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EMARKS ON EXTRACTUM JABORANDI LIQUIDUM (B.P.)

BY

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THE CHEMISTRY OF THE JABORANDI ALKALOIDS.*

BY H. A. D. JOWETT, D.Sc.

PILOCARPINE was discovered by Gerrard and by Hardy in 1875, and shortly afterwards two other alkaloids (pilocarpidine and jaborine) were isolated from the mother liquors. Of these latter alkaloids, pilocarpidine had a similar physiological effect to pilocarpine but weaker, whilst jaborine had an antagonistic effect, resembling atropine in its action. The properties and reactions of these alkaloids were studied by various chemists, but their results were incomplete and in some cases contradictory, and left the subject in a confused state. The complete investigation of this group of alkaloids was undertaken by the author in order to remove this confusion, and a brief account of the results obtained is here given. The original source of pilocarpine was the leaves of true jaborandi, but of late years this variety has become extremely scarce, and the leaves at present on the market are those of *Pilocarpus pennatifolius* and the Maranham jaborandi or *Pilocarpus microphyllus*. The leaves vary considerably in the amount of pilocarpine they contain, rarely more than 0.5 per cent. and sometimes none at all. In addition to pilocarpine, an isomeric alkaloid, isopilocarpine, is found in both varieties of leaves, but pilocarpidine has been found in small quantity only in the true jaborandi leaves (*P. jaborandi*). A most careful search has

* Being three Papers read in the Section of Pharmacology and Therapeutics at the Annual Meeting of the British Medical Association, held at Ipswich, July-August, 1900.

been made for jaborine in the three varieties of jaborandi above mentioned, but no trace of any substance with an atropine-like action has been discovered. The jaborine of commerce was found to consist of colouring matter with a small quantity of the above-mentioned alkaloids, and it had, as was to be expected, a mild pilocarpine physiological effect. Pilocarpine $C_{11} H_{16} O_2 N_2$ is the principal alkaloid of the series, and is a thick syrup becoming thinner on warming; it yields crystalline salts, and of these the most useful are the nitrate and hydrochloride. The nitrate occurs as distinct crystals, fairly soluble in water (1 in 7) but somewhat sparingly soluble in strong alcohol. The hydrochloride occurs as large cube-shaped crystals, very soluble in water and moderately so in alcohol (1 in 10). Of these salts the nitrate is the most convenient to use in medicine on account of its stability in the air, the hydrochloride being hygroscopic in moist air. The nitrate of commerce is fairly pure but contains a varying amount of the isomeric isopilocarpine nitrate.

I have shown good reason why this salt should replace the galenical preparations of the leaves. The character and tests, therefore, become of great importance, and since isopilocarpine nitrate, the most likely impurity, has been shown by Marshall to have a similar though weaker effect to pilocarpine, a suitable standard can be adopted. From further experience I would suggest that the melting point of the salt should be from 173° to 178°, and its specific rotation $+80°$ to $+83°$. These factors would be correct for a salt containing a very small trace of isopilocarpine, and which would be "commercially producible yet medically reliable."[1] As additional tests and characters, the behaviour of a strong solution with excess of ammonia and the formation of a crystalline picrate of sharp melting point (147°) might be suggested.

Isopilocarpine is isomeric with pilocarpine, and is formed by the action of heat or alkalis on this base. It was discovered, but incorrectly named pilocarpidine, by Petit and Polonowsky. Pilocarpine is converted quantitatively into its isomer by heating with water in a sealed tube at 180° for four hours. Isopilocarpine is very similar in its chemical properties to pilocarpine; it is a syrup which can be distilled unchanged in a vacuum, and forms crystalline salts. The nitrate is not quite so soluble as the pilocarpine nitrate, but the difference in solubility is so

small that they cannot readily be separated by fractional crystallisation unless one salt is present in only a small quantity. Isopilocarpine nitrate is present as an impurity in the commercial pilocarpine nitrate, and this impurity has been confused with pilocarpidine nitrate, which is not present, as it is easily separated owing to its much greater solubility, and remains in the mother liquors. Isopilocarpine occurs in small quantity in the leaves, but the greater portion is formed during the process of manufacture in a similar manner to the conversion of hyoscyamine into atropine. It is curious that as the optically active hyoscyamine is converted in alkaline solution into the inactive atropine, so in alkaline solution the optically active pilocarpine is converted into the optically inactive isopilocarpin (in neutral solution isopilocarpine is optically active).

