

THE PLATINOCHLORIDE TEST FOR CHOLINE IN HUMAN BLOOD.

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INTRODUCTION.

HALLIBURTON and Mott have recently published (³ and ⁴) extensive researches on the chemistry of nerve degeneration; and from the fact that lecithin, an important constituent of nerve tissue, may be broken up into choline and glycerophosphoric acid, they inferred that choline would appear in recognisable quantities in the circulating fluids of persons in whom nerve degeneration was taking place. To investigate the possible presence of this substance in blood and cerebro-spinal fluid they employed, among other tests, that with platinum chloride.

The details of this test will be found in an article by Mott (⁶). The principle is to extract the blood several times in succession with absolute alcohol, in the belief that the choline will thereby be freed from potassium, to precipitate the choline as choline platinochloride, and to crystallise the latter from 15 per cent. alcoholic solution. The crystals are yellow octahedra, readily seen under the low power of the microscope.

In confirmation of this test, Halliburton and Mott examined other properties of the crystals, and also observed the physiological action of the material they extracted from the blood and cerebro-spinal fluid. Upon these points the present authors have not touched; their desire was merely to test the clinical value of the platinum chloride reaction.

METHOD USED IN THE PRESENT RESEARCH.

An attempt was made to obtain comparable results in all the experiments by using, as far as possible, the same volumes of solvent at the corresponding stages of the process. The full method is as follows:—

Sixty minims, or about 4 c.c., of blood were drawn from the vein of the patient and ejected into 30 c.c. of absolute alcohol. The mixture was allowed to stand for half an hour, being stirred from time to time. It was then filtered. The pale yellow filtrate was evaporated at 40° C. When dry, the residue was rubbed up with 7 c.c. absolute alcohol and filtered; there was always a yellowish-

brown insoluble deposit, which was washed with a further 3 c.c. absolute alcohol, a total of 10 c.c. being thus used for the extraction. The filtrate was evaporated to dryness at 40° C. This step was twice repeated. The dry residue was then rubbed up with 7 c.c. absolute alcohol, filtered, and the gummy residue further washed with 3 c.c. absolute alcohol. To the filtrate at least five drops of a 10 per cent. solution of pure platinum chloride in absolute alcohol were added, and the turbid mixture evaporated at 40° C. The yellow-brown residue was rubbed up with 7 c.c. absolute alcohol to redissolve the excess of platinum chloride; the mixture was filtered, and the residue washed with 3 c.c. absolute alcohol. There were thus obtained :—

(1) *A filtrate*:—This, which throughout the paper is referred to as the “final filtrate,” contains the excess of platinum chloride, and those double platinochlorides, which are the more soluble in absolute alcohol. It was evaporated to dryness at 40° C., redissolved in 3 c.c. absolute alcohol, filtered, and set to crystallise spontaneously. Crystals obtained from it we term “crystals from the final filtrate.”

(2) *A residue*:—This, which we refer to as the “final residue,” contains the less soluble double platinochlorides. Amongst these would be that of choline. This residue was washed three times with the same 3 c.c. of 15 per cent. alcohol in distilled water. The double platinochlorides, insoluble in the absolute alcohol, but now dissolved in 15 per cent. spirit, were evaporated to dryness at 40° C., redissolved in 3 c.c. of 15 per cent. spirit, filtered, and allowed to crystallise spontaneously. Crystals so obtained we term “crystals from the final residue,” and amongst them would be those of choline, were any originally present in the blood. The absolute alcohol employed throughout had a strength of 99.6 per cent., in order to take up as little of the inorganic salts in the blood as possible; the importance of this has been well pointed out by numerous observers (2).

RESULTS OF APPLYING THE TEST TO HUMAN BLOOD IN HEALTH AND DISEASE.

Mott states that the yellow octahedral crystals are obtainable from 5 c.c. or less of blood from persons in whom active nerve tissue destruction is taking place; whereas “using 10 c.c. of normal human blood the results are practically negative, although frequently a few small octahedra may be found on careful examination” (8). From a clinical point of view this would be of great value in diagnosis, and we proceeded to test it.

Typical crystals were readily obtained from 60 minims of the blood of four male patients suffering from general paralysis of the insane, three male and two female patients suffering from locomotor ataxia, two male patients suffering from disseminated sclerosis, two male patients suffering from compression myelitis, one male patient suffering from lateral sclerosis of the cord, one female patient suffering from chorea, and from one male patient suffering from combined sclerosis of the cord.

On testing perfectly healthy persons in the same way, however, yellow octahedral crystals were obtained with equal facility. This was so in the case of each of the authors of this paper and of three robust medical students. At first we hoped that, in cases of nervous

degeneration, there would be more abundant crystals than in normal subjects; but even of this we could not convince ourselves. Two of the healthy students yielded as plentiful a crop of typical yellow octahedral crystals as did any one of the patients suffering from nervous disease.

Sources of Fallacy in the Test.—It was suggested from the results just described that there was some fallacy in the test. From further investigations we conclude that some of the yellow octahedral crystals consist of an inorganic platinochloride, probably the double chloride of platinum and potassium.

Incineration.—The experiments from which we draw this conclusion are those in which we incinerated the alcoholic extract of the blood.

