XXI. A NOTE ON DENATURED GLOBIN.

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THE formation of a protein material insoluble at the isoelectric point in a solution of native protein is known as denaturation, and the protein is said to be denatured. The physical criterion of insolubility has been widely applied to decide whether a protein has been denatured or not by some extraneous influence. Besides this physical test the formation of an —SH (or of an —S—S—) group may likewise be used; this latter standard has the advantage that it can be applied to the changes undergone by keratins where solubility alterations cannot be studied. When the nitroprusside test of Mörner [1901] is applied to denatured proteins it is found that these can be divided into three classes:

- (1) those which give a reaction for an —SH group;
- (2) those which give a reaction for —S—S—;
- (3) those which do not give a reaction for either free —SH or —S—S—.

Egg-albumin [Heffter, 1907] typifies the first group, and serum-proteins the second. Ovomucoid [Harris, 1923] and globin [Meldrum and Dixon, 1930] yield neither sulphhydryl nor disulphide groups and accordingly belong to the third class.

Anson and Mirsky [1931] state that Meldrum and Dixon's observation is erroneous and claim to have obtained a preparation of denatured globin which gave tests for both —SH and —S—S— groups.

The author has re-examined the matter: he has confirmed the original observations of Meldrum and Dixon [1930], and has succeeded in explaining those of Anson and Mirsky. Ox and sheep globins were prepared by three different methods:

- (1) the Schulz [1898] method,
- (2) according to Hill and Holden [1926],
- (3) by the acetone method of Anson and Mirsky [1930].

The first two methods consist, in essence, in removing haematin from globin by the use of ether, the aqueous layer being acid. The last process consists in precipitating free globin by adding excess of acid acetone to an acidified solution of carboxyhaemoglobin. Schulz's method yields entirely denatured globin, the others a mixture of denatured and native material².

Globin preparations having been made in these three ways, the acid aqueous

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² It is best to use haemoglobin dialysed free from the glutathione present in blood-corpuscles.

solutions were boiled, or saturated with urea, and allowed to stand. The denatured material was ground moist with ammonium sulphate and sodium nitroprusside, and alkali was added. The test was negative with globins made by the methods of Schulz and of Hill and Holden. Thus the sulphhydryl group appears to be absent from denatured globin made by the use of ether. In the case of acetone-globin a faint purplish tint appeared. This differed from the typical nitroprusside test for $-\text{SH}$ in being of a different colour. It was more stable and did not disappear with time as does the typical nitroprusside reaction for $-\text{SH}$. When globin made in these three ways was ground with $(\text{NH}_4)_2\text{SO}_4$ and potassium cyanide the nitroprusside test was again negative with ether-globin, and again a faint purplish tint appeared with acetone-globin, identical in appearance with that obtained before treatment with cyanide. The statement of Anson and Mirsky that denatured globin contains the normal amount of $-\text{S}-\text{S}-$ groups could not be confirmed.

Now the Rothera test for acetone bodies resembles the nitroprusside test for sulphhydryl closely. Grote [1931] has described a method by which the nitroprusside test for $-\text{SH}$ can readily be distinguished from that due to acetone. When the solution to be tested is saturated with sodium bicarbonate and sodium nitroprusside added the typical colour is developed when sulphhydryl is present, but acetone bodies do not react. On applying this test to acetone-globin no colour develops, but the typical purple appears when ammonia is used as alkali.

The following experiment was devised to analyse the system further. A 5 % solution of acetone-globin was slowly neutralised as described by Anson and Mirsky [1931]. The precipitate of denatured globin yielded a pale purple colour with the nitroprusside reagent in the presence of ammonia and ammonium sulphate, but not in saturated bicarbonate. The solution of native globin obtained by filtration from the denatured fraction was electro dialysed for 8 hours in the apparatus of Stadie and Ross [1926], the current density being 0.2–0.3 amp. A solution of native globin thus obtained yielded no observable nitroprusside reaction after it had been denatured by boiling or treatment with urea.

SUMMARY.

1. The observation of Meldrum and Dixon [1930] that denatured globin does not yield a nitroprusside reaction for free $-\text{SH}$ or $-\text{S}-\text{S}-$ groups has been confirmed.
2. The statement of Anson and Mirsky [1931] that denatured globin contains the amount of $-\text{SH}$ and $-\text{S}-\text{S}-$ groups normal to most denatured proteins could not be confirmed.
3. Only in the case of globin prepared with acetone does any colour appear on treating with nitroprusside. This colour differs from the normal given by sulphhydryl, and does not appear when the conditions are chosen to obviate reaction between nitroprusside and acetone.

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