Pilocarpidine $C_{10}H_{14}O_2N_2$ is found only in the leaves of true jaborandi, and differs from pilocarpine and isopilocarpine in its chemical composition and in other respects. The nitrate is much more soluble in alcohol or water (1 in 2 parts of water) than the other nitrates. I have definitely proved it to be absent in the pilocarpine nitrate of commerce and the Maranham jaborandi. From 7 lbs. of true jaborandi leaves I obtained a very small quantity of a very soluble nitrate, which appeared from its melting point (137°) and its crystalline form to be pilocarpidine nitrate. The yield was 0.017 per cent. of the leaves taken, and as the supply of genuine jaborandi leaves is almost exhausted nothing further can be done with this alkaloid. It is now, however, definitely proved that this alkaloid exists, and is found only in small quantity in the leaves of the true jaborandi and not in the other varieties.

Jaborine.—I had previously shown that no alkaloid answering to the description of jaborine occurs in the Maranham jaborandi or in the jaborine of commerce, so a very careful search was made to ascertain whether such an alkaloid is present in true jaborandi. Seven pounds of genuine jaborandi leaves yielded 0.35 per cent of pure pilocarpine nitrate, a small quantity of isopilocarpine nitrate, and an amorphous substance soluble in excess of acid but precipitated on adding ammonia insufficient to neutralise the excess of acid. This substance was not alkaloidal, and proved on examination to have no atropine action. This amorphous product obtained was 0.016

per cent. of the leaves taken. No substance having an atropine like action has been found in any of the leaves examined. It is thus proved that no such alkaloid as jaborine occurs in either the true or Maranham jaborandi, and that the jaborine of commerce is impure pilocarpine.

The Constitution of Pilocarpine.—Hardy and Calmels have assigned a constitutional formula to pilocarpine and pilocarpidine, and have claimed to have synthetised these alkaloids, obtaining a synthetic product identical in its physiological action with the natural alkaloid pilocarpine. As the work of these chemists has been proved to be inaccurate by the author, and also by Pinner and Kohlhammer[2] and others, no further reference need be made to it, but for many years their work was accepted, and even now finds a place in all textbooks and dictionaries. On account of the interest attached to the relation between physiological action and chemical constitution an attempt is being made to determine the constitution of pilocarpine, and considerable progress has been made. Since pilocarpine readily changes into its more stable isomeride isopilocarpine, experiments are first being made with this base, as the nature of the isomerism would probably follow the determination of the constitution. Isopilocarpine acts not only as an ammonia base, but as a lactone, and forms salts with hydrates, and can even be titrated by hot alkali. On oxidation it yields ammonia, methylamine, acetic acid, and an acid of the formula $C_7H_{10}O_4$, which is probably the lactone of hydroxy-isobutyl-malonic acid. Isobutyric acid is formed by the fusion of pilocarpine with caustic potash. By the action of potash on methyl-isopilocarpine methylamine alone is formed, and further experiments prove that the nitrogen atoms occur as the groups :NH and :NCH_3. There are thus indications of the existence of the following groups in isopilocarpine$(CH_3)_2$:CH.CH.CH(CO_2)C,:NH,:NCH_3, leaving three atoms of carbon and of hydrogen to be accounted for. It is hoped that results will be obtained which will throw some light on the reason of the physiological action of this group of alkaloids.

REFERENCES.

[1] *Digest of B. P. Researches*, p. 85. [2] *Ber.*, 1900, xxxiii, 1424.

THE PHARMACOLOGY OF THE JABORANDI ALKALOIDS.

