Biophysical characteristics of small intestine ephithelial cells with particular reference to the effect of Aldosterone and Hydrocortisone.

A Thesis prepared by Hugh MacAskill Noble

for presentation to the University of Aston in Birmingham in application for the degree of Doctor of Philosophy.

Declaration

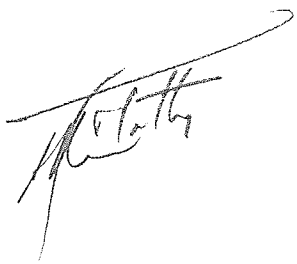
I hereby declare that the following thesis is based on the results of the investigation conducted by me, that the thesis is my own composition, and that it has not been previously presented for a higher degree.

The research was carried out at the University of Aston in Birmingham, Department of Biological Sciences under the supervision of Professor A.J. Matty.

Hugh L M Noble.

Supervisor's Certificate

I certify that Hugh MacAskill Noble has spent the equivalent of twelve terms at research work in the Department of Biological Sciences, University of Aston in Birmingham, under my supervision, that he has fulfilled the conditions of the University and that he is qualified to submit this thesis for the Degree of Doctor of Philosophy.



Academic Record

I obtained a B.Sc. in pure science at Glasgow University in 1959, and the degree of M.Sc. at St. Andrews University in 1965. I was admitted to the course for the course for the degree of Doctor of Philosophy in October, 1965 at the University of Aston in Birmingham and since then I have been engaged on research under the supervision of Professor A.J. Matty.

Acknowledgements

I would like to thank my supervisor Professor A.J. Matty for continuous help, encouragement and forebearance. I would also like to thank a number of other people in the Biological Sciences Department for helpful discussions, the University Animal House attendants and Mr. W. Armstrong of the Mathematics department who gave me a great deal of useful advice on the computer programmes.

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1. Introduction.

The subject of this thesis is the electrical phenomena which are associated with the absorption of ions, water and hexose by the small intestine of the Rat. A method will be described by which the short-circuit current of small intestinal segments may be measured *in vivo* and results will be presented, having been obtained by this method using both normal rats and rats with an experimentally altered hormonal status.

The historical development of *in vivo* and *in vitro* methods for the study of intestinal absorption has been traced by T.H. Wilson in his monograph entitled 'Intestinal Absorption' (1) and any repetition of this would be superfluous. *In vitro* methods are popular with experimenters because there is much greater control over environmental conditions than is possible *in vivo*, but it is important that data from both types of experiment should be continually reviewed and compared. After describing the work of Crabbe and of Edelman on the effects of Aldosterone on the toad bladder *in vitro* Mulrow wrote (149):

"If the results of these *in vitro* studies with toad bladder can be extrapolated to other tissues *in vivo*, an important start has been made in understanding the biological effects of Aldosterone."

Part of the work reported in this thesis is an attempt to carry out just such an extrapolation. Despite several reports that the hormone exerts an influence on Rat jejunum, hindsight makes it clear that jejunum is a poor choice of tissue with which to study the action of Aldosterone. A large literature has succeeded in producing a rather confusing picture of very complicated physiological mechanisms, with the result that a good deal of the 'Discussion' of this thesis concerns physiology, unaffected by hormones.

I intended to study the effects of hormones on the zeta potential of isolated mucosal cells because I felt that insufficient attention had been given, by other workers, to the local ionic environment of the cell

membrane, which can differ markedly from that of the bulk medium. Accordingly, two types of apparatus were constructed, the first for measuring the electrophoretic mobility of isolated mucosal cells and the second apparatus was for short-circuit current measurement in vivo. The latter was used to screen hormones for intestinal effects. While working for my M.Sc. thesis I had found great difficulty in maintaining the viability of isolated segments for more than one hour. The in vivo method allows a 'physiological' application of the hormones and it makes it possible to detect effects with latent periods of 2 or 3 hours. The observations of the electrophoretic mobility of normal mucosa showed that the cells orientate themselves in an electric field (or hydrodynamically) so that the surface charge which produces the electrophoretic motion belongs to the side wall (part of the serosal surface). This means that the brush-border, the most interesting and important part of the surface, is effectively inaccessible. This finding led to a concentration of effort on the short circuit current technique and it was realised that the method provided a convenient way of extending the work of Crane et al (§5.8(2)) to see if Aldosterone could alter the kinetics of the Sodium-Hexose interaction. It was found that although the hormone is able to restore to normal the transmural potential difference of jejunum after adrenalectomy, the effect can be mimicked to some extent by Hydrocortisone. Neither hormone had a significant effect on the Hexose-dependency of the potential and the data on this relationship in vivo did not fit well the saturation kinetics suggested by Crane. In an attempt to explain these observations attention was directed to the known behaviour of the jejunum and to the unknown origin of the potential difference. An explanation has been put forward which is in general accord with the views of Crabbe (§5.6) but in addition the explanation has been placed within the context of a hypothesis concerning intestinal physiology. The hypothesis is tentative but has the merit that it suggests new lines of experimental research.

SUMMARY

This thesis describes a method for the measurement of the potential difference (P.D.) and Short-circuit current (Isc) of Rat small intestine, and presents data which was obtained using the method. The P.D. and Isc were recorded by a novel technique which allows a semi-automatic and continuous record of both P.D. and Isc and therefore also of tissue resistance. The technique was used to screen a small number of hormones which were injected intravenously into the anaesthetised animal and also to follow the increase in P.D. and Isc which is observed when various hexoses are added to the fluid in the intestinal lumen.

A computer programme was devised and used to find the Michaelis-Menton kinetic parameters which provided the best statistical fit to the data. The result highlights a general inadequacy of the Michaelis-Menton scheme as a model for the Hexose dependency of P.D. and Isc. Linear regression coefficients were also used to describe the hexose dependent and hexose-independent terms in the total P.D. and Isc. These coefficients were then used to compare data from a number of groups of animals which had had various treatments designed to alter their hormone status particularly with regard to Aldosterone. The only demonstrable effect was that both Aldosterone and Hydrocortisone were able to restore to normal the hexose-independent P.D. and Isc which were depressed by adrenalectomy. Other treatments designed to vary the endogenous secretion of Aldosterone appeared to be explicable in terms of nutritional status rather than in terms of plasma levels of Aldosterone. It is suggested that the Aldosterone effects which have been reported by others are a reflection of increased plasma concentrations of hexose and electrolytes.

A method is also described for the measurement of the Zeta potential (ζ) of the intestinal mucosal cell surface. Data is presented concerning the variation of ζ with the pH of the suspending medium. These results are compared with other data on the "pore-phase"

which is obtained from data on the streaming potentials of intact intestine. It is concluded that the fluid transfer which generates the streaming potential probably does not pass through the lateral spaces.

Finally a comprehensive but tentative hypothesis is put forward concerning the absorptive functioning of the jejunum. An alternative explanation of the effect of Glucocorticoids on absorption is outlined in terms of specific enzymic interference in the Embden-Meyerhof pathway of Glycolysis, while Aldosterone is considered in this explanation to directly affect the membrane transfer of Sodium.

2 MATERIALS and METHODS.

2.1 Animals

(1) Normal Rats.

Male albino rats of about 300 g were used. These were taken from stock having been fed and watered ad libitum up until sacrifice. The diet was Diet 41 B. supplied by Pilsbury Ltd. An analysis of this diet is given in Appendix 3.

(2) Adrenalectomized Rats.

These were bought as such from Scientific Products Ltd. from whence they were despatched one or two days after the operation. On arrival at the University animal house they were put on a normal diet with saline drinking water (0.9% NaCl). They were maintained thus for at least a week before sacrifice.

(3) Salt Depleted Rats.

These were subjected to a procedure described by Edmonds (277) which briefly is as follows:- The animals are given an intraperitoneal injection of Glucose solution (5ml./100 g. body weight, concentration 5% w/v). This is removed 2 hours later. Edmonds found that 0.4 mM Na/100 g. body weight was removed in this way, representing about 8% of the rats 24 hr. exchangeable Na^+. The rats are then put on a Sodium-free diet consisting of 80 - 100 g. boiled rice daily for 7 to 10 days. Boiled rice:- 100 g. boiled in 200 ml. distilled water with the addition of 3 mM KCl/100 g. dry rice.

The control group were given 0.9% NaCl solution for drinking water.

Edmonds found that such rats remained generally healthy. This was not my subjective opinion and after one group had been sacrificed the experiment was not repeated.

(4) Salt loading.

Some otherwise normal rats were given 0.9% NaCl solution as drinking water for over 7 days.

2.2 Hormone treatment

(1) Vasopressin.

"Pitressin", 3mU/0.2 ml. distilled water injected intravenously to otherwise normal anaesthetised rats during the experiment.

(2) Insulin

0.5 IU in 0.9% NaCl solution pH 6.5. 0.5 ml of solution injected intravenously.

(3) Aldosterone. Ciba alcohol free.

Two dose levels were used 10 μg and 50 μg/rat, given by intraperitoneal injection in 0.5 ml. distilled water, 24 - 30 hrs. before sactifice. The second dose level was the standard dose in all the experiments reported.

(4) Hydrocortisone. 'Efcortelan' soluble.

0.5 mg. in 0.5 ml. distilled water given by intraperitoneal injection 24 - 30 hrs. before sacrifice (in some cases an additional injection was given 4 hrs. before sacrifice.)

2.3 THE SHORT CIRCUIT CURRENT APPARATUS

(1) Electrode Assembly.

The apparatus, which is illustrated in fig. 1. was essentially the same as that described by others who used the everted sac preparation (2, 9, 190). It consisted of a central or axial silver/silver chloride wire, about 1.5 mm. in diameter mounted in, and projecting about 5 cm. from the nozzle of a cannula which was tied into a short segment of intestine. The outer or serosal electrodes were two half cylindrical silver/silver chloride plates. These fitted loosely round the serosal or peritoneal surface and made electrical contact with it by means of two pads of polystyrene foam soaked in physiological saline solution. The transmural electrical potential difference (PD) was monotored using conventional salt bridges (agar/KCl in polythene capillary tubing) which were positioned, one inside the segment of intestine and the other lying between the polystyrene pads and the peritoneal surface of the intestinal muscle. The salt bridges passed into two balanced $Hg/HgCl_2$ half cells and these in turn were connected to a valve voltmeter (Pye-dynacap, input resistance $10^{12}\Omega$). An expanded scale attachment to this instrument allowed the PD to be read to within 0.2 mV.

The lower end of the internal silver electrode (the axial wire) was wound into a tight helix which, when sheathed in polythene, performed the double service as a convenient point to ligature the lower end of the segment under test and also as a mount for a second (outlet) cannula. The hydrostatic pressure of the luminal contents was controlled by the height of the outlet oraface.

(2) Recording. (See Fig.2).

The P.D. as already mentioned, was measured by a valve voltmeter. The output of the voltmeter was displayed on one channel of a multipoint pen records (Devices M4). The applied current, (see Principle of the S.C.C. technique, § 5.4(1)) was derived from a dry cell and was made to oscillate slowly (period 30 sec.) between zero and a maximum value.

This maximum value was set manually at a value which was a little greater than that necessary to eliminate the PD. The current was monitored on the adjacent channel of the pen recorder so that both PD and ISC were recorded alternately and semi-automatically. PD. was read at those points where ISC equalled zero and the ISC was read at those points on the trace where PD equalled zero. Not only is this a convenient arrangement but also gives a continuous measurement of tissue resistance.

The double connection to the pen recorder gave some trouble with ground loops. These were eliminated by linking the ISC leads to the pen recorder through a DC to DC converter in which the signal was chopped, amplified, transformer coupled and finally rectified and smoothed. The chopper oscillations were filtered out of ISC.

A complete schematic block diagram of the system is shown in Fig.2 and circuit diagrams of the various components in Figs. 3,4,5 and 6.

(3) Amplifiers.

Details of amplifiers used in Pen Recorder (Devices M4)

1. ISC. Devices DC3 with sub unit 5
(after amplification by the DC to DC converter (see fig. 2) the output of the pen drive unit DC5 was calibrated periodically using a moving coil ammeter (EEL 0 - 1000 μa resistance 280 Ω) which was placed in the ISC leads.

2. PD. Devices DC3 with sub-unit 5.
The output to the pen drive unit (DC5) was short circuited to earth by means of a 64 μF electrolytic capacitor to filter out a persistent 50Hz hum.

3. Temperature. Devices DC2 Sub unit 2.
Full scale deflection $\pm$ 1 mV (See § 2.4(5))

(4) Insulation.

The bench top was covered by a sheet of insulation board (Paxalite) and this was given a thin coating of silicone grease. Fluid spilt on this surface tended to coalesce into discrete bubbles rather than form a conducting film.

Rubber gloves were worn for all procedures which required touching the animal after the short circuit current and the voltmeter had been switched on.

These precautions were necessary to avoid inadvertent 'ground loops'.

2.4 Surgical Procedure and Location of Test Segment.

(1) Anaesthetic.

The procedure followed was entirely dictated by the booklet "An Introduction to the Anaesthesia of Laboratory Animals" by P.G. Croft (UFAW) 1964 (189).

'Nembutal' was given by intraperitoneal injection 0.1 ml/100 g. body weight. For most experiments lasting 2 hours this was sufficient to maintain surgical anaesthesia throughout (as judged by lack of foot reflex and very much reduced eye reflex). Occasionally ether was given cautiously as a supplement and additional doses of 'Nembutal' (half the original does) were given by spraying the fluid into the peritoneal cavity if the experiment was to be prolonged.

At the end of the experiment the animal was killed by either exsangination or by means of an overdose of ether.

It has been reported that Sodium Pentobarbitol and Sodium Hexobarbitol anaesthesia have an inhibitory effect on the Adrenal Hydroxycorticosteroid secretion in the dog (279), in contrast to ether anaesthesia, which raises the secretion rate (278).

(2) Location.

After the animal had been anaesthetised the visceral cavity was opened. The pylorus was located and a small incision made. A cannula was introduced at this point and through it 5 ml. of Krebs-ringer-bicarbonate (at 37°C) was injected. The contents of the small intestine were thus forced down toward the caecum and it was found that 5 ml. of fluid cleared the duodenum completely and a small part of the jejunum. The jejunum could, therefore, be easily located with a reasonable margin of error. (see Table No.2).P43 At the end of the experiment after the animal had been killed the entire small intestine was dissected out and the location of the test segment was expressed as a fraction

$$= \frac{\text{length from pylorus to 1st. incision of segment}}{\text{total length of small intestine}} \times 100$$

although the jejunum is difficult to distinguish from the ileum (no satisfactory definition of these seems to exist) the duodenal and jejunal flexure is easy to recognise and it can be said with confidence that none of the test segments included tissue from the duodenum.

(3) Inserting the Electrodes.

When the appropriate region had been located another cut was made in the intestine through which the inner part of the electrode assembly, complete with cannula, was inserted and secured with a ligature round the cannula. The intestine was then ligatured to the lower end of the electrode, ("Polythene bulb" fig.1). A second incision was made just below the bulb and the outlet cannula was pushed in and into the 'bulb'. A complete perfusion loop was thus established with a test segment some 5 cm. long (without tension) defined and isolated by the two ligatures yet still connected to an intact blood supply. The outer electrodes were then placed in position with their plastic pads (soaked in Kreb-ringer-bicarbonate at 37°C). The whole animal, except for head and neck was covered by a polythene sheet, held in place by a weighted pliable rod.

(4) Perfusion Method.

Two methods of introducing the perfusate were tried. The first, which was only partly successful, involved the use of a 20 ml. hypodermic syringe 5 ml. aliquots were injected from this into the intestinal segment via a polythene capillary. The act of injection was usually accompanied by a transient surge in the potential and short circuit current. A similar effect on water transport has already been reported by Blickenstaff, Bachman, Steinberg and Youmans (192) who attributed it to a transient increase in surface area due to the mechanical disturbance of the villi. The second method was a simple gravity fed supply from a 50 ml. reservoir held in clamp and stand which was adjustable for height over the range 0 - 30 cm. by means of a screw jack attachment. This eliminated the transient surge effect and is similar to the perfusion method of Sols and Ponz (193). The outlet cannula was held at a constant

3 cm. above the test segment. 3 cm. water was therefore the normal hydrostatic pressure in the lumen. But at high perfusion rates, or when there was a blockage of the outlet cannula, the luminal pressure rose, the maximum rise being dictated by the level of the supply reservoir. The reservoir was raised, and the flow rate increased, during the change over from one perfusate to another, but after about 5 ml. had passed through the reservoir was lowered to some 5 or 6 cm. above the test segment which gave a perfusion rate of one drip/10 secs or 1 ml./min. approximately.

(5) Temperature Control.

50 ml. quantities of the various stock perfusates were heated in a water bath set at 37°C. The reservoir which held the perfusate was surrounded, as was the experimental animal, with an atmosphere of warmed moist air supplied by a fan heater. The temperature was subjectively judged to be approximately 37°C. The temperature of the solution in the reservoir was monitored and if the temperature fell below 33°C the fan heater was readjusted to give more heat. The length of tubing (Portex PP50) which connected the reservoir to the cannula entering the intestinal segment under test, was enveloped for most of its length by a plastic water jacket through which was circulated water at 37°C from the neighbouring water bath. This arrangement ensured that the temperature of the perfusate was 37°C as it entered the test segment, even although the temperature of the solution in the reservoir may have dropped a few degrees below 37°C.

The temperature of fluid emerging from the test segment was monitored by means of an insulated thermocouple junction which pierced the outlet cannula about 3 cm. below the point at which the cannula left the segment. The reference thermocouple junction was placed in the water bath and the output of the thermocouple system was displayed on the third channel of the pen recorder.

All exposed parts of the viscera, including the test segment of intestine, the complete electrode assembly and saline soaked pads were

covered by a small sheet of polythene to minimise evaporation. The temperature of the saline pads was the most difficult part of the system to control. Although the surrounding air mass was at about 37°C and moist, a certain amount of evaporation did occur and hence some cooling of the pads was inevitable. The intact blood supply was the least exposed part of the apparatus and this would to some extent compensate for any cooling of the less well protected regions.

2.5 Perfusion Solutions

(1) Replacement Media.

Three perfusate solutions were used. Two of these were based on 0.9% NaCl solution and the third on Krebs-ringer bicarbonate. In all of these the sodium and the hexose concentrations were varied but the osmolarity of the solution was maintained by means of a suitable replacement solute.

Perfusate 1. designation Na/Choline (Tris buffer)

Basic solution Na^+ = 144 meq/1 Cl^- = 144 meq/1

Glucose 30 mM

Tris Bicarbonate 25mM

Trisbicarbonate was prepared by titrating Tris with CO_2 until pH 7.4 was reached.

Replacement of Na^+ by $Choline^+$ and of Glucose by Mannitol to give

Na = 0, 24, 48, 72, 96, 120, 144 meq/1

Glucose = 0, 5, 10, 15, 20, 25, 30 mM

Perfusate 2. designation Na/K (Sodium Bicarbonate)

Basic solution (Na^+ = 144 meq/1)* Cl^- = 120 meq/1

Glucose 24 mM

Sodium Bicarbonate 24mM

*including contribution from $NaHCO_3$

Replacement of Na^+ by K^+ and of Glucose by Mannitol to give

Na = 24, 48, 72, 96, 120, 144 meq/1

Glucose = 1, 2, 4, 8, 16, 24 mM

Perfusate 3. designation Krebs/K

Basic solution Kreb-bicarbonate ringer

with Glucose or Galactose = 0.5% w/v

or 27.7 mM

Replacement of Na^+ by K^+ and of Glucose (or Galactose) by Mannitol to give

Na = 24, 48, 72, 96, 120, 144 meq/l

Glucose (or Galactose) = 0, 5.56, 11.12, 16.68, 22.24, 27.7 mM

or as % w/v = 0, 0.1, 0.2, 0.3, 0.4, 0.5%

The pH of each perfusate was adjusted to pH 7 - 7.5 before use. It is normal practice to gas such solutions with O_2/CO_2 (95/5%) before use. This was done on some occasions but on others the solution was allowed to equilibrate with the air so that much of the tissue oxidation was supplied by the animal's blood stream as it would be in the intact animal.

(2) Composition of Krebs-Ringer-Bicarbonate.

Table No.1 Composition of Krebs-Ringer-Bicarbonate

Sodium	1.433×10^{-4}	eq/ml.
Chloride	1.283×10^{-4}	"
Potassium	5.923×10^{-6}	"
Calcium	5.200×10^{-6}	"
Magnesium	2.4×10^{-6}	"
Bicarbonate	2.5×10^{-5}	"
Sulphate	2.388×10^{-6}	"
Phosphate	2.035×10^{-6}	"

(see Ref.191, p.149).

2.6 Cell Electrophoresis

(1) Apparatus.

The apparatus is identical in principal to that described by Bangham et al (220). A cylindrical chamber (precision capillary tubing) with its axis horizontal is filled with a cell suspension and then sealed at both ends. When such a cell is subjected to an axial electric field the solution will move with respect to the chamber walls, and, because the chamber is sealed, this bulk movement will be cancelled by an equal but opposite bulk movement down the axial line. The hydrodynamics of such a situation has been examined by Lamb (197). It is evident that there is a "stationary point" where the velocity of the solution with respect to the walls is zero and it has been shown by Bangham et al (220) using Lamb's analysis that this stationary level occurs at a point 0.707 R units from the axis where R is the radius of the chamber, or more conveniently 0.293 R from the wall. It is at this "Stationary point" that the electrophoretic velocity of the cells due to the applied field can be observed without the complication of fluid movement.

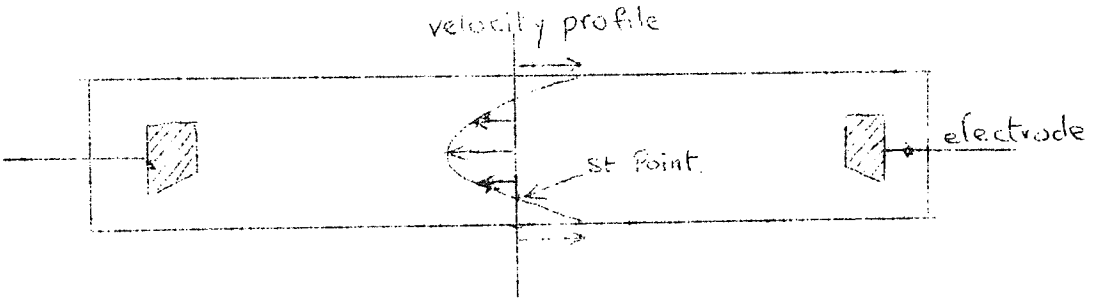


Figure 8 shows the electrophoresis chamber. For cleaning purposes this was made so that the perspex "side blocks" could be disconnected from the short length of precision capillary tubing of "Verida" glass. Electrodes of silver wire were introduced through tight fitting rubber bungs inserted in the openings AA'. The sample of suspended cells was injected from a syringe through the opening B. B and B' were then closed by means of nylon screws. A small side aperture in B' (not shown) allowed

the final sealing of B' to be accomplished without at the same time expelling the bungs from A, A'. The complete chamber, as shown (fig.8) fitted tightly inside a perspex box which was mounted on the stage of an upturned microscope (fig.9). The objective of the miscroscope penetrated the perspex box through a thin rubber diaphram (Durex' London Rubber Co.,) and the box was filled with liquid paraffin.

The chamber could be filled with a sample of suspended cells (or flushed out) without removing it from its position on the microscope stage. Only occasionally was it necessary to disemble it and clean the tubes with pipe cleaners.

The high voltage was obtained from the H.T. sockets of a "Labgear" power pack and the current was monotored by a suitably shunted micrometer. The meter was calibrated by connecting the circuit in series with an Avo electronic universal test meter and comparing the readings at several current values. The rize of the current could be controlled both by changing the H.T. supply and by means of a wirewound potentiometer. A reversing switch was also incorporated into the circuit to allow selection of field polarity.

As described by Bangham et al (220) an "optical flat" is ground on the cylindrical surface of the capillary tubing which allows the objective lens to make a close approach to the internal tube.

(2) Preparation of the cell suspension.

Healthy young adult female rats were killed with a blow on the head and the small intestine flushed out with ice-cold freshly oxygenated krebs-ringer-bicarbonate solution (§2.5 (2)). The whole small intestine was then everted and quartered. The segments of intestine were rinsed to remove intestinal flora and fauna and placed in separate petri dishes of fresh ice-cold ringer (krebs). The intestinal mucosa were then scrapped off the segments by pulling them through between the prongs of a pair of forceps held in a nearly closed position. The cell preparation was washed by centrifugation at 500g. for ten minutes and resuspension

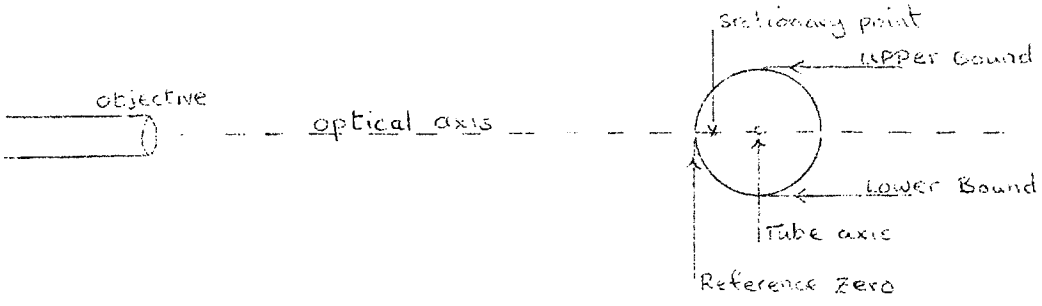
(cf ref. 221). When it was required to store the cell preparation overnight, the tube containing it was placed in a refrigerator at 5°C.

