XXXIII. THE LIPOCHROME OF ADIPOSE TISSUE IN MALIGNANT DISEASE.

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INTRODUCTION.

IN spite of the fact that in the course of the last ten years a considerable amount of research work has been carried out on the substances pigmenting fatty and other tissues of the body, there still seems to be a lack of unanimity regarding their chemical nature and function.

Thudichum [1869] who was the first to study the chemical character and distribution of these so-called lipochromes in the body, came to the conclusion that the "luteine" which he had isolated as a crystalline substance of a fatty nature, was responsible for the pigmentation of the corpus luteum and egg yolk. He also observed that this compound was to be found firmly held by protein substance in blood serum, in the cells of adipose tissue, and in the yellow fat of the secretion of the mammary gland or butter. In mammals it occurs abnormally in ovarian cysts and in serous effusions; in the plant world, he asserted it was present in seeds such as maize, in the husks and pulp of fruits such as anatto, in roots such as carrots and in the stamina and petals of a great variety of flowers of which he gave a list in his interesting paper. While Thudichum's contribution was of great importance in opening a new avenue of research, the term by which he designated or grouped together the various pigments worked on is rather unfortunate, being one rather of generic than of specific significance as it may be inclusive in many of his own instances of such widely differing substances as carotene, the *L*-, *B*- and *Y*-xanthophylls of Schunck [1903] and the anthocyanin and flavone flower pigments [Dufour, 1887; Devaux, 1888]. Later investigators, notably, Schunck, Escher, Willstätter, Newbigin, Palmer and Eckles, have helped considerably to make our knowledge of the lipochromes more definite.

Newbigin [1898] has isolated a typical lipochrome from the muscles and ovaries of the salmon. The pigment of cow fat does not, however, give the common lipochrome reactions according to this investigator.

Palmer and Eckles [1914] in a series of studies on cow milk and fat pigmentation came to the conclusion, on the other hand, that carotene and xanthophyll were invariably associated together in the pigmented localities,

and surmised that the pigment of human fat was derived exogenously from vegetable food and was of a carotinoid nature.

The present paper is chiefly concerned with the pigment of human adipose tissue, and I shall refer to it as adipochrome in order for descriptive purposes to differentiate it from such pigments as occur physiologically in the corpus luteum, in the epithelium of the seminal vesicles, testicles and epididymis, in the Kupffer cells of the liver, and pathologically in renal epithelium and in cases of brown atrophy of the heart.

There is much difference of opinion regarding the constitution of fat pigment. Some investigators identify it with carotene in spite of the fact that it does not give the lipocyanin reaction with concentrated sulphuric acid¹; others state that it contains a carotene-xanthophyll complex, while a third group maintains that many fat pigments accumulated under pathological conditions are merely "waste pigments" (lipofuscins) of non-vegetable origin and are closely related in structure to the fat-like substances from which they are held to be derived.

SCOPE OF RESEARCH.

The purpose of this research was to determine if the pigment of circumcarcinomatous fat was in any way different from that of normal fat, and in view of the divergence of opinion expressed regarding the constitution of normal fat pigment, it became necessary either to investigate the nature and properties of adipochrome, or to compare, without elucidating its structure, certain of its outstanding chemical properties with those of the pathological fat pigments worked on. I have not carried out an extensive investigation on the structure of adipochrome, although an empirical formula and certain information relative to its chemical constitution are given. A rigid comparison of chemical and physical properties was made not only between pigment from normal and pathological fat, but between these and carotene and xanthophyll from plant sources, and the results are incorporated in this paper.

EXPERIMENTAL.

Isolation of adipochrome.

The pigment-containing fat was obtained—post-operative and post-mortem—chiefly from the breast and abdominal wall. Adipochrome was isolated by various methods, *e.g.* by the well-known saponification method, and by a process outlined by the author in a separate paper [1924] which seemed to possess some advantages over the potash method, which tends to produce resinification of pigments and condensation products of certain impurities present in traces in the alcohol. The aldehyde resins produced from alcohol and possibly from fatty acids are highly coloured, inert substances, closely

¹ Carotene on treatment with concentrated sulphuric acid gives a blue colour and small blue crystals of lipocyanin are formed.

simulating adipochrome in their chemical behaviour and their presence with the latter substance may modify to a considerable extent its characteristic response to chemical or physical agents.

Some properties of adipochrome.

