ASPECTS OF THE ASSIMILATION OF SUGAR IN *ROUSETTUS AEGYPTIACUS*

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DECLARATION BY CANDIDATE

I hereby declare that this thesis is my own work, and that it has not been submitted to any other university.

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ABSTRACT

The fruit bat Rousettus aegyptiacus is a voracious eater of soft ripe fruit and will ingest 70% or more of its own body weight of bananas during the night. Although the bat consumes these large quantities of fruit and has a very rapid transit time of 46 minutes for bananas, the amount of sugar present in the faeces is very small, indicating that the bat has a very rapid rate of sugar assimilation. The rapid rate of glucose and fructose assimilation was confirmed in experiments, where it was shown that the conscious bat assimilated glucose and fructose three and four times faster than the rat respectively.

The transfer of glucose from the intestinal lumen to the blood in the bat does not appear to be either an active transport mechanism or one of simple diffusion. In all the in vivo and in situ assimilation experiments done, the transport of glucose against a concentration gradient was always present in the rat but never in the bat. No active transport system for fructose could be demonstrated in either the bat or the rat. If, however, glucose is slowly infused into the jejunum, then the addition of glucose into the stomach will cause a two to three fold increase in the rate of assimilation of glucose from the intestine. This increase in the rate of assimilation is probably due to the stimulation of a facilitative diffusion mechanism.

The bat digests sucrose very efficiently. The activity of the sucrase is not a limiting factor as both glucose and fructose were found to be present in the intestinal lumen during the digestion of sucrose. Samples of blood taken from the portal vein, during these experiments,

showed that no sucrose entered the blood and that there was very little conversion of either glucose or fructose to some other product prior to assimilation.

Intravenous glucose tolerance tests showed that the very high blood glucose levels of 700 mg and more of glucose per 100 ml of blood recorded during the oral glucose tolerance tests were not due to an insulin deficiency but possibly the result of this very rapid rate of glucose assimilation.

The structure of the rat's gastro-intestinal tract showed that firstly it had no large intestine and secondly, its small intestine was shorter than the rat's. The scanning electron microscope showed that the villi were longer, slimmer and closely packed together, thus increasing the luminal surface area. The study of the mucosal cells under the electron microscope showed that the microvilli were very long and slender and so increased the luminal surface area of the mucosal cell by a factor of 30 times compared to the 18 times in the rat.

Limitations imposed by the inherent variability of biological systems were recognised and attempts were made to compensate for this by using the best statistical methods in the treatment of the experimental results. Certain misinterpretations could, however, arise from this method.

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1. INTRODUCTION

1.1. The assimilation of glucose and fructose

At the beginning of the century Hendon (1900) reported That the different sugars ingested, were assimilated at different rates. Cori (1925) measured the rates of assimilation of a large number of sugars in the conscious rat and arranged these sugars in order of the rates at which they were assimilated. He showed that galactose was the most rapidly assimilated followed by glucose, with fructose low down on the list at a rate of 43% compared to the glucose rate. Bárány and Sperber (1939) showed that when glucose was placed in an isolated loop of rabbit intestine, assimilation of glucose continued even when the concentration had fallen below the blood glucose level. However, they failed to show that the glucose was actually transferred and not merely metabolised.

Rosenberg (1954) classified the net movement of molecules against a concentration difference as the phenomenon of active transport. Atkinson, *et al.*, (1957) using a dog showed that 3-O-methyl-D-glucose, which is not metabolised by the dog, behaved like glucose in that it continued to be assimilated against a concentration gradient.

Hestrin-Lerner and Shapiro (1953 a, b, 1954) reported from chromatographic evidence that when glucose was assimilated it did not appear in the blood as glucose but in some other form. This however, was quickly shown to be incorrect, for many workers reported that most of the glucose assimilated was recovered as glucose. Kiyasu, *et al.*, (1955) using isotopes showed that in rats, 97% of the isotopes

recovered consisted of glucose, lactate and alanine of which 82 - 9[illegible] was glucose. Similarly Atkinson, et al., (1957) using dogs showed that glucose accounted for 70 - 8[illegible]% of the isotopes collected. All the mammals studied to date, showed that glucose was actively assimilated and there was little conversion of glucose to some other subst[illegible] before it had reached the blood stream.

In the case of fructose assimilation there was however some species difference. In the early thirties Bollman and Mann (1931) showed that in th[illegible] hepatectomised do[illegible], fructose was converted to glucose by the intestine. Shonsker, et al.,(1963) confirmed this in the eviscerated dog. Similarly the rat, (Kiyasu and Chaikoff, 19[illegible]) guinea pig, (Friedmueller and Chaikoff, 19[illegible]) and the hamster (Wilson and Vincent, 1[illegible]) all converted fructose to glucose and thus assimilated the fructose. In fact the assimilation of fructose was not dependant to any great extent on the prior conversion of fructose to either glucose or lactate (Cook, 19[illegible] and Hollsworth and Tawfik, 19[illegible]).

Many investigations showed that fructose in contrast to glucose does not appear to be actively transported (Bogdanove and Baker 1[illegible], Friedmueller and Chaikoff 19[illegible]). However Grassy, et al.,(19[illegible]) showed that when the rat intestine was incubated in a media containing fructose, the concentration of fructose in the interstitial fluid increased to levels higher than that of the incubation fluid, suggesting some active transport system.

1.2. Digestion of sucrose

Following the ingestion of sucrose Gryboski, et al., (1963) showed that unless the mucosal cells were damaged

there was no trace of sucrose in the urine. This indicated that under normal conditions sucrose was not transferred from the intestinal lumen into the blood stream. Fridhandler and Quastel (1955) and Wilson and Vincent (1955) showed that sucrose, lactose and maltose were all hydrolysed by intestinal enzymes and the monosaccharides produced, glucose, fructose and galactose were then assimilated in the normal way.

Dahlqvist and Borgstrom (1961) confirmed the much earlier work of Röhmann and Nagano (1903, that the enzyme activity of the intestinal contents was not sufficient to account for the amount of disaccharide disappearing from the lumen. Pierry (1912) had suggested that the enzymes were not in the intestinal juices but in the mucosal cells. Miller and Crane (1961) demonstrated that these enzymes were present in the brush borders of the mucosal cells.

A great amount of work has been carried out on the assimilation of glucose and fructose and also on the digestion of sucrose in a number of animals like the rat, dog, hamster, mouse and man. The natural diet of these animals contains a high proportion of carbohydrates in the form of starch, but very little monosaccharides. No work, however, has been carried out on the fruit bat, whose frugivorous diet contains very high concentrations of glucose, fructose and sucrose. It was therefore decided to investigate the digestion and assimilation of sugars in the fruit bat.

1.3. Rousettus aegyptiacus

The fruit bat Rousettus aegyptiacus is one of the smaller members of the megachiroptera weighing approximately 120 g (Jacobson, 1973) and is the exception among the fruit bats in that it is a cave dweller. Not only does this bat

have excellent eye sight, similar to other fruit bats (the retina being adapted to twilight vision) (Luck, personal communication) but also an acute sense of smell (Lombard, 1961) and is a voracious eater of soft ripe fruit. The animal's natural diet consists mainly of wild figs (Ficus species) and litchis (Litchi chinensis) when available. Jacobson and du Plessis (1976) reported that they also ate:-

Water pear	Syzygium cerrardii
Water berry	Syzygium cordatum
Kaffir plum	Harpephyllum caffrum
Cape ash	Ekebergia capensis
Bitter almond	Prunus africana

A colony of these animals has been kept in captivity in the Department of General Physiology since 1972 where they have bred freely, are in excellent condition and readily put on weight on a diet of ripe bananas sprinkled with pronutro*.

The habits of these bats in the wild and in the laboratory have been described by Lyon (1967), Kulzer (1959, 1963) and Jacobsen (1972, 1976).

Van der Westhuyzen (1975) showed that these bats would eat 76% of their own body weight of bananas per night in the laboratory. Jacobsen (1976) reported that bats kept in an aviary would eat on the average 90 g of litchis during the night, that is approximately half of their body weight. Because of these large amounts of food eaten by the bat during the night, some modification to its digestive system

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*Pronutro: Balanced breakfast cereal manufactured in South Africa by Bind Bros and Co. Ltd.

could be expected.

Early observations on these bats showed that when eating bananas, they masticated the food thoroughly and then used the tongue to press the bolus against the hard palate so extracting the fruit juices. The initial suggestion was that the bat swallowed the juice and spat out the fibrous remains. This hypothesis would explain why there was no solid material in either the bat's stomach or the intestine, when it was killed on returning to the cave after a night's feeding. However, the amount of solid material that was spat out by the bats in captivity was insignificant, and following a meal, the floor of the cage was littered mainly with hard fibrous faeces. A few partially eaten pieces of banana were also present but very few pellets resulting from the spitting out of sectioned bananas could be found. It would therefore appear that in captivity the bat ingested large amounts of solid materials and the reason why the stomach and the intestine were empty a few hours after a meal should be investigated. Also, since the fruit bat ingested 70% or more of its own body weight of bananas during the night, very large quantities of sugar were potentially available in the intestine for assimilation and the fate of these sugars should be of great interest.

Due to these interesting observations on these bats kept in captivity, the present study was carried out to investigate the different aspects in the assimilation of the sugars in these fruit bats. At the same time, it was decided to compare some of these observations with those found in the rat (Rattus norwegicus) where a great deal of work had already been done.

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2. METHODS

2.1. Methods for the determination of the concentrations of sugar and lactic acid in the blood and other solutions

2.1.1. Glucose concentration

The concentration of glucose in the blood and in the different solutions was determined using the "Blood Sugar G.O.D.-Perid Method"* This is a glucose oxidase method based on that of Werner (1970).

The blood or solutions were deproteinized by adding 0,1 ml of sample to 1,0 ml of 0,3 M perchloric acid. The solutions were centrifuged for 5 minutes at 3000 r.p.m. and then 0,7 ml of the supernatant was added to 2 ml samples of the buffer solution which also contained POD,** GOD** and ABTS.** The green colour which developed was directly proportional to the amount of glucose in the solution. The concentration of the glucose in the blood or solutions was measured directly using the concentration mode on a Hilger and Watts "Uvichem" spectrophotometer at a wavelength of 578 nm.

2.1.2. Glucose and fructose concentrations

The method used for the determination of the concentration of fructose in the blood and other solutions was based on that of Schmidt (1971). This method used hexokinase

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* A Biochemica Test Combination product by Boehringer Mannheim GMBH.

** POD - Peroxidase
GOD - Glucose oxidase
ABTS - 2,2'-Azino-di-(3 ethyl-benzthiazoline sulfonate)

for the estimation of the glucose* concentration and phosphoglucose isomerase for the fructose concentration.**

The method consisted of deproteinizing the solution with 0,33 M perchloric acid and centrifuging the solution as in the glucose estimation.

The "Biochemica Test Combination" solution containing the buffer, NADP*** and ATP**** was placed in a quartz cuvette having a light path of 1 cm and 0,[illegible] ml of the supernatant added. The solution was mixed and the optical density of the solution was read at a wavelength of 340 nm using air as the reference in a Hilger and Watts "Uvichem" spectrophotometer. Once the optical density was constant, usually within 5 minutes, the value was recorded, 0,0[illegible] ml of the hexokinase solution added and the solution mixed. The optical density was read at 5 minute intervals until there was no further increase, and the maximum optical density recorded. The [illegible] between these two readings represented the [illegible] of glucose in the solution. Once this value was recorded then 0,02 ml of phosphoglucose isomerase was added and the solution mixed. The optical density was recorded until no further changes occurred. The difference between this reading and the previous reading (i.e. the reading after the hexokinase had been added) represented the amount of fructose in the [illegible]on. Standard

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* Glucose Hexokinase method "Biochemica Test Combination" Boehringer Mannheim G.M.B.H.

** Phosphoglucose isomerase. Boehringer Mannheim G.M.B.H.

*** NADP - Nicotinamide-Adenine dinucleotide phosphate.

**** ATP - Adenosine-5-triphosphate.

curves were prepared for glucose and fructose and from these, the concentrations of the sugars were calculated.

2.1.3. Sucrose concentration

The method for sucrose determination was similar to that of glucose and fructose. In the determination of the sucrose concentration the plasma proteins could not be removed by precipitation with perchloric acid, as the latter caused the hydrolysis of the sucrose leading to false results. Therefore in this method the protein was precipitated by mixing 0,1 ml of the blood or solution with 1,0 ml of physiological saline and placing it in a water bath at 85° C for 10 minutes. A glass marble was placed over the mouth of the test tube to prevent loss of water vapour. This method resulted in no hydrolysis of the sucrose and a protein free supernatant.

The concentrations of glucose and fructose in the supernatant were estimated as in the previous method using hexokinase and phosphoglucose isomerase. Once the optical densities of the glucose and fructose had been determined the sucrose in the cuvette was hydrolysed by adding 0,02 ml of β-fructosidase.* The final change in the optical density was proportional to the concentration of sucrose in the cuvette.

2.1.4 Lactic acid concentration

The method for estimating the blood lactate concentration was that based on Hohorst (1963). It is available as a "Biochemica Test Combination".**

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* Product of Boehringer Mannheim G.M.B.H.

** Boehringer Mannheim G.M.B.H.

The method was modified in a number of ways. Firstly, the blood volumes recommended were too large to be taken from the bat, so instead of adding 0,5 ml of blood to 1,0 ml of 0,6 M perchloric acid, only 0,1 ml of blood was added to 1,0 ml of 0,33 M perchloric acid and centrifuged. This meant that the glucose, fructose and lactate could all be determined using samples from the same supernant solution. Secondly, to increase the concentration of the lactate, 0,45 ml of the supernatant was used instead of the recommended 0,2 ml. Thirdly, in the experiments where sucrose was used the plasma proteins were precipitated by adding 0,1 ml of the blood sample to 1,0 ml of saline and heating the solution at 85° C for 15 minutes in a waterbath without hydrolysis of sucrose occurring.

2.2. Blood sampling technique

In the experiments carried out on anaesthetised rats and bats the blood samples were taken from polythene cannulae that had been placed in the right jugular vein and passed down the vein until the tip was in the right atrium. Thus this blood sample was representative of mixed venous blood.

In the experiments on conscious animals however different methods had to be used on the bat and the rat.

2.2.1. The bat

The restraining cage used to hold the bat consisted of a perspex cylinder with an internal diameter of 5,25 cm and 20 cm long (Fig. 1). The cylinder had a slot 1,5 cm wide and 14 cm long starting at the proximal end of the tube and running along the length. The sterile of the distal end of the tube was sealed with a perspex plug which was attached to a retort stand. A number of 0,7 cm diameter

holes were made in the cylinder at the distal end so that the bat would not suffocate.

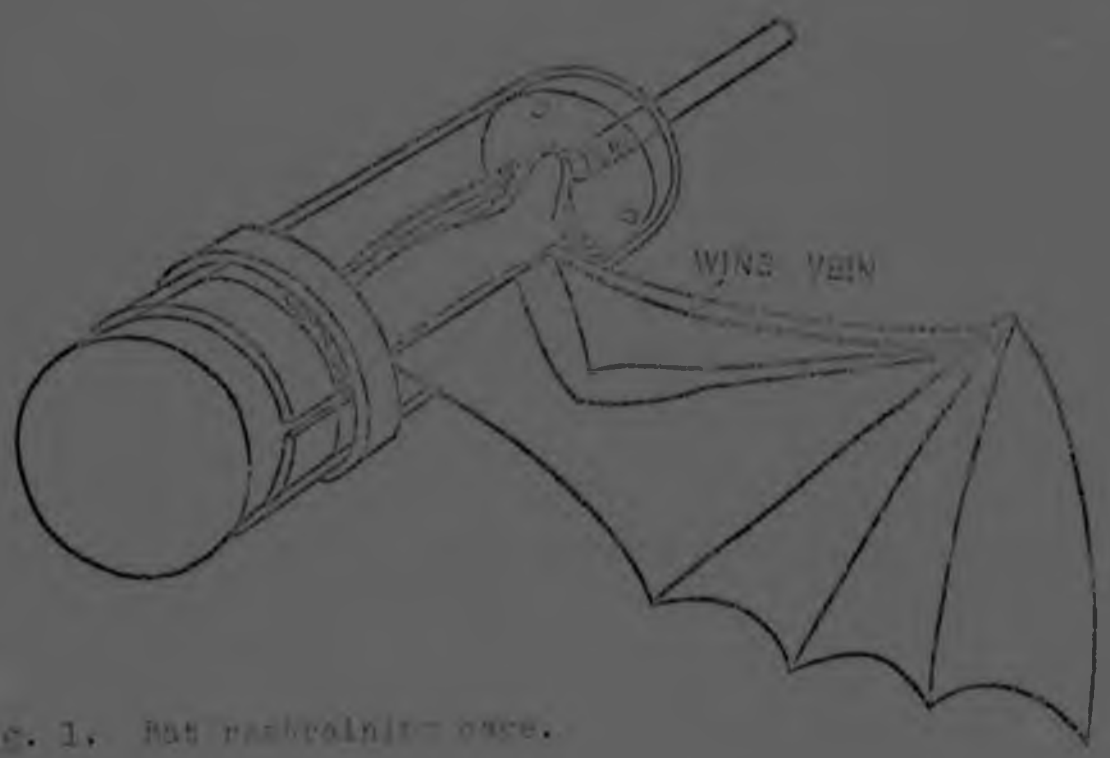

Fig. 1. Bat restrained in case.

A tightly fitting ring about 1 cm wide was made which could slide over the entire length of the outside of the cylinder. The ring was removed and the bat placed head first into the cylinder, keeping the one wing folded and the other open, the open wing passing through the slot in the cylinder. The ring was slid over the cylinder, so preventing the wing from being drawn back into the cylinder. The proximal end of the tube was closed with a rubber bung. The wing was extended and the blood samples were taken from the vein running along the leading edge of the wing with a microsyringe. ("Allar" supplied by Wellcome).

This method for collecting the blood samples in the bat

worked well and the animals appeared completely tranquil.

2.2.2. The rat

The rat restraining cage consisted of a wire mesh cage 5 cm x 5 cm x 19 cm, with a wire door at both ends. To be able to shorten the length of the cage there were wooden blocks 4,5 cm x 4,5 cm x 2,5 cm which could be placed inside the cage before the rat was put inside. The rat was placed head first into the cage with its tail sticking out through the wire door. The amount of space in the cage was adjusted so that the rat had no room in which to move and so withdraw its tail into the cage. A convenient way of quickly collecting blood samples was to chop off the distal 3 cm of the tail with a wood chisel. The rat did not appear to suffer any discomfort from this procedure and 0,15 ml samples of blood could be collected from the stump. To prevent coagulation occuring too quickly the animal was heparinised by giving it 1 000 units of heparin intraperitoneally at least half an hour before the experiment was started. Sometimes the wound did not bleed very freely in which case either the tail was gently "milked" or a further centimeter of the tail was chopped off.

2.3. Anaesthetics used in these experiments

A number of different anaesthetics were tried during this investigation. Finally only the phenobarbitone, pentobarbitone mixture was used (see 2.3.4).

2.3.1. Pentobarbitone (Nembutal) sodium solution

Pye (1967) reported that this was the anaesthetic of choice to be used with bats even though the dose required for anaesthesia varied greatly from animal to animal. The

anaesthetic solution was made up by mixing 1 ml of pentobarbitone sodium (Sagital 60 mg/ml May and Baker) with 9 ml of 10% ethyl alcohol.

The animals were anaesthetised by injecting 0,66 ml of the anaesthetic mixture per 100 g body weight intraperitoneally. In these experiments done on both the bat and the rat it was found that the dose of anaesthetic needed varied and that after an hour the anaesthetic began to wear off and no second dose of the anaesthetic had to be given.

2.3.2. Thalamonal*

Thalamonal is a mixture of anaesthetics and is classified as a neuroleptanalgesic. The animals were given 0,1 ml/ 100 g body weight intraperitoneally. However this drug proved unsuitable for the study as the amount of sugar assimilated after this treatment was much less than normal.

2.3.3. Urethane

The urethane anaesthetic was injected subcutaneously, 0,4 ml per 100 g body weight (100 mg per 100 g body weight). This anaesthetic however increased the resting blood glucose level by a factor of 2 to 3 and was thus rejected.

2.3.4. Phenobarbitone and Pentobarbitone mixture

A stock solution of the anaesthetic containing 1,7 g of phenobarbitone sodium and 0,24 g of pentobarbitone sodium dissolved in 100 ml of a 50% propylene glycol/water solution, could be stored in the refrigerator at 4° C for three months. The stock solution was diluted 10 times with water and 0,66 ml/ 100 g body weight of the diluted anaesthetic solution

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* "Thalamonal" 0,05 mg/ml fentanyl and 2,5 mg/ml droperidol, made by Janssen Pharmaceutica, Belgium.

injected intraperitoneally.

Kündig (personal communication) reported that this anaesthetic worked very well in rats and in this investigation it was found that it also gave excellent results in bats.

2.4. Transit time for bananas in bats

In this estimation 47 bats of both sexes were used.

The bats were fed normally the night before the experiment. The following morning all food and water was removed from the cage. Bats will not eat during the day unless they are starving.

On the night of the experiment the bats were placed individually in a small wire cage so that their activity could be observed. A piece of banana, weighing approximately 5 g, was placed in the cage and the time recorded when the animal started to chew the banana, this time, was taken as the time when swallowing actually occurred. The transit time was taken as the period between this time and the time when the first voiding occurred. Since it was impossible to observe how long the food remained in the mouth before the first swallowing occurred, it is possible that the transit times recorded could be slightly longer than the real transit time for bananas. The evidence that the faeces were the result of the ingested banana, was the observation that when the bats were killed approximately three hours or more after a meal there was no food material in either the stomach or small intestine and these bats had had no food for at least 10 hours.

2.5. Oral glucose tolerance test

These tests were carried out on anaesthetised bats and rats.

The night before the experiment the animals were isolated in separate cages from the rest of the colonies. They were deprived of all food but had free access to water. The following morning the animals were weighed and anaesthetised with the sagital/ethyl alcohol anaesthetic (see page 11).

A freshly prepared solution of glucose was made containing 10 g/100 ml of water and kept in a waterbath at 37° C. A 10 ml luer lock syringe with a hyperdermic needle attached was filled with approximately 11 ml of the sugar solution, the needle was then sealed with a rubber bung and the syringe placed in a waterbath at 37° F until it was required.

A Jacques catheter (charrier 8) was shortened to approximately 20 cm from the tip and a 18G luer lock needle tied firmly into the end of the tube. A mark was made on the catheter, so that when the tube was being introduced down the oesophagus, the mark would coincide with the incisors when the tip of the catheter was in the stomach.

In the rats the right jugular vein was exposed and cannulated so that blood samples could be collected. The rats were heparinized with 500 units intravenously. In the bats blood samples were taken from the wing vein and no heparinisation was found necessary.

Resting blood samples of approximately 0,15 ml were collected from the animals. All blood samples were collected in heparinised micrometer syringes ("Aglar" Burroughs Wellcome) and 0,1 ml pipetted into 1,0 ml of 0.6 M perchloric acid. The blood glucose concentration was estimated using the G.O.D. Perid technique (page 6).

After the resting blood samples had been taken, the needle from the prepared syringe containing the glucose

was removed and the stomach tube connected to the syringe. The stomach tube was then filled with glucose solution from the syringe and the volume in the syringe adjusted so that 500 mg of glucose would be given per 100 g body weight. To prevent any loss of fluid from the syringe the stomach tube was closed with a bulldog clip.

The animal was held in the left hand, head uppermost and with the use of the left thumb and index finger the mouth was opened and the stomach tube, lubricated with liquid paraffin, was passed down the oesophagus into the stomach. The position of the tip of the stomach tube being indicated by the mark previously made on the tube. The bulldog clip was removed and the warm glucose solution injected into the stomach over a period of 30 seconds. The bulldog clip was then replaced, insuring that 500 mg of glucose per 100 g body weight had been given. The stomach tube was slowly withdrawn from the animal. If it was removed too quickly it often resulted in the animal regurgitating some of the sample. In the event of this happening the animal was immediately withdrawn from the experiment.

In some animals 10 ml of distilled water was given instead of the sugar solution to see if a large volume of fluid had any effect on the blood levels of glucose.

Further blood samples were taken 5, 10 and 20 minutes after the administration of the glucose and the concentrations of glucose estimated. After the animals had recovered from the anaesthetic they were returned to their colonies.

Graphs were drawn of the mean blood glucose levels, with the standard error of the mean against the respective

times of collection.

2.6. Intravenous glucose tolerance tests

The bats and rats were removed from the stock cages the night before the experiment and placed in smaller cages. All the animals were deprived of solid food but had free access to water and an isotonic glucose solution. The following morning the animals were weighed and anaesthetised with Saggital/ethyl alcohol (page 11). A fresh glucose solution was prepared containing 5,25 g of glucose per 100 ml of water and placed in a waterbath at 37° C.

In the experiments performed on the rats the right jugular vein was exposed and cannulated using a polythene tube (Portex 47 Poly). The rats were then heparinized with 500 units of heparin intravenously.

Resting blood samples were collected in the bat from the wing vein and from the jugular vessels in the rat. The blood was collected with a heparinised "Aclar" syringe, aliquots of 0,1 ml of blood sample were added to 1,0 ml of 0,33 M perchloric acid and the concentration of glucose estimated using the G.O.D. Perid method (page 7).

The animals were then given 1,45 ml of the 5,25 g/100 ml glucose solution per 100 g body weight intravenously.

In the case of the bat the glucose was injected into a wing vein and in the rat into the jugular cannula. After the glucose solution had been injected into the rat, the solution still remaining in the cannula was washed into the animal with 0,2 ml of saline. Blood samples were taken in every case at 5, 10, 20 and 40 minutes after the administration of the glucose solution.

As controls, two bats were given 2,0 ml per 100 g body

weight of warm physiological saline intravenously. Blood samples were only taken at 2,5 and 5 minutes as this was the time when the effects on the blood glucose levels were found to be the greatest.

2.7. Assimilation of sugars in the conscious animal

The technique used to estimate the amount of sugar that had been assimilated from the intestine was based on that of Cori (1925).

The basis of this method was to give the animal a known amount of sugar with a stomach tube and then to kill the animal after a known length of time and estimate how much of the sugar was still present in the gastrointestinal tract. The difference between the amount of sugar given and the amount recovered would be the amount of sugar assimilated, mainly from the intestine.

In these experiments where the volumes of concentrated sugar solutions were to be measured it would be an advantage if there were no food materials in the stomach and intestines. This necessitated the need for preparing the animals at least one night before the experiment. Cori deprived his animals of all food the night before the experiment but they did have free access to water. This method, was found to be unsatisfactory with respect to the bats for they showed signs of weakness following 24 hours without food and seldom survived 40 hour periods of starvation. To ensure that the animals, especially the bats were not in this deleterious state, the method used by Fisher and Parsons (1949) was followed where the animals had not only access to water but also to a solution containing 5 g of glucose per 100 ml of water the night before

the experiment.

The following morning the animals were weighed and placed in restraining cages (page 9, 11), in which they showed little signs of struggling and appeared tranquil. The rats were given an intraperitoneal injection of 500 units of heparin to prevent coagulation of the blood when the samples were taken. In the bat this was not necessary as the blood never coagulated in the time available.

A solution of sugar containing 15 g of the sugar (either glucose, fructose or sucrose) per 100 ml of water was prepared as stock and kept in a waterbath at 37° C. A 10 ml syringe containing glucose was prepared as before (page 14) and placed in the same waterbath. In the experiments where a mixture of glucose and fructose was given then the solution contained 7,5 g of each of the sugars per 100 ml of water.

Resting blood samples were collected from the wing veins in the bats and in the rats from the tail stump (pages 9, 11).

All blood samples were collected in heparinised micrometer syringes, and the glucose concentration was estimated by the G.O.D. Perid method (page 6). The fructose concentration was estimated using the hexokinase method in conjunction with phosphoglucose isomerase (page 6). The concentration of sucrose was then estimated as described on page 8.

Having collected the resting blood samples, 10 ml of the warm sugar solution was injected into the bat and rat as previously described (page 15).

(In the rat, if difficulty was experienced in giving this volume, these animals were then immediately removed from the experiment.)

The animals were then returned to their respective restraining cages and blood samples taken at 5, 10, 20, and 30 minutes after the administration of the sugar. During the experiment if any material was defaecated that animal was taken out of the experiment.

