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A COMPARISON OF THE PROPERTIES OF CERTAIN TISSUE EXTRACTS HAVING DEPRESSOR EFFECTS.

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The number of recent papers on the depressor substances in the various tissues of the body reveals an increasing interest in the subject; Frey [1929], Lange [1930], v. Euler and Gaddum [1931], Zipf [1931], Drury and Szent-Györgi [1929], and Felix and Putzer-Reybegg [1932]. It seemed desirable to compare the extract with which we have been working [1929, 1930] with the substances obtained by these workers.

In our experiments we have used extracts of brain, liver, lung and pancreas, prepared in an identical manner, although more experiments were carried out with brain extract than with the other extracts. The experiments on blood-pressure were carried out under light ether anæsthesia.

Methods of preparation of the extracts.

Ten kg. of brain, liver, lung or pancreas were mixed with 10 l. of acetone and the mixture stirred vigorously for a period of an hour. After filtration the filtrate was evaporated to dryness and taken up with 200 c.c. of distilled water. This preparation will be referred to in the future as the crude extract of brain, liver, etc.

Crude extracts were thus obtained from various portions of the brain, and Fig. 1 shows that extracts from the basal ganglia are more active than extracts from certain other parts of the brain.

The effect of the various crude extracts after treatment with "norit" is shown in Fig. 2. This treatment, shaking up 25 c.c. of crude extract with 2 g. of norit, removes all the activity of the lung extract, 90 p.c. of the activity from the liver extract, 50 p.c. from the pancreas extract and only a small amount from the brain extract. Since this treatment removes histamine this is strong evidence that the activity of the lung

extract and most of the activity of the liver extract is due to histamine or histamine-like substances, as was shown by Best, Dale, Dudley and Thorpe [1927], and that the activity of the brain extract is not due to histamine.

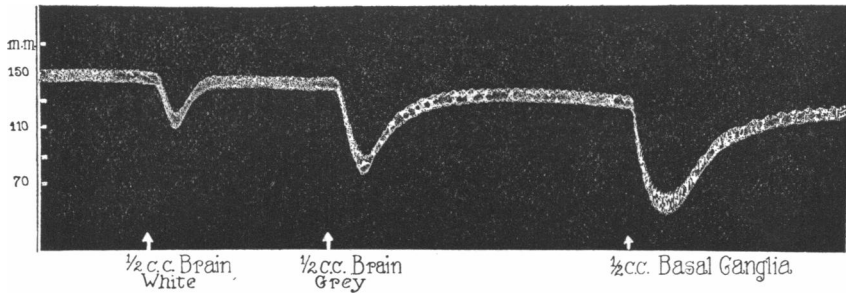

Fig. 1. Curves showing the depressor effect of extracts from various parts of the brain. 1 c.c. of extract represents 0·5 g. of brain tissue.

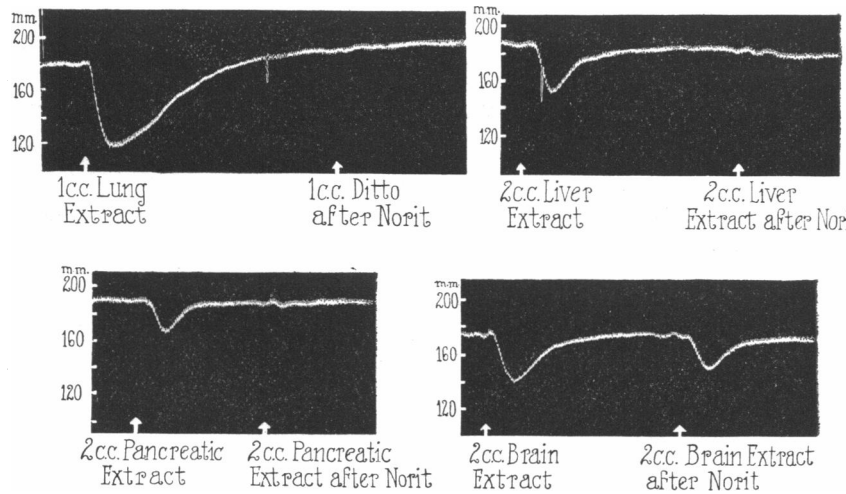

Fig. 2. The effect of treating the extracts with norit. Norit removes all of the depressor activity of the lung extract and most of the activity from extracts of liver and pancreas. Brain extract is only slightly affected by this treatment.

The behaviour of the crude extract after treatment with phosphotungstic acid is shown in Fig. 3. Treatment with phosphotungstic acid removes approximately 95 p.c. of the activity of the liver extract, 25 to 50 p.c. of that of the pancreatic extract, 95 p.c. of that of the lung extract and approximately 50 p.c. of that of the brain extract.

