dye.

Liver: Unstained: yellow and brown pigment in liver cells; small amount in Kupffer cells. Scharlach R stained some pigment red, some only a rusty yellow. Blue and green pigment present with Nile blue. Much of the pigment is blackened by silver nitrate; some only moderately darkened. Most of it can be bleached. It is negative to dopa, fat solvents and alcoholic potassium hydroxide, except that some seems to have been dissolved. Much of it is stained by basic fuchsin but some is only tinged by this dye. Some gives the iron reaction. Lipochrome could be extracted (carotin 1 to 6 mg. per cent).

Spleen, Intestine, Seminal Vesicles, Prostate, Testicle: The reactions in these organs were essentially the same as in the heart and liver. In all the pigment was tinged red with Scharlach R, and most often green, that is to say, not stained, with Nile blue sulfate. The pigment could be darkened by silver nitrate and where dense masses of it occurred it appeared black. Most of the pigment could be

bleached out. In all, the dopa reaction, reaction to fat solvents and alcoholic potassium hydroxide were negative, except that sometimes the alkali appeared to dissolve some of the pigment. Two constant results were obtained with basic fuchsin, namely, a deep red-staining substance in droplets and granules, and a more granular material which was only tinged with the dye. In all except the testicle more or less iron-containing pigment could be demonstrated. Only the spleen yielded lipochrome upon extraction (carotin, a trace to 2.1 mg. per cent). The pigment of the interstitial tissue of the seminal vesicles appeared to be the same as in the epithelial cells. Some of this was scraped away, treated with absolute alcohol, in which it was insoluble, then taken up in dilute ammonia water in which it became soluble. This gave a positive guaiac reaction, but contamination with blood could not be excluded, though the tissue had been well washed previous to treatment. In the testicle the pigment was always present in interstitial cells, and nearly always took an intense red stain with fuchsin. A rare blue dot could be found in tissues examined for iron, but the reaction was, on the whole, negative in this organ.

Adrenal Cortex, Corpus Lutem, Carotin Lesion: These tissues gave the same reactions. Unstained, the pigment of the adrenal cortex and corpus luteum occurs in such fine particles that it cannot be resolved in the microscope. The tissue appears uniformly yellow, and all the pigment is obscured when fat stains are used. It is dissolved out by chloroform after dehydration with acetone, is readily bleached with ferric chloride and potassium hydroxide, and when treated with alcoholic potassium hydroxide and formalin the pigment collects in small aggregates and in visible yellow to reddish yellow crystals. It is negative to dopa. Fuchsin stains the fat in which it is dissolved, but after treatment with fat solvents neither fat nor pigment remains to be stained. Silver nitrate precipitates in the cytoplasm of the fat-laden cells, but in the carotin lesion where large masses of lipochrome occurred, a darkening of the pigment occurred with silver nitrate. The pigment, upon extraction, proved to be carotin (adrenals: carotin, 4.25 to 15.6 mg. per cent; corpus luteum: carotin, 4.1 mg. per cent; this also contained an alcohol-soluble pigment, possibly xanthophyll). No pigment could be demonstrated or extracted from the adrenals of a 3 months' old infant.

Adrenal Medulla: The pigment here is brown in unstained sections, takes a rusty tinge with Scharlach R, and green with Nile blue. It becomes intensely black with silver nitrate and can be bleached. It could not be seen that dopa increased the amount of pigment present, though it darkened the existing pigment somewhat. It was negative to fat solvents, alcoholic potassium hydroxide, basic fuchsin and the iron reaction. Chemical examination could not be made.

