V. ACETYLCHOLINE, A NEW ACTIVE PRINCIPLE OF ERGOT.

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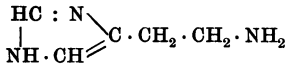
Investigations carried out during the past few years have resulted in the isolation from ergot of several active principles, the presence of which adequately accounts for those actions of the drug which have been regarded as specially related to its therapeutic effects, each type of action having been advocated by one observer or another as a basis for its physiological standardisation.

The principles in question are (1) the alkaloid ergotoxine $C_{35}H_{41}O_6N_5$ (the hydroergotinine of Kraft [1906]) which was isolated by Barger and Carr [1907] and further investigated by Barger and Ewins [1910]; (2) *p.* hydroxyphenylethylamine, $OH.C_6H_4.CH_2.CH_2.NH_2$ [Barger 1909; Barger and Dale 1909], and (3) β-iminazolylethylamine



[Barger and Dale 1910], the last two being amines derived respectively from the amino-acids tyrosine and histidine by decarboxylation.

In addition, however, to those of apparent therapeutic importance, certain other effects are shown in a more or less marked degree by all samples of the drug and by some with marked intensity. These effects have been familiar to the pharmacological workers in these laboratories for some years, but apparently have not been the subject of exact description or investigation. Conspicuous among these is an inhibitor effect on the heart, suggesting an intense though curiously evanescent muscarine action. Finding that the prominence of this effect in the action of different specimens of ergot ran closely parallel with their stimulant action on intestinal muscle, and that both effects were abolished by atropine, Dr Dale was led to suspect the presence in ergot of a principle producing both these effects. He was also able to

devise a convenient method for its physiological estimation by the use of a loop of rabbit's intestine isolated according to Magnus's method. When an opportunity recently occurred of obtaining an adequate supply of a preparation which exhibited this type of action with marked intensity, he suggested to me that an attempt should be made to identify the supposed new principle, and has followed the successive steps of its isolation with the physiological control. This paper deals with the chemical procedure by which the principle in question was isolated and identified. The details of its action will be described elsewhere by Dale.

The physiological effects described above resemble somewhat closely those described for muscarine, especially as the action in both cases is completely abolished by small amounts of atropine. On other grounds also it appeared not improbable that ergot might contain muscarine, since choline has long been recognised as one of its constituents [Brieger 1887], while Böhm [1885] had shown many years ago that fungi other than *Amanita muscaria* contain muscarine. Preliminary experiments carried out with a view to the isolation of the base, lent further support to this idea, since it was always found associated with the choline fraction. Thus it was completely precipitated from alcoholic solution, and partially precipitated from concentrated aqueous solution by mercuric chloride, and when the extract was fractionally precipitated by means of silver and baryta (Kutscher's method) only the last (choline) fraction contained any of the active base with which we were concerned.

The actual isolation of the active base was finally accomplished by the method which is set out in detail in the experimental portion of this paper. In the first instance there was obtained only a very small quantity of a crystalline platinichloride; 2·8 milligrams in all. The base from this was found to be extremely active in producing the physiological effects with which we were concerned. Such chemical comparison as was possible with this minute quantity of material tended to support the identity of the unknown base with muscarine. The melting point, for example, was practically the same as that of synthetic muscarine platinichloride. A physiological comparison made by Dale with both natural and synthetic muscarines[1] showed however that the new base could not be either of these.

The clue to its identity was furnished by the observation, made repeatedly during the process of its isolation, that it was very susceptible to the action of alkali. In fact a dilute solution of the active base, if made distinctly alkaline with caustic soda at the ordinary temperature and neutralised again

[1] For the former we are indebted to the kindness of Dr O. Rosenheim.

almost immediately, loses nearly all its original activity. This fact, and its constant association with choline, suggested that it might be a choline ester. This was the more probable since Hunt and Taveau [1911] had already described a number of choline derivatives, many of which were physiologically considerably more active than choline itself. Of these derivatives acetylcholine had been indicated as one of the most active bases, and was also on general grounds the most likely to occur naturally. A small quantity of acetylcholine was therefore prepared by the method originally described by Nothnagel [1894]. Comparison of the physiological action of this base with that isolated from the extract of ergot showed that it was qualitatively and quantitatively the same. Further, by working up a larger quantity of the ergot extract, there was obtained sufficient of the crystalline platinichloride of the active base, to establish beyond doubt, by melting point and analysis, its identity as acetylcholine.

Acetylcholine therefore exists in ergot and is the base responsible for the physiological action described at the commencement of this paper. That it occurs as such in the original ergot grains, is shown by the fact that a fresh extract made by boiling the drug with dilute alcohol, produces the effects shown by the extract prepared according to the directions of the British Pharmacopoeia. The presence of acetylcholine is consequently not due to fermentative or other changes taking place during the preparation of the extract.

Experimental.

With the help of the physiological control above mentioned the following method was ultimately adopted and led to the isolation of the active base.

