CLXXXVIII. THE QUANTITATIVE EXTRACTION OF HISTAMINE FROM TISSUES BY ELECTRODIALYSIS.

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DURING an investigation involving a large number of histamine assays, it occurred to us that the chemical manipulation could be greatly simplified if the base could be extracted by electrodialysis. A further advantage of the method would be the elimination of all but basic substances of low molecular weight from the extract. In the method we had been using [Best *et al.*, 1927] the tissue is extracted with 96 % alcohol for 24 hours, the alcohol removed and the meat re-extracted with 60 % alcohol for a further 24 hours. The combined extracts are concentrated *in vacuo* and fat removed by extraction with ether. The extracts so prepared were usually yellow and often slightly cloudy, although free from biuret products.

By electrodialysis in a three compartment cell a water-clear colourless extract is obtained in less than 2 hours after receipt of the tissue: this means a great saving of time when many samples have to be worked up. In a large number of experiments during the last 3 years we have never found an electrodialysate giving a smaller histamine value than an equivalent dose of an extract prepared by the alcohol method. (The histamine value of the extracts was obtained by matching against a standard of pure histamine on the blood pressure of an anaesthetised cat.) Further, histamine added to blood, which contains no histamine, has been repeatedly extracted without loss. Histamine added to tissues which contain histamine has also been recovered without detectable loss.

The process of electrodialysis does not subject the tissues in the middle compartment to any drastic conditions. The temperature does not rise above 40°, and there is no great change in the p_H of the liquid in the middle compartment. The method therefore supports the alcohol method not only quantitatively but also in the suggestion that the histamine in the tissues is, if not free, very loosely combined.

On a few occasions we have also extracted a portion of the tissue by the more drastic method of Best and McHenry [1930], in which the tissue is heated with 10 % HCl at 95° for 1 hour and then evaporated to dryness *in vacuo*; alcohol is added and distilled off to remove some of the acid, and the residue is taken up in water, neutralised and filtered. These extracts, which are clear but dark brown in colour, usually give a value about 30 % higher than extracts prepared by either of the above methods. In view of the agreement between the alcohol and dialysis methods and our quantitative recovery of added histamine, we feel that it is highly significant that extracts prepared by the Best and McHenry method give a strong biuret and several other protein reactions

not given by the alcohol extracts or electrolysates of the same tissue; especially since we have observed an increased histamine value when a membrane passed biuret products.

It might be argued that the lower value obtained by the dialysis is due to loss of histamine by autolysis [Best, 1929] in the dialysis cell, whilst the lower value by the alcohol method is due to incomplete extraction. Apart from the coincidence of the errors, it should be noted that the time for which the tissue is in the region of 37° rarely exceeds half an hour. This short time would not be sufficient, judging from Best's curve, to cause any perceptible loss in histamine. Again, Best *et al.* [1927] were unable to detect any difference in the histamine content of a dog's lungs worked up immediately after removal from the animal or after keeping 2 hours; and MacGregor and Peat [1931] found no change in the histamine in a lung mince which had stood overnight.

EXPERIMENTAL.

Description of apparatus.

Cells. The cells used for these experiments were, with one exception, made from wood to the pattern of those of Foster and Schmidt [1923]. Small cells of 100 cc. capacity in each compartment were carved from solid blocks of well-seasoned beech or Oregon pine. Larger cells of 250 cc. capacity (Foster and Schmidt size) were built up from pieces of thick wood. The wood was well impregnated with high-melting paraffin wax. The waxing is of some importance since a badly waxed cell often gives up during dialysis an alkali-soluble substance giving a strong Pauly reaction. This substance is insoluble in neutral or acid solution. Still larger cells used in some of the preliminary experiments were made from three glass museum jars, faces $7" \times 5\frac{1}{2}"$, with $3\frac{1}{2}"$ holes cut in the appropriate faces. The capacity of each compartment varied from 600 cc. to 1500 cc. according to the width of the jars used. For the wooden cells the gaskets were made of ordinary red rubber sheet $\frac{1}{16}"$ thick. Owing to their fragility the glass cells required special soft rubber gaskets $\frac{3}{8}"$ thick. These were specially made for us by Messrs C. Macintosh and Co., Ltd., Manchester.

Electrodes. The cathodes were made from pure nickel sheet slightly smaller than the cross section of the cell. The anodes were made from one or two $6" \times 2" \times \frac{1}{4}"$ carbon plates according to the size of the cell.

Membranes. In the experiments with lung and blood ordinary parchment dialysing-paper or cellophane sheets were found quite satisfactory. With liver and heart, especially the former, these membranes passed traces of "biuret" products, and the dialysates gave histamine values perceptibly higher than those obtained from the corresponding alcohol extracts. The passage of such substances is prevented by using cathode membranes of collodion. We found it simpler to make sacs by the inside tube method in $10" \times 1\frac{1}{2}"$ test-tubes than to make sheets direct by the plate or mercury method. The sacs were then cut up into sheets and kept in distilled water until required. 14 % pyroxylin in equal parts (by weight) of ether and absolute alcohol [Walpole, 1915] gave sufficiently robust membranes.