By C. R. MARSHALL, M.A., M.D.,
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In the previous paper Dr. Jowett has stated that until recently three alkaloids were supposed to exist in jaborandi leaves—pilocarpine, pilocarpidine, and jaborine. Pilocarpine exerts a stimulating influence on the nerve terminations of involuntary muscle and most secreting glands; pilocarpidine was believed to produce a similar but somewhat weaker effect; while jaborine was said to antagonise these substances. The last-named compound was isolated by Harnack and Meyer. They did not obtain it in a pure form, although they state that in constitution it is closely allied to pilocarpine. Jowett, as already stated, has been unable to isolate any such body, and from the crude products he has sent me I have failed to obtain any atropine effect. As Harnack and Meyer undoubtedly obtained an atropine action I am at present unable to explain their results, but criticism I shall leave until my own experiments are published in detail.

From different specimens of jaborandi leaves, Jowett has isolated pilocarpine and a small amount of an isomeric alkaloid isopilocarpine, and from true jaborandi leaves a small quantity of pilocarpidine. Jaborine has been carefully looked for but without success. The substances submitted to me as possibly containing this body invariably turned out to be mixtures of pilocarpine and isopilocarpine with some resinous substance. And this seems to be the composition of the jaborine sent out by Merck; it gives a weak pilocarpine effect, no atropine action.

As both pilocarpine and atropine affect the same structures —certain nerve terminations, or what we may regard as such— in various parts of the body, it is evident that a comparative investigation of such substances might be carried out in a variety of ways. I have chosen their influence on the heart as being the simplest and, I think, the most exact. These results were controlled by observations on the excretion of saliva, and by experiments with minimal doses on myself. The animals used were rabbits and cats. Both were anæsthetised with chloroform before the operation was commenced. In both the blood pressure was taken from the right carotid artery, and the injection cannula tied into one of the right facial veins, so that its mouth projected into the external jugular vein. Sometimes one vagus, sometimes both vagi were ligatured and cut. After obtaining a constant vagus effect, a solution of the drug was injected and the vagus again stimulated.

After the injection of pilocarpine the heart beats became slower and the blood pressure fell, and after a short but variable interval both gradually returned to normal, the blood pressure sometimes slightly beyond it. During the rise of the blood pressure the vagus was generally less irritable, but on reaching its normal height it became, for a short time, slightly more sensitive. This effect is produced by an injection of $\frac{1}{2}$ to 1 c.cm. of a 1 in 5,000 pilocarpine solution; such a dose also produces distinct salivation. After somewhat larger doses the heart ceases to beat for a time, and then gradually returns to the normal. After still larger doses the heart is stopped permanently and the respiration is paralysed. By careful gradation of the dose, however, the vagus terminations may be paralysed, and then large doses can be given without much effect upon the heart.

Isopilocarpine produces a similar but much weaker effect to pilocarpine. In the rabbit its action on the heart seems to be comparatively less powerful than its influence on the salivary glands, but in cats and man this is not so obviously the case. Roughly, we may place its activity as one-eighth to one-tenth that of pilocarpine.

Pilocarpidine is very slightly active; it may almost be said to be inactive. Solutions of 1 in 200 injected into the circulation cause a slight fall of blood pressure and in cats slight salivation.

In all experiments the nitrates of the alkaloids were employed, but in all cases the dose is given as pure alkaloid.

In the experiments on myself the substances dissolved in water were taken by the mouth. My endeavour was to obtain the least dose causing salivation and perspiration ; and under the conditions of the experiment this was obtained by pilocarpine 0.005 gram, isopilocarpine 0.04 gram, jaborine (Merck) 0.09 gram. Pilocarpidine 0.38 gram (4 grs.) did not produce distinct salivation or sweating, but it caused slight nausea. It may also be said that the resin of jaborandi in doses of 2 grams (31 grs.) is inactive.

Certain derivations of pilocarpine have been investigated, but these are of no practical interest, and their action will therefore be described elsewhere.

REMARKS ON EXTRACTUM JABORANDI LIQUIDUM (*B.P.*).