On account of the extremely small quantities of adipochrome isolated, the tests performed were mainly of a qualitative rather than of a quantitative nature, and, in consequence, the accumulation of data of value in the interpretation of function was considerably limited in scope and application.

The elemental composition of the pigment was first ascertained. It was found that carbon and hydrogen were present. In some cases there was a very faint trace of iron, but this was subsequently found to be present as an impurity. Nitrogen was found absent (by Castellana's modified method); phosphorus was also absent (Neumann's test). There was no indication of the presence of calcium or of halogens. Sulphur was absent.

The absence of phosphorus and nitrogen indicates that there is no lecithin residue or structural unit in adipochrome. The closely associated cholesterol is also absent as the substance gives no colour response to the Liebermann reagent.

The pigment as obtained from the sources previously mentioned was a reddish brown paste with a heavy sweat-like odour. It was difficultly crystallisable. On making a slide preparation with alcohol a magma of acicular crystals was obtained. The melting point of fat pigment isolated from a carcinomatous lesion was 35.5°.

An aqueous calcium chloride solution of pigment was decolorised by bromine water. Further confirmatory evidence of the unsaturated nature of the adipochrome molecule was forthcoming on making an osmic acid slide preparation, when a characteristic blackening took place.

The substance under investigation seemed to be of a weakly acid nature. An aqueous alcoholic solution after a very long interval produced a slight colour change towards the red in blue litmus paper.

The original calcium "salt" of adipochrome is insoluble in ether, light petroleum, benzene, chloroform, chloroform + 1 % alcohol (slight purplish colour developed in alcoholic chloroform layer), toluene, carbon tetrachloride, nitrobenzene, quinoline, ethyl alcohol and amyl alcohol. It is soluble in glycerol, ethyl benzoate, acetic anhydride and pyridine.

With selenic acid, formic, citric and tartaric acids, a slight "urochrome" blush is obtained.

Solubility tests were carried out on a porcelain plate with small quantities of adipochrome.

Adipochrome is soluble in aniline, carbon disulphide, ethyl alcohol, amyl alcohol, light petroleum, toluene, benzene, ether, nitrobenzene and carbon tetrachloride.

In concentrated sodium hydroxide it is comparatively easily soluble, a brown

solution which is unchanged by acetic acid, in contradistinction to that of the anthocyanins, being obtained. It is also soluble in concentrated ammonia, concentrated nitric acid and concentrated sulphuric acid. With the latter solvent no violet coloration is obtained¹.

The fat pigment is more easily soluble in ammonia than in caustic soda and a slightly darker brown solution is obtained. When potassium ferri-cyanide is added to this ammoniacal solution, an immediate colour change to yellow is observed, and on testing this yellow substance with ferric chloride and concentrated hydrochloric acid a deep Prussian blue colour is obtained, showing that in the presence of alkali the pigment substance is able to convert ferric iron to the ferrous state. It acts, therefore, in the presence of alkali as an oxygen distributor of singular potency.

The sulphuric acid solution of pigment on the addition of yellow potassium ferrocyanide shows an immediate colour change to olive green.

Adipochrome is easily precipitated from alkaline solution by tannic acid.

When Cole's solution (copper sulphate and glucose) was added to an ammoniacal solution of pigment there was a retention of the blue colour, but this does not necessarily mean that there is no carbohydrate structure in the substance, since the retention is primarily due to the formation of the cuprammonium ion. By other methods, however, *e.g.* the osazone microscopic method, it was found that there was no sugar group present.

Adipochrome gives (1) no colour reaction with ferric chloride, (2) is not bleached by sulphur dioxide; (3) gives no odour of benzene on heating with soda lime, thus implying the absence of an aromatic group; (4) gives a slight saturation action with zinc dust; (5) does not react with sodium bisulphite. When suspended in a 5 % solution of cane sugar, it gives a very faint pink colour to the solvent after several days.

A characteristic reaction is with H_2SO_4 , menthol and alcohol. Equal quantities of acid and alcohol are placed in a test tube containing a single crystal of menthol and a small quantity of adipochrome. Immediately a deep orange, almost red, colour is produced. In a control with the same reagents without adipochrome, a deep rich pink is produced, but only after a comparatively long interval. In the first tube, the colour development is instantaneous and progressive.

Cotte [1903] believes that adipochrome contains a cholesterol group, but we have not found the presence of this group made manifest either by the Liebermann test or by means of the Kahlenberg anhydrous arsenic chloride reagent [Kahlenberg, 1922].

