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A BRONCHODILATING PRINCIPLE ISOLATED FROM EARTH WORM

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Earth worm (*Lumbricus Spencer*) has been claimed to possess antipyretic and diuretic actions [Li, 1596; Mizoguchi, 1914]. It has also been used empirically against asthmatic attacks. Yagi [1911] in 1911 isolated from it a hemolytic principle, which he gave the name "Lumbritin". Its cadmiun salt was assigned the formula $C_{259}H_{528}O_{125}N_{47}SP_3(CdCl_2)_{19}.3H_2O$. Sewaka [1914] obtained a toxic principle *"Telestrolumbrilysin"*, and showed that this principle also produced hemolytic effect. Tanaka and Nukada [1915] investigated the antipyretic action of earth worm, and from their experiments came to the conclusion that the substance responsible for the antipyretic action was a derivative of tyrosine. It was precipitated from the aqueous extract of earth worm by alcohol, and was given the name *"Lumbrofebrin"*. Murayama and Aoyama [1921; 1922] made a study of the chemical constituents of earth worm, and obtained fats, fatty acids, cholesterol, choline, various amino-acids and derivatives of nucleic acid.

It appears then that there is some evidence relating to the presence of antipyretic substance in earth worm. Derivatives of nucleic acid may possess some diuretic effect, but at the moment there is no indication that their influence upon the secretion of urine is a marked one. Concerning the antiasthmatic property of the earth worm constituents no study has been made. The present work deals only with the question as to whether earth worm contains anything which could substantiate the claim that it is useful against asthma. The outcome of our experiments was of

such interest that we believed it worthwhile to study the problem in detail.

EXPERIMENTAL

Methods.

The earth worm used was purchased from the native drug stores, commonly called "Kwang-ti-lung", which means goods coming from the province of Kwangtung. It came in dry form, and measured about 20 cm long in average. After their intestinal contents had been removed, they were washed clean with water, further dried and powdered. In the first few experiments, a simple aqueous extract was used. Good results were obtained. Later it was felt desirable to remove as much protein material as possible; this was accomplished by treating the concentrated aqueous extract with 95 per cent alcohol, filtering off the precipitate, and finally removing the alcohol by evaporation. The extract, thus obtained, gave identical results.

Unless otherwise stated, the experiments were confined to the perfusion of lungs according to the method of Sollmann and Von Oettingen [1928] with slight but unimportant modifications. The extract tested was introduced slowly with a syringe, always 1 cc at a time, into the connecting tube immediately above the lung. Control injections of the same amount of Locke's solution, produced no discernible effect in the rate of perfusion flow.

Perfusion of lungs with crude extract.

Experiments were carried out on both rat and rabbit lungs, and yielded similar results. Lungs were perfused with Locke's solution, which contained NaCl 0.92, KCl 0.042, $CaCl_2$ 0.012, $NaHCO_3$ 0.015 per cent and was freshly prepared for each occasion. The same result was obtained when van Dyke and Hastings' solution [1927] was employed, which has the following composition: NaCl 0.659, KCl 0.046, $CaCl_2$ 0.005, $MgCl_2$ 0.009, NaH_2PO_4 0.01, Na_2HPO_4 0.008, $NaHCO_3$ 0.252 per cent.

Altogether 13 experiments were performed on normal lungs, 2 with 20 percent, 2 with 50 percent and the rest with 100 percent extract. As has been said, the dose injected was always 1 cc in volume. None of them failed to elicit a bronchial dilatation. The maximum increase in the number of drops, usually occuring immediately after the drug had reached the lung, could be anything between 30 to 80 per cent of the control readings. It was also found possible to counteract broncho-constrictors without reference to the site of their actions. This is illustrated in fig. 1, which shows the antagonistic effect of earth worm ex-

tract toward histamine which is a muscular stimulant and toward pilocarpine which is a parasympathetic stimulant.

Isolation of active principle.

The extract whether treated with alcohol or not, was always acid in reaction. The objection might be raised that the dilating effect was due to acid [Bray, 1931] rather than to any specific substance in it. This objection was over-ruled by the observation that when neutralized or rendered alkaline, it gave similar results.

