mixture was passed through a "Zeo-carb 225" 4.5 % crosslinked cation-exchanger in the hydrogen form, neutralized with barium hydroxide, evaporated to dryness in a desiccator and finally taken up in the solvent used in the chromatography.

Uronic acid and galactosamine are present in equimolar proportions thus there is strong evidence for the presence of chondroitin sulfuric acid. However, the sulfur content is much too high. Another interesting feature is the presence of glucosamine and galactose in equimolar proportions. This, together with the high sulfur content, strongly suggests the presence of a compound similar to the keratosulfuric acid recently described by Meyer *et al.*⁸ to occur in the cornea. The sulfur content is sufficient to account for both these polysaccharides.

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On the Mechanism of the Heparin-induced Lipemia Clearing Reaction

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The clearing of alimentary lipemia by injection of heparin, first discovered by Hahn¹, is due to the production *in vivo* of an active substance (Anderson & Fawcett²) called the clearing factor. For the formation of this substance a factor from tissues and another from plasma are needed in addition to heparin (Anfinsen *et al.*³). By the action of clearing factor on (lipemic) serum the concentration of lipoprotein particles of the lowest density decreases, while a concomitant increase

Fig. 1. Ability of electrophoretically purified serum protein fractions to clear a purified clearing factor — olive oil emulsion mixture (= electrophoretic pattern of the lipid acceptor-protein). Clearing/30 minutes/mg protein: albumin 0.010, β globulin 0.032.

occurs in the more dense low-density lipoproteins and alpha lipoproteins (Graham *et al.*⁴, Anfinsen *et al.*³, Boyle *et al.*⁵, Nikkilä⁶). This means that new protein molecules enter into combination with lipids. The lipid acceptor-protein, if identical with the "coprotein" of Anfinsen *et al.*³, is claimed to be localized in Fraction III—0 of the conventional ethanol fractionation scheme (Anfinsen *et al.*³). Recently, serum albumin has been reported to increase the clearing rate, probably by binding the fatty acids, which are known to be released under the action of clearing factor (Shore *et al.*⁷) and which inhibit the clearing reaction (Gordon *et al.*⁸). This paper reports briefly on some experiments carried out to throw more light on the mechanism of the clearing reaction.

As is to be expected in view of the necessity of an acceptor-protein, a tween-stabilized olive oil emulsion is not cleared upon the addition of purified (Nikkilä⁶) clearing factor. When normal serum is added to this system the clearing rate increases directly proportionally to the serum concentration up to a certain limit (20–30 % of the total volume of the mixture) but declines on the further increase of the amount of added serum, thus suggesting the presence of some slightly inhibiting substance(s) in serum. To elucidate

the nature of the acceptor-protein supplied by serum, the protein fractions prepared from a normal human serum by the continuous electrophoretic technique of Grassmann and Hannig¹⁰ were tested for their ability to initiate clearing of a purified clearing factor-olive oil emulsion mixture. Clearing took place only in samples containing albumin or beta globulin. Calculated on the basis of protein content the activities of the two fractions were in a ratio of about 3 to 1, respectively. Commercial preparations of bovine (Armour) and human albumin (Orion) possessed some acceptor-protein activity but this was clearly inferior to that of electrophoretically prepared albumin. Whole normal serum added to a system containing fat emulsion, purified clearing factor and electrophoretic albumin enhanced the clearing exactly to the extent implied by its albumin content. We believe these results make it evident that the main acceptor-protein required in the lipoprotein transformation caused by the clearing factor is serum albumin or a protein intimately related to it. No evidence is found to support the view of Gordon *et al.*⁸ on the need of a second cofactor, their result being explicable, *i. a.*, by the poorer activity of chemically than of electrophoretically prepared albumin. The possibility remains, however, that the second cofactor is contained in our clearing factor preparation.

The mode of action of albumin in the clearing reaction may quite well be, as suggested by Gordon *et al.*⁸, the binding of the fatty acids produced by the clearing factor. This binding, however, is probably something more essential than the mere elimination of an inhibiting factor. This is shown by the fact that an emulsion of oleic acid (tween-stabilized) is instantly cleared on addition of electrophoretic albumin but not of the other fractions. These findings provide one explanation for the clearing reaction, the primary role of the clearing factor then being to act as a lipase or a lipase activator.

Further valuable information on the clearing mechanism was accumulated from our attempts to prepare the "tissue factor" from rat pylorus, as suggested by Brown & Kauffman¹¹. It appeared namely, that cell-free saline extracts of rat duodenum (not of stomach) possessed high clearing activity without any addition of heparin. Also this clearing reaction required the

presence of normal serum, which, however, could be replaced by sodium glycocholate. The initial clearing rate as well as the net decrease of turbidity were directly proportional to concentration of the bile salt. An emulsion of oleic acid is cleared at once by glycocholate. In the clearing reaction induced by the clearing factor from post-heparin serum, glycocholate not only was incapable of substituting serum (albumin) as the fatty acid acceptor but it also inhibited the reaction in the presence of serum. On the basis of these observations it seems to us highly likely that the active substance of rat duodenum is not a "tissue factor", and not even identical with the clearing factor of serum, but is a lipase. The different behaviour of the clearing activities of postheparin serum and duodenal extract against bile salt shows that the clearing factor of serum is probably not a lipase in nature but can be a lipase activator or that the heparin-induced clearing reaction does not involve fat splitting as a primary cause but only as a secondary outcome of the change in the physicochemical state of fats. Further studies are in progress and a more detailed description of our experiments will be published shortly.

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