The chemical nature of lipochrome.

It has been remarked by some observers [Palmer and Eckles, 1914] that the fat pigments of adipose tissue are of a carotinic or xanthophyllic nature, and many interesting conclusions regarding their biological significance have

¹ See footnote, p. 236.

been developed from the primary assumption. In the literature we have consulted on the subject there is very little direct reference as to the manner in which the ascription has been made.

The characteristic test for carotene is the development of a blue colour with concentrated sulphuric acid. It also forms a deep violet addition compound with iodine and gives a characteristic spectral absorption in the blue and the violet. It melts at 174°.

Xanthophyll gives the sulphuric acid reaction, forms addition compounds with the halogens, loses its colour on exposure to air and gives a characteristic three band absorption in the blue. It melts at 173°.

Fucoxanthin agrees very closely in chemical and physical properties with the members of the carotene group. It has a melting point 159°.

The evidence adduced in the previous section of this paper is sufficient to show that fat pigment is not of a carotinoid nature. The members of the carotene family have high melting points, ranging from 159° to 174°. Adipochrome has an extremely low melting point, *i.e.* 35.5°; the latter substance is crystallographically indefinite in its structural configuration giving a magma of acuminate crystals occasionally embedded in spheroidal or ellipsoidal aggregates of the same substance, while the carotenes crystallise regularly in the rhombohedral system.

Carotene is soluble in chloroform, carbon disulphide, benzene, difficultly soluble in light petroleum and ether. It is almost insoluble in cold alcohol, but goes into solution on prolonged boiling. Its behaviour is similar with methyl alcohol. Xanthophyll follows carotene closely in its solubility behaviour.

Adipochrome, on the other hand, is comparatively easily soluble in the solvents above enumerated. Even in light petroleum its solubility coefficient is so high as to render its complete separation from binding fat a matter of extreme difficulty.

Adipochrome like the carotenes contains carbon and hydrogen only and is an unsaturated compound. One would then expect that because of its unsaturated nature it would be peculiarly liable to undergo chemical change on exposure to air. On standing, however, there is no progressive increase in absorption of iodine and there is no evidence of autoxidation. It is, therefore, *in vitro*, particularly inert, though possibly in the body it lends itself to ready change in response to specific stimuli. Carotene on exposure to air undergoes chemical change and loses colour; adipochrome is also slightly chromolabile, but, on the other hand, the tendency is towards an increase of intensity of colour on exposure. This increase in colour intensity was noticeably in evidence in the case of a sample of pig fat.

The pigment of pig fat.

The fat, which was removed from a tumour-bearing animal assumed, on straining, a greenish tinge, probably due to a chlorophyllic break-down product. The freshly deposited fat did not seem to contain much pigment. It was poured into a beaker and allowed to stand for several

weeks, when at the circumference of the fat disc formed, deeply embedded in the fat substance and as far removed as possible from the influence of surface oxidation, a zone of reddish brown pigment was observed. There was a gradual shading away of pigmentation towards the fat surface, and at a certain point on the circumference of the ring (on the under side), where there was ample macroscopic evidence of fungal attack, the pigmentation was most intense. The mould was the common *Penicillium glaucum*. Moulds and bacteria are known to produce pigments which are classified on a differential basis of solubility in water and alcohol [Muir and Ritchie, 1919]. The pigment here produced did not give the lipocyanin reaction and was in other respects identical with adipochrome. It would seem that the pig fat pigment is endowed with an inherent chromolability. Micro-organisms may influence the chromogen or pro-chromogen. We would, however, think that the mould depends in the main on fat for its energy supply, and that it employs the already present adipochrome for a subsidiary purpose.

This view is partly borne out by the fact that the pig fat situated in a region of high *Penicillium* concentration is differently saturated from that of the remote areas where the mould distribution is on a sparser scale. The former fat (containing fat pigment) is harder, *i.e.* has a lower iodine value or is more saturated and is less soluble in ether. Adipochrome may possibly be used by the mould as a director of synthesis from a fatty substrate. With pig fat, fatty acid saturation is accompanied by increased adipochrome production.

