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# AN ANTIHISTAMINE-LIKE SUBSTANCE (OR SUBSTANCES) IN EXTRACTS PREPARED FROM HUMAN TISSUES

BY

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A method for the extraction of an antihistamine-like substance (or substances) from human tissues is described. The method has demonstrated the presence of this active principle(s) in a large variety of human tissues, so far without exception. The substance(s) has a wide range of activity in antagonizing the *in vitro* effects of histamine, 5-hydroxytryptamine and bradykinin and the Schultz-Dale reaction on the guinea-pig ileum and rat uterus preparations.

One of us (Kovacs, 1950) was the first to report the presence of an antihistamine-like substance in extracts of human tissues (eosinophil-rich leucocyte suspensions). In 1951, Karady, Kovacs, Kovacs, Szerdahelyi & Vajda showed that extracts prepared from different animal organs such as rat and guinea-pig liver, lung and spleen, and from human urine inhibited the action of histamine on isolated smooth muscle preparations. Vercauteren (1953) obtained similar results with extracts prepared from granules of horse eosinophils. Francis & Melville (1958) reported that extracts of human and dog gingival tissues exerted a strong antagonism of histamine on the guinea-pig ileum preparation. Archer (1960) showed that watery extracts of eosinophils antagonize the local oedema formation produced by histamine. Feldberg & Kovacs (1960) and Archer, Feldberg & Kovacs (1962) confirmed the presence of a histamine-antagonizing substance in eosinophil-rich leucocyte suspensions, using the effects of histamine aerosol on guinea-pigs as a test preparation. Kovacs & Melville (1962, 1963) reported that extracts of normal human urine exerted a wide range of activity in antagonizing the *in vitro* effects of histamine and 5-hydroxytryptamine and the Schultz-Dale reaction ; and Francis, Melville & Douglas (1962) showed that extracts of human colon also contained a histamine antagonist. On the basis of these observations, it seemed important to find out whether the active principle (or principles) was present in a few selected tissues only, such as eosinophils, gingiva and colon, or whether it was distributed in the body widely like histamine.

In this paper, a method is described for the extraction of an antihistamine-like substance from a variety of human tissues. It has been successfully applied to demonstrate that extracts of many different human tissues contain a substance (or substances) with a wide range of activity in antagonizing the *in vitro* effects of histamine, 5-hydroxytryptamine and bradykinin, and the Schultz-Dale reaction.

## METHODS

### *Preparation of extracts*

Fresh specimens of various tissues obtained at operation were weighed, frozen and stored at −10° C. When used for extraction, the tissues were first thawed and cut into small pieces. To each g of tissue, 30 ml. of 0.9% saline were added and the mixture homogenized using a Virtis 45 homogenizer. The homogenate was acidified with concentrated hydrochloric acid to a *p*H of 1.5 to 2.0, and incubated for 30 min at 56 to 60° C. It was then neutralized with 5N-sodium hydroxide solution to a *p*H of 6.8 to 7.2 and extracted first with ether and then with a mixture of ether and chloroform (3:1) using 200 ml. of ether and of the ether-chloroform mixture per g of tissue. The pooled ether and ether-chloroform extracts were evaporated to dryness by distillation in a water-bath at 40 to 50° C at a reduced pressure. The residue was re-extracted three times with water-free ether using 10 ml. of ether per g of tissue for each extraction. The ether filtrate was evaporated to dryness and the residue stored at −10° C in flasks filled with argon and closed with well-greased stoppers. Immediately before use, the dried ether-soluble material was dissolved in Tyrode solution (1 ml. of Tyrode solution per 0.2 to 1.0 g of original tissue) and filtered, and its *p*H checked and adjusted to 7.2 to 7.4.

### *Biological methods*

*Isolated smooth muscle preparation of the guinea-pig ileum.* Guinea-pigs weighing 200 to 250 g were killed by a blow on the head. Immediately afterwards, the terminal segment of the ileum was removed and a piece 6 to 7 cm long was suspended in Tyrode solution at 34° C in a 15 ml. organ-bath. Acetylcholine or histamine dihydrochloride (weights refer to the bases) was added at 3 min intervals, left in contact for 20 sec and then washed out. Synthetic bradykinin was added at 4 min intervals and left in contact for 40 sec.