In order to find out whether oxidation has any influence on the dilatating effect of the earth worm extract, it was treated with hydrogen peroxide and such treatment did not alter the result. In view of the

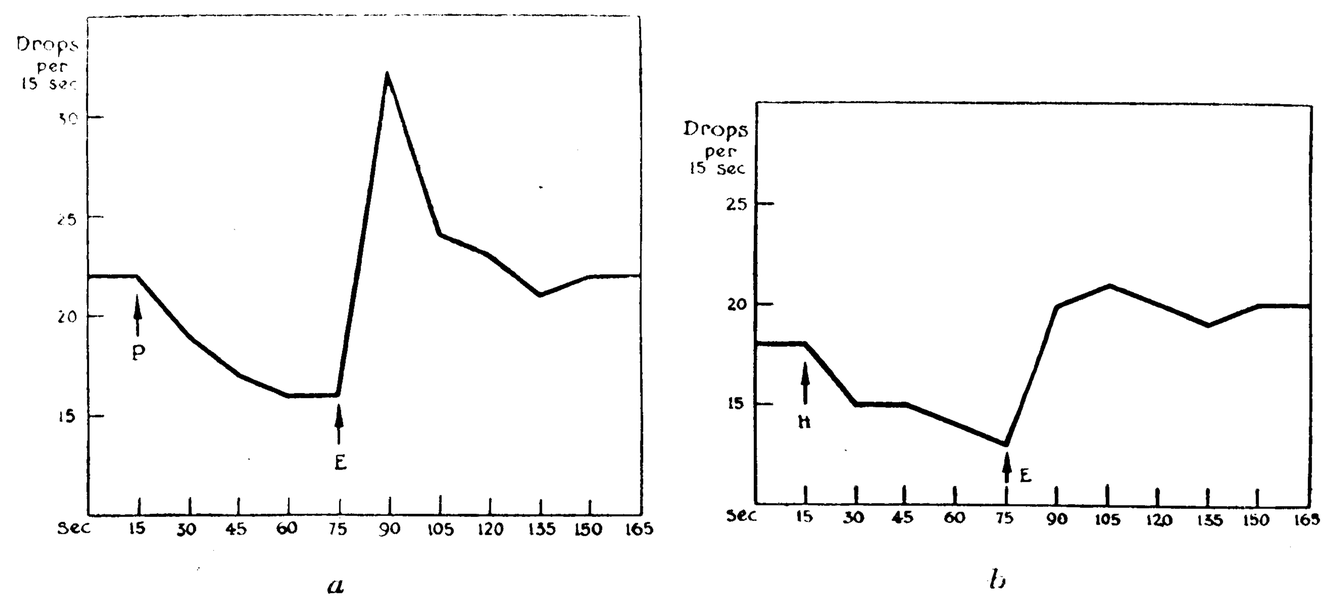

Fig. 1. Effect of earth worm extract on histamine and pilocarpine treated lungs. H, histamine, P, pilocarpine E, earth worm extract.

above findings we were led to believe that earth worm appeared to contain some constituent or constituents which could dilate the bronchial muscles.

After the above preliminary orientation, we made an attempt to isolate the active principle.

One kilo of dried earth worm was finely powdered and boiled with 90 percent alcohol for a day. The alcohol extract was filtered, distilled to recover the alcohol and finally concentrated on the water bath to a syrup. The residue was taken up with water and filtered from any insoluble part. The aqueous solution was treated with a solution of lead acetate. The lead precipitate, after being filtered and well washed, was