The synthetic activity of the mould in manufacturing adipochrome is not of a very high order. In pathological fats, *e.g.* in cancer, the increase in pigmentation in fat areas near the growth is not so much ascribable to an increase in the synthetic activity of some specific enzyme as to the change in the solvent glyceride-, or free fatty acid-containing pigment. The concentration of oleic acid is increased in circum-carcinomatous fat, and there is a corresponding increase in the solubility of adipochrome. The phenomenon of increased pigmentation in the fat round cancerous areas goes hand in hand with that of increased fluidity of the pigment-containing fat. In this case *desaturation* activity is also accompanied by increased adipochrome production.

Absorption spectra of adipochrome.

The absorption spectra of the adipochrome from various sources further emphasise the difference between it and carotene and allied compounds. The spectrograph used for spectral examination was the Heele instrument, with a three prism train of uviol glass, and the light source was a copper arc. Table I (below) gives the necessary data for constructing a spectrum map for the instrument.

Table I.

Scale reading	10.1	10.4	12.4	13.45	15.3	21.25	28.6	29.4	37.8
$\lambda\lambda =$	6708	6409	5896	5700	5351	4608	4062	4023	3650

The nature of the absorption of adipochrome (in this instance, from a case of fibro-adenoma) is shown in Fig. 1 (Plate I). There is no selective absorption in the blue or violet region of the spectrum. In this plate, there seems to be a general absorption in the green and red end of the spectrum. This, however, is not so, as there was ample visual evidence of the presence of a continuous group of lines in this region. The exposure of the arc was much shorter in this instance, and this may account for the inability of the plate to pick out the missing lines. The general absorption, confined to the violet end of the spectrum, was characteristic of the human fat pigments examined.

In the second spectrophotograph (Fig. 2, Plate I) the first picture is that of the copper arc spectrum extending from $\lambda = 5770$ to the ultra violet;

the second is of pig fat pigment; the third is the absorption spectrum of snake fat dissolved in ether; the fourth is that of tomato pigment (lycopin) in alcohol, and the last is that of fat pigment removed from a case of fibrosarcoma.

A brief examination of this plate will show that absorption is least with snake fat pigment. It is harder than human fat, being of the same degree of consistency as mutton fat, and, in common with the latter, follows the general rule that saturation increase of the containing fat leads to a decrease in the intensity of pigmentation. Its fat pigment gives an absorption band between $\lambda = 5050$ and $\lambda = 4860$, and from $\lambda = 4050$ there is general absorption.

The adipochrome from pig fat, which gave a greenish tinge on straining, shows a distinct absorption band between $\lambda = 4860$ and $\lambda = 5050$; also between $\lambda = 4300$ and $\lambda = 4050$. Beyond $\lambda = 4050$ there is general absorption. There is also a suggestion of absorption between $\lambda = 4300$ and $\lambda = 4400$. Chlorophyll in living nettle leaf gives a corresponding absorption between $\lambda = 4300$ and $\lambda = 4510$. The absorption of pig fat pigment is very similar to that of urobilin in chloroform-alcoholic solution. With this solvent, urobilin exhibits one band between $\lambda = 5050$ and $\lambda = 4860$ with violet and ultra-violet absorption commencing at $\lambda = 4300$. Carotene in alcohol gives two bands; the first lies between $\lambda = 5000$ and $\lambda = 4810$ and the second between $\lambda = 4650$ and $\lambda = 4500$. From $\lambda = 4290$ there is general absorption. Xanthophyll in the same solvent shows absorption between $\lambda = 4550$ and $\lambda = 4450$. General absorption commences at $\lambda = 4150$. With lycopin there is a diffuse absorption in the blue and violet with general absorption in the ultra-violet; and in the last photograph—the mammary fat pigment from a case of fibrosarcoma (solvent, ether)—there is an absorption between $\lambda = 4860$ and $\lambda = 5050$. Beyond $\lambda = 4500$ there is general absorption.

A careful analysis of the above absorption ranges and an examination of the plate will demonstrate that pathological human fat pigment, as also several cases of fat pigment from other animal sources, presents few absorption features in common with carotene, xanthophyll, or fucoxanthin. One case is cited above of absorption in the visible region of fat pigment isolated from pathological human fat, but this phenomenon is rather the exception than the rule. In this laboratory, many pathological fats and several normal fats have been examined during the course of the year by the direct vision spectroscope and the human fat pigment above referred to stood alone in giving selective absorption, which was found to correspond closely to that of urobilin. Otherwise, the absorption was of the general nature shown in Fig. 1, Plate I. This type of absorption emphasises the diactinic nature of lipochrome, and, in this respect, the latter corresponds closely with the unsaturated aliphatic hydrocarbons.