Skin: Yellow to brown pigment was present in many of the basal cells in the unstained section. In addition a yellow crystalline pigment could be seen in some sections in the cornified outer layer, quite sparse in most specimens and absent in some. The two pigments gave different reactions. The first, in the basal cells, was not stained by Scharlach R, but became green with Nile blue. It was intensely blackened by silver nitrate, was negative to fat solvents, alcoholic potassium hydroxide and basic fuchsin, and did not give the iron reaction. Dopa increased the amount of pigment present and slightly darkened the existing pigment. The second pigment was tinged with Scharlach R, not stained with Nile blue, was darkened slightly with silver nitrate, and unaffected by dopa. It was more easily bleached than the first pigment but both bleached finally. Most of the outer pigment was soluble in fat solvents, negative to basic fuchsin and the iron reaction, and, being already crystalline, it was not affected by alcoholic potassium hydroxide. These were obviously different pigments, the deeper being melanin, the outer, lipochrome. (Some of the outer pigment might also have been melanin, as all was not dissolved out.)

Old Hemorrhage: Abundant yellow and light brown pigment was present in the connective tissue and endothelial cells surrounding the cyst. Some of this was tinged red with Scharlach R, and green with Nile blue. Some of the pigment was definitely blackened with silver nitrate. It was unaffected by dopa, not soluble in fat solvents, but some seemed to have been dissolved out by alcoholic potassium hydroxide. Much of the pigment stained intensely red with fuchsin and about half gave the iron reaction. Some yellow pigment was tinged red with fuchsin and some was not stained at all. Chemical examination was not made.

Discussion

It seems somewhat difficult to bring order out of the chaotic state in which one is left after a study of the varying opinions recorded above. It is obvious that too much reliance has been placed upon what are considered to be specific histologic reactions. There are a few points, however, upon which almost universal agreement exists, and if one concedes that silver nitrate, a notoriously unreliable agent, does upon occasion darken or even blacken other pigments besides melanin, a correlation can be made which is compatible with most observed facts and with some opinions. If silver nitrate blackens melanin only, then one must conclude that this pigment is normally and usually present in the liver, spleen and hemorrhagic foci, and develops in granulomatous lesions (such as those produced by the injection of carotin), a conclusion which seems entirely unreasonable. In fact, no pigment seems to be completely unaffected by this salt, all of them showing the same brownish tinge which intercellular substances exhibit. If the pigments occur in densely packed masses the density of color is increased proportionately and the appearance of blackening becomes an optical rather than an actual effect. This is probably the case in the liver and spleen where comparatively large amounts of pigments are normally found.

All pigments except hemosiderin can be bleached, lipochrome the most readily, and practically all are lightly stained with Scharlach R. Nile blue sulfate, as pointed out by Hueck, gives a greenish appearance to pigments, not because of actual staining, but because of the mixture of yellow pigment and blue dye. These dyes, and others, seem merely to be adsorbed to the surfaces of the pigments.

The dopa reaction is not expected to differentiate pigments once fully formed. The results recorded here are therefore not conclusive, but do confirm other work in that no substance was found in heart muscle, liver and other organs, except the skin, and possibly the adrenal medulla, which was capable of forming melanin from dioxyphenylalanine.

Fat solvents, properly used, effectively remove true lipochrome and alcoholic potassium hydroxide affects this pigment only, causing it to aggregate and crystalise from its usually dispersed particles.

The two most constant reactions have been those produced with basic fuchsin stain and the test for iron. Two reactions to fuchsin

were present; (1), an intense red produced in a substance which appeared frequently as droplets rather than granules, and (2), a red tinge in definitely granular pigment. In the hemorrhagic focus one can hardly escape the conclusion that these substances represent stages in the transformation of hemoglobin into hemosiderin, a conclusion previously reached by Mallory. The intense red pigment seems to be a semiliquid substance which by condensation becomes granular and so gradually loses its affinity for the fuchsin stain. If this is true for the hemorrhagic focus, it seems likely that it is also true in other places where a similar association of pigments is found.