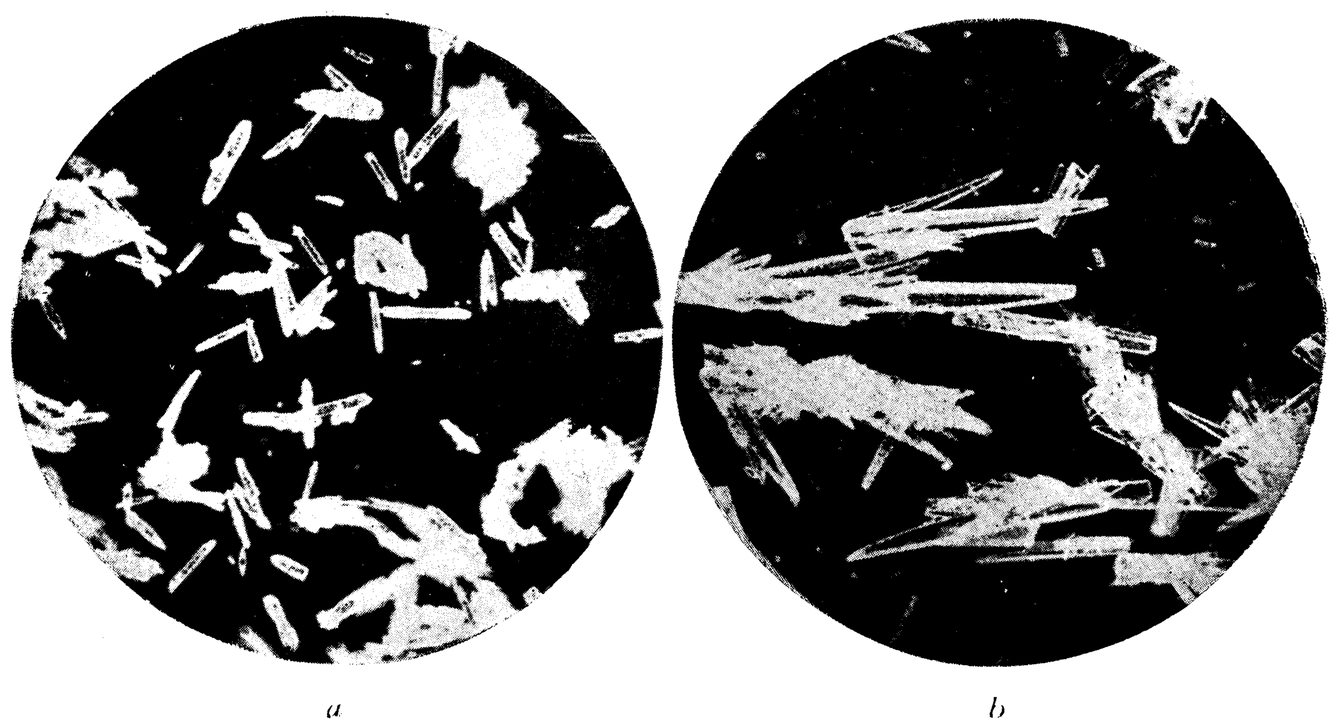

Fig. 2. Bronchodilating principle isolated from earth worm. *a*, base, m.p. over 320°C; *b*, hydrochloride, m.p. over 320°C.

suspended in water and decomposed by a current of H_2S. When filtered from lead sulphide, the solution was concentrated to a small volume from which a neutral principle has been isolated. It contains nitrogen, but its aqueous solution reacts neutral to litmus paper, it crystallies from methyl alcohol or water in group of small prisms (fig. 2) m.p. over 320°C.

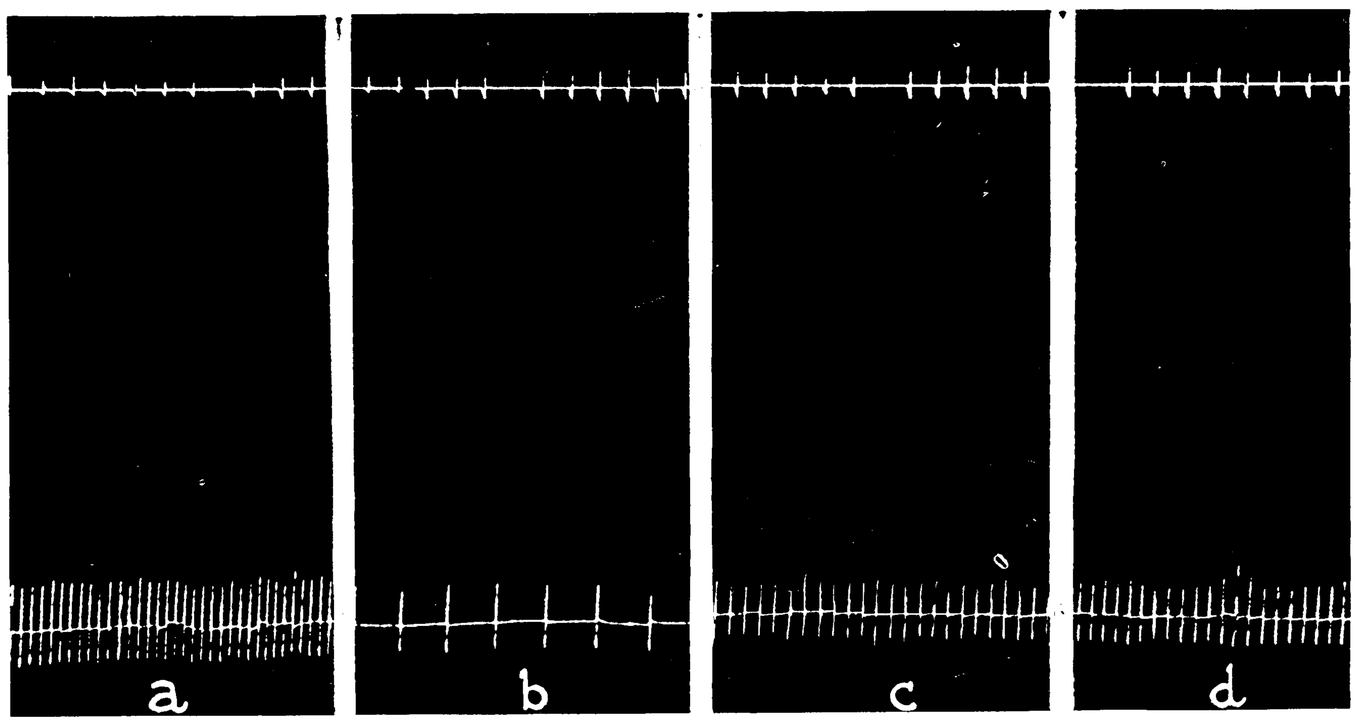

Fig. 3. Perfusion of the rabbit's lung with (a) Locke's solution, (*b*) histamine, (*c*) earth worm principle and (*d*) histamine again. Tracings from above downward: Time in 5 sec, perfusion pressure and outflow in drops.