Four c.c. of blood were taken, extracted with 30 c.c. absolute alcohol; dried, extracted with 10 c.c. absolute alcohol exactly as in the process already detailed; re-extracted with 10 c.c. absolute alcohol a second and a third time. The dried residue from this last extraction was then incinerated at a red heat for 15 minutes, after which it was extracted with 10 c.c. absolute alcohol; platinum chloride solution was added, and the last steps of the process continued exactly as if no incineration had been done. From the final residue quantities of typical yellow octahedral crystals were obtained on every occasion.

We think the numbers of crystals were fewer than those given by the same patient's blood without incineration; but that crystals were obtained at all, showed that at least some of the crystals obtained by the test are not those of choline.

Swale Vincent ⁽¹⁰⁾ expresses his opinion that the octahedra obtained by the "choline test" are due to ammonium-platinochloride; but that this is not entirely the case is also proved by the above experiments, for incineration must have destroyed both choline and ammonia. Control experiments with pure choline and with ammonium chloride gave no crystals after incineration.

It is necessary, therefore, to consider what are the inorganic salts of the blood which contains normally the following ⁽⁹⁾:—

Sodium	0.185 per cent.
Potassium	0.182 "
Calcium as oxide } in pig	0.014 "
Magnesium as oxide }	0.098 "
Iron	0.059 "
Ammonia	0.003 "

The double platinochlorides of all these can form yellow octahedra indistinguishable microscopically from those of the choline salt. We therefore took a minute quantity of the chloride of each separately and carried it through the "choline process," with the following results:—With the chlorides of sodium, calcium, magnesium, and iron the "final filtrate" yielded on crystallisation some yellow octahedra, while the "final residue" gave none.

Hence the solubility of the platinochloride of each of these in absolute alcohol is such that none of the salt remains to yield crystals in the "final residue." With the chlorides of potassium and ammonium the "final filtrate" gave a few yellow octahedra, while the "final residue" showed many such crystals. Hence not only are these chlorides sufficiently soluble in absolute alcohol to be carried on to the final stage of the extraction, but their double platinochlorides are so little soluble in absolute alcohol that much of them remains to form crystals in the "final residue." It is these which, in our opinion, vitiate the process as a reliable test for choline in the blood.

To complete the control experiments we carried a specimen of pure choline through exactly the same procedure, and found—

In the "final filtrate" abundance of needles due to excess of platinum chloride, and a considerable number of yellow octahedral crystals.	}	In the "final residue" many octahedral crystals.

Therefore choline remains in the "final residue" in the "choline process," but its platinochloride is not so insoluble in absolute alcohol but that some of it is removed in the "final filtrate." Indeed, we thought that the proportion of octahedral crystals in the "final filtrate" and "final residue" respectively suggested that the solubilities of the double platinochlorides in absolute alcohol were as follow:—

Sodium platinochloride .	}	So soluble in absolute alcohol that all the octahedra were in the "final filtrate"; none were in the "final residue."
Calcium platinochloride .		
Magnesium platinochloride .		
Iron platinochloride .		
Choline platinochloride .	{	Not so insoluble in absolute alcohol but that many octahedra appeared in the "final filtrate," though many also remained in the "final residue."
Potassium platinochloride .	{	Even less soluble in absolute alcohol, than is choline platinochloride, so that though some octahedra appeared in the "final filtrate," more appeared in the "final residue."
Ammonium platinochloride .		

It may be pointed out that although potassium chloride is usually said to be insoluble in absolute alcohol, 100 parts of the latter can dissolve 0.034 parts of this salt at 19° C. (1).

Now 4 c.c. of normal blood contain 0.014 grm. of potassium chloride, and 10 c.c. of absolute alcohol—the amount used in extracting each residue—can dissolve 0.0034 grm., which corresponds to 0.011 grm. of potassium platinochloride. This would require 133 c.c. of absolute alcohol for solution (1). The 10 c.c. actually used for

washing the precipitate will therefore leave much of the potassium platinochloride to appear in the "final crystallate."

CONCLUSIONS.

From our experiments we conclude therefore—

1. That the obtaining of yellow octahedral crystals from blood by the platinochloride test is not of itself sufficient proof of the presence of choline.

2. That when 4 c.c. of blood are taken and carried through the "choline process," yellow octahedral crystals are obtained as readily from the blood of some healthy persons as from that of the subjects of various nervous disorders.

3. That of a mixture of choline, potassium, ammonium, sodium, calcium, magnesium, and iron chlorides, the first three alone will appear as yellow octahedra in the final stage.

4. That yellow octahedral crystals may be obtained from blood even after incineration, and that these are the platinochloride of potassium.

Finally, it may be pointed out that these experiments were performed upon blood alone. Donath⁽²⁾ has affirmed the presence of choline in the cerebro-spinal fluid of epileptics and of those suffering from various acute nervous disorders. Upon this point we can venture to express no opinion. The crystals figured in the paper referred to have not, however, the shape characteristic of choline platinochloride.

We wish to thank Dr. Pembrey for the facilities which he afforded us in carrying out our work, a preliminary account of which was given to a meeting of the Physiological Society in November 1903. We also thank Professor Halliburton and Dr. Mott for criticisms and suggestions upon further work on choline in human blood. The expenses were defrayed by a grant from the Royal Society to Dr. Pembrey, and the work was done during the tenure by Herbert French of the Gillson Scholarship of the Society of Apothecaries of London.

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For references to the pure chemistry of choline, see Donath's paper, *supra*.