*Schultz-Dale reaction.* Guinea-pigs were sensitized with two intraperitoneal injections of 0.5 ml. of a 10% solution of egg white given on two successive days. The animals were killed 21 to 23 days later. Immediately after killing each animal, a segment of ileum 15 to 20 cm long was removed. It was then cut into strips 5 to 6 cm long, and one strip was used as a control and one for testing. The challenging dose of antigen consisted of 0.1 ml. of a 10% egg white solution added to the organ-bath.

*Rat uterus.* The uteri of virgin rats (100 to 120 g) injected subcutaneously 24 hr earlier with stilboestrol (0.2 mg) were suspended in Jalon solution in a 15 ml. organ-bath at 29° C. The action of 5-hydroxytryptamine creatinine phosphate was always tested at 5 min intervals; it was left in contact for 90 sec and then washed out. The contractions of smooth muscle preparation were recorded on a smoked drum by a frontal lever, using the same lever and magnification throughout.

Tissue extracts tested against contractions induced by histamine or acetylcholine were kept in contact with the preparation for 2 min; against contractions induced by bradykinin or 5-hydroxytryptamine for 3 min; and with the Schultz-Dale reaction for 6 min.

## RESULTS

### *The effect of human tissue extracts on isolated smooth muscle preparations*

Seventy-two extracts have been examined for antihistamine, anti-5-hydroxytryptamine and antibradykinin activity. Each tissue extract tested contained a substance which, in varying degree, antagonized these agents. A typical example of the results obtained by use of tissue extracts on contractions induced by histamine is shown in Fig. 1. After the administration of an extract corresponding to 0.5 g of colon (wet weight), the preparation did not respond to a subsequent dose of 0.2 μg of histamine. Even after repeated washing, the sensitivity of the gut was

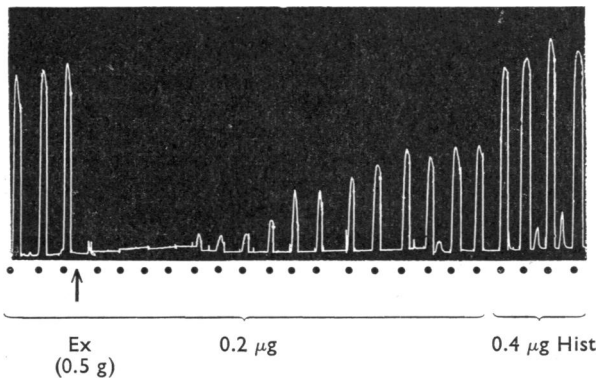


Fig. 1. Responses of a guinea-pig ileum preparation to histamine (Hist, at black dots) before and after the addition of an extract of colon (Ex, at the arrow) corresponding to 0.5 g of original tissue. Intervals of 3 min elapsed between administrations of histamine. In each instance, the drum was stopped temporarily after washing out the organ-bath and restarted 20 sec before the next test dose. The contact times were 20 sec for histamine and 2 min for the extract.

still much reduced and it was necessary to double the original dose of histamine to obtain responses similar to those of the initial controls.

Fig. 2 shows that when the extracts were applied, in minimal effective concentrations, to the gut, a well-defined dose/response curve could be obtained. The tissue extract in this experiment was added in three different doses to the preparation