This brown pigment (hemofuscin, Mallory; lipofuscin, Borst; fetthältige abnutzungspigment, Sehrt; abbaupigment, Lubarsch; alterspigment, Oberndorfer;[18] lipomelanin, Kutschera-Aichbergen;[19] sometimes lipochrome in American literature) is constantly associated with hemosiderin in the normal heart, liver, spleen, smooth muscle of the intestine, seminal vesicles and prostate; is present, with hemosiderin, in increased amount in brown atrophy of the heart and liver; increases proportionately with hemosiderin in all these organs and others in hemochromatosis (Mallory); and is always associated with hemosiderin in local hemochromatosis (pseudomelanosis, ochronosis) of the large intestine (Lubarsch). Hemosiderin was present in very small amount in the testicle, and not at all in the heart of the 6 weeks' old infant, though pigment which stained with fuchsin was present in both these organs.

A word of caution is needed here. One assumes, without justification perhaps, that most material of a granular nature which is shown to contain iron is hemosiderin (that is, iron derived from hemoglobin). Where this substance is found associated with hemorrhage, the conclusion that it comes from hemoglobin seems obvious. But Sprunt, Colwell and Hagan,[20] have apparently shown that iron may be derived from other proteins during autolysis, and that an iron-containing pigment is not necessarily a product of hemoglobin decomposition. Hueck has said that hemosiderin is, in fact, an inorganic iron compound by the time it is recognizable in tissues, and so has no property by which its origin can be traced. The only point one can make here is that, whatever the source, it seems to be the same for both hemofuscin and its iron-containing satellite.

True lipochrome (carotin) can be demonstrated histologically in the adrenal cortex, the corpus luteum, in atheromatous plaques of

the aorta and the skin. It cannot be differentiated from other pigments in the liver and spleen, although it was shown to be present in these organs by chemical examination. Also, because of the nature of the tissue, it is not demonstrable histologically in fat, but can be extracted from adipose tissue. It is to be noted that lipochrome was not present in any of the tissues of the infants studied.

This pigment should offer no morphological difficulty. Only in the outer layers of the skin can it be seen as a granular pigment. In all other places it occurs in solution in lipoids, giving the tissue a yellow coloration, but is not visible as a particulate substance by the microscope. The granules in the skin are probably formed by condensation and precipitation in the outer layers as the epidermal cells are pushed outward and become keratinized. It is difficult to find the pigment in this tissue probably because it is actually scant in normal skin, and because a large part of it becomes oxidized and is then invisible.

Melanin could be demonstrated beyond a reasonable doubt only in the skin, although a definite effort to locate this pigment in all tissues where it might occur was not made. The pigment of the adrenal medulla gave all the reactions of melanin, and none of those which have been found to distinguish other pigments. If this work and that of Bloch and Masson are conclusive, they show that melanin is a constituent of certain special cells only, and cannot be regarded as the product of degenerative processes in tissues.

Conclusions

A revision of the characteristics of these several pigments must be made. So, we have:

1. *Melanin:* A brown pigment occurring in certain cells of ectodermal or nervous tissue origin (ectodermal melanoblasts) and, in man, in what are essentially cell rests, in cells of mesodermal origin (mesodermal melanoblasts) which form Mongolian spots and certain blue nevi. In addition it may occur in phagocytic cells (melanophores, chromatophores) in the corium. There is no definite evidence that true melanin occurs elsewhere. The pigment is intensely blackened by silver nitrate and is revealed, in cells which are capable of forming it, by the dopa reaction. It is insoluble in acid and alkaline solutions, and in fat solvents. In common with all other pig-

ments except hemosiderin it is bleached by oxidizing agents. It occurs pathologically in melanotic tumors.

2. *Hemosiderin:* An iron-containing pigment, insoluble, may be darkened with silver nitrate, does not bleach, stains irregularly with fat stain, is lightly stained with basic fuchsin. It occurs in phagocytic cells and intercellular tissues (after degeneration of the cells which originally contained it), normally in the spleen and liver (epithelial and Kupffer cells), and in advancing age in the muscle of the heart, intestine, seminal vesicles, prostate and blood vessels. It is increased in the heart and liver in brown atrophy of these organs; and in these and many other organs in hemochromatosis; is present in the mucosa, submucosa and muscle of the intestine in pseudomelanosis, or local hemochromatosis. It is always present in the vicinity of old hemorrhages.

