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ACUTE INSULIN DEFICIENCY PROVOKED BY SINGLE INJECTIONS OF ANTI-INSULIN SERUM

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After prolonged treatment with insulin, guinea-pigs and some other animals are known to yield sera which will abolish the hypoglycaemic effects of insulin injected simultaneously into mice (Moloney & Coval, 1955; Arquilla & Stavitsky, 1956; Moloney & Goldsmith, 1957; Moloney & Aprile, 1959), and the stimulant action of the hormone upon glucose consumption by the isolated rat diaphragm (Wright, 1959*a*). These effects are thought to be due to the presence of insulin antibodies which combine with the hormone and so prevent its action. The antibodies may also neutralize the effects of endogenously secreted insulin, for such serum is known to induce hyperglycaemia in mice (Moloney & Coval, 1955; Moloney & Goldsmith, 1957). The observations now reported strongly suggest that the serum of insulin-treated guinea-pigs will neutralize the effects of endogenously secreted insulin in the rabbit, rat and cat, but not in the guinea-pig itself; a preliminary account has been published (Wright, 1959*b*).

METHODS

Production of anti-insulin serum. Recrystallized bovine insulin (10 mg, 22·2 u./mg; Boots Pure Drug Co. Ltd.) dissolved in 10 ml. aqueous phenol 0·3 % (w/v) acidified with HCl to pH 2·6 approx., was mixed with liquid paraffin B.P. 7 ml. and anhydrous lanolin (*adeps lanae* B.P.) 3 ml. and the mixture emulsified in a Waring blender.

Groups of 5–8 albino guinea-pigs were injected subcutaneously at monthly intervals with 2 ml. of this thick emulsion, 1 ml. being injected between the shoulders and 0·5 ml. into the inner aspect of each thigh. Two weeks after the third and each subsequent monthly injection of antigen about 10 ml. of blood was withdrawn from each animal under light ether anaesthesia by cardiac puncture. The serum specimens from animals in each group were pooled and kept frozen ($-10°$ C) until required; pooled sera from different groups of animals were kept separate.

No hypoglycaemic reactions were observed at any time after the injections of insulin antigen. Most animals remained in good health for periods of 6–17 months. The majority of deaths occurred immediately after cardiac puncture and were due to haemorrhage into the pericardial or pleural cavities; a few animals became thin and died for no apparent reason.

In the following description, serum obtained in this way is termed anti-insulin serum. Serum from guinea-pigs treated for 3 months under similar conditions with the same

emulsion, but containing no added insulin, is termed control serum. Normal untreated guinea-pigs were used to obtain normal serum.

Experimental animals. Serum was injected intravascularly in single doses into conscious rats and rabbits and into anaesthetized rats, rabbits, a guinea-pig and a cat; Professor J. M. Robson of the Pharmacology Department of this school carried out the experiment on the cat. All these animals were fed on standard diets. When necessary, food, but not water, was withdrawn 24–48 hr beforehand.

The rats, each weighing 200–300 g, were albino animals of a Wistar strain originally supplied by the Chester Beatty Research Institute and bred at this school. The rabbits, with half to three-quarter lop ears, usually weighed 2–3 kg. One ear was deprived of its sympathetic nerves by excising the ipsilateral superior cervical and stellate ganglia at least 1 week beforehand; the vessels of such denervated ears provide a sensitive indicator for the presence of vasoconstrictor substances in the blood stream. Adrenalectomy of the rabbit was performed by a one-stage operation, the animal being subsequently maintained in good health by injections of cortisone or DOCA (deoxycortone acetate B.P.) (Armin & Grant, 1959).

Anaesthesia was induced with pentobarbitone sodium (30 mg/kg = 0·5 ml./kg veterinary Nembutal; Abbott Laboratories Ltd.) and maintained by smaller doses repeated as required. Local procaine 2% (w/v) anaesthesia was also used during the introduction of catheters. For intravascular catheterization of the rat and guinea-pig, fine polythene tubing (internal diameter 0·011 mm; size PE 10; Clay Adams Co. Inc., New York) was used; larger tubing (internal diameter 0·023 mm; size PE 50) was preferred for the rabbit and cat. Such catheters were usually inserted into the femoral artery or vein; in the conscious rabbit into the marginal vein or central artery of the ear. Patency was maintained by the slow infusion of NaCl solution 0·9% (w/v) from a perfusion pump. A Perspex three-way tap (Armin & Grant, 1953) inserted in the length of the catheter allowed the injection of serum, withdrawal of blood samples and measurement of blood pressure by capacitance or mercury manometer. In the rabbit the temperature of the sympathectomized ear was measured by thermocouple.

