XII. NOTE ON THE DETERMINATION BY DISTILLATION OF VOLATILE CONSTITUENTS IN BLOOD, WITH SPECIAL REFERENCE TO THE ESTIMATION OF ALCOHOL.

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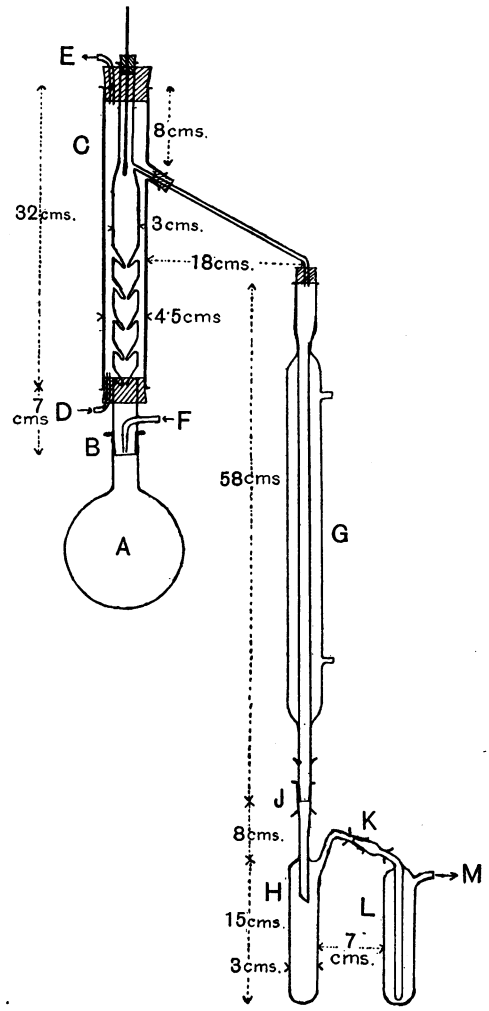
To workers in this field, the frothing of blood on distillation has always proved a difficulty. When, as so often happens, the operation is carried out in an ordinary distilling flask with side tube, it is difficult to obtain a distillate which is absolutely free from haemoglobin, for although no visible drops may be carried over, yet in most cases some of the fine spray generated by the breaking of the blood films passes into the receiver.

The writer during an investigation of the normal volatile reducing substance in blood and also while determining the concentration of alcohol under various conditions in blood, has found the still-head useful which is represented in the accompanying figure. The parts and dimensions of the complete apparatus[1] are given in the hope that they may be of service to others.

The blood or other body fluid is distilled from *A*, a round-bottomed, ring-necked, 1 litre pyrex flask, which is heated in a water-bath (not shown). *A* connects with the lower end of the Lapworth column at *B*, by means of a ground glass joint which is well vaselined to enable the flask to be removed after each distillation. The Lapworth column is surrounded by a glass jacket *C*, in which water at about 75° is placed or made to circulate by the inlet and outlet tubes *D* and *E*. *F* is a tube for introducing (by means of the usual screw clamp and rubber tube) a small current of air to help carry over the vapour into the condenser *G* and also to regulate the pressure. The distillate falls into the receiver *H* which is connected with the lower end of the condenser by a ground glass joint *J*. *H* is connected by means of another ground glass joint *K* with a similar tube *L* which, containing about an inch of distilled water, serves to trap any vapour which has escaped condensation. *M* connects to the water pump to enable the distillation to be carried out under reduced pressure. Tubes *H* and *L* are surrounded by an ice-salt mixture. The upper end of the Lapworth column can be closed by a cork with thermometer if desired. The distillation is carried out with the pressure reduced to 7–8 cm. of mercury. In the case of blood, distillation is carried on till the sticky stage is reached.

[1] Made for me by Mr F. Hartwig of the Scientific Glass Blowing Co., Manchester.

With this still-head there is the great advantage that blood distillations can be left to themselves, for the blood films are rapidly broken up, partly by the heat from the surrounding water in the jacket, and partly by the projections into the lumen of the column. These cones also help to prevent the passing over of fine spray loaded with haemoglobin.



The method and estimation follow in the main those adopted by Pringsheim [1908], who used a modified form of Cotte's [1897] method of estimation. The former heated the solution of alcohol to be estimated with a known amount in excess of $N/20$ potassium dichromate (to which was added 1 cc. of sulphuric acid per 5 cc. of dichromate solution) for 1–1½ hours in the water-bath. After cooling the excess of dichromate was estimated by $N/20$ solution of ferrous ammonium sulphate (to which had been added sulphuric acid to the extent

of 5 % to stabilise). Potassium ferricyanide was used as indicator. In Pringsheim's paper no mention is made of the necessity to have the final volume of the fluid constant before oxidising. It might be expected that this would be an important point, since the extent of the oxidation depends in a large measure on the concentration of the dichromate and sulphuric acid used [see Van Slyke, 1917]. The following figures show the need for this:

cc. of 1 % alcohol taken	cc. standard dichromate	cc. conc. H_2SO_4	Final volume in cc.	cc. stand. ferrous am. sulphate to reduce excess of dichromate
4	20	4	45	8·5
4	20	4	90	10·8
4	20	4	135	14·6
4	20	8	135	9·5

It is evident that as the final volume varies important differences appear in the amounts of ferrous ammonium sulphate used. If the final volume be made the same, both in standardisations and in actual estimations comparable results are obtained as the following tables show:

Table I. *Standardisation of the ferrous ammonium sulphate solution against known amounts of alcohol.*

Equivalent number of cc. of absolute alcohol taken	Equivalent number of cc. of standard ferrous ammonium sulphate solution used
0·01	14·3
0·01	14·2
0·02	28·6
0·02	28·6
0·02	28·6
0·02	28·55
0·03	42·5
0·03	42·4
0·04	56·4

In each of the above estimations 20 cc. of standard dichromate and 10 cc. of concentrated sulphuric acid were taken, the final volume being made up to 150 cc. It is obvious that the estimations are accurate up to quantities of 0·03 cc. of alcohol. In any experiment, if there is reason to suspect a greater amount of alcohol than 0·03 cc. in a distillate the alcohol in a known fraction of this is estimated.

Table II. *The amount of alcohol recovered when known amounts are added to blood and the latter then distilled in the above described apparatus.*

Equivalent number of cc. of absolute alcohol added to blood	Equivalent number of cc. of standard ferrous ammonium sulphate solution required
0·01	14·15
0·02	28·4
0·03	42·4

The alcohol was in each case added to about 10 g. of rabbit's blood.

If blood containing alcohol be allowed to clot it is impossible to recover by the above means the whole of the added alcohol, even if the clot be broken up.

It may be added that the writer finds it much easier to add excess of ferrous ammonium sulphate and titrate this back with standard permanganate solution than to use potassium ferricyanide to determine the end point.

In conclusion it may be stated that it is hoped shortly to publish the results of experiments dealing with the various problems of alcohol metabolism in which the apparatus described is being used.

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