action of amidine derivatives *in vitro*, the diamidines being more active than the monoamidines.

Many of the diamidines studied in this paper cause an increase in the blood histamine concentration (MacIntosh & Paton, 1949), due to the release of histamine from the tissues. Some of the compounds which were particularly active as inhibitors of histaminase in our experiments were also highly active in MacIntosh & Paton's experiments. There is no general parallelism between antihistaminase activity and histamine release, but in view of the observations here reported the possibility must be considered that some of these substances exert an antihistaminase effect also in the living animal.

SUMMARY

1. The inhibitory action of amidine derivatives on the histaminase of pig kidney has been studied.
2. Diamidines, diguanidines and diisothiurea derivatives have an inhibitory action.
3. Stilbamidine and dimethylstilbamidine are strong inhibitors of the enzyme.
4. The relation between chain length and inhibition has been studied and the results are discussed.

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A New Type of Preparative Electrophoresis Cell

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The electrophoresis apparatus devised by Tiselius (1937) is now available in many laboratories. It is predominantly used for analytical work, but can also be used for preparing electrophoretically pure materials in small quantities by virtue of the fact that after the migration of the various components of a mixture has taken place for a time, the fastest and slowest may be isolated by lateral displacement of the cell sections, the boundaries having been compensated to suitable positions. The disadvantages of this method are twofold. In the first place, at the conclusion of one such separation the whole apparatus must be taken out of the refrigerated bath, and at least partially dismantled in order that the desired material may be removed, and if more material is required, the apparatus must be re-assembled, refilled, and replaced in the bath before another run can be started. In the second place, when removing components obtained in this way, it is difficult to avoid dilution with the buffer which fills the remainder of the cell compartment. These difficulties have to a certain extent been overcome by a semi-continuous procedure developed by MacLagan & Bunn (1947) with the insertion of capillary tubes inside the channel of the long centre

section Tiselius U-tube, but the method is not entirely satisfactory to operate.

EXPERIMENTAL

Description of cell. The type of cell described here enables both the disadvantages mentioned to be overcome, while still using the standard auxiliary equipment belonging to the analytical cell. In principle it is based on the type of cell first described by Svensson (1942) and later simplified and modified by him (Svensson, 1946). Fig. 1 shows that the cell is identical with the standard Tiselius model with the long centre section, except for the fact that five capillary openings are provided, two at the top of either arm of the cell, two at the bottom, and one in the base section which joins the two arms. These openings allow material to be introduced into and withdrawn from the cell, while the apparatus as a whole remains in the refrigerated bath. In order that this should be possible, arms of capillary tubing are attached to the outlets either by rubber sleeves or by ground-glass joints, and by means of taps in these tubes the flow of liquid into and out of the cell may be controlled. The formation of protein-buffer boundaries in this cell takes place by flow of liquid, the

protein solution being introduced to the cell through the opening in the base section, rising in either arm stabilized by density difference, until it reaches the opening in the cell which is acting as an outlet. The boundary may be sharpened by techniques described below.

Fig. 1. Longitudinal section of the cell components.

Fig. 2 shows the cell assembled with the side arms in position. The sections are squeezed together after greasing the ground-glass surfaces as in assembling the normal cell. Since no lateral movement of the section takes place once the assembly is completed, a rather more viscous grease can be used than with the analytical cell; ordinary vaseline has proved satisfactory. This is of advantage in preserving a water-tight seal when the apparatus is subjected to mild internal hydrostatic pressure in the course of a separation. For the same reason, the downward pressure provided by the metal spring strips on top of the cell should be greater than with the analytical model. The capillary side arms, if attached by means

of rubber sleeves as shown here, should be pressed firmly in place so that the flattened end of the arm is flush with the end of the projection on the cell, in order to reduce the volume of the liquid contained in the arm to a minimum. The bore of the tubing used should also be as small as possible; a bore of 1 mm. has been found satisfactory.

It will be seen that the stand in which the cell is assembled differs from the stand for the analytical cell in that the pneumatic pumps, or alternative devices by which lateral movement of the standard cell sections is effected, are replaced by metal plates which hold this cell firmly in position when it has been assembled. These plates are removable, and are adjusted by means of a bolt and wing nut projecting through a slot in the base of the stand; they also supply some support and alignment for the capillary side arms. It is with respect to this stand only that the accessories for this cell differ from those of the normal analytical model.

The electrode vessels are attached to the upper part of the cell in the usual manner (Fig. 3). The Ag/AgCl electrodes are fitted through rubber bungs so that the apparatus can be sealed, tubes being provided through which saturated KCl may be introduced to surround the electrodes. Air vents with taps are also provided in each bung to allow all the air in each vessel to be readily displaced. The two inlets to the connecting arms of the electrode vessels, through which hydrostatic equilibration occurs in the analytical apparatus, are connected by a Y-piece to a container from which buffer may be introduced into either arm of the cell at will.

