

THE ALLEGED OCCURRENCE OF ACETYLCHOLINE IN NORMAL OX BLOOD.

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BOTH chemical and biological determinations of the amount of acetylcholine present in normal ox blood have yielded widely divergent results to different workers. The position down to 1933 is set out in the papers by Dudley [1933] and by Chang and Gaddum [1933]. Since then Gollwitzer-Meier [1934], using eserized leech muscle in a study of whole blood, plasma and corpuscles, has reported that the amount of acetylcholine present is not greater than 0.013 mg. per litre of blood. Kahlson and Römer [1934] have examined the products obtained by a precipitation of blood extracts with Reinecke acid, as described by Kapfhammer and Bischoff [1930] and found no indication of the presence of acetylcholine; the gold salt obtained later by decomposition of the precipitate was shown by analysis and m.p. to be choline aurichloride. These experiments provide confirmation by chemical means of the results which Kahlson [1934] has obtained by various physiological methods.

The work described in this paper was undertaken in order to provide further evidence on this disputed question. The method adopted has been to divide the total alcoholic extract from 2 litres of blood into two equal portions; one of these was concentrated directly, while to the other were added prior to concentration 40 mg. of acetylcholine chloride dissolved in 0.5 c.c. of water. The two alcoholic extracts were then worked up simultaneously and received similar treatment throughout.

A number of samples of blood were treated thus and no difficulty was experienced in recovering acetylcholine from those alcoholic extracts to which it had been added; in every such case the aurichloride had the m.p. and gold content of acetylcholine aurichloride. Those extracts, on the other hand, to which no acetylcholine had been added, all yielded gold salts which had the m.p. and gold content of choline aurichloride.

The recovery of added acetylcholine suggests that amounts greater than 15 mg. per litre of blood should be readily isolated and identified chemically.

EXPERIMENTAL.

The technique described by Dudley [1933] and used by him with positive results in Freiburg and with negative results in England was followed rigorously. Ox blood was precipitated and extracted with alcohol within 25 min. of its being drawn from the animals and oxalated at the slaughter-house. The aurichlorides were recrystallized from hydrochloric acid solutions of pH 4, which is the pH for maximum stability of acetylcholine [Hofmann, 1930].

Details of two typical experiments are recorded below:

Aurichlorides isolated from alcoholic extracts of blood with and without added acetylcholine.

| | Alcoholic extract from 1 litre of normal ox blood with the addition of 40 mg. of acetylcholine chloride | Alcoholic extract from 1 litre of normal ox blood |
|---|--|---|
| <i>Exp. 1.</i> Wt. of aurichloride after recrystallization | 1st crop 36 mg., M.P. 164° C. 2nd crop 26 mg., M.P. 162° C., mixed M.P. 162° C. | 41 mg., M.P. 261° C. |
| Gold content | 40.9 p.c. Au | 44.6 p.c. Au |
| Recovery | 58 p.c. | |
| <i>Exp. 2.</i> Wt. of aurichloride after recrystallization | 45 mg., M.P. 166° C. | 48 mg., M.P. 260° C. |
| Gold content | 40.7 p.c. Au | 44.6 p.c. Au |
| Recovery | 42 p.c. | |
| Choline aurichloride | M.P. 268° C., 44.5 p.c. Au | |
| Acetylcholine aurichloride | M.P. 168° C., 40.6 p.c. Au | |

SUMMARY.

Attempts to repeat the isolation of acetylcholine from normal ox blood, in the form of its aurichloride, as described by Kapfhammer and his co-workers [1930, 1931*a, b*] and by Vogelfanger [1933], have been unsuccessful. The gold salt obtained from such blood was choline aurichloride, a result in agreement with those of Dudley [1933] and Kahlson and Römer [1934]. On the other hand, when 40 mg. of acetylcholine were added to the alcoholic extract from 1 litre of blood, no difficulty was encountered in isolating acetylcholine aurichloride.

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