After the 30 minute blood sample had been taken the animal was removed from the restraining cage, stunned and killed. The abdomen was opened and ligatures tied around the oesophagus where it enters the stomach, around the pyloric sphincter and the distal end of the ileum. In the rat the fluid had sometimes reached the colon in which case the fluid was squeezed back into the ileum before tying the ligatures. The stomach and intestine were removed, the fluid contents in each were extracted and using a 10 ml measuring cylinder, the volumes were measured. The stomach and intestine were individually rinsed out with saline, the washings together with the original contents of each organ were pooled and the volume made up to 100 ml. Aliquots of 0,1 ml of these solutions were used to determine the concentration of the sugars present.

As the amount of sugar given and the amount of sugar recovered was known the amount of sugar assimilated could be calculated.

The stomach and the intestine were weighed and the length of the intestine measured. In the latter case the intestine was left in a saline solution at room temperature for 30 minutes, this appeared to give more consistent lengths as the intestine was stretched to some degree while it was being removed and this period allowed it to return to normal.

The amount of sugar assimilated could be expressed in

absolute terms or relative to the intestinal length or weight.

2.8. In vitro assimilation of glucose

2.8.1. The gut sac

The method used was that originally described by Wilson and Wiseman (1954).

The animals, both rats and bats, were removed from their respective cages and fasted overnight of solid food. They had access to water and a glucose solution (page 17). The following morning all the animals were given 500 units of heparin intraperitoneally. After 30 minutes the animals were stunned and killed. The abdomen was quickly opened and the intestine removed and placed in Krebs bicarbonate solution (Umbreit, et al., 1949). The solution was gassed continuously with a mixture of 95% oxygen and 5% CO_2. Five sections were cut from the intestine each approximately 5 cm in length and representing the duodenum, jejunum, proximal ileum, mid-ileum and the distal ileum. A glass rod 3 mm in diameter and 20 cm long was used to evert the different sections of the intestine and the sections once everted were returned to the Krebs solution. This process from killing to everting the gut was carried out as quickly as possible, usually taking less than 2 minutes, so that the period of hypoxia for the mucosal cells would be kept to a minimum. A cotton ligature was tied firmly around the one end of the intestine while a second ligature was tied loosely around the other end. A small glass bead was attached to the first ligature to act as a weight so that when the intestine was placed in the incubation fluid it would be kept below the surface of the fluid. The intestine was removed

from the Krebs solution and the excess water on the surface of the intestine was removed by lightly blotting the intestine with a paper face tissue. The intestine was weighed together with the ligature and glass bead. A blunt hypordermic needle (G 18) was passed down the now serosal lined lumen from the open end of the intestine. Once the needle was in position the ligature which was already in place was tied firmly with a single knot. A syringe filled with the Krebs bicarbonate solution containing [illegible] g of glucose per 100 ml was connected to the needle and the incubation fluid slowly injected into the gut sac. Care was taken to see that when the intestine was filled the villi were just free and the intestine not overdistended. A small bubble of oxygen was sometimes injected into the gut sac so as to ensure that the fluid remained fully oxygenated. This latter step was found to be unnecessary as it had no effect on the results. The needle was slowly withdrawn and once its tip passed the site of the ligature, the latter was finally tied. The excess fluid on the serosal surface removed by blotting it off and the intestine weighed with the fluid inside. The difference between the two weights, was the weight of the Krebs solution added to the gut sac. The gut sacs were placed in a water jacketed tissue bath containing 500 ml of the Krebs bicarbonate solution at 37° C. A 95% Oxygen and 5% CO_2 gas mixture was continuously bubbled through the Krebs bicarbonate solution. The gassing not only oxygenated the fluid but also kept the solution stirred so that no stagnation of fluid could occur in the vicinity of the intestine. After 30 minutes the intestine was removed and the excess water removed by blotting the mucosal surface

with face tissues and the intestine weighed. The difference in weight between this weight and the final weight of the intestine after the luminal fluid had been drained would be the weight of Krebs bicarbonate solution in the gut sac at the end of the experiment. The fluid inside the sac was collected and the concentration of glucose determined using the G.O.D. Perid method (page 6).

The length of the intestine between the two ligatures was measured so that the amount of glucose transferred could be expressed relative to the length of the intestine used.

Knowing the concentration of the glucose as well as the volume of Krebs bicarbonate solution in the gut sac both before and after the experiment, the amount of glucose in the gut sac both before and after the experiment could be calculated. The difference between the original and final amounts of glucose will be the net gain or loss of glucose with respect to the serosal surface in 30 minutes. This value was then divided by the length of intestine used giving the amount of glucose transferred per cm/cm/30 minutes.

2.9.7. Deamination across the everted gut

The apparatus used for this investigation was based on the one used by Casey and Wade (1960). The experiment consisted of everting the gut and then circulating the serosal compartment with Krebs bicarbonate solution. The mucosal compartment was kept separate from the serosal compartment and also contained Krebs bicarbonate solution. Samples of the incubation fluid could be withdrawn from either compartment at any time.

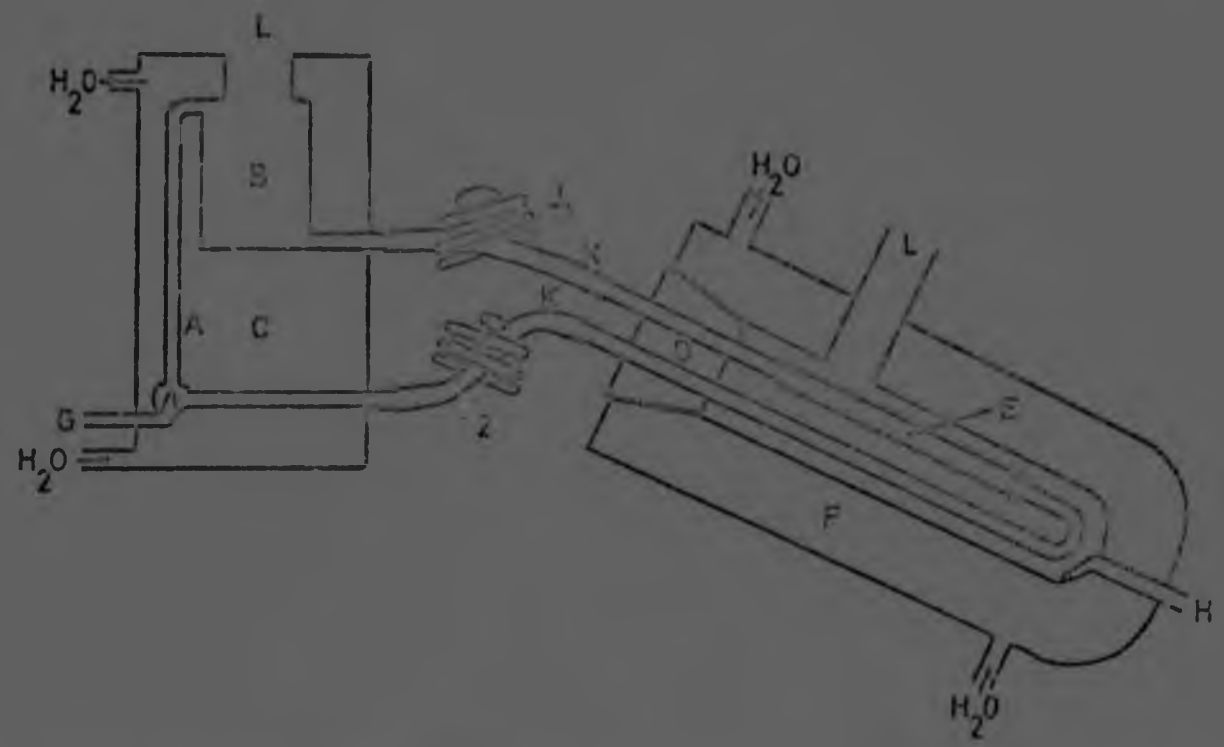

Fig. 2. The apparatus for the recirculation of the serosal fluid through the everted intestine.

A: gas lift pump. B: reservoir containing serosal fluid. C: water-jacket surrounding gas lift pump and reservoir. D: oil plast cannula unit. E: eversted intestine. F: tissue holder (containing mucosal fluid) and water-jacket. G and H: gas inlets for a mixture of 95% oxygen and 5% carbon dioxide. J, and K: screw clips. L: silastic tubing. L: gas outlet.

The apparatus in Fig. 2 consists of three separate parts: a gas lift pump/reservoir "A, B, C", a cannula unit "D" with the two cannula and a tissue holder "E". Both the tissue bath and the pump/reservoir were surrounded by water-jackets so as to keep the Krebs solution at a constant 37° C.

The apparatus was prepared by closing the two silastic tubes* "K" connecting the gas lift pump to the cannula unit with screw clips "J" and "J". The cannula unit was disconnected from the tubes and held in a clamp ready for the cannulation. The reservoir (serosal) and tissue holder (mucosal) were filled with known volumes of Krebs bicarbonate solution containing approximately 500 mg glucose/100 ml.

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* Silastic - Dow Corning

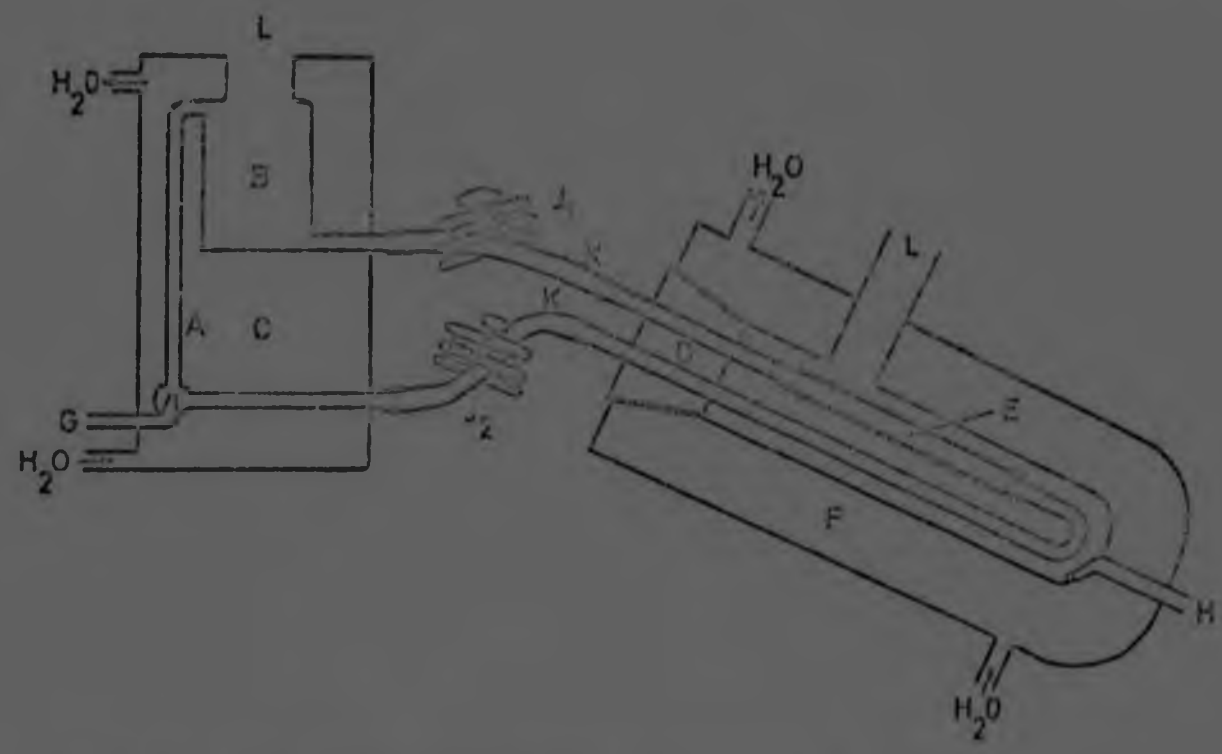

Fig. 2. The apparatus for the perfusion of the serosal fluid through the everted intestine.

A: gas lift pump. B: reservoir containing serosal fluid. C: water-jacket surrounding gas lift pump and cannula. D: oil phase cannula unit. E: everted intestine. F: tissue holder containing mucosal fluid and water-jacket. G and H: gas inlets for a mixture of 95% oxygen and 5% carbon dioxide. J, and J: screw clips. K: silastic tubing. I: gas outlet.

The apparatus in Fig. 2 consisted of three separate parts: a gas lift pump/reservoir "A, B, C", a cannula unit "D" with its two cannulae and a tissue holder "E". Both the tissue bath and the pump/reservoir were surrounded by water-jackets so as to keep the Krebs solution at a constant 37° C.

The apparatus was prepared by closing the two silastic* tubes "K" connecting the gas lift pump to the cannula unit with screw clips "J" and "J". The cannula unit was disconnected from the tubes and held in a clamp ready for the cannulation. The reservoir (serosal) and tissue holder (mucosal) were filled with known volumes of Krebs bicarbonate solution containing approximately 100 mg glucose/100 ml.

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* Silastic - Dow Corning

The screw clips at "G" and "H" were adjusted so that there was a steady flow of a water saturated gas mixture of 95% oxygen and 5% carbon dioxide, through both the mucosal compartment and the gas lift pump. If the gas was not water saturated loss of water by evaporation occurred,

The animals were fasted of all solid food the night before but had access to a glucose solution and water. The following morning both the rats and the bats were given 500 units of heparin intraperitoneally and half an hour later were stunned and killed. The intestine was rapidly removed from each animal and placed in an oxygenated Krebs bicarbonate solution at 37° C. Sections approximately 6 cm long were taken from 5 regions of the gut representing the duodenum, jejunum, proximal ileum, mid-ileum and distal ileum. All these gut sections were quickly everted using a glass rod 0,3 cm in diameter and 20 cm long; and then replaced in the Krebs bicarbonate solution. The everted sections were cannulated "D" making sure that the proximal end of the intestine was tied to the inner cannula of the cannula unit. Any excess length of tissue could be folded over the cannula so that there was 2 cm of gut between the two cannulae. When the gut had been tied in position the cannula unit was reconnected to the gas pump/reservoir tubes and the screw clips released, the reservoir screw "J" first so that the Krebs solution started to flow in the correct direction. The solution then flowed from the reservoir through the serosal lumen of the gut to the gas lift pump and back to the reservoir again.

The cannula unit was then placed in the tissue holder and the level of fluid in the reservoir adjusted so that

the luminal pressure was 7 cm of water. Two samples, each of 0,1 ml of the Krebs bicarbonate solution were taken, one from the serosal and the other from the mucosal compartments. The concentration of glucose in both samples was determined.

One hour after taking the initial samples of Krebs bicarbonate solution further samples of 0,1 ml were taken from both compartments and the glucose concentrations determined. The gas mixture was switched off and the cannula holder removed from the tissue bath, allowing any fluid on the mucosal surface to run back into the tissue bath. The volumes of fluid in both compartments were measured and the length and weight of the intestine recorded.

The actual volume of fluid in both compartments at the start of the experiment was the difference between the volume of fluid added and the 0,1 ml samples taken at the start of the experiment for the glucose analysis. The volume at the end of the experiment was the final volume measured plus the 0,1 ml samples taken at the end of the experiment for the estimation of the glucose concentration. Knowing the glucose concentrations and the volumes of fluid in both compartments, the amounts of glucose present in both compartments at the start and at the end of the experiment were calculated. From this information the amount of glucose assimilated, transferred and utilized can be calculated and expressed as mg of glucose per cm length of intestine per hour.

As soon as the duodenal tissue had been set up and the samples of fluid taken, the jejunum, proximal ileum, mid-ileum and distal ileum were set up in turn.

2.8.3. Recirculation of the isolated intestine

The method used in this technique was based on that of Fisher and Parsons (1949).

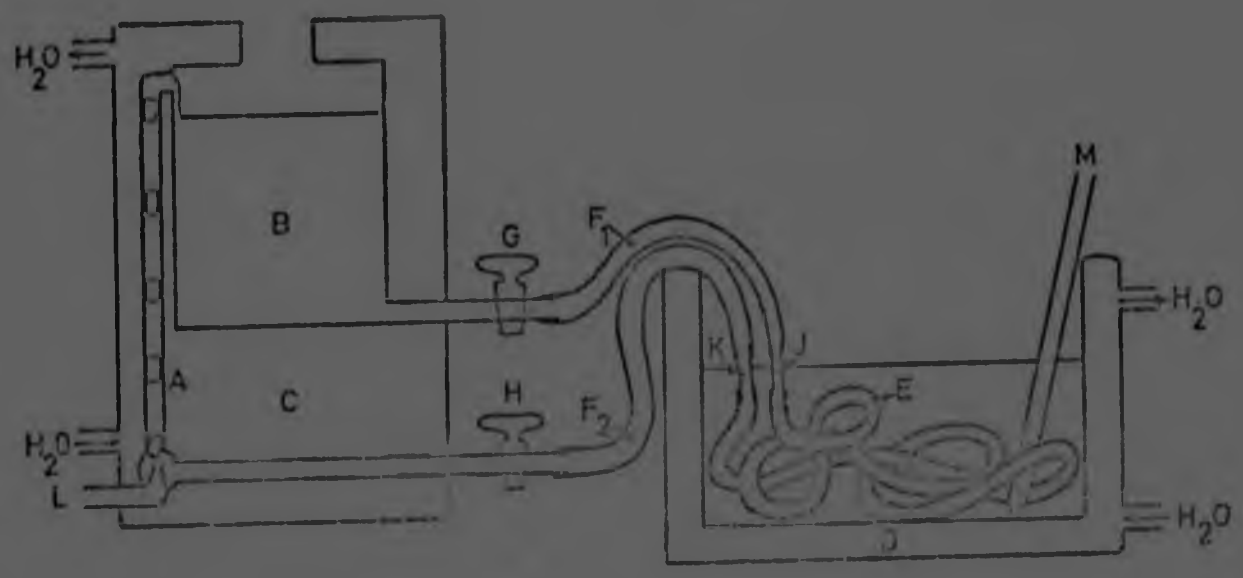

Fig. 3. The apparatus for the recirculation of the small intestine in vitro.

A: gas lift pump. B: reservoir containing Krebs-Ringer fluid. C: water-jacket surrounding gas lift pump and reservoir. D: tissue bath containing warmed fluid. E: intestine. F and F1: polarographic tubing. G: carbon tap. H: inlet tap. J: platinum cannula. K: dissecting cannula. L and M: gas inlets for mixture of 95% oxygen and 5% carbon dioxide.

The apparatus (Fig. 3 consisted of two glass units. The first, "A, B and C" was a combined gas lift pump and reservoir enclosed in a water-jacket, similar to the one used in the previous experiment. This apparatus, however, had a 80 ml reservoir and the inlet and outlet tubes had glass stopcocks (G and H). The second unit consisted of a water-jacketed tissue bath (D) in which was placed a glass tube to saturate the Krebs solution with a gas mixture of

95% oxygen and 5% CO_2. Water at 37° C was circulated through both the water jackets.

The animals were fasted overnight of solid food but had access to water and an isotonic glucose solution.

An hour before the start of the experiment, each animal was anaesthetised intraperitoneally with the phenobarbitone, pentobarbitone mixture (page [illegible]). The taps (G and K) were closed and the reservoir and the tissue bath were filled with known volumes of the Krebs bicarbonate solution containing approximately 100 mg of glucose/100 ml. The solutions were gassed continually with a [illegible] mixture of oxygen and carbon dioxide. Two lengths of silastic tubing were attached to the taps while the distal ends of the tubes were connected to each other with a short length of polythene tubing. The taps were opened and the gas lift pump set in motion which kept the solution circulating and fully oxygenated.

Once the animal was asleep, the hair over the abdomen was removed with a depilator and the abdomen opened along the midline. The duodeno-jejunal junction was identified and two ligatures were placed loosely around the junction. The cranial of the two ligatures was tied tightly preventing any loss of blood from the intestine when it was cannulated. Two further ligatures were placed around the ileum about 1 cm proximal to the ileocolic junction in the rat and 4 cm from the rectum in the bat. The caudal of these two ligatures was tied firmly. The ileum was then cannulated, using polythene tubes (J and K) 2 cm in length and having

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* Noir - Natal.

an outside diameter of 3 mm, just distal to the jejunal ligature and proximal to the ileal ligature, using the loose ligatures to tie the cannula firmly into position. A blunt needle connected to a 10 ml syringe was inserted into the proximal cannula and the intestine gently washed out with 10 ml of saline at 37° C.

Polythene tubes were used as cannulae as they had the largest inside diameters for a fixed outside diameter due to their thin walls and so minimised the changes of blockage as well as keeping the resistance to flow of Krebs solution in the tubes to a minimum.

The stopcocks (G and H) were closed and the two silastic tubes separated. The tube (F_1) connected to the reservoir was connected to the proximal cannula inserted into the jejunum while the second tube (F_2) was connected to the distal ileal cannula. The stopcocks were opened, first (G) the one from the reservoir then the other (H). The height of the reservoir was adjusted so that there was a continuous flow of Krebs bicarbonate solution through the intestine.

The intestine was perfused for two minutes, then the mesentry cut and the intestine removed from the body. Great care was taken to ensure that the mucosal perfusion was maintained continuously so that at no time was the mucosa deprived of oxygen.

The blood on the outside of the intestine was washed off with glucose free Krebs bicarbonate solution and the intestine was then placed in the tissue bath (D). Aliquots of 0,1 ml of both mucosal and serosal fluids were taken for glucose estimation. After 60 minutes the experiment was terminated and the volume of fluid in the mucosal and serosal

compartments measured. The weight and length of the small intestine were measured.

2.9. Effect of [illegible] on assimilation of [illegible]

2.9.1. The effect of [illegible] on the assimilation of glucose in the rat

The animals had been fasted overnight of solid food but had access to water. The animals were anaesthetised intraperitoneally the following morning with one of the following anaesthetics:-

a. Thalamonal

b. Urethane

c. Phenobarbitone [illegible]

Once the rats were asleep, the hair was removed from the neck and abdomen. The right jugular vein was exposed and cannulated with a polythene tube (6 cm length) (Portex PP) filled with heparin (Leo) (5 000 units/ml). The oesophagus was exposed and cannulated using a Jacques catheter (Ch 8) filled with a 1% g per 100 ml glucose solution and clamped with a mosquito clip. Care was taken to make sure that the tip of the catheter was in the stomach before tying the catheter in the oesophagus.

The rat's abdomen was opened with a midline incision and the distal end of the ileum cannulated using silastic tubing (OD - 0,1"). Any effluent from this cannula was collected in an Erlenmeyer flask.

The animal was heparinised by injecting 0,1 ml of saline into the jugular cannula, this washed the heparin that had been in the cannula into the body.

The oesophageal catheter was then connected to a syringe containing 10 ml of a warm glucose solution containing

1,5 g of glucose.

The pre-administration blood sample of 0,15 ml was collected from the jugular vein to estimate the blood glucose level. The bulldog clip on the stomach tube was removed and the 10 ml of the glucose solution slowly injected into the stomach over 30 seconds. Once the 10 ml had been injected the oesophageal catheter was once again closed with the bulldog clip, insuring that exactly 10 ml had been injected and also preventing the loss of any fluid from the catheter. Blood samples were taken from the jugular catheter 5, 10, 20 and 30 minutes after the glucose had been given.

When the 30 minute blood sample had been taken, the abdomen was quickly reopened and a double ligature placed around the pyloric sphincter, a few millimeters apart. Two further ligatures were placed around the cardiac sphincter once the oesophageal cannula had just been withdrawn from the stomach, and the second around the ileum just proximal to the distal cannula. The stomach and intestine were removed, and the volumes of the contents in the stomach and intestine were removed and measured. The stomach and intestine were individually rinsed out with saline, the washings together with the original contents of each organ were pooled and the volumes made up to 100 ml. Aliquots of 0,1 ml of the solutions were added to 1,0 ml of 0,33 M perchloric acid, the glucose concentrations in these solutions were measured and the amounts of glucose recovered from each organ calculated. From this information the amount of glucose assimilated was calculated. The stomach and the intestine were weighed and the length of the intestine measured.

2.9.2. Comparison between the conscious and anaesthetised bats in their ability to assimilate glucose and fructose.

The bats were fasted overnight of solid food but had free access to water and an isotonic glucose solution.

In the first two experiments, four bats were given 1,5 g of fructose in a solution containing 15 g/100 ml. In the last two experiments, two of the bats received 0,5 g of glucose while the other two received 0,5 g of fructose, this time however the concentrations of the solutions were 5,25 g/100 ml. The reason why the lower concentration was used in the last two experiments was because the fluid was going straight into the jejunal lumen by-passing the stomach and should therefore be isotonic to the blood. This was done although, as already discussed on page [illegible], it appears that in the bat, maintenance of the osmolarity of the lumen of the intestine relative to blood is of minor importance.

Each experiment consisted of two bats, one conscious, the other animal anaesthetised.

The method used on the conscious bat was the same as previously described on page 17. The other animal was anaesthetised with an intraperitoneal injection of phenobarbitone pentobarbitone mixture (page 18) and the experiment carried out using the method described on page 20. (With the exception that in the low concentration experiments the solution was introduced directly into the duodenum).

As the bat has no large intestine it was not necessary for the abdomen to be opened. The distal end of the intestine was cannulated by passing the silastic catheter up

the anus and tying it in position.

Blood samples were taken as before; the first was taken prior to the administration of the sugar, while the others were collected 2,5 5, 10, 20 and 30 minutes after the administration of the sugar. The dose of 10 ml of the sugar was given either into the stomach or duodenum as before depending on which technique was used. After the 30 minutes blood sample had been taken the animals were killed, the abdomens opened and the amounts of the sugars still present in the stomachs and intestines were estimated as before. Knowing the amount of sugar administered and the amount recovered then the difference would be the amount of the sugars assimilated.

2.10. Method for the infusion experiments

The method used for the infusion experiments was based on that of Jacobs and Luper (1957).

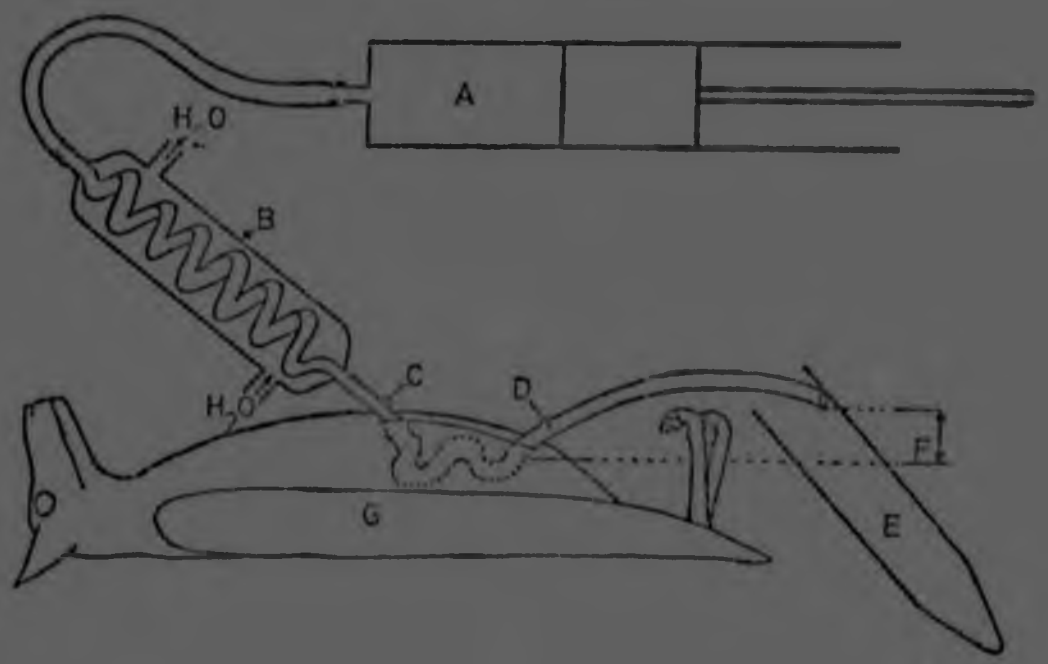

Fig. 4. Apparatus for the infusion of sugars at a constant rate through the jugular vein.

A: 50 ml syringe fitted in a constant rate infusion pump. B: condenser at 37° C. C: proximal cannula. D: distal cannula. E: centrifuge tube. F: height the distal end of the distal cannula is above its proximal end. G: bat.