Operation of cell. In use, the apparatus is first assembled and filled with the buffer in which the protein separation is to be carried out. The bulk of the filling may be done through the open top of an electrode vessel, but the final filling is best done through the capillary leading to the base of the cell, in order to remove any bubbles from the U-tube. The side arms and the air vents on the electrode vessels are then opened in turn and washed through to ensure that no air remains in the apparatus. When filling is complete, all the outlets are closed and a hydrostatic pressure of 20–30 cm. buffer imposed on the system through the capillary to the base of the cell for 2–3 min. to reveal any leaks in the rubber connexions or ground-glass surfaces. If no leaks occur, the pressure is removed and the apparatus placed in the refrigerated bath at 0.2–0.5°. One of the air vents in the top of the electrode vessels is opened to allow for contraction of the buffer. After 30–45 min. 25 ml. saturated KCl, precooled to 0.2–0.5°, is run in to surround each electrode through the tubes provided, the displaced buffer first filling the air vent that was opened to allow for thermal contraction, and later flowing out through any one of the side arms of the cell opened for the purpose.

The apparatus, now in thermal equilibrium with the bath, is again sealed.

The protein-buffer boundaries are formed for the start of the electrophoresis run by introducing the protein solution through the inflow capillary to the base of the cell, and displacing buffer through the appropriate side arm. In general, when carrying out operations with this cell, it is advisable to open the outflow point before the inflow, and to close the inflow point before the outflow; this prevents a static pressure building up in the apparatus which, because of a slight elasticity in the system, may give rise to unwanted boundary displacements when released.

A container such as a graduated pipette is attached to the inflow capillary; one of the side arms to the lower outlets is opened, and the tap in the inflow tube is gently opened until the displaced buffer emerges from the side arm at a rate of about one drop every 2 sec. When 3–4 ml. solution have run in, a very diffuse boundary rises from the bottom of the tube towards the open lower outlet. When this outlet is reached, the boundary sharpens until the diffuse region in which the protein solution and buffer are mixed is displaced. The inflow is now shut off, and the outflow side arm closed. The same process is now repeated on the other side of the cell, but the side arm to the upper outlet rather than the lower is opened, and the protein, rising from the bottom of the tube, forms a boundary at the upper level.

These two boundaries may be sharpened by allowing buffer to flow down from the pipette attached to the Y-piece into the U-tube and out through the capillary at the level of the boundary concerned. In general, boundaries formed by the flow-through method will initially be more diffuse on one side than on the other, and may be sharpened by a stream of material on the diffuse side, boundaries diffuse at the upper side being sharpened by buffer, and those diffuse at the lower side by protein solution.

In this way, boundaries are produced in the U-tube in the usual positions for the start of an electrophoresis run, one at the top of a limb, and one at the bottom of the opposite limb. Two precautions must be observed. The separated components are removed through the upper outlets at the end of the run, and it is desirable that these should be free from the protein mixture; consequently they must be washed through with buffer as a final step in boundary formation at the upper level. Also, unless the lower side arms are filled with protein solution at the beginning of a run, convections occur when the protein solution in the cell migrates above the level of the outlet. Formation of the lower boundary is thus completed by a flow of protein as a final step.

If the current is turned on, electrophoretic migration now takes place in the usual way. This is allowed to proceed until the front edge of the leading

component in the ascending limb reaches the level of the upper outlet capillary. To enable this level to be observed optically, two small holes are drilled in the slotted metal diaphragm fitted in front of the cell to cut off light other than that passing through the channels of the U-tube. These holes are at the levels of the pairs of outlet capillaries, and allow a thin pencil of light to pass through the optical system at each of these levels. On the plane at which the usual electrophoretic boundary pattern appears, these light beams give rise to two horizontal streaks of light in positions corresponding to the level of each outlet capillary (Fig. 4). These act as markers, and enable the position of the migrating components relative to the outlets to be seen. A mask on the outer bath window makes it possible to cut off these rays of light if a photograph of the boundary pattern is required for analytical purposes. In the model supplied by Messrs Hilger and Watts, the light marker beam device is replaced by the following arrangement. Two lines engraved on a grey screen positioned in the plateholder, are aligned to the capillary openings, the position of the lines being set to pointers attached to the cell mount.

