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EXPERIMENTAL DIABETES IN RATS PRODUCED BY PARENTERAL ADMINISTRATION OF ANTI-INSULIN SERUM

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We have shown (Armin, Grant & Wright, 1960) that the intravascular injection of single small doses of guinea-pig anti-insulin serum produces transient hyperglycaemia in the rat, rabbit and cat. This is thought to be due to the neutralization of endogenously secreted insulin by antibodies in the injected serum. We now show that when larger doses are administered to the conscious rat by intraperitoneal injection or prolonged intravenous infusion, anti-insulin serum produces a diabetic syndrome characterized amongst other things by hyperglycaemia, polyuria, glycosuria and ketonuria.

The rat was chosen for these observations, rather than a larger and experimentally more suitable animal, because of the smaller volumes of serum required; potent anti-insulin serum was in short supply. Moreover, the rat is omnivorous and has already been much used for the study of experimental diabetes produced by alloxan and by pancreatectomy.

METHODS

Details of the methods used for the production and assay of anti-insulin serum have already been reported (Armin *et al.* 1960). Briefly, such serum was obtained from guinea-pigs treated with an emulsion containing recrystallized bovine insulin and its potency was deduced from the hyperglycaemic effect which it evoked in rats on intravenous injection. Control serum was obtained from guinea-pigs treated similarly with insulin-free emulsion, and normal serum was derived from normal untreated guinea-pigs. The rats into which these sera were injected or infused in the present experiments were of an albino Wistar strain and were fed on a standard rat cake diet; unless otherwise stated, food and water were freely available.

Anti-insulin serum. Four batches were used, their potencies being estimated to be such that 1 ml. neutralized approximately 2·3 u. (batch 16204); 3·4 u. (batch 14108); 2·1 u. (batch 227328); and 1·8 u. insulin (batch 424). Control (batches 40 and D15) and normal sera were also used.

Intraperitoneal injection. The rats (male, 190–205 g) were kept in warm cages. Serum (1–5 ml.) was injected through a fine short-bevelled needle into the peritoneal cavity at a point a little to the right of the mid line and half way between the xiphisternum and the

symphysis pubis. Piercing the abdominal wall at this point seldom resulted in injury to, or loss of serum into, the gut. At regular intervals (1–2 hr) after each injection the rats were removed from the cage and placed on a clean glass plate; here they usually passed urine, especially if the end of the tail was compressed. Samples of blood (0·2 ml.) were collected from the cut end of the tail at the same time. At the end of the experiment the rats were reweighed, decapitated and examined.

Intravenous infusion. With the apparatus and method described in the appendix, polythene catheters were inserted into the jugular vein and carotid artery. The rats (male, 230–305 g) were anaesthetized with pentobarbitone sodium.

As soon as the catheters were in place saline infusion was begun, the total rate of intravascular infusion being 0·5 ml./hr. The saline solution consisted of 0·9% (w/v) sodium chloride in glass-distilled water containing heparin (1000 i.u./100 ml.; Injection of Heparin, Boots Pure Drug Co. Ltd.) and an antibiotic mixture (penicillin, 1000 u. and streptomycin, 100 μg/100 ml.). When placed in the cage the rats recovered consciousness, ate, drank and moved about normally within 1–2 hr. They soon learned to avoid turning round too often in one direction, which caused twisting of the loose skin of the neck around the lower end of the coiled spring.

On the following day, 21–26 hr after insertion of the catheters, guinea-pig serum containing an antibiotic mixture (penicillin, 1000 u. and streptomycin, 100 μg/15 ml.) was infused intravenously for 20 hr. The total fluid infusion rate was increased to 0·82–0·98 ml./hr by adjusting the intra-arterial rate of saline infusion.

When the serum infusion ended, fluid infusion was continued intravenously and intra-arterially at varying rates until the end of the experiment. In most cases saline was infused at total rates ranging from 0·4 to 0·9 ml./hr. In a few experiments (mentioned in detail later) Krebs–Ringer solutions (Umbreit, Burris & Stauffer, 1945) were used to provide potassium or buffering action. At the end of the experiments the rats were killed with an overdose of barbiturate, reweighed and examined.

Investigations

Blood samples (0·2 ml.) were obtained from the tails of injected animals or withdrawn through the arterial catheter of infused rats after discarding the first drops, which were admixed with saline. To avoid excessive blood loss, samples were drawn only when significant changes in blood-sugar concentration were expected. The sugar content was estimated by a modification (Wright, 1957) of the method described by King (1951); the glucose oxidase method (Huggett & Nixon, 1957) was used for some samples and gave essentially the same results.

Urine. All urine passed by infused rats was collected and examined qualitatively; a few quantitative investigations were carried out. Samples passed by injected rats and drops issuing from the funnels under the cages of infused rats immediately after they had micturated were examined qualitatively. Glucose was considered to be present when the urine turned glucose-oxidase test papers ('Clinistix'; Ames and Co.) a deep blue within 30 sec, and ketone bodies if it turned 'Acetest' tablets (Ames and Co.) to a deep violet colour within 10 sec. Slower or less intense colour reactions were considered negative in both tests. To avoid contamination of urine specimens by those passed previously by infused rats, the funnels under the cages were washed and replaced at regular intervals; this was especially important at times when an abnormal component of the urine was expected to disappear.

The urine passed by infused rats was collected in batches over individual periods ranging from 2 to 30 hr and was preserved by the addition of 1 drop of 10 N-HCl at the beginning of each collection period. The volume of each batch was measured and the sugar and ketone-body contents were determined qualitatively by the standard Benedict, Rothera & Gerhardt tests (King, 1951). In some instances the absolute sugar contents were determined

with Benedict's quantitative reagent (King, 1951) and the glucose oxidase method (Huggett & Nixon, 1957); both gave essentially the same results.

Water intake. In all but the first few serum infusion experiments the water bottles fitted to the cages were weighed and refilled at regular intervals. The water consumed by injected rats was not measured.

Food intake. Weighed amounts (50 g) of solid rat cake were placed in the cages of infused rats at regular intervals, the weight remaining at the end of each period giving an indication of the amount eaten. A small amount of broken cake, however, was lost into the funnel. The food consumed by injected rats was not measured.