Phosphotungstic acid should remove histamine and choline and so evidence is furnished that the activity of brain extract is not due to these substances. Felix and Putzer-Reybegg, by fractional precipitation

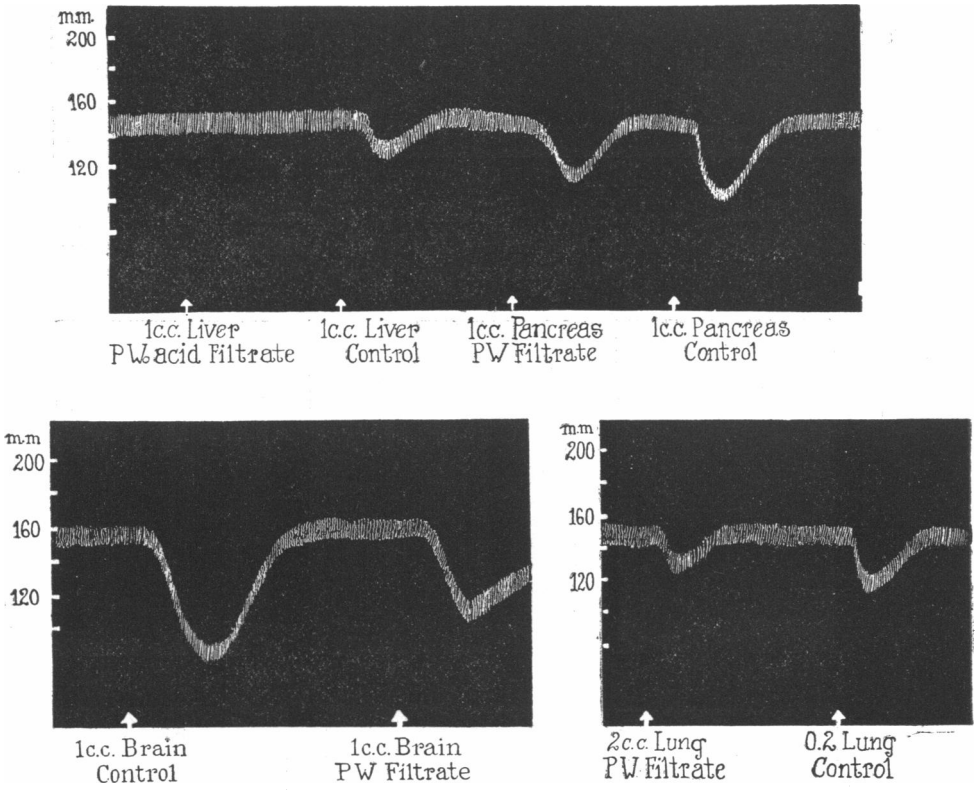

Fig. 3. The effect of treating the extracts with phosphotungstic acid. This treatment removes 95 p.c. of the depressor activity of the liver extract, 50 p.c. of the activity of the pancreatic extract, practically all of the activity of lung extract and less than 50 p.c. of the activity from the brain extract. Note that the amount of the lung extract control was only one-tenth of that used after treatment with phosphotungstic acid.

with silver, have brought evidence to show that 50 p.c. of the depressor activity of the precipitates from the kidney and mesentery was due to choline. Fig. 4 shows the effects of choline and of a silver filtrate of the brain extract, and that atropine abolishes the action of choline but has no effect upon that of the brain filtrate.

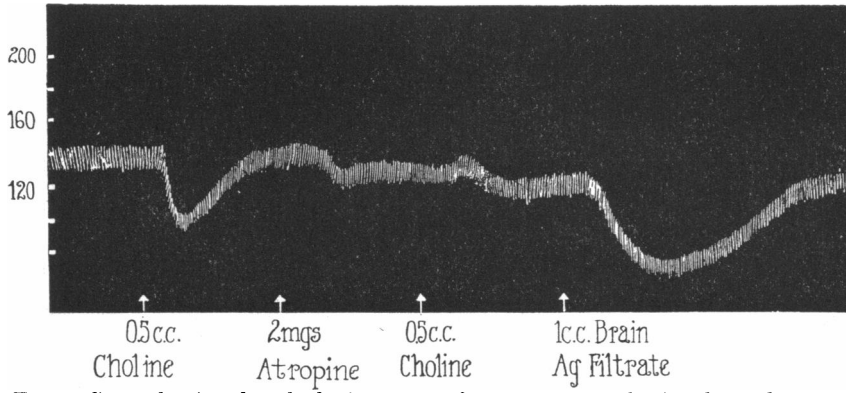

Fig. 4. Curve showing that the brain extract after treatment with silver has a depressor effect upon an atropinized animal.