When the run has been completed, the electrophoretic fractions may be removed, the fastest moving components in the ascending limb and the slowest in the descending limb being displaced through the upper side arms by feeding protein solution into the base of the U-tube. To remove the leading components, for example, the tap in the upper side arm on the ascending limb is opened, and the protein inflow adjusted to give a slow displacement of liquid through this arm. The electrophoresis pattern will be seen to move towards the upper marker light, forming a new protein-buffer boundary at this level as the components flow out through the side arm (Fig. 4). This displacement is continued until the leading edge of the peak corresponding to the first undesired component reaches this marker level; the protein inflow is then turned off to stop unwanted material from entering the side arm. During this process the liquid emerging from the arm is collected; the first fraction of this, however, consists of buffer with which the arm was filled before starting the run, so that a volume of liquid equal to that of the side arm (here 1 ml.) is rejected. At the end of this displacement, the side arm contains a part of the desired material; this is obtained by allowing buffer to flow into the U-tube on the ascending side from the pipette attached to the Y-piece until the liquid in the side arm is displaced. This completes the removal of the fractions on the ascending side.

From the descending limb the slower moving components can be removed in the same way through the upper side arm. If the slowest component has travelled an appreciable distance away from the upper marker, it is unnecessary to start collecting

the displaced material until the trailing edge of the boundary has moved up to the side arm. After allowing for the volume of the arm itself as before, the protein fractions can be collected.

On account of the narrow bore of the capillary side arms there is very little mixing of buffer with separated protein components during the sampling procedure. Consequently the pure components are obtained at effectively the same concentration as that with which they occur in the protein mixture.

To continue the separation, new boundaries must be formed. In order to prevent progressive migration of the potassium chloride from the electrode vessels ultimately reaching the U-tube and making further runs impossible, alternate runs are carried out in opposite directions, with reversal of current. Consequently, the initial boundary positions must be inverted, the upper boundary being formed in the limb that was previously the ascending and vice versa. The lower boundary is formed by washing residual boundaries through the lower outlet with buffer from above, and the upper by washing out through the upper outlet with protein solution from below. They are sharpened as described above, and a second run started with reversed current.

General remarks

For the first run about 15 ml. of protein solution are generally needed to form the boundaries, and for subsequent runs about 7–10 ml. are needed in order to remove separated components and re-form the boundaries in the new positions.

It has been found when working with human plasma and plasma fractions at concentrations of 2–3 g./100 ml. in phosphate buffer, pH 8, I 0.2, that currents in the region of 20–30 mA. can be used without convections occurring. This enables at least two separations to be carried out in the course of a day, for with proteins of high mobility such as albumin and α -globulin each run is complete in 2.5–3 hr., depending on the current used. The apparatus can also be operated overnight at currents of 4–10 mA.

The normal manipulation of the apparatus may be

modified in many ways. It is possible, for instance, to continue the run until the leading component has passed the level of the upper outlet, and to stop the current when the marker line comes between the first and second components. The first component may then be obtained by displacing with buffer from above, or the mixture of first and second components by displacing with protein from below. If the protein mixture being separated is not plentiful, a certain amount can be saved during the re-forming of the boundaries by letting downward displacement by buffer on one side produce an upward displacement on the other. The potentialities of the apparatus and its flexibility in various situations are realized more fully as experience is gained of it in use.

The limitations of the apparatus are those common to all in which electrophoretic migration takes place in solutions stabilized by gravity. The main disadvantage is that in a protein solution consisting of more than two components, only the fastest and the slowest of these may be obtained electrophoretically pure after a single migration. Separation of proteins with closely similar mobilities is made difficult by the limitation on the migration distance imposed by the length of limb available, and the occurrence of the stationary delta boundary. In the absence of the latter, the migration distance could be increased by a displacement of liquid in the system in the direction opposite to that of protein migration, but the delta boundary may not be displaced beyond the base of the U-tube without upsetting the density equilibrium.

This tube finds its most useful application when relatively large quantities of material are available, so that the semi-continuous separation effected can be used to full advantage.

SUMMARY

1. A modified form of electrophoresis U-tube is described, which is particularly suitable for semi-continuous preparative operation.
2. Details of the method of operating the apparatus are given.

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EXPLANATION OF PLATES

PLATE 2.

Fig. 2. The cell assembled on metal stand with side arms in position.

Fig. 3. The complete U-tube assembled with electrode vessels and filling pipettes.

PLATE 3.

Fig. 4. Ascending limb photographs of a protein mixture obtained from a horse antitoxic serum. Total protein 3 g./100 ml. in phosphate buffer pH 8, I 0.2. Migration 3.5 hr. at 20 mA. (a) Initial boundary. (b) Initial boundary and capillary level marker lights. (c) Electrophoretic pattern of protein mixture at completion of migration, and prior to removal of components. Peaks, left to right: albumin, α_1 - and α_2 -globulins, β_1 - and β_2 -globulins, δ -boundary. (d) As (c) but after removal of the fraction consisting of albumin, α_1 - and α_2 -globulins.

Fig. 3.

Fig. 2.

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Fig. 4.

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