The preparation available for investigation was a liquid extract, prepared according to the directions of the British Pharmacopoeia. 1600 cc. of this extract were concentrated under reduced pressure on the boiling water-bath to remove the alcohol. The residual syrupy liquid was diluted with water to 480 cc. and aqueous mercuric chloride added until no further precipitation occurred. The amount required was 1100 cc. of a saturated aqueous solution. The precipitate was filtered off, washed with water and, since it was found to be almost physiologically inactive, discarded. From the filtrate and washings the slight excess of mercury was removed as sulphide, and the excess of sulphuretted hydrogen by means of a current of air. The solution was then neutralised with sodium carbonate and concentrated on the boiling water-bath

under reduced pressure to a thin syrup. This was poured into strong alcohol (92–95%) and allowed to stand for a few hours. The gummy precipitate was filtered off, washed with alcohol, and, being found to be practically inactive, discarded. The alcoholic filtrate and washings were taken to dryness and the residue dissolved in a small quantity of pure methyl alcohol, in which it was almost completely soluble. The methyl alcoholic solution was again precipitated by the addition of four or five volumes of absolute alcohol. The precipitate was filtered off, the filtrate evaporated to dryness, and the residue completely extracted with small quantities of absolute ethyl alcohol. If necessary, precipitation by means of excess of alcohol was repeated, until the alcoholic solution obtained gave no further precipitate on addition of a large volume of alcohol.

To the alcoholic solution (240 cc.) so obtained alcoholic mercuric chloride (560 cc.) was added until no further precipitate was produced. The mixture was allowed to stand over night. The precipitate, which contained practically the whole of the active base originally present in the alcoholic solution, was filtered, washed with alcohol, and then extracted four times with boiling water, 150 cc. of water being used for each extraction. The portion of the mercury precipitate insoluble in hot water was found to be inactive and was therefore discarded. The hot aqueous extract on cooling deposited a further small quantity of precipitate which was very slightly active and was neglected. The clear, light yellow filtrate was then concentrated *in vacuo* on the water-bath. A precipitate, which was for the most part crystalline, soon commenced to separate. Concentration was continued until the volume was about 50 cc. The solution was then cooled and the precipitated mercuric chloride compound filtered off, when there was obtained 17 grams of a mixture consisting for the most part of the mercuric chloride compounds of choline and the active base. The salt was finely ground in a mortar, suspended in about 200 cc. of water, and decomposed by sulphuretted hydrogen. The mercuric sulphide was filtered off, re-suspended in water, and again treated with sulphuretted hydrogen, the process being repeated until decomposition was complete. The combined filtrates and washings were freed from sulphuretted hydrogen by a current of air, and the strongly acid solution treated with freshly precipitated silver carbonate until free from chlorine ions. The slight excess of silver in solution as carbonate was removed as sulphide and excess of sulphuretted hydrogen again removed by air. The slightly alkaline solution was then neutralised with tartaric acid, and a further amount of the latter, equal to that required for neutralisation, was added. The solution was then taken to dryness *in vacuo* on the water-bath at 60°–70°, and the residue

completely extracted with absolute alcohol. The alcoholic solution was concentrated to small volume (15 cc.) and allowed to stand. After about 18 hours the acid tartrate of choline which had separated[1] was filtered off, and, when dry, weighed 0·3 gram. It was identified as choline by:—

(*a*) the mercuric chloride compound m.p. 249–250°,

(*b*) the aurichloride m.p. 261–262°,

(*c*) the platinichloride m.p. 245°.

An analysis of the aurichloride gave the following result:

0·1625 gave 0·0722 Au. Au = 44·43 per cent.
Calculated for $C_5H_{14}ONCl \cdot AuCl_3$. Au = 44·47 per cent.

The alcoholic filtrate from the choline tartrate was next treated with an alcoholic solution of platinum chloride until no further precipitate was produced. The precipitated platinichlorides were filtered off and dried. The weight obtained was 1·3 grams. A small quantity of this platinichloride was decomposed by evaporating its aqueous solution to dryness with an excess of potassium chloride, extracting with absolute alcohol, evaporating off the alcohol, and dissolving the residue in a little water. This solution when tested physiologically was extremely active and it was found that practically the whole of the active base had been precipitated, as platinichloride. In order to separate the platinichloride of the active base from that of choline, which was still present, the main portion of the platinichlorides was treated with 2 cc. of boiling water in which it completely dissolved. The solution was then cooled to about 35°, when a small quantity of imperfectly formed polyhedra separated, which were quite different in appearance from the platinichloride of choline, which at the temperature indicated remained completely in solution. The crystals were filtered off, washed with a little cold water and dried at 100°. There was thus obtained 0·205 gram of a platinichloride melting at 253–254°. On decomposing a few milligrams by the method already described, the solution of the free base obtained was qualitatively and quantitatively indistinguishable in physiological action from a specimen of acetylcholine prepared by Nothnagel's method.

The platinichloride was recrystallised from a little hot water. It is considerably less soluble in water than choline platinichloride. On recrystallising, however, a certain amount of hydrolysis occurs, as was pointed out by Hunt and Taveau [1911]. On cooling there was obtained 0·060 gram of platinichloride, which in solubility, crystalline form, and melting point

[1] This method was employed by Honda [1911] for the separation of choline for muscarine.

(256–257°) was identical with that of acetylcholine prepared from choline by the action of acetylchloride.

Analysis gave the following results:—

0·0383 g. gave 0·0109 g. Pt.	Pt = 28·4 per cent.
Calculated for acetylcholine platinichloride $(C_7H_{16}O_2NCl)_2PtCl_4$.	Pt = 27·8 per cent.

SUMMARY.

An active principle of ergot, recognisable by its inhibitor action on the heart and its stimulant action on intestinal muscle, has been identified as acetylcholine.

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