The apparatus consisted of a 50 ml hypodermic syringe filled with the fluid to be infused through the apparatus. The syringe was driven by a stepper motor so that once the speed of the motor had been adjusted, a constant rate of infusion was maintained even if the load on the motor altered. The syringe was connected by a short length of silastic tubing to a small condensor, through which water at 37° C was circulated. The proximal cannula, about 5 cm long, was connected directly to the condensor and was made

from the tip of a Jacques catheter (CH 8). The distal cannula 15 cm in length was also made from the Jacques catheter.

The animals were fasted of solid food overnight but had free access to water and an isotonic glucose solution. In the morning the animals were anaesthetised with the phenobarbitone/pentobarbitone mixture and the hair removed from the neck and abdomen. The syringe, tube, condensor and cannula were filled with the infusion fluid to be used and the volume adjusted to 50 ml. To prevent the loss of any fluid from the apparatus, the tube from the syringe to the condensor was clamped.

The animal was placed on its back on a warmed operating table. The right jugular vein was exposed and cannulated using an 8 cm length of polythene tubing (portex 47 poly) filled with heparin. The cannula was passed down the jugular vein until the tip was in the right atrium so as to obtain samples more similar to those of arterial blood with respect to the concentration of the sugars.

The abdomen was opened with a midline incision and the jejunum identified. Two ligatures were placed loosely around the proximal end of the jejunum. At a distance of about 6 cm distal to these ligatures, two further ligatures were placed and the intestine cannulated with the distal cannula. The proximal end of the jejunum was then cannulated, adjusting the condensor so that there was no interference with the intestinal blood supply. The distal cannula was also adjusted, making sure that the intestine and blood vessels were not occluded. The end of the distal cannula was placed in a 10 ml centrifuge tube so as to collect the effluent. The tip of the cannula was adjusted

so that it was a few millimetres higher than the intestine. This meant that the intestine was slightly distended with fluid due to a hydrostatic pressure.

The animals were only heparinised at this stage so that if any damage had been done to the mucosa resulting in haemorrhaging the normal coagulation processes could cause haemostasis. This precaution resulted in the effluent always being clear of blood after the first sample. The patency of the jugular cannula was checked to see if blood samples could be withdrawn quickly and freely from the cannula, if not, the cannula was adjusted until they could be taken quickly.

The blood sample was taken from the jugular cannula and either the glucose or glucose plus fructose concentration determined in the blood.

The infusion pump was switched on and the jejunum infused at a rate of 0,3 ml per 2,5 minutes. The centrifuge tubes, in which the effluent was collected, were replaced at 2,5 minute intervals and the blood samples taken at regular intervals. The effluent collected during the first 10 minutes was discarded as the volumes varied at the beginning but usually reached a steady state within the first 10 minutes.

The volumes in the centrifuge tubes were measured and the concentrations of the sugars in each sample estimated. From this information the amount of sugar recovered was calculated and knowing the amount of sugar infused in 2,5 minutes the amount of sugar assimilated was calculated.

At the end of the experiment the abdomen was opened and the length of the intestine used was carefully removed,

the cannulae were taken out and the intestine placed in a saline solution. After 10 minutes the length of the jejunum used was measured, this gave sufficient time for the intestine to have reached a constant length following stretching when removing the tissue. The intestine was then dried of excess fluid and weighed. The amount of sugar assimilated was expressed as mg/cm/2,5 minutes.

2.10.1. Assimilation of glucose against a concentration gradient in vivo

The method used in this experiment was based on that used by Sheff and Smyth (1960).

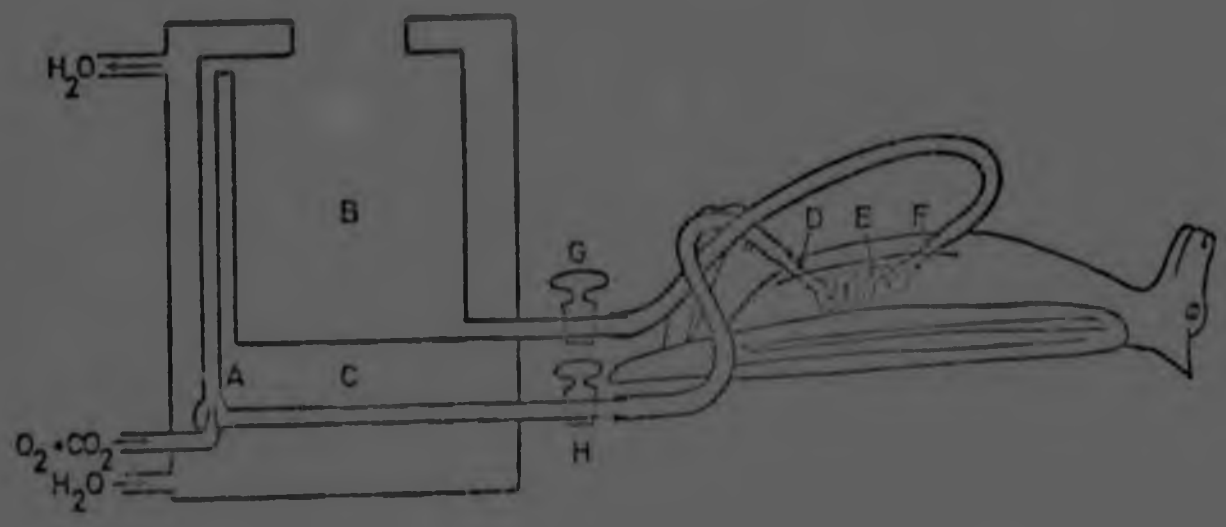

Fig. 5. The apparatus for the perfusion through an isolated length of small intestine in vivo.

A: gas lift pump. B: reservoir. C: water-jacket surrounding gas lift pump and reservoir. D: distal cannula. E: jejunum. F: proximal cannula. G: reservoir tap. H: gas lift pump tap.

This experiment was carried out on three bats and three rats.

The experimental procedure was essentially the same as that used for the infusion experiments except that instead of the infusion pump, a reservoir was used and the effluent returned to the reservoir by a gas lift pump. The length of intestine used was also different in that, in these experiments the intestine from the sphincter of Oddi to the distal end of the ileum was perfused.

The reservoir was first filled with an isotonic glucose solution (5,25 g/100 ml) and the height of the apparatus adjusted so that the level of the fluid in the reservoir was 10 cm above the level of the animal's abdomen. This height of the reservoir resulted in a hydrostatic pressure sufficient to [illegible] the intestine perfused. The taps (G and H) on the perfusion apparatus were opened and the intestine perfused with the glucose solution for 15 minutes to raise the blood glucose level. At the end of the 15 minutes the apparatus was disconnected from the cannulae, the glucose solution discarded and the apparatus rinsed out with saline. The reservoir was then filled with saline and reconnected to the proximal cannula. The intestine was then washed out with 100 ml of saline, the effluent from the ileum being discarded.

Once all the saline had been used the reservoir tap (G) was closed and 50 ml of the glucose solution (100 mg/100 ml) was placed in the reservoir. Blood and perfusate samples of approximately 0,1 ml were taken from the jugular cannula and the reservoir respectively and the glucose concentration estimated. The apparatus was reconnected to the distal cannula and the perfusion started again. The time interval between stopping the perfusion of the 5,25 g/100 ml and

starting the 100 mg/100 ml glucose solutions was approximately 15 minutes. At 15 minute intervals during the perfusion samples of blood from the jugular cannula and perfusate from the reservoir were taken for glucose determination. The perfusate samples taken at the start and the end of the experiment were carried out in duplicate.

After the 60 minute samples had been taken, the perfusion was stopped, the abdomen opened and the intestine removed. The volumes of fluid in the reservoir and intestine were measured and the glucose concentrations estimated. The length of the intestine used was measured.

The difference between the amount of glucose recovered and the amount of glucose initially placed in the reservoir was the amount of glucose assimilated or secreted by the intestine in one hour.

This experiment was not carried out using fructose as the blood fructose level could not be increased to levels above the concentration of fructose in the perfusion fluid.

2.10.2. Permeability of the intestine to the movement of glucose from the blood to the lumen

This investigation was carried out on one bat and one rat. The animals were fasted of solid food overnight but had free access to water and an iso'onic glucose solution.

The method used was similar to that used in the infusion experiments. But in this investigation saline was used instead of a sugar solution as the infusion fluid. Also, longer lengths of jejunum were used, 11 cm and 12 cm in the bat and the rat respectively.

The animals were anaesthetised with the phenobarbitone/ pentobarbitone mixture and the experimental procedure

carried out as previously described on page 33. After the preinfusion blood samples had been collected, the infusion pump was switched on and the physiological saline perfused through the gut at a rate of 0,6 ml per 10 minutes. The effluent tubes were changed every 10 minutes.

In the experiment carried out on the bat, 40 minutes after the infusion of saline had begun, 105 mg of glucose was given intravenously as an isotonic glucose solution. This initial glucose loading dose was followed by repeated intravenous injection doses of 52 mg of glucose every 10 minutes. This investigation was terminated after 80 minutes with an intravenous injection of 0,5 units of insulin followed by a second injection of 4 units of insulin 8 minutes later.

In the rat the initial intravenous glucose load was 265 mg and this was followed by an intravenous glucose infusion at a rate of 42 mg of glucose per 10 minutes. After 120 minutes the saline infusion fluid was replaced with saline containing 0,5 % phloridzin.

The bat and the rat were killed 140 and 150 minutes respectively after the infusion of the intestine had started. The abdomens were re-opened and the lengths and weights of the jejunums used were measured. The volumes of the perfusate in the effluent tubes were measured and the glucose concentrations in the solutions estimated.

2.10.3. Estimation of the concentrations of sugars and lactic acid in the portal and portal blood during the assimilation of a sugar

In this investigation only bats were used. They were fasted overnight of solid food but had free access to water

and an isotonic glucose solution. The animals were anaesthetised and the same experimental procedure followed as had been used in the infusion experiments (page 33) except that in these experiments no distal catheter was used. As a result the whole jejunum and a large part of the ileum were perfused, by which time all the fluid was assimilated.

In the bat the cannulation of the portal vein, so as to get portal blood samples, was difficult as all the larger vessels are short. The portal blood samples were therefore taken directly from the portal vein about 4 - 5 mm from the liver. This was done using a 26G hypodermic needle connected to an "Aglar" syringe. The disadvantage of this method was that a portal blood sample could only be taken once, for on withdrawing the needle the animal bled to death. No blood samples could therefore be taken before the infusion was started.

The bat's intestine was infused with solutions of 5,25 g/100 ml of either glucose, fructose or sucrose. After 45 minutes 0,3 ml samples of systemic blood were collected from the jugular cannula and 0,3 ml of portal blood from the portal vein. All estimations were done in duplicate. In all the experiments the concentration of blood lactate was measured using the Isolab LP analyser (page 6). In the glucose infused animals the blood glucose concentration was estimated using the G.O.D. method (page 6) while in the other two experiments glucose was estimated using the hexokinase method. The blood fructose and sucrose concentrations were estimated using the hexokinase method in conjunction with phosphoglucose isomerase and β fructosidase (pages 6 and 9). In the sucrose experiments the plasma

and an isotonic glucose solution. The animals were anaesthetised and the same experimental procedure followed as had been used in the infusion experiments (page 33) except that in these experiments no distal cannula was used. As a result the whole jejunum and a large part of the ileum were perfused, by which time all the fluid was assimilated.

In the bat the cannulation of the portal vein, so as to get portal blood samples, was difficult as all the larger vessels are short. The portal blood samples were therefore taken directly from the portal vein about 4 - 5 mm from the liver. This was done using a 23G hypodermic needle connected to an "Aglar" syringe. The disadvantage of this method was that a portal blood sample could only be taken once, for on withdrawing the needle the animal died to death. No blood samples could therefore be taken before the infusion was started.

The bat's intestine was infused with solutions of 5,25 g/100 ml of either glucose, fructose or sucrose. After 15 minutes 0,3 ml samples of systemic blood were collected from the jugular cannula and 0,3 ml of portal blood from the portal vein. All estimations were done in duplicate. In all the experiments the concentration of blood lactate was measured using the lactate DH method (page 8). In the glucose infused animals the blood glucose concentration was estimated using the G.O.D. Perid method (page 6) while in the other two experiments glucose was estimated using the hexokinase method. The blood fructose and sucrose concentrations were estimated using the hexokinase method in conjunction with phosphoglucose isomerase and β fructosidase (pages 6 and 5). In the sucrose experiments the plasma

proteins were not precipitated with perchloric acid but by boiling in a saline solution (page 9).

2.10.4. The effect of the presence of glucose in the stomach on the rate of assimilation of glucose from the jejunum

Only bats were used in this investigation, they were fasted overnight of solid food but had free access to an isotonic glucose solution. The following morning the animals were anaesthetized with sodium pheno barbitone/pentobarbitone mixture (page 17). Once the animal was asleep the right jugular vein was cannulated (page 22) and the oesophagus posterior to the larynx was identified, cleaned and prepared for cannulation. A Jacques catheter (C.I.C.), approximately 20 cm long, attached to a 10 ml syringe were both filled with the isotonic glucose solution to be administered. To prevent the loss of any fluid from the syringe and catheter, the latter was plugged firmly with a bulldog clip. The syringe was then placed in an infusion pump apparatus and the oesophagus cannulated with the catheter making sure the tip of the catheter was in the stomach.

The abdomen was opened along the midline and a double ligature placed firmly around the pyloric sphincter, to prevent the loss of any fluid from the stomach. The jejunum was identified and prepared for the infusion of a sugar solution (page 24).

In the control experiments a single pre-infusion blood sample was taken and the glucose concentration determined. The intestinal infusion pump was then switched on and the jejunum perfused with glucose at a rate of 0,5 ml/ ,5 minutes. Blood samples were taken at 5 and 10 minutes

after the start of the infusion and then at 10 minute intervals. The effluent was collected for every 2.5 minutes and the experiment terminated after the intestine had been infused for one hour. Although the oesophagus had been cannulated the stomach infusion pump was not switched on and so no glucose was infused into the stomach.

In the actual experiment glucose (5.25 g/100 ml) was used for both the stomach and jejunal infusions. Blood samples were collected prior to and 5, 10, 20 and 30 minutes after the intestinal infusion had been started. After the 30 minutes blood sample had been taken, the bulldog clip was removed and the stomach also infused with glucose at 0.8 ml/2.5 minutes. Further blood samples were collected at 35, 40, 50 and 60 minutes. The experiment was terminated after the stomach had been infused for 31 minutes.

The animals were killed after the experiment and the lengths and weights of the jejunum infused were recorded.

The volumes and concentrations of the glucose in the effluent tubes were measured and the amounts of glucose assimilated were calculated (page 56). Graphs and histograms were drawn to show the concentrations of the sugars in the venous blood and the amounts of glucose assimilated.

2.11. Results

2.11.1. Normal fasted rats

The animals, both rats and bats, were fasted of all solid food overnight, but they did have free access to water and an isotonic glucose solution. The following morning the animals were killed and the abdomen opened by midline incision and the intestine removed as quickly as possible and placed in cold saline. The intestine was cut into

sections, opened up and pinned out on a wax plate made up of pink modelling wax*. The surface of the mucosa was washed free of mucous and any other waste material with saline.

The method used in the preparation was similar to that used by Hale, et al., (19[illegible]) and consisted of fixing the pinned-out tissue with 2,5% glutaraldehyde solution for 24 hours then post fixing it in 1% osmic acid for an hour. The tissues were then dehydrated through graded ethyl alcohol and graded amyl acetate in absolute alcohol until they were in 100% amyl acetate. The tissues were then dried by the critical point technique, mounted and coated with gold palladium and viewed with a Cambridge scanning electron microscope.

2.11.2. Preparation of electron microscope

In these investigations a comparative study of the structure of the intestine of the bat and the rat was made. The animals were fasted of solid food overnight but had free access to both water and an isotonic glucose solution.

The following morning the animals were killed, the intestine quickly removed and cut into lengths of about 1 cm, and immediately fixed in a cold (4° C) solution of 2% glutaraldehyde in a cacodylate/glucose buffer (pH 7,2 and 415 - 420 mOsm). After one hour the tissues were removed and cut into 1 mm cubes and then returned to the fixative. At the end of four hours the specimens were removed from the fixative and washed in a 0,1 M cacodylate buffer and

---

*Pink modelling wax "Associated Dental products" toughened N-4.

post fixed in 1% osmium tetroxide in cacodylate buffer for a further four hours after which they were washed in the cacodylate buffer and left overnight in this buffer. The specimens were dehydrated in ethyl alcohol containing 2% uranyl-acetate and finally placed in propylene oxide. The tissues were embedded in araldite/epon mixture.
Thick sections 1 - 2 µm were first cut and stained with toluidine-pyronin so that the block could be orientated and trimmed so that the mucosal cells were cut approximately along their long axis. The thin sections were cut approximately 60 nm thick and stained first with uranyl acetate and finally with lead acetate. The sections were examined with a Joel Jem 100 C electron microscope.

The measurements of the lengths, diameters and distances between the microvilli were made directly from the negatives. A number of measurements were made from each negative and the mean of three readings used in the final calculations. The thickness of the plasma membrane was measured both on the negative and the print and here again a number of measurements were made, the mean being used in the final calculations.

3. RESULTS

3.1. Transit time for bananas

The mean transit time for bananas in the 47 bats used in the investigation was 46 minutes. The standard deviation was found to be ± 24 minutes.

3.2. Oral glucose tolerance tests

Tables 17 and 18 (see appendix) and figures 6 and 7 show the changes in the blood glucose concentrations following the oral administration of either 10 ml of distilled water or 500 mg of glucose per 100 g body weight in the anaesthetised rat and bat.

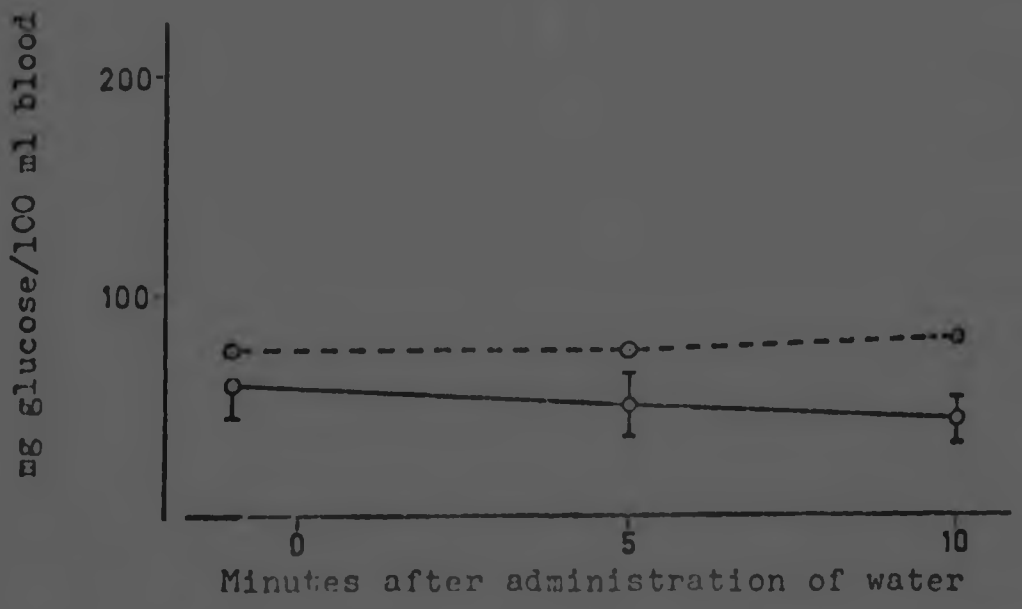

Fig. 6. The blood glucose concentration in the unanaesthetised bat and rat following the oral administration of 10 ml of distilled water.

(Solid line _____ represents the bat and the broken line ----- the rat results. Circles are known the mean value and the vertical bars - the standard error of the mean.).

From table 17 and fig. 6 it can be seen that after the administration of 10 ml of distilled water there were no significant changes in the blood glucose concentration in

either the bat or the rat.

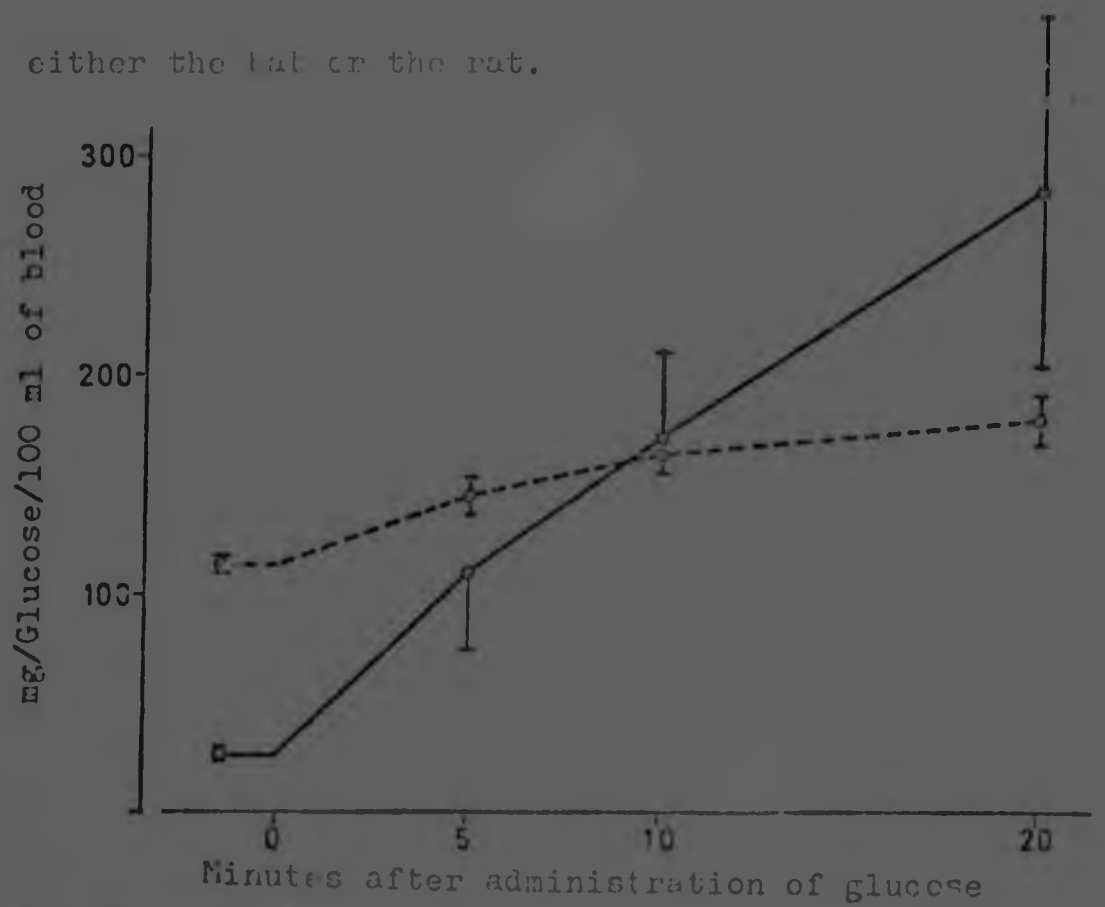

Fig. 7. The blood glucose concentration in one anaesthetised bat and rat following the oral administration of 500 mg of glucose per kg body weight.

(Solid line _____ represents the bat and the broken line ----- the rat results. Circled dots show the mean value and the vertical bars - the standard error of the mean).

Fig. 7 and Table 1 (see appendix) show that after the administration of glucose, the blood glucose concentration increased in both groups of animals. In the rat this increase was from a resting level of 113 mg/100 ml of blood to a value of 180 mg/100 ml of blood in the 20 minute blood sample. In the bat however this increase was far greater, starting at a level of 77 mg/100 ml of blood and increasing to 284 mg/100 ml of blood in the 20 minute blood sample.

To see if there was a significant difference between the results obtained from the rat and bat, a test was made for the significant difference between the slopes of the

curves recorded for the bat and the rat glucose tolerance test. (See appendix [illegible] page [illegible]). This was found to be highly significant at the 0,2% level.

3.3. Intravenous glucose tolerance test

Table 19 (see appendix) and fig. 8 show the changes in the blood glucose concentration following the intravenous glucose tolerance test.

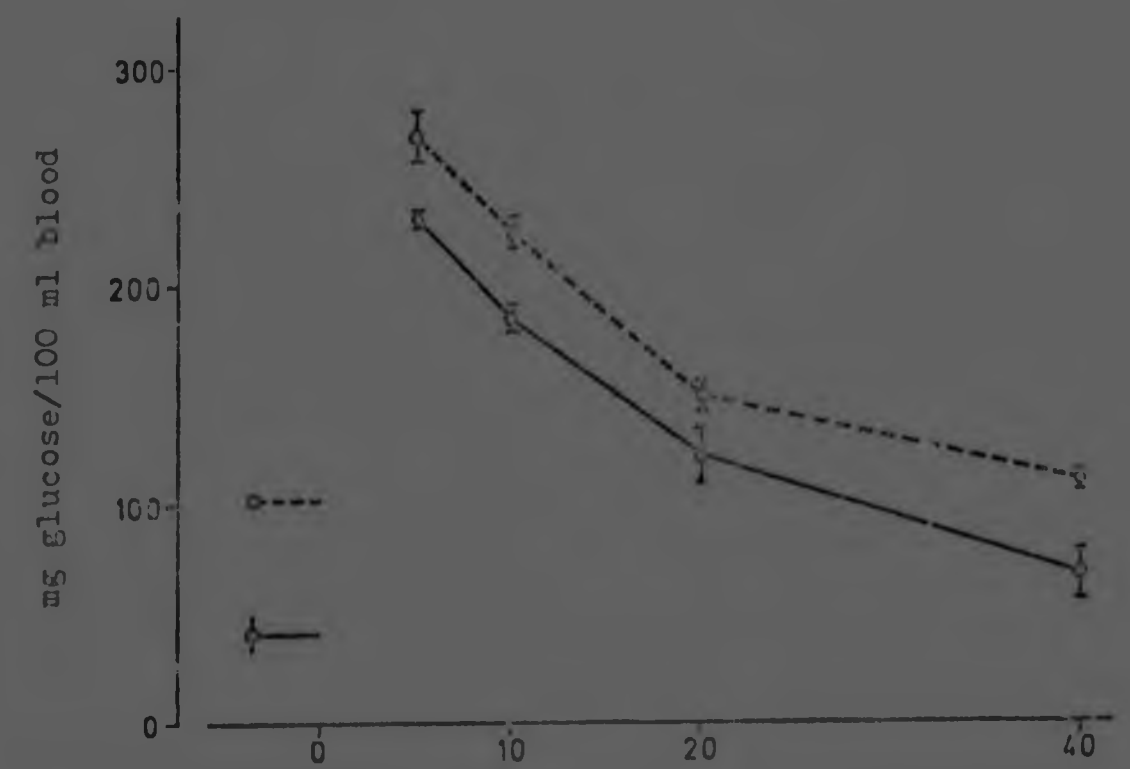

Fig. 8. The blood glucose concentration in the anaesthetised bat and rat following the intravenous injection of 75 mg of glucose per 100 g body weight.

(Solid line _____ represents the bat and the broken line ----- the rat results. Circled dots shows the mean value and the vertical bars ± the standard error of the mean).

The bat, as usual, had a lower resting blood glucose level than the rat. The amount of glucose given to each animal caused roughly the same increase in the blood glucose level from the resting blood glucose levels. The

important observation, however, is that the decay of the slope of the two graphs is identical.

In the control experiments where the animals were given an intravenous injection of saline of equivalent volumes, it was found that there was no change in the blood glucose concentration.

3.4. Assimilation of sugars in the conscious bat and rat

3.4.1. Assimilation of glucose

Table 1. Glucose assimilation in the conscious bat and rat in 30 minutes.

Glucose	Bat (6)*	Rat (4)*
Administered (mg)	1487	1540
Recovered from stomach (mg)	459 ± 65**	702 ± 179**
Entering the intestine (mg)	1028 ± 80	838 ± 179
Recovered from intestine (mg)	263 ± 59	561 ± 174
Amount assimilated (mg)	765 ± 60	278 ± 17
% assimilated to available	75 ± 12	34,5 ± 5

* Number of animals shown in the brackets.
** Mean and standard error of the mean.