Perfusion of lungs with crystalline principle.

The effect of the active principle on the lung was found quite similar to that produced by the crude extract: It dilated the normal lungs and relieved the constricting effect of both pilocarpine and histamine. Fig. 3 illustrates some of these findings. As seen from it, the lung was first perfused with Locke's solution (*a*) and then with Locke's solution plus histamine (*b*). The difference between *a* and *b* in the number of drops of perfusate was quite marked. As soon as the earth worm principle was introduced (*c*), relaxation occurred. It is interesting to note that the relaxation was so persistant that the second dose of histamine (*d*) was no longer able to counteract it.

TABLE 1.

The effect of earth worm principle on the survival of guinea pigs after histamine injection

G. P. No.	Weight	Treatment	Survival time *(min)*
1	445 *g*	Histamine 0.6 *mg/kg*	4
2	636	,, ,,	4
3	462	,, ,,	5
4	715	,, ,,	5.5
5	450	E. W. principle 20 mg followed 15 sec later by histamine 0.6 *mg/kg*	Survived
6	490	,,	7
7	460	,,	6
8	355	,,	Survived

Influence of active principle on histamine shock.

Judging from our result with excised lungs which showed dilatation in every instance, regardless of whether the preparation was treated with a bronchoconstrictor or not, we felt justified in concluding that the principle which we have isolated from the earth worm had a definite bronchodilating effect. Attempt was then made to determine if the principle could relieve in intact animals the bronchospasm artificially produced by such agent as histamine. For this purpose, guinea pigs were used as experimental animals. In one set of experiments, histamine alone in a

dose of 0.6 mg/kg was injected intravenously; and in another set, 15 seconds before histamine injection, earth worm principle in a dose of 20 mg was administered as a protective agent. The number of animals which survived and the time of survival were noted. It will be seen from table 1, that of 4 untreated animals, none survived, and of 4 treated ones, two survived, and that the time of survival of the other two treated animals was slightly longer than that of untreated.

Discussion.

Now on examining the literature regarding the anti-asthmatic efficiency of various agents, we found that Barlow and Beams [1933] reported that the efficiency in a descending series was as follows: atropine, epinephrine and sodium nitrite. According to them injection of 10 mg of atropine protected about 60 percent of the pigs tested. If our results are comparable with theirs, we are of the opinion that the antiasthmatic efficiency of the earth worm principle is approximately equal to atropine and certainly greater than epinephrine.

In addition to its action on the bronchial muscle, the earth worm principle was found to lower the blood pressure of a cat and a guinea-pig when injected intravenously, in a dosage of 10 mg and to inhibit the tone of isolated segments of the rabbit's intestine.

These properties together with its bronchial effect resemble those of adenosine [Bennet and Drury, 1931; Drury and Szent-Gyorgyi, 1927] a derivative of nucleic acid. The question was then raised if the principle which we were dealing here was not adenosine. This was answered in negative, because adenosine melts at 229°C [Levene and Bass, 1931], whereas the earth worm principle did not melt when heated to 320°C. Whether this is a new or known substance we can not say at present and it will be a subject of further investigation.

SUMMARY

An active principle containing nitrogen but neutral to litmus paper in its aqueous solution has been isolated from "Kwang-ti-lung", i.e. the earth worm obtained from the province of Kwangtung. The principle causes dilatation of bronchial muscles in normal and in histamine and pilocarpine treated lungs of rats and rabbits. This effect together with its depressor effect on the blood pressure and the inhibitory action on

intestine has led us to suspect that the substance with which we were dealing is adenosine. That it is not adenosine is proved by the fact that the earth worm principle does not melt when heated to 320°C, whereas the melting point of adenosine is 229°C.

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蚯蚓中之舒展支氣管的物質

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自廣地龍(即廣東產之蚯蚓乾)中，提得一種含氮之有效成分，其水溶液對于石蕊試紙呈現中性反應．將此物質試諸灌注之白鼠肺及家兎肺，發生顯著之舒展支氣管作用，即于預經組織毒或疋羅卡品處理之灌注肺，仍能表現此種舒展作用．又於活體之荷蘭豬，此物質之靜脉注射，能保護百分之 50 之動物，使不死于致死量之組織毒．此種支氣管舒展作用，及其降低血壓及抑制小腸緊張力作用，與腺糞苷頗爲相似，但其化學性質則不同．蚯蚓中所得物質，于攝氏三二〇度時尙不熔化，而腺糞苷之熔點，則爲二二九度．