RESULTS

Intraperitoneal injection

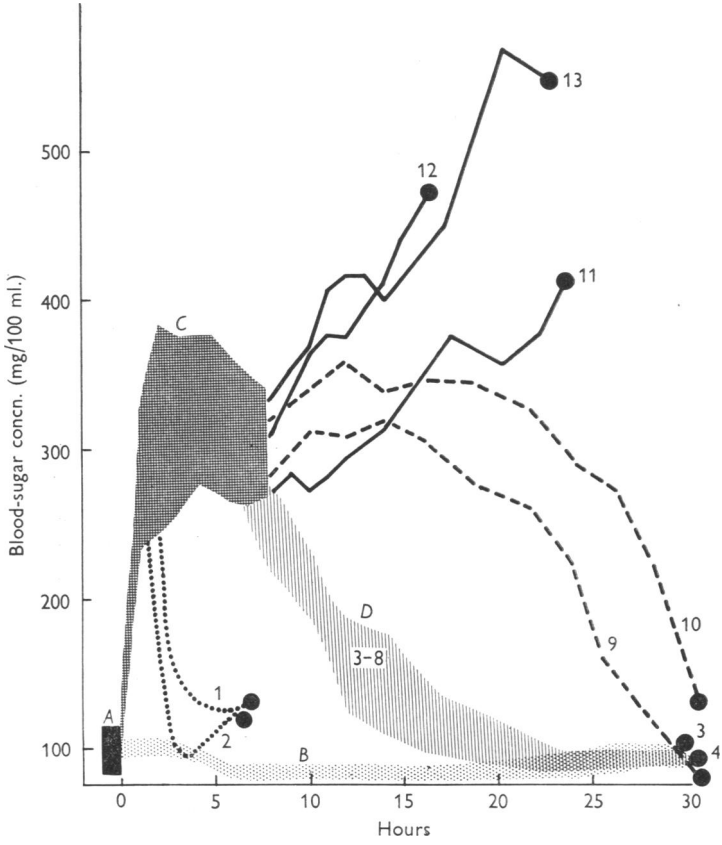
The effects of intraperitoneal injection of guinea-pig serum into groups of conscious rats are shown in Text-fig. 1. Normal and control serum caused no rise in blood-sugar concentration, but marked hyperglycaemia developed within an hour of injecting anti-insulin serum (batch 227328).

Normal and control serum. In two rats injected with 5 ml. normal serum and in two with 5 ml. control serum (batch 40), the blood-sugar concentrations fell slightly but irregularly during the first 8 hr and remained at or a little below the pre-injection level up to 30 hr (Text-fig. 1; area *B*); no observations were made thereafter. During the first 4 hr all rats lost weight (10–15 g) and this was probably due to the unusual amount of faeces passed by them and other injected rats at this stage. Thereafter they maintained weight, eating and drinking water at the same rates as uninjected animals. No abnormality was detected when these rats were killed and examined 55 hr after serum injection.

Anti-insulin serum (1·0 *ml.*). In two rats (Text-fig. 1; rats 1 and 2) injected with this small dose the blood-sugar concentrations rose to maxima (248 and 319 mg/100 ml.) in 1–2 hr but returned almost to the pre-injection levels in 2½–3½ hr. Transient glycosuria occurred in both rats but neither developed ketonuria. Both rats behaved normally throughout and had lost only 10 g weight when killed 6 hr after injection. No abnormality was detected at necropsy.

Anti-insulin serum (3·0 *ml.*). Four rats (Text-fig. 1; rats 5–8) injected with this medium dose, but otherwise untreated, showed the same initial rapid rise in blood-sugar concentration to maxima ranging from 280 to 370 mg/100 ml. About 5–7 hr after injection the levels began to fall and were fast approaching normal after 10–12 hr. Glycosuria was found in 1–2 hr and ketonuria followed 4–5 hr later. Unlike normoglycaemic rats, they drank frequently and passed urine at about hourly intervals for the first 10 hr. After 10–14 hr, however, all four rats moved about very little and seemed weak; they did not reach up to drink from the water bottles, though they drank when water was brought to their

mouths. They seldom passed urine, and blood samples were obtained with increasing difficulty as blood issuing from the tip of the tail often clotted at once. Despite these signs of dehydration and collapse all four rats recovered. Glycosuria and ketonuria ceased 11–15 hr after injection. After 24 hr two rats from which blood could be obtained were normoglycaemic and all had lost weight (10–20 g); this they regained in the next 3 days, by which time they were behaving normally.



Text-fig. 1. Blood-sugar concentrations in male rats (180–220 g) injected intraperitoneally at zero time with guinea-pig serum. Two rats received control serum (5 ml.) and two were injected with normal serum (5 ml.). The doses of anti-insulin serum injected were 1 ml. (rats 1 and 2), 3 ml. (rats 3–8) and 5 ml. (rats 9–13). Unbuffered Krebs–Ringer solution (1 ml.) was injected subcutaneously after 7 hr every 2 hr into rats, 3, 4, 9 and 10 until they became normoglycaemic again. The shaded areas include all blood-sugar concentrations found (*A*) before serum injection, (*B*) after injection with normal or control serum, (*C*) up to 8 hr after anti-insulin serum injection unless a progressive fall had occurred earlier and (*D*) subsequently in those animals receiving 3 ml. anti-insulin serum. The blood-sugar concentrations at the time of death in animals killed or dying less than 30 hr after injection are indicated by the large dots.

It was thought that the weakness and other symptoms observed in these animals were due to acute dehydration and acidosis. Therefore two more rats (Text-fig. 1; rats 3 and 4) were given the same dose of anti-insulin serum and in addition, starting 7 hr later, were injected subcutaneously every 2 hr with 1 ml. of unbuffered Krebs–Ringer (stock) solution. Essentially the same changes in blood-sugar concentration occurred as in the first four rats, normal levels being re-established in 14–18 hr. Glycosuria started in 1–2 hr with ketonuria following 5–7 hr later; both subsequently ceased 12–14 hr after serum injection. These animals also became weak and stopped eating or drinking, but when glycosuria and ketonuria passed off they began to eat and drink again. Blood samples, however, were easily obtained throughout and the animals urinated every 1–2 hr. When killed 30 hr after injection they still seemed slightly weak and had lost about 20 g weight, but nothing abnormal was found at necropsy.

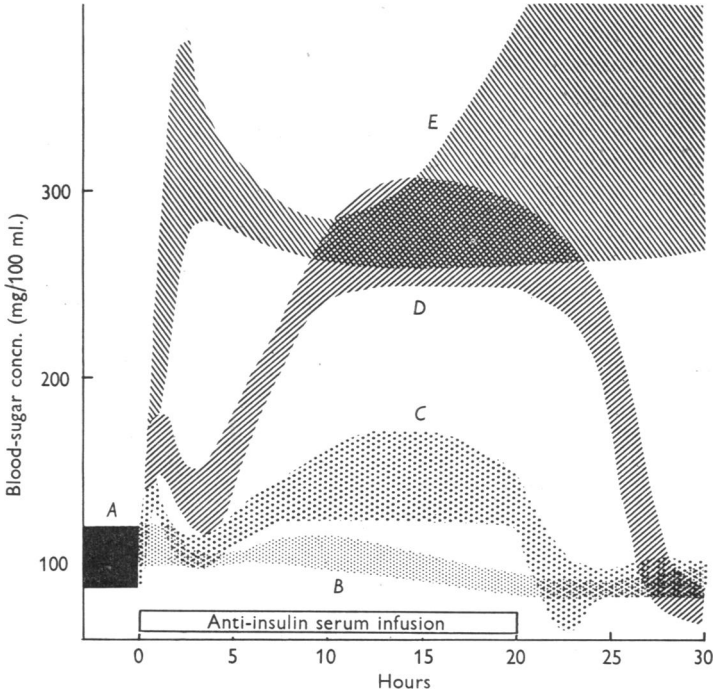
Anti-insulin serum (5 *ml.*). Of three rats (Text-fig. 1, rats 11–13) injected with this large dose, but otherwise untreated, one died about 17 hr later and two were killed after 24 hr when they seemed about to die. Blood-sugar concentrations rose rapidly, remained between 270 and 370 mg/100 ml. from 2 to 8 hr and then rose to levels greater than 400 mg/100 ml. at death. In the initial stages polyuria and polydipsia occurred; glycosuria appeared within 2 hr with ketonuria 5–7 hr later, both persisting till death. After about 10 hr the animals became weak, but still appeared to be thirsty. Gradually their general condition deteriorated; they became limp, cold to the touch and sunken-eyed. They passed urine seldom and blood was difficult or impossible to obtain. In the 2–3 hr before death their respiratory rates rose to over 140/min. At death they had lost weight (20–30 g); their livers were shrunken and yellow and their stomachs grossly dilated with clear colourless fluid.

