LXXI. THE ISOLATION OF HISTAMINE FROM THE HEART.

BY WILLIAM VEALE THORPE.

*From the Physiological Department, The University,
Edmund Street, Birmingham.*

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IN connection with some problems on the physiology of the heart under investigation in this laboratory, the question arose as to whether the readily extractible depressor substance was the base histamine.

It is well known that simple aqueous or alcoholic extracts of almost any tissue cause a fall in blood-pressure on injection into the circulation of the cat. The heart is not an exception. In fact, it yields an extract with powerful depressor activity.

The marked similarity in the physiological behaviour of tissue extracts and of solutions of histamine gave rise to the view that histamine was present in these extracts, a view which has been confirmed recently by the isolation of pure histamine, in the form of its dipicrate, from extracts of liver and lung [Best, Dale, Dudley and Thorpe, 1927], of muscle [Thorpe, 1928], and of spleen [Dale and Dudley, 1929]. It therefore seemed reasonable to expect that the depressor substance of heart extracts would be histamine. This expectation has, in fact, been realised.

Apart from giving additional confirmation to the view that extracts of most tissues contain histamine, proof of the presence of histamine in heart extracts is of significance in connection with recent work on the so-called "heart hormone." Many workers have observed that heart extracts have a stimulating action on the frog's and rabbit's heart but there is much disagreement as to the nature of the substance responsible for these effects. Some workers [Demoor, 1924; Haberlandt, 1927] are of the opinion that the substance is formed in certain regions of the heart and is a specific heart hormone. Others, however [Rigler and Tieman, 1927; Rigler, 1929; Oppenheimer, 1929], deny the specificity of this substance, and have obtained extracts producing similar effects not only from any region of the heart but also from a variety of other tissues. Further, they have produced similar effects with pure histamine alone. Rigler, in fact, on physiological grounds puts forward the view that the substance is actually histamine. A full discussion of the work in this field is beyond the scope of this paper. The main features are briefly but effectively summarised by Oppenheimer [1929], who gives references to the more important papers.

The experiments described in the present communication prove that histamine is present in extracts of ox ventricles. Further, these extracts appear to contain relatively large quantities of histamine. In view of the presence of this large amount of histamine in heart extracts, it seems probable that the cardiac stimulating effect of heart extract may be due wholly or in part to histamine, and it is clear that unless histamine is removed from such extracts no conclusion can be drawn with regard to the presence of a specific heart hormone. In the absence of evidence for a specific heart hormone, chemical proof of the presence of histamine in heart extract tends to support Rigler's view.

The vasodilator activity of different tissue extracts varies considerably [Thorpe, 1928]. Lung extracts are particularly rich in histamine compared to other tissue extracts. Heart extract was found to have activity of a high order, being second only to lung extract.

Extracts prepared from four ox hearts (1.3, 1.5, 1.2, 1.7 kg.) gave values equivalent to 9.4, 18, 9.6, 8.1 mg. histamine per kg. of fresh tissue respectively when estimated by comparison with pure histamine on the blood-pressure of the cat under chloralose. The action was all of a histamine-like type and there was neither chemical nor physiological evidence to suggest the presence of a choline-like substance.

The above values apply to the ventricles of the heart. In two cases the auricles were worked up giving the interesting result that the activity, weight for weight of fresh tissue, was approximately double that of the ventricles of the heart in question. Auricle extracts therefore have nearly the same order of activity as lung extracts (40 mg. per kg.).

The technique employed for the isolation of histamine from heart extract was similar to that employed previously, alcohol being used for the extraction. In the final stages difficulties arose similar to those encountered in dealing with muscle extracts [Thorpe, 1928]. Heart muscle contains considerable quantities of creatine which during the silver fractionation is converted to creatinine and methylguanidine. The presence of these substances made the isolation of histamine picrate very difficult. The total amount of bases in the histidine and arginine fractions was small and it was found that the alcohol extraction of these could be omitted.

With regard to the freshness of the material, the hearts were obtained in the laboratory within half an hour of the animals' death at the slaughter house and the same precautions were taken to ensure sterility as in previous work.

EXPERIMENTAL.

The ventricles from a fresh ox heart (1.5 kg.) were minced and extracted for 24 hours in 3.5 l. 96 % alcohol, the extract was filtered off in a press and the meat re-extracted with a further 3 l. 60 % alcohol. The combined extracts, after addition of sulphuric acid (0.36 cc. 50 % per l.) which produced a slight precipitate, were filtered and concentrated *in vacuo* below 30° to about 250 cc.

