114. THE IRON OF THE PLASMA

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THE iron content of the plasma is small but it is probably of great importance as transport iron. In the normal subject the range is narrow and results obtained by various workers within recent years have shown general agreement, the figures ranging from about 0.1 to 0.2 mg. Fe per 100 ml. [Barkan, 1927; Fowweather, 1934; Tompsett, 1934; Moore, 1937].

Barkan [1925] found that plasma Fe was not ultrafiltrable but became so after incubation of the plasma with dilute HCl at 37°. Tompsett [1934] found that only a small fraction of the plasma Fe could be recovered in trichloroacetic acid extracts and that added Fe (as iron alum) could not be recovered quantitatively in such extracts. Fowweather [1934] made a similar observation. The writer has found that plasma Fe and added Fe could be recovered quantitatively in trichloroacetic acid extracts if thiolacetic acid, a reducing agent, had previously been added. It has also been found that whereas ferrous Fe could be recovered quantitatively in trichloroacetic acid extracts of materials containing phosphatides or phosphoproteins, ferric Fe could not. Plasma Fe was shown to be in the ferric state *in vitro*. In later work, Barkan [1937] noted that the plasma Fe could be recovered quantitatively in trichloroacetic acid extracts provided that the plasma had previously been incubated with dilute HCl at 37° for 24 hr.

The following experiments were carried out in order to obtain a clearer insight into the nature of the plasma Fe and the Barkan phenomenon.

In the first experiments, the influence of plasma on the dialysis of ferrous and ferric salts was studied. To 5 ml. of plasma was added 1 mg. Fe (ferrous sulphate, iron alum, iron ammonium citrate) and the mixtures were dialysed against 15 ml. water for 4 hr. At the end of this period the Fe contents of the dialysates were estimated with thiolacetic acid and NH_3 [Tompsett, 1934]. Typical results are shown in Table 1, where it will be seen that whereas ferrous Fe dialysed easily, ferric Fe dialysed hardly at all.

Table 1. *Typical results obtained in a study of the dialysis of added Fe from plasma*

1 mg. Fe added; the figures represent mg. Fe that have dialysed in 4 hr.

Ferrous sulphate	0.30
Iron alum	0.01
Iron ammonium citrate	0.01
Iron alum (plasma containing 0.8% HCl)	0.18

Tompsett [1940] has shown that certain foodstuffs are capable of reducing ferric Fe to the ferrous state, particularly at acid reactions. It was suggested that proteins were chiefly responsible for this phenomenon. It seemed possible that serum and plasma could have this property. 1 mg. Fe (as iron alum) was added to 5 ml. plasma. On standing no appreciable reduction occurred. On standing at acid reactions reduction did occur, as evidenced by the fact that

after standing 1 hr. in the presence of 0.8 % HCl, a red colour developed on the addition of $\alpha\alpha'$ -dipyridyl.

The dialysis of ferric Fe from acidified plasma (0.8 % HCl) was then examined. As will be seen from the results shown in Table 1 ferric Fe appeared to dialyse readily under these conditions. Upon examination with $\alpha\alpha'$ -dipyridyl, it was found that the whole of the Fe in the dialysates was in the ferrous state.

The question naturally arises as to the nature of the substances producing this reduction. It might be due to ascorbic acid; the writer, however, is rather of the opinion that the plasma proteins are responsible. Ascorbic acid has been found to reduce ferric Fe as readily in neutral as in acid solution, whereas in the case of plasma an acid reaction appears to be necessary or to be very definitely favourable. This problem requires further investigation.

DISCUSSION

The Fe present in samples of plasma obtained under conditions which do not prevent the re-oxygenation of haemoglobin is in the ferric state. This accounts for the fact that the plasma Fe, unless subjected to special treatment, is not ultrafiltrable. The phosphatides are probably more concerned with this phenomenon than the proteins.

It is now possible to explain the "Barkan phenomenon". It has been shown that the plasma Fe becomes reduced to the ferrous state when the plasma is acidified and allowed to stand. Ferrous Fe is ultrafiltrable.

Barkan has referred to the plasma Fe as the "easily split iron". This term would suggest that such Fe is present as a compound distinct from complexes of Fe which are generally recognized. The Fe of the plasma is probably present as a salt, although undissociated, with such substances as phosphatides, proteins etc. There is no reason to suggest that it is combined with any particular substance or group of substances and it is quite likely distributed amongst a number of them. In view of the facts discussed above, the writer is of the opinion that the term plasma Fe is a sufficient description.

The Fe of the plasma has been found to be wholly in the ferric state and as such undissociated. This does not indicate that this is the permanent state of the plasma Fe *in vivo*. It seems very probable that at some stage the plasma Fe will, at least in part, be changed into the ferrous state, i.e. become capable of dissociation and ultrafiltrable. That this must occur seems obvious, for otherwise it seems difficult to visualize how the plasma Fe, if it is to be regarded as transport Fe, is to become utilizable.

SUMMARY

The "Barkan effect" observed in plasma and serum has been shown to be due to the reduction of the plasma Fe to the ferrous state with a consequent change from a non-ultrafiltrable to an ultrafiltrable condition.

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