¹ In this laboratory we have fed several mice exclusively on carrots over a period of six weeks, and on examination of the intestinal contents after death, abundant evidence, both spectroscopic and chemical, was forthcoming of the presence of carotene. No trace of this substance was obtained in the depot fat of the mouse.

concentration of isomers would, if present, offer an insuperable obstacle to spectroscopic differentiation. We have to be content with taking the spectroscopic evidence, with the melting point, for instance, as a criterion of purity and as an index of a certain structural individuality. Beyond that we cannot go; the remaining chemical evidence tends to show that the adipochrome is of a paraffinoid nature and very closely related in structure to the fat with which it is associated.

BIOLOGICAL SIGNIFICANCE.

It cannot be said that animal fat pigments are so amenable to classification as are plant pigments like anthocyanins, and the lack of coherent data for the correlation of their function and chemical structure impedes progress in marked degree in the ascertainment of their biological significance. In the body, pathological change manifests itself in many diverse pigmentary ways, *e.g.* in the red degeneration of submucous fibroids of the uterus, in the increased pigmentation of fat, bone marrow, and melanotic growths, in myeloid degeneration, in the ochronosis of cartilage, in chloroma, in hemochromatosis and in icterus.

In the already pigmented areas, increase in pigmentation may be due in some cases to an increase in concentration of the pigmenting substance; in other cases it may be due to the accession of a new structural entity; in others again there may be a working combination of both factors to produce a certain pathological end.

An example of the first condition is given by fat pigment local to a carcinomatous or sarcomatous lesion. In the second instance we may refer to the colour change produced in the skin after a heavy X-ray dosage. The mechanical effect of X-ray bombardment, coupled with X-ray absorption is the chief stimulus contributory to melanin change. The pigment accession at the site of a bruise is analogous to the pigmentation produced in plants by an unnatural accumulation of synthetic products, by trauma, by the puncture of insects in the plant leaf or stem, by fungal attack, or by blocking of the translocation current.

We can, however, hardly regard a tumour mass as a traumatic or a blocking factor in the modification of the comparatively inert adipochrome, for there seems to be no visible interference with the ordinary course of fat supply and distribution. It would seem that the increased pigmentation is partly due to bacterial attack intracarcinomatously, the associated pigment formed being transported to the neighbouring seat of storage. Thus, increased pigmentation in fat near to a malignant growth is of epiphenomenal rather than of prophenomenal import, and we can say that the manifestations of hyperchromatism or of chromidiosis in the tumour cell produce but a negligible response in the distant fat areas.

Adipochrome as an incitant, or cell proliferant, must, therefore, be ruled out of the category of such substances as Sharlach R (azo-*o*-tolueneazo- β -

naphthol) which produces an abundant epithelial proliferation in which both normal somatic and reduced mitoses occur [Barratt, 1907] and its gradual accumulation in its fat solvent might be regarded as a compensatory provision for the exothermic changes which the nutrient fat undergoes in response to the increased demand of the rapidly proliferating cancer cell.

SUMMARY.

1. Adipochrome is an unsaturated aliphatic hydrocarbon.
2. It is not related to carotene, xanthophyll, fucoxanthin, the anthocyanins, or the melanins.
3. It would seem to be manufactured from associated fat.
4. An account of its properties is given. *In vitro* it is comparatively inert chemically.
5. It is not a direct cell proliferant but may aid proliferation indirectly in virtue of its structural relationship to fatty acid.
6. Adipochrome of the pathological cases worked on (fats invaded by carcinoma, sarcoma) is not different from adipochrome in normal fat.
7. In cancer, there is an increase in adipochrome concentration in fat local to the growth.
8. The increased pigmentation is not due to chromoclasia but to an accessory chromosynthetic activity.
9. In the causation of cancer adipochrome is of epiphenomenal rather than of prophenomenal import, and bears no relationship to the chromidiosis of the tumour cell.

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DESCRIPTION OF PLATE.

- Fig. 1. Fat pigment from a case of fibro-adenoma. No selective absorption in evidence; general absorption in violet.
- Fig. 2. The first spectrophotograph is that of the copper arc; the second, that of pig fat pigment dissolved in ether; the third, that of snake fat, also dissolved in ether; the fourth, that of lycopin dissolved in alcohol; the last is that of fat pigment (mammary origin) from a case of fibro-sarcoma. Solvent, butyl alcohol.

Fig. 1.

Fig. 2.