Early attempts to measure the electrophoretic velocity of intestinal mucosa in krebs-ringer-bicarbonate failed due to the fact that the cells settled out of suspension very rapidly. It was found that a more dense, viscous suspending medium was required and for this purpose the Sodium Chloride of Krebs-ringer-bicarbonate was replaced by isoosmotic Sucrose solution.* Assuming total dissociation of the ionic components the various media had an osmolarity of approximately 300 mosm. The sample of cells was resuspended twice in the Sucrose medium after centrifugation at 500 g for ten minutes.

It was found by trial and error that a fairly dispersed suspension was the most satisfactory a 1 ml pellet of cells being dispersed in about 100 ml. of sucros-medium. The experiments were carried out at room temperature.

(3) Recording Results.

After a sample of suspended cells had been introduced into the electrophoresis chamber, air bubbles expelled and the chamber sealed, the microscope was focused on the capillary tubing. The mid-point was found by using the vertical stage vernier to bisect the distance between the upper and lower bounds of the horizontal tube. The microscope was then focused on the nearside inner wall of the tube using the course focus control with the fine focus control set at zero. This point is labelled "Reference Zero" below.



*A similar suspension medium 0.2M Sucrose buffered with Potassium Phosphate was used by Stern and Reilly in their investigation of the respiration of intestinal mucosa cells (96).

In this position the optical axis passes through the sample tube axis. The fine focus control, which had been calibrated, was used to move the focus point inwards to the stationary point. It was found that when the cells were highly dispersed it was difficult to find a sufficient number of cells to make up a statistically satisfactory sample. On the other hand if the suspension was congested the cells near to the nearside wall obscured those at greater depth. A compromise was reached using a moderately dispersed suspension (as mentioned in the previous section § 2.6 (2)). It was then possible to find a number of cells (about 20) whose positions straddled the "stationary point". When the electrophoretic velocity of these had been found the "stationary point" value was calculated as described below (§ 3.3.). After the reference zero point had been found the following procedure was followed in chronological order.

(1) The current was switched on and adjusted to a standard mark corresponding to 1.5 ma. The current was switched off.

(2) The microscope was racked inwards to focus on a single mucosal cell, recognisable by its brush border.

(3) The current was switched on again and the movement of the cell across a fixed number of divisions of the eyepiece graticule was timed using a stop watch.

(4) The current was read and switched off.

(4) The following data was noted (in this order) Current, direction of movement, orientation of cell, distance moved (number of divisions), time taken (to the nearest tenth of a second), depth (reading on the fine focus control), polarity (switch position).

(6) The polarity of the field was then reversed in readiness for the next measurement.

Bangham et al (220) state that there is little danger of contamination by electrode products if the current (2.0 ma in this case) lasts only five times that required for the measurements.

(4) Calibrations.

(i) The eyepiece graticule of the microscope was calibrated by means of a microscope stage micrometer.

(ii) The graduations on the fine focus control knob were calibrated thus:

A number of pieces of glass ranging in thickness from a microscope cover slip upwards were broken to provide a jagged edge. The edge was then observed under the microscope, focusing first on the upper and then on the lower edge and the scale graduation was noted The thickness of the glass was then measured using an engineers micrometer gauge. The readings were repeated at least 12 times and the mean value taken.

According to Bangham et al (220), Henry's correction applied to Verida Glass (refractive index 1.47) amounts to only 0.01 R when the tube is in contact with water (where R is the capillary tube radius) and the correction may accordingly be ignored.

3. Calculations

3.1 The Calculation of the Linear Regression Coefficients.

The coefficients of the linear regression line $y = A + Bx$ (where y = P.D. or Isc and x = hexose concentration.) were calculated by the method of least squares for each individual animal. These individual coefficients were then used to calculate the overall A and B for a treatment group and also the standard error of the mean. The number of degrees of freedom is equal to the number of animals minus the number of parameters.

3.2 Calculations of Michaelis Menton Kinetics Parameters.

(1) Introduction.

As has been already mentioned Michaelis Menton kinetics have been used by a number of workers to analyse the flux rates of various transported materials. In particular the work of Lyon and Crane (230) investigated the relationship between hexose concentration and the P.D. using the classical Lineweaver - Burke Plot (an inverse plot) to obtain estimates of Km and Vmax. Their data for the jejunum did not in fact fit the inverse plot conspicuously well (230, Fig.7) and in any case Lineweaver-Burke plot is itself highly suspect (147). The defects in the plot can be partially corrected by using weighted points provided the errors associated with the points are small and are reasonably constant. This method was used to find first approximations for the parameters (Vmax and Km) using data points which were the mean points for all rats in a single treatment group. Unfortunately this method although giving moderately good estimates of Vmax and Km. gives no information about the precision of the estimates so that it is impossible to compare the calculated parameters associated with two treatments using any kind of statistical test for significance. An alternative method based on the "Prindipal of Maximum Likelihood" has become popular since the advent of computers. A good description of the method has been given by Cleland (146). In addition he gives a full computer programme, unfortunately in Fortran, which the Elliot 803 installation at Aston University will not accept. Nor were the staff of the computer centre able to undertake the rewriting of this programme. Instead I have written a similar, but not identical programme, using the method of Least Squares, in Elliot 803 Algol suitable for 5 - hole tapes. A description of this and the preliminary programme to obtain first approximations are given below and the full text of the programme appears in appendix No. 2(2).

The Michaelis Menton kinetic analysis deals with the reaction.

$$S + X \underset{R_2}{\overset{R_1}{\rightleftharpoons}} (SX) \xrightarrow{R_3} \text{Products (produced at rate V)}$$

Where S is a substrate, (S_X) is a substrate/carrier or substrate/enzyme complex, X is the free species of carrier or enzyme and "concentrations" are implied throughout.

A maximum velocity Vmax is imposed on the reaction because of the limited supply of carrier $X_o = X + (SX)$. At steady state the rate V and substrate concentration S are related by the equation.

$$V = \frac{V_{max} \cdot S}{K_m + S} \qquad \left[\text{where } K_m = \frac{R_2 + R_3}{R_1} \simeq \frac{R_2}{R_1} \quad \text{since } R_2 \gg R_3 \right]$$

$1/K_m$ described the affinity of S for X. Km is numerically equal to the concentration of S which gives the "half maximum" velocity.

In the situation described here X is presumably some carrier species (§5) while V is the P.D. or Isc presumably proportional to the rate of transfer of some ionic species. Doubt can be cast upon the validity of this treatment but these doubts will be discussed later.

Inversion of equation (2) gives.

$$1/V = \left(1/V_m\right) + \left(\frac{K_m}{V_m}\right) 1/S \quad \text{ie} \quad y = A + Bx \quad \text{(a straight line)}$$

$$\text{where} \quad y = 1/V \qquad A = 1/V_m \qquad B = K_m/V_m \qquad x = 1/S$$

(2) Programme No. 1

The classical Lineweaver – Burke method plots Y against X to obtain a straight line of gradient = B and Y-intercept = A. If the points are scrupulously accurate this is a valid and convenient method for finding Km and Vmax. Unfortunately, however, if the points are subject to an experimental error the inversed data is subjected to highly distorted error. That is, it is no longer distributed normally about its mean and is no longer constant for all points along the line. If the "error" is

measured by the variance (σ^2) then

if $y = 1/v$

$$\sigma_y^2 = 1/v^4 \, \sigma_v^2$$

(ref. 148, P. 187)

That is the variance of Y is equal to the variance of V modified by a factor ($1/v^4$). To correct for this the values of Y must be weighted a factor V^4 before finding the best straight line by the method of least squares. This is the technique which was used by Programme No. 1 used to find the first approximations to Vmax and Km. This programme operates on mean data only. It is not suitable for plotting a curve from all points drawn from a treatment group otherwise the resulting curve is biased in favour of rats yielding high values of V. It could be used on a single set of points from a single rat, but it was found that the individual values were so variable and with only six points per rat that the results were often nonsensical since a single erroneous point might imply a Vmax of infinity (indicated by negative values of Vmax and Km.) The mean points on the other hand gave a satisfactory curve. The programme also assumes that the values of S are not subject to error, and no weighting correction is applied to $1/S$.

The full text of this programme appears in appendix No. 2 (1)

DATA TAPE. N.

(S1, V1), (S2, V2), - - - - (Sn. Vn)

where N = No. of Pairs of Data

(S1, V1) = Pair No.1. Substrate conc., Velocity (PD) respectively

Note that S = 0 is impermissible because $1/S = \infty$.

These are Hexose - Dependent data only and the base level must be subtracted. Similarly if V = 0 is required it is preferable to convert this to a small number. Since these are Hexose - Dependent data V = 0 should not occur.

Output $A = 1/Vm$

$B = Km/Vm$

(3) Programme No. 2.

The programme follows a procedure similar to that described by Clelland (146) (p5 - 6). The principal is stated perhaps more succinctly by Paradine and Rivett (148, p.263). The technique is to generate the equations:

$$\left.\begin{aligned} \delta a \frac{\partial^2 L}{\partial a^2} + \delta b \frac{\partial^2 L}{\partial a \partial b} &= -\frac{\partial L}{\partial a} \\ \delta a \frac{\partial^2 L}{\partial a \partial b} + \delta b \frac{\partial^2 L}{\partial b^2} &= -\frac{\partial L}{\partial b} \end{aligned}\right\} \quad \text{Eq 4.}$$

Where L = function $\left(S, \alpha, \beta\right) = \sum \left(\frac{\alpha S}{\beta + S} - V\right)^2$

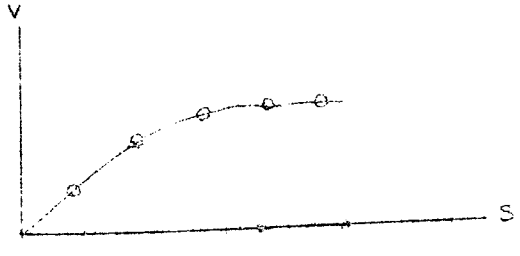
S = substrate, α, β are required parameters

a, b are estimates of α, β respectively.

The equations are then solved for δa, δb which are corrections to be applied to a and b.

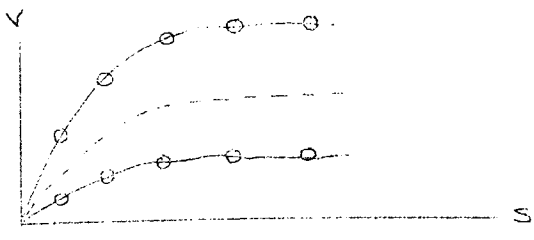
Estimates for a and b are obtained from Programme No.1.

The variance of a and b are given by the diagonal elements of the matrix obtained by multiplying the inverse of the square matrix on the left hand side of equation (4) by σ^2. Where σ^2 is the mean squared deviation of the residuals. The calculation of σ^2 presents some difficulties. Clelland (146) states that required degree of freedom (DF) is (P - n) where P = number of points, n = number of parameters to be extracted from the data (2 in this case). However, as already explained the calculation of a curve for each individual rat proved impractical and the calculation of a curve from meaned data would give an accurate curve but an over optimistic estimate of the precision of the estimates of parameters obtained in this way. That this is so can be seen by reference to the diagrams below



Mean points giving perfect Saturation Curve

Variance of Vmax = Virtually nil.



Scatter Diagram showing two sets of Data. The True Variance of Vmax is not equal to Zero.

It is required then to calculate the curve parameters from the original scatter diagram, This requirement introduces problems in deciding the DF. The total number of points (P) appear in sets belonging to individual animals (N sets of K) and within a set it cannot be said that the points are truly independent. The figure (P -n) would therefore also give an over optimistic value for DF. Conversely the number of data points (K) (i.e. values for S used to plot the curve) cannot be accepted as this would ignore the number of animals used which is the most important factor. I have been unable to find any advice as to the procedure to follow in these circumstances and so have adopted a compromise. The DF used in programme No. 2. is given by the relationship.

$$DF = P - Nn + n - K$$

D.F. = Degrees of freedom.

Where P. = total number of points

N. = number of amimals.

K. = number of data from each animal (K = 5)

n. = number of parameters.

I can supply no rigorous theoretical for this relationship, but I am of the opinion that it is a reasonable one on the basis of the following argument.

For a single set of points (i.e. from one animal) one would use the formula D.F. = (K - n)

But these are N. animals. If parameters could be calculated for each individually the over all mean would be calculated using D.F. = (N -1). Combining these we have

$$\begin{aligned} D.F. &= (K - n)(N - 1) \\ &= (KN - Nn) - (K - n) \\ &= (P - Nn) - (K - n) \\ &= P - Nn + n - K \end{aligned}$$

Numerically this means, for example, that if 10 animals are used and 5 items of data measured on each, and that the calculated curve has two

parameters. Then:-

$P_o = 50,\ N_o = 10,\ K_o = 5,\ n_o = 2$

$D.F_o = 50 - 20 + 2 - 5 = 27.$

Which is appropriately midway between the optimistic (50 - 2) and the pessimistic (10 - 2).

The programme has been tested on a set of data which was generated from a ficticious set of parameters. Since the parameters were known the true mean values and standard deviations of these parameters could be calculated and compared directly with the computed results. The computed standard deviations are in good agreement with the true values but the parameter values are low. This is due to the fact that those curves with higher Vmax are truncated to a greater extent than those with smaller Vmax. The computed curve however, is almost identical with the "curve of means". This phenomenon, of differential truncation, would appear no matter what scheme was used for calculating the parameters because in the case where the curves were calculated for individual rats the curves with higher Vmax would require the greatest extrapolation and therefore be associated with the least precision. In all cases the overall means will be weighted in favour of the lowest values.

It was found in practice that the first approximations of A and B were not sufficiently good for the method outlined above (and characterised by equation 4) to be immediately applicable. For this to be the case the errors δa and δb must be less than unity otherwise it is quite impermissible to ignore the higher terms (in powers of δa, δb) which occur in the Taylor series used in forming equations Eq.(4). Accordingly the programme first scales all input data by factors to bring these data to lie between zero and unity. A damping term "Divisor" is also introduced which reduces the calculated error and forces the computer "to take small steps" initially. The magnitude of "Divisor" is progressively reduced to unity after 100 iterations.

Four forms of instability occurred.

1) Progressive increase to extremely large values of Vmax and Km This can be interpreted as a straight line relationship.

2) Negative values of Vm and Km which implies that the curve is concave upwards and rises to infinity at S = -Km.

3) When the relationship between L (the error) and the parameters is plotted as a map (with contour heights in L), the map often resembles a long narrow trough. In its attempt to find the lowest point the Programme sometimes enters the trough and progresses down it in a series of infinitessimal steps. This is a common failing of hill climbing techniques.

4) If the error in spite of scaling exceeds unity the programme diverges from the true end point.

To minimise the problem of these types of error the programme automatically terminates after 150 steps and selects the best values encountered during the computation, but in these cases the calculated variances are highly suspect since the gradients of the error/parameter surface are not Zero at the at the point defined by the best values.

Another modification which was introduced in later computations was the use of a lower limit to the parameters. These lower limits were set on the data tape.

Date tape:-

Data Ref. No.

Approximate Vmax (from programme 1)

Approximate Km (from programme 1)

Lower limit Vmax (by inspection)

Lower limit Km (by inspection)

Data for each individual rat consists of a series of pairs of numbers referring to the hexose concentration and the PD or Isc respectively. The series must begin with a pair with concentration = 0, since this zero is used as a recognition of individual rats and

separates the data used to calculate the parameter C (the hexose - independent PD or Isc).

When the data for all rats has been given the programme commences to compute the parameters on reading the terminating pair

10,000 10,000

The complete programme is concluded by the Data Reference Number 101.

The computer will then output the first approximations and all succeeding approximations with the computed error. On reaching the desired 'end point' (or after 150 steps) the computer will output the final Vm and Km and C, with variances and the corresponding "Best Plots" so that the curve can be drawn to fit the equation.

$$y = \frac{Vm\ x}{Km + x} + C$$

where y = PD or Isc

x = Hexose concentration

C = Independent parameter

(i.e. $y = C$ when $x = o$).

Vm and Km are as described above.

The computer will then continue to input data and repeat the above process until the Data Ref. No. greater than 100 is reached.

(4) Theoretical Note

Because the data is subject to a large error and the curves computed from the data of a single animal is oversensitive to error even in a single point, it was not considered practical to attempt to compute separate curves for each animal and then to find the Mean Parameters from these. This, however, is theoretically the correct method for finding the parameters. It is not theoretically sound to find mean data points and then to compute a single curve from the meaned data. It is of interest, however, to compare the results obtained by these two methods.

Let the two curves so obtained be given by the expressions:-

$$y = \frac{Ax}{B + x} \qquad \text{------ Eq (1)}$$

$$y = \frac{A' x}{B' + x} \qquad \text{------ Eq (2)}$$

where A,B are the <u>mean parameters</u> (Vm + Km resp). 1st method.

and A', B' are the parameters from the <u>mean curve</u>. 2nd method.

$$A = \sum_{i=1}^{n} a_i/n \quad , \quad B = \sum_{i=1}^{n} A_i/n$$

where Ai and bi are the parameters corresponding to the curves of individual animals.

We require the relationship between A and A' ; B and B'.

Now as $x \to \infty$; $y \to A$; $y' \to A'$

$$y' = \sum_{i=1}^{n} \left[\frac{a_i x}{b_i + x} \right] \Big/ n \;\underset{\text{as } x \to \infty}{\to}\; \sum_{i=1}^{n} \left(\frac{a_i x}{x} \right) \Big/ n = \sum \frac{a_i}{n} = A$$

$$\therefore A' = A$$

i.e. both methods give the same estimate of Vmax.

Again as $x \to 0$; $y \to \frac{A}{B} x$; $y' \to \frac{A'}{B'} x$

$$\therefore \left(\frac{dy}{dx} \right)_{x=0} = \frac{A}{B} \quad ; \left(\frac{dy'}{dx} \right)_{x=0} = \frac{A'}{B'}$$

now $$\frac{A}{B} = \frac{\Sigma a_i/n}{B} = \frac{1}{n} \left(\frac{a_1}{B} + \frac{a_2}{B} + \frac{a_3}{B} + \cdots \right)$$

but $$y' = \sum_{i=1}^{n} \left(\frac{a_i x}{b_i + x} \right) \Big/ n \;\underset{\text{as } x \to 0}{\to}\; \sum_{i=1}^{n} \left(\frac{a_i}{b_i} x \right) \Big/ n$$

$$\therefore \left(\frac{dy'}{dx} \right)_{x=0} = \sum_{i=1}^{n} \left(\frac{a_i}{b_i} \right) \Big/ n$$

$$= \frac{1}{n} \left(\frac{a_1}{b_1} + \frac{a_2}{b_2} + \frac{a_3}{b_3} + \cdots \right)$$

now since $A = A'$ then $\frac{A}{B} = \frac{A'}{B'} \iff B' = B$ (and conversely)

and

$$B = B' \iff \left(\frac{a1}{b1} + \frac{a2}{b2} + \ldots\right) = \left(\frac{a1}{B} + \frac{a2}{B} + \frac{a3}{B} \quad \ldots\right)$$

which is not true in general.

As mentioned above the first method is theoretically sound, but this is only so if A and B are independent of one another. The true values of A and B may indeed be independent but the best estimates of A and B are not. The independent parameter is $\frac{ai}{bi} = \phi i$.

It is the second method which treats this parameter as independent, since

$$\left(\frac{dy'}{dx}\right)_{x=0} = \frac{\Sigma\, ai/bi}{n} = \frac{\Sigma \phi i}{n} = \phi_{mean}$$

For this reason it is felt that the second method is valid and may even be prererable to the first.

3.3 Electrophoretic Velocity

The value of the electrophoretic velocity at the "Stationary Point" (§ 2.6 (1).) was calculated from a series of values which straddled the stationary point, by fitting a quadratic curve to the data. This is the theoretical velocity profile (Poisseiulle's Law). The curve was fitted using orthogonal polynomials by an Elliot Library Computer Programme (803 - Y2). The Stationary Point value was then calculated using the output parameters of the best curve.

4. Results

4.1 Hexose Dependence of Bioelectric Phenomena.

The data are presented mainly in tabular form. The figures 10 - 15 are intended to be illustrative only.

Table 2 shows the data on lengths and positions of the intestinal segments. The method of finding and representing the position data has been described in § 2.4 (2). The mean values are given with the standard error of the mean and "N" the number of animals involved. It should be noted however that the lengths (in centimetres) represent the "stretched" length, that is, the length measured after the whole small intestine had been excised and layed out. The mean value is approximately 10 cm while the distance between the ligatures of the segment *in vivo* was 5 cm. There is always a difficulty in deciding what criterion to use in estimating the "amount" of tissue involved in such experiments. Objections can be made about any criterion e.g. length, dry weight, wet weight or weight of mucosal cells after scraping. In this case the stretched length measurements are intended to provide a measure of the degree of standardization within a treatment/control pair and are not intended to be an absolute measurement. In fact the lengths were remarkably consistant. This consistancy is probably an indication that the muscle tone of animals dying under an overdose of anaesthetic is relatively uniform.

TABLE 2.

	Position	Length	N
Normal	16.7 ± 1.47	10.9 ± 0.28	10
Adr'x	13.1 ± 0.85	10.2 ± 0.66	5
Adr'x + Aldo	17.8 ± 2.39	11.6 ± 0.98	7
Adr'x + Hydroc.	22.4 ± 3.81	10.4 ± 0.43	7
Sod. Depl.	34.8 ± 7.40	10.5 ± 0.29	4
Sod. Depl. Control.	30.7 ± 3.19	11.3 ± 0.92	4
Sod. Load.	17.7 ± 1.48	10.7 ± 0.71	6
Sod. Load + Aldo.	22.6 ± 3.43	10.7 ± 0.52	7
Adr'x + Hydroc (Gluc.)	20.4 ± 2.29	10.8 ± 0.79	8
Adr'x + Hydroc + Aldo (Gluc.)	21.8 ± 2.51	11.7 ± 0.64	9

Table 3 confirms these comments. The groups of animals have been divided into 10 treatment/control pairs for the purposes of comparison. The figures appearing in the body of the table are the "P" values - the probability of the difference between the means, calculated by the Students t-test. A rather unusual notation has been used. The P value has been converted to a percentage and the two figures given are the upper and lower bounds of the interval in which P lies.

Thus:

$60/_{50}$ indicates $60\% > P > 40\%$

This notation is more compact for use in tables and does not involve symbols which are absent from a standard typewriter.

The following abbreviations have also been used:

Adr'x - - - - - - - - - Adrenalectomy.

Aldo. - - - - - - - - - Aldosterone.

Hydroc. - - - - - - - - - Hydrocortisone.

Sod. Depl. - - - - - - - - Sodium depletion.

Sod. Load. - - - - - - - - Sodium loaded.

Gal. - - - - - - - - - - Galactose.

Gluc. - - - - - - - - - - Glucose.

Mannitol/Galactose is the substrate combination unless otherwise stated.

TABLE 3. Values of P. The probability of the Student's t

Treatment/control Pair	Position	length.
Normal Adr'x	$^{20}/_{10}$	$^{20}/_{10}$
Adr'x Adr'x + Aldo	$^{20}/_{10}$	$^{30}/_{20}$
Adr'x Adr'x + Hydroc.	$^{10}/_{5}$	$^{90}/_{80}$
Adr'x + Aldo Adr'x + Hydroc.	$^{40}/_{30}$	$^{30}/_{20}$
Sod. Depl. Sod. Depl. Control	$^{70}/_{60}$	$^{50}/_{40}$
Sod. Load Sod. Load + Aldo	$^{30}/_{20}$	$/_{90}$
Adr'x + Hydroc. (Gal.) Adr'x + Hydroc. (Gluc.)	$^{70}/_{60}$	$^{70}/_{60}$
Adr'x + Hydroc. (Gluc.) Adr'x + Hydroc. + Aldo (Gluc.)	$^{70}/_{60}$	$^{40}/_{30}$

P as a percentage $^{60}/_{50}$ indicates $60\% > P > 50\%$

Table 4 gives data on the P.D. measured in each treatment group with various perfusates. The term "Krebs" has been used as an abbreviation for Krebs - Ringer - Bicarbonate Solution.

