10729 P

Preparation of Blood Lipid Extracts Free from Non-Lipid Extractives.

JORDI FOLCH AND DONALD D. VAN SLYKE.

From the Hospital of the Rockefeller Institute for Medical Research, New York.

Deficiencies in Purification of Lipids by Petrol Ether. Resolution in petrol ether has been a classical procedure for analytical purification of extracted fats. Thus the blood fats extracted with Bloor's[1] efficient alcohol-ether mixture are, for certain analyses, dried and purified by resolution in petrol ether.[1, 2, 3] The non-lipid extractives, such as urea, glucose, amino acids, and inorganic salts, dissolve in varying amounts in the alcohol-ether, but they do not by themselves dissolve in petrol ether.

It has been recognized, however, that the petrol ether solutions show higher N:P ratios than could be expected from any of the known phosphatides. Several attempts have been made to identify the extra nitrogen.[4, 5, 6]

The present writers have been able to identify most of it as urea, determinable with urease and other urea regeants. Urea by itself is insoluble in petrol ether, but dissolves measurably in it when the blood lipids are present. Measurable amounts of amino acids, determinable by the specific amino acid carboxyl method of Van Slyke and Dillon,[7] are also present.

On the other hand, petrol ether fails to redissolve the phosphatides completely. A fraction of them remains in the undissolved residue. It is slight in normal plasmas, but in certain pathological ones it may represent 40% of the phosphatides. It has the following properties suggestive of sphingomyelin: soluble in alcohol, insoluble in petrol ether, N/P ratio of 2, C/P ratio of about 45. All the other lipids seem to be completely redissolved by the petrol ether.

Proposed Extraction. The proteins and lipids are precipitated together by colloidal iron, and the water-soluble extractives are

1 Bloor, W. R., *J. Biol. Chem.*, 1928, **78**, 53.

2 Boyd, E. M., *J. Biol. Chem.*, 1933, **101**, 323; 1935, **110**, 61.

3 Kirk, E., Page, I. H., and Van Slyke, D. D., *J. Biol. Chem.*, 1934, **106**, 203.

4 Channon, H. J., and Collinson, G. A., *Biochem. J.*, 1929, **23**, 663.

5 Page, I. H., Pasternack, L., and Burt, M. L., *Biochem. Z.*, 1930, **223**, 445.

6 Van Slyke, D. D., Page, I. H., Kirk, E., and Farr, L., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 837.

7 Van Slyke, D. D., and Dillon, R. T., *Compt. rend. Lab. Carlsberg*, 1938, **22**, 480.

washed away. The lipids are then extracted by stirring up the wet precipitate with alcohol and ether.

To one volume of plasma in a centrifuge tube one adds in succession, with stirring, 15 volumes of water, 1.25 volumes of colloidal iron solution (Merck's "Dialyzed Iron" with 5% Fe_2O_3), and 0.65 volume of a 1:1 aqueous solution of $MgSO_4.4H_2O$. The precipitate is centrifuged for 5 minutes, and is then washed by centrifugation with 15 volumes of water plus 0.65 volume of the 1:1 $MgSO_4.4H_2O$ solution. The washing can be repeated as many times as necessary for desired completeness. The last washing can be done without adding the $MgSO_4$. For routine analyses 2 washings suffice.

The washed precipitate is transferred to a volumetric flask marked to contain 10 times the volume of the plasma sample. For the transfer 4 volumes of absolute alcohol and 4 volumes of ether are used as follows. The precipitate is suspended in 2 volumes of the alcohol, and transferred as completely as possible to the flask. To finish the transfer one then uses in succession 1 volume of alcohol, 1 volume of alcohol, 2 volumes of ether, and 2 volumes of ether, finally filling to the mark with ether. The mixture is filtered.

For complete extraction of the lipids the presence of water is necessary in about the ratio of 1 volume to 6 of alcohol-ether, which is approximated by the above conditions. If a larger proportion of alcohol-ether per volume of plasma were taken, water would have to be added also in order to insure quantitative extraction of the lipids.

The clear filtrate contains all the plasma lipids, and we have not found in it any evidence of non-lipid extractives.

10730 P

Desoxycorticosterone Acetate Is Estrogenic in the Human Female.

UDALL J. SALMON.

From Mount Sinai Hospital, New York.

It has previously been shown that women, after surgical castration, excrete estrogens.[1] Furthermore, it was noted that in some

[1] Frank, R. T., Goldberger, M. A., and Salmon, U. J., PROC. SOC. EXP. BIOL. AND MED., 1936, **33**, 615.