Conscious rabbits were restrained in the box already described (Armin & Grant, 1957). In conscious rats, serum in doses of up to 1 ml. was injected into a tail vein by a technique similar to that described by Lazarow & Palay (1946). To keep the tail veins dilated the rats were kept in a warm cage, and just before injection the tails were immersed in warm water (40–50° C). Blood samples were obtained from the severed end of the rat's tail and collected on watch-glasses containing dried anticoagulant solution (1 drop 3·0% potassium oxalate and 1 drop 0·3% potassium fluoride). For serum injection and blood sampling conscious rats were restrained in boxes from which the tail protruded. The blood sugar concentration was determined on 0·2 ml. samples by a modification (Wright, 1957) of the method described by King (1951).

RESULTS

Hyperglycaemia induced by anti-insulin serum

The anti-insulin serum injected into rats, rabbits and the cat provoked a transient hyperglycaemia in each instance, but in the guinea-pig did not alter the blood-sugar concentration. No significant change in blood-sugar concentration followed the injection of normal or control serum into any of these animals.

Guinea-pig. Into one anaesthetized animal 1 ml. normal serum was injected by way of the femoral artery, and this was followed half an hour later by 1 ml. anti-insulin serum (batch X). No appreciable change in blood-sugar concentration was found in samples drawn at 5 min intervals

from the time of the normal serum injection up to 30 min after injection of the anti-insulin serum.

Cat. The intra-arterial injection of 3 ml. normal serum into the anaesthetized cat quickly provoked a strong but transient reaction (presumably due to the foreign protein), shown by sneezing, coughing, dyspnoea and cyanosis, together with a sharp rise of blood pressure and heart rate;

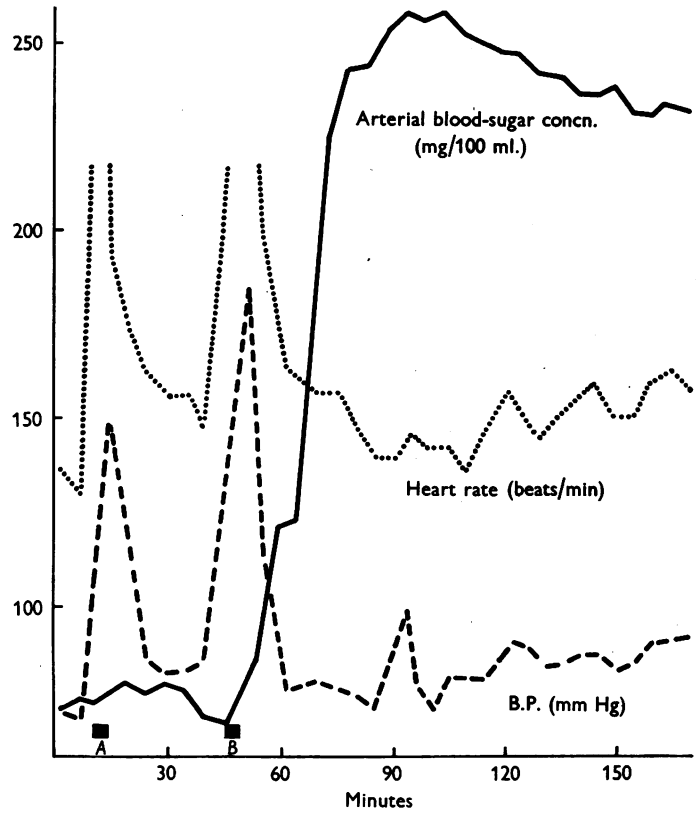

Fig. 1. Cat, 3 kg, anaesthetized. Records of arterial blood-sugar concentration, mean arterial blood pressure and heart rate. before and after intra-arterial (femoral) injection at *A* of normal guinea-pig serum (3 ml.) and at *B* of anti-insulin serum (3 ml.; batch X).

a similar reaction followed the injection 35 min later of 3 ml. anti-insulin serum (batch X). As Fig. 1 shows, however, no significant change in blood-sugar concentration followed the first injection, whereas following injection of the anti-insulin serum the level rose steeply to a maximum of about 250 mg/100 ml. in 45 min and was still high (230 mg/100 ml.) when the experiment was ended after 2 hr.