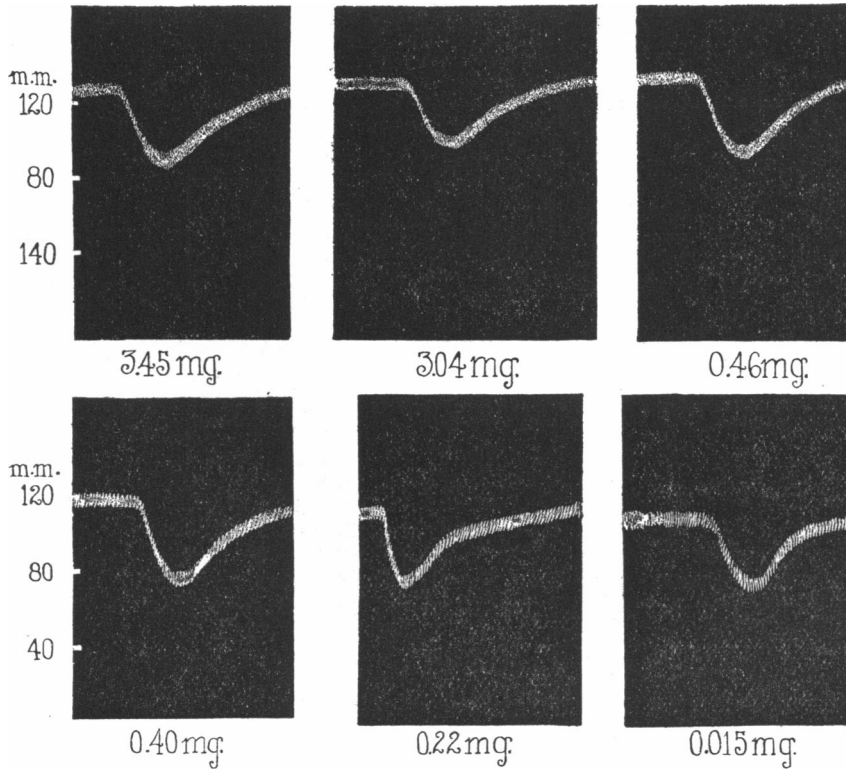

Fig. 5. Tracings showing purification of brain extract without loss of activity. Figures in terms of mg. of solids injected. The curve labelled 0·015 mg. shows the depressor effect of 0·015 mg. of histamine.

For the purification of the brain extract we have also proceeded as follows: the crude brain extract is treated with a saturated alcoholic solution of mercuric chloride and made alkaline; the abundant precipitate after removal of mercury can be divided into two fractions, one soluble in water, the other not; the active depressor principle with which we are working is in the former.

Fig. 5 shows the effects of purification upon the activity of the extract. The first curve shows the activity of the mercuric precipitate after removal of the mercury. The dose employed contained 3·40 mg. of solids. After treatment with phosphotungstic acid this extract shows the same activity when a dose containing 3·04 mg. of solids was injected. This solution was then made acid to Congo Red and treated with Lloyd's reagent, filtered, and the precipitate ground with barium hydroxide, water added, and again filtered. The barium was removed from the filtrate with sulphuric acid. The activity of an amount of the resulting solution which contained 0·46 mg. of solids is shown. When this solution was made alkaline and treated with norit, an amount of the filtrate containing 0·4 mg. of solids exhibits the depressor effect shown in the curve. This solution was evaporated to dryness and extracted with 90 p.c. alcohol, the alcoholic solution evaporated and taken up in water; an amount containing 0·22 mg. of solids has the activity shown in the curve, which is approximately equivalent to that produced by 0·015 mg. of histamine in aqueous solution.

We have also pharmacological evidence that these extracts do not owe their activity to either histamine or choline. In Fig. 6 the activity of the extract purified by treatment with Lloyd's reagent and alcohol is tested against a solution of histamine. The quantity of brain extract employed had about six times the depressor activity of the histamine used when tested on a dog under ether anæsthesia. The curve shows that the brain extract has no effect upon a virgin guinea-pig uterus, while the solution of histamine produced its characteristic response. This purified brain extract was next tested against the isolated intestinal loop of a rabbit. The intestinal loop showed the greatest response to choline, less to histamine, and showed no response whatever to the brain extract (Fig. 7).