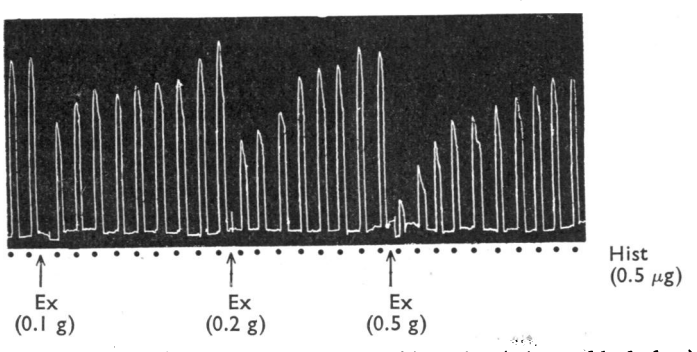


Fig. 2. Responses of a guinea-pig ileum preparation to histamine (Hist, at black dots) before and after the addition of three different doses of stomach extract (corresponding to 0.1, 0.2 and 0.5 g of original tissue) at the arrows (Ex). Intervals of 3 min elapsed between administrations of histamine. In each instance the drum was temporarily stopped after washing out the organ-bath and restarted 20 sec before the next test dose. The contact times were 20 sec for histamine and 2 min for the extracts.

starting with the minimal effective dose. The addition of the extract to the organ-bath in a dose corresponding to 0.1 g of stomach produced about a 30% reduction in the contractions due to 0.5 μg of histamine. When a dose, corresponding to 0.2 g of stomach, was added to the preparation, it produced about a 50% reduction and, after the addition of the extract in a dose corresponding to 0.5 g of stomach, there was almost complete inhibition.

When tested against contractions induced by either 5-hydroxytryptamine or bradykinin and the Schultz-Dale reaction, the efficacy of a tissue extract was the same as against histamine, but it only slightly reduced the contractions induced by acetylcholine.

Fig. 3 illustrates the effect of tissue extracts on contractions of the rat uterus preparation induced by 5-hydroxytryptamine. The addition of an extract corresponding to 0.8 g of colon nearly completely inhibited the contractions elicited by 0.5 $\mu$g of 5-hydroxytryptamine. After repeated washing, the sensitivity of the preparation returned almost to that of the initial control.

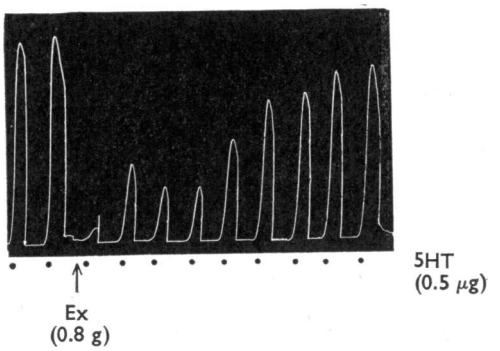


Fig. 3. Responses of a rat uterus preparation to 5-hydroxytryptamine (5HT, at black dots) before and after the addition of an extract of colon (corresponding to 0.8 g of original tissue) at the arrow (Ex). Intervals of 5 min elapsed between administrations of 5-hydroxytryptamine. In each instance the drum was temporarily stopped after washing out the organ-bath and restarted 20 sec before the next test dose. The contact times were 90 sec for 5-hydroxytryptamine and 3 min for the extract.

The activity of tissue extracts in inhibiting contractions of the guinea-pig ileum induced by bradykinin is shown in Fig. 4. After the addition of an extract corresponding to 0.8 g of colon, the preparation did not respond to a subsequent dose

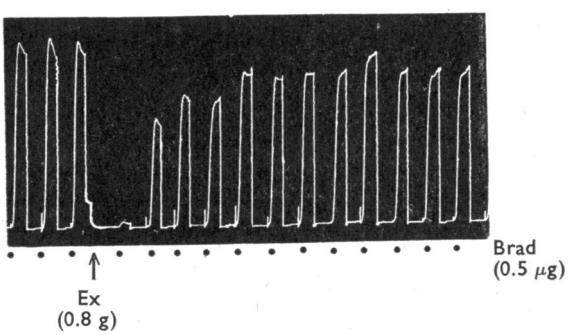


Fig. 4. The responses of a guinea-pig ileum preparation to bradykinin (Brad, at black dots) before and after the addition of an extract of colon (corresponding to 0.8 g of original tissue) at the arrow (Ex). Intervals of 4 min elapsed between administrations of bradykinin. In each instance the drum was temporarily stopped after washing out the organ-bath and restarted 20 sec before the next test dose. The contact times were 40 sec for bradykinin and 3 min for the extract.

of 0.5 $\mu$g of bradykinin. Fig. 4 also shows that the initial sensitivity of the gut to bradykinin could be restored much faster than with histamine or 5-hydroxytryptamine.