The results (Table 1) shows that the bat differs from the rat in a number of ways. The first was that the bat assimilated 765 mg of glucose compared to the 278 mg of the rat during this 30 minute period, thus this under similar circumstances the bat's rate of assimilation was 2,8 times faster than the rat.

The second difference was that in the 30 minute period

more glucose left the bat's stomach than the rat's. Finally, in the bat the percentage of the amount of glucose assimilated compared to the amount of glucose available in the intestine for assimilation was 75% as compared to 36,5% for the rat.

Table 2. The volumes and concentrations of glucose found in the stomach and small intestine 30 minutes after the oral administration of glucose.

	Bat (4)*	Rat (3)*
Volumes (ml)		
Initial	10	10
Stomach	3,4 ± 0,5**	6,9 ± 0,5**
Intestine	4,0 ± 1,3	8,6 ± 0,9
Concentration g/100 ml		
Initial	14,87	15,4
Stomach	11,8 ± 0,3	13,3 ± 0,9
Intestine	7,5 ± 0,5	4,9 ± 0,4

* Number of animals in brackets.
** Mean and standard error of the mean.

Both the rats and the bats were given 10 ml of the 15 g/100 ml glucose solution (Table 2). In the rat a total volume of 15,5 ml of fluid was recovered from the stomach and the intestine compared to 7,4 ml in the bat. In the bat no measurable volume of fluid was observed in the distal two thirds of the intestine whereas in the rat the fluid occupied the entire length of the small intestine and sometimes had entered the large intestine.

The concentration of glucose in both animals' stomachs, showed that the glucose solution had been diluted, but that

it was still more than twice the osmolarity of the plasma. The surprising comparative difference between these two animal species was that in the bat the osmolarity due to the glucose in the intestine was 40% greater than that of the plasma whereas in the rat it was less than isotonic.

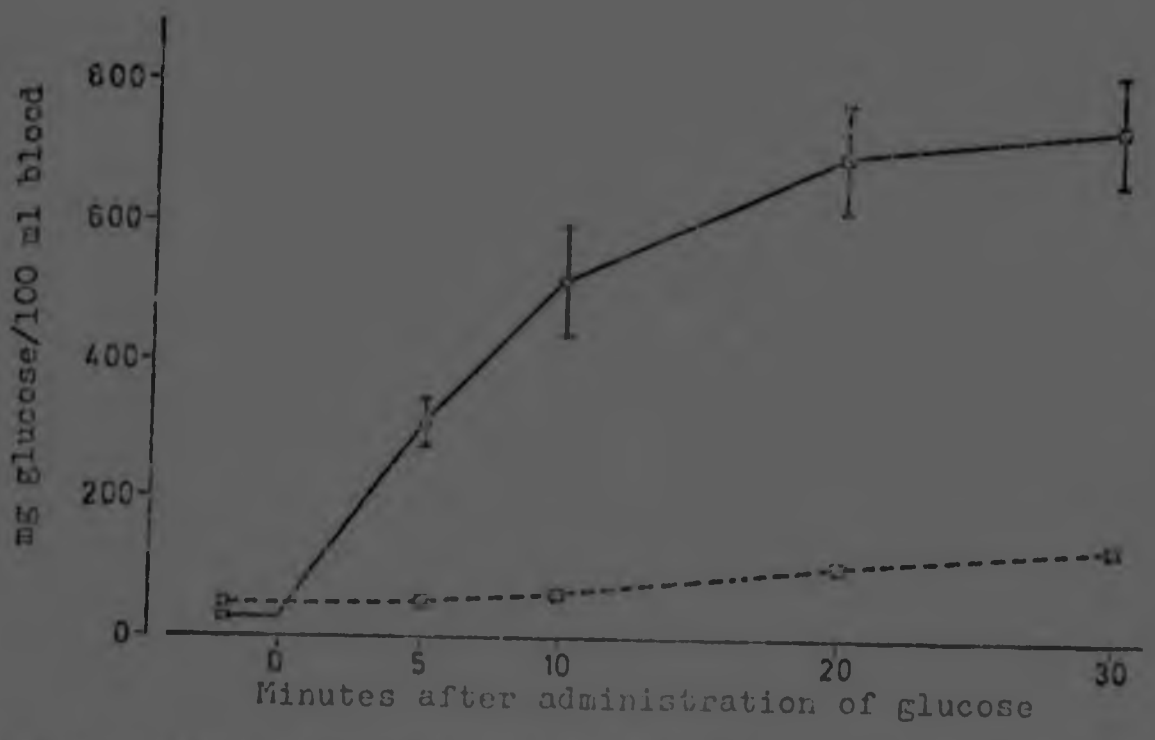

Fig. 9. Blood glucose levels in the conscious bat and rat following an oral dose of 1.5 g of glucose.

(Solid line _____ represents the bat and the broken line ---- the rat results. Circled dots shows the mean value and the vertical bars ± the standard error of the mean).

Table 20 (see appendix) and Fig. 9 show the mean venous blood glucose concentration found in the conscious bat and the rat before and after the oral administration of glucose. Prior to the start of the experiment the blood glucose level in the bat was 70 mg/100 ml compared to 53 mg/100 ml in the rat. In the bat after the administration of the glucose there was a rapid increase in the blood glucose level which

started to level out after 20 minutes and reached a mean concentration of [illegible] mg/100 ml after 30 minutes. In the rat by contrast, the blood glucose level increased slowly from 53 mg/100 ml to reach a level of 133 mg/100 ml after 30 minutes.

3.4.2. Assimilation of Fructose

Table 3. Fructose assimilated in the noctuous bat and rat in 30 minutes.

Fructose	Bat (6)*	Rat (4)*
Administered (mg)	1491 ± (**)	1500 **
Recovered from stomach (mg)	[illegible]	617 ± 95
Entering the intestine (mg)	1158 ± 50	883 ± 95
Recovered from intestine (mg)	82 ± 27	615 ± 82
Amount assimilated (mg)	1076 ± 71	268 ± 40
% assimilated to available	92 ± 3	30 ± 4

* Number of animals investigated.
** Mean and standard error of the mean.

The results, Table 3, showed that the bat assimilated four times more fructose than the rat. The amounts of fructose assimilated were 1076 and 268 mg for the bat and rat respectively for the 30 minute period. In the bat not only had more fructose left the stomach to enter the intestine, when compared to the rat, but of this fructose 92% was assimilated, whereas in the rat only 30% was assimilated.

Table 4. The volumes and concentrations of fructose found in the stomach and small intestine 30 minutes after the oral administration of fructose.

	Bat (4)*	Rat (4)*
Volumes (ml)		
Initial	10	10
Stomach	2,5 ± 0,6**	5,7 ± 1,1**
Intestine	1,1 ± 0,5	11,4 ± 1,3
Concentrations g/100 ml		
Initial	14,9	15,0
Stomach	12,2 ± 0,6	11,2 ± 0,8
Intestine	8,5 ± 0,8	5,4 ± 0,2

* Number of animals in brackets.
** Mean and standard error of the mean.

The animals were given 10 ml of a fructose solution (15 g/100 ml) intragastrically. It was found that in the rat a total volume of 17,1 ml of fluid was collected from the stomach and intestine compared to 3,6 ml in the bat.

The concentrations of fructose in both the stomach and intestinal samples was greater in the bat when compared to the rat.

The mean venous concentrations of glucose and fructose found in the bat and rat following the administration of 1,5 g of fructose are recorded in Table 4 and Figs. 10 and 11.

Fig. 10 shows that following the administration of fructose the blood glucose level in both species increased, in the case of the bat from a pre-administration level of

60 mg/100 ml to reach 261 mg/100 ml in the 30 minute blood sample. In the rat the blood glucose level increased from 70 mg to 100 mg/100 ml. There was no fructose in the blood prior to the administration of the fructose in either species (Fig. 11). After the administration of the fructose in the bat, there was a very rapid increase in the blood fructose level which reached a maximum value of 132 mg/100 ml in the 5 minute blood sample and then slowly decreased. In contrast only a trace of fructose was found in the 20 minute blood sample from the rat and this increased to 9 mg/100 ml in the 30 minute blood sample.

The statistical analysis to test the significance between the amounts of glucose and fructose assimilated by the bat showed a significant difference at the 0.5% level. (See appendix d, page [illegible]).

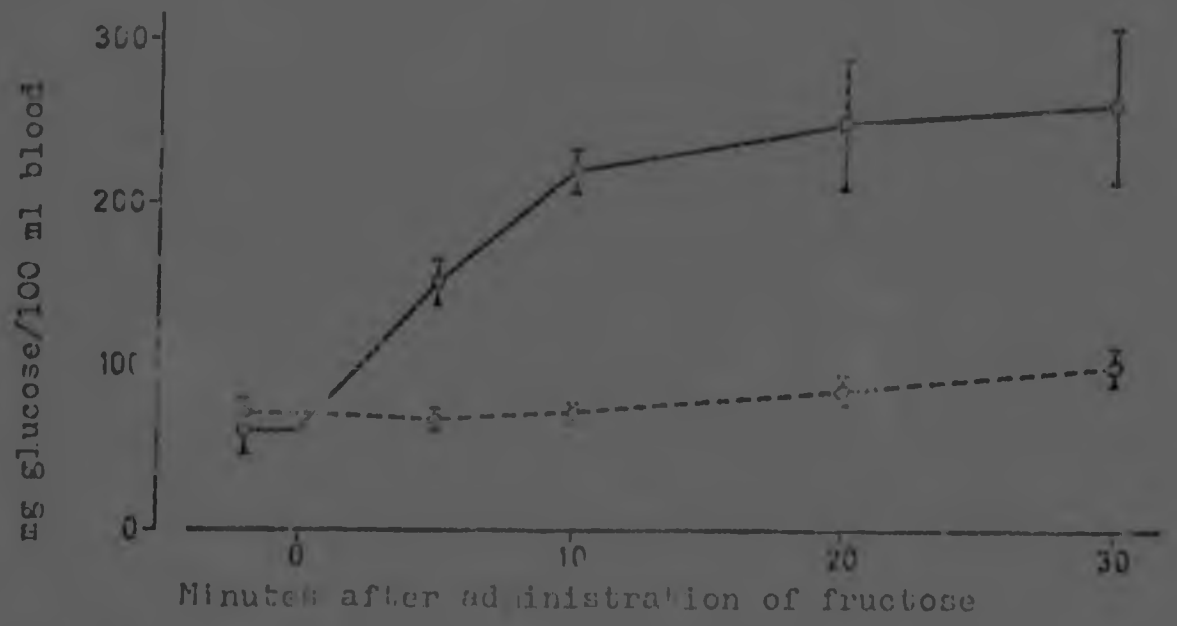

Fig. 10. Blood glucose levels after the administration of 1.5 g of fructose.

(Solid line _____ represents the bat and the broken line ------ the rat respectively. Circled dot shows the mean value and the vertical bars ± the standard error of the mean).

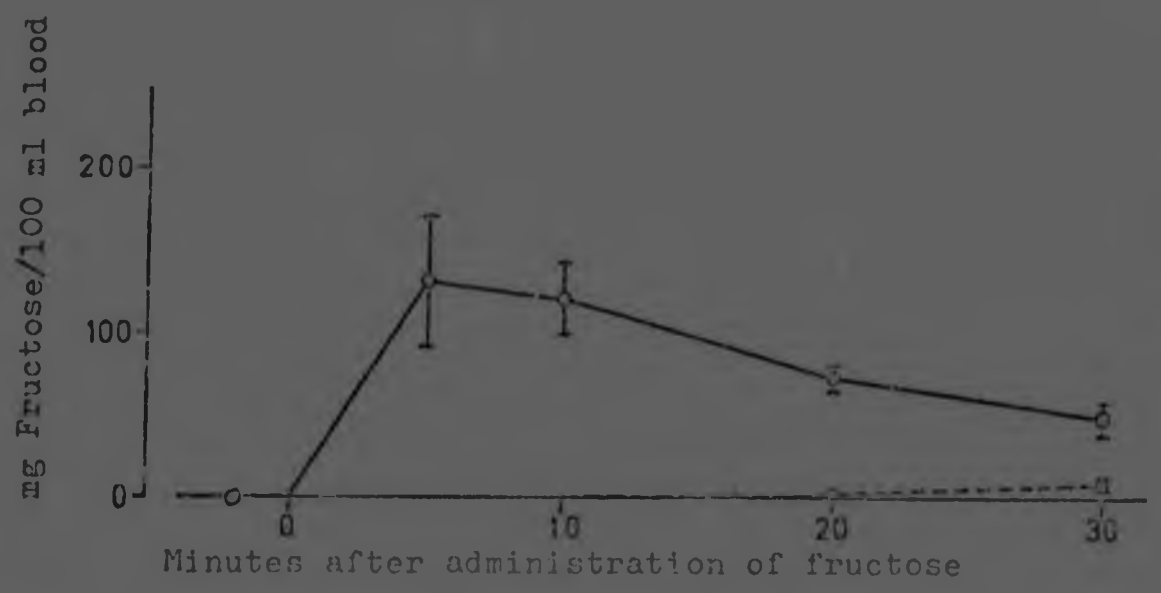

Fig. 11. Blood fructose levels after the administration of 1,5 g of fructose.

(Solid line _____ represents the test and the broken line ----- the reference. Each dot shows the mean value and the vertical bars - the standard error of the mean).

3.4.3. Assimilation of a mixture of glucose and fructose in the test

Table 3. The assimilation of a mixture of glucose and fructose in the test in 30 minutes.

	Glucose (6)*	Fructose (6)*
Administered (mg)	750 ± 2**	748 ± 2**
Recovered from stomach (mg)	[illegible] ± 39	218 ± 38
Entering the intestine (mg)	[illegible] ± 39	530 ± 38
Recovered from intestine (mg)	111 ± 30	65 ± 20
Amount assimilated (mg)	[illegible] ± 55	465 ± 50
% assimilated to available	78 ± 6	87 ± 4,3

* Number of animals in brackets.
** Mean and standard error of the mean.

Table 5 shows that the amounts of glucose and fructose assimilated in 30 minutes following the administration of a mixture of these two monosaccharides were 41 mg and 365 mg respectively. In each experiment more fructose than glucose was assimilated. The student "t" test could not be used to test whether there was a significant difference between the rates of assimilation, because of the large variations in the amounts of glucose and fructose assimilated in the different experiments. Therefore a test was done to see whether the mean of the differences was significantly different from zero.

Table 6. The amounts of glucose and fructose assimilated in 30 minutes and their differences.

	Sugar assimilated mg/30 minutes		
Bat N°	Fructose	Glucose	Difference
1	404	327	77
2	355	329	26
3	549	534	15
4	311	[illegible]	74
5	580	563	17
6	592	514	78
			Σx [illegible]
			$\bar{x}$ 47,8

Compute $t_{n-1} = \frac{\bar{x}}{\frac{s.d.}{\sqrt{n}}}$

(paired sample t-test)

where $\bar{x}$ = 47,8

S.D. = 31,5

n = 6

so $t_5 = \frac{47,8}{31,5} \times \sqrt{6} = 3,718$

$t_{5,1}$ 0,99 = 3,365

So significant at the 1% level.

The statistical analysis of these experiments showed that there was a highly significant difference between the amounts of fructose and glucose assimilated. These results were further emphasized by the fact that although the same amounts of glucose and fructose had entered the intestine, 8% of said intestinal fructose was assimilated compared to the glucose value of [illegible].

Table 7. The volumes and concentrations of glucose and fructose found in the stomach and intestine in the bat 30 minutes after the administration of a mixture of 750 mg glucose and 750 mg fructose.

	Bat (6)*
Volumes (ml)	
Initial	10 **
Stomach	3,5 ± 0,6
Intestine	2,9 ± 0,9

	Glucose (6)*	Fructose (6)*
Concentrations g/100 ml		
Initial	7,5 **	7,5 **
Stomach	6,4 ± 0,1	6,2 ± 0,1
Intestine	4,1 ± 0,3	2,1 ± 0,2

* Number of animals in brackets.
** Mean and standard error of the mean.

The bats were given intragastrically 10 ml of the mixture of [illegible]. Thirty minutes later the animals were killed and it was found that the stomach still contained approximately a third of the initial volume but that the concentrations of both glucose and fructose had decreased. There was still a relatively large volume of fluid in the intestine and as expected the fructose concentration was less than that of glucose.

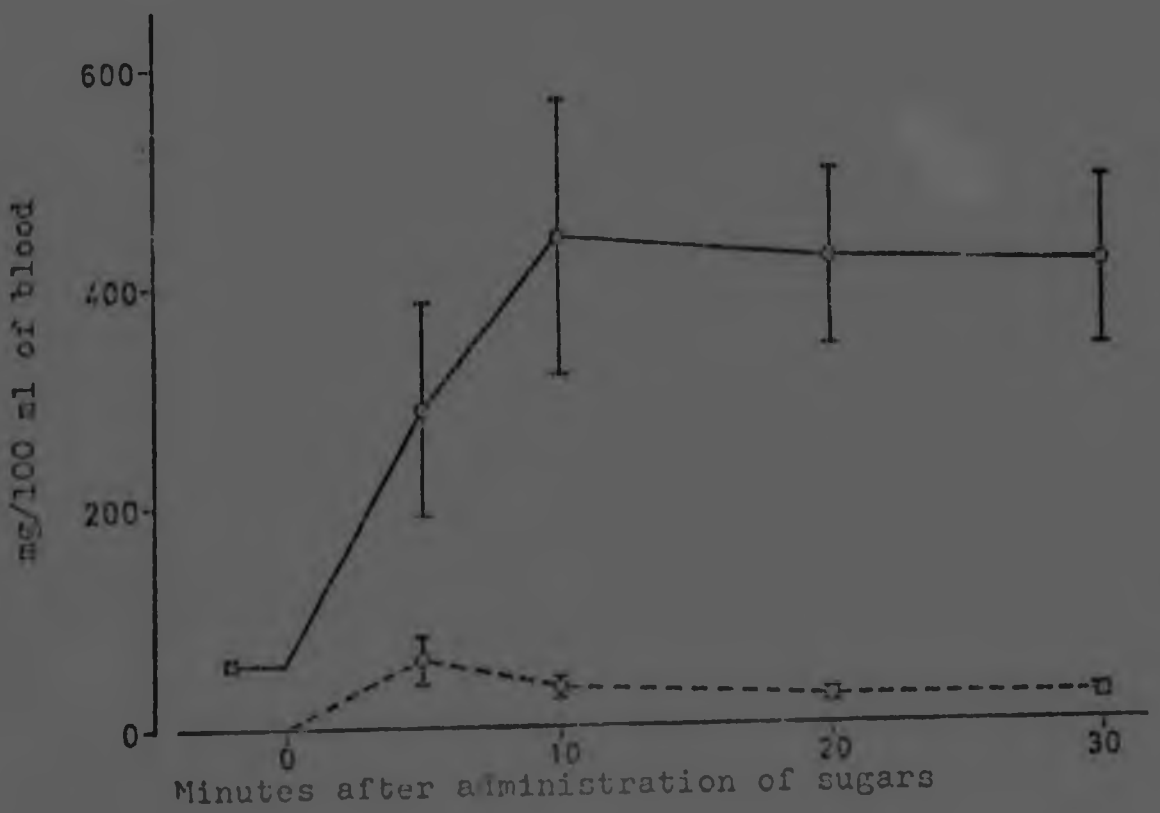

Fig. 12. Blood glucose and fructose concentrations in the [illegible] bat after the administration of a mixture of 750 mg of glucose and 750 mg of fructose.

(Solid line ——— represents the fructose and the broken line ----- the glucose content. Circled dot shows the mean value and the vertical bars - the standard error of the mean.

Table 22 (see appendix) and fig. 12 show the mean venous blood glucose and fructose concentrations in the bat following the administration of a solution containing equivalent amounts of glucose and fructose. Prior to the administration of the monosaccharides there was no fructose in the blood and the blood glucose level was [illegible] mg/100 ml. After the sugars had been given the blood glucose concen-

tration increased very rapidly and reached a maximum level of 442 mg/100 ml in the 10 minute sample and then slowly decreased. Fructose also appeared in the blood and reached a maximum concentration of 61 mg/100 ml in the 5 minute sample and then slowly decreased.

The patterns recorded for the blood glucose and fructose concentrations during this experiment were very similar to those recorded when either of these monosaccharides were administered separately, except that the sugar concentrations were lower (glucose fig. 9 and fructose fig. 11).

3.4.4. Digestion of sucrose in the rat

Table 8. The amount of sucrose digested and recovered from the stomach and small intestine in the rat, 30 minutes after the administration of 1,500 mg sucrose.

	Sucrose (-)*
Administered (mg)	1500 **
Recovered from stomach (mg)	508 ± 33
Recovered from intestine (mg)	60 ± 19
Total sucrose recovered (mg)	568 ± 43
Amount of sucrose digested (mg)***	932 ± 26
% digested to administered	62 ± 2

* Number of animals in brackets.
** Mean and standard error of mean.
*** Assumed that none to be recovered from absorption of sucrose.

Table 8 shows that 30 minutes after the sucrose had been given only 508 mg of the sucrose was still in the stomach. This meant that approximately 1000 mg of sucrose entered the small intestine of which only 60 mg was re-

covered. As no sucrose was found in any of the blood samples, it is reasonable to assume that the remaining sucrose was hydrolysed to glucose and fructose.

Table 9. The amounts of glucose and fructose formed and assimilated in the 30 minutes following the administration of 1.5 g sucrose.

	Glucose (6)*	Fructose (6)*
Sugar formed (mg)	490 ± 14**	490 ± 14**
Recovered from stomach (mg)	5 ± 2	5 ± 2
Recovered from intestine (mg)	116 ± 39	91 ± 35
Total sugar recovered (mg)	121 ± 42	96 ± 37
Sugar assimilated (mg)	369 ± 50	394 ± 46
% assimilated to available	75 ± 9	79 ± 8

* Number of animals in brackets.
** Mean and standard error of the mean.

The hydrolysis of 925 mg of sucrose resulted in the formation of 490 mg of both glucose and fructose in the 30 minutes (Table 9). The amounts of glucose and fructose assimilated were 369 and 394 mg respectively. The results showed that 75% of the available glucose and 79% of the available fructose were assimilated. Once again fructose was assimilated faster than glucose and a statistical analysis was carried out to see whether the mean of the differences, between the amounts of glucose and fructose assimilated, was significantly different from zero (see method p. 52). The results showed that the amount of fructose assimilated was significantly greater than for

glucose at the 0,[illegible] level.

Table 10. The volume and concentrations of the sugars found in the stomach and intestine of the bat 20 minutes after the oral administration of 1,[illegible] g of sucrose.

	Fluid Volume (ml) (6)*	Concentration g/100 ml		
		Sucrose (6)*	Fructose (6)*	Glucose (6)*
Initial	10 **	15 **	0 **	0 **
Stomach	4,1 ± 0,[illegible]	12,[illegible] ± 0.4	0,1 ± 0,05	0,11 ± 0,05
Intestine	4,2 ± 1,[illegible]	1,1 ± 0,[illegible]	1,9 ± 0,17	2,7[illegible] ± 0,15

* Number of animals in brackets.
** Mean and standard error of the mean.

An initial volume of 10 ml of a 1[illegible] g/100 ml sucrose solution was placed in the stomach. After 20 minutes, (Table 10) there was still 4,1 ml of fluid in the stomach, the sucrose concentration had been diluted to 12,[illegible] g/100 ml. There were also traces of glucose and fructose in the stomach fluid.

The volume of fluid in the intestine was 4,2 ml and the concentrations of the sugars were 1,1, 1,9 and 2,77 g/100 ml for sucrose, fructose and glucose respectively. Thus the equivalent osmolarity due to these sugars is approximately 294 mOsm or nearly isotonic with the bat's blood.

Table 2[illegible] (see appendix) and Fig. 1[illegible] show the blood levels of glucose and fructose during the experiment. At no time during the experiment was any sucrose found in the venous blood. The blood glucose level increased after the admi-

nistration of the sucrose and reached a value of 330 mg/ 100 ml by the 30 minute sample. Fructose was not found in the blood prior to the administration of the sucrose but appeared in the 5 minute blood sample and reached a maximum value of 21 mg/100 ml of blood in the 10 minute blood sample and then slowly decreased.

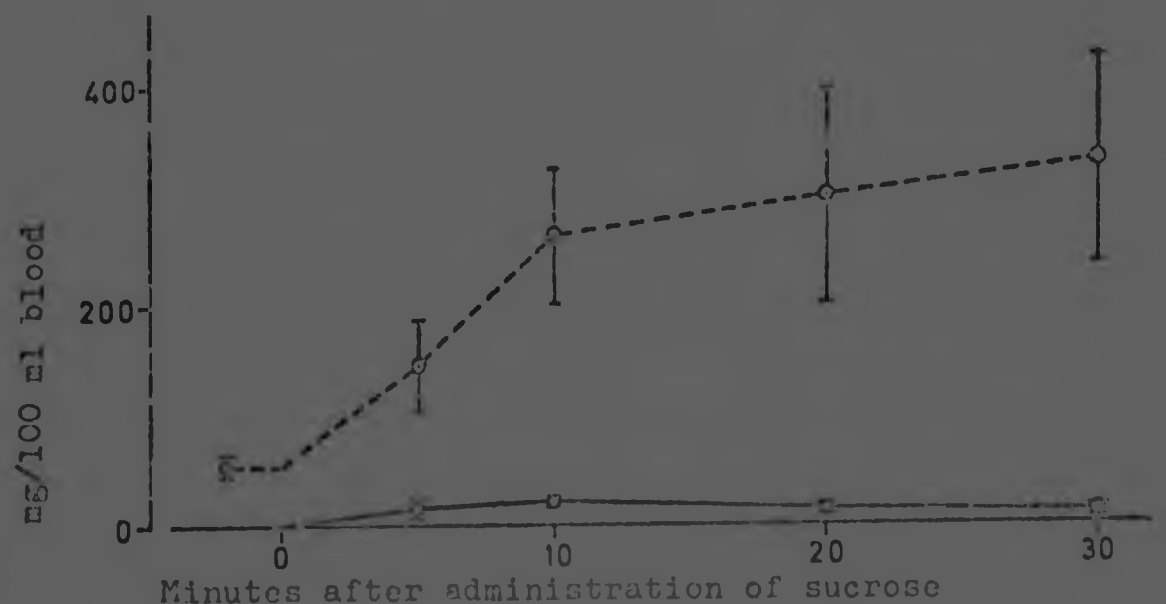

Fig. 15. Blood glucose and fructose concentrations after the administration of 1,5 g of sucrose.

(Solid line ——— represents fructose and the broken line ----- the glucose concentrations. Circled dot shows the mean value and the vertical bars - the standard error of the mean).

3.5. The in vitro permeability of glucose and fructose in the rat and the rat

3.5.1. Rat gut

The glucose transferred by the small intestine is shown in Table 11. In the rat there was a net gain of 40 % of glucose in the serosal compartment. In contrast in the rat there was a net loss of glucose from the serosal com-

partment.

Table 11. The quantity of glucose transferred from the mucosal to the serosal surface in the bat and the rat using the gut sac.

Animal	Glucose transferred µg/cm/30 minutes
Rat (5)*	40 ± 12,3**
Bat (5)*	-52 ± 11,0

* Number of animals in brackets.
** Mean and standard error of the mean.

3.5.2. Recirculation through the everted sac

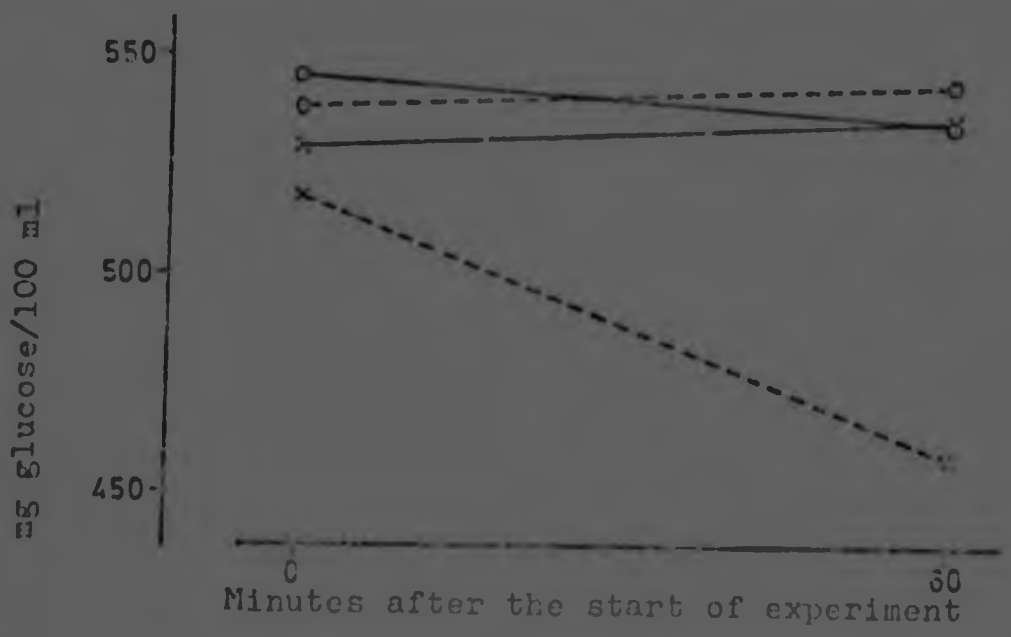

Fig. 14. The concentration of glucose in the serosal and mucosal compartments before and after the recirculation of glucose through the everted intestine.

