CXXXIX. THE DETERMINATION OF SOAP IN BLOOD.

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It has long been known that the fatty acids of the blood occur not only in combination as fats and cholesteryl esters, but also in small amounts either free or as simple soaps. Whether or no soaps exist as such, is problematical, since it appears probable that such substances, under the conditions present in blood, would undergo salt hydrolysis, with consequent formation of free fatty acid. It is, of course, this salt hydrolysis which renders impossible the titration of free fatty acid with alkali in aqueous solution. In the presence of alcohol, however, the hydrolysis does not take place, and titration is possible. When, therefore, blood, which is slightly alkaline, is poured into a large excess of alcohol, soap is present, whether it existed in the blood previously as such or as free fatty acid.

Exact figures as to the total amount of soap present and also suitable methods for determining this amount have not hitherto been readily available. Yet some relatively simple method of estimation might prove of value in assisting to complete the picture of the changes occurring in the organism during fat metabolism. The usual methods for the estimation of soaps in biological fluids involve extraction with some solvent in which the soap is especially soluble and the oxidimetric titration of the fat radicle [Bang, 1918; Condorelli, 1922].

Such methods are open to considerable objection since it is impossible to get a complete separation of fats from soaps, by means of selective solvents—in none of the usual fat solvents is either of these substances absolutely insoluble—and the amounts of fat and soap present in a biological fluid may be very small indeed. Thus there is always the possibility that some of the substance finally oxidised during the titration may not necessarily be soap and the results obtained must therefore be regarded as qualitative rather than quantitative, or at best, roughly quantitative through compensation of errors. Gravimetric estimation [Lemelland, 1921] is open to the same objection although larger amounts of material must be used for analysis. A satisfactory method for the determination of soap must therefore avoid dependence on selective solubility, and it would seem, therefore, that the point of attack should be, not the fatty acid portion of the molecule which is common to soap and fat, but the metallic part which is peculiar to the soap. Were it then

possible to titrate the base combined with the fatty acid without affecting that combined with other acids in the alcohol-ether extract we should have a relatively simple means of estimating the soap content of the blood.

We therefore carried out a series of experiments to find with what degree of accuracy it was possible to determine small amounts of soap, using such volumes of fluid as could be titrated with the Rehberg burette. 0.5 cc. of alcoholic solution of pure soap was evaporated to dryness in a hard glass test-tube (6 by 1.5 cm.), 0.5 cc. $N/350$ H_2SO_4 added and the mixture titrated with $N/70$ NaOH with 0.2 cc. bromocresol purple as indicator. The end-point was taken at the first colour change. The difference between a blank titration with 0.5 cc. $N/350$ H_2SO_4 and the titration in the presence of soaps gave the amount of alkali combined with fatty acid. Various indicators other than bromocresol purple were tried but offered no advantages.

In the course of these experiments we found it essential to keep the amount of alcohol at a minimum—hence the evaporation to dryness of the soap solution. Besides interfering with the sharpness of the end-point even in the control titrations, excess of alcohol dissolved the fatty acid, which, in solution, was sufficiently dissociated to prevent the titration of the mineral acid alone. In the absence of alcohol, and with the indicator chosen, the end-point is sufficiently sharp, and occurs when the free mineral acid has been completely neutralised and the fatty acid remains untouched.

A further source of error was the extraction of estimatable amounts of alkali from the glassware during the evaporation of the alcoholic soap solution. Since this error was appreciable even during the evaporation of such small amounts, and was, moreover, variable, it would obviously have proved fatal to the method when applied to blood with the consequent concentration of larger volumes. We found it possible, however, to eliminate it entirely by the use of hard glass test-tubes (clear silica tubes, of course, would be ideal) with silica chips to prevent bumping during the evaporation. In applying the method to blood later we used silica beakers for the evaporation of the alcohol-ether extract. The amount of heating involved in the preparation of the alcohol-ether extract did not, we found, introduce any appreciable error. A specimen series of analyses is given in Table I.

Table I.

cc. soap	cc. $N/350$ H_2SO_4	cc. NaOH (0.0149 N)	cc. NaOH equivalent to fatty acid	Sodium oleate (mg.)		% error
				Found	Present	
0.0	0.5	0.0890	—	—	—	—
0.0	0.5	0.0890	—	—	—	—
0.1	0.5	0.0685	0.0205	0.0927	0.095	-3.0
0.2	0.5	0.0463	0.0427	0.1930	0.190	+1.5
0.4	0.5	0.0075	0.0815	0.3690	0.380	-3.0

The percentage error varied from +1.5 % to -3 % within the range of error of measurement of the solutions to be titrated which were delivered from a graduated 1 cc. pipette.