Rabbit. The intravascular injection of normal or anti-insulin serum in

doses of 1 ml./kg body weight into conscious or anaesthetized animals provoked no obvious reaction. Mean blood pressure and heart rate remained unchanged, the temperature of the sympathectomized ear did not fall and the ear vessels remained dilated. No appreciable rise in blood-sugar concentration followed injections of normal serum but anti-insulin serum evoked a conspicuous increase, detectable within 5 min. In five rabbits the blood sugar concentrations rose initially at rates ranging from 2·4 to 3·7 mg/100 ml./min (mean 3·04) and reached maxima of 200–300 mg/100 ml.; hyperglycaemia persisted for 3–5 hr. Figures 2 and 3 illustrate these results. Bilateral adrenalectomy with subsequent maintenance on cortisone or DOCA did not significantly alter the degree or duration of the response to anti-insulin serum injection.

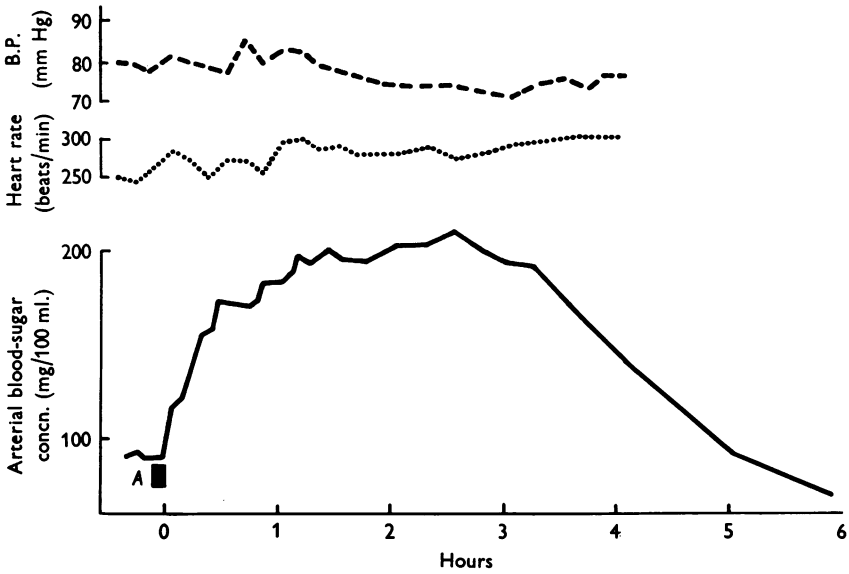

Fig. 2. Rabbit, 2·5 kg, fed, anaesthetized. Records of arterial blood-sugar concentration, mean arterial blood pressure and heart rate before and after intra-arterial (femoral) injection at *A* of 2·5 ml. guinea-pig anti-insulin serum (batch X).

When the injection of anti-insulin serum was repeated once or more at intervals of about a week, a strong foreign-protein reaction was provoked in both normal and adrenalectomized rabbits, whether conscious or anaesthetized. During this reaction, which lasted about half an hour, mean blood pressure and heart rate rose, the temperature of the sympathectomized ear fell and its vessels became constricted; the conscious animal became slightly restless and sneezed. The hyperglycaemic response, however, was similar to that of the normal animal receiving its first dose

(see Fig. 3). In one instance (Fig. 4), the fourth such dose of anti-insulin serum was preceded by an injection of normal serum. This normal serum provoked a strong foreign-protein reaction, including vasoconstriction in the sympathectomized ear, but only a slight transient increase in blood-sugar concentration. Anti-insulin serum injected half an hour later caused no such general reaction, but more than doubled the blood-sugar concentration.