(Solid line ——— represents the serosal fluid and the broken ----- the mucosal fluid. The circled dot shows the mean glucose values for the bats and the crosses the mean glucose values for the rats.

The results recorded when glucose was recirculated through short lengths of small intestine of the bat and

rat are shown in tables 12 and 24 (see appendix) and fig. 14. In the rat the glucose concentration in the mucosal compartment fell from [illegible] mg to [illegible] mg/100 ml in the 60 minutes while in the serosal compartment the glucose increased from 5,8 mg to [illegible] mg per 100 ml. When the volumes of fluid in the different compartments and the lengths of intestine were taken into consideration, then the amounts of glucose assimilated, transferred and utilized were 4,0, 0,96 and 3,[illegible] mg/cm/hr. respectively. (Table 12).

Table 12. The fate of glucose in the *in vitro* experiments, where the everted gut was recirculated with a solution of Krebs bicarbonate solution containing approximately 500 mg of glucose per 100 ml.

		Fate of glucose (mg/cm/hr.)*		
Animal	Number	Assimilated	transferred	Utilized
Bat	4	1,0 ± 0,03	-1,0[illegible] ± 0,18	2,06 ± 0,17
Rat	4	4,0 ± [illegible]	0,9[illegible] ± 0,2	3,04 ± 0,39

* Mean and standard error of the mean.

In the bat (table 12) the glucose concentration in the mucosal compartment increased but due to the movement of water there was the assimilation of 1,0 mg/cm/hr. of glucose. There was also a decrease in the glucose level in the serosal compartment and this represented a net loss of 1,06 mg/cm/hr. Therefore there was a net loss of glucose from both compartments in the gut.

3.5.3. Recirculation through the isolated intestine

The results of the recirculation of glucose through the isolated intestine are shown in fig. 14 and tables 13 and 25 (see appendix).

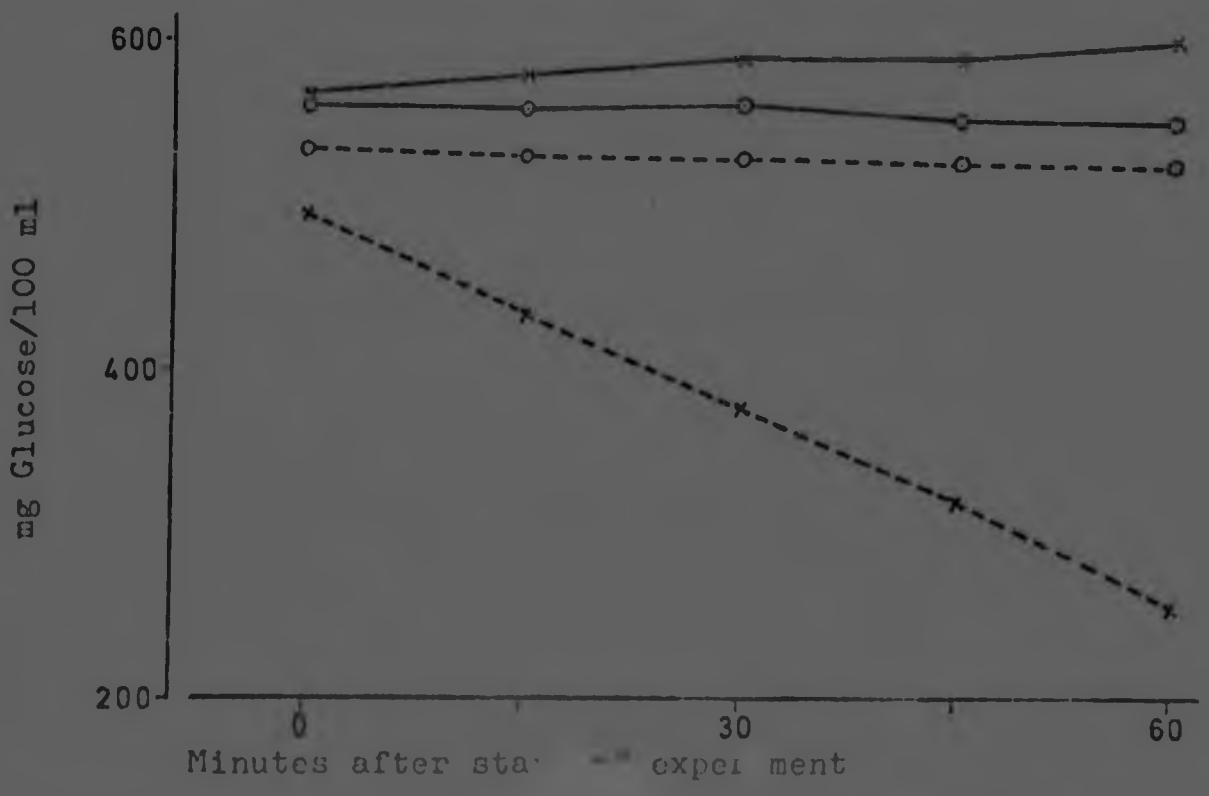

Fig. 14. The mean concentration of glucose in the mucosal and serosal fluids while recirculating the small intestine in vitro.

(Solid line ——— represents the serosal fluid and the broken line ----- the mucosal fluid. The circles denote the mean glucose values for the bats and the squares the mean glucose values for the rats).

In the rat the concentration of glucose fell from 404 to 257 mg/100 ml in the 60 minutes while in the serosal compartment the glucose concentration increased from 0 to 509 mg/100 ml. At no time during the experiment did the mucosal glucose concentration exceed the serosal concentration. Thus any net movement of glucose from the mucosal to the serosal surface, would by definition be

active transport.

In the bat the glucose concentration decreased in both the compartments, in the mucosal from [illegible] to [illegible] and in the serosal from '59 to '5[illegible] mg/10[illegible] ml.

Table 13. The amounts of glucose assimilated, transferred and utilized in the recirculated isolated small intestine.

Animal	Number	Glucose mg/cm/hr.* Assimilated	Transferred	Utilized
Rat	2	[illegible]	0,93	1,3
Rat	2	0,3	-0,3[illegible]	0,[illegible]

* Mean value.

Table 1[illegible] shows that the rat assimilated, transferred and utilized [illegible], [illegible] and 1,[illegible] mg. of glucose/cm/hr. respectively. These values were similar to those reported by Fisher and Parsons (1[illegible]). In the bat however glucose was lost from both of the compartments and 0,[illegible] mg/cm/hr. of glucose was metabolised.

3.6. The effect of anaesthetics

3.6.1. The effect of different anaesthetics on the assimilation of glucose in the rat

Table 14 shows that the quantity of glucose assimilated by the rat varied with anaesthetic used. The largest amount of glucose was assimilated when the phenolarbitone, pentolarbitone mixture was used. The effect urethane had on assimilation was very similar to that of the pheno/pentolarbitone. The thalamorbal anaesthetised animal

Table 14. The effects of the different anaesthetics on the quantities of glucose assimilated in 30 minutes following the oral administration of 1.5 g of glucose.

Anaesthetic.	Glucose assimilated. mg/30 min.
Thalamonal	159 (1)
Urethane	289 (5)
Pheno/Pentobarbitone	294 (2)

* Number of animals in brackets.

assimilated the least amount glucose.

Table 26 (see appendix) and fig. 16 show the blood glucose concentration before and after the administration of 1,5 g of glucose.

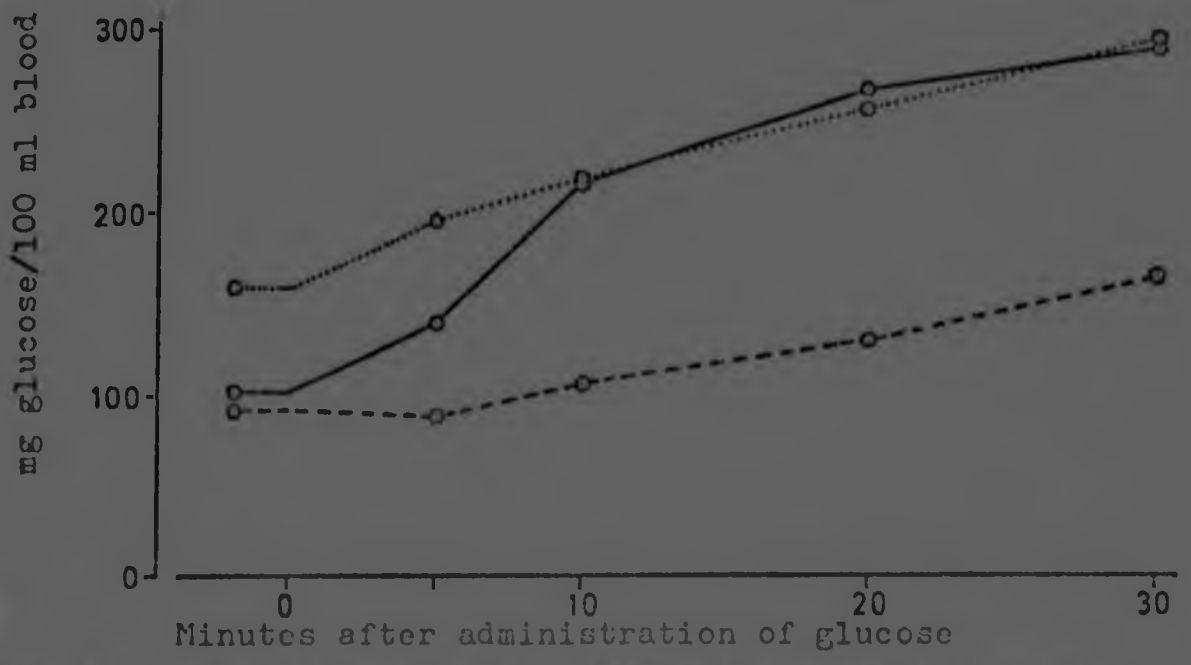

Fig. 16. The effects of the different anaesthetics on the blood glucose levels before and after the administration of 1,5 g of glucose.

(Solid line ——— represents phano/pentobarbitone, the broken line ----- thalamonal and the dotted line ..... urethane anaesthetics. The circled dot shows the glucose concentration.

After the administration of the glucose the blood glucose levels rose very rapidly in the phano/pentobarbitone anaesthetised animal. The increase in the blood glucose levels, with the other two anaesthetics were of the same order. All the rats that had received the urethane anaesthetic had resting blood glucose levels approximately 50 mg higher than the usual resting levels.

3.6.2. Comparison between the control and the anaesthetised rat

The amounts of sugar assimilated in 30 minutes by the

Table I5. The comparison of the rates of assimilation of sugars in the conscious and anaesthetised rat.

Rat		Sugar	Dose mg	SUGAR (mg) Recovered from stomach	Entering intestine	Recovered from intestine	Sugar assimilated mg/30 min.	% Assimilated to available
I	Cons.	Fructose	1487	223	1264	22	1242	98
II	Cons.	Fructose	1463	186	1277	51	1226	96
III	Anaes.	Fructose	1487	916	571	16	555	97
IV	Anaes.	Fructose	1463	1164	299	14	275	91
V	Cons.	Glucose	590	86	504	41	463	91
VI	Anaes.	Glucose	590	127	463	41	422	91
VII	Cons.	Fructose	540	4	536	2	534	99
VIII	Anaes.	Fructose	540	121	419	12	407	97

conscious and anaesthetised bats are shown in Table 15. The results showed that while the conscious bat assimilated over 1200 mg of fructose in 30 minutes, the anaesthetised animals assimilated 555 and 275 mg of fructose in the same time. However, when either isotonic glucose or fructose solutions were administrated then the amounts of sugar assimilated in the conscious and anaesthetised animals were very similar. All the animals at the end of the experiment had only small quantities of sugar in the intestine, irrespective of the amount that was still in the stomach. In all the animals over 90% of the sugar that had entered the intestine had been assimilated. This means that the limiting factor was not so much the rate of assimilation of the sugar but the rate and the amount of sugar that left the stomach to enter the intestine during the 60 minutes.

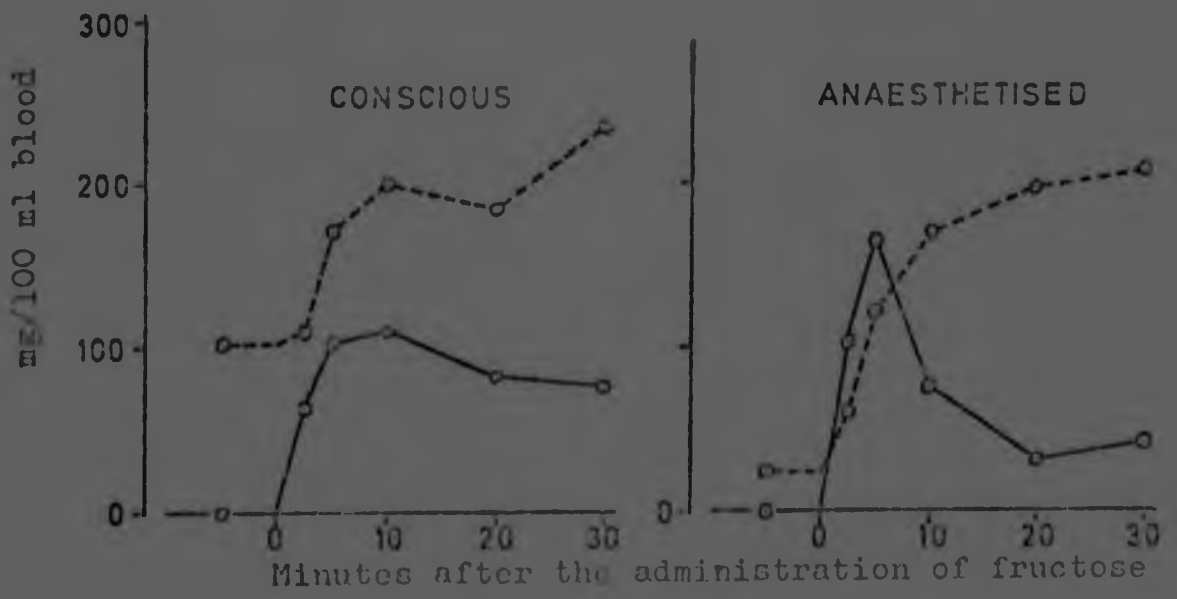

Fig. 17. The average blood glucose and fructose levels following the administration of 1,500 of fructose to two conscious and two anaesthetised bats.

(The solid line ——— represents the fructose and the broken line ----- the glucose concentrations.

The blood glucose and fructose levels following the administration of 1,5 g of fructose to two conscious and two anaesthetised rats are shown in fig. 17. The average resting blood glucose level in the anaesthetised animal was 25 mg/100 ml, which was similar to previously recorded resting blood glucose levels. In this experiment however a value of 117 mg/100 ml for the resting blood glucose concentration was recorded. Why the resting blood glucose levels in the conscious rats in this experiment were so high can not be explained as these experiments were carried out at the same time of the day under similar circumstances as in previous experiments.

After the administration of the fructose, the maximum blood glucose levels were similar for both the conscious and anaesthetised animals, which was surprising for the conscious animal had a higher resting blood glucose level and had assimilated more than twice as much fructose as the anaesthetised rats.

The blood fructose levels were not the same in the conscious and anaesthetised rats. In the former, blood fructose levels reached a maximum of 110 mg/100 ml after 10 minutes and then decreased slowly to reach a value of 77 mg/100 ml by the 30 minute sample. In the anaesthetised animal, which had assimilated only 25 % as much fructose, the blood fructose concentration reached a maximum of 104 mg/100 ml in the 5 minute sample and then decreased to reach a value of 42 mg/100 ml in the 30 minute sample.

3.7. Infusion experiments

3.7.1. Isolated rat gut preparation in a concentration gradient

Following the initial perfusion of the small intestine, the blood glucose concentration at the beginning of the second perfusion was raised above the normal resting levels in both the rat and the bat.

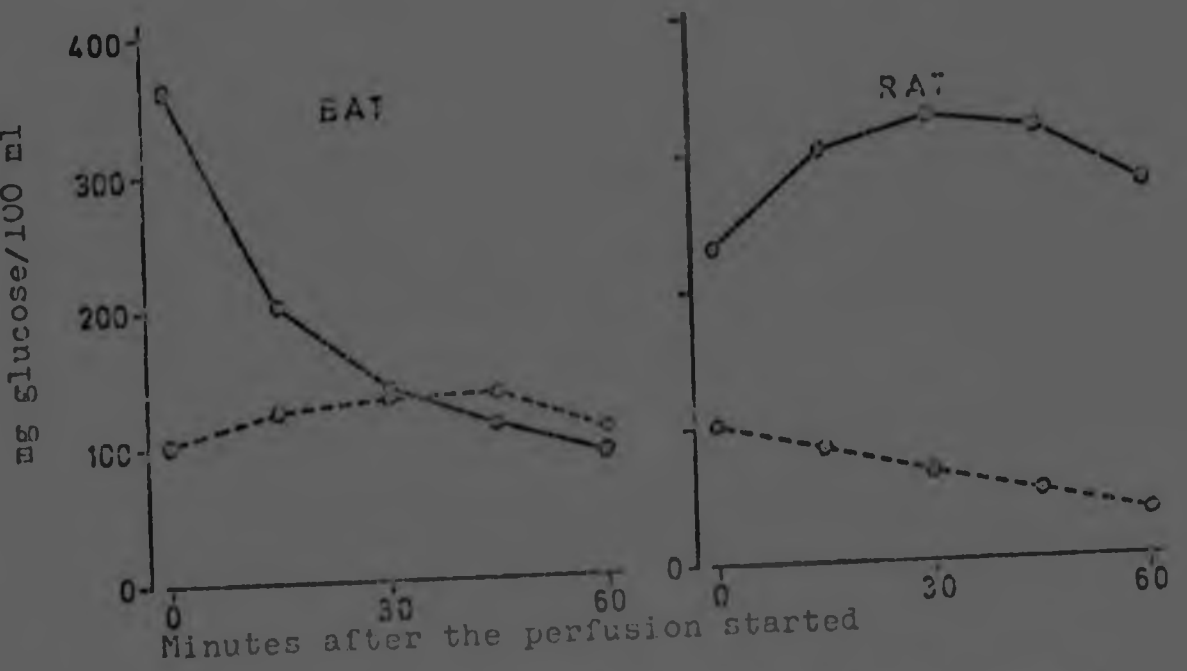

Fig. 18. The concentrations of glucose in the blood and perfusion fluid when the intestine was being perfused with a glucose solution.

(The solid line ——— represents the blood and the broken line - - - - the perfusion fluid. The circled dot shows the zero glucose concentration.)

Fig. 18 and Table 29 (see appendix) showed that at the start of the perfusion, when using the dilute glucose solution, the blood glucose levels were 5.5 and 231 mg/100 ml in the bat and the rat respectively, while the concentration of the glucose in the intestinal lumen was 10[illegible] mg/100 ml. During the experiment the blood glucose levels in the rats increased for the first 30 minutes to reach 429 mg/100 ml and then started to decrease. The glucose concentration in the perfusion fluid decreased from the start of the experiment and after 60 minutes had fallen from 10[illegible] to 35 mg/

100 ml. Therefore throughout these experiments on the rats the glucose concentration in the blood was greater than that of the perfusion fluid. During this period 31,9 mg or 60% of the glucose in the perfusate was assimilated by the small intestine.

The results carried out on the bat were not conclusive. After the start of the experiment the blood glucose level decreased and after approximately 30 minutes had fallen to below the glucose concentration in the perfusion fluid. In the meantime the glucose concentration in the perfusion fluid had increased and only started to decrease once the concentration in the lumen was greater than the blood glucose level. This meant that in these experiments on the bat, the blood glucose level was greater than that of the perfusion fluid for only the first half of the experiment. In the bat there was no assimilation of glucose, but an actual increase of 11,6 mg of glucose into the perfusion fluid.

5.7.2. Intravenous injection of glucose

The blood glucose concentration before the intravenous injections of glucose had been given was 13-30 mg/100 ml of blood. Fig. 15 and table 28 (the appendix) show that during this initial stage there was a trace of glucose in the saline effluent from the intestine. However, after the intravenous glucose injections were given the blood glucose levels increased to 173 mg/100 ml and within 10 minutes of giving the intravenous glucose injections, glucose had appeared in the perfusate and after 50 minutes had reached a maximum concentration of 130 mg/100 ml. After the injection of insulin both the blood and perfusate glucose levels

decreased.

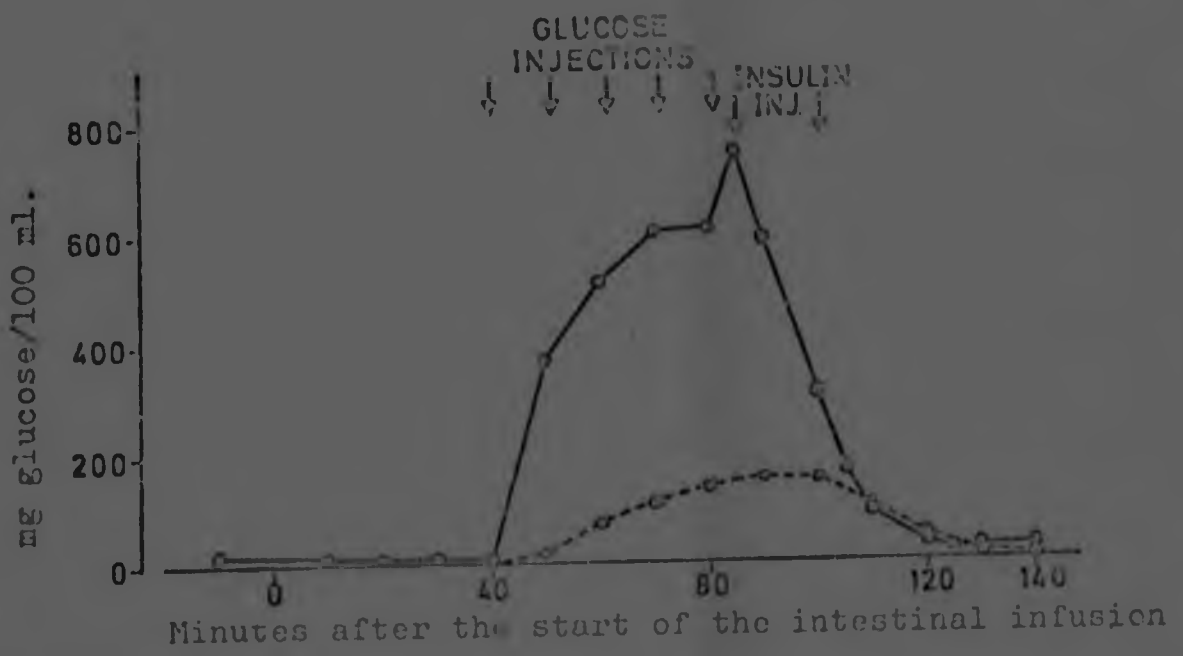

Fig. 19. The glucose concentrations in the blood and perfusion fluid after repeated intravenous glucose injections.

(Solid line ——— represents the blood and the broken line ----- the perfusion fluid. The circled dot shows the glucose concentration.)

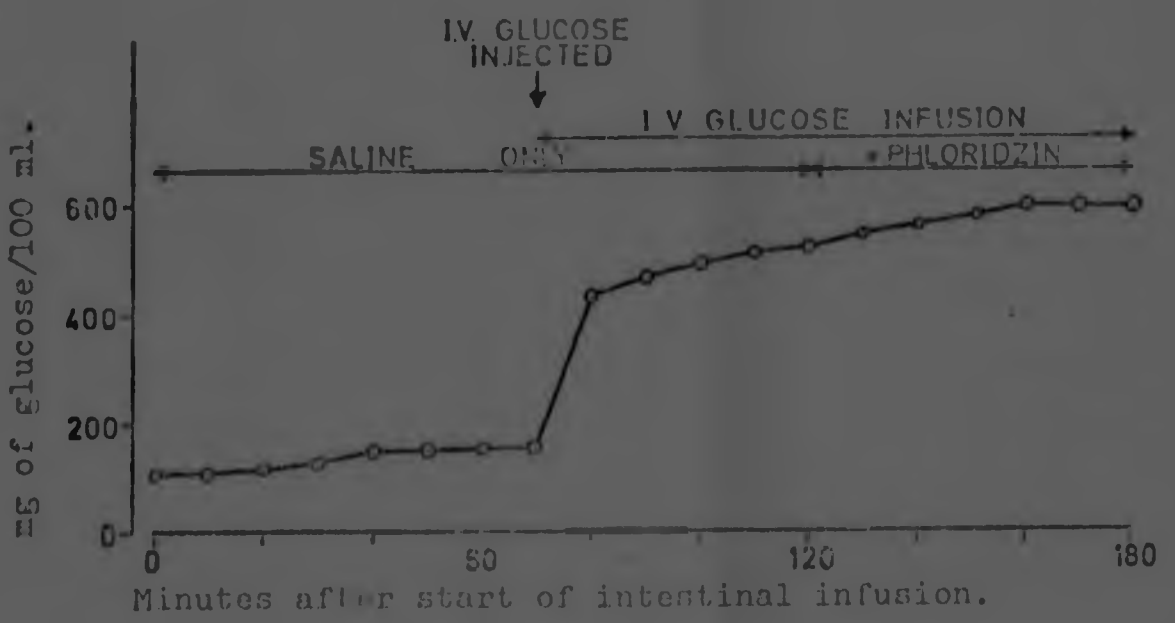

Fig. 20. The blood glucose concentrations in the rat following an intravenous injection and infusion of glucose.

(The circled dot shows the blood glucose concentration).

Table 29 (see appendix) and Fig. 20 show that in the rat the resting blood glucose level was over 100 mg/100 ml. After the intravenous glucose had been given the blood glucose rose to over 500 mg/100 ml of blood. At no stage was there any glucose in the saline effluent from the intestine. The saline perfusate was then exchanged for a saline solution containing phloridzin (0,5 mM) and still no glucose appeared in the effluent even when the blood glucose level had reached a value just short of 500 mg.

3.7.3. The concentrations of the sugars and lactate in the systemic and portal blood of the rat during the assimilation of the sugar

Table 1[illegible] shows the concentrations of glucose, fructose and lactate in the systemic and portal blood while the intestine was being infused with either glucose, fructose or sucrose.

The blood glucose level in the portal blood was 34% higher than in the systemic blood while glucose was being infused. The blood lactate too was 9[illegible] higher in the portal blood.

When fructose was infused the blood concentrations for glucose, fructose and lactate were 1[illegible], [illegible] and [illegible] higher respectively in the portal compared to the systemic blood.

In the sucrose experiment no trace of sucrose was found in either the systemic or the portal blood samples. However, the concentrations of glucose, fructose and lactate in the portal blood was higher than in the systemic blood by 20%, 90% and [illegible] respectively.

Table 16. The concentrations of blood glucose, fructose and lactate during the assimilation of some sugars.

Sugar infused.	Blood concentrations mg/100 ml.**					
	Glucose		Fructose		Lactate	
	Systemic	Portal	Systemic	Portal	Systemic	Portal
Glucose (4)*	280 ± 10,5	348 ± 1-2	-	-	6,57 ± 0,34	7,17 ± 0,89
Fructose (5)*	139 ± 13,0	141 ± 14,7	68 ± 13,5	185 ± 16,6	35 ± 0,25	39 ± 1,2
Sucrose (5)*	263 ± 10,6	317 ± 11,0	29,0 ± 1,5	78,0 ± 7,5	23,5 ± 0,79	25 ± 1,54

* Number of animals in brackets.
** Mean and standard error of the mean.