Again, two rats (Text-fig. 1; rats 9 and 10) injected with this same large dose (5 ml.) of anti-insulin serum were also given 2-hourly injections (1 ml.) of Krebs–Ringer (stock) solution from 7 hr onwards. The initial response was the same but in neither did the blood-sugar concentration subsequently rise above 360 mg/100 ml. at any time. Marked hyperglycaemia persisted until 16–22 hr, after which time the blood-sugar concentrations fell at first slowly but later rapidly towards normal levels (28–31 hr). Polyuria occurred throughout this hyperglycaemia; glucose appeared in the urine in less than 2 hr, ketone bodies being detected 3–5 hr later. Again the animals became weak but seemed less ill than those which had not received saline injections; their respiratory rates did not rise above 120/min. As the blood-sugar levels fell glycosuria and ketonuria ceased. When the rats were killed (30 hr) they were still

weak and had lost weight (*ca.* 20 g). Necropsy showed that the livers were shrunken and yellow but the stomachs were not distended.

Intravenous infusion

The effects of intravenous infusions of guinea-pig serum into seventeen male rats at various rates for 20 hr are summarized in Text-fig. 2. Normal and control serum had no appreciable effect, whereas anti-insulin serum produced hyperglycaemia of a degree and duration depending upon the rate of serum infusion. When hyperglycaemia persisted for a sufficient time it was accompanied by polyuria, glycosuria, ketonuria and in severe cases led to oliguria, acidosis, marked loss of weight and ultimately to death.



Text-fig. 2. Blood-sugar concentrations in male rats (230–305 g) infused from zero time for 20 hr with guinea-pig serum. The shaded areas include all blood-sugar concentrations found in rats (*A*) before infusion; and up to 30 hr after the start of infusions (*B*) with normal or control serum (0·5–0·6 ml./hr) or with anti-insulin serum at rates of (*C*) 0·14, (*D*) 0·28 and (*E*) 0·5–0·6 ml./hr.

After introduction of the catheters fifteen rats received intravascular saline (0·5 ml./hr) for periods ranging between 21 and 25 hr; two were infused with serum immediately. During this time twelve of these, for which adequate measurements were made, passed urine (mean = 0·50 ml./hr, S.D. = 0·20) and ate food (mean = 0·59 g/hr, S.D. = 0·14) at comparable

rates, but their water intakes (mean = 0·43 ml./hr, S.D. = 0·25) were more variable. Blood-sugar estimations carried out less than 3 hr before infusion with serum showed that all seventeen animals were normoglycaemic (mean blood-sugar concentration = 103 mg/100 ml., S.D. = 8·3), none having levels greater than 120 or less than 89 mg/100 ml.

Control and normal sera (0·5–0·6 *ml./hr*)

Two rats were infused with normal serum and two with control serum (batch D15) for 20 hr. During this time their blood-sugar concentrations fluctuated irregularly about the pre-infusion level but did not rise above 120 mg/100 ml. (Text-fig. 2; area *B*). The animals moved about, ate, drank and passed urine normally. The urine remained free from glucose and ketone bodies. After infusion with normal serum one animal was infused with saline for 54 hr but was otherwise untreated, whilst the other was starved for 44 hr and infused with saline.

Saline infusion (0·9 *ml./hr*). The blood-sugar concentration never rose above 120 mg/100 ml. The animal continued to pass urine, eat and drink normally; its urine remained free from glucose and ketone bodies. When killed 74 hr after the start of the normal serum infusion the rat had lost some weight (17 g) but necropsy revealed no significant abnormality.

Saline infusion (0·5 *ml./hr*) *and fasting* (44 *hr*). For the first 4 hr this animal was infused with saline alone whilst having free access to food. No significant change in blood-sugar concentration was observed in specimens of blood drawn at regular intervals (cf. changes occurring in animals after infusion with anti-insulin serum at low rate). Thereafter all food, but not water, was withdrawn and the blood-sugar concentration fell steadily over 44 hr to 55 mg/100 ml. Ketone bodies were not detected with 'Acetest' tablets during this period of fasting, but urine collected over the last 24 hr gave a definite pale purple ring in the Rothera test. When food was restored the rat ate hungrily and the blood-sugar concentration rose to 130 mg/100 ml. in less than 2 hr. When killed 79 hr after the start of the serum infusion and 6 hr after the end of the fasting period, some loss of weight (33 g) was noted but necropsy revealed no other abnormality.

Anti-insulin serum

Three groups of rats were infused at different rates with anti-insulin serum, after which they were infused with saline unless otherwise stated.

Low rate (0·14 *ml./hr*). Three rats were infused with serum (batch 227328) at a rate estimated to neutralize approximately 0·29 u. insulin/hr. Text-figure 2 (area *C*) shows that in the first hour the blood-sugar concentration rose by 20–30 mg/100 ml., after which it fell to pre-infusion

levels. At 3 hr a second increase began, the blood-sugar concentration rising 20–70 mg/100 ml. before the serum infusion was stopped. During the serum infusion the rats ate less than usual (0·18–0·30 g/hr) but passed urine and drank at essentially normal rates; they received saline intravenously at a rate of 0·76 ml./hr. None developed glycosuria or ketonuria.

When serum infusion was stopped the blood-sugar concentrations (120–150 mg/100 ml.) fell rapidly by 50–60 mg/100 ml. in 3–4 hr; in each case the pre-infusion level was reached or passed in 3 hr (Text-fig. 2; area *C*). The animals continued to receive intravascular saline (0·51 ml./hr) and though they ate rather less than usual (0·14–0·57 g/hr) they otherwise behaved normally until they were killed 48 hr after the start of serum infusion. By this time they had lost some weight (20–35 g) but nothing abnormal was found at necropsy.