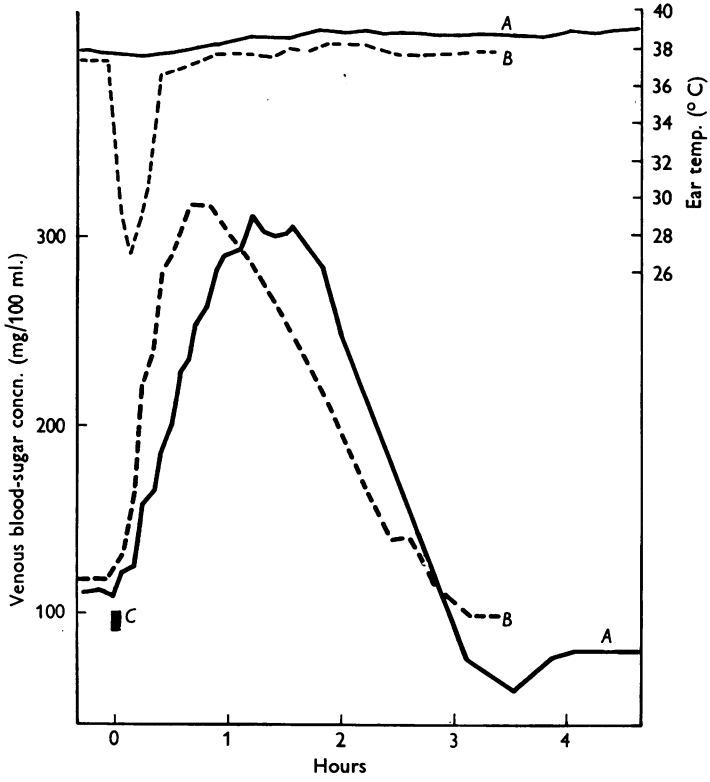

Fig. 3. Rabbit, 2·7 kg, fed, conscious; ear sympathectomized 2 months before first observations. Records of venous blood-sugar concentration (left-hand ordinate), and sympathectomized ear temperature (thermal junction, right-hand ordinate) before and after intravenous (marginal ear) injection at *C* of guinea-pig anti-insulin serum (1 ml./kg; batch X). Curves *A* show responses to 1st dose (2·7 ml.) and curves *B* show responses to 5th dose (2·8 ml.) 42 days later.

Rat. Normal, control and anti-insulin sera provoked no obvious foreign-protein reactions. No change in arterial blood pressure, pulse or respiration rates followed injection of control or anti-insulin serum (0·5 ml.) into an anaesthetized normal animal. Following normal or control serum the blood-sugar concentration fluctuated slightly and irregularly about the

pre-injection level, but showed no sustained change. Thus the maximum blood-sugar concentration observed in normal, fed, conscious rats after injection with control serum (four rats) or normal serum (three rats) was no more than 28 mg/100 ml. above the pre-injection level.

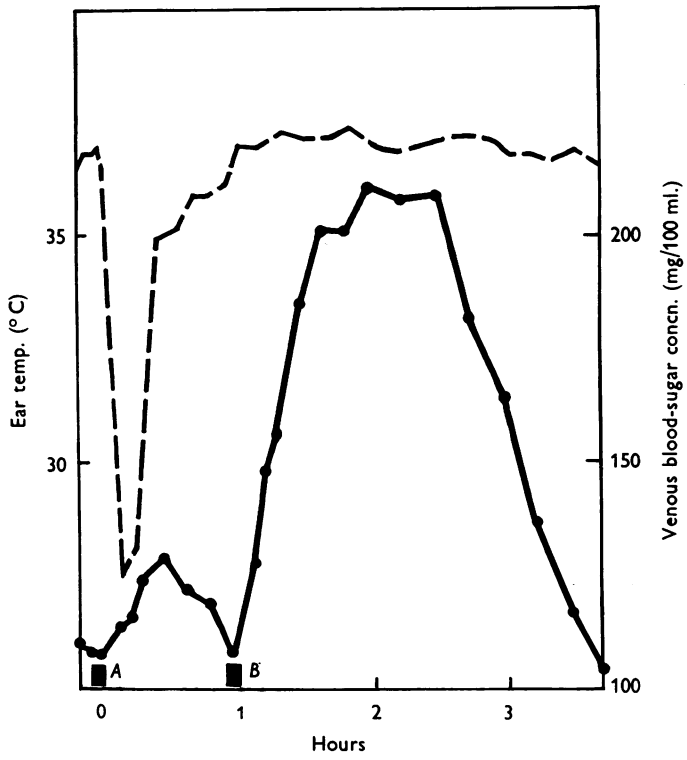

Fig. 4. Rabbit (same as in Fig. 3). Records of venous blood sugar concentration and sympathectomized ear temperature before and after intravenous (marginal ear) injection at *A* of normal guinea-pig serum (1 ml./kg) and at *B* of fourth dose of anti-insulin serum (1 ml./kg; batch X), 33, 19 and 5 days after 1st, 2nd and 3rd doses respectively of the anti-insulin serum.