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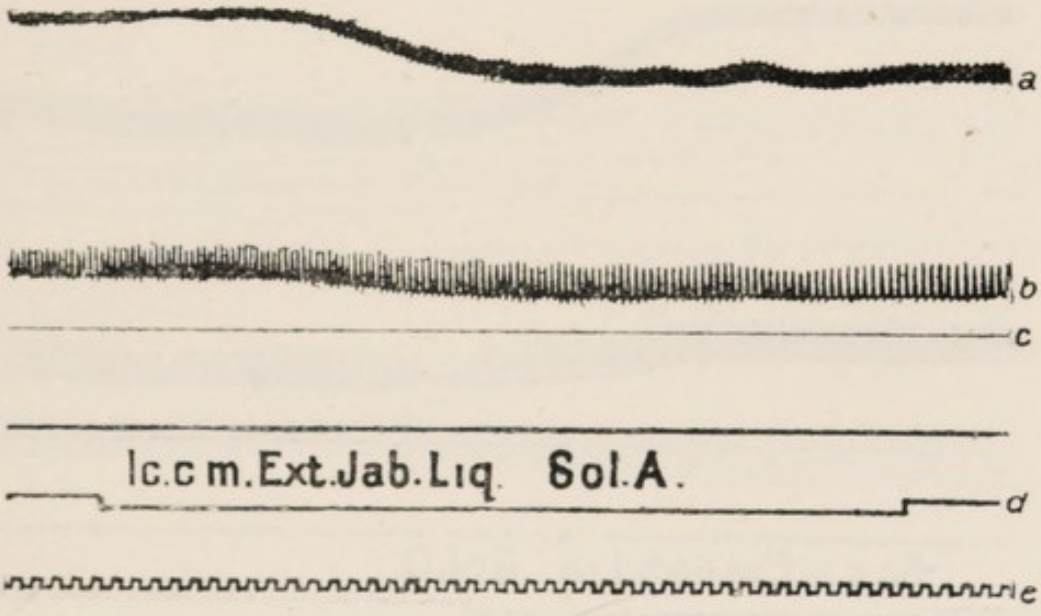
Investigations with this preparation were made in order to determine whether any active principle other than pilocarpine and its allies was present in jaborandi leaves. Earlier investigators had noticed that crude products sometimes gave results different to those of pilocarpine, and consequently they had concluded that at least two active substances, antagonistic in action, were present in this plant. We have, however, failed, as stated in the previous communications, to detect chemically or physiologically any substance antagonistic to pilocarpine, and in none of the liquid extracts examined have we found an atropine-like effect. But apart from this question of the active ingredients, the action of the preparation itself is of some interest. It is the most important galenical preparation of jaborandi in our *Pharmacopœia*, and we believe, although we do not know to what extent, it is used in practice.

As samples, three specimens were obtained from three large manufacturing druggists. They were assayed by a method described in the *Year Book of Pharmacy* (1899, p. 438) for "total alkaloid" and pure alkaloid (chiefly pilocarpine) with the following result :

Sample.	Total Alkaloid.	Crystalline Nitrate (chiefly Pilocarpine).
A	0.21 per cent.	*Nil.*
A_2	0.34 ,,	*Nil.*
B	0.50 ,,	0.082 per cent.
C	0.25 ,,	0.082 ,,

The supply of A was exhausted before the experiments were finished, and consequently another sample, A_2, which proved to be similar in composition, was obtained from the same source. Owing to the small quantities at our disposal, the comparatively slight amounts of alkaloids, and the presence of resin, the assay could not be expected to give accurate results. The "total alkaloid" invariably contains resin, and this interferes with the crystallisation of the alkaloidal salt. A and A_2 may have contained a small amount of pilocarpine or its allies (0.01 to 0.02 per cent.), and B, which contained a large quantity of resin, may have contained more than that given.