3.7.4. The effects of the presence of glucose in the stomach on the assimilation of glucose from the jejunum

In the control experiments (fig. 21) it can be seen that the blood glucose level has increased in the 5 minute blood sample and that the concentration continued to increase throughout the experiment.

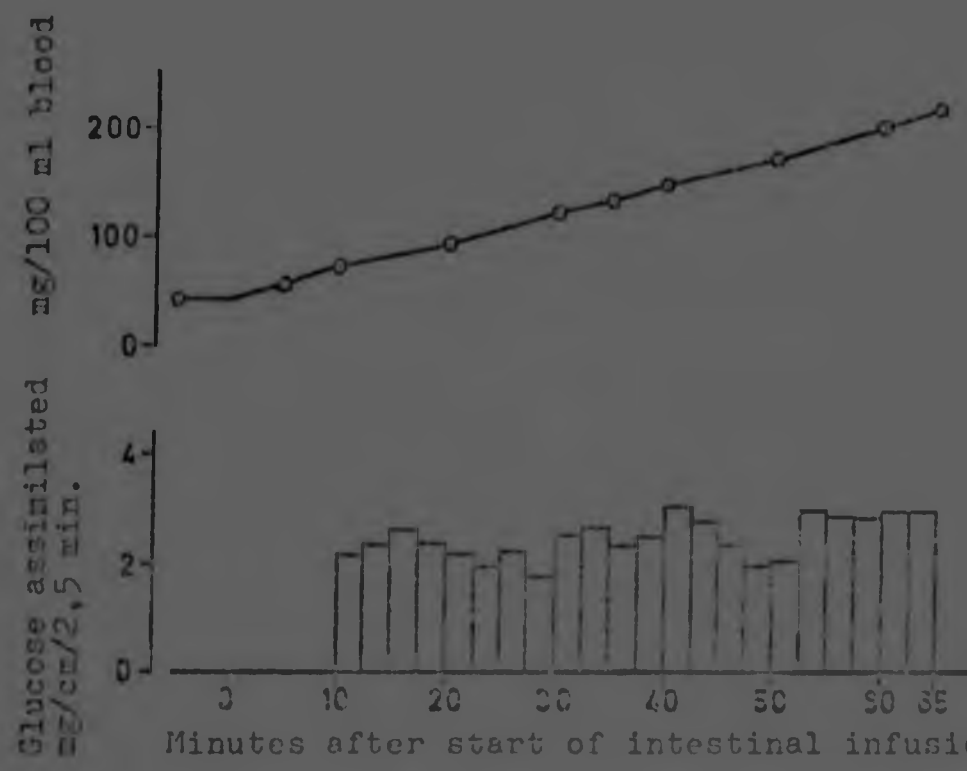

Fig. 21. A: the blood glucose concentration and B: the amount of glucose assimilated while infusing the jejunum with glucose.

The amount of glucose assimilated was approximately 2,0 mg/cm/2,5 minutes. 10 minutes after the intestinal infusion had begun, the rate slowly increased during the experiment to reached approximately 3,0 mg/cm/2,5 minutes at the end of the experiment.

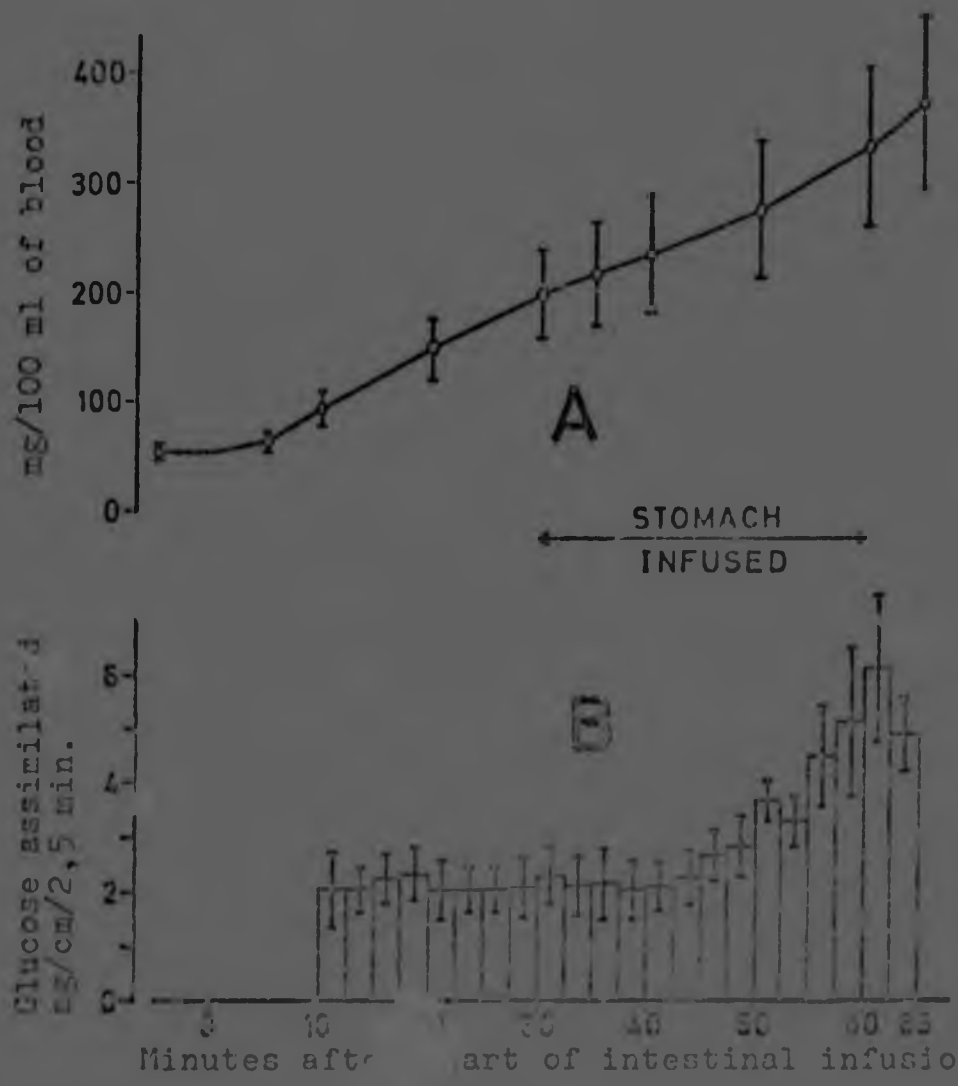

Fig. 22. A: the blood glucose concentration and B: the amounts of gluc. assimilated while infusing the stomach and jejunum with glucose. (Height of histogram represents the mean value and the vertical bars the standard error of the mean of five experiments).

The results of the experiments when the stomach and jejunum were infused with glucose are shown in fig. 22. Soon after the jejunal infusion had begun the blood glucose level increased and then showed signs of levelling out. However when the stomach infusion started there was a second rise in the blood glucose level. The

avera e amount of g'u o e as i ilated during the period from 10 - 30 minut s wa .0 mg/cm/2,5 minut s. After the start of the tomach infu ion th re was a gradual increase in the rate of glu ose assimi ation wh reach d a maximum valu of ,11 mg/cm/2, minut s and then lowly decreased.

3.8. Struct r

3.8.1. Scanning electron micr

Fig. 23. Scanning electron micrograph of the rat's jejunal mucosa.

Figs. 23 and 24 show the surface of the jejunal mucosa in the hamster and the rat. The interesting observation is that in the bat the villi are far more finger-like while the villi in the rat are more leaf-like. Also in the bat the villi are more closely packed together than in the rat.

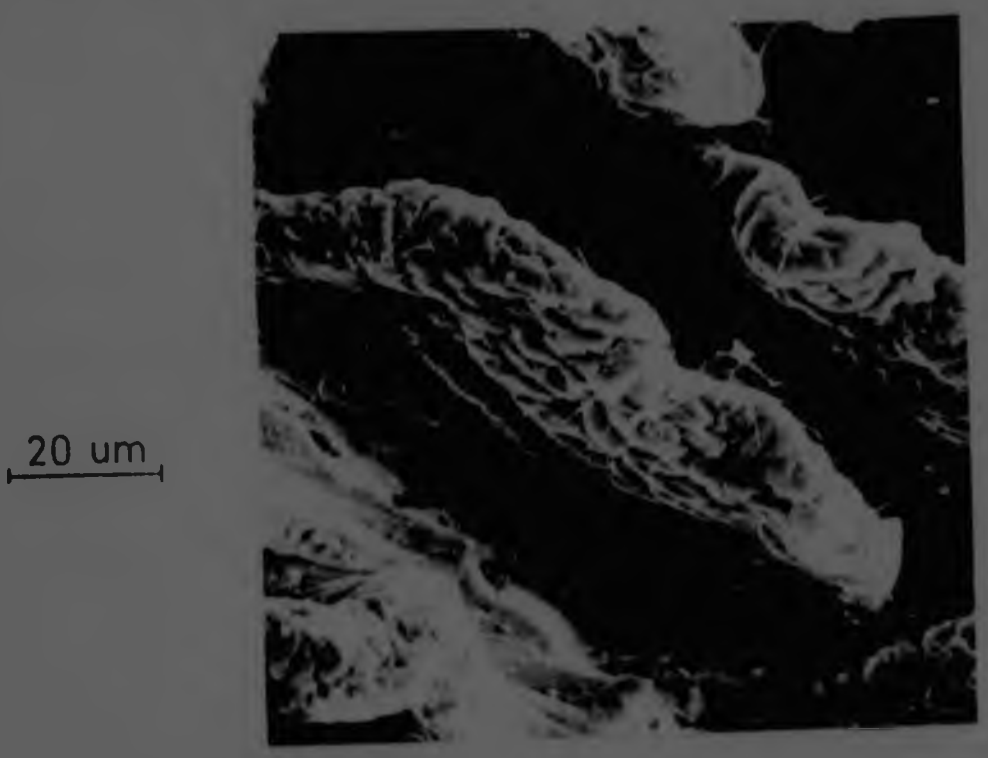

Fig. 24. Scanning electron micrograph of the rat's duodenal mucosa.

3.8.2. Electron microscopy

Fig. 25 and 26 are typical photomicrographs of the microvilli found in the bat and the rat small intestine, cut in longitudinal section.

The mean lengths of the microvilli were 0.6 μm and 1,14 μm for the bat and the rat respectively, while the diameters were 0,09 μm and 0,1 μm (table 5; see appendix).

Fig. 27 and 28 show microvilli cut in cross section so that the distance between the microvilli as well as the diameters could be measured. In the bat and the rat the distance between the microvilli was 0,07 μm and 0,08 μm respectively.

The cross section micrograph showed that there was a roughly hexagonal arrangement between the microvilli and that there were approximately 97 and 84 microvilli per μm^2

in the bat and rat respectively.

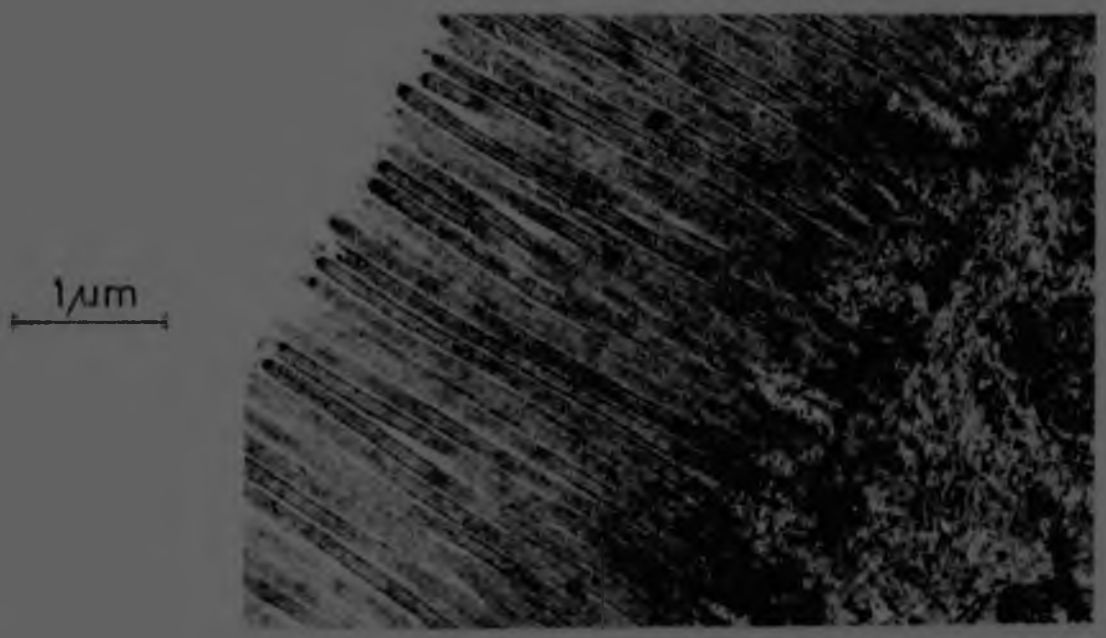

Fig. [illegible]. Microvilli on the rat's jejunal mucosal cell.

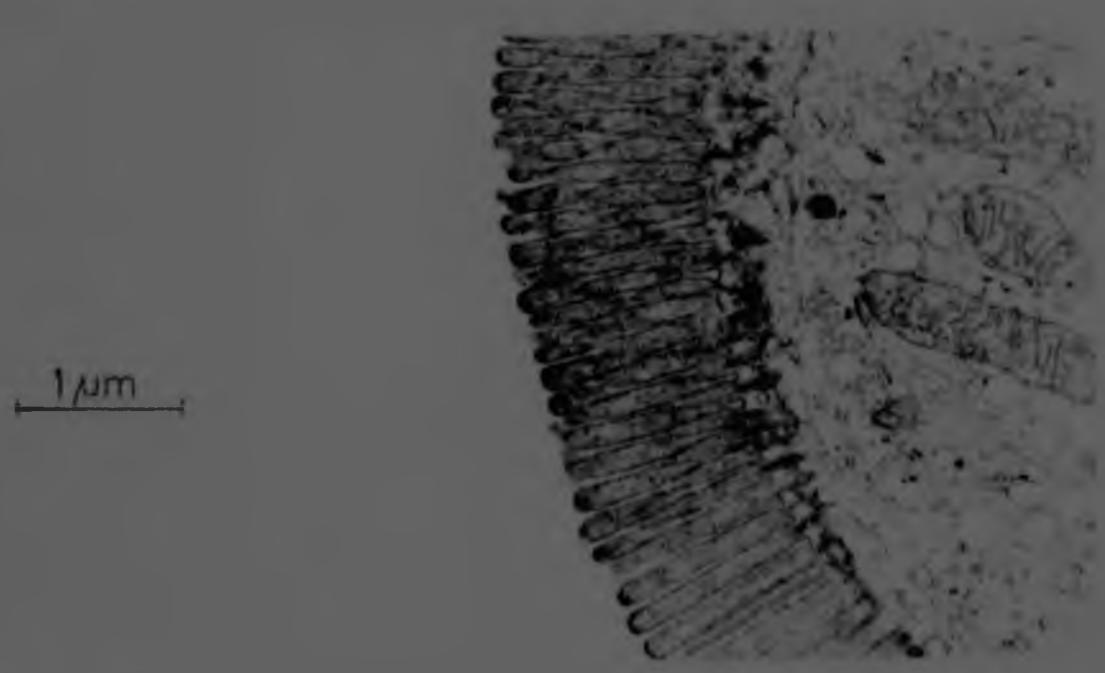

Fig. [illegible]. Microvilli on the rat's jejunal mucosal cell.

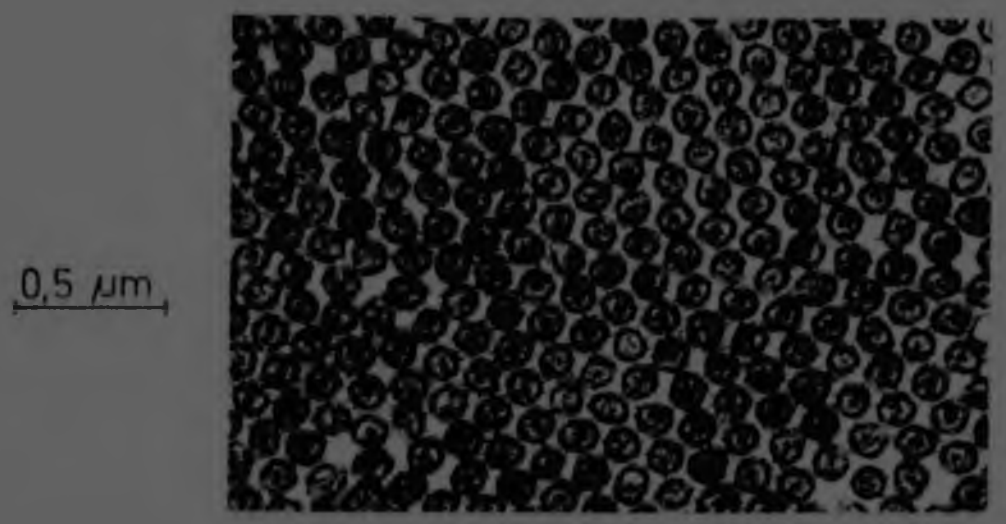

Fig. 2?. Cross section of the microvilli in the bat jejunum.

Fig. 2?. Cross section of the microvilli in the rat jejunum.

4. DISCUSSION

4.1. Transit time for bananas

Three to four hours after a meal, a bat when killed was found to have no food material in the gastro-intestinal tract. Noctilio leporinus does not have a large intestine and anatomically the intestine changes from the ileum to the rectum.

The absence of food material in the intestine raised two interesting possibilities. The first was that the animal only swallowed the juice of the fruit it was eating spitting out the fibrous remains. This however, could be discarded as defaecation of solid material was always observed, when the animal was on a banana diet. The other possibility was that the bat had a very rapid transit time. The experiments (page 15) and results (page 55) showed that a conservative estimate of the mean transit time for bananas in the bat was 45 minutes. This transit time, however, was much longer than that reported by Lombard (1961) who reported transit times in excess of 15 minutes. In the rat on the other hand Sanford and Smyth (1936) using phenol red given by stomach tube, showed that after 60 minutes no dye had reached the distal fifth of the ileum and only 25% of the dye had reached this part of the ileum after 120 minutes. These experiments show that the transit time for food was much shorter in the bat than in the rat. This very rapid transit time coupled with a rapid rate of digestion and assimilation of food would be of immense advantage to the bat. It would mean that the bat would not have to fly home, after feeding, possibly 20 - 30 kilometers, handicapped with any excessive and useless weight. This is

especially true when it is realised that the bat consumes 70% and more of its own weight in bananas per night (J. van der Westhuyzen, 1975).

Indirect evidence to support the theory that the bat has a rapid and effective process of digestion and assimilation, is that although the average size bat consumed at least 84 g of bananas per night, which would contain 4,8 g glucose, 3,2 g fructose and 5,5 g of sucrose (Widdowson and McCance, 1935) the faeces contained very little glucose (van der Westhuyzen, 1975).

4.2. Oral glucose tolerance tests

Oral glucose tolerance tests were carried out, to observe the rates of increases in the blood glucose levels. A more rapid increase in the blood glucose levels could indicate a more rapid rate of assimilation of glucose in the bat than in the rat.

In the graph showing the mean blood glucose levels in the bat and the rat during the oral glucose tolerance test, (figure 7) showed that in the case of the rat the blood glucose level increased from a resting level of 113 mg/100 ml of blood to 150 mg/100 ml of blood at the 20 minute sample. Whereas in the bat the mean rise in the blood glucose was far more rapid from a resting level of 27 mg/100 ml to 284 mg/100 ml. In the rat the individual results at a specific sampling time showed little variation, with the result the standard error of the means was not very large. On the other hand, the bat results were very variable resulting in large standard error of the means. Due to those large standard error of the means found in the bat it was decided to process, statistically, the two sets of results. This was carried out

by testing for the significant difference between the slopes of the two glucose tolerance curves. This test showed that the difference between the two curves was highly significant at the 0,[illegible] level.

These animals had all received the same amount of glucose per body weight. Therefore, if the rates of assimilation and utilization of glucose were the same in both animals, the slopes of the increase in the blood glucose concentrations during a glucose tolerance test would be the same. However, in this experiment the slopes were not the same, with the bat having a steeper slope. This means that either glucose was being assimilated faster in the bat than in the rat, or that the bat was not able to handle the assimilated glucose as effectively as the rat. To eliminate or confirm the latter possibility the animals were given intravenous glucose tolerance tests.

4.3. Intravenous glucose tolerance tests

The results obtained after carrying out intravenous glucose tolerance tests on the bat and the rat are shown in figure 8 and table 19.

The results showed firstly that the bat had a lower resting blood glucose level than the rat. Secondly the increase in the blood glucose concentration in the initial blood samples following the intravenous glucose injection was approximately the same in both the animals indicating that the volume of the extracellular compartment in both animals was approximately the same, relative to its body weight. However, the more important observation was that the decay in the levels of the blood glucose concentrations was the same for both groups of animals. This means that

the rate of removal of glucose from the extracellular space must be the same for both animals.

These results therefore excluded the possibility that the rapid rise in the blood glucose level, in the bat when compared to the rat following an oral glucose tolerance test, was due to an inability on the part of the bat to utilize the assimilated glucose. Thus the steep rise in the blood glucose level in the bat was a good indicator that the bat was able to assimilate glucose at a faster rate than the rat.

4.4. Assimilation of sugars in the anaesthetised animal

The results of the oral and intravenous glucose tolerance tests indicated strongly that the bat assimilated glucose at a faster rate than the rat. In order to ascertain if indeed the bat had a faster rate of glucose assimilation, an accurate and direct experimental method for the measurement of the rate of assimilation had to be used. This method had to be as physiological as was possible but stil allow the maximum accuracy.

One such method was that of Cori (1925). Although some workers, Mackay and Clark (1942) and Pauls and Drury (1942) have questioned his results, nevertheless this method has a number of great advantages over other methods. Firstly the animals were not anaesthetised, but were fully conscious, so that any possible actions the anaesthetic may have had on the assimilation of glucose were eliminated. Secondly, the animals underwent no surgical procedures so no damage or irritation to the intestine or blood vessels could occur and so hence adversely effect the assimilation of the sugars. Thirdly, although unfortunately the Ani-

mals were force fed, on the credit side the using of the stomach tube meant that the exact volume of the sugar solution administered was placed accurately into only the stomach.

Cori used very high concentrations of 25, 50 and 80 g of glucose per 100 ml, so that he had only small volumes of the sugar solution to administer. These high concentrations of sugars are not normally found in nature, so these animals were being subjected to an unaccustomed diet.

Fenton (1945) who also used this method used both low and high concentrations but like Cori he also administered small volumes, less than 2 ml. This resulted in small quantities of the sugar being given when the low concentration sugar solutions were used. This would result in less sugar being administered than the animal was theoretically able to assimilate.

In this investigation it was decided to use a concentration similar to that found in the ripe banana, which is the staple diet of these baboons kept in captivity. Widdowson and McCance (1943) gave the concentrations of fructose, glucose and sucrose in ripe bananas as 3,8 0,6 and 5,5 g per 100 g of banana respectively. This meant that the potential sugar from a banana diet was 16,2 g per 100 g of banana. As bananas contain approximately 80% water, (van der Westhuysen, 1975) the concentration of the total sugars in the banana juice is of the order of 20 per 100 ml. As the sucrose has first to be digested before assimilation can occur, it was decided to use a standard concentration of 15 g of sugar per 100 ml.

Having decided what concentration of sugar solution to use, two further factors had to be taken into consideration.

The first was the length of time the experiment would last and the second the volume of the sugar solution to be administered.

The oral glucose tolerance tests were carried out for 20 minutes and as the blood glucose levels were still increasing, it was decided that the experiment would be run for 30 minutes.

Knowing the concentration of the sugar solution to be used and the length of time the experiment would last only the volume of fluid had to be decided. In preliminary experiments carried out on the bat it was found that approximately 1,5 g of sugar needed to be placed in the bat's stomach to be sure that after 30 minutes there was still some sugar left in the stomach. This meant that 10 ml of the sugar solution had to be administered. No difficulty was experienced giving this volume of fluid to the bat, although, in a few cases in the rat, there was regurgitation of the fluid. These animals were discarded from the experiment.

4.4.1. Glucose assimilation in the intestine

The results showed, table 1, that the bat assimilated 765 mg of glucose compared to 278 mg by the rat in 30 minutes. Thus under the same experimental conditions the bat assimilated glucose at a rate 2,8 times faster than the rat. This rapid rate is all the more impressive when it is taken into consideration that the bat weighs less than half as much as the rat.

The rates of assimilation in these experiments were expressed as mg per animal per 30 minutes and not in terms of the length or weight of intestine or the weight of the

animal. This was because, firstly in the bat there was no accurate way in which to measure the length of the intestine involved in assimilation as approximately only the proximal third of the intestine contained any measurable fluid when the animals were killed at the end of the experiment. Secondly, unlike Cori (1925) no correlation between the amount of glucose assimilated and the body weight could be found.

The rate of glucose assimilation in this investigation for the rat was 80% and 90% higher than the values reported by Cori (1925) and Fenton (1945). Cori reported values of 162 - 178 mg/100 g body weight per hour compared to 222 mg/100 g body weight per hour in these experiments. The only two factors different in this investigation and Cori's was that larger volumes of fluid and more dilute glucose solutions were used. The use of 10% glucose solutions should have had the opposite effect as Fenton (1945) showed that with increasing concentrations, the rate of assimilation increased. How the volume could increase the rate of assimilation is difficult to explain. Fenton reported values of only 146 mg of glucose assimilated in 30 minutes per animal. He, however, had used a 12,5 g/100 ml solution and had only given 1,75 ml. This meant that the total amount of glucose placed in the stomach was only 437 mg, which is less than the amount assimilated in this investigation. These small quantities of glucose being placed in the stomach would certainly explain in part the low assimilation rates he recorded but even when he did use larger quantities he recorded values of only 400 mg in one hour.

Mackay and Clark (1942) and Pauls and Drury (1942) pointed out that if the rat assimilated glucose at a rate

of only 180 mg/100 g body weight, as Cari's results showed then the animal would have to assimilate food throughout the 24 hours of the day to meet its basic energy requirements. This continuous assimilation in the rat is possible as there is always food material in the stomach and in the intestine. In these experiments, however, values of approximately 220 mg/100 g body weight/hour were recorded which would place the rat in a far better energy balance.

Having established that the rate of assimilation in the rat is comparable to other reported values then the rate of assimilation in the bat becomes a most interesting observation. This rapid rate of glucose assimilation in the bat cannot be simply explained by the possibility that the bat may have a larger intestinal surface area over which assimilation can take place, because although their intestinal diameters are of the same order only the proximal third of the bat's intestine, about [illegible] cm, contained any measurable fluid at the end of the experiment, while in the rat the glucose solution usually filled the entire length of the small intestine (96 cm).

In the bat at the end of the experiment the concentration of the glucose in the intestine was [illegible] g/100 ml, whilst in the rat it was 4,[illegible] g/100 ml. This meant that in the bat there appeared to be a lesser need to dilute the glucose solution in the stomach to make it isotonic to blood before it entered the small intestine. Thus in the bat the glucose concentration gradient between the intestinal lumen and the blood was 50% greater than in the rat. Could this increase in the glucose concentration gradient be a possible explanation for the more rapid assimilation

of glucose in the bat compared to the rat or is it that the bat has a more active glucose transport system.

This very rapid rate of glucose assimilation by the bat when compared to the rat, would certainly cause the very rapid increase in the blood glucose levels seen both in this experiment and in the oral glucose tolerance tests. The blood glucose levels in these experiments (fig. 9) followed a similar pattern to those recorded in the oral glucose tolerance tests (fig. 7), the difference being that in the latter experiments the blood glucose levels did not reach such high levels. This difference was probably due to the fact that in the oral glucose tolerance tests the bats were anaesthetised and smaller quantities of glucose had been used.