Intravascular injection of anti-insulin serum (0·25–1·0 ml.) caused a marked but transient hyperglycaemia which was of about the same degree in both anaesthetized and conscious fed animals, either male or female. As Fig. 5 shows, after equal doses of anti-insulin serum the degree and duration of hyperglycaemia varied considerably in different animals though the initial rates of increase and subsequent maximum rates of fall in blood-sugar concentration were about the same. In eleven such experiments in which forty-four conscious, fed rats (180–220 g) were injected intravenously in groups of four with differing doses of six batches of anti-insulin serum, the mean rates of increase in blood-sugar concentration

during the first, second and third 15 min ($3{\cdot}31 \pm 0{\cdot}13$; $3{\cdot}12 \pm 0{\cdot}20$; $3{\cdot}13 \pm 0{\cdot}17$ mg/100 ml./min, respectively) were not significantly different from one another or from the mean rate measured over the whole period, namely $3{\cdot}16 \pm 0{\cdot}11$ mg/100 ml./min. Calculated from the maximum falls subsequently noted in 15 min in each of these animals, the mean maximum rate of fall in blood-sugar concentration ($5{\cdot}36 \pm 0{\cdot}24$ mg/100 ml./min) was significantly greater ($P < 0{\cdot}01$). In some animals a secondary rise in blood-sugar concentration occurred before or sometimes after the pre-injection level was regained.

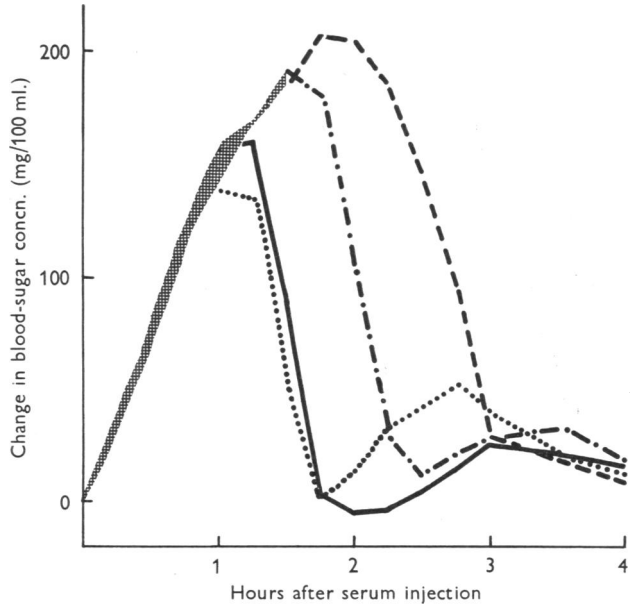

Fig. 5. Rats, female, conscious, fed. Changes in blood-sugar concentration in four animals (195–204 g) following injection into the tail veins of guinea-pig anti-insulin serum (1 ml.; batch Y).

Increasing doses of injected anti-insulin serum prolonged the period of hyperglycaemia and raised the maximum blood-sugar concentration induced. Figure 6 exemplifies the effects produced by three doses of one batch of anti-insulin serum in three groups each of four fed and conscious rats. The mean blood-sugar concentrations rose and subsequently fell at the same rates in each group but the maximum levels reached, and hence the periods of hyperglycaemia, increased with the dose injected.

A period of fasting before injecting anti-insulin serum reduces the degree but increases the duration of hyperglycaemia. Figure 7 shows the effect of fasting upon the response of female rats from the same litters to a dose of anti-insulin serum; male rats responded in the same way. In three

groups each of eight rats (four male and four female) the mean rate of rise in blood-sugar concentration in the first 45 min was significantly less ($P < 0{\cdot}01$) in the group starved for 24 hr ($1{\cdot}38 \pm 0{\cdot}08$ mg/100 ml./min) than in the fed animals ($3{\cdot}22 \pm 0{\cdot}15$ mg/100 ml./min); in the group starved for 48 hr ($1{\cdot}76 \pm 0{\cdot}15$ mg/100 ml./min) it was slightly greater ($P < 0{\cdot}05$) than that in the group starved for 24 hr.