This is what we found physiologically. When injected into the circulation of animals these preparations, although crude and containing 45 per cent. of alcohol, were found to be fairly well borne. Fig. 1 shows the effect of the three samples on the blood pressure of a small rabbit. It is the first experiment made. The animal had been chloroformed, the right carotid artery joined to the manometer, a cannula introduced into the right external jugular vein, and the right vagus ligatured, cut, and stimulated several times before the injections were commenced. Extractum jaborandi liquidum A (1 c.cm.) undiluted was first injected.



[*For description see next page.*]

The blood pressure, which was rather low, fell somewhat, and the heart instead of beating 17 in 5 seconds only beat 12. Afterwards the blood pressure rose considerably above its previous height, and then fell slightly. The vagus was also

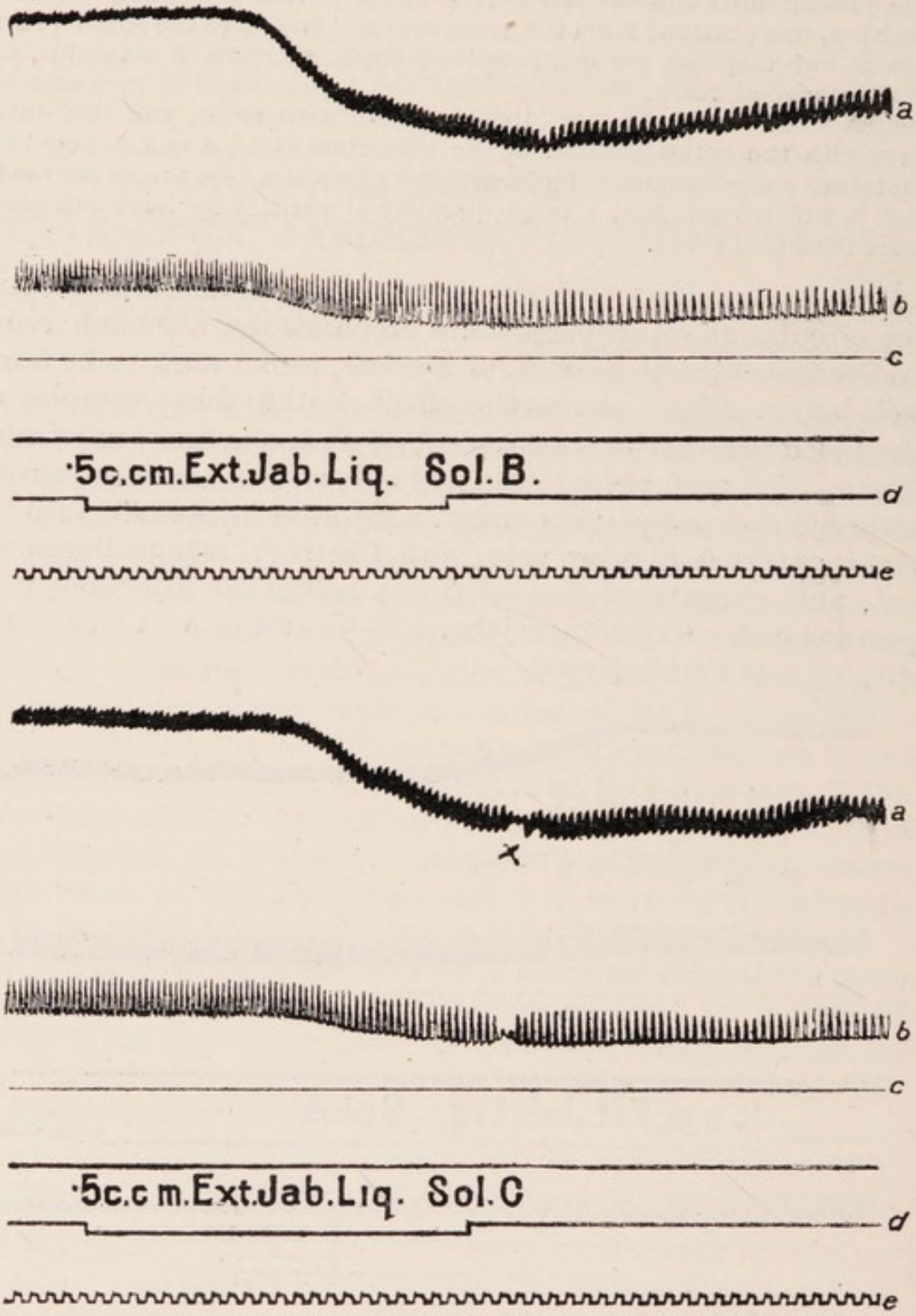


Fig. 1.—Action of extractum jaborandi liquidum (*B.P.*)—three samples—on blood pressure of rabbit. Tracing taken by Hürthle's manometer. *a*, Ordinary blood pressure; *b*, same showing individual heart beats; *c*, zero line; *d*, time of injection; *e*, time in seconds.

less irritable than before the injection, but there was nothing to suggest an atropine action. No salivation occurred. The fall of blood pressure may have been due to the crude nature of the solution, as a slight fall occurs on injecting alcoholic preparations of this kind into the veins, but it is not unlikely that a minute amount of pilocarpine was present.

About 17 minutes afterwards 0.5 c.cm., Sample B, was injected. This time a more marked fall of blood pressure and slowing of the heart (17 to 9 beats in 5 seconds) occurred. Salivation also quickly followed, and the vagus, after the return of the blood pressure to normal, was slightly more sensitive to stimulation than before. All these are pilocarpine actions. A similar but less powerful effect was produced by the injection of the same dose (0 5 c.cm.) of Sample C. There was a fall of blood pressure, slowing of the heart, and a slightly increased sensitiveness of the vagus, which at once suggested pilocarpine or isopilocarpine. Afterwards 1 c.cm. of Sample C

