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Separation of Lipids in Gravimetric Acetone Method for Plasma Total Protein.

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In the method of Bierry and Vivarro for the estimation of plasma total protein as modified by Guillaumin, Wahl and Laurencin, proteins are precipitated by adding 3 ml of plasma to 10 ml of acetone, centrifuging and washing the precipitate twice with acetone. Commenting on this method, Peters and van Slyke[1] state, "This is the most rigidly exact of plasma protein methods, because the acetone treatment removes lipoids. . . . In the other methods . . . the lipoids are precipitated with the proteins, and their nitrogen content probably causes a slight plus error in the protein figure."

Boyd[2] has shown that when cold, fat solvents are used to extract plasma lipids, a minimal initial volume of solvent is required per volume of plasma if complete extraction is to be obtained. With Bloor's extracting fluid, 3:1 alcohol-ether which is generally conceded to be the best lipid extractor, a minimum of 20 volumes of solvent per one volume of plasma were found necessary. On checking the protein method of Bierry and Vivarro as outlined by Peters and van Slyke,[1] it was found, as was to be expected, that lipids are not completely removed by the acetone. A few typical examples are given in Table I to show that lipid values in alcohol-ether extracts by Boyd's method[2] are appreciably and consistently higher than lipid values of the combined acetone mother liquors in the protein method.

The acetone protein method does not, therefore, remove all the lipids and the presumption is that the portion left is weighed later with the proteins. In plasma with normal amounts of protein and lipids, this would result in a plus error of 2 to 3% for the protein fraction. In the plasma of patients with nephrosis, the error would be much greater. I have encountered total lipid values as high as 2,000 mg % in nephrotic patients and assuming such occurred along with a true plasma protein value of say 2%, and assuming the same relative extraction of lipids (it might easily be lower in such cases), the plus error for protein could be as high as 25%.

On the other hand, if protein were estimated as nitrogen, the error

1 Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry*, Vol. II, p. 688, 1932.

2 Boyd, E. M., *J. Biol. Chem.*, 1936, **114**, 223.

TABLE I.
A Comparison of Lipids Found by Boyd's Extraction and in the Acetone Mother Liquors of Human Plasmas.
(Values are expressed in mg %.)

Sample	Total Lipid		Total Cholesterol		Phospholipid	
	Boyd	Acetone	Boyd	Acetone	Boyd	Acetone
1	527	378	145	71	149	133
2	471	323	123	72	136	113
3	642	498	159	114	193	167
4	528	392	135	94	159	155

would be much less—that is, using the acetone method but estimating the nitrogen of the precipitate rather than its weight. Lipid nitrogen is contained in the phospholipid fraction. It represents but a small part of the phospholipid fraction. The phospholipid fraction represents but one quarter of the total lipid. And phospholipids are more readily extracted by moist acetone (in the presence of other lipids and without magnesium chloride) than other lipids such as cholesterol.

The protein method of Bierry and Vivarro may be suitably modified to remove all of the lipids by simply diluting the plasma in a volume of acetone corresponding to the proportion of alcohol-ether used by Boyd.[2] It has been found that when plasma is diluted in 20 or 25 volumes of acetone, the acetone extract contains amounts of lipids identical to those in the alcohol-ether extract of the same sample of plasma. The suggested modification of the Bierry and Vivarro method as outlined by Peters and van Slyke[1] is thus to add 3 ml of plasma to 75 ml of acetone instead of 10 ml in the initial step of the procedure.

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Physiological Contraction of Double Hearts in Rabbit Embryos.

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The purpose of this paper is to show that the primitive lateral hearts in rabbit embryos are physiologically active and independent for some hours before they fuse into a single heart.

Knowledge concerning the early development of the mammalian heart has been obtained, for the most part, by the study of fixed