After removal of fat by shaking with ether, the fat-free aqueous layer was concentrated to 160 cc. After removal of 2.5 g. creatine which separated on standing, the liquid was neutralised with NaOH and treated with basic lead acetate until no further precipitate was produced. The filtrate and washings from this precipitate, after removal of lead as sulphide, were concentrated *in vacuo* to 100 cc. when a further 1.65 g. creatine separated.

After removal of creatine, 50 % sulphuric acid was added to produce a concentration of 5 %. Phosphotungstic acid (25 % in 5 % sulphuric acid) was then added until precipitation was complete, 400 cc. being required. The precipitate was decomposed in the cold with baryta in the usual way, excess of baryta being removed as sulphate. The solution of bases was concentrated to 100 cc. and fractionated by the Kossel-Kutscher method. 15 cc. 40 % silver nitrate were added and the resulting precipitate ("purine" fraction) removed. Hot saturated baryta was then added cautiously until the white precipitate was just tinged with brown. The precipitate ("histidine" fraction) was filtered off. Saturation of the filtrate with baryta precipitated the "arginine" fraction. The filtrate from this precipitate forms the "lysine" fraction.

The purine and lysine fractions had no appreciable activity. Silver was removed from the histidine and arginine fractions by suspending in sufficient dilute sulphuric acid to render the liquid just acid to Congo red and treating with hydrogen sulphide. After removal of silver sulphide excess of sulphuric acid was removed by baryta and the solution of the bases from each fraction evaporated to dryness *in vacuo*.

A biological assay of the solutions before evaporation had indicated that the depressor activity of the histidine and arginine fractions was equivalent to 3.2 and 4.8 mg. histamine respectively.

The histidine fraction was treated with picric acid which immediately gave a crystalline precipitate. After a long fractionation of the picrate 0.25 g. potassium creatinine picrate and 0.1 g. creatinine picrate were separated in pure condition. The residues showed a powerful histamine-like activity but it was not found possible to isolate histamine picrate in a pure state from the creatinine picrate still remaining.

The arginine fraction contained relatively little material and was also converted to picrate. This picrate (180 mg.) was subjected to a long fractional crystallisation similar to that employed when dealing with muscle extract. The mixture consisted mainly of the picrates of methylguanidine and histamine and finally after resorting to mechanical separation of the crystals a total of 5 mg. pure histamine dipicrate was obtained. At the same time 50 mg. methylguanidine picrate were obtained.

A solution of 3.2 mg. of this histamine dipicrate in 100 cc. water had the same depressor effect on the cat's blood-pressure as a similar solution prepared from a known sample of pure histamine dipicrate. The Pauly reactions were quantitatively equal. The picrate from heart, pure histamine dipicrate and a mixture of the two all melted together at 240° with decomposition.

The yield of histamine from heart.

Throughout the purification samples were taken at the end of each stage for assaying on the cat's blood-pressure in comparison with pure histamine. The values obtained are given in Table I.

Table I.

Stage	Activity in mg. histamine
Crude extract	27
After basic lead acetate	22
Phosphotungstate	14
Purine fraction	— 0.3
Histidine fraction	— 3.2
Arginine fraction	4.8
Lysine fraction	— 0.0
Histamine isolated as pure picrate	1.0

Although rather better than that from muscle, the yield of pure histamine from heart extract is poor. But if the yields are considered at the end of each stage in the purification the losses in the earlier stages are reasonable, and comparable with those obtained in experiments with other tissues, only the loss in the silver fractionations being rather larger than usual (41 % instead of about 25 %). The yield from the picrate stage is certainly low, but it must be remembered that the experiment was performed on a very small scale. Consequently, since sufficient volume of liquid had to be left for manipulation, relatively less creatine could be removed by crystallisation in the early stages than in large-scale experiments such as those with muscle. Thus a relatively greater interference by creatinine and methylguanidine is to be expected in the picrate stage.

The physiological assays show a continuous fall in activity throughout the purification, a fall which would be expected if histamine was adsorbed by the relatively large precipitates removed during the manipulation. The losses are of the same order as in previous experiments with other tissues, and it would appear that the losses in activity are due to the removal of histamine and not to the removal of another depressor substance. This is supported by the fact that the biological assays gave no hint of the presence of any substance other than one closely resembling histamine, so that it seems justifiable to conclude that the depressor substance of heart extract prepared as described in this paper is the base histamine.

SUMMARY.

- (1) Extracts of ox ventricles show a powerful depressor activity corresponding to 8 to 18 mg. histamine per kg. assayed biologically.
- (2) Pure histamine, in the form of its dipicrate, has been isolated from such an extract and it is suggested that this substance is responsible for the depressor activity.
- (3) The significance of these findings in relation to recent work on the so-called heart hormone is discussed.

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