Table 5 gives equivalent data on the short circuit current (Isc.)

These tables (4,5) give the meaned data for each point ± the standard error of the mean. No correction has been applied for variations in length of segment since it is not clear what such variations are caused by. The data associated with any segment was however rejected if the corresponding length or position varied from the mean by greater than twice the standard deviation of the population.

TABLE 4

Potential Differences. (mV)

Treatment	Krebs without Hexose	Sodium % w/v Galactose (with Mannitol replacement) 0.0	0.1	0.2	0.3	0.4	0.5	N
Normal	1.42 ± 0.22	0.20 ± 0.25	0.86 ± 0.27	1.58 ± 0.28	2.21 ± 0.28	2.82 ± 0.24	3.32 ± 0.25	14
Adrenal'x	1.30 ± 0.43	0.46 ± 0.35	0.04 ± 0.37	0.80 ± 0.28	1.50 ± 0.30	2.08 ± 0.30	2.64 ± 0.33	5
Adrenal'x + Aldo.	3.13 ± 0.24	1.91 ± 0.51	2.86 ± 0.37	3.16 ± 0.35	3.90 ± 0.26	4.53 ± 0.19	4.84 ± 0.22	7
Adrenal'x + Hydroc.	2.84 ± 0.41	1.90 ± 0.37	2.60 ± 0.41	3.31 ± 0.63	3.67 ± 0.61	4.21 ± 0.61	4.67 ± 0.56	7
Sodium Depletion	2.58 ± 0.46	0.70 ± 0.26	1.60 ± 0.62	1.82 ± 0.72	1.96 ± 0.26	2.80 ± 0.63	3.86 ± 0.62	5
Sod. Depletion Controls.	3.08 ± 0.39	1.50 ± 0.48	2.05 ± 0.55	2.68 ± 0.47	3.05 ± 0.39	3.63 ± 0.27	4.13 ± 0.19	4
Sodium Loaded	2.55 ± 0.73	1.45 ± 0.78	2.03 ± 0.82	2.88 ± 0.63	3.20 ± 0.61	3.37 ± 0.38	3.68 ± 0.30	6
Sodium Loaded + Aldo.	2.88 ± 0.39	1.57 ± 0.38	2.19 ± 0.34	2.90 ± 0.32	3.32 ± 0.30	3.82 ± 0.42	4.16 ± 0.52	9
				Glucose				
Adrenal'x + Hydroc.	2.9 ± 0.45	2.1 ± 0.70	2.8 ± 0.70	3.3 ± 0.70	3.6 ± 0.54	3.8 ± 0.53	4.3 ± 0.58	8
Adrenal'x + Hydroc + Aldo	2.4 ± 0.44	1.13 ± 0.35	2.1 ± 0.37	2.7 ± 0.45	8.5 ± 0.42	3.9 ± 0.48	4.4 ± 0.49	9

TABLE 5.

Short Circuit Current (μa)

	Treatment	Normal Krebs Ringer	Sodium % w/v Galactose 0.0	0.1	0.2	0.3	0.4	0.5	N
1	Normal	250 ± 61	93 ± 82	191 ± 71	412 ± 128	501 ± 118	595 ± 100	689 ± 112	10
2	Adrenal'x	72 ± 17	-40 ± 34	7.5 ± 38	62 ± 22	136 ± 24	176 ± 31	231 ± 33	5
3	Adrenal'x + Aldo	896 ± 224	620 ± 223	805 ± 223	879 ± 267	966 ± 211	1189 ± 251	1131 ± 168	7
4	Adrenal'x + Hydroc	740 ± 148	353 ± 84	534 ± 84	707 ± 180	734 ± 176	878 ± 178	1022 ± 228	7
5	Sodium Depletion	269 ± 60	81 ± 46	166 ± 72	208 ± 63	194 ± 11	284 ± 40	350 ± 50	4
6	Sod. Depletion Controls	395 ± 47	159 ± 47	230 ± 72	323 ± 91	373 ± 101	478 ± 109	536 ± 117	4
7	Sodium Loaded	1082 ± 378	316 ± 169	565 ± 148	950 ± 213	1079 ± 190	1465 ± 426	1742 ± 630	6
8	Sodium Loaded + Aldo	456 ± 74	226 ± 84	324 ± 63	424 ± 38	551 ± 47	677 ± 59	820 ± 90	8
9	Adrenal'x + Hydroc	516 ± 106	514 ± 168	709 ± 226	709 ± 223	843 ± 230	846 ± 237	845 ± 175	8
10	Adrenal'x + Hydroc. + Aldo	519 ± 86	200 ± 76	392 ± 59	525 ± 89	644 ± 111	776 ± 190	808 ± 160	9

Tables 6 and 7 give the linear regression coefficients for P.D. vs Hexose and Isc vs Hexose curves respectively. The parameter A is a measure of the PD (or Isc) when 0.5% Mannitol is present in the perfusate which parameter B is the gradient of the Galactose (or Glucose) dependent increase.

ie $y = A + Bx$

where y = PD or Isc

x = Concentration of Galactose (or Glucose) % wv

The basic data from which the regression lines were calculated is not obtained from tables 4 and 5 but instead is obtained from the data of individual animals as described in § 3.1

Tables 8 and 9 give the Michaelis-Menton kinetic parameters Vm and Km. The method of calculation has been described in § 3.2. Data for which the computer programme failed to find a satisfactory 'end point' have been indicated **. In these cases no standard error has been given since the best values of Vm and Km obtainable do not correspond to points on the error/parameter surface where the gradient is zero. The parameter "C" is equivalent to A of the linear regression line (given in tables 6 and 7) and is approximately equal to it.

The equation to which the data has been fitted is

$$y = \frac{Vm.x}{Km + x} + C$$

where y = PD (or Isc)

x = concentration of Galactose (or Glucose) % w.v.

Vm, Km are the Michaelis-Menton Parameters

C = a constant.

Table 10 presents the P values using the same notation described for table 3. Values of P which are below the 5% level (commonly described as "Significant") have been indicated *.

Table 11 gives P values for the linear regression coefficients. Regrettably the data on Vm and Km are not sufficiently precise to allow a similar comparison for these parameters.

TABLE 6 Linear Regression Coefficients of the PD/Substrate curve.

PD

	A	B
Normal	0.26 ± 0.27	6.40 ± 0.40
Adrx	-0.49 ± 0.28	6.38 ± 0.29
Adrx + A	1.67 ± 0.29	6.97 ± 0.43
Adrx + H	2.01 ± 0.42	5.44 ± 0.69
Sod.D	0.73 ± 0.50	6.60 ± 0.56
Sod. DC	1.53 ± 0.57	5.23 ± 1.20
Sod. L	0.84 ± 0.37	5.68 ± 0.66
Sod. L + A	1.58 ± 0.38	5.85 ± 0.51
Adrx + H (Gluc)	2.29 ± 0.72	4.11 ± 1.19
Adrx + H + A (Gluc)	1.36 ± 0.37	6.38 ± 1.06

where PD = A + B (% [Hexose])

TABLE 7 Linear Regression Coefficients of the ISC/Substrate curve.

Isc

	A	B
Normal	110 ± 84	1177 ± 101
Adrx	-40 ± 30	548 ± 51
Adrx + A	661 ± 247	1077 ± 289
Adrx + H	392 ± 85	1254 ± 337
Sod. D	94 ± 56	481 ± 113
Sod. DC	158 ± 65	765 ± 234
Sod.L	287 ± 184	3212 ± 1397
Sod. L + A	161 ± 107	1314 ± 164
Adrx + H	588 ± 201	627 ± 233
Adrx + H + A	250 ± 72	1232 ± 451

where PD = A + B (% [Hexose])

TABLE 8. Michaelis-Menton Parameters of the PD/Substrate curve.

PD	Vm	Km	C	N
Normal	22.0 ± 4.5	3.0 ± 7.0	0.2 0.26	14
Adrx **	77	11 x 10^6	-0.46	5
Adr'x + Aldo	10.75 ± 5.5	1.31 ± 0.86	1.914 ± 0.93	7
Adr'x + Hydroc	9.6 ± 3.6	1.25 ± 0.61	1.9 ± 0.85	7
Sod. Depletion **	5.2 x 10^6	0.1 (lower limit)	-	5
Sod. Depl. Cont. **	26.4	4.55	1.5	4
Sod. Loaded	4.4 ± 1.06	0.48 ± 0.2	1.45 ± 1.0	6
Sod. Loaded + Aldo	8.62 ± 2.01	1.15 ± 0.36	1.56 ± 0.67	9
(Adr'x + Hydroc	4.62 ± 1.8	0.60 ± 0.375	2.08 ± 1.05	8
(Adr'x + H + Aldo.	8.75 ± 2.13	0.85 ± 0.30	1.133 ± 0.53	9

$$\text{where P.D.} = \frac{Vm\ x}{Km + x} + C$$

and x = % Hexose

In the computation for Sodium Depletion the programme reached the arbitary lower limit for Km = 0.1 and proceeded into unstable oscillations. It is thought that its true value would have been negative.

TABLE 9. Michaelis-Menton Parameters of the ISC/Substrate curve.

Isc	Vm	Km	C	N
Normal	2268 ± 557	1.38 ± 0.44	89 ± 80	10
Adrx **	8081	14.248	-40.8	5
Adrx + A	1430 ± 882	0.80 ± 0.62	620 ± 335	7
Adrx + H	2371 ± 1670	1.354 ± 1.22	356 ± 168	7
Sod. D **	7755	18.2	81	4
Sod. D.C. **	11089	13 x 10^6	159	4
Sod. L. **	58317	20.5	316	6
Sod. L + A	neg values	neg values	-	8
Adrx + H	458 100	0.167 ± 0.10	514 ± 240	8
Adrx + H + A **	1486	0.679	200	9

$$\text{where Isc} = \frac{Vm\ x}{Km + x} + C$$

and x = % Hexose

TABLE 10.

Probability of the Students "t"

	Normal Krebs		0.5% Mannitol		0.5% (Galatose or Glucose)	
	PD	Isc	PD	Isc	PD	Isc.
Normal Adr'x	$^{80}/_{70}$	$^{10}/_{5}$	$^{20}/_{10}$	$^{30}/_{20}$	$^{20}/_{10}$	$^{2}/_{1}$*
Adr'x Adr'x + Aldo	$^{2}/_{1}$*	$^{2}/_{1}$*	$^{1}/_{0.1}$*	$^{5}/_{2}$*	0.1/*	$^{1}/_{0.1}$*
Adr'x Adr'x + Hydroc.	$^{5}/_{2}$*	$^{2}/_{1}$*	$^{1}/_{0.1}$*	$^{1}/_{0.1}$*	$^{2}/_{1}$*	$^{2}/_{1}$*
Adrx + Aldo Adrx + Hydroc.	$^{60}/_{50}$	$^{60}/_{50}$	$/_{90}$	$^{30}/_{20}$	$^{80}/_{70}$	$^{80}/_{70}$
Sod. Depl. Sod. Depl(control)	$^{50}/_{40}$	$^{20}/_{10}$	$^{20}/_{10}$	$^{30}/_{20}$	$^{80}/_{70}$	$^{20}/_{10}$
Sod. Load Sod. Load + Aldo	$^{70}/_{60}$	$^{10}/_{5}$	$^{90}/_{80}$	$^{70}/_{60}$	$^{50}/_{40}$	$^{20}/_{10}$
Adr'x + Hydroc. Adr'x + Hydroc(Gluc)	$/_{90}$	$^{30}/_{20}$	$^{90}/_{80}$	$^{50}/_{40}$	$^{70}/_{60}$	$^{60}/_{50}$
Adr'x + H (Glucose) Adr'x + H + A	$^{60}/_{50}$	$^{90}/_{80}$	$^{30}/_{20}$	$^{10}/_{5}$	$^{90}/_{80}$	$^{90}/_{80}$

P values as percentage the expression $^{60}/_{50}$ $60\% > P > 50\%$

TABLE 11.

P values.	PD		Isc	
	A	B	A	B
Norm Adrx	$^{20}/_{10}$	$/_{90}$	$^{30}/_{20}$	$^{1}/_{0.1}$ *
Adrx. Adrx + Aldo	0.1/ *	$^{40}/_{30}$	$^{5}/_{2}$ *	$^{20}/_{10}$
Adrx. Adrx + Hydroc.	$^{1}/_{0.1}$ *	$^{40}/_{30}$	$^{1}/_{0.1}$*	$^{20}/_{10}$
Adrx + Aldo Adrx + Hydroc.	$^{60}/_{50}$	$^{10}/_{5}$	$^{40}/_{30}$	$^{70}/_{60}$
Sod. Depletion Sod. Depl. Control.	$^{40}/_{30}$	$^{40}/_{30}$	$^{50}/_{40}$	$^{40}/_{30}$
Sod. Loaded Sod. Load + Aldo	$^{30}/_{20}$	$^{90}/_{80}$	$^{60}/_{50}$	$^{30}/_{20}$
Adrx. + Hydroc (Gal.) Adrx. + Hydroc (Gluc.)	$^{80}/_{70}$	$^{40}/_{30}$	$^{50}/_{40}$	$^{30}/_{20}$
Adrx. + Hydroc (Gluc.) Adrx + H + Aldo (Gluc.)	$^{30}/_{20}$	$^{20}/_{10}$	$^{20}/_{10}$	$^{30}/_{20}$

P as a percentage $^{60}/_{50}$ indicates $60\% > P > 50\%$

4.2 The Effect of Replacing Sodium by Potassium.

Table 12 repeats some of the data from table 4 but places these data beside the corresponding data obtained when Potassium replaced the Sodium in the perfusate. The columns headed "Mannitol" and "Galactose or Glucose" mean that the added substrate was 0.5% Mannitol (i.e. no actively transported Hexose) or 0.5% Galatose (Glucose in the case of the last two rows of data).

It will be seen from the table that Potassium replacement increases the P.D. in every case where data are available. When an actively transported Hexose is present, however, the effect is less dramatic or absent in some cases.

Table 13 is equivalent to table 12 but gives data on the Isc.

As for PD there is an increase in Isc when Potassium replaces Sodium provided Mannitol is the Hexose present. When an actively transported Hexose is present, however, there is a decrease in Isc except in the case of the second last row (Adr'x + Hydroc (Gluc.)).

TABLE 12. The Effect of Potassium replacement on the P.D.

	Mannitol		Galactose or Glucose	
	Sodium	Potassium	Sodium	Potassium
Normal	0.20 ± 0.25	-	3.32 ± 0.25	-
Adrx	-0.46 ± 0.35	-	2.64 ± 0.33	-
Adrx + Aldo	1.91 ± 0.51	3.94 ± 0.25	4.84 ± 0.22	4.56 ± 0.59
Adr'x + Hydroc	1.90 ± 0.37	3.60 ± 0.61	4.67 ± 0.56	3.87 ± 0.64
Sod. Depl.	0.70 ± 0.26	3.33 ± 0.99	3.86 ± 0.62	4.36 ± 0.75
Sod. Depl.Contr.	1.50 ± 0.48	3.48 ± 0.90	4.13 ± 0.19	5.60 ± 0.77
Sod. Load	1.45 ± 0.78	-	3.68 ± 0.30	-
Sod Load + Aldo	1.57	2.65 ± 0.60	4.16 ± 0.52	4.88 ± 0.55
Adr'x + Hydroc (Gluc.)	2.1 ± 0.70	4.3 ± 0.58	4.3 ± 0.58	4.7 ± 0.49
Adr'x + Hydroc + Aldo (Gluc.)	1.13 ± 0.35	4.4 ± 0.49	4.4 ± 0.49	5.6 ± 0.54

TABLE 13. The Effect of Potassium replacement on Isc.

	Mannitol		Galactose or Glucose	
	Sodium	Potassium	Sodium	Potassium
Normal	93 ± 82	-	689 ± 112	-
Adr'x	-40 ± 34	-	231 ± 33	-
Adr'x + Aldo	620 ± 223	821 ± 193	1131 ± 168	979 ± 246
Adr'x + Hydroc	353 ± 84	530 ± 98	1022 ± 228	544 ± 122
Sod. Depl.	81 ± 46	350 ± 50	350 ± 30	338 ± 38
Sod. Depl.Contr.	159 ± 47	323 ± 116	536 ± 117	528 ± 148
Sod. Load	316 ± 169	-	1742 ± 630	-
Sod. Load + Aldo	226 ± 84	497 ± 90	820 ± 90	790 ± 25
Adr'x + Hydroc. (Gluc.)	514 ± 168	836 ± 74	845 ± 175	836 ± 74
Adr'x + Hydroc + Aldo (Gluc.)	200 ± 76	650 ± 163	808 ± 160	931 ± 260

4.3 The Electrical Resistance of Jejunum

The electrical resistance is calculated from the simple equation associated with Ohm's Law.

$$\Delta\psi = IX$$

Where $\Delta\psi$ = open circuit potential difference. (in volts)

I = short circuit current. (in amps)

X = Resistance (in Ohms)

Table 14 gives the collected data on Tissue resistance but it may be found that Figure 15 gives a less confused impression of the salient features, which are

i) A striking increase in resistance due to the replacement of Sodium by Potassium.

ii) An increase due to Adrenalectomy.

iii) An apparent increase in resistance due to the Sodium depletion diet. This increase can be partially explained as an artifact, however. Reference to TABLE 2 will show that the position measurements of the Sodium Depletion pair one greater than those of other groups.

eg. Normal position = 16.7

Sod. Depl. position = 34.8.

No error is involved provided comparisons are made within treatment/control pairs but when comparison is made as above the difference in position must be taken into account. As described in § 2.4 (2) the segment was located by injecting 5 ml. of solution into the intestine at the pylorus. The different lengths of intestine flushed out by this 5 ml. can be attributed to differing radii of intestines. The intestinal segments will therefore have differing surface areas even when the segment lengths are equal. The position measurement can, however, be used to correct for this effect.

A volume V of solution flushes the intestinal with cross-sectional area πr^2 to the position P.

$$\therefore V = P \times \pi r^2$$

$$r = \frac{1}{\sqrt{P}} \times \text{const}$$

The surface area of a segment is proportional to the circumference
The surface area is therefore proportional to $1/\sqrt{P}$
Since the electrical resistance of the segment is inversely proportional to the surface area, the resistance/segment data can be standardized to resistance/unit area by multiplying by the factor $1/\sqrt{P}$.
Standardization of this kind reduces (but does not eliminate) the apparent difference in tissue resistance between normal Rats and those on a Sodium depletion diet but it enhances the increase in resistance due to adrenalectomy.

Table 15 compares the standardization to the standardized mean resistances. These mean data were taken from Figure 15 and ignores the data for Potassium ringer fluid.

TABLE 14 The resistance in ohms/segment.

Galactose/ Glucose conc.	0.0	0.1	0.2	0.3	0.4	0.5	Without Hexose
Normal	2.15	4.48	3.86	4.41	4.74	4.82	5.7
Adr'x	8.7	5.3	12.8	11.0	11.8	11.4	18.6
Adr'x + Aldo	3.07	3.55	3.60	4.03	3.84	4.28	3.5
Adr'x + Hydroc.	5.38	4.86	4.68	5.00	4.80	5.25	3.83
Sod. Depl.	8.63	9.64	8.70	10.01	9.85	11.00	10.5
Sod. Depl. Control.	9.42	8.90	8.30	8.16	7.59	7.71	7.8
Sod. Load.	4.59	3.60	3.03	3.26	2.30	2.11	2.35
Sod. Load + Aldo.	4.18	4.64	6.77	5.03	4.80	4.54	6.3
Adr'x + Hydroc.(Glue)	4.08	3.95	4.55	4.26	4.48	5.07	5.6
Adr'x + Hydroc. + Aldo (Glue)	5.5	5.35	5.15	5.44	5.25	5.45	4.6

TABLE 15 The standardization of Segment resistance.
P = Position

	Unstandardized	P	Standardized
Normal	4.5	16.7	1.1
Adr'x	11	13.1	2.8
Adr'x + Aldo	3.5	17.8	0.8
Adr'x + Hydroc.	4.5	22.4	0.9
Sod. Depl.	9	34.8	1.5
Sod. Depl. controls.	8	30.7	1.5
Sod. Load.	3	17.7	0.7
Sod. Load + Aldo	4.5	22.6	0.9
Adr'x + Hydroc. (Glue)	4.0	20.4	0.8
Adr'x + Hydroc. + Aldo (Glue)	5.0	21.8	1.1

It has been observed (79) that the resistance of Rat jejunum increases as do the P.D. and Isc. with increasing concentration of Glucose. Figure 15 shows that this is also true of normal animals (column J) when Galactose is the actively transported Hexose. The Linear Regression Coefficient of the data (Table 14) is equal to 4.2 and the Correlation Coefficient of 0.79 indicates that the regression Line is significant at the 5% level.

The pattern of increasing resistance is not repeated, however, in all other groups. (columns A - I in Figure 15). It is perhaps significant that the two columns (C and F) which have a negative Regression Coefficient are associated with groups of animals which had saline drinking water (Sodium Loaded and the Sodium Depleted Control group) without having any compensating factor such as Adrenalectomy.

Figure 16 shows that when an overdose of ether is given not only does the P.D. fall exporentially to zero but the tissue resistance increases. This increase in resistance may be due to a fall in blood pressure associated with death.

TABLE 16 The Streaming Potential and Current when 28mM Mannitol is added to the perfusate.

	$\Delta\psi_s$ (mV)	ΔI_s (μa)
Normal	- 1.22	- 159
Adr'x	- 1.76	- 112
Adr'x + Aldo	- 1.22	- 276
Adr'x + Hydroc	- 0.93	- 134
Sod. Depletion	- 1.88	- 148
Sod. Depletion controls	- 1.58	- 136
Sod. Load	- 1.10	- 766
Sod. Loaded + Aldo	- 1.31	- 230
Adr'x + Hydroc. + Aldo	- 1.30	- 319

4.4 The Effect of Intravenous injections of some Hormones.

The results of these experiments, in which a solution of a hormone was injected into the femoral vein of an anaesthetised rat and the PD and Isc of the perfused Jejunum was recorded, were mainly negative. This is not altogether unexpected but there was the possibility that the failure to elicit effects on the everted sac in vitro could have been due to the inability of the large hormone molecules to penetrate either the mucosal surface of the epithelial cells on the thick muscle layers of the intestine. For the sake of completeness the results are given below.

(1) Insulin (dose: 0.5 I.U.) no noticeable effect within 30 minutes of injection.

Perfusate: Krebs-ringer-bicarbonate solution with 0.25% Glucose.

Number of experiments: 3.

(2) Aldosterone (dose: 3mg in H_2O). no noticeable effect within 2 hours of injection.

Perfusate: Krebs-ringer-bicarbonate solution with 0.25% Glucose.

Number of experiments: 2.

(3) Vasopressin (dose: 3 mll in 0.2 ml distilled water).

There was a transient increase in P.D. (no record of Isc) 3 to 4 minutes after injection which lasted for about 5 minutes. The amplitude of this pulse was variable (32% to 59%) on a base line of approximately 2 mV.

Perfusate: Krebs-ringer-bicarbonate solution milk 0.25% Glucose.

Number of experiments: 3.

This last effect (of Vasopressin) has been observed by Field, Plotkin and Silen (95) for Rabbit ileum which had been stripped off the longitudinal muscle. If it were not for this report one would be tempted to explain the effect observed in the present experiments as being due to an increase in vascular pressure. The observation of

Field et al (95) that subsequent applications of Vasopressin produced no effect was also confirmed in the present experiments (in this case even when the dose was increased by a faction of 10).

Figure 17 is a redrawn trace of the Vasopressin 'Effect'.

4.5 The Electrophoretic mobility of Mucosal cells.

Table 17 gives the values of U the velocity of mucosal cells as observed in eye piece divisions/second and the pH of the suspending medium (Sucrose Buffer).

Figure 18 represents these results graphically.

It has been suggested with reference to Red Cells that the electrophoretic mobility varies greatly with storage times at very low pH values (263). Bearing this in mind Figure 18 emphasises that the most reliable (and important) section of the graph lies in the physiological region pH 5-8. Taking these data alone the regression line of velocity on pH has parameters:

$A = -1.680$ $\quad B = 0.372$

where: velocity = A + B (pH).

The Correlation Coefficient = 0.767

which indicates a Probability of less than 2%, that is the regression line is "Significant".

The regression line indicates that the velocity will be equal to zero (isoelectric point) at pH 4.5. Both the regression line and the curve on Figure 18 indicate a velocity of approximately 0.75 at pH 7. and it is this value which has been used to calculate the mobility in cm/second and hence the Zeta potential (ζ).

Calibration data used to calculate the mobility (U) and ζ.

(1) 1 eye piece division = 1.09×10^{-3} cm.

(2) The applied current (I) = 1.5 ma. = 1.5×10^{-3} amps.

(3) The viscosity of Sucrose Buffer at 18°C. (η) = $2. \times 10^{-2}$ poise. (extrapolated from reference tables (275)).