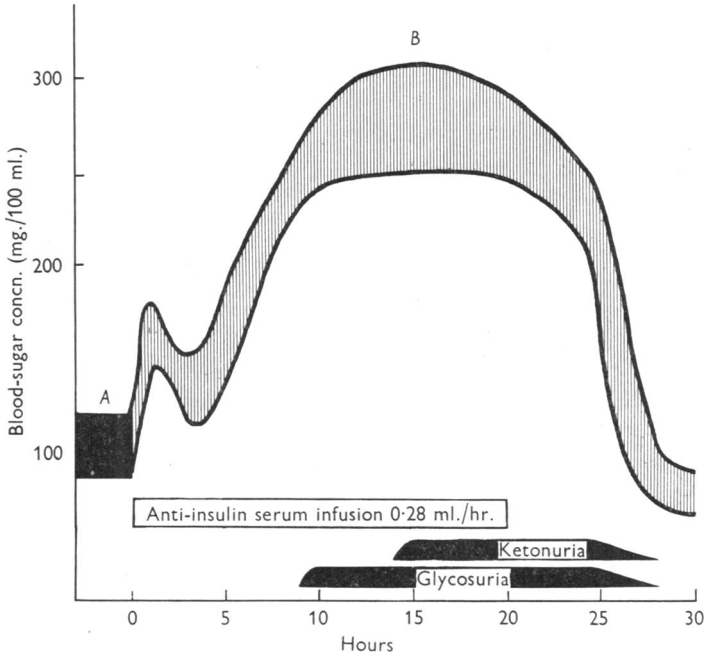
Medium rate (0·28 *ml./hr*). Three rats were infused with serum (batch 16204) at a rate estimated to neutralize approximately 0·64 u. insulin/hr. Text-figures 2 (area *D*) and 3 show that the blood-sugar concentration rose in the first hour to 150–180 mg/100 ml. and then fell. At 4 hr, however, the blood-sugar level began to rise steeply again, reaching 250–280 mg/100 ml. at 10 hr; it remained within the range of 260–310 mg/100 ml. until the infusion was stopped. During infusion with serum the rats ate less (0–0·4 g/hr) and passed more urine (0·59–0·92 ml./hr) but still drank at very variable rates (0·05–0·53 ml./hr); they received saline intravascularly at a rate of 0·62 ml./hr. In each case glucose was first detected in the urine after 9 hr when the blood-sugar concentrations had risen to 230–260 mg/100 ml.; ketone bodies appeared 6 hr later.

When serum infusion was stopped (Text-fig. 3) the blood-sugar concentration remained above 220–250 mg/100 ml. for 3–4 hr before falling sharply to the pre-infusion levels, which were reached at 25–28 hr. Glycosuria and ketonuria ceased almost simultaneously as the blood-sugar concentrations fell below 220 mg/100 ml. Until they were killed 26–33 hr after the end of the serum infusion the rats received intravascular saline (0·6–0·9 ml./hr); they ate (0·66–0·75 g/hr) and drank (0·68–1·24 ml./hr) slightly more than usual and passed variable amounts of urine (0·32–0·92 ml./hr). When killed they had all lost weight (20–40 g) but at necropsy the livers appeared normal and no depletion of body fat was noted.

High rate (0·5–0·6 *ml./hr*). Serum of batches 14108 (four rats), 16204 (one rat) and 424 (two rats) was infused at rates calculated to neutralize approximately 1·1–1·7 u. insulin/hr. During the infusions all seven rats responded in similar fashion, but thereafter their fates varied; six either died or were killed and only one recovered.

Text-figures 2 (area *E*) and 4 show that a rapid uninterrupted increase in blood-sugar concentration occurred in the first 2 hr of the infusion. By

this time levels of 260–380 mg/100 ml. were reached, but subsequently the concentration fell until at 10–12 hr it lay between 260 and 290 mg/100 ml. In five rats the blood-sugar concentration remained between 260 and 310 mg/100 ml. until the end of the infusion but in two others (Text-fig. 4; rats *D* and *F*) higher levels were reached (360 and 390 mg/100 ml.). All the animals moved normally about the cage during the infusion but none of them ate. They passed much urine (0·8–1·9 ml./hr)



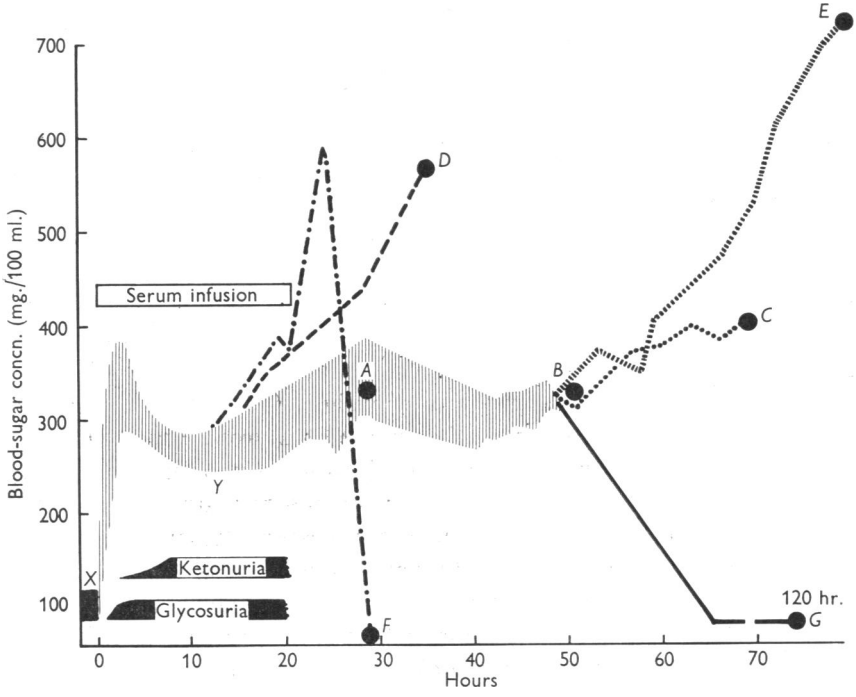
Text-fig. 3. Blood-sugar concentrations, glycosuria and ketonuria in three male rats infused from zero time for 20 hr with anti-insulin serum (0·28 ml./hr). The shaded areas include all blood-sugar concentrations found (*A*) before and (*B*) up to 30 hr after the start of infusions.

and at least one animal drank large volumes of water (1·33 ml./hr). Glucose appeared in the urine within 3 hr and during the infusion its concentration in urine obtained from three rats was about 5 % (Benedict's method). Ketone bodies appeared 3–4 hr after the onset of glycosuria. These animals also received saline (0·3–0·4 ml./hr) intra-arterially.

Some time after the infusions were stopped all but one of the animals died or were killed. The animal which survived (Text-fig. 4, rat G) was the first to be infused and was not observed as closely as the others. The blood-sugar concentration of this animal remained between 260 and 330 mg/100 ml. for a total of at least 50 hr. During this time it passed urine at a high rate (0·97 ml./hr) containing ketone bodies and glucose, the

latter in a concentration of 5–7 %. By 50 hr the animal looked ill and had obviously lost much weight; it was breathing rapidly and its fur was staring. At 65 hr the blood-sugar concentration was normal (80 mg/100 ml.) but the animal still moved slowly and seemed weak. In the next 24 hr it ate and drank well and when killed 5 days after the serum infusion began it was moving actively about its cage. It was not weighed but necropsy showed depletion of retroperitoneal and other fat depots. The liver was mottled and the tissues generally pale and wasted.