Stirring. The liquid in the middle compartment was kept stirred mechanically to prevent any local aggregation of mince. Some tissues, especially heart muscle, tend to form a layer on the cathode membrane which increases somewhat the electrical resistance of the cell and thus slightly increases the time required for dialysis. This layer was broken up from time to time by a glass rod.

Current. The cell was connected to 230 volt D.C. mains in series with a resistance of a bank of 4 carbon filament lamps in parallel. Each lamp passed about 1 amp. and could be switched into or out of the circuit as required. For the smaller cells 2 or 3 lamps were generally sufficient. The mean consumption of the 100 cc. cells was about 50 watt-hours, and of the 250 cc. cell about 120 watt-hours.

Temperature. The temperature was kept down by providing each compartment with glass cooling coils supplied with a rapid stream of cold water. In this way the temperature even in the smallest cell could be kept below 40° when the current passing was 1 amp. The temperature only reached this value while a heavy current was passing. This rarely occurred for more than half an hour. A typical curve showing the current and temperature changes is given in Fig. 1.

p_H . The p_H change in the middle compartment was not sufficient to warrant alteration, e.g. by addition of baryta. With tissues, the p_H in the middle compartment, which is initially just above 7, usually falls to about 4.6. We have never observed a p_H more acid than this. With blood, the p_H does not usually fall below 6.

End-point. Theoretically, when the resistance of the cell has attained a steady value and the titratable alkalinity is constant, all the histamine should have passed to the cathode chamber. In view, however, of the difficulty of accurate current measurement without special instruments and the minute amount of histamine present relative to other electrolytes, we found it more reliable to use the Pauly reaction as an indication of the completion of the dialysis. Samples were withdrawn from the cathode chamber at 15 minute intervals until two successive samples gave the same colour. For this purpose we have adapted Gebauer-Fülnegg's [1930] modification of the reaction as a quantitative method. 1 cc. of test solution is mixed with 1 cc. $N/2$ Na_2CO_3 and to this are added 2 cc. of fresh diazo-reagent prepared by mixing 1 cc. of 0.125 % *p*-nitraniline in $N/10$ HCl with 1 cc. 0.37 % $NaNO_2$. The colour gradually deepens and then fades, finally becoming cloudy. The maximum colour intensity, which usually appears in about 1 minute, is recorded in red and yellow units in a Rosenheim and Schuster [1927] colorimeter. Concentrations from 1/33,000 to 1/300,000 can be read off directly without dilution. Plotting colorimeter red units against histamine concentration gives an approximate straight line over this range. The method gives consistent results and for our purpose is quicker and more convenient than the Koessler and Hanke [1919] method which we used in our early experiments. But it must be stressed that this, like other adaptations of the Pauly reaction, is not specific for histamine and cannot be used for the estimation of the base in tissue extracts. In every case our Pauly values have been higher than would be expected from the blood pressure assay. The reaction could be used as an indicator for the extraction from tissues of any alkali-stable base which passes to the cathode with the same ease as histamine.

Since the convenience of this end-point depends upon the use of a Rosenheim and Schuster colorimeter, we have studied the current relations throughout the experiments with a view to giving an alternative method for determination of the end-point. Measurements of the voltage across the cell and amperage of the circuit were noted every 15 minutes. From the area of a watt-time curve drawn from these data the total consumption in watts was obtained. In 60 experiments with 4 different cells we have found the ratio WT/ad to be approximately constant for a given tissue. T is the time taken to attain a constant

Pauly value, a the area of the cathode in cm. and d the distance in cm. of the cathode from the centre of the middle compartment. The mean values of this ratio are given in Table I. (In only 5 cases were the values more than ± 0.1 from the mean.) As would be expected these values are roughly proportional to the mean values for the titratable alkalinity of the cathode liquids. The latter values, expressed as cc. N acid per 100 cc. of dialysate, and the weight of tissue corresponding to this volume are also included in the table.

Table I.

Tissue	$\frac{WT}{ad}$	Titratable alkalinity cc. N acid	Weight of tissue g.	Number of experiments
Blood	0.63	3.9	30	15
Heart-muscle	0.49*	2.6	20	18
Liver	0.43	2.6	20	11
Lung	0.53	3.3	20	16

* This value is probably relatively high, since with heart-muscle there was a much greater accumulation of tissue on the cathode membrane than with any other tissues examined.

Since we had no indication in any experiment that prolonging the dialysis caused any loss of histamine, it should be safe to assume the complete passage of any equally stable and dialysable base when sufficient current has been passed to satisfy the equation $WT/ad = 0.6$ for the tissues or $WT/ad = 0.7$ for blood.

Method.