(4) The equivalent conductance (Λ) of Sodium Bicarbonate Solution, the most abundant ion in the Sucrose Buffer (§2.6 (2)).

$= 72.5$ $ohms^{-1}$

(interpolated from reference tables (275)).

(5) Radius of the capillary tube which formed the electrophoresis cell = 1 mm.

(6) The dielectric constant of the medium has been taken to be equal to that of pure water = 80.

The Field strength (E) is given by

$$E = IR$$

where I = current in amps

R = resistance in ohms/cm

E = Volts/cm.

$$\text{also } R = \rho \frac{L}{\pi r^2}$$

where ρ = Specific Resistance

L = length = 1 cm.

r = radius = 0.1 cm.

$$\text{and } \rho = \frac{10^3}{\Lambda C}$$

where C = concentration (gm formula wts/litre)

Λ = equivalent conductance

Substituting the data given above in these formulae

$E = 26.3$ volts/cm.

$= 26.3/300$ esu/cm.

The mobility $U = 1.5 \times 1.09 \times 10^{-3}$ cm/s

$= 1.63 \times 10^{-3}$ cm/s.

Substituting these values in the formula

$$\zeta = \frac{4\pi\eta u}{ED}$$

$= 5.83 \times 10^{-5}$ esu

$= 5.83 \times 10^{-5} \times 300$ volts

$= 17.5$ mV.

TABLE 17

Electrophoretic speed vs pH.

Speed is in units of eye-piece divisions/second.

pH	speed
3.25	0.62
3.5	1.00
5.5	0.41
6.0	0.51
6.0	0.37
6.0	0.89
6.4	0.51
7.1	1.27
7.2	0.79
7.4	0.70
7.5	0.76
7.9	1.54

5. REVIEW AND DISCUSSION.

5.1 Introduction.

This chapter is devoted to a review of the literature on electrolyte and Hexose absorption by the small intestine, and to a discussion of my own experimental results. Inevitably such a review will cover ground which has already been reviewed by established authorities on the subject (1, 2, 3, 4). My aim therefore, has been to keep the review fairly brief in general and to expand only those aspects to which my own results have particular relevance.

5.2 Fluid and Electrolyte.

(1) General Characteristics.

Clarkson (145) has identified two types of epithelial tissue according to their absorptive properties.

(a) Tissues, which in vivo separate solutions of very different ionic strength and composition, possess a high resistance, a large open-circuit voltage and a low short-circuit current (see § 5.4)

(b) Tissues, which in vivo separate solutions of similar composition have low resistance and open-circuit voltage but relatively high short circuit currents.

The small intestine falls into the second category. The high permeability of the tissue in comparison to (say) Frog skin, makes it difficult to distinguish between transport phenomena which are directly powered by metabolic energy (Active Transport) and those which are secondary (or Passive), being driven by the conditions of disequilibria set up by the active transportation. Three types of disequilibrium are frequently encountered.

(a) A spontaneous electric-potential difference (PD or $\mathcal{E}$) is found to exist across the epithelial sheet due to the transport of ions.

(b) Concentration gradients.

(c) The bulk flow of fluid through water filled pores.

Since an epithelial sheet is a multi compartment system of membranes, local disequilibria, of the kinds described above, might easily escape detection, with the result that a particular transport phenomenon will wrongly be attributed to metabolic energy. Indeed, in a situation like this, to distinguish "Active" from "Passive" transport becomes a matter of semantics (200).

One of the main features of intestinal absorption is the rapid flux of Sodium, Chloride and water which is described as 'fluid' transfer rather than water transfer. Hydrostatic and osmotic pressure gradients

are fairly obvious candidates for the role of controlling factor.

(2) Hyrdostatic Pressure.

In the absence of Hexose, the absorption of fluid by the intestine is not assisted to any great extent by a favourable hydrostatic pressure gradient up to and including 85 cm.H_2O, (38) which is greater than the intraluminal pressures normally encountered in vivo (196). However, an adverse hydrostatic pressure gradient of as little as 4 cm.H_2O, can effectively prevent fluid absorption (101). It has been suggested that this effect may be due to physical collapsing or kinking of various structures on the serosal side of the tissue (198).

During in vivo experiments it has been observed that there appears to be an optium intraluminal hydrostatic pressure (192) but it was thought that this was due to stimulation of the intestinal villus motion. When the supply of oxygen was interrupted motor activity was observed when an intraluminal pressure of 35 cm.H_2O was applied (107).

These data refer to gross hydrostatic pressures. There is little information about local internal hydrostatic pressures although these have been invoked to explain one step in the solute-linked fluid transfer (199). The observed accumulation of fluid in the intestinal wall during Glucose-stimulated fluid absorption (13, 201) seems to imply that fluid traverses at least the last stage of its journey through the muscle layers (in vitro) with the assistance of a hydrostatic pressure.

(3) Osmotic Pressure.

There is no doubt that osmotic pressure has a considerable influence on water movement in the colon (106) and in the small intestine, (64, 140, 200) the effect being more pronounced in the jejunum than in the ileum (201). Nevertheless fluid absorption continues to some extent in the presence of suitable substrate despite an unfavourable osmotic pressure gradient (106, 200, 201, 202). Whether this can be described as 'Active transport' or not, is, as Parsons et al have remarked (200), a matter of definition. The osmotic pressure required to eliminate the

net fluid movement is about 5 stmospheres (38).

(4) Local Osmosis.

There is a strong correlation between the movement of fluid and the movement of certain solutes (particularly sodium) in a number of intestinal preparations (69, 140, 38, 90, 122, 203, 204, 205, 206, 207, 209, 210, 211). But there is a problem in deciding which of these two fluxes represents the primary flux. The view of Curran and Solomon (213, 140) is that the Sodium flux is primary with water movement being created by local osmosis. This mechanism is explained in principle by Curran and MacIntosh (199). It requires a system of two semipermeable membrances in series. The first has a high Staverman Relection Coefficient with respect to a single solute species, and the second membrane has a much lower Reflection Coefficient. The solute molecule is pumped across the first membrane into the space enclosed between the two membranes. Water is then drawn into the intermembrance space by the local osmotic pressure across that membrane and the resultant local hydrostatic pressure forces both solute and solvent through the second membrane. In this way a movement of total fluid across the membrane system is achieved by means of a solute pump. The model has been successfully operated using synthetic membrances by Curran and MacIntosh.

A similar suggestion for fluid transport in the gall bladder has been made by Diamond and Tormey (214) but in this case the 'intermembrane space' is considered to lie in the long extracellular channel. "The first membrane" (the osmotic barrier) is the serosal surface of the epithelial cells and the "second membrane" is a transient effect produced by opening and closing the serosal end of the extracellular space. Since Sodium is thought to be expelled from the epithelial cells across their serosal surface (discussed later) this is an attractive theory.

(5) Sodium and Local Osmosis.

The view that the Sodium pump is the most important agent in bringing about the movement of fluid has accumulated some powerful support.

(a) Under normal conditions sodium is transported in the same or slightly greater concentrations as it is present in the mucosal fluid (from which it originated) while the concentration of other solutes vary (38, 54).

(b) It is possible to obtain net Sodium absorption while net water secretion is created by an unfavourable osmotic gradient (208).

(c) When the tissue is short-circuited (see section § 5.4) thereby removing the restraining influence of the transmural electric potential, the flux of Sodium increases without a concomitant increase in fluid transport (72).

(6) Sugars.

Fluid transport in the jejunum is profoundly influenced by the presence of certain Hexoses. This phenomenon will be discussed in the section devoted to Sugars (section § 5.5).

(7) Bicarbonate and pH.

The presence of Bicarbonate is necessary for fluid transport in Rat jejunum (109) and this has been confirmed for Human jejunum (91). Carbonic Anhydrase inhibitor 6063 depresses the absorption of fluid and electrolytes from jejunum and reverses the direction of CO_2 net movement in ileal segments (90) where it is normally secreted (unlike the jejunum where it is absorbed) (90, 92). In small intestine the luminal contents are normally acidic in the duodenum and become progressively more alkaline in the distal regions (e.g. dog, 215). Hydrogen ions are secreted into the mucosal fluid in the jejunum as a bye-product of Lactic acid production in glycolysis (23, 216). This is diminished but not abolished in the presence of Oxygen (80). The effect of acidification (of the lumen) on fluid, Sodium and Potassium flux rates were examined by Code et al (170) in Dog duodenum. They observed that all flux rates increased, including secretion rates and concluded that this was a mechanism whereby the duodem could rapidly achieve

equilibration of luminal contents with plasma before the total solution was reabsorbed by the more distal regions.

McHardy and Parsons examined the effect of pH variations on the absorptive behaviour of Rat jejunal segments. In the region pH 4 to 8 the flux rate of fluid rose in approximately linear fashion with pH. The flux rate of Sodium was parallel to that of fluid below pH 7 but showed a distinct peak at pH 7 (148).

(8) Diffusion Potentials.

The small intestine generates a PD. of a few millivolts (0-10 mV) in vivo and in vitro, even when identical media bathe the two sides of the Tissue. The PD. is therefore due ultimately, if not primarily, to metabolic processes within the tissue. The transfer of any ionic component across the membrane will generate the PD. which will continue to rise until a counter current, driven passively by the PD, is equal and opposite to the first current. Calling these two currents The Active current (Ia)* and the reverse current (Ir) we have

$$Ia + Ir = o$$

$$\text{and } \Delta\psi = Ir.X \quad \ldots\ldots\ldots\ldots \text{Eq.(1)}$$

where X is an ohmic resistance.

Both Ia and Ir however consist of a number of subcurrents I_i where i denotes a number of ionic components.

Therefore, although at steady state Ia = -Ir, it does not follow that for each component $(I_i)_a = -(I_i)_r$

The fact that the Active and Reverse currents may be equal in magnitude but not identical in structure is probably the explanation of the anomalous findings by Wright (176) that the diffusion permeability, coefficient ratio Sodium : Chloride = 10:1 although it has been experimentally observed that Chloride ions are absorbed at a rate approximately equal to that at which Sodium is absorbed (56, 148, 213).

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* "Active Current" is used here to imply "non-diffusional". It is not intended to restrict the term to the conventional functional definition of Active Transport.

It would appear that the Chloride flux is a major component of Ia but not of Ir and is therefore not described adequately by the conventional equation for diffusion potentials.

An equation often used to describe diffusion potentials is:-

$$= R.T. \ln \frac{P_K K_2 + P_{Na} Na_2 + P_{Cl} Cl_2}{P_K K_1 + P_{Na} Na_1 + P_{Cl} Cl_1} \quad \ldots\ldots \text{Eq.(2)}$$

See list of Symbols.

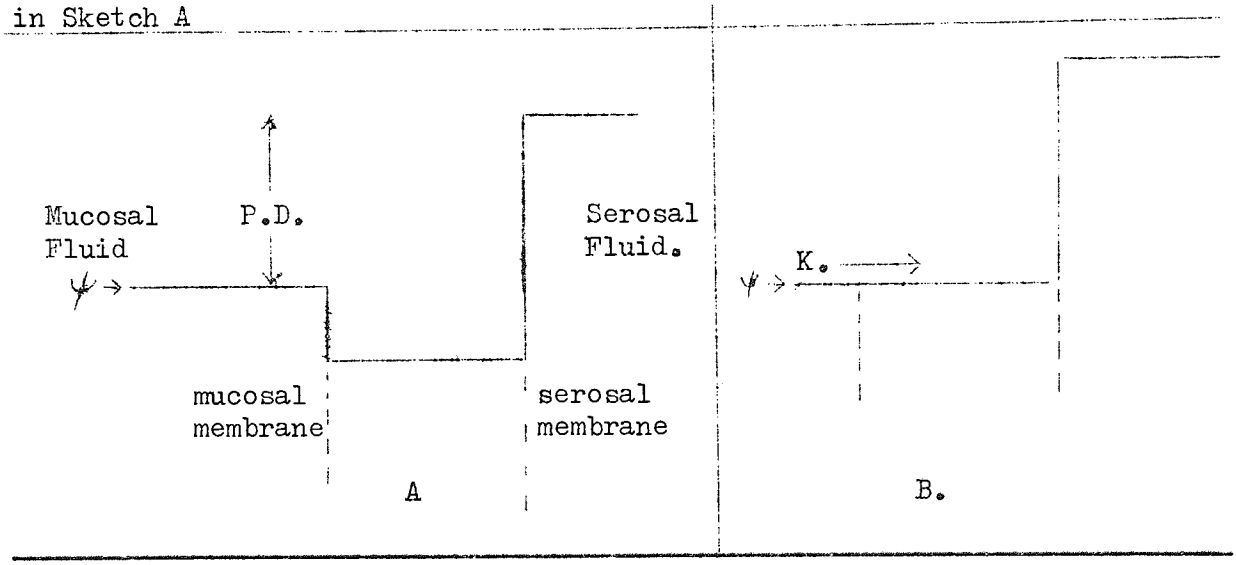
which was derived by Hodgkin and Katz (136) using an analysis due to Goldman (180). As well as assuming that Potassium, Sodium and Chloride are the only important contributers to the diffusion current, this equation depends on the fundamental Goldman assumption, that the gradient of potential is constant throughout the membrane. This assumption most definitely does not hold in the case of small intestine as has been shown by micro electrode puncture (83). The Goldman-Hodgkin-Katz equation, however, will still hold even if the Goldman assumption is not made, provided certain other restrictions are not violated (262). These restrictions require that the membrane should separate solutions of equal ionic strength. The technique of replacing Sodium with a non-ionic solute, such as Mannitol, will therefore give results which are misleading quantitatively, although the qualitative interpretation may still be valid.

It has been observed that the replacement of Sodium by Potassium leads to an increase in the PD. (with 0.5% Mannitol; table 12), and to an increase in Isc (table 13). These observations would be readily explained as a diffusion potential with Potassium having a greater permeability coefficient than Sodium (176) if it was not for the fact that these changes are also accompanied by an increase in the tissue resistance (Figure 15). On a number of occasions the direction of the applied current was reversed in order to check on the possibility that the jejunum possess the property of rectification (§ 5.2 (13)), but there was no indication of rectification the ratio $\Delta\psi / \Delta I$ being identical to that obtained in the 'forward' direction.

It does not appear, therefore, that the increased PD, associated with Potassium perfusate, can be due to an increased flux of cations through water filled pores or by way of any mechanism which is highly susceptable to changes in the P.D. The observed changes may, however, be explained by one of the following:

(a) Potassium, entering readily into the cytoplasm of the mucosal cells from the luminal fluid would eliminate the 'downhill' step in the potential profile at the musocal surface. To explain this more fully,

Wright (83), by micro-electrode puncture, has measured the profile of potential across the jejunum. Schematically it is as shown in Sketch A

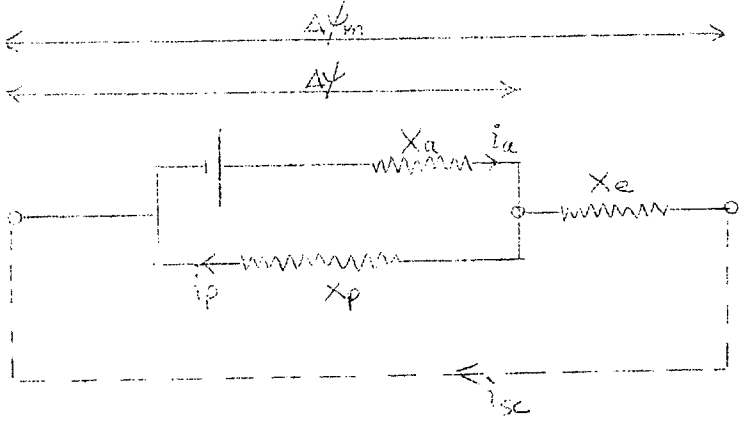


Perfusion by Potassium fluid, however, may produce the conditions shown in sketch B thus increasing the overall PD. by eliminating the reversed mucosal step. In addition the high cytoplasmic concentration of Potassium may increase the resistance of the serosal membrane. If the high step at the serosal membrane is due to the extrusion of Sodium this would necessarily be supplied by Sodium from the serosal fluid.

(b) An alternative explanation is based on the suggestion (which has often been put forward, but most specifically by Clarkson (145)), that there exist two channels for ion transport in the small intestine. One is described as an active channel and the other passive (145). The passive

channel is extra cellular. In addition to these functional channels there exists within the preparation, as described, a barrier including the intestinal muscle layers. This barrier has an electrical resistance which is not present in the physiological functioning of the intact animal and has only been introduced by the positioning of the electrodes. It is, therefore, described as the "error resistance" (Xe).

From this an equivalentcircuit can be drawn to represent the experimental conditions.



where $\Delta\psi, \Delta\psi_m$ = real and 'measured' PD. respectively.

V = equivalent voltage of active transport.

X = resistance

i = current.

suffices a,p,e,sc,m = active, passive, error, short-circuit and measured respectively.

<u>In open-circuit conditions:</u> ($i_{sc} = o$.)

then $i_a = i_p$ and $\Delta\psi_m = \Delta\psi = i_p X_p = i_a X_p$ . . . . . Eq.(1)

<u>In short-circuit conditions:</u> ($\Delta\psi_M = o$)

$\Delta\psi = i_p X_p = i_{sc} X_e$ $\therefore$ $i_p = i_{sc}(X_e/X_p)$

Also $i_{sc} = i_a - i_p = i_a - i_{sc}(X_e/X_p)$

$\therefore$ $i_{sc} = i_a/(1 + X_e/X_p)$ . . . . . Eq.(2)

The term $1/(1 + Xe/Xp)$ is therefore the correction factor for i_{sc}.

Now the measured resistance $Xm = \frac{\Delta\psi_m(\text{open-circuit})}{i_{sc}(\text{sh-circuit})}$

$\therefore$ using Eq. (1) and Eq. (2)

$$Xm = \frac{i_a Xp}{i_a}(1 + Xe/Xp) = Xp + Xe \quad \text{Eq.(3)}$$

(as might be expected)

Consideration of Equations (2) and (3) will show that both i_{sc} and Xm will increase as Xp increases.

This effect might be put in words thus: "As the passive channel resistance increases, not only does the measured tissue resistance increase but the short circuit current appears to increase, due to the fact that that part of the active current which is 'lost' to the short-circuit current by returning passively through the membrance is gradually eliminated".

(9) Solvent Drag.

Ussing has described the phenomenon known as "solvent drag" (137) whereby solute molecules are carried through the membrane system in a stream of solvent. Kedem and Katchalsky (138) have given a more generalised treatment with a physical interpretation in terms of friction coefficients. The fluid movement may arise in three ways

(a) movement of some other ionic species (solute drag)

(b) osmotic pressure gradients

(c) hydrostatic pressure gradients

(10) Mechanical Filtration.

An 'equivalent' pore size of 4Å radius (an average value) has been calculated for intestine from a comparison of the molecular size of neutral solutes and their permeability (139). Five years earlier Curran and Solomon used a value of 36Å radius (140) based on electron-micrographs by Granger and Baker (141) but these pictures are poor by present day standards. The discrepancy may be explained by the observations of Tidball (142) and Tidball and Cassidy (143) that

Calcium and Magnesium ions control the aqueous permeability of the intestine. It has been suggested that this influence is exerted at the junctions between the mucosal cells (144). Such a mechanism may well open up large pores leaving the 4Å radius pore in the cell membrane proper. Clarkson has suggested that in rat ileum, exfoliation of the mucosal cells will open pores whose radius is measured in microns rather than Angstrom Units (145). Such a pore would allow relatively free access of the mucosal fluid to the basement membrane. Clarkson, using an analysis based on that of Kedem and Katchalsky, has presented evidence that 'friction with the ions in the membrane' presents the greatest barrier to electrolyte movement across the intestine rather than friction with the membrane itself (138).

(11) Ionic Filtration.

There seems to be general agreement that the pores in the cell membrane are negatively charged (145, 140, 88). If fluid is forced through such pores, negatively charged ions will tend to be restrained, while positively charged ions will pass through with relative ease. Fluid movement in the intestine, driven by osmotic or hydrostatic pressure gradients are associated with an electric potential which can be explained by such a mechanism (streaming potential). Such observations have been used to calculate an equivalent pore radius of 4Å which agrees with the figure arrived at independently by Lindemann and Solomon (139).

The relationship between the hydrostatic pressure (or osmotic pressure) and the streaming potential which it generates is given by

$$\Delta\psi_s = \frac{-P\epsilon\zeta}{4\pi\eta L} \qquad \text{. . . . . Eq. (1)}$$

where $\Delta\psi_s$ = streaming potential (esu volts) P = pressure (dynes/cm^2)

η = viscosity (poise) L = Specific conductance (esu ohms^{-1})

ϵ = dielectric constant ζ = Zeta potential (esu volts)

Table 4 shows that the PD. falls when Mannitol is added to the perfusate (28 mV). If this effect is attributable to a streaming potential generated by the osmotic pressure gradient then the ζ-potential can be calculated.

$P = 0.68 \text{ atm} = 0.69 \times 10^{6} \text{ dynes}/_{cm^2} = 6.9 \times 10^{5} \text{ dynes/cm}^2$

$\epsilon = 80$

$\Delta\psi_s = 1.2 \times 10^{-3} \text{ volts} = 0.4 \times 10^{-5} \text{ esu volts} = 4 \times 10^{-6} \text{ esu volts.}$

$\eta = 6.9 \times 10^{-3}$ poise (water at 37°C)

$L = \Lambda c \times 10^{-3} \text{ (ohm cm)}^{-1} = 120 \times 144 \times 10^{-6} = 1.73 \times 10^{-2} \text{ (ohm cm)}^{-1}$

$= 1.73 \times 10^{-2} \times 9 \times 10^{11}$ esu reciprocal units

$= 1.56 \times 10^{10} \text{ (esu ohms)}^{-1}$

Substitution of these data in Eq. (1) give a ζ potential of 28 mV. This figure of 28mV is not so greatly different from the 50 mV calculated by Smyth and Wright (88). If the value of viscosity is changed to agree with the value of 10×10^{-3} poise used by them, then the calculated potential increases to 41 mV. Although 6.9 millipoise is the more correct value for pure bulk water at 37°C there is considerable doubt as to whether such figures are relevant to the electrolyte within the double layer. Smith and Wright (88) examined the streaming potential caused by equal concentrations of solutes and by using Mannitol as standard calculated their reflection coefficients. The result was an estimated, pore size of 4Å. In a pore of this size the assumption (that the double-layer thickness is much smaller than the pore radius) which was used to derive Eq. (1) is no longer tenable. Rice and Whitehead (264) have studied the electrokinetic behaviour of very narrow capillary tubes. Quantitatively their conclusions are valid for values of ζ less than 50mV. One result is that the ζ potential calculated from Eq. (1) (ζ-apparent, denoted ζ_a) is less than the true ζ-potential. By inserting the present data into the appropriate equations of Rice et al. (264) an inverse Debye - double layer thickness (K) = 1.23×10^{7} is obtained and the product Kr = 0.465 where

r is the radius of the pore in centimetres. (This is much smaller than anything considered by Rice et al.) Entrapolating from their Figure 4 the surprising result is obtained.

$$\frac{J \text{ apparent}}{J \text{ true}} = 0.05 \text{ approximately}$$

This result is probably incorrect since at the order of Angstrom Units the Poisson-Boltman formula for the potential distribution adjacent to a charged surface will not hold. Nevertheless the result indicates that there is probably a gross under-estimation of the true pore surface potential. Similarly the volume transfer due to electro-osmosis as calculated by the classical formula is an over-estimation which underlines the conclusion of Smith and Wright (88) that fluid transfer in Rat small intestine cannot be due to electro-osmosis. This statement was based on the fact that their various data did not fit the general relationship.

$$\frac{\Delta\psi_s}{P} = \frac{V}{I} \quad \ldots\ldots\ldots\ldots \text{Eq. (2)}$$

where V = volume transfer and I the current both due to electro-osmosis when P = o (266).

While the failure of Eq. (2) to fit the data implies that the fluid transfer is <u>not</u> driven electro-osmotically by a transmural potential (in this case because the fluid transfer is far in excess of that expected from the observed values of $\Delta\psi_s/P$ and I the current), the converse is not tested by this equation i.e. that the potential is generated by fluid transfer.

If it is assumed that fluid transfer takes place through water-filled pores which also determine the electrical resistance of the tissue then using the classical formulae for flow in a right-circular pore we have:-

$$\text{Resistance X} = \frac{l}{L\pi R^2 N} \quad \ldots\ldots\ldots\ldots \text{Eq. (3)}$$

where ℓ = length of pore L = Specific conductivity of the medium.

R = Radius of the pore N = number of pores/cm^2 tissue.

and the Volume transfer

$$V = \frac{PR^3}{6\eta\ell} N \quad \ldots\ldots\ldots\ldots \text{Eq. (4)}$$

where P = driving pressure in dynes/cm^2 η = viscosity (poise)

Eq. (3) gives an expression for $N/\ell = 1/L\pi R^2 X$

Substituting this in Eq. (4) and re-arranging

$$P = \frac{V\ 6\eta\ L\pi\ X}{R} \quad \ldots\ldots\ldots\ldots \text{Eq. (5)}$$

This is an expression for the pressure required to produce of volume flow V $cm^3/sec.cm^2$.