3. *Hematoidin:* Yellow crystalline or semiliquid (dissolved in tissue juices), soluble, is darkened with silver nitrate, bleaches readily, contains no iron and does not stain with fat or basic dyes.

4. *Hemofuscin:* Yellow or brown semiliquid or crystalline; insoluble in fat solvents, partly soluble in alkali; does not give the iron reaction, but darkens with silver nitrate; stains lightly with fat stains (by adsorption); in early stage of formation it stains intensely with basic fuchsin, but as it becomes crystalline, it stains only lightly with this stain, and bleaches less readily than hematoidin, melanin and lipochrome. *Hemofuscin is present in all places and under the same conditions as hemosiderin,* in usually greater amount. It, with hemosiderin, constitutes the "wear-and-tear" pigment of the body, and is probably a product of the metabolism of hemoglobin, both from blood and muscles.

5. *Lipochrome (Carotin, Xanthophyll):* A diffuse, finely dispersed yellow pigment, associated with fat. It does not stain with fat stains, is soluble in fat solvents, bleaches readily and darkens with silver nitrate (when crystalline). It is aggregated, or becomes crystalline, when treated with alcoholic potassium hydroxide. It occurs naturally as crystals only in the outer layers of the skin. It is present normally in the adrenal cortex, corpus luteum, liver, spleen, fat and skin; increases in these tissues with increased ingestion of food, or when, for some reason, lipemia is present. It occurs in pathologic processes in atheromatous plaques of arteries, and in xanthomas. It is not present in the tissues of infants.

There are therefore two pigments which accumulate in the body tissues in advancing age, and seem to be increased in cachectic conditions. Only one of these, however, (hemofuscin, which apparently slowly becomes hemosiderin), can be regarded as a "wear-and-tear" pigment, the result of slow or rapid disintegration of a tissue protein. (The fact that this pigment is present in the heart of infants is dismissed by Rössle [21] with the statement that "Man is old before he is born.") When lipochrome is present in increased amount it is due to increased ingestion, or to the absorption of the fat containing it. In the latter condition, no more lipochrome is present than formerly, but the proportion to the amount of remaining solvent is greater.

Summary

A review of the characters which are said to differentiate the various pigments of the body was undertaken in conjunction with the chemical examination of certain organs for lipochrome (carotin and xanthophyll). Tissue from the normal heart, liver, spleen, skin, intestine, seminal vesicles, prostate and testicle, as well as a lesion produced by carotin injection in a guinea pig and an old hemorrhagic focus, were treated in identical ways histologically, and where feasible, chemically. Two groups of pigments were found to accumulate in the body with age, namely, lipochrome and hemofuscin (with hemosiderin). The former of these is exogenous in origin; the latter constitutes the real "wear-and-tear" pigment, and possibly is derived from muscle hemoglobin.

From the results of this inquiry the pigments present in the various tissues studied seem to be:

1. *Skin:* Melanin and lipochrome; also, in hemochromatosis; hemofuscin and hemosiderin.
2. *Fat:* Lipochrome.
3. *Heart, Intestinal Muscle, Seminal Vesicles, Testicles, Prostate:* Hemofuscin and hemosiderin. Increased in old age, brown atrophy and hemochromatosis.
4. *Liver and Spleen:* Hemofuscin and hemosiderin besides bile pigment. Increased in old age, brown atrophy and hemochromatosis.
5. *Adrenal Cortex:* Lipochrome.
6. *Adrenal Medulla:* Probably melanin.
7. *Corpus Luteum:* Lipochrome.