The cell having been assembled, the cathode and anode compartments are filled to capacity, say 250 cc., with distilled water. The middle compartment is filled with 200 cc. distilled water and 50 g. of the minced tissue. It is not necessary to add an electrolyte to the distilled water. After turning on the cooling water and starting the stirrer, the current is switched on. If the small cells are used, the current and temperature in the middle compartment, which rise rapidly at first, fall after about 1 hour. The end-point is then determined as already described (p. 1396). The cathode liquid is then removed and the chamber washed out. The combined liquid and washings after neutralisation with $N H_2SO_4$ and adjustment to appropriate volume are ready for the physiological assay.

Physiological assay. The extracts were compared as to their depressor effect when injected into the femoral vein of an anaesthetised cat. At least two definite matches were obtained before the pairs of extracts were recorded as equal. Histamine values were obtained by matching against a standard histamine solution [Best *et al.*, 1927; Burn, 1928]. The cats were anaesthetised with medinal (0.42 g. per kg. body weight) after induction with ether.

RESULTS.

Comparison of alcohol and electrodialysis methods. Three histamine-containing tissues were examined. In all the experiments the tissue was minced and well mixed. Samples were then worked up by the dialysis method and the alcohol method and assayed physiologically. There was no detectable difference. These experiments are summarised in Table II, which also shows the extreme histamine values for each tissue.

Table II.

Tissue	Source	Number of experiments	Extreme histamine values in mg. per kg.
Heart (ventricular muscle)	Ox	8	8-25
Liver	Dog	2	13-40
	Ox	2	10-20
	Pig	2	13
	Sheep	3	2.5-6
Lung	Dog	3	10-13
	Ox	11	30-60

In the case of the liver which contains choliné, the dialysates and the alcohol extracts were equidepressor both before and after injection of atropine into the cat, indicating, as would be expected, that choline passes to the cathode like histamine.

Fig. 1. Electrodialysis of 20 g. lung in 100 cc. cell.

- × ——— × Histamine values of cathode liquid.
 ○ ——— ○ Pauly values of cathode liquid.
 Δ ——— Δ Titratable alkalinity values of cathode liquid.
 ● ——— ● Temperature of mixture in centre compartment.
 + ——— + Watt values.

Reliability of end-point. In nine experiments samples were withdrawn at 15 minute intervals, neutralised and injected into an anaesthetised cat. The results from one of these experiments are shown in Fig. 1. Similar curves were obtained in the other experiments. They showed that:

1. The Pauly value never attained a maximum before the histamine concentration.
2. The titratable alkalinity value usually reached a maximum before the histamine concentration.
3. The current usually but not always reached a minimum after the histamine concentration became maximum.

Thus the Pauly reaction was the only reliable method of the three for determining the end-point.

Recovery of added histamine.

1. *From histamine-free material.* A known volume of histamine solution was added to a 30 % blood solution and electro dialysed. The same volume of histamine was added to an appropriate volume of water. The dialysate was then

concentrated *in vacuo* to this volume, and the two solutions were sterilised and preserved until compared on the cat's blood pressure. The amounts of histamine added were such as to give a similar concentration in the cell to that given when a tissue was being dialysed, *i.e.* 2, 1 or 0.5 mg. were added to the 250 cc. cell and 0.8, 0.4 or 0.2 mg. to the 100 cc. cell. These amounts correspond to tissues containing 40, 20 and 10 mg./kg. respectively. In nine experiments the base was recovered quantitatively so far as could be detected by the physiological assay. It has already been stated in a preliminary communication [MacGregor and Thorpe, 1933] that a large amount of histamine (0.06 g.) added to blood was easily recovered in the form of its picrate in good yield.

2. *From tissues containing histamine.* Failure to recover completely histamine which has been added to tissues has often been reported. These experiments have been carried out by adding histamine to one portion of the tissue and working up another portion without added histamine. The difference between the two extracts is then estimated by matching them against a standard histamine solution on the cat's blood pressure, and hence the amount of added histamine is calculated. The method, especially when the differences are great, is liable to big errors owing to the large multiples involved in the calculations. In our experience the stronger solutions tend to be underestimated. Being of the opinion that this was largely responsible for the failures to recover added histamine, we planned our experiments so as to use equipotent solutions and to eliminate all calculations.

A known volume of a histamine solution was added to a portion of the tissue and the mixture dialysed. An equal portion of the same tissue was dialysed in another cell and the same volume of histamine added to the dialysate after removal from the cell. The two dialysates were then neutralised, adjusted to the same volumes and equal doses compared on the cat's blood pressure. In five experiments no difference could be detected between the two solutions. The histamine had been recovered quantitatively within the limits of the physiological assay.

SUMMARY.

1. A rapid method for the quantitative extraction of histamine from tissues by electro dialysis is described.
2. The method gives results in complete agreement with the alcohol method.
3. Added histamine is recovered quantitatively within the limits of the physiological assay.

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