4.4.2. Fructose assimilation in the bat and the rat

The results of the assimilation of fructose were even more startling than those for glucose. The animals had all been given approximately 1.2 g of fructose. The results (table 3) showed that the bat assimilated 1076 mg of fructose compared to the 268 mg of the rat in 30 minutes, i.e. four times as much as the rat under identical circumstances. Cori (1925) reported assimilation values for fructose in the rat of 77 mg/100 g body weight/hour, this is equivalent to approximate 100 mg/30 min. in an average size rat. Why the assimilation rated in this experiment should be so much greater than Cori's values is not known. As discussed when dealing with glucose assimilation the only two factors different in the two techniques were the more concentrated solutions he used coupled with the smaller volumes.

Of the initial volumes of fructose placed in the stomach, only 2,5 ml of fluid was collected from the bat's stomach at the end of the experiment compared to 5,7 ml in the rat. Therefore in the bat far more fructose entered the intestine than in the rat, but of this fructose that entered the intestine 92% was assimilated in the bat compared to 30% in the rat. The small amount of fructose assimilated in the rat cannot therefore be because there was no fructose in the intestine. Similar to the bat glucose experiments, no measurable fluid was found in the distal two thirds of the intestine and the concentration of the fructose in the intestine was 8,5 g/100 ml again resulting in a large fructose concentration gradient between the intestinal lumen and the blood. Could this high fructose gradient which was 50% higher in the bat than the rat be the cause of this very rapid rate of fructose assimilation?

The blood sugar levels showed that in the rat the glucose and fructose levels increased slowly, whereas in the bat these levels increased very rapidly. In the bat the blood glucose levels rising to over 250 mg/100 ml of blood in the 30 minute sample, the fructose level, however, reached a maximum value of 150 mg/100 ml of blood in the 5 minute sample and then slowly decreased. It is highly unlikely that the blood fructose level is an indication only of the rate of fructose assimilation, for there was still a large quantity of fructose in the intestine 5 minutes after the start of the experiment and also the blood glucose level had continued to increase. This decrease in the blood fructose levels may be due to the fact that after the initial rise in the blood fructose level, some reaction

for the conversion of fructose to glucose was stimulated. This could be in the intestine at the site of assimilation or it may be the liver. Further experiments involving the sampling of the portal blood will have to be carried out to elucidate this observation.

In the bat the rate of fructose and glucose assimilation was 1076 mg/30 minutes and 765 mg/30 minutes respectively. The difference between these two groups of experiments was highly significant at the 0,[illegible] level. These results were surprising as no other worker had reported an animal that assimilated fructose faster than glucose. Cori (1925) reported assimilation rates of 43% for fructose compared to glucose. Other workers, Holdsworth and Dawson (1964) and Fordtan and Ingelfinger (1968) pointed out that fructose assimilation was more rapid than would be expected if assimilation was only passive diffusion but still the rate was less than the glucose assimilation rate.

Fisher and Parsons (1[illegible]), Wilson and Wiseman (1954), Sheff and Smyth (1955) and many other workers have shown that the rat intestine is able to transfer glucose against a concentration gradient.

An active transport system for glucose in the intestine has been demonstrated not only in the rat but in all the mammalian species that have been studied. The bat, especially as it assimilated glucose at a rate three times faster than the rat, could not be expected to be an exception and have no active transport system. Once it had been postulated that there was an active transport system for glucose, and as fructose is assimilated at a statistically faster rate than glucose then an active transport

system for fructose must also be postulated. The circumstantial evidence against such a postulate is very great as the active transport of fructose in the intestine has not been demonstrated either *in vivo* or *in vitro*. The only possible supporting evidence in favour of an active system for fructose was that of Gracey *et al.*, (1972). They showed in *in vitro* experiments using the rat intestine, that the concentration of fructose was higher in the tissue water than in the incubation fluid.

These results of the rates of assimilation of glucose and fructose suggested that the bat had an active transport system for both of these sugars.

4.4.3. Assimilation of a mixture of glucose and fructose in the bat

The rates at which glucose and fructose were assimilated from the mixture of glucose and fructose administered was 417 and 465 mg respectively in the 30 minutes. The fructose was assimilated significantly faster than glucose ($P < 0,01$) which confirmed the previous observations when comparing the rates of assimilation of these two sugars when they were given separately. In this experiment fructose was assimilated 12% faster than glucose, which was less, when compared to the 40% faster rate when these sugars were administered separately.

When glucose or fructose were given separately, then the bat assimilated 10[illegible]6 and [illegible] mg of fructose and glucose respectively in the 30 minutes. Therefore if these two sugars were given as a mixture, but each at half the original concentration then if assimilation were passive it would be expected that the rate of assimilation of each

of the sugars would be half the original rate, i.e. 538 and 383 mg of fructose and glucose respectively. In this experiment however 465 mg of fructose and 417 mg of glucose were assimilated, which meant there was a 11% reduction in the rate of fructose assimilation and a 13% increase in the glucose rate. Could this decrease in the fructose and an increase in the glucose rates of assimilation, possibly be explained by Stein's dimer theory (19[illegible]).

During this experiment the same quantities of glucose and fructose left the stomach and as fructose was more rapidly assimilated than glucose, its not surprising that 87% of the available fructose compared to [illegible] glucose was assimilated.

Similar to the experiments where glucose or fructose were administered, the combined concentrations of the intestinal sugars in this experiment was [illegible] monosaccharides/ 100 ml, which meant that not only were the intestinal contents hypertonic, but there was a high sugar concentration gradient which would favour assimilation.

The blood glucose and fructose levels in this experiment rose rapidly after the administration of the sugar mixture. The blood glucose levels recorded during assimilation were greater than when fructose was given alone, but as expected, the blood glucose and fructose concentrations did not increase as much as when the individual sugars had been administered separately.

4.4.4. Digestion of sucrose in the gut

The amount of sucrose in 100 g of ripe bananas is approximately 5,5 g (Widdowson, 193[illegible]) and so accounts for [illegible]% of the total monosaccharides and disaccharides available in

the bat's diet. The animal's capacity to digest this sugar and assimilate the resulting fructose and glucose was therefore of great interest.

The results showed that of the 1,5 g of sucrose administered 508 mg was still present in the stomach 30 minutes after the sugar had been given. There was also traces of both glucose and fructose in the stomach, probably the result of acid hydrolysis. The quantity of sucrose that entered the intestine was 98? mg and of this 932 mg or 94% was digested, if the assumption that sucrose can not be assimilated is correct (see page 114). The digestion of sucrose resulted in the formation of 490 mg of both glucose and fructose, which were then available for assimilation. The results showed that of these amounts, 3?4 mg and 369 mg of fructose and glucose respectively were assimilated leaving the balance of 9? mg of fructose and 121 mg of glucose in the intestinal lumen. Similar to all the bat experiments where glucose and fructose assimilation was compared, fructose was assimilated at a significantly faster rate than was glucose ($P < 0,00?$).

When the rates of assimilation of glucose and fructose in this experiment were compared to the experiment where the animals were given a mixture of the two sugars, it was found that although the mean values were lower in the sucrose experiment there was no significant differences between the results obtained for the two groups of experiments. The results of the sucrose experiments showed that more than 20% of the monosaccharides formed and 6% of the sucrose were still present in the lumen. Hence the digestion of sucrose is not a limiting factor in the processes involved

in the digestion of sucrose and the ultimate assimilation of its products. These results showed that the bat had a very efficient sucrase enzyme system.

The combined concentrations of sugars in the intestine at the end of the experiment resulted in an osmotic pressure isotonic to the blood. This was in contrast to the other assimilation experiments, carried out on the bat, where the tonicity of the fluid was greater than that of the blood.

In these sucrose experiments the volume of fluid in the intestine at the end of the experiment was 4.2 ml, which was more than had previously been observed in the other experiments and as a result the sucrose solution was found in most of the proximal half of the intestine. The reason for this large volume of fluid was due partly to the large quantity of glucose, fructose and sucrose still present in the intestine and partly to the lower concentrations of these sugars.

The blood glucose and fructose levels increased after the sucrose had been administered, but these increases were not as great as seen when the mixture of glucose and fructose was given. This was to be expected since not as much of the monosaccharides were assimilated in this experiment as in the mixture experiment.

4.4.5. Urine formation during the assimilation of the monosaccharides

An interesting observation was that during these assimilation experiments the bats very seldom passed any urine and when killed the bladders contained only small volumes of fluid. When this urine was tested for glucose using

"test tape"* very little glucose if any was found to be present. It is of interest to note that when van der Westhuyzen (1975) gave 800 mg of glucose per 100 g body weight in oral glucose tolerance tests, glucose was demonstrated in 5 of the 6 urine samples. Whether the higher glucose load given in these assimilation experiments caused a complete shutdown of the kidney is not known. A glucose load of 1,5 g, as already discussed (page 88) was not excessive. The shutdown of the kidney would theoretically be a great advantage to the bat, for during the period of feeding the blood glucose would rise to very high levels without the loss of any of the glucose in the urine. However, in captivity, when the bat was voluntarily eating a banana diet, the urine produced, containing 0,1-2 g of glucose/ 100 ml of urine.

4.5. The assimilation of sugars in vitro

The results from the assimilation experiments showed that both glucose and fructose were far more rapidly assimilated from the bat's intestine than from the rat's. Secondly the bat assimilated fructose at a faster rate than glucose. This evidence together with the knowledge that in the rat glucose was actively transported, has led to the postulation that both glucose and fructose are actively transported in the bat. To confirm an active transport system there must be evidence that the sugar involved is transferred against a concentration gradient.

4.5.1. Glucose assimilation into the gut sac technique

In the experiments carried out using the gut sacs to

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* "test tape" urine sugar analysis paper produced by Lilly Laboratories (S.A.) Pty. Ltd.

measure the rate of transfer of glucose, it was found that when the initial concentration of glucose was 300 mg/100 ml then the rate of transfer of glucose in the rat was 40 µg/cm/30 minutes. This rate when compared to the value of approximately 30 µg/cm/30 minutes (value calculated) reported by Wilson and Wiseman (1954) showed that the results recorded when using this technique were similar to other published results. However, the results obtained for the bat showed that there was a net loss of glucose from the serosal compartment of 12 µg/cm/30 minutes.

This loss of glucose from the serosal compartment could be due to a number of factors. The first was that the tissue had been damaged by the initial hypoxia while preparing the gut sac and was no longer capable of active transport. The second was that as quickly as the glucose was transported from the mucosal to the serosal surface it was diffusing back into the mucosal compartment. A last possibility was that in the bat there was no active transport mechanism for glucose and the loss of glucose was due to metabolism.

Before trying out other *in vitro* methods, the gut sac experiments were repeated on the bats, but this time the glucose concentrations were altered and the experiments were carried out for longer periods of time. Finally the temperature of the Krebs bicarbonate solution used, when the gut sac was being prepared, was altered. The results obtained after all these modifications still showed a net loss of glucose from the serosal compartment.

One disadvantage of this technique was the very small volume of fluid inside the gut sac compared to the mucosal

compartment. The error in the measurement of the quantity of glucose in the mucosal compartment would be far greater than the actual amount of glucose in the serosal compartment, so no accurate measurement of glucose metabolism could be made. It was therefore decided to use a method where the volumes in the two compartments were similar.

4.5.2. Recirculation through the everted gut

In the experiments carried out on the rat the mean concentrations of glucose, at the beginning and at the end of the experiment, in the mucosal compartment were 518 and 455 mg/100 ml respectively, whilst in the serosal compartment they were 522 and 545 mg/100 ml respectively. At no time during this experiment was the concentration of glucose in the mucosal compartment greater than in the serosal compartment. During the experiment there was a marked decrease in the glucose concentration in the mucosal compartment and an increase in the concentration in the serosal compartment. In terms of the actual movement of glucose, 4 mg/cm/hr of glucose had left the mucosal compartment and of this 1,0 mg/cm/hr had entered the serosal compartment against a glucose concentration gradient. The remaining 3 mg/cm/hr must either have been metabolised or trapped in the tissues.

No records of similar experiments using the everted gut were found but if the assimilated results from this investigation of 4 mg/cm/hr were compared to the 0,0 mg/cm/hr recorded in the gut sac experiments then there was a 50 fold increase in the rate of assimilation. This showed that the recirculation method gave assimilation rates closer to a possible physiological value.

Fisher and Parsons (1949) recorded a discrepancy be-

tween the rate glucose disappeared from the mucosal compartments and the appearance of glucose in the serosal compartments of 1,1 mg/cm/hr, while in this experiment a value of 3 mg/cm/hr was recorded. Fisher and Parsons reported, that if the tissues were deprived of oxygen while setting up the experiment, then there was a shift in the ph of the mucosal fluid to the acid, suggesting lactic acid formation. This would mean that for the same amount of energy more glucose would have to be metabolised. In the Fisher and Parsons' experiments the mucosa was at no time deprived of oxygen, whereas in the present experiment, where the gut had to be everted, there was a period of approximately one minute while the intestine was removed from the animal and the tissue everted before it was placed in the oxygenated Krebs solution. This period of lack of oxygen to the mucosal surface would probably explain the difference in the results obtained for these experiments and those of Fisher and Parsons.

The results nevertheless did demonstrate that although the tissue had probably been damaged due to the lack of oxygen it was still capable of the active transport of glucose.

In the rat the concentrations of glucose in the mucosal compartment at the beginning and at the end of the experiment were 500 and 552 mg/100 ml respectively, while in the serosal compartment they were 500 and 503 mg/100 ml. Therefore in the rat there was an increase in the concentration of the glucose in the mucosal compartment and a decrease in the serosal compartment. The experimental results showed that 1,0 mg/cm/hr of glucose had actually left the mucosal

compartment, so the increase in the concentration of glucose in the mucosal fluid during the experiment must be explained by the transfer of water from the mucosal to the serosal compartment. As the concentration of glucose in the mucosal compartment was higher than in the serosal compartment at the end of the experiment, any net movement of glucose from the mucosal to the serosal compartment was not evidence for an active transport system for glucose. However, as there was no net gain, but a loss of 1,0 mg/cm/hr of glucose from the serosal compartment, it would appear that there was no net transfer of glucose from either of the compartments and that the glucose difference in each of the compartments was due to the metabolic needs of the tissue.

The results of this experiment showed that in the rat the active transport of glucose by the intestine was clearly demonstrated, while in this case no evidence was found.

Because of the large amounts of glucose metabolised and the possible damage done to the mucosa due to hypoxia, these experiments to demonstrate the presence of an active transport system for glucose were repeated but this time the method used was similar to that used by Fisher and Parsons (1949) where the mucosal cells were not subjected to any period of hypoxia.

4.3.3. Experiments to eliminate cell damage

The great advantage of this *in vitro* method as compared to the two previous *in vitro* methods used, was that in this method the mucosal cells were at no time subjected to hypoxia, for the blood supply to the intestine was only terminated once the recirculation of the intestinal lumen with

oxygenated Kreb's solution had been well established.

In the rat, the concentration of glucose at the start of the experiment was less in the mucosal than in the serosal compartments. This was due to the fact that some glucose had been assimilated from the intestine before the blood supply had been terminated and the intestine placed in the tissue bath. Thus from the very start of the experiment there was a concentration gradient opposing the movement of glucose from the mucosal to the serosal compartments. After 60 minutes the glucose concentration in the serosal compartment had increased from 567 to 597 mg/100 ml and had fallen in the mucosal compartment from 494 to 257 mg/100 ml. Three changes in the glucose concentration resulted in a further increase in the concentration gradient, but in spite of this increase there was still a net transfer of approximately 1 mg/cm/hr of glucose from the mucosal to the serosal compartments. This experiment proved conclusively the presence of an active transport system for glucose in the rat intestine.

In the rat experiment the amount of glucose utilized was approximately 1,3 mg/cm/hr, a value similar to that recorded by Fisher and Parsons (1950), but about half the amount utilized in the previous experiment when the intestine had been everted. Yet in this experiment the glucose transferred was the same as in the recirculated everted experiments.

The results obtained for the bat were similar to those recorded in the recirculated everted intestine. As with the rat, the concentration of the glucose at the start of the experiment was less in the mucosal than serosal compart-

ments. In the bat, however, in contrast to the rat, there was very little change in the concentration of glucose during the experiment. Similar to the rat experiments, the concentration of glucose in the serosal fluid remained higher than in the mucosal fluid, throughout the experiment. In the bat there was no net movement of glucose from the mucosal to the serosal surface and so no evidence that there was an active transport system for glucose.

The amount of glucose metabolised by the bat in this experiment was about 40% of the amount recorded in the recirculated everted sac. The reduction in the utilisation of glucose by both the bat and the rat in these experiments compared to the everted experiments was probably due to the fact that the mucosal cells had not been damaged by hypoxia and so was more efficient in the catabolism of glucose. Fisher and Parsons (1949) showed that with hypoxia to the mucosal cells there was an increase in the lactic acid production.

The number of bats used in these *in vitro* experiments were kept to the minimum. The reason for this was that there is not sufficiently abundant supply of these animals, to repeat experiments that were giving negative results. However as all three of the *in vitro* methods showed the active transport of glucose in the rat but not in the bat, it would appear that in the bat an active transport mechanism for glurose can not be demonstrated.

4.6. The effects of anaesthetics on the assimilation of sugars

To examine the assimilation of sugars in more detail *in vivo* it was necessary to anaesthetise the animals.

The anaesthetic used up to this stage was a pentobarbitone and ethyl alcohol mixture. This anaesthetic mixture suggested by [illegible] (1967) seemed to [illegible] but the depth of anaesthesia was very variable and often it was necessary to give further doses of the anaesthetic mixture, which meant that the animal was not in a steady state. Therefore it was decided to look for another anaesthetic. Stevens *et al.*, (19[illegible]) showed that rats when under barbiturate anaesthesia had an elevated blood glucose level following an oral dose of glucose.

[illegible]-Brown ([illegible]) showed that Urethane hydrochloride had no effect on the resting blood glucose level, while pentobarbitone affected it, which depended on whether the animal was fed or starved, in the former there was no increase while in the latter there was an increase in the resting blood glucose level. Although these results showed that these anaesthetic agents had an effect on the resting blood glucose levels as well as the levels of glucose and insulin following an intravenous glucose tolerance test, they do not show what effect the anaesthetic had on assimilation.

4.4.1. <u>The effects of different anaesthetics on the assimilation of glucose in the rat</u>

Some anaesthetic agents were tested on rats to see what effect they had on the assimilation of glucose. The anaesthetics tried were "Hypnorm"* urethane and a pentobarbitone/[illegible] mixture.

The normal rate for glucose assimilation in the unanaesthetised rat was [illegible] mg/30 min. (SEM ± [illegible] mg/30 min.)

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* "Hypnorm" a mixture of fentanyl and fluanisone, Janssen Pharmaceutica.

The anaesthetic used up to this stage was a pentobarbitone and ethyl alcohol mixture. This anaesthetic mixture suggested by Pye (1[illegible]) worked on these bats but the depth of anaesthesia was very variable and often it was necessary to give further doses of the anaesthetic mixture, which meant that the animal was not in a steady state. Therefore it was decided to look for another anaesthetic. Stevens et al., (1963) showed that rats under barbiturate anaesthesia had an elevated blood glucose level following an oral dose of glucose.

Aynsley-Green (1972) showed that Ketamine hydrochloride had no effect on the resting blood glucose level, while pentobarbitone effected an increase on whether the animal was fed or starved, in the former there was an increase while in the latter there was no increase in the resting blood glucose level. Although these results showed that these anaesthetic agents had an effect on the resting blood glucose levels as well as the levels of glucose and insulin following an intravenous glucose tolerance test, they do not show what effect the anaesthetic had on assimilation.

4.6.1. The effects of different anaesthetics on the assimilation of glucose in the rat

Some anaesthetic agents were tested on rats to see what effect they had on the assimilation of glucose. The anaesthetics tried were 'Thalamonal'* urethane and pentobarbitone/phenobarbitone mixture.

The normal rate for glucose assimilation in the unanaesthetised rat was 277 mg/30 min. (± 1[illegible] mg/30 min.)

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* 'Thalamonal' a mixture of fentanyl and droperidol, Janssen Pharmaceutica.

page 48. In this experiment it was found that the rat assimilated 294 and 289 mg/30 min. under the pentobarbitone/phenobarbitone mixture and urethane respectively, while under thalamonal the assimilation of glucose was 159 mg/30 min. (Table 14). Due to the lower rate of assimilation under thalamonal anaesthesia this anaesthetic was discarded.

In the graph of the blood glucose levels (fig. 16) it can be seen that the barbiturate and thalamonal did increase the resting blood glucose levels slightly but not to the same degree as urethane which increased it to over 160 mg/100 ml of blood.

To confirm this elevated resting blood glucose concentration an experiment was carried out on 12 rats. Blood samples taken before and when under urethane anaesthesia showed that the anaesthetic increased the blood glucose level from an average resting level of 79 mg/100 ml (SEM ± 4) to a mean level of 165 mg/100 ml (SEM ± 9). For this reason urethane was not used and for the rest of this investigation the pentobarbitone/phenobarbitone anaesthetic was used.

4.6.2. Restrictions to fructose absorption and the anaesthetised bat

In the first group of experiments, where the tonicity of the fructose was approximately three times greater than that of blood, the anaesthetised bats assimilated less fructose than the conscious animals.

These results suggested that the anaesthetic had a marked inhibitory effect on the assimilation of fructose. This however was not so, for Table 15 shows very clearly that more than 90% of the fructose that had entered the

intestine in both the anaesthetised and conscious animal had in fact been assimilated. The cause of this discrepancy between the amounts of fructose assimilated in the conscious and anaesthetised bats lies in the fact that in the anaesthetised animal most of the fructose solution that had been given was still present in the stomach, at the end of the experiment and had never reached the intestine where assimilation could take place.

The effect of the anaesthetic was therefore not to cause a decrease in the rate of assimilation but to delay the emptying of the contents of the stomach. The mechanisms involved in this are not known.

The experiments carried out using isotonic glucose and fructose solutions emphasized that the tonicity of the solution was a critical factor, for in these experiments there were no signs of the animals failing to settle normally. Secondly, in these experiments the amounts of glucose and fructose assimilated in the conscious and anaesthetised animals were of the same order.

The experiments carried out on the conscious and anaesthetised bats stressed that when the animals was anaesthetised, the stomach had to be bye-passed so as to get results comparable to those in conscious animals.

In all further experiments carried out on anaesthetised animals, the assimilation of the sugars was determined using the method described by Jacobs and Lupor (1957).

4.7. Infusion experiments

4.7.1. Assimilation of glucose against a concentration gradient

In all the *in vitro* experiments the active transport

of glucose was demonstrated in the rat, whereas in the bat it was not. A possible explanation for this failure to demonstrate an active transport system in the latter was that when the intestine was removed from the animal, the resulting loss of circulation had caused irreversible damage to the transport system.

An in vivo method similar to that used by Sheff and Smyth (1955) was designed where the concentration of glucose in the lumen was less than in the blood. Any assimilation of glucose from the intestine against this gradient, would be evidence for an active transport mechanism for glucose.

In the bat unfortunately, the fasting or resting blood glucose level was low and this would mean that large volumes of a dilute perfusate would have to be used, so as to have measurable amounts of glucose to work with. To overcome this disadvantage the blood glucose level was increased by perfusing the intestine first with an isotonic glucose solution for 15 minutes. Then while the blood glucose level was still high, the experiment was carried out by perfusing the intestine with a solution having a low glucose concentration.

The results from these experiments carried out on the rat showed that throughout the experiment the blood glucose concentration was many times greater than that of the perfusate and yet 91,9 of glucose was assimilated. This amount of glucose assimilated was 60 of the available glucose and confirmed that in the rat intestine, there was an active transport system which was able to assimilate glucose against a concentration gradient.

In the bat however, the blood glucose concentration remained higher than that of the perfusate for only the first 30 minutes. This was because the blood glucose concentration decreased rapidly during the experiment and secondly, unlike the rat, the concentration of glucose in the perfusate increased during the first 45 minutes of the experiment.

These observations can be explained by the hypothesis that the bat's intestine was freely permeable to glucose. The evidence supporting such a hypothesis was that at the start of the experiment, when the concentration of glucose was greater in the blood than in the intestinal lumen, the concentration of glucose in the lumen increased and as no large changes in the volume occurred, then the increase in the concentration must be due to an increase in the movement of glucose from the blood into the lumen. Similarly in the latter part of the experiment when the concentration of glucose was higher in the lumen than the blood, then glucose must have been transferred from the lumen to the blood so as to reverse the decrease in glucose concentration in the lumen.

This failure to show any evidence that glucose was transferred against a concentration gradient, suggest that in the bat there was no active transport system for glucose.

This experiment could not be repeated using fructose because even a relatively low blood fructose level could not be maintained in either the bat or the rat.

4.7.3. Permeability of the intestine to glucose

The increase in the concentration of the glucose in the intestinal lumen, as observed in the previous experiment carried out on the bat was due to an increase in the

quantity of glucose in the lumen as there had been no decrease in the volume. This increase in the amount of glucose could have come from either of two sources. The one was that there was still glucose in the intestine after the initial perfusion when the concentrated glucose solution was used. This was most unlikely because the intestine had been rinsed out with saline prior to the final infusion. The second possibility was that the intestine in the bat was freely permeable to glucose.

This experiment was based on the infusion experiments of Jacobs and Lyman (1957). The method was designed to see if the intestine was freely permeable to the movement of glucose from the blood into the intestinal lumen. The method entailed perfusing *in situ* a length of either the bat's or the rat's intestine with saline and then raising the blood glucose levels with intravenous glucose injections and infusions.

In the bat the blood glucose level before the intravenous injections of glucose was less than 70 mg/100 ml and there was a trace of glucose in the effluent. This trace was of no significance as it was at the limits of the sensitivity for this method for glucose analysis.

After the intravenous injection of glucose, the blood glucose concentration increased and reached 172 mg/100 ml of blood 10 minutes later. The glucose concentration in the effluent had also increased from a trace to 93 mg/100 ml (Table 22 and fig. 19) in the same time period.

In the graph it would appear that there was a lag period between the glucose concentration in the blood and the effluent but it must be remembered that the effluent

collected had been in the cannula some time before collection and so would lag behind what was actually occurring in the experiment.

To investigate what would happen to the intestinal glucose if the blood glucose level decreased, insulin was given intravenously to bring down the blood glucose level. This resulted in a fall in the glucose level in the intestinal perfusate.

From this result it was clear that in the bat the intestine was freely permeable to the movement of glucose from the blood to the lumen. No report in the literature could be found, where any animal had an intestine permeable to the movement of glucose from the blood to the lumen. An interesting hypothesis was that in other animals any glucose that did diffuse across the intestinal wall would, immediately, be actively transported back into the body. The bat not having an active transport system would thus accumulate glucose in the lumen.

To test this hypothesis the same infusion experiment was carried out on a rat. In this experiment the blood glucose level was increased by giving an intravenous glucose injection and then an intravenous glucose infusion. By this method the blood glucose level was increased to 600 mg/100 ml of blood and at no time was there any glucose in the effluent. When the blood glucose level had reached 500 mg/100 ml the saline infusion fluid was substituted with a saline solution containing 0,' mM phloridzin. This concentration has been shown not to adversely affect cellular metabolism (Newey et al., 1959) but will inhibit the active transport of glucose. In this experiment even with

the presence of phloridzin in the lumen there was still no glucose in the luminal fluid. Therefore it would appear that as phloridzin did not result in glucose appearing in the lumen, there was no movement of glucose from the cell to the lumen and so it would appear that the intestine of the rat is not permeable to the movement of glucose from the blood to the lumen.

4.7.3. The concentrations of glucose, fructose and lactate in the portal and systemic blood during the assimilation of sugars in the rat

As the venous blood samples were taken from the right atria, the concentration of glucose, fructose and lactate in these samples must be very similar to that found in the arterial blood supplying the intestine. A comparison between the concentrations of these substances in the systemic and the portal blood would therefore supply valuable information about the mechanisms involved in the assimilation of the sugars.

Glucose

Table 16 showed that the concentration of glucose in the portal and systemic blood samples were 388 and 289 mg/100 ml respectively. This was an increase of almost 100 mg of glucose per 100 ml in the portal samples. This marked increase in the glucose concentration in the portal blood was strong evidence to support the theory that glucose was being assimilated as glucose and that it was not being converted to another substance which was then assimilated. In this respect the bat was similar to other reported species (Kiyasu et al., 1960 and Alvin, 1967).

The slight rise in the blood lactate level could be due

to the conversion of glucose to lactate during assimilation but this has not been demonstrated in other animals (Crane, 1960). This increase in the lactate concentration was probably the result of the normal metabolism of glucose which occurs in the intestinal tissue. This was supported by the observation that in all the experiments, irrespective of the sugar administered or the systemic lactate concentration, the increase in portal lactate level was 0,5 to 3 mg/100 ml higher than the systemic blood.