Table I presents a comparison between the brain extract with which we have worked and the depressor substance described by other investigators. The "hormone" of Frey was isolated from the urine, the depressor substance of Felix and Lange was isolated from several organs, particularly the kidneys and the mesentery, while the depressor

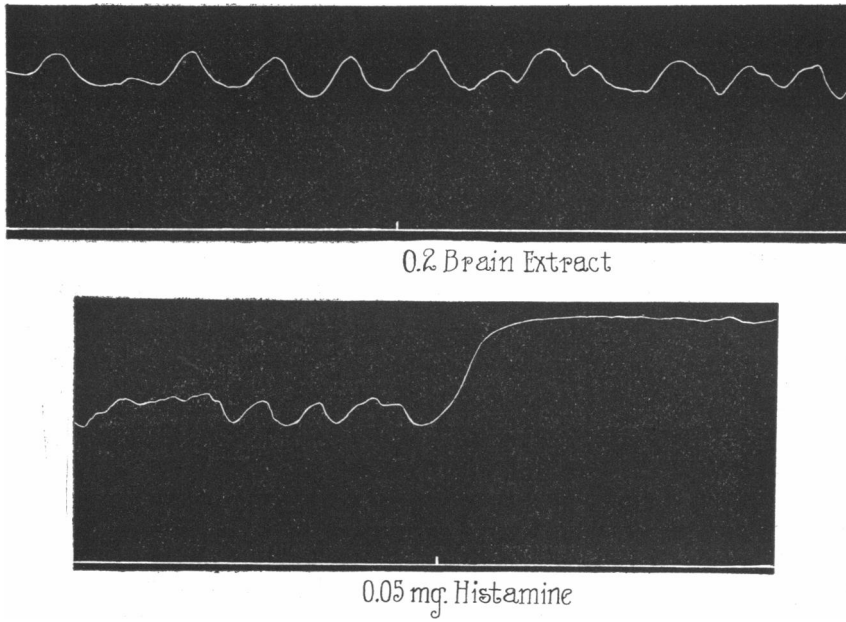

Fig. 6. Curve showing that the purified brain extract has no effect upon the virgin guinea-pig uterus, while histamine produces a characteristic contraction.

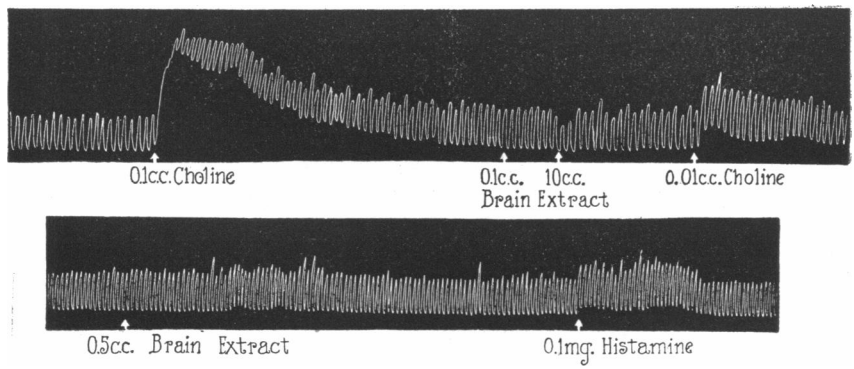

Fig. 7. Curve showing that the purified brain extract has no effect upon the isolated intestinal loop of the rabbit, while choline produces a marked contraction and histamine a lesser contraction.

extract of Euler and Gaddum was also obtained from several organs, extracts from the small intestine and brain apparently containing the largest amounts of the depressor principle.

TABLE I.

Treatment	Histamine	Choline	Adenosine	Frey's hormone	Euler and Gaddum	Felix and Lange	Brain
Silver precipitation	–	+	–	.	.	–	+
Phosphotungstic acid precipitation	–	–	–	–	–	–	+
Norit absorption in alkaline solution	–	.	.	.	.	.	+
Mercury precipitation in alcoholic solution (acid)	–	.	.	.	.	–	+
Mercury precipitation in alcoholic solution (alkaline)	–	–	–	.	–	–	–
H_2SO_4 5 p.c. Boiling 5 minutes	+	+	–	–	.	+	+
NaOH *N*/1. Boiling 5 minutes	+	.	+	–	–	.	+

+ or – indicates presence or absence of activity in the filtrate after indicated treatment

If the data given by these other observers are constant, we are apparently working with a different depressor substance.

SUMMARY.

The extract of brain tissue with which we have been working has a powerful effect. It is not precipitated by silver, phosphotungstic acid or mercury in acid alcoholic solution. It is not absorbed by norit from an aqueous solution, but is precipitated by mercury in alkaline alcoholic solution. It is not destroyed by boiling in 5 p.c. sulphuric acid or in normal sodium hydroxide for 5 minutes. It is active in atropinized animals and does not show a Pauly reaction. In one of our previous communications the statement was made that the purest solution we had obtained which contained this depressor principle showed a positive Sakaguchi reaction, indicating the presence of a guanidine compound. Since the publication of that paper we have obtained an extract which is quite active but gives a negative Sakaguchi reaction.

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