LETTERS TO THE EDITORS

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Behaviour of Lipoids in Human Serum

FILTERED human serum gradually becomes turbid through the formation of a precipitate which is mainly lipoid. The presence of approximately 7 gm. of lipoid in 1 litre of human serum is shown by shaking with a mixture of ether and alcohol[1], when the protein is precipitated and the lipoid goes into solution. When ether alone is used, the protein is not precipitated, and negligible amounts of lipoid are contained in the ether extract. It has not hitherto been believed possible to remove lipoids in any quantity from serum by the use of ether alone.

If serum is shaken with ether and the mixture frozen below −25° C., on subsequent thawing a coloured ether layer forms on the surface containing large amounts of lipoid. Freezing at temperatures above −20° C. fails to produce lipoids in the ether layer, and freezing at temperatures down to −70° C. does not increase the amount in the extract. There is no apparent advantage in freezing for longer than is required to bring the bulk of frozen material uniformly to below −25° C. From 1 litre of human serum shaken with 300 ml. ether and frozen to −25° C., 3·5 gm. lipoid is obtained in the extract which forms on the surface after thawing in cold water and allowing to stand at room temperature for 6–8 hours. The underlying serum is siphoned off and will give another 0·85 gm. lipoid if shaken with 100 ml. ether and frozen. On repeating this, another 0·2 gm. lipoid is obtained, each successive extract containing approximately one quarter the lipoid of its predecessor.

A litre of serum which has been exhaustively extracted in this way gives a further 2·5 gm. lipoid on treatment with ether-alcohol. The serum separated at each stage is not clear to transmitted light but may be clarified by centrifuging. The final product contains 11 per cent dissolved ether, which can be removed in various ways. Freezing once more and thawing after removal of the ether causes the deposition of a gelatinous material of low solubility in cold water, and if this is removed by filtration in the cold through an ordinary filter paper the siphoned as well as the centrifuged product is perfectly clear at all temperatures and filters readily through a bacteria-retaining filter.

Electrophoresis photographs of human serum before and after the extraction show only one difference, namely, that the concentration of β-globulin has been reduced by the extraction. It follows that much of the apparent β-globulin of human serum is a lipoid-β-globulin complex so orientated that the protein determines the surface properties of the complex; or alternatively, that β-globulin itself is a compound of lipoid and a serum protein having surface properties different from those of its constituents. It is not possible to decide between these views on the evidence available, but it is at least clear that in the β-globulin fraction of human serum much lipoid is associated with a hydrophylic substance in such a way that it behaves atypically, that is, as a water-soluble and ether-insoluble material.

In the region of the eutectic temperature (−23° C.) of sodium chloride, serum becomes truly solid and, after thawing, the previously clear serum is cloudy. Similarly, serum which has been dried from the frozen state but not from the liquid state is reconstituted with water to form a milky suspension. This suggests that the association of lipoid with a stabilizing substance depends on the presence of liquid water and is destroyed by freezing. On subsequent thawing, unprotected lipoid is able to aggregate to visible particles or droplets. These, however, soon re-acquire the same stabilizer from solution, because the initial rate of aggregation is not maintained and the formed particles can be observed with the naked eye to migrate with the β-globulin boundary. The lipoid of these particles is also not extractable with ether unless accompanied by freezing.

After freezing and thawing, it must be assumed that a lipoid phase has been created in equilibrium with the aqueous phase, and if dissolved ether is present in the serum it will redistribute itself between the two. Lipoid aggregates containing dissolved ether rise to the surface and merge with the excess of ether there to form a solution of lipoid in ether. It may be suggested as an alternative to this that ether droplets containing dissolved lipoid are formed between the ice crystals on freezing, and owing to the low miscibility of ether in water these are able to rise to the surface carrying lipoid with them without re-solution of the ether taking place. This is contra-indicated by the observations that an extracted serum saturated with ether does not form an ether layer after freezing and thawing, and an unextracted serum saturated with ether but having no excess of it on the surface has the appearance after freezing and thawing of an emulsion and on standing a fatty layer collects at the surface.

The method of extraction has been used to clarify blood-grouping sera which had become cloudy, and these showed little or no reduction in agglutinin titre and remained clear in ether for nearly a year. Extracted human serum after drying from the frozen state is reconstituted with water to form a clear solution, and there is no evidence that the physical properties of the serum proteins have been affected by either process. For reasons not understood, most of the excess fat in a lipæmic serum is extractable with ether without freezing and the same has been found for the serum of nephrotics[2]. Details of the application of the method to citrate plasma are being published elsewhere.

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March 31.

[1] Hardy, W. B., and Gardiner, Mrs. S., *J. Physiol.*, **40**, 68 (1910).
[2] Longsworth, L. G., and MacInnes, D. A., *J. Exper. Med.*, **71**, 77 (1940).

Metabolic Products of 3 : 4-Benzpyrene

IN 1936, Peacock[1] observed that animals receiving intravenous injections of 3 : 4-benzpyrene excreted in the bile an alkali-soluble product (*BPX*) possessing specific fluorescence bands which differed from those of the parent hydrocarbon. Subsequent investigations by Chalmers[2,3] demonstrated the existence of a similar phenolic derivative in the fæces, and this led to its isolation and purification. From recent crystallographic analysis[4], this metabolic product is considered to be a monohydroxy-benzpyrene.

In a study of the oxidation of benzpyrene by ascorbic acid *in vitro*, Warren[5] has found, in addition to the two known quinones (5 : 8- and 5 : 10-), a phenolic substance, soluble in alkali, giving a strong