One animal (Text-fig. 4; rat *B*) was killed 30 hr after the end of the infusion. This rat was infused with saline at a low rate (0·38 ml./hr) but



Text-fig. 4. Blood-sugar concentrations, glycosuria and ketonuria in seven male rats (*A–G*) infused from zero time for 20 hr with anti-insulin serum (0·5–0·6 ml./hr). The shaded areas include the blood-sugar concentrations found in all rats (*X*) before infusion; and at any one time in the majority of rats (*Y*) during and up to 50 hr after the start of serum infusion. Where the blood-sugar level in a rat differed at any time from those of the majority it is shown separately. The blood-sugar concentrations in individual rats at the time of death are indicated by the large dots. Rats *D* and *F* received Krebs–Ringer solutions intravascularly after infusion with serum and rat *F* also received 12·5 u. insulin I.V. at 24 hr. Ketonuria and glycosuria persisted until normoglycaemia was re-established (rat *G*) or until death; rat *F* was anuric at the time of death and the last urine which it passed at 23 hr contained glucose and ketone bodies.

continued to pass large volumes of urine (1·5 ml./hr) containing glucose in high concentrations (5–7 %) and ketone bodies. Food was still refused and the rat became weak; it was sunken-eyed and limp but continued to respond to stimuli. The blood-sugar concentration remained at 260–340 mg/100 ml. and towards the end of this period some increase in respiratory rate (120–130/min) was noted. When killed 50 hr after the start of the serum infusion the rat had lost much weight (80 g), its liver was small and yellow and the fat depots were depleted. The stomach was markedly distended.

Two other rats (Text-fig. 4; rats *C* and *E*) survived for more than 50 hr and up to that time they behaved similarly. One (rat *E*) was infused slowly with saline (0·12 ml./hr) but also continued to pass urine at a high rate (0·6–0·8 ml./hr) until, at about 58 hr, the rate of urine formation fell rapidly. Up to this point this animal had shown only signs of weakness and dehydration, but then it became progressively more sluggish in its movements. In the last 20 hr of life the blood-sugar concentration rose steadily to more than 700 mg/100 ml. For the last 10 hr no urine was passed. The respiratory rate rose to 130/min and fell again just before the animal was killed. At the time of death, 79 hr after the start of the serum infusion, this rat was thought to be comatose, and was the only one which became so; it lay quietly on its side breathing rapidly and would not respond to stimuli. Apparently about to die, this rat was killed and necropsy revealed the usual findings, a small pale liver, depleted fat depots, a dilated stomach and marked loss of weight (66 g). The other animal (Text-fig. 4; rat *C*) was infused with saline at a greater rate (0·9 ml./hr) and for 30 hr after the end of the serum infusion it too maintained a high urine output (1·4–0·9 ml./hr) and a steady blood-sugar concentration (310–390 mg/100 ml.). Thereafter, however, it passed less urine and became anuric 8 hr before death, which followed a sudden and severe bleeding from the mouth. In these last hours the blood-sugar level rose steadily to more than 380 mg/100 ml., the respiration rate increased (140–150/min) and movements became very sluggish. Necropsy again showed a pale shrunken yellow liver, depleted fat depots and a distended stomach containing blood. There was only slight loss of weight (20 g) but the pleural and peritoneal cavities contained free fluid and the lungs and other tissues generally were grossly oedematous.

One other animal (Text-fig. 4; rat *A*) received only saline (1·1 ml./hr) when serum infusion was stopped. This animal died after two convulsions 29 hr after the start of the serum infusion; necropsy revealed massive haemorrhages into the pericardial and pleural cavities. The rat had lost weight (40 g), its liver had a blotchy appearance and the stomach was slightly distended. Before death the blood-sugar concentration had not

risen above 320 mg/100 ml. and it had been passing urine at a high rate (0·8–1·2 ml./hr).

Since the animals which died in an anuric state showed evidence of potassium depletion and acidosis, attempts were made to prevent these in two other rats. One rat (Text-fig. 4; rat *D*) was infused with unbuffered Krebs–Ringer (stock) solution after the end of the serum infusion when it was seen that the blood-sugar concentration was rising. The rate of infusion (1·5 ml./hr) was calculated to replace fluid lost during the previous infusions of serum and saline. Despite this, however, the blood-sugar concentration continued to rise, anuria developed and the respiratory rate rose until the animal died about 35 hr after the start of the serum infusion. At necropsy the liver had a typical nutmeg appearance and the stomach was slightly distended; the rat had lost weight (43 g). The remaining animal (Text-fig. 4; rat *F*) also had a rapidly increasing blood-sugar concentration at the end of the infusion period. It was, therefore, infused with Krebs–Ringer bicarbonate buffer (1·2 ml./hr). The rat became anuric, its blood-sugar concentration continued to rise, and the blood pressure fell rapidly. Since it seemed certain that this animal would die, it was given insulin (12·5 u.) intravenously, sufficient to counteract half of the anti-insulin serum infused. Within 4 hr the blood-sugar concentration reached hypoglycaemic levels (28 mg/100 ml.) and the blood pressure, already low (45 mm Hg), fell even lower. Six hours after the insulin and 30 hr after the serum infusion began, the rat died in a hypoglycaemic, anuric and hypotensive state, having lost weight (42 g). Necropsy revealed a grossly distended stomach filled with clear colourless fluid, a blotchy congested liver and sparse fat depots.

DISCUSSION

Whether infused intravenously or injected intraperitoneally with anti-insulin serum, all rats whose blood-sugar concentrations rose above about 200 mg/100 ml. developed glycosuria which persisted as long as the hyperglycaemia remained of this degree; the glucose content of such urine ranged between 4 and 7 % (four rats). About 4 hr after the onset of glycosuria ketonuria occurred in every animal whose hyperglycaemia lasted long enough, and passed off again about as soon as did the glycosuria. In the infusion experiments the onset of glycosuria was accompanied by polyuria and probably also by polydipsia. In every case in which serum was given in large amounts the rats stopped eating, but this of itself was not responsible for the ketonuria; even 44 hr fasting provoked but slight ketonuria in an otherwise normal rat.