Thus a satisfactory degree of accuracy can be obtained in the determination of small amounts of pure soap solution, the error being no greater than that involved in the measurement of small volumes of fluid.

In applying the method to blood, the usual mixture of alcohol (3 vols) and ether (1 vol.) was used for the extraction of the fatty substances. 2 cc. of blood were added drop by drop to 30 cc. of the mixture in a 50 cc. flask. The flask was shaken throughout the addition of the blood, and was then heated on the water-bath until the ether began to boil. It was then cooled and alcohol-ether was added to the 50 cc. mark. After thorough mixing, the sediment was allowed to settle. 20 cc. of the supernatant liquor were pipetted off and filtered through a small plug of ether-cleansed glass wool into a silica beaker. The plug was washed with alcohol-ether, and the washings were run into the beaker. The contents of the beaker were evaporated to dryness on the water-bath (overheating must be sedulously avoided) and the residue was thrice extracted with about 1 cc. of hot absolute alcohol. The combined extracts were evaporated to dryness in a hard glass test-tube with silica chips to prevent bumping and 0.5 cc. $N/350$ H_2SO_4 was added to the residue. The liquid was cloudy because of the presence of fats and free fatty acids and titration was impossible owing to turbidity and to adsorption of the indicator on the fat. To overcome this difficulty we added 0.5 cc. of a saturated solution of pure sodium chloride and about 1 cc. of pure light petroleum. After standing overnight in the ice-chest the liquid cleared and titration was possible. The results of several such determinations in duplicate are given in Table II.

Table II. *Determination of soap in whole blood.*

The cc. $N/70$ NaOH used represents the difference between the control titration and that of the specimen.

	Vol. of blood in extract (cc.)	cc. $N/70$ NaOH used	Mg. oleic acid per 100 cc. blood
Blood 1	1.0	0.0859	34.6
" 1	1.0	0.0854	34.4
Blood 2	1.0	0.0661	26.6
" 2	1.0	0.0659	26.5
Blood 3	0.4	0.0400	40.3
" 3	0.4	0.0420	42.3

It is impossible to prove directly that the substance determined is soap, though from indirect evidence it appears highly probable that this really is the case. In the first instance, soap added to blood could be determined with reasonable accuracy (Table III).

Table III. *Determination of added soap.*

Alcoholic solution of pure sodium oleate (equivalent to 19.3 mg. oleic acid per 100 cc.) added to alcohol-ether extract of blood.

	cc. $N/70$ NaOH used	cc. $N/70$ NaOH used for added soap	Mg. oleic acid per 100 cc. soap solution
10 cc. blood extract (0.4 cc. in extract) ...	15.5	—	—
10 cc. blood extract + 0.04 cc. soap solution	35.0	19.5	19.6
10 cc. blood extract + 0.08 cc. soap solution	53.4	37.9	19.1

Further, the substance determined cannot be a salt of phospholipin, or diphosphoglyceric acid, since its concentration is greater in the plasma than in the corpuscles (Table IV).

Table IV. *Determination of soap in whole blood and plasma.*

	Mg. oleic acid per 100 cc.		Plasma. Vol. % of whole blood	Mg. oleic acid whole blood calc. from plasma content
	Whole blood	Plasma		
1. Normal	34.5	46.7	60	28.0
2. Normal	57.4	98.7	60	59.2
3. Polycythaemia	45.3	112.8	40	45.1

It cannot be a salt of lactic acid, acetoacetic acid, or hydroxybutyric acid since the sodium salts of these acids added to blood are not determined by the method (Table V). The dissociation constants of these last two acids are so similar to that of butyric acid that this statement may be taken as correct, although the experiment has not actually been carried out owing to the difficulty of obtaining these substances. Moreover, the amount of soap actually found in normal blood is greater than would be accounted for by the small amounts of "acetone bodies" known to be present.

Table V. *The non-interference of added lower fatty acid salts and of added fat.*

	Mg. oleic acid per 100 cc. blood
Blood alone	18.82
Blood + 400 mg. sodium lactate per 100 cc. ...	18.88
Blood alone	31.00
Blood + 400 mg. sodium butyrate per 100 cc. ...	30.30
Blood alone	28.60
Blood + 500 mg. tripalmitin per 100 cc. ...	28.30

Incidentally it might be remarked that such a method as this which we have described might be useful in following the activity of lipase in blood.

SUMMARY.

A method is described for the determination of soap (or free fatty acid) in blood. For normal human blood the concentration calculated as oleic acid is 18-40 mg. per 100 cc., for plasma 46.7-98.7 mg. per 100 cc. in the specimens we have so far examined.

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