REFERENCES

1. Willstätter, R., and Stoll, A. Untersuchungen ueber Chlorophyll. Julius Springer, Berlin, 1913.
2. Van den Bergh, A. H., and Snapper, J. Die Farbstoffe des Blutserums. *Deutsches Arch. f. klin. Med.*, 1913, cx, 540.
3. Palmer, L. S. Carotinoids and Related Pigments. Chemical Catalogue Co., New York, 1922.
4. Connor, C. L. Studies on lipochromes III. The quantitative estimation of carotin in blood and tissues. *J. Biol. Chem.*, 1928, lxxvii, 619.
5. Connor, C. L. Studies on lipochromes II. The identification of carotin, xanthophyll, and associated lipoids in tissues. *Am. J. Path.*, 1928, iv, 235.
6. Hueck, W. Pigmentstudien. *Beitr. z. path. Anat. u. z. allg. Path.*, 1912, liv, 68. Also, Die pathologische Pigmentierung. Handb. d. allg. Path. Krehl-Marchand, 1921, Bd. 3.
7. Sehrt, E. Zur Kenntniss der fetthältigen Pigmente. *Virchows Arch. f. path. Anat.*, 1904, xvii, 248.
8. Lubarsch, O. Ueber fetthältige Pigmente. *Centralbl. f. allg. Pathol. u. path Anat.*, 1902, xiii, 881; *Ibid.* Ueber das sogenannte Lipofusin. *Virchows Arch. f. path. Anat.*, 1922, ccxxxix, 491.
9. Brahn, B., and Schmidtmann, M. Pigmentstudien. Zur Kenntnis des Melanins und des braunen Abnutzungspigments. *Virchows Arch. f. path. Anat.*, 1920, ccxxvii, 137, *Ibid.*, Zur Pigmentfrage, 1922, ccxxxix, 488.
10. Salkowski, E. Ueber die Darstellung und einige Eigenschaften des pathologischen Melanins. *Virchows Arch. f. path. Anat.*, 1920, ccxxvii, 121.
11. Staemmler, M. Untersuchungen über autogene Pigmente. *Virchows Arch. f. path. Anat.*, 1924, ccliii, 459.
12. Bloch, B., and Ryhiner, P. Histochemische. Studien in überlebendem Gewebe über fermentative Oxydation und Pigmentbildung. *Ztschr. f. d. ges. exper. Med.*, 1916–17, v, 179.
    Bloch, B. Chemische Untersuchungen über das spezifische pigmentbildende Ferment der Haut, die Dopaoxydase. *Ztschr. f. physiol. Chem.*, 1916–17, xcviii, 226. Bloch, B., and Schaaf, F. Pigmentstudien. *Biochem. Ztschr.*, 1925, clxii, 181.
13. Masson, P. Les naevi pigmentaires, tumeurs nerveuses. *Annal. d'Anat. Path. et d'Anat. Normal.*, 1926, iii, 417, and 657.
14. Wells, H. G. Chemical Pathology, Ed. 5, 1925, 526.
15. von Recklinghausen, F. Handb. d. allg. Path. des. Kreislaufs u. d. Ernährung, Deutsch. Chir. II and III, 1883.
16. Mallory, F. B., Parker, F., Jr., and Nye, R. N. Experimental pigment cirrhosis due to copper and its relation to hemochromatosis. *J. Med. Res.*, 1921, xlii, 461.
17. Mallory, F. B. The relation of chronic poisoning with copper to hemochromatosis. *Am. J. Path.*, 1925, i, 117.

18. Oberndorfer, S. Die pathologischen Pigmente. *Ergebn. d. allg. Pathol. u. path. Anat.*, 1921, xix Abt, II, 47.

19. Kutschera-Aichbergen, H. Ueber Melanin und über das braune Abnutzungspigment. *Frankfurt. Ztschr. f. Path.*, 1922, xxvii, 21.

20. Sprunt, T. P., Colwell, H. S., and Hagan, H. J. Pigment formation in the liver during autolysis and its relation to the pigmentation of hemochromatosis. *J. Exper. Med.*, 1912, xvi, 607.

21. Rössle. Über das Altern. *Naturwissenschaftl. Wchnschr.*, 1917, xviii, 241.