This syndrome of polyuria, glycosuria, ketonuria (and probably polydipsia) associated with hyperglycaemia differs in at least two respects from

experimental diabetes induced by alloxan in the rat, but is very like that provoked by pancreatectomy. In the first place, permanent hyperglycaemia is not established in the alloxan-treated rat until several hours have elapsed; during this time the blood sugar first rises and then falls, often to hypoglycaemic levels (see Lukens, 1948). Secondly, the persistent hyperglycaemia found in the alloxan-diabetic rat, though accompanied by polyuria, polydipsia and intense glycosuria, is not usually associated with ketonuria, which has been reported by only a few authors (Dunn & McLetchie, 1943; Lyman-Duff & Starr, 1944; Bailey, Bailey & Leech, 1944) or with loss of appetite. Ketonuria also appears to be the exception rather than the rule in alloxan-diabetic rabbits (Lewis, Moses & Schneider, 1947; Duffy, 1945) and dogs (Goldner & Gomori, 1943; Thorogood & Zimmerman, 1945). To study ketonuria, Janes & Myers (1946) chose alloxan-diabetic rats since, as they say, they are usually on the borderline of ketosis and respond more readily to any treatment that alters carbohydrate metabolism than do normal animals. Thus ketonuria and acidosis have been induced in alloxan-diabetic rats by fasting (Guest, Mackler & Knowles, 1952) and by administration of nicotinic acid (Janes & Myers, 1946). Alloxan-diabetic dogs, which also survive for long periods with little evidence of ketonuria (Goldner & Gomori, 1943), rapidly develop ketonuria and fatal acidosis if the pancreas is then removed (Thorogood & Zimmerman, 1945). This latter rapid sequence of events also occurs in the rat after removal of 99·5% of the pancreas (Scow, 1957). Death occurs within 48 hr unless insulin therapy is administered and at the time of death the 'totally' pancreatectomized rat exhibits weakness and prostration, over-ventilation and hypothermia; the stomach also becomes grossly dilated. This is also the picture seen just before death in rats treated with anti-insulin serum and at necropsy the stomach is also grossly distended.

After pancreatectomy or treatment with alloxan the persistent diabetic state is due to more or less complete destruction or removal of the β-cells which secrete insulin. It seems, though the evidence at present is only circumstantial, that the diabetic state induced by anti-insulin serum is not due to any failure of insulin production by the pancreas but rather to neutralization of all endogenously secreted insulin by the injected antibody. It is known that injected labelled antibodies persist in decreasing amounts in the circulation (Coons, 1954) and so presumably an animal injected with anti-insulin serum will remain diabetic until its pancreas has secreted sufficient insulin to neutralize the antibody. In the mean time secondary metabolic changes develop which may cause death. Dehydration and weakness, which prevented voluntary drinking in the intraperitoneally injected rats, resulted in acidosis and death when the dose exceeded 3 ml. Life was prolonged and recovery facilitated by administration of fluid

subcutaneously. Saline infusion prolonged the lives of rats infused with larger doses of anti-insulin serum to 70–80 hr and one rat recovered. All those which died or were killed after prolonged hyperglycaemia became oliguric and then anuric by which time they showed signs of acidosis. Diabetic acidosis is associated with potassium depletion; therefore two rats (Text-fig. 4; rats *D* and *F*) were infused with solutions containing bicarbonate buffer or potassium as soon as the blood-sugar concentration was found to be rising rapidly. This treatment, however, did not seem to prolong life or to restore renal function. It may be that these particular animals, which were used in midwinter, were not so physically fit as those which were infused in the late summer (Text-fig. 4; rats *B*, *C*, *E* and *G*) and which developed oliguria at a much later stage. All that can be said at the moment is that it is possible to maintain diabetic rats alive for about 30 hr and that on occasion they have been kept alive for almost 80 hr. The factors which facilitate recovery are not yet known, but at least one of the animals used in the present experiments did recover after being diabetic for at least 50 hr whilst receiving only intravenous saline.

The responses to different doses of anti-insulin serum suggest that the islet cells of the pancreas react in two phases; first, they release stored insulin and then they secrete the hormone as fast as it is required or can be synthesized. Such a store of hormone has been inferred from the presence of granules which disappear from the β-cells when glucose is administered (see Lazarow, 1957). It could also be inferred from the fact that the effects produced by anti-insulin serum were not directly proportional to the dose injected. Thus 5 ml. injected intraperitoneally provoked hyperglycaemia lasting approximately 30 hr whilst that following 1 ml. lasted only about 3 hr (Text-fig. 1). Further, infusions at the lower rates (0·14 and 0·28 ml./hr) caused an initial rise in blood-sugar concentration which was followed by a temporary fall and then a greater and more persistent hyperglycaemia (Text-fig. 2). We interpret these observations to mean that the initial hyperglycaemia induced by the injection or infusion of the anti-insulin serum causes the release of insulin stored in the β-cells; this neutralizes small injected doses and halts the progressive increase in blood-sugar concentration induced by serum infused at low rates. Once this store of insulin has been released and neutralized, the blood-sugar concentration again rises in the infused animals if endogenously secreted insulin is insufficient to neutralize the infused antibody and supply the requirements of the tissues.

Assuming that this interpretation is true, that the estimates of insulin-neutralizing power of the serum are correct and that all the antibody in the injected serum remains available to neutralize insulin, we may calculate the rate at which the rat pancreas can secrete the hormone. Rats weighing

approximately 250 g when infused with serum at a rate sufficient to neutralize about 0·3 u. insulin/hr, developed slight but persistent hyperglycaemia towards the end of the infusion period, after which the blood-sugar concentration fell immediately (Text-fig. 2; area *C*). This implies that they were producing sufficient insulin to neutralize the infused antibody but not quite enough to maintain normoglycaemia as well. Higher rates of infusion sufficient to neutralize about 0·64 u. insulin/hr produced a more marked secondary hyperglycaemia, which persisted for several hours after the end of the infusion (Text-fig. 2; area *D*). In this case the pancreas was unable to secrete enough insulin even to neutralize the infused antibody. It seems, then, that the rat pancreas can secrete insulin at a rate between 1·2 and 2·6 u./kg/hr. The effects produced by intraperitoneal injection of the serum support this conclusion. Hyperglycaemia lasting about 15 hr was induced by 3 ml. serum, whilst 5 ml. elevated the blood-sugar level for 30 hr (Text-fig. 1). The additional 2 ml. of serum, sufficient to neutralize about 4·2 u. insulin, prolonged hyperglycaemia for 15 hr in rats weighing 200 g. Thus towards the end of the hyperglycaemic period these rats were secreting insulin at a rate of about 1·4 u./kg/hr. These rough estimates of the probable maximum rate at which the pancreas is capable of synthesizing and secreting insulin are much greater than the 0·33–0·50 u./kg/hr of soluble insulin which Spiro & Hastings (1958) found necessary to maintain normal metabolism in alloxan-diabetic rats once adequate control of the diabetes had been established. If this latter rate of insulin dosage represents its normal insulin requirements, then the rat can apparently increase its insulin secretion rate three- to fivefold and can maintain this rate for many hours. It remains to be seen, however, whether more prolonged stimulation of the pancreas by hyperglycaemia will exhaust and finally destroy the secretory capacity of the β-cells.

Similarly the reserve of insulin in the pancreas can be calculated roughly. When anti-insulin serum is infused at a rate sufficient to neutralize 0·64 u. insulin/hr, the initial rise in blood sugar concentration is halted after 1 hr for 2–3 hr before the secondary rise begins (Text-fig. 2; area *D*). Presumably, therefore, the pancreas has released sufficient insulin to neutralize the antibody infused during this period. On this basis the reserve of insulin available in such rats (250 g) is about 5–8 u./kg. Further, the injection of serum sufficient to neutralize 2·1 u. insulin produced hyperglycaemia in 200 g rats lasting only 3 hr (Text-fig. 1; rats 1 and 2); insulin must therefore have been secreted at a rate of 3·5 u./kg/hr. Thus even if the pancreas had been synthesizing and secreting insulin at its maximum rate (1·4 u./kg/hr) it must also have secreted an additional 6·3 u./kg during this time, and this may be taken to represent a low estimate of the insulin reserve in the pancreas. For comparison with these

rough estimates, it may be noted that the mean extractable insulin in the pancreas of the Wistar rat is 7·5 u./kg and ranges between 5·8 and 8·4 u./kg (Best, Haist & Rideout, 1939).