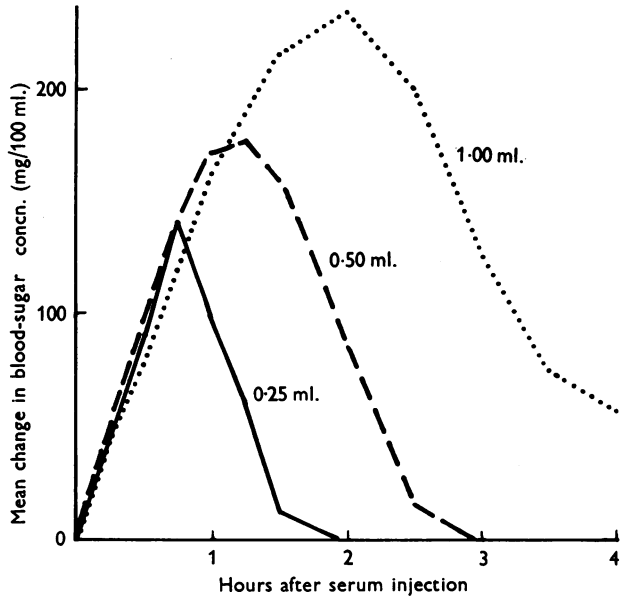

Fig. 6. Rats, female, conscious, fed. Mean changes in blood-sugar concentration in three groups of four animals of similar weights (178–218 g) following injection into the tail veins of 0·25, 0·50 and 1·0 ml. of guinea-pig anti-insulin serum (batch 6).

Anaesthesia has no appreciable effect upon the response of fed rats but reduces and prolongs the hyperglycaemic response of starved animals. Thus two rats deprived of food for 24 hr and then anaesthetized were each injected with 0·5 ml. of a batch of anti-insulin serum which provoked a conspicuous hyperglycaemia in fed, anaesthetized animals. In both rats the blood-sugar level rose steadily but at the very slow rates of 0·24 and 0·27 mg/100 ml./min for the period of 2 hr during which regular samples were taken.

When anti-insulin serum (2 ml./kg; batch 34943) was repeatedly injected intravenously into four normal male conscious rats 6, 19 and 27 days after the first dose, the hyperglycaemic response to the last was greater than those observed previously. Control serum (batch 48) injected in the same dose 34 days after the first serum injection increased the blood-sugar concentrations by 40–50 mg/100 ml. in 30–45 min. One of these animals

was therefore injected under anaesthesia 7 days later, first with control serum (0·5 ml.) and then 1 hr later with anti-insulin serum (0·5 ml.). The control serum produced an immediate transient general reaction, shown by a fall in blood pressure and pulse rate, laboured respirations and a marked increase in blood-sugar concentration to 220 mg/100 ml. in 30 min. Subsequent anti-insulin serum injection, however, caused only a rise in blood-sugar concentration.

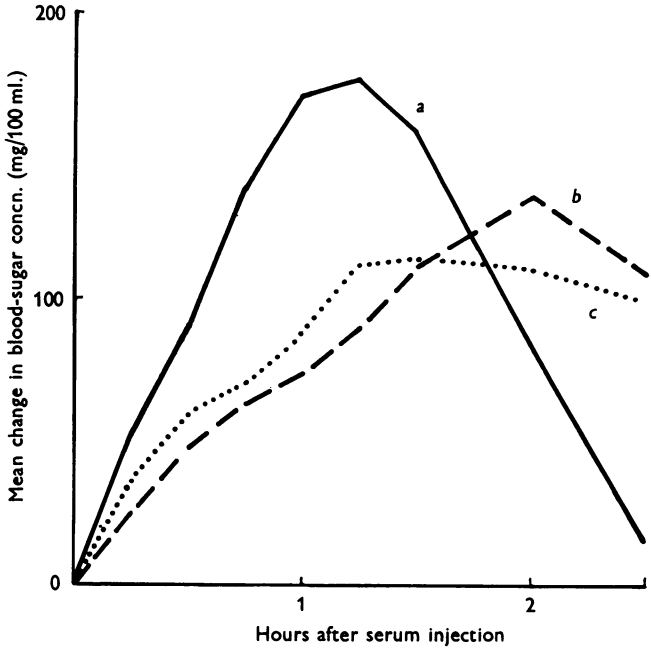

Fig. 7. Rats, female, conscious. Mean changes in blood-sugar concentration in three groups each of four animals of equal weights (210–218 g) following injection into the tail vein of anti-insulin serum (0·5 ml; batch 6) in the fed state (*a*) and after 24 hr (*b*) and 48 hr (*c*) fasting.

