CXXX. ON THE PIGMENT OF THE FAT OF CERTAIN RABBITS.

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ALTHOUGH the adipose tissue of man and certain farm animals is characterised by the presence of varying amounts of pigment, in certain other species of mammals, such as rodents, it appears to be almost entirely absent. Reference to the literature confirms this view [Palmer, 1922, p. 132], although Lewkowitsch [1922] states that rabbit fat is of a "dirty yellow colour." It was therefore both interesting and surprising to find, in the course of certain studies on genetics by other workers [Pease, 1928], that occasionally rabbits do exhibit adipose tissues that are deeply pigmented. Moreover, Pease has shown that this fat pigmentation follows the Mendelian laws and has demonstrated the existence of two types, one having yellow fat and the other white. The heterozygous animal has white fat, so that the white factor is the dominant.

From the biochemical point of view two problems suggested themselves for further study, namely, the nature of the colouring matter in the "pigmented" rabbit, and the reasons for its absence from the "unpigmented" rabbit. Only the former problem was investigated here and somewhat unexpectedly [*vide* Palmer, 1914] the pigment present proved to be xanthophyll, derived from the food. As it was not possible to carry the investigation further it was decided to publish the results obtained. The latter problem has since been investigated by Bernheim [1928]. He confirmed the presence of xanthophyll in the fat of the "pigmented" rabbit and found that its absence from the "non-pigmented" rabbit was due, not to an inability to absorb the pigment, but to the fact of its oxidation in the liver by a thermolabile system. This system was apparently absent from, or inhibited in, the liver of yellow-fatted rabbits.

EXPERIMENTAL.

The adipose tissue from a "pigmented" male rabbit (*Lepus cuniculus*) was carefully dissected off yielding in all some 175 g. The animal had been fed on the usual diet of bran, oats, hay and cabbage leaves without restriction. The character of the food is important because the pigment will only appear in the "pigmented" rabbit when some greenstuff is included in its rations. On the other hand, no matter what food is fed, the "non-pigmented" animal never develops pigment. In the case of the "pigmented" animal the food supply is thus the source of the pigment.

The fatty tissue was minced three times giving 170 g. of material with a colour of about the same intensity as that of New Zealand butter. It was then rendered in an oven at 65°, 134 g. of clear melted fat being decanted. The Sp. Gr. of the rendered fat was 0.930 (15.5°). The residue consisted of darker-coloured material, part of which was obviously tissue other than fat, and this was rejected. The pigmented fat was then immediately saponified with colourless 20 % alcoholic potash, using 2 cc. per g. of fat. In order to avoid the formation of resins the ethyl alcohol employed in the preparation of the alcoholic potash had been carefully purified by treatment with silver nitrate and unslaked lime. After 1 hour's gentle boiling under reflux the solution was cooled and diluted and the pigment extracted with purified ether in the usual way. The ethereal extract, which was deep yellow in colour, was then washed with distilled water until the washings no longer reacted alkaline to phenolphthalein. The washed extract was then dried over anhydrous sodium sulphate and the solvent removed under reduced pressure since the pigment is readily oxidised in ether. A residue of clean orange globules remained and these were dissolved in pure carbon disulphide and kept in the dark, and a portion was reserved for chromatographic analysis.

The unsaponifiable matter was readily soluble in the usual fat solvents (light petroleum, chloroform, etc.) giving yellow-coloured solutions. In carbon disulphide an orange solution was obtained, whilst in formic acid a green tint was noticeable on dilution. With antimony trichloride a slaty-blue colour was obtained. A portion of the unsaponifiable matter was taken up in 80 % alcohol and shaken with light petroleum (B.P. 50–60°) in the phase test. No pigment was given up to the light petroleum layer. These results suggested that the colouring matter present was probably xanthophyll.

The chromatographic analysis of Tswett [1906] provides another method of distinguishing xanthophyll pigments from carotin. Calcium carbonate, previously dried for several hours at 150°, was used as adsorbent. A glass adsorption tube of 2 cm. diameter and 12 cm. length was drawn out to a point at one end so as to leave an orifice of 2 mm. diameter. The narrow end was then closed by a tight-fitting plug of cotton-wool which was followed by the dry calcium carbonate. Great care was taken to fill the tube as evenly and tightly as possible. It was then connected with a small filter flask, suction applied and a stream of pure carbon disulphide passed through the column. A little of the pigment solution in carbon disulphide was now poured into the tube with gentle suction. This was followed by more of the pure solvent so as to establish a stream through the adsorbing column. Different adsorption rings were observed but the largest and best defined was of light orange colour and presumably represented xanthophyll *a*. No evidence was obtained for the presence of carotin. It was concluded therefore that xanthophyll pigments must be present.

Parallel with the saponification of the pigmented fat, a control experiment was carried out with the fat from an "unpigmented" male rabbit of about the

same weight as the "pigmented" animal. 145 g. of white fat were saponified. The unsaponifiable matter was isolated in exactly the same way as for the pigmented fat already considered. The dried ethereal extract containing the unsaponifiable matter was practically devoid of colour, and on removal of the solvent the solid matter was only faintly pigmented. Carotinoïd pigments were also found to be absent when the blood-serum of the same rabbit was examined by Palmer's method [1922, p. 207]. These results showed that the colouring matter of the pigmented fat could not have been developed during the saponification process but was derived from the animal's tissues.

SUMMARY.

1. Certain rabbits exhibit deeply pigmented adipose tissues.
2. The colouring matter present has been identified as consisting of xanthophyll pigments.

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