Fig. 5 shows that the tissue extracts can also inhibit the Schultz-Dale reaction. Fig. 5A illustrates the typical reaction elicited by the addition of 0.1 ml. of a 10% solution of egg white to the organ-bath, while Fig. 5B illustrates the response of

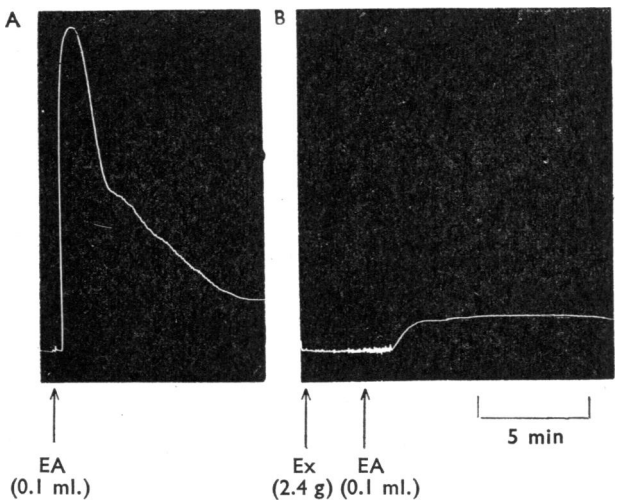


Fig. 5. Effect of an extract of liver on the Schultz-Dale reaction. A shows the response of a sensitized guinea-pig ileum preparation to the addition of the challenging agent (0.1 ml. of 10% egg white solution) at the first arrow (EA). B shows the response of a similar strip from the same animal; a tissue extract (corresponding to 2.4 g of liver) was added to the organ-bath at the second arrow (Ex) 6 min before the addition of the challenging agent (0.1 ml. of 10% egg white solution) at the third arrow (EA). The time scale refers to the two contractions due to egg white.

a similar piece of ileum from the same animal when a tissue extract corresponding to 2.4 g of liver had been added to the bath 6 min before the addition of egg white.

These tissue extracts furthermore abolished contractions induced by histamine without significantly reducing the responses to acetylcholine (Fig. 6). As can be seen, when an extract corresponding to 2.0 g of stomach was added to the bath, the preparation did not respond to a subsequent dose of 0.2 $\mu$g of histamine but the contraction elicited by 0.1 $\mu$g of acetylcholine was only slightly reduced.

*The distribution of the active principle (or principles) in human tissues*

Since the chemical nature of the active principle (or principles) in human tissue extracts is not yet known, it was necessary to establish some arbitrary method by which the activity of the extracts, obtained from different tissues, could be compared and expressed. Under the standard conditions, described in Methods, the sensitivity of the ileum preparation to histamine showed surprisingly small variations and the size of the contractions due to 0.2 to 0.5 $\mu$g of histamine was practically equal in each experiment (6 to 8 cm on the kymograph). Using this response as a basis, the

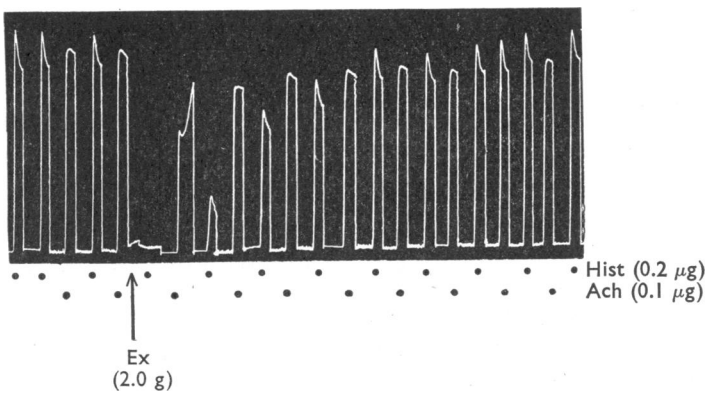


Fig. 6. Responses of a guinea-pig ileum preparation to histamine (Hist) and acetylcholine (Ach) before and after the addition of an extract of stomach (corresponding to 2.0 g of tissue) at the arrow (Ex). Intervals of 3 min elapsed between administrations of drugs which are marked by the black dots. In each instance the drum was temporarily stopped after washing out the organ-bath and restarted 20 sec before the next test dose. The contact times were 20 sec for histamine and acetylcholine and 2 min for the extract.

amount of tissue, expressed in terms of its wet weight, capable of completely suppressing such a contraction was determined and the activity contained in this is referred to as one unit.