By substituting the appropriate values for L and η and using

$$\left.\begin{aligned} V &= 3 \times 10^{-5}\ cm^3/sec\ cm^2 \\ R &= 4 \times 10^{-8}\ cm \end{aligned}\right\} \text{See ref. 88}$$

$$X = 25\ ohms/cm^2 = 25/9 \times 10^{11}\ esu\ ohms.$$

the surprisingly low figure of

$$P = 55\ dynes/cm^2 \simeq 55 \times 10^{-6}\ atmospheres$$ is obtained.

Such a pressure difference would be created by a difference in concentration of 2×10^{-6} M. That much larger fluid flux rates are not observed must be due to the 'Electroviscous' effect described by Rice and Whitehead (264). This is a clear indication of how firmly fluid transfer is under the control of the pore-phase Zeta potential. It also demonstrates that although the electro-potential does not cause fluid transfer it may well in turn be caused by fluid transfer.

Smyth and Wright (88) found that the apparent value of ζ declined with decreasing pH as one would expect if the ζ- potential is due to dissociated weak acidic groups. In view of what has been said above therefore it is surprising to note that water transfer decreases with decreasing pH (§ 5.2 (7)).

The value of 28 mV. calculated here for the pore-phase ζ- potential is considerably above that of 17.5 mV measured by electrophoresis(§ 4.5)

of the cell surface particularly if, as expected, the surface potential decreases as the ionic strength increases. It therefore seems unlikely that the lateral wall the mucosa form the "pores" through which the fluid passes to generate the streaming potential. It is still possible however that small more highly charged pores exist in the region of the terminal bar.

(12) Zeta Potential.

A charged solid surface in contact with an electrolytic solution induces in the solution an electric counter-charge which takes the form of a diffuse layer adjacent to the surface (Gouy - Chapman layer). When such an electrical double layer is placed in a tangential electric field the two layers are, as a result of their opposite polarities, driven in opposite directions. The result is a relative motion of surface and solution. Shearing at the interface takes place within the solution leaving a very thin layer of molecular dimensions clinging to the solid surface and it is the electric potential difference between this layer and the bulk solution (reference zero) which controls the electrophoretic velocity of suspended particles. It is called the "Zeta" potential (ζ). For a cylindrical particle with axis parallel to the applied field the following relationship holds

$$\zeta = \frac{4\pi \eta u}{ED} \times 9 \times 10^4$$

where ζ = Zeta Potential (volts)

η = coeff. of viscosity (poise)

u = electrophoretic velocity (cm/sec)

E = Field Strength (volts/cm)

D = Dielectric const. of the medium (dimensionless) (D taken as 80 for water)

(for derivation see for example Glasstone (254)).

The factor 9×10^4 converts both ζ and E from esu terms to volts and volt/cm respectively.

This relationship has been used by many workers in the field (256, 257, 258), and applies to non-conducting cylindrical cells only. The electrical resistance of rat small intestine is considerably greater than that of the free solution so that the isolated cells may be regarded as "non-conducting" despite their ability to maintain high rates of electrolyte transport.

A study of the ζ potential is important for several reasons. Clarkson (145) has outlined a hypothesis regarding the movement of electrolyte through the spaces vacated by exfoliated mucosal cells. The electric charge on the surface of the surrounding cells would therefore play an important part in the generation of streaming potentials and of related phenomena. Clarkson calculated, from his data on water (volume) transport against imposed electric current, that the ζ potential was about 14 mV. The figure of 17.5 mV calculated here is in remarkably good agreement. It should be borne in mind that the experiments described in this thesis (§ 2.6 (2)) were carried out at a subnormal ionic strength (with Sucrose replacing Sodium in normal Krebs ringer) and that it has been found that the ζ potential of Toad bladder cells is proportional to the inverse of the square root of the ionic strength of the medium (256) and by analogy one would expect the ζ potential of intestinal mucosa to decrease as the ionic strength is increased to normal. It is unwise, however, to be catagorical at this stage in view of the amount of work which remains to be done on this problem, and it should be noted that an increase of ζ with increasing ionic strength has been found for red cells (262). It can be stated firmly that the surface of the intestinal mucosa carries a negative sign at physiological pH values.

The sign and magnitude of the surface charge density can have an influence over the ionic composition and pH of the medium adjacent to the surface. The Gibbs-Donnan equilibrium relationship has been used successfully to calculate the pH of the surface layer of Cetyl Sulphate

micelles (265). The same workers also investigated the Na/Ca ratio in the surface layer. As anticipated the negative surface layer favoured the divalent ion but the effect was even more pronounced than the prediction made using the Gibbs-Donnan relationship. This was attributed to binding of Calcium to the surface (265).

The pH of the surface layer associated with epithelial transporting cells is of crucial importance in the transportation of weak organic acid and in considering the transport of ions (and Sodium in particular) it is necessary to know that concentration of these ions the transporting cell "sees". The important region of the surface of intestinal mucosal cells is the brush-border. It was observed that in an electric field the cells, besides moving towards the positive pole, also habitually orientated themselves with brush-border pointing at the negative pole. There are two possible explanations for this:-

(i) The brush-border is less negative than other regions (or is positively charged).

(ii) The orientation is due to the dart-like shape of the mucosal cell.

It does not appear possible to establish which explanation is correct with the experimental apparatus and cell preparation described here. Preparations of brush-borders only have been described (93) but the method of preparation, involving Tris disruption, does not appear likely to leave the ζ potential unaltered. Alternatively the ζ potential might be measured in situ using a longitudinal electric field to drive a stream of fluid along a section of the intestinal lumen. The technical problems, however, seem formidable.

(13) Rectification.

The phenomenon of membrane "rectification" is analogous to rectification in electronics. A membrane which rectifies has a resistance to the passage of water and solutes which varies with direction. For example, it has been shown for Toad bladder that a

large fluid flux can be produced in the mucosal to serosal direction by a favourable osmotic pressure gradient while an equal but opposite osmotic gradient produces only a small fluid flux in serosal to mucosal direction (159).

Oki (161) has made a theoretical examination of the conditions required for rectification. He has concluded that rectification will occur when:

(a) The membrane is pierced by changed pores.

(b) The two surfaces of the membrane carry surface changes of opposite polarity.

(c) The surface change density is in excess of the ionic charge concentration of the bathing media.

The situation can be explained qualitatively thus: The membrane is sandwiched between two oppositly charged "atmospheres" of counter ions (The Gouy-Chapman layers) and only one of those has a change sign favourable to a passage through the changed pores.

Reference to the possible existance of Rectification in jejunum has been made in §5.2 (8) §5.6 (2) and §6. It should be noted however that the opposite poles of the mucosa could each possess rectifying properties with opposite orientations which would give an overall high ohmic resistance.

5.3 The Sodium Pump

(1) Introduction.

Present knowledge of this ubiquitous phenomenon owes much to research on nerve axons, red cells and amphibian epithelia. Baker (247) has written an excellent short review of its subject. Only those aspects which are relevant to the present discussion have been noted here.

(2) A summary of the Properties.

(i) The Sodium Pump has an energy requirement which is supplied by Adenosine triphosphate (ATP) in Squid Axon (268)

(ii) Located on Red cell membranes is an enzyme system often referred to as "Na-k-membrane ATPase" which is sensitive to Sodium and Potassium and Hydrolyses ATP to ADP. (248)

(iii) Similar Membrane - ATPase systems have been isolated from a wide variety of tissues. (249)

(iv) Reversal of the Sodium pump in Red cells (by alteration of the Sodium - Potassium concentration gradients) can result in the incorporation of inorganic phosphate into ATP. (250)

(v) The oxygen uptake of kidney Homogenates in relation to the intracellular concentration of Adenosine Diphosphate (ADP) is regulated by cell membrane ATPase (251)

(In addition Potassium is able to stimulate oxygen uptake by another mechanism, possibly a direct effect on the mitochondria)

(vi) In transporting intestinal epithelial cells the functioning Sodium pump is thought to be located on the serosal membrane since the electropotential profile as examined by microelectrode seems to indicate that the thermodynamic 'uphill' step occurs at this point.

The situation is more complicated, however, in Frog skin and Toad bladder where two positive or uphill steps have been detected and in some cases more. (252, 154)

(vii) ATP stimulates the P.D. of Rat jejunum. (85)

(viii) Sodium transport is diminished but not abolished by lack of oxygen in turtle bladder. Under anaerobic conditions it has been found that Sodium transport is linked stoichiometrically with glycolysis (269)

(ix) It is also suggested that Sodium controls the aerobic glycolysis of Glucose and some other substrates by controlling the supply of ADP. in pig ciliary processes. (270)

Although some of the above remarks are tentative there appears to be a growing body of opinion that the transport of Sodium is linked to Glycolysis through the supply and conversion of ATP to ADP. It might be said that the Sodium pump acts as an escapement mechanism to the Glycolytic pathways.

5.4 The Short Circuit Current Technique.

(1) General.

The Short Circuit Current technique is a method for measuring the ionic current traversing a membrane which is separating two accessible bodies of fluid. By means of two electrodes and an externally energised circuit the electric potential difference (PD) (which is often found to exist across biological membranes) is cancelled to zero. The electric current required to maintain this condition is exactly equal to the net ionic current traversing the membrane. Ussing and Zerhan (134) who introduced the term 'short circuit current' (Isc) used identical bathing media on either side of the membrane (frog skin). In such a case the net ionic current is moving between media of identical electrochemical potential and is, therefore, the "active transport current", by definition. If the media are not identical then part of the ionic current is driven by the thermodynamic disequilibrium. The condition of zero PD is, however, still important, not least because the electrical resistance of the tissue is defined by the relationship:

$$\Delta\psi = I\dot{X}$$

where $\Delta\psi$ is the open circuit potential (PD)

I is the current at zero potential (Isc)

X is the resistance.

A useful form of this relationship is $X = \frac{\Delta\psi}{\Delta I} = \frac{d\psi}{dI}$

where $\Delta\psi$, ΔI are the related changes in PD and current respectively and $d\psi/dI$ is the associated differential (136)

(2) Short Circuit Current and Toad Bladder.

Using a double labelling with radioactive isotopes ^{22}Na and ^{24}Na Ussing and Zerhan were able to show that Isc equalled the net flux of Sodium. It was concluded that sodium was actively transported and was responsible for the spontaneous transmural PD (134).

(3) Short Circuit Current and Small Intestine.

This experiment has been the inspiration for a number of similar experiments designed to find the origin of the PD generated by small and large intestine (75, 81, 94, 79, 72, 74, 73).

In Rat ileum the net Sodium transfer and the short circuit current (Isc) are almost exactly equal (72). This is also the case in Rabbit ileum (73, 74). In rat jejunum, however, the relationship is more complex. The PD and/or Isc of rat jejunum is greatly enhanced by the presence of the actively transported hexoses (20, 21, 22, 61, 62, 70, 75, 76, 77, 78, 79, 81, 82). With Glucose in the bathing medium the net Sodium flux can account for about $\frac{3}{4}$ of the Isc, but when Galactose replaces Glucose the net Sodium flux is only equal to about $\frac{1}{4}$ of the Isc (75).

So far no stoichiometric relationship has been found between Isc and any transferred component of the bathing medium (78).

5.5 Absorption of Sugars.

(1) Transfer Steps

The transport of monosacharides through the intestine is thought to take place in at least two identifiable steps (33):-

i) an entry mechanism which transports hexose across the cell membrane and by itself would achieve thermodynamic equilibrium with the environment in terms of the transported material.

ii) an active transport mechanism which results in the transported material being concentrated within the cell (24). A third step is implied by this scheme, namely the exit of the hexose through the serosal border of the mucosal cells (33). There is little information about the nature of this exit mechanism. It has frequently been assumed that it is governed by simple diffusion. On the other hand there is considerable data relating to steps i) and ii).

(2) Specificity and Energetics.

Step(i) is thought to be a carrier mediated process with a wide specificity for sugar molecules. L-Arabinose, L-Rhamnose and L-Fructose enter the cell in this way along with D-Glucose, D-Galactose, 3-O-Methyglucose and a variety of others (32). The entry step is probably unaffected by lack of Oxygen or the presence of DNP (33,130,50) Analysis of the mechanism by means of the Michaelis-Menton Kinetic scheme (35, 55, p.227) shows that all the sugars tested have the same maximum rate of transfer and that there is competition between them (34, 35) which suggests strongly that there is only one species of carrier (32).

Step (ii) has a much narrower specificity than step (i) (32) and produces the active transport of certain sugars across the intestinal epithelium. Step (ii) is inhibited by lack of

Oxygen and the presence of DNP (33), and is therefore dependent on metabolic energy. It has been suggested that in particular the Citric Acid Cycle provides enough for step (ii) (33,37,126).

The presence of Sodium has profound influence on the behaviour of hexose transfer. This phenomenon will be discussed in detail later (§5.8).

(3) Localisation.

The location of these steps in relation to morphology has been the subject of a number of studies. Phlorrhizin is a glycoside which has been shown to have an inhibitory effect on hexose transfer (40,33,31,53, 41,43,45). This effect is demonstrable at concentrations of phlorrhizin lower than that at which it inhibits endogeneous metabolism (43). Phlorrhizin, a known inhibitor of hexose absorption (39), inhibits the transfer of hexose, not merely the concentration thereof (i.e. step (i) rather than step (ii)) (33) and an analysis of the inhibition on the basis of Michaelis-Menton Kinetics suggests that it competes with the hexose for sites on the carrier molecule responsible for step (i) (45,51). High resolution autoradiography of phlorrhizin -^{3}H in rings of Hamster small intestine has shown that the glycoside accumulates in the brush-border of the mucosa (47,48). From this evidence Stirling (48) has calculated that there are some 2.6 x 10^{6} transporting sites per epithelial cell or 1700 carrier molecules per μ^{2} of cell membrane, with a turnover number of 21 molecules/carrier/second. This is fairly convincing evidence that step (i) is located in the brush border.

The location of step (ii) is more speculative but the fact that Galactose can be shown to accumulate within the cell indicates that the site is probably near the luminal pole of the mucosa (48, 24, 29). It has been shown that there is a considerable local concentration of enzymes such as Alkaline Phosphatase in the brush border of the mucosa (93,97,98,226,227) but the significance of these observations is debateable (135).

(4) Specificity and Mechanisms.

Until recently there has been widespread agreement that the chemical specificity for the active transport of sugars (i.e. step (ii)) is:-

a) a D-pyranose ring

b) a Methyl or substituted methyl group at carbon 5

c) a Hydroxyl group in the glucose formation at carbon 2. (6,7)

Two recent observations have necessitated a modification of this view.

1. D-Xylose is avtively transported to a slight extent in the small intestine of both Bullfrog (8) and Hamster (9,28)
2. It has also been found that L-Glucose undergoes active transport in Rat small intestine under conditions where endogenous D-Glucose is either lacking or greatly reduced (10).

These observations apart, the sugars which undergo strong active transport include:-

D-Glucose, D-Galactose, 3-O-Methylglucose, 1-Deoxyglucose and 6-Deoxyglucose. (11,6,7,5,12,13,18,17). D-Glucose is metabolized in the intestinal mucosa (27,80,14,23,15) but the bulk of the transported glucose appears in the mesenteric blood relatively unscathed (14,15,16,17,23,26,30). A popular theory was that the Hexose is first phosphorylated and then dephosphorylated in the process of being actively transported (119,118,53). However, experiments have shown that in Hamster an insignificant proportion of transported Glucose passes through the pool of Glucose-6-phosphate (15,26).* It is generally held, therefore, that while a

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* On this subject Crane (46) made an interesting comment in 1962:

"The experiments of Landau and Wilson [(15)] have also been frequently cited as evidence against phosphorylation-dephosphorylation as a possible mechanism of sugar active transport. However, with our present knowledge of the location and specificity of the active transport process, it is clear that the observation that actively transported glucose does not pass through a pool of glucose-6-phosphate labelled with C^{14} through the metabolism of C^{14}-galactose actually does not bear on the question of whether phosphorylation is involved in active transport, ----- what has been disproved is a specific hypothesis for phosphorylation-dephosphorylation involving the cell as a whole, not the possibility per se if it were restricted in distribution to the brush-border region. There are no experiments known to us that test this possibility."

fraction of the absorbed Glucose contributes to the general metabolism of the mucosa cells (15), the bulk is transported by a mechanism which does not require any chemical alteration of the molecule. This view depends largely on the assumption that there is only a single active transport mechanism. Experiments using the other sugars which share the hypothetical single mechanism have eliminated a number of possible molecular transformations (16,17,11). It would appear that, if the transferred sugars are obliged to form some kind of bond or link with a carrier molecule, then the bond does not involve the removal of any atoms from the hexose molecule; a Hydrogen bond perhaps.

(5) Sugars and Fluid Absorption.

It has been known for a decade that the presence of Glucose in the intestinal lumen stimulates the absorption of fluid (202,37,38,56,69,91,105, 113,115). Fisher (202) concluded that the absorption of water was an "active process" with Glucose acting as a nutrient. Although the question of active water transport has been the subject of semantic argument, the role of glucose as a nutrient has subsequently been confirmed in a number of ways.

(a) The presence of Glucose in the serosal fluid will support in <u>in vitro</u> preparations, provided it is present at a sufficiently high concentration. (109)

(b) Fluid transport is supported by Fructose (which is metabolized by jejunal mucosa but not actively transported, as such) (38,120)

(c) Fluid transport is not supported to any extent by Galactose*

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*This does not appear to be the case for Human jejunum where Galactose does support fluid transport (218) and in dog (219). These differences could, however, be due to differences between <u>in vitro</u> and <u>in vivo</u> experiments. It is possible that Galactose is only able to support fluid transport only when there is in addition an endogenous supply of substrate. According to Holdsworth and Dawson (218) Fructose is not able to support fluid absorption to the same extent as Glucose or Galactose in Humans.

(which is actively transported but not metabolized to any extent by Rat jejunal mucosa) (38).

Accepting that carbohydrate metabolism plays a role in the transport of fluid the question arises as to whether it is possible to discover the precise section of its many possible metabolic pathways. In Rat jejunum Pyruvate, Succuiate and Citrate do not stimulate fluid transport (115), unlike ileum where Pyruvate has a stimulatory effect (54). It would appear then that the energy for fluid transport is not derived from the Citric Acid Cycle at least in the jejunum. It has been shown that there are at least two types of fluid transfer in small intestine (125) both being dependent the presence of oxygen but the first a "glucose dependent mechanism" predominates in the jejunum and may derive energy from glycolysis or the monophosphate shunt (116,126) while the second mechanism is "Glucose-independent" and probably obtains energy from the citric acid cycle. This second mechanism predominates in the ileum (126,116). Observations on the effect of phlorrihizin which blocks hexose entry (§ 5.5 (3)) are in agreement with this scheme (40,125,128).

5.6 Aldosterone and Electrolyte Transport.

(1) General.

Mulrow (149) in a comprehensive review of the metabolic effects of Aldosterone used these words in his introductory paragraph:

> "After a decade of occupying a central role in the hypothesis concerning salt and water metabolism, aldosterone is now being placed in its rightful role, an important but limited one; it is necessary for the fine regulation of Sodium and Potassium excretion and, in a permissive way, in the formation of edema".

In mammals the kidney is the organ which is most obviously concerned with the excretion and retention of electrolytes, but it is a difficult tissue to work with and for some time toad bladder has been used as a simple analogue of mammalian kidney (181). So far most of the evidence about the mechanism of action of Aldosterone is due to experiments with toad bladder and this information will be reviewed below. The alimentary canal, however, is also deeply involved in physiology of electolyte balance. The colon in particular is a site of Sodium and water retention and not surprisingly the hormone Aldosterone has been found to exert an influence on this tissue. That the hormone also has a demonstrable effect on jejunum is perhaps more surprising. This region of small intestine has different energy requirements from ileum and colon for its transport mechanisms (see section § 5.5) and is much more permeable to the two-way movement of fluid. In the jejunum a considerable amount of equilibration takes place between the luminal contents and the plasma under the influence of osmotic forces (170). The physiological significance of an 'Aldosterone effect' is therefore obscure but its study may nevertheless throw some light on energy requirements of Aldosterone action.

(2) Toad Bladder.

It has been established that, for toad bladder, the short circuit current is equivalent to the actively transported Sodium current (150) (for an

explanation of these terms see section § 5.4). Crabbé, making use of this fact, showed that the Sodium transport of isolated toad bladder was enhanced by injections of Aldosterone given to the intact animal before sacrifice (151). A similar effect was obtained by keeping the animal in a low Sodium environment (151), which raised the endogeneous Aldosterone secretion. Incubation of the isolated tissue in a medium containing the hormone also enhanced Sodium transport after a delay of up to 2 hours (152). This increase of Sodium transport was accompanied by an increase in the 'tissue pool' of radiosodium derived from the medium bathing the mocusal surface (156). To undersatnd the significance of this we must consider how Sodium moves through Toad bladder under normal conditions. (see § 5.3).

Sodium appears to enter the mucosal surface of the transporting epithelial cells in a passive manner (that is 'downhill' in the thermodynamic sense), while the active step (uphill) occurs at the serosal border of the epithelial cells (153, 154, 155). The Sodium pump can therefore be thought of as an "extraction pump" as distinct from a "force pump" (which would concentrate the substrate within the cell). An increase in the activity of the Sodium pump would therefore be expected to lower the amount of Sodium within the cell and an increase in cellular Sodium indicated an increase in the passive entry rate. Accordingly, Crabbé and De Weer reached the conclusion that Aldosterone increased the premeability of the mucosal surface of the ephithelial cells, thereby providing more substrate for the Sodium pump which continued normally at a submaximum rate (156, 157, 182). Fanestil, Porter and Edelman dispute this view (158). They subjected an isolated toad bladder to an artificial PD sufficiently high to make Sodium outflux exceed Sodium influx. The ratio Na(out)/Na(in) was found to decrease under the influence of Aldosterone. They argue that a mere increase of passive permeability could not account for this and suggest instead that the hormone stimulates the Sodium pump (158).

These observations appear to be mutually contradictory on the basis of the elementary model of a single sheet of epithelial cells. They can be reconciled, however, if Sodium outflux takes place mainly through extracellular spaces. It is also possible that the individual cell membranes impose directional properties even on the passive flux of solute and solvent. If Aldosterone affects the charge density on the surface of the mucosal membrane in a way that increases the rectification effect (see § 5.2(13)) this would explain the observations noted above.

(3) Toad Colon.

Colon of toad responds to Aldosterone in very much the same way as Toad bladder (168, 169). Again an incubation period of several hours (3 hours in this case) or preinjection the evening before sacrifice is necessary for the effect to become demonstrable.

(4) Mammalian Colon.

Aldosterone injections give rise to increased Sodium and water absorption by Human colon (171) and in Rat colon Aldosterone is able to restore the electrolyte balance to normal after adrenalectomy (177). The effect on Rat colon of a prior treatment of Sodium depletion is largely as one might expect from the above, if the main effect of the treatment is to raise the endogenous secretion of Aldosterone (174, 175). One important difference between these results and those for amphibians is an increased time delay (or latent period) before the onset of the Aldosterone effect. A latent period of 24 hours was noted in Humans (171) and in rats, although the first effects on PD were noted 80–110 minutes after injection, the PD became considerably enhanced if injections were continued for several days (177). In these observations the increased PD was thought to be largely but not entirely due to increased Sodium absorption (175). One unexplained observation was the failure of a course of Aldosterone injections to alter the faecal electrolyte of either normal subjects or those with longstanding ileostomies (173).

(5) Small Intestine.

Early work by Clark demonstrated that adrenalectomy diminished the rate of Sodium Chloride absorption from the intestine (183), but this was largely attributed to lack of Glucocorticoids which influenced Glucose absorption, and through this, salt balance (184). Other workers have confirmed that Glucocorticoids promote Glucose absorption (185). Aldosterone, injected into anaesthetised rats altered the two-way flux of both Sodium and Potassium in such a way, that Sodium exchange increased, Potassium exchange decreased, and the net effect was nil on the electrolyte balance (186). These rats were not adrenalectomized, however, and the segment of intestine studied was subjected to a preparatory rinsing of distilled water. It has already been shown that such treatment considerably alters the absorption of water (187). Crocker and Munday (172, 183) were abable to find any direct effect of Aldosterone on isolated sacs of jejunum, but once again, prior injection (5 μg) of intact animals followed by a delay of more than 24 hours produced an increase in Sodium and water absorption. These workers related the long latent period to the functional life cycle of the intestinal mucosal cells which a are generated in the crypts of Lieberkuhn and migrate to the tips of the villi in 24 hours, where they are exfoliated. The animals in these experiments are intact, but were given a high Sodium diet to depress their endogenous secretion of Aldosterone. Some such treatment appears to be necessary for similar injections in normal animals produced no effect (172).