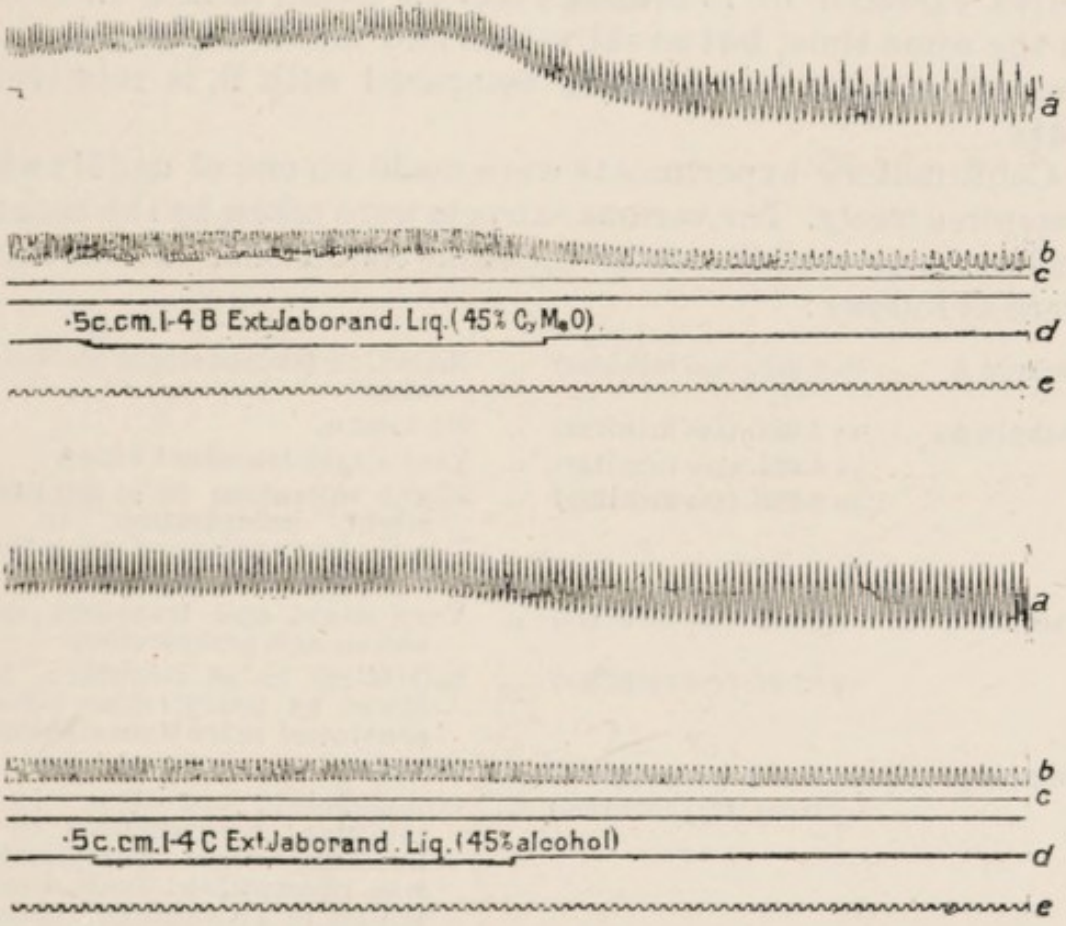


Fig. 2.—Effect of extractum jaborandi liquidum on blood pressure of cat. Letters as before.

was injected, and later 1 c.cm. Sample B. Both produced the same, but correspondingly more marked, effects as before, and B caused failure of the respiration (also a pilocarpine effect), which could not be permanently re-established until a small dose of atropine (1 c cm. of 1 in 20,000 solution) had been injected.

In later experiments the liquid extracts were diluted or otherwise modified, but the same results were obtained. In all B was found to be more powerful than C, and C than A or A_2. The relative action of B and C on the blood pressure of a cat is seen in Fig. 2. The samples were diluted with three times their volume of saline solution and 0 5 c cm. of these solutions was injected into the external jugular vein in each case. Previously twice the amount of sample A_2 (0.5 c.cm. of 1 in 2 solution) had been injected with comparatively little effect. There was slight slowing of the heart and slight salivation, but no distinct fall of blood pressure. After B the saliva excreted in 16 minutes was 2.4 c.cm., after C 2.1 c.cm. in the same time, but as salivation had not ceased when this was injected, the amount, as compared with B, is relatively large.

Confirmatory experiments were made on one of us (M) who perspires freely. The various extracts were taken by the mouth and as far as possible under the same conditions. The results were as follows :

Sample A ...	20 c.cm. (340 minims) ...	No action (except slight alcoholic one).
Sample A_2 ...	15 c.cm. (250 minims) ...	No action.