The amount of the active principle varied greatly, but it was found in all tissues investigated. Table 1 summarizes the results obtained in 72 experiments. The first column gives the number of specimens of each tissue tested. The average amount of

TABLE 1

THE AMOUNTS OF HUMAN TISSUES (IN G/WET WEIGHT) CONTAINING ONE UNIT OF ACTIVITY OF THE ACTIVE PRINCIPLE(S)

One unit is the amount required to inhibit completely the response to a standard dose of histamine

| Tissue | No. of extracts tested | Weight of tissue (g) | |
|---|---|---|---|
| | | Mean | Range (highest/lowest) |
| Brain (cerebellum) | 2 | 0·6 | 0·5/0·7 |
| Brain (frontal cortex) | 2 | 3·2 | 2·4/4·0 |
| Thymus | 2 | 0·71 | 0·09/1·4 |
| Ovary | 1 | 3·4 | — |
| Thyroid | 1 | 3·6 | — |
| Skeletal muscle | 1 | 4·0 | — |
| Lung | 5 | 3·16 | 1·2/7·4 |
| Stomach | 27 | 5·9 | 0·5/18·0 |
| Large intestine | 21 | 6·0 | 0·5/18·0 |
| Spleen | 2 | 4·3 | 0·6/8·0 |
| Skin | 5 | 8·4 | 2·4/16·0 |
| Bone marrow | 1 | 5·6 | — |
| Liver | 2 | 1·4 | 0·8/2·0 |

tissue (expressed as g wet weight) containing one unit of activity is shown in the second column. The third column shows the highest and lowest values found in each kind of tissue obtained from different individuals.

## DISCUSSION

The results of these experiments not only confirm the presence of a naturally occurring antihistamine-like substance (or substances) in human tissues, but they also show its wide range of activity and its wide distribution in the human body. The quantity of this substance(s) varies in different organs and there are large variations in the concentrations found in tissues from different persons. This variation could be due in part to pathological changes. Although the utmost care was taken to use only normal tissue as indicated by macroscopic and microscopic examination, the likelihood that there were chemical changes which were not apparent visually is supported by the finding that, in certain diseases, the amount of active principle(s) may be increased or decreased five- to twenty-fold compared with normal tissues (Pelletier, Kovacs & Rose, unpublished).

The active principle(s) in human tissues shows the same wide range of activity in preventing the *in vitro* effects of histamine, 5-hydroxytryptamine and bradykinin, and the Schultz-Dale reaction as that in normal human urine extracts. Thus the substance(s) in tissues and urine are very similar and most probably identical. It has been recently shown (Kovacs & Melville, 1963) that urine extracts injected into guinea-pigs strongly protect against a lethal aerosol of histamine, and they also prevent capillary permeability effects both of histamine and of bradykinin.

From the present results, no definite conclusion can be drawn about the chemical nature of the active principle(s) in human tissue extracts. It was found, however, that the activity of the extracts rapidly decreased when they were left in contact with air. This effect was particularly conspicuous if the extracts were dissolved in Tyrode solution. Consequently, all extracts were kept in dried form under argon at $-10°$ C. Under these conditions, the activity remained unchanged for several months.

At present we do not know what the possible physiological or pharmacological role and importance of the active principle(s) in human tissues may be. Its wide range of activity and the fact that it has been found in every tissue so far investigated suggests the possibility that it is a form of tissue hormone which in conjunction with other systems such as diamine and monoamine oxidases and the sympathetic activators tends to balance the effects of histamine, 5-hydroxytryptamine or bradykinin.

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