LI. A NOTE ON THE BLACK PIGMENT IN THE SKIN OF AN AUSTRALIAN BLACK.

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The pigment occurring in the skin of black races is stated to absorb the violet and ultraviolet rays in the solar spectrum, and thus to act as a protection against the harmful effects of tropical sunlight. This absorption has only been demonstrated in the case of the whole skin [1913], and no references are to be found in the text books on Biochemistry to any work on the absorption spectrum of any of the melanin pigments which have been prepared from various sources.

The present note contains an account of the preparation of melanin, from the skin of an Australian Black who died in the local hospital, which was prepared in order to study the absorptive action on light of the isolated pigment.

The only instance recorded in the literature of the isolation of melanin from human skin is that of Abel and Davis [1896], who prepared a black pigment from the skin of a negro. The tissue was destroyed by heating either with 5–6 % potassium hydroxide, or with concentrated hydrochloric acid, and the pigment separated in the form of granules which contained the pigment and the pigment structure. The melanin was set free from these "pigment granules" by treatment for several days with hydrochloric acid. It was then extracted with a warm solution of potassium hydroxide in which it slowly dissolved, and finally precipitated by the addition of alcohol and ether. It was purified by repeatedly dissolving in warm alkali and reprecipitating. Abel and Davis found the composition of the "pigment granules" to be:

C = 52·83 %, H = 3·86 %, N = 14·01 %, S = 3·6 %, Fe = – %

and of the free pigment after treatment with alkali:

C = 53·56 %, H = 5·11 %, N = 15·47 %, S = 2·53 %.

In a paper on the melanin in black wool, Gortner [1910] stated that this pigment was readily destroyed by continued heating with a solution of caustic alkali. He recommended for the extraction the use of hot very dilute (0·2 per cent.) sodium hydroxide which dissolved out the pigment without decomposing it. In his opinion the heating with strong alkalies or acids, employed by Abel and Davis and by other workers in the extraction of melanins from other sources, was calculated to decompose a large proportion of the pigment.

In the present instance, however, it was found that a solution of alkali of the strength recommended by Gortner was too dilute to attack the skin, and in the preparation described below 5 per cent. potassium hydroxide was employed and the melanin dissolved out by heating on the water bath with this solution. To avoid continued heating of the dissolved melanin with the alkali, the material was treated with a successive number of small quantities of the solution, each portion being only allowed to act for a short time. The melanin when purified had the composition:

C = 60·12 %, H = 6·70 %, N = 11·89 %, S = – %, Fe = 0·21 %,

thus differing from that obtained by Abel and Davis. On combustion in oxygen a small quantity of ash was left, which contained iron. Abel and Davis record the presence of iron only in the "pigment granules."

A solution of the pigment both in 5 per cent. potassium hydroxide and in concentrated sulphuric acid was examined spectroscopically. In both cases the solutions absorbed all the rays in the violet, the blue and in part of the green to a wave length of about 515μ. Beyond this point there was slight blurring of the green and orange, whilst the red was practically unaffected.

Experimental.

A piece of skin from the back, 450 grams, was cut up as finely as possible and was warmed on the boiling water bath with 400 cc. of a 5 per cent. solution of potassium hydroxide. After about half an hour the solution was filtered through glass wool, and most of the liquid squeezed out of the residue by pressing with a pestle. The residue was then treated with a fresh lot of alkali in the same manner, and this process repeated until the material had been thoroughly macerated. The bulk of the material including the pigment went gradually into solution, and the filtrates on cooling deposited a good deal of fat which could be removed by filtering through glass wool. Each lot of extract as obtained, was at once cooled, most of the fat removed,

and the mixture acidified with hydrochloric acid. The pigment was thereby precipitated as a dark brown powder, and was gradually collected by filtering each of the mixtures through the same paper.

When as much as possible had been obtained, it was purified by repeatedly dissolving in warm 5 per cent. potassium hydroxide, filtering and precipitating with hydrochloric acid. The last traces of acid were removed by repeatedly suspending in distilled water and filtering. The brown powder was then dried on the filter paper by washing with alcohol and ether, and the paper folded and extracted in a Soxhlet apparatus, first with alcohol, and then with ether. Finally it was washed with pure carbon bisulphide, then with pure ether, and dried in a vacuum over sulphuric acid.

The product was a dark brown powder, insoluble in water. It was readily soluble in warm dilute potassium hydroxide forming a brown solution. It dissolved in cold concentrated ammonia and in cold concentrated sulphuric acid, being precipitated from the latter by dilution with water.

The substance contained carbon, nitrogen, sulphur and a trace of iron.

When dried it was found to take up moisture very rapidly, so that the samples taken for analysis were dried to constant weight in a vacuum over phosphoric anhydride at the temperature of boiling toluene. After combustion in oxygen a very small quantity (0·5 %) of a red coloured ash was left, in which the iron was determined colorimetrically with potassium thiocyanate, a standard iron solution being employed for comparison.

The nitrogen was determined by Kjeldahl, but unfortunately the quantity of material available was extremely small, so that the result must be accepted with some reservation.

Sufficient material was not obtained for a quantitative analysis of the sulphur. Analysis:

0·1169 g.; 0·2577 g. CO_2; 0·0700 g. H_2O; 0·25 mgm. Fe.
0·0387 g.; 0·0046 g. N.
C = 60·12 %; H = 6·70 %; N = 11·89 %; Fe = 0·21 %.

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LII. QUANTITATIVE ESTIMATION OF ASPARTIC AND GLUTAMINIC ACIDS IN THE PRODUCTS OF PROTEIN HYDROLYSIS.

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The figures obtained by the majority of workers when estimating glutaminic acid in caseinogen by separating the hydrochloride in the usual way, are uniformly about 11 per cent. In two cases, however, very much higher yields of the recrystallised hydrochloride have resulted. Thus Osborne and Guest [1911] found 15·55 per cent., and Foreman 15·9 per cent. (unpublished result). In these two cases the most favourable conditions for the separation were obtained, but could not be clearly defined. The accuracy of the results, however, still remained in doubt, and the method is unreliable.

The ease with which the glutaminic acid hydrochloride separates seems to vary with the protein under treatment and with the amount of glutaminic acid present. Plimmer [1912] states that separation of the hydrochloride "occurs in the case of caseinogen and certain vegetable proteins which contain from 10–40 per cent. of this amino-acid." If the protein contains under 10 per cent., then, as a rule, no hydrochloride will separate. Occasionally, however, a separation is obtained; thus Hopkins and Savory [1911] working with Bence-Jones protein obtained 8·05 per cent.

When a protein contains so little glutaminic acid that separation as the hydrochloride before esterification is impossible, or any other difficulties are encountered, the estimation of this amino-acid must depend upon the working up of the higher boiling esters. The yield of glutaminic acid hydrochloride obtained in this way is always very poor when compared with that obtained by separation before esterification. An investigation of the unesterified portion will show that glutaminic and aspartic acids, probably

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