(6) Potassium.

Potassium secretion in colon of humans, rats and dogs is increased by the action of Aldosterone (177, 179) but in one experiment the effect was produced by continuous infusion of Aldosterone which implies that at least one component of the effect has a very short latent period indeed. It is possible that the hormone cuases an initial outflux of Potassium from the cellular cytoplasm (associated with the onset of protein synthesis) which is followed some hours later by an enhanced Sodium-Potassium exchange

mechanism at the cell membrane. This hypothesis is supported by the fact that, during their continous infusion experiment, Shields and Mulholland and Elmslie observed no increase in Sodium or water absorption (179). Sodium depletion has a stimulatory effect on Potassium secretion in Rat colon provided Sodium is present in the lumen (presumably available for exchange) (174, 178). Adrenalectomy has an inhibitory effect on Potassium secretion (177).

(7) Protein Synthesis.

There is no doubt that the action of Aldosterone is mediated by protein synthesis since the effect is blocked by Actinomycine D (162, 163). Puromycin and Cyclohexinide have similar effects to Acitomycin D and their effects quantitatively correlate with their inhibitory effects on RNA and protein synthesis (163). Puromycin, however, has an inhibitory effect on Sodium transport as well as blocking the action of Aldosterone (162). An RNA fraction which is rapidly labelled by a radioactive precursor in Toad bladder has been isolated, and it is found that the rate of labelling is enhanced by the presence of Aldosterone (164). A number of substrates which participate in the Citric Acid cycle have been found to be synergistic with Aldosterone (165, 166). From this, and work with inhibitors, Fimognari, Porter and Edelman have concluded that Aldosterone exerts its influence "by stimulating a step or steps in the Tricarboxylic Acid cycle at a point between Condensing enzyme and x-betoglutarate dehydeogenase" (165). Falchuk and Sharp, however, state that this conclusion is not justifiable and that the only valid conclusion on present evidence is that "the functioning of an intact Tricarboxylic Acid cycle is necessary for the full expression of the action of Aldosterone" (167).

The delay of 2 hours noted by Crabbé (152) before the onset of the 'Aldosterone effect' in Toad bladder fits well with this idea of protein synthesis via RNA.

5.7 Glucocorticoids and Intestinal Absorption.

There is ample evidence that glucocorticoids exert an influence on the enzymic components of the brush-border of intestinal mucosa in rat (221, 222, 223) and in chick (224) this is particularly true of maturation processes in young animals (221, 224). A large part of the enzymic component is mono and disaccharidases (93, 97, 98, 226, 227) but it has not been shown that these are necessarily directly concerned with the transplantation of their substrates. Indeed it has been shown that in Rat small intestine the density profile of monophosphate activity is totally different from the transport activity profile of glucose (225,117).

There is, however, some indication that glucocorticoids have a stimulatory effect on Glucose absorption (223, 228, 185). Moses and Streeten (271) have reported that Cortisol has a log-dose related stimulatory effect on the entry of Sodium into the red cells of adrenalectomized dogs in vitro. This effect is abolished by Glucose deprivation and 2-Deoxyglucose. Cortisol (150-1000 $\mu g/l$) causes a similar enhancement of Glucose utilization. These workers suggest that the hormone exerts its effect by stimulating the entry of Sodium which is linked by an ATP/ADP conversion to the Embden-Meyerhof pathway.

5.8 The Sodium-Hexose Link.

(1) Introduction.

In 1958 Riklis and Quastel made the important observation that the presence of Sodium in the lumen of Guinea-pig small intestine is necessary for the absorption of Glucose, (52) confirmed later by others using different preparations (58, 60, 63, 65, 66, 102, 68, 67). Later, it was shown that the presence of Glucose greatly increased the electric potential difference (PD) generated by Rabbit ileum and that this effect was mimiced by hexoses which were actively transported but not metabolized by that tissue (62). Similar observations were made in other species (20, 21, 22, 51, 61, 76, 81, 82, 86, 89). Since there is ample evidence to connect P.D. with Sodium transport in amphibian bladder, skin, squid axon and rabbit ileum (for example) these two phenomena have served as a springboard for a number of interesting speculations.

(2) Two Hypotheses.

Curran (217) noted that the active transport mechanisms of Sodium and Glucose are thought to be located at opposite poles of the mucosal cells (§ 5.3, § 5.5). A link between these at either site would therefore couple two solute species one of which was undergoing active transportation and the other moving downhill in a thermodynamic sense. In particular he suggested that metabolic energy is supplied to the Sodium pump only and that the coupling enabled Glucose to utilize the energy of the 'passive' Sodium entry. It follows that Glucose would have to have some secondary effect on the Sodium flux either by acting as a nutrient for the Sodium pump or by helping to eliminate some passive barrier to Sodium flux, otherwise the presence of Glucose would decrease rather than increase the Sodium flux since it would act as a secondary load on the energy of the Sodium pump. This hypothesis is similar to the one put forward by Schultz and Zalusky (111).

Crane (46) put forward a specific hypothesis concerning the link between hexose entry and Sodium. He suggested that the carrier

responsible for hexose entry (§ 5.5(2)) has receptor sites for Sodium and Potassium, and that the affinity of the carrier for suitable hexose molecules is greatly enhanced when Sodium shares the carrier. On entering the cell together Sodium is replaced by Potassium and the carrier with its affinity changed releases the hexose molecule. The stimulation of the P.D. by hexose transport would then be due to an increased supply of Sodium to the Sodium pump. He was able to show with Bosackova (59) that sugar and Sodium entry rates correlated well over a wide range but that the intracellular Sodium level appeared to be unimportant. Crane and his fellow workers have used this attractive model for a number of experiments involving kinetic analysis of of concentrations vs. PD. (20, 21, 22, 51, 70, 49), and it was Crane's work which, to a large extent, inspired a part of this thesis. However, the discovery by Barry et al (75) that the enhanced PD associated with Galactose transfer in Rat jejunum is not accompanied by an enhanced Sodium transfer, has shown that the model is inadequate, without actually disproving it. The Sodium-Hexose-Carrier complex could still exist and function as described by Crane if at least some of the Sodium is returned to the lumen as suggested by Parsons (212), but in this case the Sodium pump could not generate the P.D. and the search for the source of the P.D. must be directed elsewhere. Moreover it has been shown that when Glucose is the substrate the increase in P.D. is almost entirely associated with an increased P.D. across the senosal surface of the cell in the intestine of Greek Tortoise (83). So far as I am aware this experiment (the measurement of the P.D. 'profile' through the mucosal cells by means of microelectrodes) has not been carried out using Galactose as substrate. Such an experiment would appear to be crucial, for much of the confusion surrounding this problem is caused by the fact that Glucose is both metabolized and actively transported by jejunum (§ 5.5). By using sugars which are either metabolized or actively transported (but not both) it has been shown that the enhanced P.D. is

associated with active hexose transport while enhanced Sodium and Fluid transport is associated with hexose metabolism (75, 76, 62, 77).

Furthermore the data presented by Lyon and Crane for Rat jejunum do not fit conspicuously well to the straight line plot of the Michaelis-Menton scheme (230 , see fig. 7, p.68). In spite of these reservations the strategy of the experiments described in this thesis was based on the "Crane hypothesis" and was argued as follows:

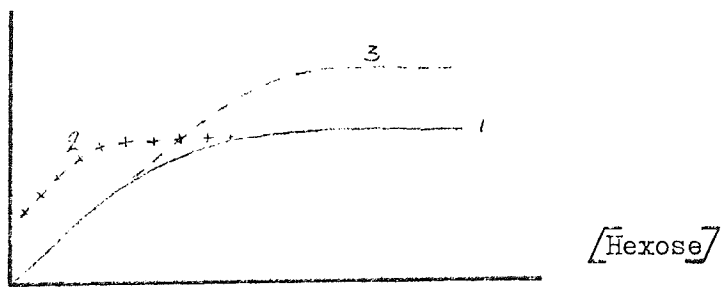
(3) Rationale.

(i) Increasing Hexose concentration increases Sodium entry and hence P.D.

(ii) Aldosterone is similarly thought, by Crabbé and others, to increase Sodium entry (§ 5.6).

(iii) Aldosterone is thought, by Edelman and others, to enhance the functioning of the Sodium Pump. (§ 5.6).

The presence of Aldosterone should therefore alter the shape of the sugar-P.D. graph (if the hormone affects jejunum (§ 5.6(5)) as is shown schematically below:-



(1) - Normal curve on adrenalectomized animals.

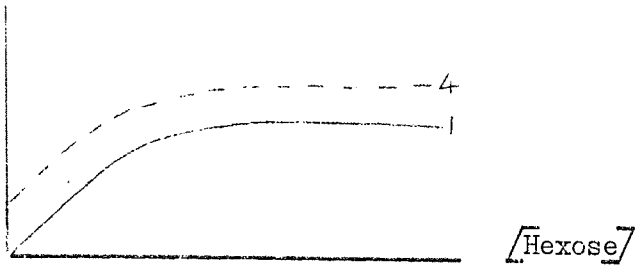
(2) - Aldosterone affects Sodium entry (Crabbé) and reduces the Hexose-dependent maximum P.D. (Vmax).

(3) - Aldosterone affects the Sodium pump (Edelman) and raises the Hexose-dependent maximum P.D. (Vmax).

§ 5.8

- - - assuming that Vmax is imposed by the saturation of the Sodium pump. If, however, Vmax is imposed by a Sodium entry mechanism the pump remaining unsaturated always, then Aldosterone will leave the Hexose-dependent curve unaltered in shape with an increase independent of hexose concentrations.

P.D. or Isc.



(4) - Aldosterone affects Sodium entry (Crabbé). Sodium pump unsaturated.

(4) Discussion of the Effect of Aldosterone and Hydrocortisone.

Figure 10 illustrates that the most dramatic effect of Aldosterone is to elevate the Hexose-independent Isc to correspond to the situation illustrated in curve (4) above. It would appear, then, that an argument confined by the limitations of the Crane Hypothesis throws doubt upon it. If the Crane hypothesis is abandoned and cognizance taken of the fact that the Galactose-dependent Isc is not due to an electrogenic Sodium pump § 5.4(3) then the explanation of the results is clear. Aldosterone promotes the transportation of Sodium as it does in Colon § 5.6(3), (4) and leaves unaltered the Galactose-dependent potential. Figure 10 appears to indicate a tendency for the Galactose-Isc to rise more steeply in Aldosterone-treated animals then it does in controls (curve C). Table 11, however, shows that this tendency is not statistically significant.

Figure 15 shows that Aldosterone restores the tissue resistance of Adrenalectomized animals to normal, which fits well with Crabbé view of Aldosterone as a "permeability hormone". Both these effects, however, are mimicked by Hydrocortisone 0.5 mg. (Figure 13).

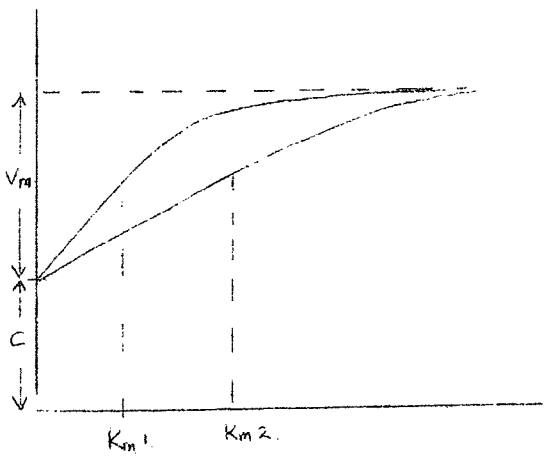
Figure 15 shows the effect of Aldosterone on an animal which has also been treated with Hydrocortisone. The Figure shows that there is a tendency for the Glucose-independent Isc to be reduced but Table 11 indicates that this is not statistically significant. It would be safe to conclude, however, that Aldosterone does not elevate the Glucose-independent Isc. It has been suggested to me that Hydrocortisone having a similar chemical structure to that of Aldosterone can exert a weak Mineralocorticoid effect when given in large doses. This may be the case with these results which would agree with the explanation put forward by Moses and Streeten (271) for the effect of Cortisol on red blood cells. Since it is very probable that Sodium expulsion and Glycolysis are intimately linked (§ 5.3) it is equally likely that a stimulation of Glycolysis will lead to an enhanced handling of Sodium. It is significant that the effect of Cortisol on Sodium entry into Red cells was inhibited by Glucose deprivation (271). An alternative explanation therefore is that Hydrocortisone and Aldosterone bring about similar results by attacking the same system of Sodium/ATP/Glycolysis but at different points. Two enzymes of the Embden-Meyerhof pathway have been found to be influenced by Glucocorticoids. These are Pyruvate Carboxylase (PC) (272) and Phosphoglyceraldehyde dehydrogenase (PGD) (273). Strictly speaking the former is not involved in the pathway but since it is involved with Pyruvate it has a direct influence on the pathway. Both these enzymes are affected within the 24 hour period which elapsed between injection and experiment, P.C. by 250% in 6 hours PGD by 180% in 4 to 8 hours. Together these enzymes would control the generation and break down of Pyruvate and thus of Lactic Acid. I know of no such effects reported for Aldosterone. The connection between the action of Aldosterone and Glycolyses is strengthened by the observation that the hormone inhibits the production of CO_2 from Glucose in Toad bladder at a time when metabolism has been stimulated by the hormone. Even when this stimulation of metabolism has been eliminated by

removing Sodium from the medium, the reduction of CO_2 production is still demonstrable (241). Similar effects have been observed in the presence of Deoxycorticosterone (241).

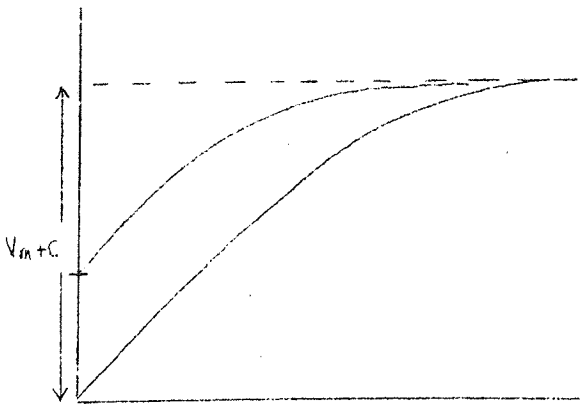
Turning to the Analysis of the Data in accordance with the Michaelis-Menton scheme. The very large variances involved prevent any firm conclusions being drawn. The failure in some cases to find a satisfactory pair of parameters to fit the experimental data, is associated with high Km values which implies that the curve is nearly a straight line. It is clearly a hopeless task to predict the maximum value and the half maximum concentration from the first short section of the curve. Since the linear regression coefficients (Tables 6 and 7) have not been significantly altered by treatment (Table 11, Parameter "B") it would appear that the overall change is not of merely increasing Km but is rather one of "straightening the line". The two sketches below indicate schematically how the change in curve shape should appear if

(a) Km is increased without changing Vm

(b) Vm + C remains constant but $Vm/_C$ is increased.



(a)



(b)

The sketch (b) illustrates more correctly the situation as observed, which would imply that the Michaelis-Menton scheme is not a satisfactory model for the relationship of PD or Isc to Hexose concentration. The sketch (b) suggests that there is an overall maximum which is approached at a more or less constant rate irrespective

of how much available capacity exists. This would be typical of an irreversible-first-step process.

That Aldosterone should stimulate the Hexose-independent PD. is in keeping with the view that it acts in conjunction with the Citric Acid cycle (§5.6). The Citric Acid cycle is thought to be responsible for the Hexose-independent fluid transfer (116). It has been reported that Aldosterone stimulates water and Sodium flux in Rat jejunum(§5.6(5)), but in this region of the small intestine the Citric Acid cycle is of only secondary importance with regard to fluid transfer (116).

It is possible that both Aldosterone and Hydrocortisone have the effect of raising the plasma concentration of various substrates such as Glucose and electrolyte and that the enhanced PD. and fluid transfer is secondary to this. It has been reported that Aldosterone can produce increased hepatic Glycogen deposition in both normal and adrenalectomized rats (276), and Hydrocortisone (15 mg/day/3 days - sc) can raise the blood Glucose concentration of adrenalectomized rats to normal (282). This dose is considerably larger than the dose used in the present experiments. The soluble form of the hormone used here, however, is claimed by the manufacturers to have a very rapid action and the dose level used (0.5 mg, ip. 24 + hrs before sacrifice) was the same as the recommended dose for use with Humans in the case of acute adrenal failure - suitably scaled for rats by weight. Further support for the view that the hormone effects (which have been reported here, and elsewhere with respect to Rat jejunum) may be secondary to enhanced substrate supply, is supplied by the data on the effect of Sodium loading (Tables 4 & 5). This treatment was intended to depress the endogenous secretion of Aldosterone which might be expected to depress PD. and Isc. Although the large variances involved make it impossible to draw firm conclusions, it is clear that the tendency is for the PD. and Isc to increase; and conversely Sodium depletion appeared to decrease the PD. and Isc, an effect which may well be due to malnutrition.

The 'nutritional' explanation of the hormone effects appears to be at variance with reports that substrates such as Pyruvate are unable to stimulate fluid transfer by jejunum (115). So far as I am aware, however, the effect of Pyruvate on the jejunum of adrenalectomized animals has not been studied.

In conclusion it can be said therefore that two explanations have been put forward described succinctly thus.

(i) Nutritional (hexose and electrolyte plasma levels)

(ii) Aldosterone increased the permeability of the membrane with respect to Sodium (c/f Crabbé, §5.6) and Hydrocortisone acts on Hexose metabolism to produce a similar overall effect on the Sodium/ATP/Hexose system.

The system just mentioned is the subject of a great deal of confusing information (§5.5) and in an attempt to clarify both the system and the possible effects of the above mentioned hormones upon it I have evolved a hypothesis which may serve as a frame-work (§6).

6. A Hypothesis

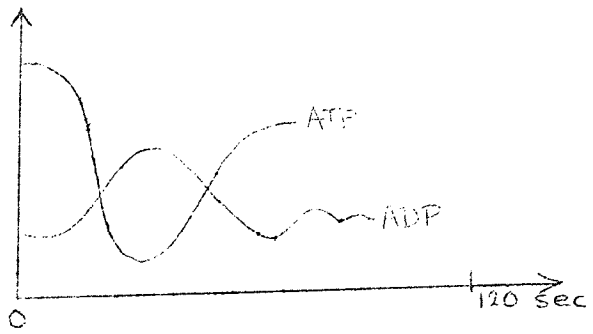
(1) Introduction

The elementary model of Sodium and Hexose transfer on which the analysis is often based, regards the mucosal cell as a simple box containing solutions differing from the external media in concentrations of its various components but essentially the same in regard to its physical properties. Sodium is pumped out through one wall of this box and it leaks in through the opposite wall. Glucose, and other hexoses, behave in a way which is the reverse - pumped in, leaked out. The Sodium-dependent P.D. is generated by the pumped exit of Sodium powered by Adenosine Triphosphatase (ATP). (5.3).

The source of the Sodium-independent P.D. is however obscure (5.4(3)). This model is probably grossly oversimplified but in the absence of experimental evidence to the contrary one would not be justified in abandoning it in favour of a more complicated one. In recent years, however, experimental evidence has been accumulating which shows that the pathways of carbohydrate metabolism may be considerably more complicated than was realised earlier.

(2) The Oscillatory Behaviour of Carbohydrate Metabolism.

In 1961, Hess and Chance showed that the concentrations of ATP and Adenosine Diphosphate (ADP) undergo very rapid changes after the sudden addition of glucose to Ascites tumor cells (231) as shown schematically below



Later Chance and his co-workers, by observing the fluorescence associated with the reduced form of coenzyme Nicotinamide Adenine Dinucleotide (NAD reduced to NADH), were able to show long lasting perturbation oscillations in both the cytoplasm and in cell free extracts of the yeast cells, S. Carlsbergensis (232, 233, 234, 235). In the presence of Trehalose these oscillations became continuous (236). Similar oscillations have been observed in E.coli and in Beef Heart extract (237, 238). The way that such oscillations can arise has been described in principle by Spangler and Snell (239) who considered an enzyme with two forms participating in two parallel reactions. The system is the chemical analogue of an electronic flip-flop circuit. In anaerobic glycolysis the crucial enzyme is Phosphofructose kinase (PFK). (232). Computer simulation of the beef heart supernatant oscillations also highlights the key role of PFK but suggests that other enzymes exert a secondary control (240). Since PFK is widespread and it is of interest to consider the possibility that such oscillations involving aerobic as well as anaerobic glycolysis occur in the intestinal mucosa and to see if any of the more puzzling aspects of intestinal absorption can be so explained.

(3). The Osmotic Oscillator

It must first be noted that if the glycolytic pathway oscillates then the concentrations of all intermediates and end products must also oscillate. One of the intermediates is ATP which suggests that the Sodium pump would occur in pulses. McAfee (242) found that the transport of Sodium and the short-circuit current of Frog skin do not agree quantitatively when these are measured at short time intervals although over long time intervals agreement is found. These observations led him to suggest that Sodium is transferred "in vesicles or other reservoirs". The observed oscillations of Sodium flux were produced by perturbation with Acetylcholine, but nevertheless their existence is relevant to this discussion.

The Pyridine Dinucleotide NAD and its phosphorylated counterpart NADP are both produced during carbohydrate metabolism, the first in the Krebs cycle and the second in the ~~glycolytic~~ pentose shunt. Both result in the production of ATP but in the absence of ADP both may react with Pyruvate to produce Lactic Acid and CO_2. The production of Lactic Acid is a feature of Rat jejunum (216). In the absence of Sodium, therefore a cell with excess ATP and a deficit of ADP will pass into acidosis. This could occur either in the mitochondria (the Krebs cycle mechanism) or in the cytoplasm (the glycolytic mechanism). The first of these two will be the more efficient however because 'respiratory control' is exercised on the production of NADP when the conversion of ADP → ATP is inhibited but the presence of a small quantity of hydrogen ions releases NAD from this control (243). Both mechanisms will however lower the pH of the cytoplasm. This will have profound repercussions. The effect will probably be to direct Phosphofructose kinase to "switch off" the anaerobic glycolytic pathway. Other enzymes are also sensitive to pH and among these is Hexokinase. This enzyme is responsible for the primary phosphorylation of Glucose and other hexose molecules. It has been suggested that the hexokinase molecule forms an initial complex with the Glucose molecule before forming the enzyme-glucose-ATP-Mg complex (in which the glucose accepts a phosphate radical from the ATP moelcule) (244). The enzyme has a pH optimum of no less than 7.2 (245) and in the event of acidosis becoming widespread the enzyme complexes will dissociate. The osmotic pressure of the cytoplasm will fall and there will be an inrush of fluid carrying with it Sodium and bicarbonate ions, especially if the hydrogen ions cancel the zeta potential of the membrane pores (see 5.2(11)). Thes ions will have separate effects. The removal of Sodium by the Sodium pump (5.3) will regenerate ADP from ATP and the Bicarbonate will promote the reaction.

Pyruvate + ATP + CO_2^* + H_2O → ADP + ortho.P. + Oxaloacetate (oAA)

(which is under the control of the enzyme pyruvate carboxylase).

The effect of these two reactions will be to end sharply the acidosis phase and the system will have completed one cycle. The enzyme hexokinase has been used here as an example because it has roughly the correct specificity to bind the group of hexose molecules known to be actively transported but it is possible that there are a number of other likely candidates for the role of binding hexose molecules.

(4) Additional features.

In order that this scheme can be made to produce the observed transportation phenomena the following unexceptional features must be added.

(i) A rectifying membrane possibly at the mucosal surface (5.2(13))

(ii) Saturating transfer mechanism (passive facilitated diffusion) for hexose at the mucosal pole of the transporting cell.

(iii) A localisation of the hexose ligand near the luminal pole.

(iv) A simple diffusional exchange of hexose molecules between the region of binding ligand and the rest of the cytoplasm.

The scheme now operates as follows:-

(5). The Scheme Phase by Phase.

Phase one. Cytoplasm is alkaline or neutral

Hexose enters the region of ligands from

(a) the cytoplasm

(b) the mucosal fluid via the saturating transfer mechanism of the mucosal membrane

(b) > (a) and both well below the maximum of (b).

The hexose -ligand bond is some loose type of bond such as a hydrogen bond. The hexose is osmotically inactive due to this bond. If the hexose is capable of being

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* Bicarbonate is the "active form" of CO_2 for this reaction (246).

metabolised then glycolysis proceeds in a limited way otherwise endogenous sources of substrate maintain glycolysis and the Krebs cycle.

Reaction ADP → ATP

Phase two. ADP is exhausted. Lactic acid is produced. Glycolysis ceases. Hexose is released and escapes from the ligand region in two ways.

(a) diffusion to the cytoplasm and thus through the serosal surface

(b) to the mucosal fluid via the saturating transfer mechanism

(a) > (b) because (b) is subject to the saturation effect. The net result of Phases one and two is the transfer of hexose from the mucosal to the serosal surface. The hexose molecules are osmotically active and the osmotic pressure of the cytoplasm falls. The falling pH cancels the zeta potential of mucosal surface pores.