	20 c.cm. (340 minims) ...	Very slight transient effect.
	30 c.cm. (510 minims) ...	Slight salivation in 20 minutes; slight perspiration in 30 minutes; continued more than an hour.
Sample B ...	5 c.cm (85 minims) ...	Very slight and transient salivation and perspiration.
	10 c.cm. (170 minims) ...	Salivation in 28 minutes; followed by perspiration; both continued more than an hour.
Sample C ...	10 c.cm. (170 minims) ...	No effect.
	15 c.cm. (255 minims) ...	Commencing salivation in 8 minutes; very distinct in 18 minutes, when perspiration was perceptible; both diminishing in 45 minutes; almost ceased after 1 hour.

In some of the experiments a little nausea and palpitation were experienced.

A point we wish to emphasise in these experiments is the enormous dose necessary to produce an effect. Of one preparation more than an ounce was required, and of the most powerful 85 minims ; $\frac{3}{4}$ oz. of another was inactive. These results, coupled with the experiments on animals, prove the extreme variability of this preparation,[1] and cast considerable doubt on its utility as a medicine. Moreover, its inclusion in the *Pharmacopœia* is unnecessary. The whole therapeutic activity of jaborandi leaves is due to the pilocarpine, or isopilocarpine, they contain, and preparations, which cannot be standardised with accuracy, only contain a variable proportion of these with alcohol and inert resinous and colouring matters. The inactivity of the preparations seems to be due to the absence of pilocarpine, and not to the presence of an antagonistic alkaloid.

NOTE.

Compare the assays of Farr and Wright (*Year Book of Pharmacy*, 1899, p. 383).