These preliminary observations suffice to show that guinea-pig anti-insulin serum provides a useful tool for producing a diabetic state and for studying insulin metabolism. More extensive experiments with animals larger than the rat would be possible if potent anti-insulin serum were available in greater amounts. At present the guinea-pig is the only animal known to yield active serum. It may be possible both to increase the titre by modifying existing production methods and also to increase the yield of serum by finding a bigger animal with an immunological response to insulin similar to that of the guinea-pig.

SUMMARY

1. Anti-insulin serum obtained from guinea-pigs treated with bovine insulin has been injected intraperitoneally and infused intravenously for 20 hr into conscious rats.

2. Provided the dose is sufficient, such serum induces a diabetic syndrome characterized by hyperglycaemia, glycosuria, polyuria, ketonuria and loss of appetite, and leading in some cases to loss of body weight, oliguria, anuria and death and in others to recovery. This syndrome is similar to that found in the 'totally' pancreatectomized rat but differs from alloxan diabetes.

3. The results suggest that the rat is capable of producing endogenous insulin at a rate of approximately 1·2–2·6 u./kg/hr and maintains a store of insulin in the pancreas (5–8 u./kg) which it can secrete rapidly.

We wish to thank Dr M. A. Floyer of the London Hospital whose infusion pump and apparatus, exhibited at a meeting of the Medical Research Society in October 1958, formed the basis of ideas incorporated in the apparatus used in the present experiments. We also wish to thank the Medical Research Council for a grant and Miss Sheila Haizelden, Miss Ann Buxton and Mr Kenneth Kilbourn for technical assistance.

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APPENDIX

APPARATUS AND METHOD FOR PROLONGED INTRAVASCULAR INFUSION OF CONSCIOUS RATS

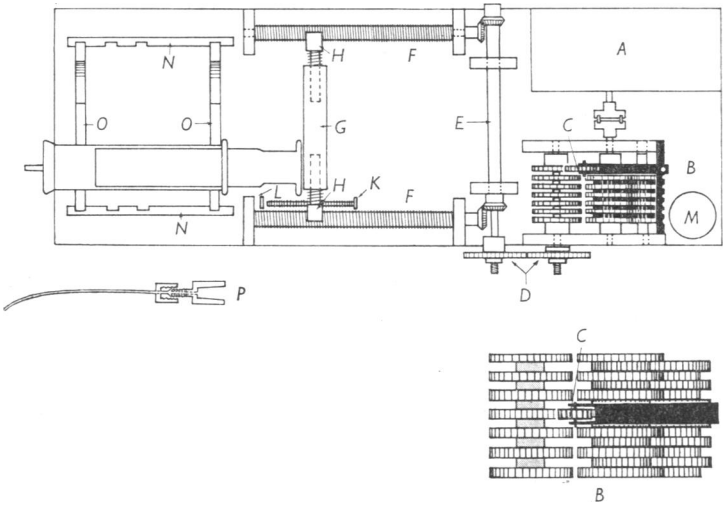
BY J. ARMIN

In each experiment two rats (230–305 g) are anaesthetized (pentobarbitone sodium, 30 mg/kg; 0·5 ml./kg Veterinary Nembutal, Abbott Laboratories Ltd.) and in each catheters are inserted in an external jugular vein and a carotid artery. They are then transferred to cages (Plate 1) and infused with saline or guinea-pig serum delivered from an infusion pump (Text-fig. 5).

Catheterization. For each rat two catheters are prepared, each consisting of polythene tubing (internal diameter, 0·011 mm; size P.E.10; Clay Adams Co. Inc., New York), 35–40 cm long, with one end bevelled and the other flared for connexion by means of a screw cap to one nozzle on a three-way Perspex tap (Armin & Grant, 1953). The second nozzle of this tap is connected by larger polythene tubing (internal diameter, 0·023 mm; size P.E.50) to the syringe of a perfusion pump (see below). To the third nozzle is attached a short length (1 cm) of tubing (size P.E.50) with a flared open end into which a blunt syringe needle may be fitted; this tubing can be connected to a capacitance manometer for blood pressure measurements and through it blood samples may be obtained or fluids

injected. The two catheters are threaded through a closely coiled stainless-steel wire spring (24 S.W.G.; coil internal diameter 3 mm; length 18 cm). The last coil at the end distal to the three-way tap is expanded to a diameter of 10 mm and covered with polythene tubing. The syringe and connected catheters are filled with saline and freed from air.

Through a $\frac{1}{2}$ in. (13 mm) incision at the back of the neck the catheters are passed through a passage in the subcutaneous tissues to a second $\frac{1}{2}$ in. incision at the front of the neck. They are inserted for a distance of 1–3 cm into the carotid artery and external jugular vein previously isolated



Text-fig. 5. Diagram of infusion pump, syringe and polythene tubing connexion; details given in text.

on one side, and each secured in place with two ligatures. The saline infusion is then started and the anterior neck wound closed. The expanded end of the spring is fixed beneath the skin in the dorsal incision by a purse-string suture and the remainder of the incision is closed. The rat is then transferred to the cage.

The cage and attachments (*Plate* 1). A wire cage ($45 \times 30 \times 15$ cm) is divided into two compartments and provided at its sloping front with a common door opening downwards. It rests on a metal stand which also supports at one side the two perfusion pumps. At the centre of the top of each compartment is a hole (3×3 cm^2) above which is a gantry consisting of two vertical brass rods (10 cm $\times$ 0·6 cm) joined at the top by a horizontal Perspex plate (Plate 1). Between, and moving up and down at its slotted ends upon these rods, is a Perspex bar, in the centre of which is a slot whose width can be adjusted with a screw; the upper end of the

coiled spring is fixed in this slot. The centre section of the horizontal bar is free to move about a horizontal axis in the plane of the vertical rods, and is suspended by a cord running over a pulley in the Perspex plate above and attached to a counterweight. Once the three-way taps have been closed and disconnected from the tubes leading to the infusion pump, they can be passed through the hole in the roof when the anaesthetized catheterized rat is transferred to the cage. The upper end of the coiled spring is fixed in the movable horizontal bar. The taps are screwed into position on the horizontal Perspex plate and reconnected to the pump for immediate infusion with saline. This system allows the rat to move about the cage, the coiled spring moving up and down freely without exerting tension or pressure on the rat's neck. The only movement which is restricted is rotation of the coiled spring about its own axis, but the animal soon learns not to turn round too often in one direction.