Phase three Fluid, Sodium and Bicarbonate enter

Reactions

(i) ATP → ADP (Sodium pump)

(ii) Pyruvate + CO_2 + H_2O + ATP → ADP + ortho.P. + OAA

Acidosis ceases

Phase one. Hexose is rebound under alkaline or neutral conditions. The osmotic pressure rises and water (or more correctly fluid) is expelled, but due to the rectification effect at either the mucosal or serosal border the net result is a movement of fluid from the mucosal fluid to the serosal fluid. This action will be assisted by the Sodium pump across the serosal surface by the mechanism described in §5.2(4). In the absence of this, i.e. lack of

Cytoplasmic ATP (as distinct from mitochondrial ATP) to drive the Sodium pump, much of the fluid may return to the mucosal fluid during the return to phase one.

(6). The Potential Difference (P.D.)

Mention has already been made of the difficulty of explaining the origin of the P.D. in Rat jejunum. So far a stoichiometric relationship has not been found between the short-circuit current and Sodium or fluid or hexose transfer (78). In this connection it is well to remember the extent of the problem. Rat jejunum has a short circuit current of about 100 $\mu a.cm^{-2}$. Since one equivalent of an ion carries 96000 (10^5 approx) coulombs, this current is equal to 10^{-9} eq/sec. According to Versar and McDougall (280) the increase in surface area of rat intestine due to the villi is approximately 8. Therefore 1 cm^2 of cylinder = 8 cm^2 mucosa. If the thickness of the mucosa layer is about 20 μ the volume of transporting cells per cm^2 is equal to $8 \times 20 \times 10^{-4}$ cm^3 = 160×10^{-4} cm^3. The concentration of isotonic saline solution is apporximately 150 mM or 300 meq/l of electrolyte including ions of both signs. If the contents of cells have a similar ionic strength, then the number of equivalents of electrolyte contained by the mucosa is equal to 300×10^{-6} eq/cm^3 x 160×10^{-4} cm^3 = 4.8×10^{-6} eq. If the short circuit current is to be carried by some molecule derived from within the cell itself then this situation could only be maintained for $4.8 \times 10^{-6}/10^{-9}$ = 4.8×10^3 seconds or 1.3 hours before the cell had lost the equivalent of its total electrolyte. Clearly the short circuit must be carried by some ionic species derived from the bathing medium.

A fraction of the short circuit current is undoubtedly carried by Sodium but there have been occasional reports that Chloride is actively secreted by mammalian jejunum under the influence of certain drugs (281). What seems to be required is a model which can adapt easily to changing conditions. The following suggestion arises out of the osmotic

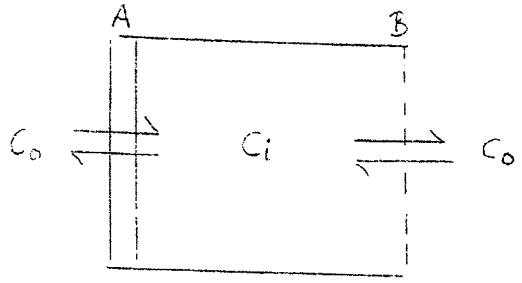
oscillator hypothesis.

In the presence of an actively transported hexose and a low level of endogenous glycolysis the cell will be alternately taking up fluid and expelling it again. It is assumed that the hexose dependent potential is generated across the serosal border of the cell. (The evidence for this is lacking. Only the glucose-dependent potential is known to be located at the serosal border). If the mucosal surface is more or less impermeable to fluid due to a high pore Zeta potential then this interchange of fluid will occur across the serosal border. Further, if due to endogenous metabolism a small amount of hydrogen ions are generated which cancel the zeta potential of the serosal pores during phase 3, fluid will enter through neutral pores and be expelled through negatively charged pores. There will be no net transfer of fluid. Only with increasing glycolysis will sufficient hydrogen ions be produced to render the mucosal pores permeable during phase 3. The result will be a net transfer of fluid. In the condition of no net transfer of fluid the cell will be accumulating Chloride ions gained from the serosal fluid which will constitute active secretion of Chloride, and when short circuited will secrete a proportion to the mucusal fluid. When net transfer of fluid takes place Sodium absorption will be the charge carrier.

In short it is suggested that variable synchronization of Hydrogen ion production and osmotic pressure gradients can produce either net fluid transfer without a concommitant electric current or an electric current with only a small fluid transfer. When Fructose is the substrate Rat jejunum corresponds to the first of these two possibilities and when Galactose is the substrate the second condition prevails

(7) Kinetics of the Hexose transfer.

To illustrate the kinetics of the proposed system a simpler system (but one which contains the essential ingredients of the proposed system) is considered.



A three compartment system as shown. Membrane (A) transfers the substrate molecules by means of a saturating mechanism described by the equation.

$$\text{rate of Flux (A)} = \frac{Vm.\ (Co - Ci)}{Km. + (Co - Ci)} \quad \ldots\ldots\ldots\ldots \text{Eq (1)}$$

And membrane (B) transfers the substrate by simple diffusion described by the equation

$$\text{rate of Flux (B)} = k\ (Ci - Co) \quad \ldots\ldots\ldots\ldots \text{Eq (2)}$$

where Co = concentration of substrate outside

and Ci = " " " inside.

Co is effectively constant but Ci exists in two conditions

in state one Ci = o

in state two Ci = 10Km (say)

let Co = Km. (say)

and also let $k = \frac{1}{10}\ \frac{(Vm)}{(Km)}$

These are arbitrary values chosen to make the calculation simple and to show that the model "works".

during state one (time duration t_1)

rate of influx (A) $= \frac{Vm\ (Co - Ci)}{Km + (Co - Ci)} = \frac{Vm\ Co}{Km + Co}$

$= \frac{Vm}{2}$ (since Co = Km)

$\therefore$ influx (A) $= \left(\frac{Vm}{2}\right) t_1$

and rate of influx (B) $= k\ (Co - Ci)$

$= k.Co = \frac{1}{10} \frac{Vm}{Km} . Km = \frac{Vm}{10}$

$\therefore$ influx (B) $= \left(\frac{Vm}{10}\right) t_1$

$\therefore$ influx (A) $>$ influx (B).

Again:

during state two (time duration t_2)

outflux (a) $= \frac{Vm\ (Ci - Co)}{Km + (Ci - Co)}\ t_2$

$= \frac{Vm\ 9\ Km}{10\ Km}\ t_2 = \frac{9}{10} Vm\ t_2$

outflux (B) $= k\ (Ci - Co)\ t_2$

$= \frac{1}{10} \left(\frac{Vm}{Km}\right) 9. Km\ t_2 = \frac{9}{10} Vm\ t_2$

and in this case outflux (A) = outflux (B).

But to preserve continuity total influx = total outflux.

$\therefore\ t_1 \left(\frac{Vm}{2} + \frac{Vm}{10} \right) = t_2 \left(\frac{9}{10} Vm + \frac{9}{10} Vm \right)$

$\therefore\ t_1 \frac{6}{10} Vm = t_2 \frac{18}{10} Vm$ $\quad \therefore \frac{t_1}{t_2} = \frac{3}{1}$ i.e. $t_1 = 3t_2$

Now comparing influx with outflux across a single membrane say (A)

$$\text{Total flux} = \text{influx (A)} - \text{outflux (A)}$$

$$= \frac{Vm}{2} t_1 - \frac{9}{10} Vm\, t_2$$

$$= \frac{Vm}{2} (3t_2) - \frac{9}{10} Vm\, t_2$$

$$= t_2\, Vm \left(\frac{3}{2} - \frac{9}{10} \right)$$

which is positive and not equal to zero.

∴ The model produces a net transfer of substrate.

The following observations can be made about this model:

(i) It is an over-simplification in that the change from stage one to stage two would not really be instantaneous and the concentration Ci would be subject to an exponential decline during stage two. Nevertheless the model illustrates in principle how a simple oscillating binding site with an assymetrical saturating supply of substrate can produce a net transfer.

(ii) An alteration of the external concentrations Co would lead lead to transfer in the reverse direction.

(iii) An alteration of the duration of the two stages (t_1 and t_2) would also greatly change the performance of the model.

(8) Hydrocortisone

It has been reported that Hydrocortisone exerts an influence on the enzymes Pyruvate Carboxylase (PC) (272) and Phosphoglyceraldehyde dehydrogenase (PGD) (273). PGD controls the production of Pyruvate by the Embden-Meyerhof pathway while PC controls the conversion of Pyruvate to Oxaloacetate (OAA). Both these reactions would be required to operate quickly if glycolysis was to produce a rapid but short-lived pulse of

lactic acid. This is put forward in conjunction with the general hypothesis as an alternative mechanism for the action of Hydrocortisone in stimulating intestinal absorptive processes.

(9) Conclusion.

As already mentioned the hypothesis is put forward tentatively. I am well aware that theory has been stretched dangerously far from established fact. My motive has been to demonstrate that the type of detailed control of glycolysis now being unravelled by Chance and others may have reperoussions in the field of membrane transport and open up a new area for experimentation. Even if the hypothesis proves to be partially correct there is no doubt that most of it would require modification in the light of future experience. Fortunately there appears to be no lack of scope for modification.

(10) Postscript.

"Sir - I have given you an argument, but I am not obliged to give you an explanation".

Dr. Johnston.

§7. References

Abreviations.

B.B.A. . . . . Biochim. et. Biophys. Acta.

B.B.R.C. . . . . Biochim. Biophys. Research Comm.

NOTE:

Certain references in this list are marked with an asterisk, and these have no corresponding reference in the text. They appeared in early drafts of this thesis but have not been eliminated because it is felt that their presence may be useful to other workers.

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SYMBOLS AND ABREVIATIONS

PD.	Potential Difference.
Isc	Short circuit current.
ψ, $\Delta\psi$	Potential, Potential Difference.
P_i	Permeability of "i".
square brackets	Concentration of "i".
C	Concentration, also a constant depending on contect.
In	Natural logarithm.
R	Gas constant.
X	Resistance.
T	Absolute temperature.
τ , t	Time symbols.
σ	Charge/unit area and also Standard Deviation.
S.E.	Standard error of the mean.
S.D.	Standard deviation.
A	Constants
B	Constant and linear regression coefficient.
Vm	Maximum Velocity (Michaelis-Menton Kinetics).
Km	Half max concentration (Michael-Menton Kinetics)
E	Electrostatic field.
η	Viscosity.
Λ	Equivalent conductance.
ζ	Zeta Potential (or electro kinetic potential).
ϵ	Dielectric constant of the medium.
ρ	Specific Resistance.
L	Specific Conductance.

APPENDIX 2.

Programme No.1

LEAST SQUARES LINE (INVERSE PLOT)

```
BEGIN INTEGER N' READ N'
      BEGIN ARRAY X,Y(1:N),P(1:2,1:2),Q(1:2)'
            REAL A,B,DELTA' INTEGER R'
            BEGIN P(1,1):=P(1,2):=P(2,1):=P(2,2):=Q(1):=Q(2):=0'
                  FOR R:=1 STEP 1 UNTIL N DO
                  BEGIN READ X(R),Y(R)'
                        P(1,1):=P(1,1)+Y(R)**4'
                        P(1,2):=P(1,2)+Y(R)**4/X(R)'
                        P(2,1):=P(1,2)'
                        P(2,2):=P(2,2)+Y(R)**4/X(R)**2'
                        Q(1)  :=Q(1)+Y(R)**3'
                        Q(2)  :=Q(2)+Y(R)**3/X(R)'
                  END'
                  DELTA:=P(1,1)*P(2,2)-P(1,2)*P(2,1)'
                  A     := (Q(1)*P(2,2)-Q(2)*P(1,2))/DELTA'
                  B     := (P(1,1)*Q(2)-P(2,1)*Q(1))/DELTA'
                  PRINT ££L2? A=?,SAMELINE,A,££L? B=?,B'
            END'
      END'
END'
```

Note in Elliott Algol "**" is equivalent to"↑"
and"!" is equivalent to";"

```
    TITLE:CURVE FIT BY ITERATION (H.M.NOBLE)'
GIN
    REAL ARRAY X,Y(1:100)'
    REAL XX,YY,BASEY,SUMC,DELTA,Z,A,B,C,SQC,BITA,BITB,W,
         SIGMA,FACTOR,VARA,VARB,VARC,COV,L,LA,LB,LAA,LBB,
        STRA,STRB,STRL,STRLA,STRLB,STRLAA,STRLBB,STRLAB,STRLBA,
         STRDEL,NEWA,NEWB,LIMITA,LIMITB,
         LAB,LBA,ALPHA,BETA,TEST,ABITA,ABITB'

    INTEGER J,DIVISOR,Q,P,R,RAT,DIRNA,DIRNB,DATA,DF'
    SWITCH S:=S1,S2,S3,S4,S5,S6,S7,S8,S9,S10,EXIT,OUTPUT,RESTART,
             DIVSET, STORAGE,CALCULATE,SCALE'
    PRINT ££L6? CURVE FIT BY ITERATION(H.M.NOBLE)?'
ESTART:  READ DATA'
    IF DATA GREQ 100 THEN
                BEGIN PRINT ££L6? NO MORE DATA ?'   GOTO EXIT'
                END
    ELSE PRINT ££L6? DATA NO.?,SAMELINE,DATA'
    RAT:=0'   SUMC:=0'  J:=1'  Q:=0'  SQC:=0'
    READ A,B'   PRINT ££L2? FIRST APPROX ?,
                      ££L ? VMAX= ?,SAMELINE,A,
                      ££L ? KM  = ?,SAMELINE,B'
    READ LIMITA,LIMITB'

CALE: IF A GREQ 1000.0 THEN BEGIN ALPHA:= 10000.0 END ELSE
      IF A GREQ  100.0 THEN BEGIN ALPHA:=  1000.0 END ELSE
      IF A GREQ   10.0 THEN BEGIN ALPHA :=  100.0 END ELSE
      IF A GREQ    1.0 THEN BEGIN ALPHA :=   10.0 END ELSE
                            BEGIN ALPHA :=    1.0 END'
      IF B GREQ  100.0 THEN BEGIN BETA := 1000.0 END ELSE
      IF B GREQ   10.0 THEN BEGIN BETA  :=  100.0 END ELSE
      IF B GREQ    1.0 THEN BEGIN BETA  :=   10.0 END ELSE
                          BEGIN BETA  :=    1.0 END'
      A:= A/ALPHA'  B:= B/BETA'
      LIMITA := LIMITA/ALPHA' LIMITB := LIMITB/BETA'
2:      READ XX,YY'  XX:= XX/BETA'  YY:= YY/ALPHA'
                     TEST:= 1000.0/BETA'
                     IF XX LESS 0.00005 THEN GOTO S3 ELSE GOTO S4'
3:      BASEY:=YY'     SUMC:=SUMC+BASEY'   SQC:=SQC+BASEY**2'
        RAT:=RAT+1'   GOTO S2'
4:      IF XX GREQ TEST THEN BEGIN
                                J:=J-1' GOTO S6' END

           ELSE GOTO S5'
5:      X(J):=XX'   Y(J):=YY-BASEY'  J:=J+1'   GOTO S2'
6:              L:= LA:= LB:= LAB:= LBA:= LAA:= LBB:= 0.0'
      BEGIN
        FOR R:=1 STEP 1 UNTIL J DO
        BEGIN     Z:=B+X(R)'
        LAA:= LAA+2*X(R)**2/Z**2'
        LBB:= LBB+6*A**2*X(R)**2/Z**4-4*A*X(R)*Y(R)/Z**3'
        LAB:= LAB+2*X(R)*Y(R)/Z**2-4*A*X(R)**2/Z**3'
        LBA:= LAB'
        LA:= LA+2*A*X(R)**2/Z**2-2*X(R)*Y(R)/Z'
        LB:= LB+2*A*X(R)*Y(R)/Z**2-2*A**2*X(R)**2/Z**3'
        L:= L+(Y(R)-X(R)*A/(B+X(R)))**2'
        END'
        DELTA := LAA*LBB-LBA*LAB'
      END'
```

```
STORAGE:  IF L GREQ STRL THEN GOTO DIVSET ELSE
            BEGIN STRL := L' STRA := A' STRB := B' STRLA := LA'
                  STRLB := LB' STRLAA := LAA' STRLBB := LBB'
                  STRLAB := LAB' STRLBA := LBA' STRDEL := DELTA'
            END'
DIVSET:
   IF Q GREQ 100 THEN BEGIN DIVISOR :=  1' GOTO CALCULATE' END ELSE
   IF Q GREQ  75 THEN BEGIN DIVISOR :=  5' GOTO CALCULATE' END ELSE
   IF Q GREQ  50 THEN BEGIN DIVISOR := 10' GOTO CALCULATE' END ELSE
   IF Q GREQ  25 THEN BEGIN DIVISOR := 25' GOTO CALCULATE' END ELSE
                        DIVISOR := 50' GOTO CALCULATE'
CALCULATE:

        BITA := (LAB*LB-LBB*LA)/DELTA'
        BITB := (LA*LBA-LB*LAA)/DELTA'
                ABITA:= ABS(BITA)'
                ABITB:= ABS(BITB)'
        IF ABITA LESSEQ 0.0001 THEN GOTO S7 ELSE GOTO S8'
S7:     IF ABITB LESSEQ 0.0001  THEN GOTO OUTPUT ELSE GOTO S8'
S8:  IF LA GREQ 0.0 THEN DIRNA:= -1 ELSE DIRNA:= +1'
     IF LB GREQ 0.0 THEN DIRNB:= -1 ELSE DIRNB:= +1'
     NEWA:= A+DIRNA*ABITA/(DIVISOR)' NEWB:= B+DIRNB*ABITB/(DIVISOR)'
      Q:= Q+1'
      IF NEWA LESS LIMITA THEN NEWA := LIMITA'
      IF NEWB LESS LIMITB THEN NEWB := LIMITB'
      A := NEWA' B:= NEWB'


       PRINT ££L? VMAX=?,SAMELINE,A*ALPHA,££S3?,KM=?,B*BETA,
             ££S3? ERROR ?,SAMELINE,L*ALPHA**2'
        IF Q GREQ 150   THEN GOTO S9 ELSE GOTO S6'
S9:     PRINT ££L2? NOT CONVERGING ?'
        A := STRA' B := STRB' L := STRL' LA := STRLA' LB := STRLB'
        LAA := STRLAA' LBB := STRLBB' LAB := STRLAB'
        LBA := STRLBA' DELTA := STRDEL'
        GOTO OUTPUT'
OUTPUT:  BEGIN FOR R:=1 STEP 1 UNTIL J DO
               SIGMA:=SIGMA+(Y(R)-X(R)*A/(B+X(R)))**2'
               DF:=J-RAT*2+2-J/RAT'
               SIGMA:=SIGMA/DF'
               FACTOR:=SIGMA/DELTA'
               VARA:=LBB*FACTOR'
               VARB:=LAA*FACTOR'
               COV:=LAB*FACTOR'
         END'
         PRINT ££L2? FINAL VMAX ?,SAMELINE,A*ALPHA,
               ££S3? VARIANCE?,VARA*ALPHA**2,
               ££L ? FINAL KM ?,SAMELINE,B*BETA,
                     ££S3? VARIANCE?,VARB*BETA**2,
             ££L ? COVARIANCE ?,SAMELINE,COV*ALPHA*BETA'
         C:=SUMC/RAT'   VARC:=(RAT*SQC-C**2)/(RAT*(RAT-1))'
    PRINT ££L? INDEPENDENT CONST,?,SAMELINE,C*ALPHA,
               ££S3?VARIANCE?,VARC*ALPHA**2'
         PRINT ££L3 ? BEST PLOT?'

         FOR W:=0.0, W+0.1 WHILE W LESS 1.1 DO
         PRINT ££L??,W,SAMELINE,££S3??,(A*W*ALPHA/(B*BETA+W) +C*ALP
         PRINT ££L3? END OF DATA SET?'
         PRINT ££L? NUMBER OF RATS ?,SAMELINE,RAT'
         GOTO RESTART'
EXIT:
END'
```

APPENDIX 3.

Diet 41B Pilsbury Ltd., Birmingham.

	%
Crude Protein	17.069
Crude Oil	2.732
Crude Fibre	4.352
Digestible Oil	2.114
Digestible Fibre	1.723
Arginine	0.801
Lysine	0.877
Methionine	0.309
Cystine	0.261
Tryptophan#	0.192
Histidine	0.326
Leucine	1.089
Tyrosine	0.362
Isoleucine	0.710
Phenylalanine	0.674
Threonine	0.555
Valine	0.837
Glycine	0.981
Ca	1.3
P	0.72
Ca : P	1 : 0.6
Na	0.575
Cl	0.154

	mcgm/lb
Vitamine B12	6.4

	ppm.
Fe	65
Mn	32
Cu	7
I	4.18
Co	0.89
Zn	8.29

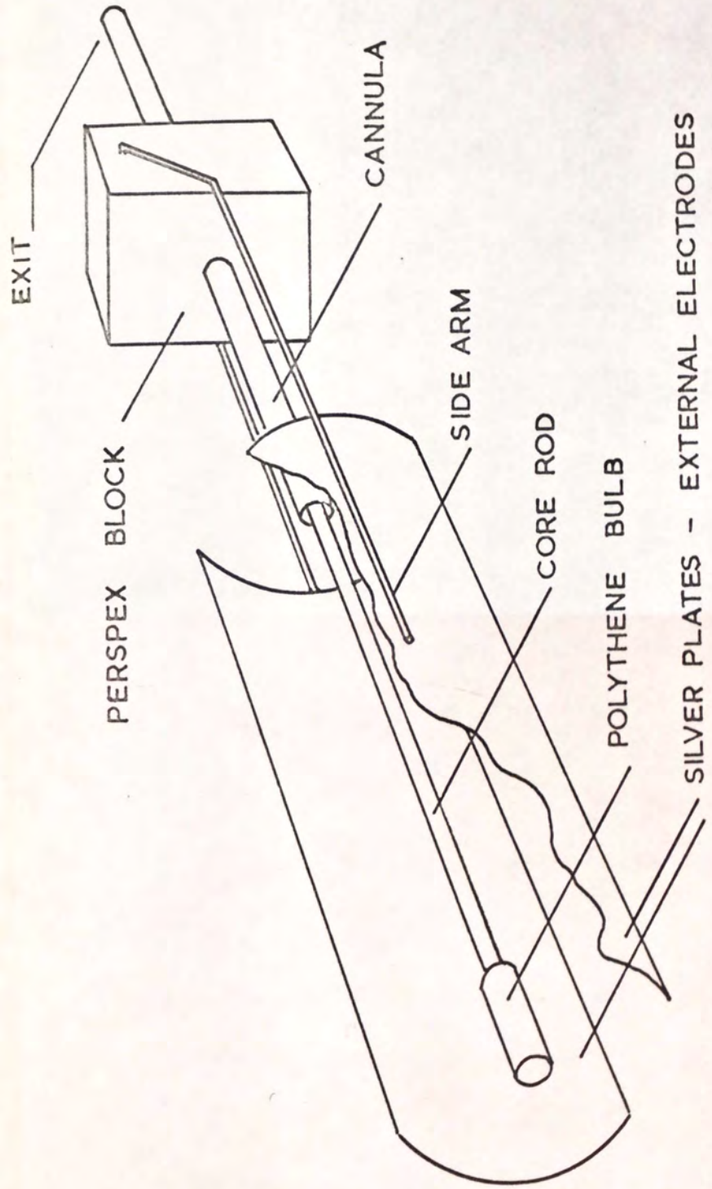
	mgm/lb.
Vitaemine E	8.698
Thiamine	2.809
Riboflavine	1.588
Niacine	25.004
Pantothenic Acid	6.801
Choline	595
Biotin	0.41
Folic Acid	0.38
Pyridoxine	2.8
Inositol	not less than 100

	I.U./lb.
Vitamine A	4641
Vitamine D_3	1160

FIG.1. The Electrode assembly. The inner section, consisting of cannula, cone rod and polythene bulb were inserted into a segment of intestine which was delineated by ties on the cannula and polythene bulb. The perfusion solution entered the segment through the cannula and made its exit through the hollow polythene bulb. The side arms were made of stainless steel and were soldered to the semi-cylindrical silver plates. The side arms, the soldered joint and the external surface of the silver plates were covered in a layer of epoxy resin. The corners of the silver plate were turned outwards and filed to sharp points on which the polystyrene foam pads were impaled after stretching them over the inner surface of the plates. The side arms moved with a hinged action (pivoting on the perspex block) which allowed the outer section to drop into place around the test segment.

A fuller description is given in section § 2.3 (1) and the procedure used when the electrodes were inserted is described in § 2.4 (3).

FIG. 1



APPARATUS FOR IN VIVO MEASUREMENT OF INTESTINE SHORT CIRCUIT CURRENT.

