V. A METHOD FOR THE QUANTITATIVE ESTIMATION OF CHOLINE IN BLOOD.

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THE chemical methods at present available for the quantitative determination of choline are unsatisfactory. Those most commonly employed depend on the compounds formed with the heavy metals. But these are fairly soluble in water, in some cases to the extent of 25 %. The aurichloride is the least soluble, one part dissolving in 75 parts of water.

The biological method of testing the acetylated product upon the frog's heart suggested by Reid Hunt [1915] and later by Fühner [1916] can at present be regarded as affording only a rough estimation of the amount of choline.

The method of detecting the presence of choline in blood and cerebro-spinal fluid described by Allen [1904] seemed to promise the possibility of being developed into a quantitative process. Staněk [1905] described such a process in which he either estimated the nitrogen of the choline compound, or decomposed the periodide with cuprous chloride. The details of the second method were criticised by Kiesel.

The following method has been devised and tested, and it appears to be free of the difficulties of Staněk's procedure.

The principle is to extract the choline from the blood or tissues and to precipitate it with iodine as periodide, to wash off any free iodine and to decompose the periodide with dilute nitric acid, finally to extract the free iodine with chloroform and estimate it using a standard solution of sodium thiosulphate.

1 cc. *N*/20 thiosulphate sol. = 0.0007 g. choline HCl.

From 20 to 50 cc. of blood are drawn off into about four times this volume of absolute alcohol and well shaken. The containing vessel is allowed to stand for about 12 hours, after which time the contents are filtered into an evaporating basin. The precipitate is rubbed up and well washed with absolute alcohol and then is added to the filtrate. The filtrate is evaporated to a syrup and taken up in distilled water. The possibility of the extraction and decomposition of lecithin with the liberation of choline at this stage is considered later. The fluid is again evaporated to a syrup and the process repeated till it is alcohol-free. The solution is then transferred to a dialysing thimble of about 15 cc. capacity and dialysed for 24 hours into 200 cc. distilled water.

It is advisable to repeat this dialysis and to combine the resulting liquids. This is then evaporated to about 5 cc. and carefully washed into a beaker keeping the bulk as low as possible. 20 to 30 vols. of saturated iodine in potassium iodide solution are now added and the beaker set aside over night to allow the choline periodide to crystallise. The crystals are then filtered through a Gooch crucible on a layer of fine asbestos wool. They are washed repeatedly with ice-cold water until free from the precipitating solution. The Buchner flask is cleared out with cold water. The crucible is placed in position for filtering and the choline periodide is now ready for decomposition. This is accomplished by a mixture of 2 parts diluted nitric acid (1 part HNO_3 to 1 part water) with 1 part chloroform.

The above mixture is then shaken and added to the crucible. The periodide is decomposed by the nitric acid and the free iodine is taken up by the chloroform and carried into the flask by the suction. This is repeated several times until the asbestos is quite white. The crucible is now removed, more chloroform is added to the flask and well shaken in order to extract any iodine dissolved in the water.

The chloroform layer is then washed acid-free by decantation with repeated quantities of cold water.

The liberated iodine in the chloroform layer is now most conveniently titrated by $N/20$ sol. of sodium thiosulphate until the pink colour just disappears.

Choline hydrochloride added to normal blood:

A. Blood, 20 cc.; choline HCl, 4.9 mg.; cc. $N/20$ thiosulphate, 6.4; recovery = $6.4 \times 0.0007 = 4.48 \text{ mg.} = 91.5 \%$.

B. Blood, 20 cc.; choline HCl, 1.9 mg.; cc. $N/20$ thiosulphate, 2.55; recovery = 94 %.

C. Blood, 20 cc.; choline HCl, 17.0 mg.; cc. $N/20$ thiosulphate, 22.0; recovery = 90.6 %.

Tests on normal blood without the addition of choline:

Blood, 25 cc.; no choline HCl.

No precipitate of periodide was produced even on long standing.

The possibility of lecithin breaking down is small if the process is carried out with reasonable speed. Tests performed on blood to which no choline was added gave negative results. If there was any free choline present its concentration was below the limit of recovery by this process.

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