Immediately beneath each compartment of the cage is fitted a removable celluloid funnel with a rectangular top of the same dimensions as the base of the compartment. Below the funnel spout stands a conical flask (50 ml.) around the mouth of which are three small teeth. Resting on these teeth is a pear-shaped glass ball whose diameter is slightly greater than that of the mouth of the flask and whose pointed end is directed into the flask. Faeces emerging from the funnel strike this ball and fall clear, whereas urine passes round and is collected in the flask beneath. To prevent urine escaping from the cage, a strip of celluloid (5 cm wide) runs round the bottom of each compartment at an angle of 45°. Above this celluloid strip the tube from a water bottle projects into each compartment.

Perfusion pump with variable gears (*Text-fig.* 5). The following apparatus has proved convenient and reliable for the prolonged infusion of small amounts of fluid into small animals. The whole is mounted on a duralumin base plate ($13 \times 36 \times 0{\cdot}5$ cm). *A* is a synchronous motor ('Sectric' heavy-duty clock motor, No. 55697; Smith, London) giving one shaft revolution per minute. The shaft is connected to *B*, a combination gear train giving 7 stages from a direct drive, by 2 to 1 reductions to a final reduction of 64 to 1. The small wheel *C* selects the gear required and transmits the drive to a pair of gear wheels *D*, which can be changed at will from direct drive, to 3 or 5 to 1 reduction or 1 to 3 or 5 increase. *D* drives the shaft *E* which in turn drives the pair of screws *F*, 20 threads to the inch. Between the screws is the ram *G* on each end of which is a spring-loaded shaft ending in a half-nut *H* which fits on to the screws. Mounted on the underside of one of the half-nuts is an adjustable screw *K*, so arranged that when the ram is near the end of its traverse, it impinges on the arm *L* of a switch (Microswitch V3ML; Burgess Products Co. Ltd., Gateshead) mounted underneath the base plate, which breaks the current to the motor and

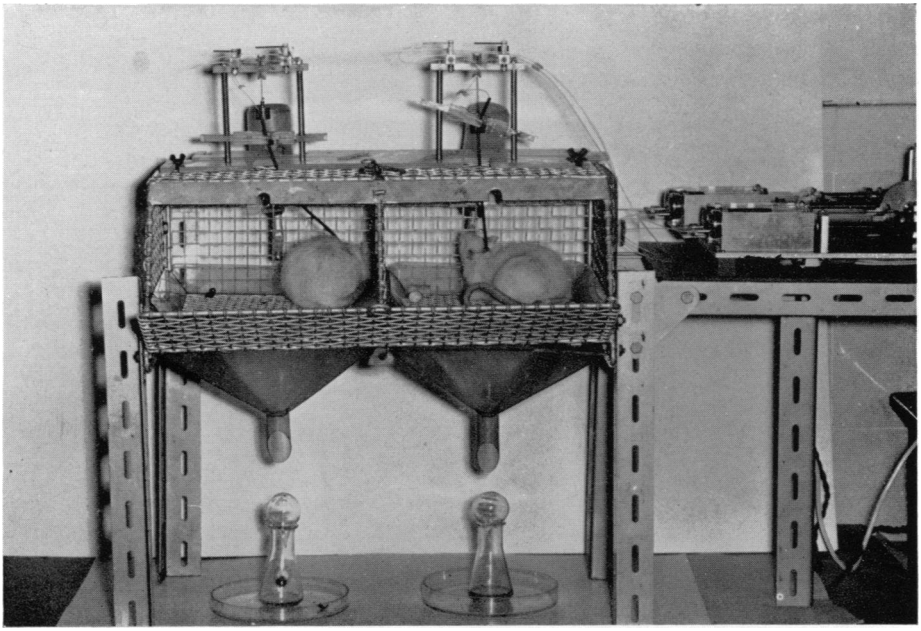


Fig. 1

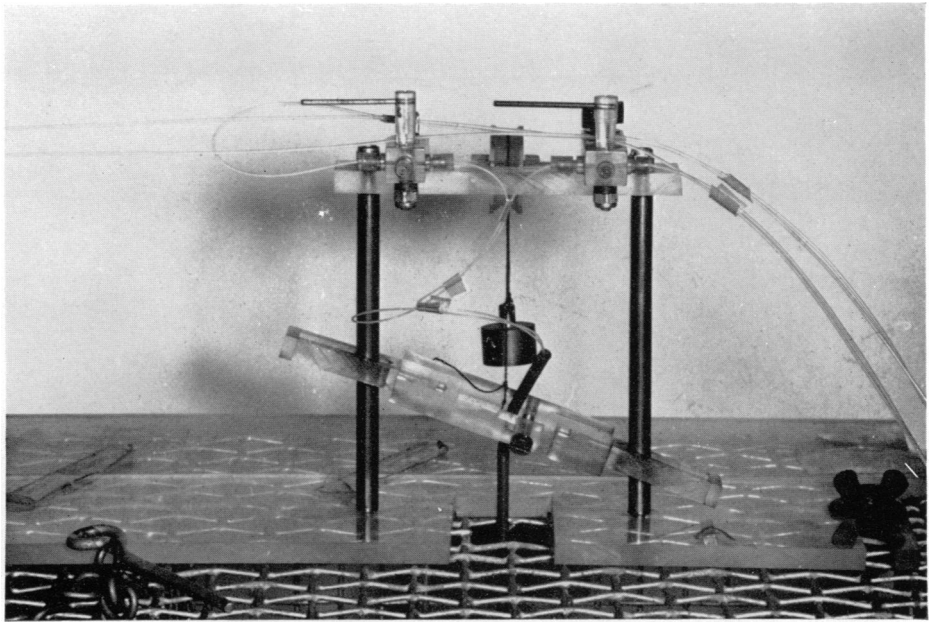


Fig. 2

lights a warning lamp *M* or rings a bell. *N* is a pair of parallel duralumin plates with slots cut in the opposing faces to hold two 0·64 cm Perspex slides *O*. The slides have U-shaped cut-outs into which glass syringes fit snugly. Those now in use can accommodate four 1 ml. syringes, three 2 or 5 ml., two 10 or 20 ml. or one 50 ml. syringe. The Perspex cap joining the syringe nozzle to the polythene tubing used for infusion is shown exploded at *P*. A Perspex cap fits over the syringe nozzle and ends in a screw. The end of the polythene tube is passed through a cap nut and flared with gentle heat. The cap nut is then screwed on to the Perspex cap. The syringe piston must fit snugly in the barrel and should be ground throughout its length. The piston is lubricated with silicone high-vacuum grease (W. Edwards and Co. Ltd., London). These measures prevent loss of fluid between piston and barrel. The gearing leads to a forward drive of the ram of from 381 to 0·238 mm/hour. The volumes delivered by the various syringes can therefore range in 210 steps from about 232 to about 0·004 ml./hr.

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EXPLANATION OF PLATE

PLATE 1

Fig. 1. Cage for infusion of conscious rats; for details see text.

Fig. 2. Gantry fixed above cage for support of coiled spring carrying catheters and attachment of three-way taps; for details see text.