FIG. 2. A schematic block diagram of the complete apparatus used for measuring and recording the electric potential and short circuit current of Rat small intestine in vivo. Separate diagrams are given for

(1) Source : Fig. 4 (2) Chopper : Fig.6

(3) Reference Oscillator : Fig.5 (4) Amplifier: Fig.3

In this diagram B, B = Salt Bridges

S = segment of intestine

E, E, E = Electrodes.

The voltmeter was a Pye Dynacap (input resistance $10^{12}\Omega$)
the recorder was Devices M4.

The Filter was a simple low pass filter consisting of a 10 Henry choke and a 0.47 μF capacity heavily screened.

The transformer rectifier was a Radio spares standard output transformer with a rectifying bridge of four OA5 diodes.
A 8 μF capacitor smoothed the output.

A description of the apparatus is given in section 2.3 (2).

FIG 2

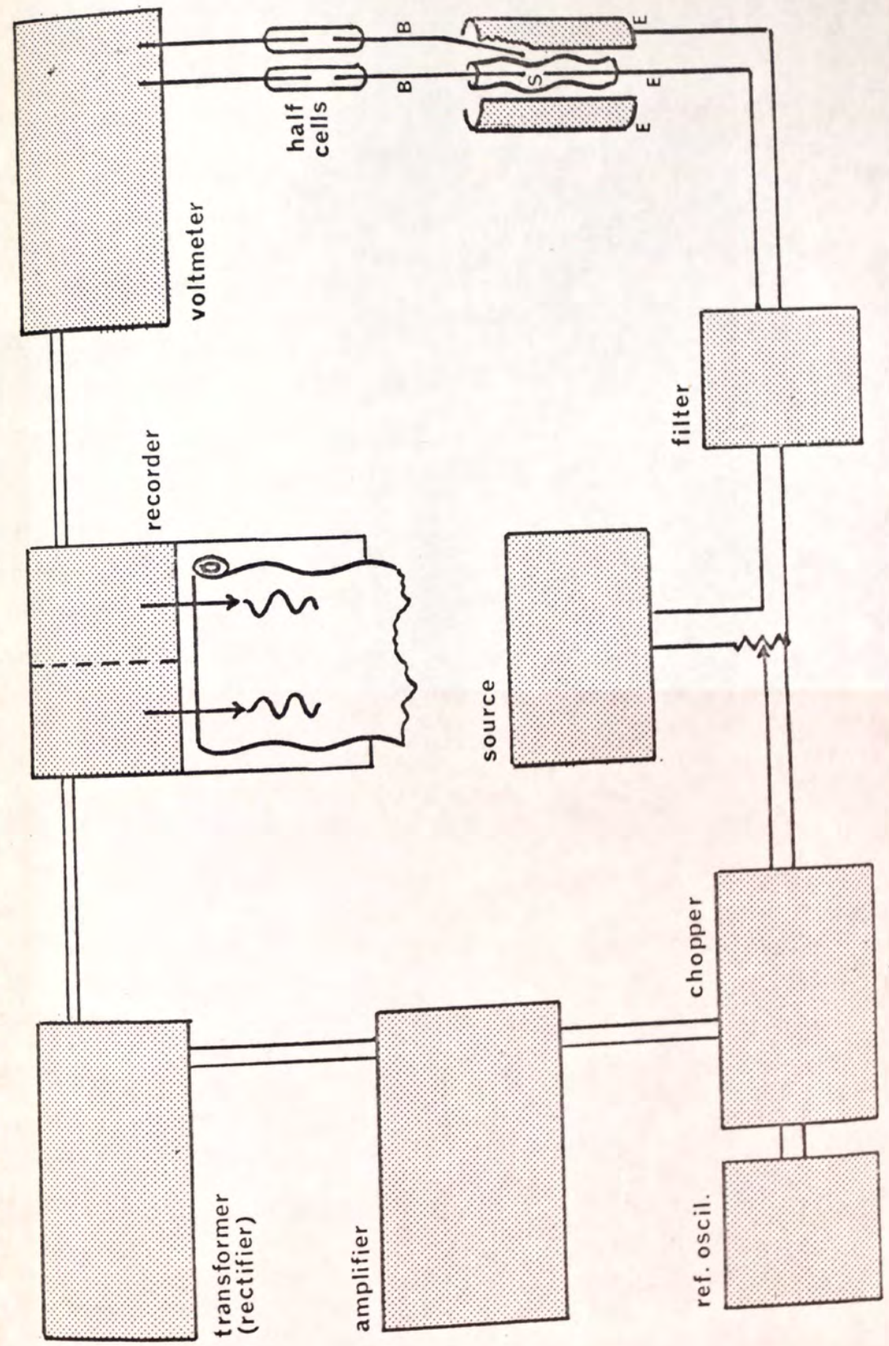


FIG. 3.

200 mW Amplifier.

This amplifier was designed by Wolfendale (274). The necessity is for any battery driven amplifier which can be isolated from earth.

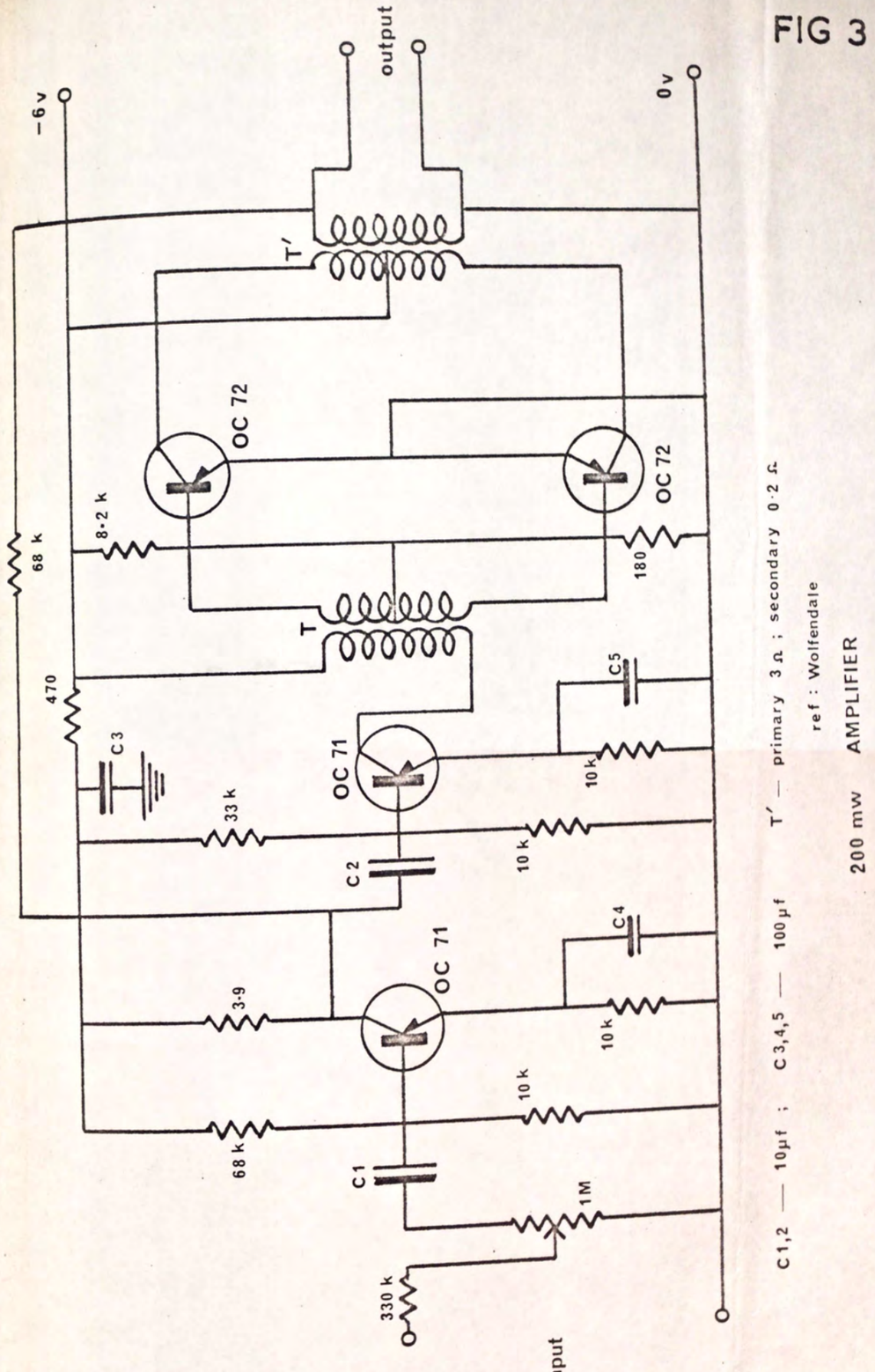


FIG 3

200 mw AMPLIFIER

T' — primary 3 Ω ; secondary 0·2 Ω

ref : Wolfendale

C1,2 — 10µf ; C3,4,5 — 100µf

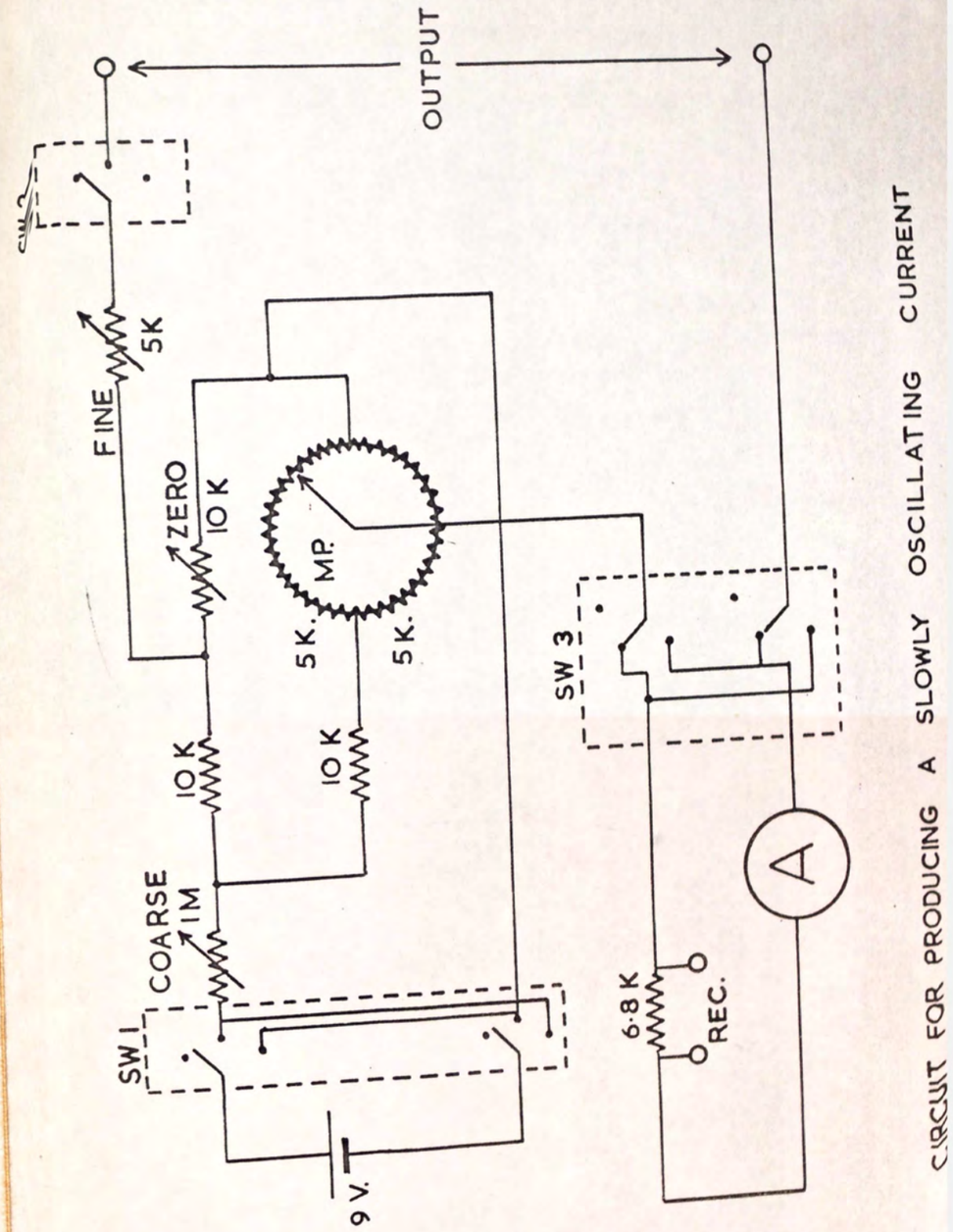
Fig. 4. The Short Circuit Current "Source".

MP = Motor-Driven Potentiometer.

The drive was obtained from a geared-down stirrer motor acting through an insulated shaft on to a modified 10K wire wound potentiometer.

REC indicates the output socket to the recording apparatus.

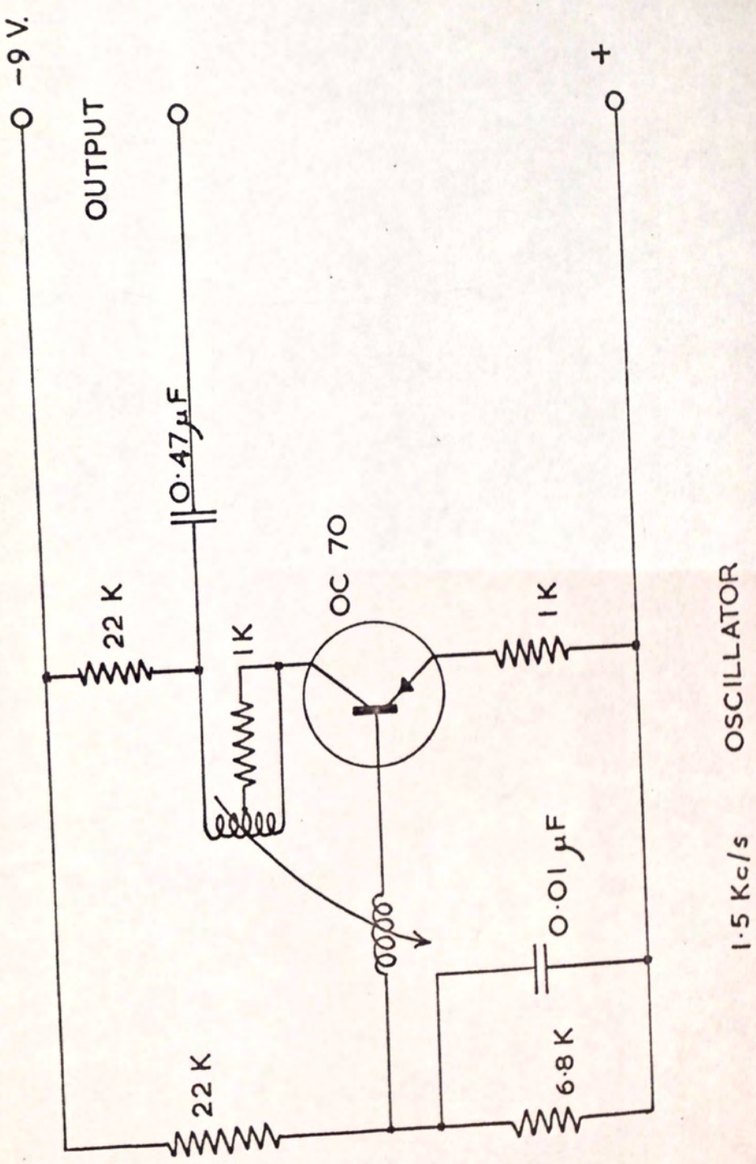
FIG 4



CIRCUIT FOR PRODUCING A SLOWLY OSCILLATING CURRENT

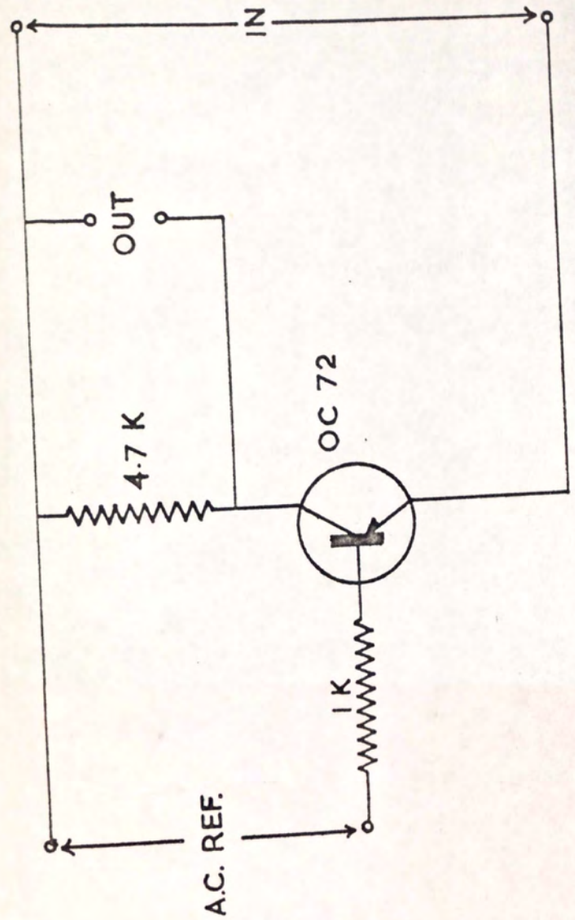
FIG. 5. The Oscillator which provided the reference AC. for the chopper. The transformer is a Radiospares, 1:1 transistor driver.

FIG 5



1·5 Kc/s OSCILLATOR

FIG. 6.



CHOPPER CIRCUIT

FIG. 6. Chopper Circuit. This is not a particularly efficient chopper and no doubt more sophisticated designs using back to back transistors would eliminate error voltages introduced by the transistor during the "on" half cycle.

FIG. 7. A typical trace. This is a photocopy of a trace obtained during an experiment. The trace is read from left to right. Minute intervals are indicated and numbered 3, 4, 5, 6, 7, 8, (just below centre). Above this are two oscillating traces. The upper trace is the transmural current with calibration marks shown on the left. These calibrations were obtained from the meter marked A in Fig.4 (of the Source). The PD. trace is just below the current trace with centre zero and full scale ± 10mV. The true open circuit P.D. is read from the crest of the wave form (current = o). Conversely the short circuit current (Isc) is read from the current trace from the points corresponding to PD = o. The vertical lines on the right show how these points were found and indicate also the time-lag which exists between the rising and falling phases of the waveform. The mean point is shown ○ and these points have been joined by hand and the line marked Isc. Below the time marker is another wave form which is a duplicate PD. with reduced sensitivity and increased full-scale deflection. This trace was used only on the rare occassions when the other PD. trace was driven to full scale.

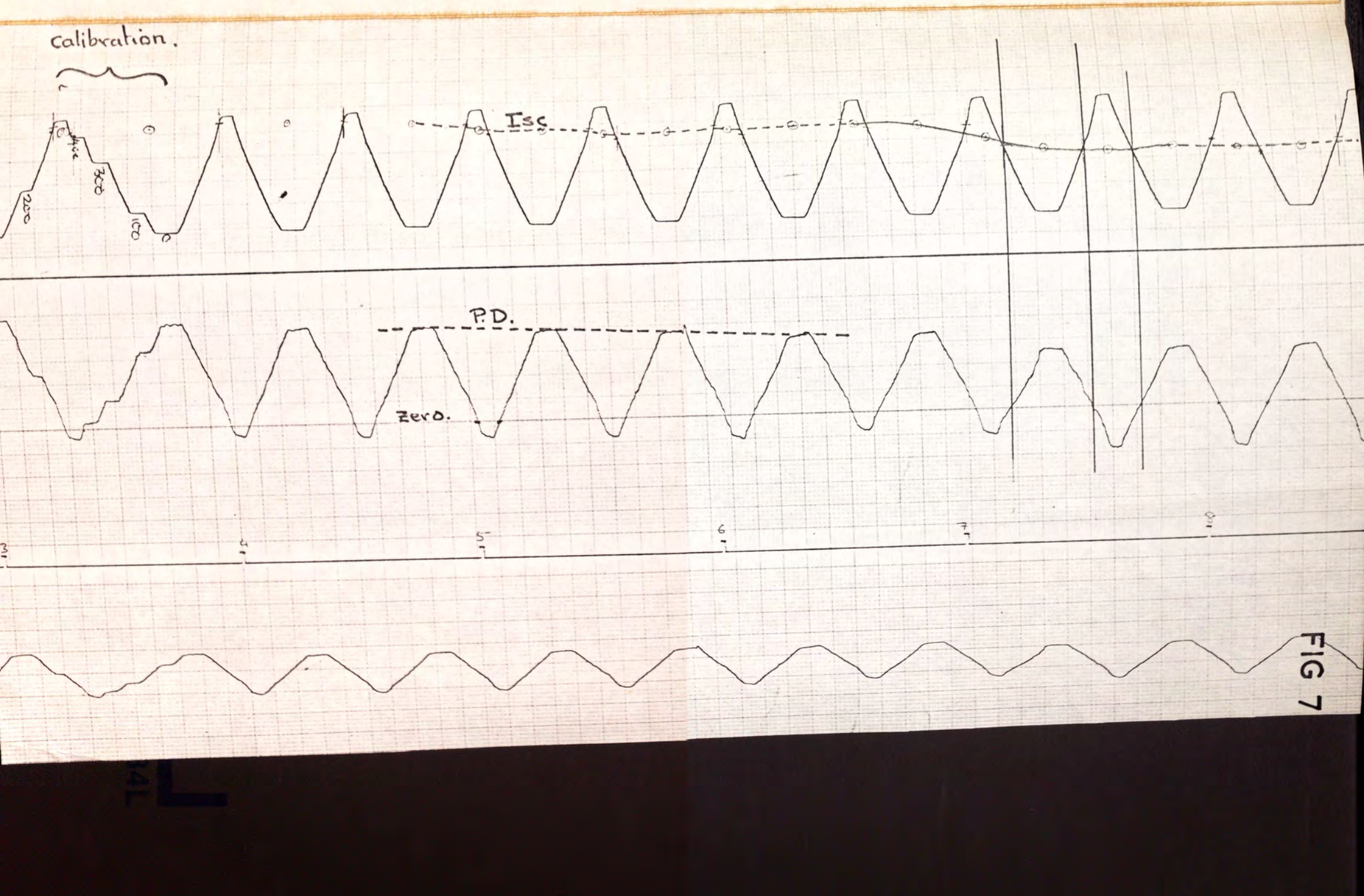


FIG 7

FIG. 8.

FIG. 8. The Electrophoresis Cell.

This diagram shows the important part of the apparatus removed from its temperature bath. A description is given in section § 2.6 (1).

The capillary diameter was 2mm.

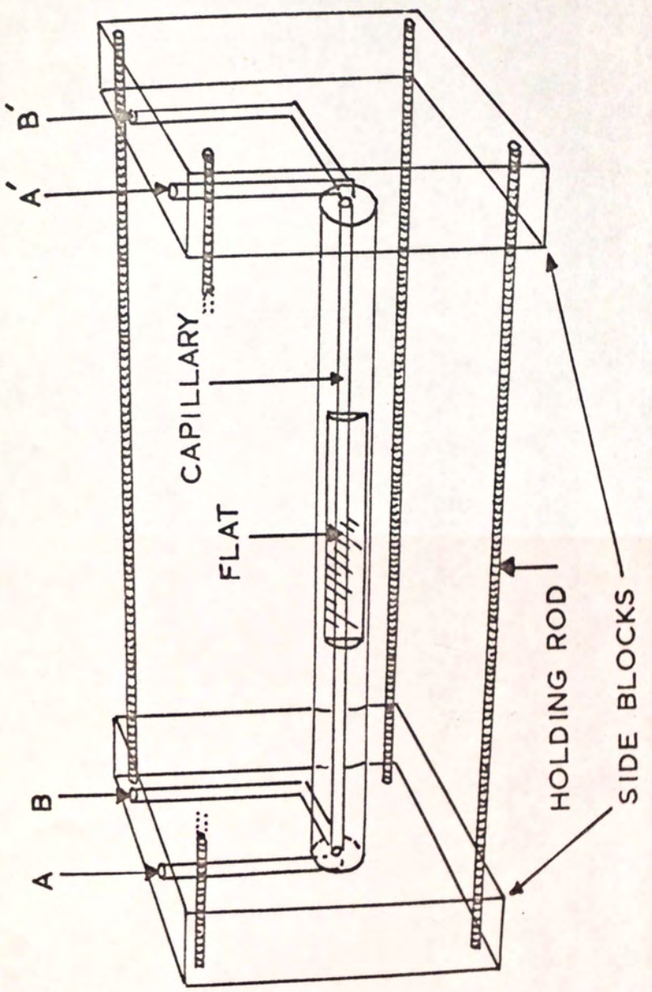


FIG. 9.

FIG, 9. The Electrophoresis Apparatus

See section § 2.6 (1) for a description.

The diagram is self explanatory.