Fructose

The concentration of fructose in the systemic and portal blood samples were 80 and 1[illegible] mg/100 ml of blood respectively (Table 16). This increase of 37 mg of fructose per 100 ml of portal blood compared to the systemic blood, showed that a large amount of the fructose being infused was being assimilated as fructose. However the difference between the systemic and portal concentrations for glucose in the glucose infusion experiment was almost 100 mg/100 ml of blood compared to 37 mg of fructose/100 ml in the fructose experiment. This decrease in the gradient between the portal and systemic fructose levels during the fructose infusion experiments compared to the glucose experiments cannot be due to less fructose than glucose being assimilated because in all the experiments carried out in the bat, fructose was more rapidly assimilated than glucose. A more probable explanation as to why the fructose gradient was about 33% that of the glucose gradient is that fructose was being converted to another substance prior to entering into the circulation.

As conversion of fructose to glucose in the intestinal

mucosa during the assimilation of fructose occurs in the golden hamster (Wilson and Vincent, 1955), the guinea pig (Riklis and Quastel, 1958) and the dog (Shoemaker *et al.*, 1963) it was not surprising that in the bat the portal blood glucose level was 2 mg/100 ml of blood higher than the systemic value of 139 mg/100 ml of blood. This slight increase in the portal blood glucose concentration was evidence that some of the fructose must have been converted to glucose. This increase in the portal blood glucose level, however was not always recorded, for in some animals the portal glucose level was less than that of the systemic blood. When this small increase in the portal glucose concentration during the fructose infusion was compared to the glucose levels recorded during the infusion of glucose, it showed that very small quantities of glucose must have been formed. Therefore it would appear that the bat was capable of converting fructose to glucose, but that it played a minor role in total amount of fructose assimilated.

The concentrations of lactate in the portal blood was slightly greater than in the systemic blood. This result is similar to that recorded when glucose was being infused and is again probably due to metabolic processes and not associated with the assimilation of fructose. However, the systemic lactate concentrations during the infusion were 35 mg/100 ml of blood which was six times higher than when the intestine was being infused with glucose. This increase in the blood lactate level is unlikely to be the result of the assimilation of lactate as the gradient is small. Therefore a more probable explanation for the increase in the blood lactate is that some of the fructose is

being converted to lactate by the liver.

Sucrose

The experimental results on the digestion of sucrose showed that the quantity of sucrose in the intestine fell rapidly and that there was a small amount of glucose and fructose present in the lumen. This result meant that either sucrose was assimilated itself or it was rapidly hydrolysed to glucose and fructose. Therefore the results of the sucrose infusion experiments showing that there was no sucrose present in either the systemic or portal blood samples indicated that the bat had a very effective sucrase system for the hydrolysis of sucrose to glucose and fructose.

In these infusion experiments both glucose and fructose appeared in higher concentrations in the portal than in the systemic blood (Table 10). Their equivalents were 52 and 30 mg for glucose and fructose respectively per 100 ml of blood. As the concentration of sucrose used in the infusion experiment was 2,75 g per 100 ml the maximum concentration for each of these monosaccharides if complete hydrolysis occurred would be 1,375 g/100 ml. Therefore the individual concentrations of these monosaccharides in the gut must always be less than half that when either glucose or fructose was infused alone. This lower glucose and fructose concentration in the intestine would result in smaller gradient increases in the concentration of glucose and fructose in the portal blood when compared to the infusion experiments where these two monosaccharides were infused separately.

The increase in the concentration of lactate in the portal blood compared to the systemic blood was very small and similar to when glucose and fructose were infused se-

parately. This increase as previously discussed was probably due to the normal metabolic processes and not due to the conversion of fructose or glucose to lactate. The systemic blood lactate level was higher than the glucose infusion experiments but less than the fructose infusion experiments again suggesting the possible role played by the liver.

These preliminary experiments suggested that glucose was assimilated as such without any further changes. In the case of fructose however there was some evidence that a small quantity of fructose was being converted to glucose which was then assimilated. To confirm these observations radioactively labelled glucose and fructose would have to be used to study the fates of these sugars.

The evidence at this stage was that there was no active transport system for either glucose or fructose and that the assimilation was a passive process controlled by the concentrations of glucose and fructose.

4.7.4. The effect the presence of glycogen stores has on the rate of assimilation of glucose from the jejunum.

In these experiments where segments of the jejunum were perfused with glucose it was found that on average the bat assimilated approximately 2 mg of glucose per cm length of intestine in 2,5 minutes. As the average length of the bat's intestine was 64 cm, then if the entire length of the intestine was able to assimilate glucose at the same rate as the jejunum, then the bat would be able to assimilate 1536 mg in 30 minutes. This value is about twice as much as the amount of glucose assimilated in the experiments carried

out on conscious bats (page 45). However, in these conscious animals fluid was only found in the proximal third of the intestine, suggesting that the intestine was able to assimilate glucose at a faster rate and that another factor had to be involved.

In the control experiments the rate of glucose assimilation increased slowly throughout the experiment. In contrast to these control experiments, when glucose was slowly infused into the tied-off stomach there was a dramatic increase in the rate of assimilation. At its peak the rate was over three times the control level. This result suggests that in some way the stomach caused an increase in the rate of glucose assimilation. The mechanism however is not known. There are a number of reports that in partial gastrectomy patients involving the pyloric area, glucose assimilation appears less efficient than in normal man (Lundh, 1958).

In all the experiments carried out so far no evidence could be found that glucose was actively transported across the intestine in the bat, but now we have a situation where the rate of assimilation can be increased. At this stage it cannot be said if it is an active transport or a facilitative diffusion system.

4.8. Summary of the Discussion

The assimilation experiments carried out on the bat and the rat (page 17) showed that the bat assimilated glucose and fructose many times faster than the rat. There were two main ways as to how this increased rate of assimilation could be achieved. The first was that the bat had a more efficient active transport system for glucose compared to

the rat and that the bat may also have an active transport system for fructose. In this investigation, an active transport system could not be demonstrated for either glucose or fructose in the bat. Therefore, if this explanation was correct the mechanisms involved were far more complicated.

The second explanation was that the surface area of the mucosa in the bat was far greater than in the rat. A study of the gross anatomy in the bat and the rat showed that the lengths of the intestine in the bat and the rat were 68 cm and 96 cm respectively while the diameters were of the same order, when the intestines were filled with fluid. This meant that from the gross examination of the intestine the bat had a smaller mucosal surface area than the rat.

4.3.1. Scanning electron micrographs

The scanning electron micrographs (Fig. 42 and 43) showed that the mucosal surfaces of the two animals were different. In the rat the villi were leaf-like with a relatively large amount of space between them, while in the bat the villi were finger-like and more closely packed. From these micrographs it would appear that there was an increase in the mucosal surface area in the bat compared to the rat. This increase however, would not be very great and no serial cross sections were made to measure the exact increase in the surface area.

4.3.2. Electron microscopy

The most striking feature when comparing the structure of the mucosal cells in the bat and the rat were the microvilli. In the bat the microvilli were long and slender with an average length and diameter of 2,6 µm and 0,1 µm respectively compared to the rat's 1,14 µm and 0,14 µm.

This length of 1,14 /um for the rat's microvilli compared well with that of 1 /um reported by other workers for the rat (Millington and Finean, 1962 and Palay and Karlin, 1959). The bat's microvilli were well over three times longer than the rat's.

The diameter of the microvilli in the bat was less than in the rat 0,099 /um compared to 0,14 /um. The microvilli appeared to be arranged in a hexagonal pattern (fig. 27 and 28) and the distances between the microvilli in the bat were 0,043 /um and 0,038 /um in the rat. As the length, diameter and distance between the microvilli was known the total surface area of an individual microvillus and its supporting plasma membrane could be calculated. In the bat this area was found to be about 1,8 /um^2 whilst in the rat the area was 0,5 /um^2. From the theoretical area of the hexagon supporting the entire microvilli the number of microvilli per /um^2 could be calculated. The number of microvilli per /um^2 was calculated to be approximately 57 in the bat and 36 in the in the rat. These calculated values were approximately the same number of microvilli counted in the cross sectional specimens.

As the surface area of the microvilli units and the number of microvilli per /um^2 were known, the total surface area of the microvilli and supporting plasma membrane per /um^2 of the theoretical apical area could be calculated. The results (Table 5) showed that as a result of the microvillus structure there was a 57 and a 18 fold increase in the mucosal surface of the intestinal cell in the bat and the rat respectively. Palay and Karlin (1959) reported a 24 fold increase in the rat. Thus in the bat the

assimilation surface of the small intestine was 5 times greater than in the rat. This meant that if the plasma membrane was the limiting factor with regard to the rate of assimilation then the very rapid assimilation of the monosaccharides observed in the bat could be accounted for in part by this increase in the assimilation area.

The fuzz on the microvilli, suggested by Hamilton and McMichael (1968) and Prichard (1969) to act as a possible barrier to the movement of glucose back into the intestinal lumen after the hydrolysis of maltose is poorly developed. In the bat it would appear that this structure was not important for the rate at which the bat assimilates the products of hydrolysis of sucrose is of the same order as were a mixture of glucose and fructose of equivalent concentrations were given.

5. CONCLUSIONS

All animals require energy to be able to live. This energy is obtained from the chemical breakdown of food. The range of food eaten by different animals varies very greatly. At the one extreme there are the carnivores, whose food consists primarily of protein, while at the other extreme there are the herbivores, with a high carbohydrate diet. Although their diets differ, all animals however, have a common problem, in that the food has to be digested and the end products must be assimilated. The assimilation of these products of digestion is complicated. Some molecules are transferred across the intestine into the blood by simple diffusion; others by a facillatative mechanism, while in a third group it is an active transport mechanism.

The fruit bat is of special interest, firstly because it belongs to the only Order of mammals that is capable of true flight and secondly its diet consists entirely of fruit, containing large amounts of glucose, fructose and sucrose.

This investigation set out to see what adaptations had occurred in the bat, so that it could overcome the handicap of having to process large amounts of food and still be capable of flying away from any predator.

This study showed that this bat does differ markedly in its handling of the ingested food compared with other animals. Firstly it has a very short transit time and secondly it has a very rapid and efficient system for the assimilation of sugars from the intestine. As a result, although the food spends a very short period of time in the

gastro-intestinal tract, most of the sugar is assimilated and the weight of food in the animal is kept at a minimum.

The fruit bat Rousettus aegyptiacus will ingest 70% or more of its body weight in fruit during a night's feeding. If the fruit eaten is bananas then approximately 20% of the ingested food consists of mono and disaccharides, while over 70% is water. Since the animal may have to fly 30 kilometres or more from its feeding ground to its roosting cave, the rapid assimilation and storage of the nutritional contents of the meal and the excretion of the water and undigestable food material will be of a great advantage.

In the laboratory, the transit time for bananas in the bat was shown to be very rapid with a mean value of 40 minutes. This can be partly explained by the fact that the bat does not have a large intestine. Nevertheless this transit time is rapid compared with the transit time of food through the small intestine only of the rat, which takes hours. This rapid transit time means that the bat must either have a very efficient digestive and assimilation system for handling these large quantities of food so quickly, or it must loose large amounts of potential food in the faeces. Van der Westhuyzen (1975) showed that there was very little sugar present in the faeces so therefore the bat must have an efficient absorption system.

In this thesis, this rapid rate of assimilation of both glucose and fructose was confirmed; when the rates of assimilation of these sugars were measured using Cori's method (1925), the experiments showed that the bat (which weighs less than half the rat) assimilated glucose and fructose three and four times faster than the rat. A further

observation in these experiments was that the bat assimilated fructose faster than glucose. This observation was also recorded when either a mixture of these two monosaccharides were given or when sucrose was given and the rate of assimilation of the resulting glucose and fructose studied.

No evidence could be found in the bat to show that either glucose or fructose could be actively transported across the intestinal wall whereas in the rat the active transport mechanism for glucose was demonstrated in all the experiments. Not only could no active transport mechanism be found in the bat, but it was observed that when the blood glucose level rose to 300 mg/100 ml, then glucose entered into the intestinal lumen. This showed that the intestine was freely permeable to glucose.

The results in this thesis showed, that in the bat, there is no active transport system for either glucose or fructose and that the intestine is freely permeable to glucose and fructose. Therefore any movement across the intestinal membrane appears to be one of diffusion. The assimilation of glucose is not one of simple diffusion, where the rate is governed by the concentration gradient. This was demonstrated by the fact that when the jejunum is being infused with an isotonic glucose solution at a steady rate, then the rate of assimilation of glucose from the jejunum could be increased by a factor of three times, when isotonic glucose was added to the tied-off stomach. This, showed that there could be another mechanism enhancing assimilation which is stimulated by the presence of glucose in the stomach of the bat.

The structure of the bat's intestine also plays a role

in the rapid rate of assimilation of sugars from the intestine. Macroscopically the rat has the longer small intestine but microscopically the bat has a much larger mucosal surface area. The bat has a greater number of villi and microvilli, which are longer and more closely packed together than the rat and this gives the bat the much larger and hence a more effective absorption surface.

The value of these adaptations; the rapid transit time, the rapid and effective assimilation of glucose and fructose to the bat, is clear when one considers the animal's mode of life, where long distances have to be crossed between dusk and dawn in search of food. Of particular interest would be to study Epomorphorus wahlbergi another species of fruit bat, which also lives on the same diet, but does not or very rarely covers any long distances of flight, as they roost in or near the trees where they are feeding.

This investigation was concerned with the modes of storage of the assimilated sugars, obviously a potentially interesting field.

in the rapid rate of assimilation of sugars from the intestine. Macroscopically the rat has the longer small intestine but microscopically the bat has a much larger mucosal surface area. The bat has a greater number of villi and microvilli, which are longer and more closely packed together than the rat and this gives the bat the much larger and hence a more effective absorption surface.

The value of these adaptations; the rapid transit time, the rapid and effective assimilation of glucose and fructose to the bat, is clear when one considers the animal's mode of life, where long distances have to be covered between dusk and dawn in search of food. Of particular interest would be to study Epomophorus wahlbergi another species of fruit bat, which also lives on the same diet, but does not or very rarely covers any long distances of flight, as they roost in or near the trees where they are feeding.

This investigation was not concerned with the modes of storage of the assimilated sugars, obviously a potentially interesting field.

6. APPENDIX

6.1. Additional tables

Table 17. The blood glucose concentration in anaesthetised bats and rats before and after the oral administration of 10 ml of distilled water.

Time of blood sample.	Blood glucose concentration mg/100 ml	
	Bat (4)*	Rat (2)*
Resting	60** ± 15***	75**
5 minutes	51 ± 14	75
10 minutes	44 ± 9	80

* Number of animals in brackets.
** Mean.
*** Standard error of the mean.

Table 18. The blood glucose concentration in anaesthetised bats and rats before and after the oral administration of 500 mg of glucose per 100 g body weight.

Time of blood sample.	Blood glucose concentration mg/100 ml	
	Bat (6)*	Rat (6)*
Resting	27** ± 4***	113** ± 5***
5 minutes	110 ± 35	145 ± 8
10 minutes	171 ± 40	± 9
20 minutes	284 ± 80	180 ± 11

* Number of animals in brackets.
** Mean.
*** Standard error of the mean.

Table 19. The blood glucose concentration in anaesthetised bats and rats following the intravenous injection of 75 mg of glucose per 100 g body weight.

Time after the i.v. glucose injection (min.)	Glucose concentration mg/100 ml of blood.	
	Bat (9)*	Rat (7)*
Preinjection	41** ± 8***	102** ± 3***
5	231 ± 4	269 ± 11
10	186 ± 6	225 ± 7
20	124 ± 12	150 ± 7
40	68 ± 11	111 ± 5

* Number of animals in brackets.
** Mean.
*** Standard error of the mean.

Table 20. Blood glucose concentrations in the conscious bat and rat following an oral dose of 1,5 g of glucose.

Animal	Time after glucose administered minutes	Blood glucose concentration mg/100 ml.
Bat (6)*	Before administration	29** ± 4***
	5	307 ± 32
	10	518 ± 74
	20	697 ± 79
	30	733 ± 78
Rat (4)	Before administration	53 ± 4
	5	54 ± 2
	10	60 ± 9
	20	104 ± 12
	30	133 ± 13

* Number of animals in brackets.
** Mean.
*** Standard error of the mean.

Table 21. Blood glucose and fructose concentrations in the conscious bat and rat following an oral dose of 1,5 g of fructose.

Animal	Time after glucose administered (minutes)	Blood concentrations	
		Glucose	Fructose
Bat (6)*	Before administration	60** ± 15***	0
	5	154 ± 14	132** ± 38***
	10	220 ± 13	122 ± 22
	20	248 ± 42	74 ± 8
	30	261 ± 47	51 ± 11
Rat (4)	Before administration	70 ± 8	0
	5	66 ± 7	0
	10	72 ± 7	0
	20	86 ± 9	4 ± 2
	30	100 ± 12	9 ± 3

* Number of animals in brackets.
** Mean.
*** Standard error of the mean.

Table 22. Blood glucose and fructose concentrations in the conscious bat following an oral dose of a mixture containing 750 mg glucose and 750 mg fructose.

Animal	Time after administration of monosaccharides. Minutes.	Blood concentrations mg/100 ml Glucose	Fructose
Bat (6)*	Before administration	58** ± 3***	0
	5	289 ± 96	61** ± 23***
	10	442 ± 127	34 ± 9
	20	423 ± 81	23 ± 2
	30	418 ± 74	25 ± 4

* Number of animals in brackets.
** Mean.
*** Standard error of the mean.

Table 24. Blood glucose and fructose concentrations in the conscious bat following an oral dose of 1,5 g of sucrose.

Animal	Time after sucrose administered. Minutes.	Blood concentrations mg/100 ml Glucose	Fructose
Bats (5)*	Before administration	55** ± 9***	0
	5	147 ± 44	17** ± 9***
	10	264 ± 60	21 ± 7
	20	300 ± 97	15 ± 3
	30	331 ± 97	12 ± 1

* Number of animals in brackets.
** Mean.
*** Standard error of the mean.

Table 24. The concentration of glucose in the serosal and mucosal compartments before and after the recirculation of glucose through the everted intestine.

	Glucose concentration mg/100 ml			
	Rat (4)*		Rat (4)*	
When sample taken	Serosal	Mucosal	Serosal	Mucosal
Beginning of experiment	528** ± 1,4***	518** ± 2,8***	545** ± 1,3***	538** ± 1,3***
End of the experiment	535 ± 5,5	458 ± 5,5	534 ± 2,2	543 ± 3,1

* Number of animals in brackets.
** Mean.
*** Standard error of the mean.

Table 25. The mean concentrations of glucose in the mucosal and serosal fluids while recirculating the small intestine in vitro.

Time.	Glucose concentration mg/100 ml.			
	Rat (2)*		Rat (2)*	
	Serosal	Mucosal	Serosal	Mucosal
0	559**	536**	567**	494**
15	558	533	579	433
30	560	528	586	376
45	554	526	588	319
60	555	524	597	257

* Number of animals in brackets.
** Mean.

Table 26. The effects of the different anaesthetics on the blood glucose concentration following the administration of 1,5 g of glucose.

Time	mg of glucose per 100 ml blood		
	Thalamonal Urethane (5)*		Phero/ Pentobarbitone (2)*
Preadministration	93**	160**	102**
5	88	195	140
10	109	218	216
20	130	257	270
30	165	296	290

* Number of animals in brackets.
** Mean.

Table 27. The glucose concentration in the blood and perfusate during the perfusion of the small intestine.

Time Minutes	Glucose concentration (mg/100 ml)			
	Bats (3)*		Rats (3)*	
	Blood	Perfusate	Blood	Perfusate
0	363** ± 182***	102** ± 1***	231** ± 45***	102** ± 1***
15	202 ± 103	123 ± 30	304 ± 33	87 ± 16
30	142 ± 62	135 ± 40	329 ± 45	68 ± 15
45	112 ± 33	138 ± 43	317 ± 39	51 ± 12
60	90 ± 23	107 ± 18	276 ± 19	35 ± 12

* Number of animals in procedure.
** Mean.
*** Standard error of the mean.

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	Bats (3)*		Rats (3)*	
	Blood	Perfusate	Blood	Perfusate
0	363** ± 182***	102** ± 1***	231** ± 45***	102** ± 1***
15	202 ± 103	123 ± 30	304 ± 33	87 ± 16
30	142 ± 62	135 ± 40	329 ± 45	68 ± 15
45	112 ± 33	138 ± 43	317 ± 39	51 ± 12
60	90 ± 23	107 ± 18	276 ± 19	35 ± 12

* Number of animals in brackets.
** Mean.
*** Standard error of the mean.

Table 28. The concentration of glucose in the blood and the perfusate, in the bat following repeated intravenous glucose injections.

Time	Glucose Conc. mg/100 ml		Time	Glucose Conc. mg/100 ml		Time	Glucose Conc. mg/100 ml	
	Blood	Perfusate		Blood	Perfusate		Blood	Perfusate
0	20	0	60	511	77	105	169	-
10	17	0	70	600	109	110	93	98
20	15	1	80	605	139	120	34	45
30	14	1	85	673	-	125	26	-
40	13	1	90	591	152	130	21	19
50	372	23	100	308	150	140	26	9

Table 28. The concentration of glucose in the blood and the perfusate, in the bat following repeated intravenous glucose injections.

Time	Glucose Conc. mg/100 ml		Time	Glucose Conc. mg/100 ml		Time	Glucose Conc. mg/100 ml	
	Blood	Perfusate		Blood	Perfusate		Blood	Perfusate
0	20	0	60	511	77	105	169	-
10	17	0	70	600	104	110	93	68
20	15	1	80	605	139	120	54	45
30	14	1	85	673	-	125	26	-
40	13	1	90	591	152	130	21	19
50	372	23	100	308	150	140	26	9

Table 29. The blood glucose concentrations in the rat following the intravenous injection and infusion of glucose.

Time	Glucose Conc. mg/100 ml	Time	Glucose Conc. mg/100 ml	Time	Glucose Conc. mg/100 ml
0	100	70	454	140	561
10	111	80	432	150	583
20	119	90	465	160	595
30	126	100	490	170	594
40	146	-	510	180	598
50	151	120	521		
60	156	130	549		

Table 30. The summary of the morphology and the surface areas of the microvilli in the bat and the rat.

	BAT	RAT
Length of microvilli (µm)	3,6 (15)*	1,14 (10)*
	S.D. ± 1,0	S.D. ± 0,2
	S.E.M. ± 0,258	S.E.M. ± 0,0[illegible]
Diameter of microvilli (µm)	0,099 (22)*	0,14 (7)*
	S.D. ± 0,01	S.D. ± 0,01
	S.E.M. ± 0,003	S.E.M. + 0,004
Distance between microvilli (µm)	0,043 (22)*	0,038 (5)*
	S.D. ± 0,005	S.D. ± 0,001
	S.E.M. ± 0,001	S.E.M. ± 0,0006
Surface area of microvilli (µm²)	1,0 µm²	0,5 µm²
Number of microvilli per µm²		
calculated	57	36
counted	56,5 (9)*	35 (5)*
	S.D. ± 2,7	
Therefore surface area increase due to microvilli	57	18

*Number of observations in brackets.

6.2. Statistics

To test for a significant difference between the slopes of two curves recorded for the bat and rat glucose tolerance tests

This was carried out in each case by fitting a straight line through the recorded points by least squares and then comparing the slopes by means of a Student's test.

$$b = \frac{\sum_{-n}^{n} \bar{y}_j \, (\bar{x}_i - \bar{x}_o)}{\sum_{n=1}^{n} (\bar{x}_i - x_o)}$$

Where $\bar{x}$ and $\bar{y}$ are the average values at the i th poin

and $\bar{x} = o \quad \bar{x}_2 = 5 \quad \bar{x} = 10 \quad \bar{x}_4 = 20$

$$\therefore \quad \bar{x}_o = \frac{0 + 5 + 10 + 20}{4}$$

$$= 8,75$$

$$\hat{b} = \frac{27\,(0-8,75) + 110\,(5-8,75) + 171\,(10-8,75) + 284\,(20-8,75)}{(0-8,75)^2 + (5-8,75)^2 + (10-8,75)^2 + (20-8,75)^2}$$

$$= \frac{-235,25 - 413,2 + 213,7 + 3195}{76,5625 + 14,0625 + 1,5625 + 126,5625}$$

$$= \frac{2760}{218,75}$$

$$\hat{b}_{bats} = 12,617$$

$$\hat{b}_{rats} = \frac{113(0-8,75) + 145(5-8,75) + 163(10-8,75) + 180(20-8,75)}{(0-8,75)^2 + (5-8,75)^2 + (10-8,75)^2 + (20-8,75)^2}$$

$$= \frac{-988,75 - 543,75 + 203,75 + 2025}{76,5625 + 14,0625 + 1,5625 + 126,5625}$$

$$= \frac{696,25}{218,75}$$

$$= 3,183$$

$$s_p^2 = \frac{1}{n_{bats} + n_{rats} - 4} \left\{ \sum_{bats} (\bar{y}_i - \bar{y}_o)^2 + \sum_{rats} (\bar{y}_i - \bar{y}_o)^2 \right\} - \left\{ \hat{b} \sum_{bats} (\bar{x}_1 - \bar{x}_o)^2 + \hat{b} \sum_{rats} (\bar{x}_1 - \bar{x}_o) \right\}$$

where Sp is the pooled SD of both lines

$$\bar{y}_{o\ bats} = \frac{27 + 110 + 171 + 284}{4}$$

$$= 148$$

$$\therefore \sum_{bats} (\bar{y}_i - \bar{y}_o)^2 = (27-148)^2 + (110-148)^2 + (171-148)^2 + (284-148)^2$$
$$= 35110$$

$$\text{and } \sum \bar{y}_{o\ rats} = \frac{113 + 145 + 163 + 180}{4}$$

$$= 150,25$$

$$\therefore \sum_{rats} (y_i - y_o)^2 = (113-150,25)^2 + (145-150,25)^2 + (163-150,25)^2 + (180-150,27)^2$$

$$= 2462,75$$

$$\hat{b}^2_{bats} \sum(\bar{x} - \bar{x}_o)^2 = 12{,}617^2 \times 218{,}75$$
$$= 34822{,}52$$

and $\hat{b}^2_{rats} \sum(x - x_o)^2 = 3{,}183^2 \times 218{,}75$
$$= 2216{,}26$$

$$\therefore \quad s_p^2 = \frac{1}{4} \quad (35110 + 246[illegible],75) - (3422{,}52 + 2216{,}26)$$
$$= 133{,}49$$

$$\therefore \quad Sp = 11{,}[illegible]54$$

$$"t"_4 = \frac{\hat{b}_{bats} - \hat{b}_{rats}}{Sp \sqrt{\frac{1}{\sum(\bar{x} - \bar{x}_o)^2_{bats}} + \frac{1}{\sum(x_1 - x_o)^2_{rats}}}}$$

$$= \frac{12{,}617 - [illegible]}{11{,}554 \sqrt{\frac{2}{[illegible]}}}$$

$$= 8{,}54$$

and comparing this value with tabulated values of the "t" distribution with 4 degrees of freedom we find this to be significant at the 0,[illegible] level.

(t_4 : 0,999 - 7,173 - [illegible] sided test)

Thus the difference between the two curves is highly significant at the 0,[illegible] level showing that the two results are different.

6.3. Statistical analysis

To test the significance between the amounts of glucose and fructose assimilated by the lat

Fructose assimilated $(\bar{x})$ = 1076 mg

$\Sigma(x - \bar{x})^2$ = 156251

S.D. = 176,5

and glucose assimilated $(\bar{x})$ = 765 mg

$\Sigma(x - \bar{x})^2$ = 106484

S.D. = 145,9

$$t_{n_1 + n_2 - 2} = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{(x_1 - \bar{x}_1)^2 - (x_2 - \bar{x}_2)^2}{n_1 + n_2 - 2}} \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

$$t_{10} = \frac{1076 - 765}{\sqrt{\frac{1 + 106}{10}} \sqrt{\frac{1}{6} + \frac{1}{6}}}$$

$$= \frac{311}{\sqrt{} \sqrt{,33}}$$

$$= \frac{311}{\text{x } 0,}$$

$$= 3,37$$

$t_{10\ 0,995}$ = 3,169

∴ Significant difference at the 0,5% level.

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