Assay of anti-insulin activity

One specimen of anti-insulin serum was kindly assayed for us by Messrs Boots Pure Drug Co. Ltd., by the mouse convulsion method; they assayed the residual insulin activity after mixing serum (2 ml.) with an excess (3 u.) of recrystallized bovine insulin. Of this sample (batch Y), 1 ml. neutralized the effects of 1·3 u. of insulin. The hyperglycaemic effects of graded doses (0·25, 0·50 and 1·00 ml.) of this and other batches of anti-insulin serum in groups of four conscious, fed rats were compared. Table 1 shows the batches of serum used, the doses injected and the areas under the response curves produced in each rat. Figure 5 illustrates the individual

responses produced in a typical experiment. The areas, A, were calculated by using the equation

$$A = \Sigma \Delta BS \times 15 \text{ mg/100 ml.} \times \text{min},$$

where ΔBS is the difference between the blood-sugar concentration at each 15 min interval from the time of serum injection to the time at which the concentration returns to the pre-injection level (or begins to rise again) and the pre-injection level itself.

TABLE 1. Areas under dose–response curves of female, fed, conscious rats injected intravenously with anti-insulin serum

Serum batch	Dose (ml.)	Area ($A \times 10^{-4}$)* Rat 1	Rat 2	Rat 3	Rat 4	Mean	Anti-insulin potency (u./ml.)
Y	0·25	0·45	0·31	0·27	0·43	0·37	1·3
	0·50	0·93	0·70	0·84	0·84	0·83	
	1·00	0·98	2·45	0·84	1·59	1·45	
	0·77 + 0·5 u.*	0·51	0·58	0·62	0·47	0·54	
6	0·25	0·56	0·90	0·84	0·52	0·71	2·5
	0·50	1·25	0·91	1·58	2·58	1·58	
	1·00	2·60	4·41	3·10	3·14	3·31	
16204	0·25	0·86	0·57	0·56	0·91	0·73	2·3
	0·50	1·74	1·56	1·00	1·52	1·46	
14108	0·25	1·34	1·03	0·72	1·48	1·14	3·4
	0·50	2·86	0·96	2·08	2·14	2·01	
227328	0·50	1·32	0·94	1·76	1·24	1·32	2·1
424	0·50	1·50	1·03	0·95	0·89	1·09	1·8

* See text.

The area thus calculated is directly related to the dose of anti-insulin serum injected. For serum batch Y, for example, the relation between area and volume injected is given by the formula

$$A = 0{\cdot}89 + 1{\cdot}42\ (D - 0{\cdot}58),$$

where D is the volume (ml.) of serum injected.

Since 1 ml. of this serum contains sufficient antibody to neutralize 1·3 u. insulin, 0·5 u. should neutralize half the antibody in 0·77 ml. serum and leave unaffected antibody sufficient to produce a response equivalent to an area of 0·62. As is shown in Table 1, the mean area under the curves produced in four rats by this mixture was 0·54, a sufficiently good approximation to the estimated figure for our purpose. This example suffices to show that this method may be used to determine approximately the insulin-neutralizing potencies of the sera. Assuming that the area under a response curve is directly proportional to the insulin-neutralizing potency of the injected serum and that inactive serum produces no effect, the anti-insulin activity of the other batches was calculated from the results shown in Table 1.

Disappearance of insulin from the blood

An attempt was made to demonstrate the disappearance of insulin from the blood of an injected rabbit by using the isolated rat-diaphragm method of plasma insulin assay (Wright, 1957). The insulin-like activity of undiluted plasma obtained before was slightly greater than that of plasma drawn 45 min after injection of anti-insulin serum. It was thought, however, that the difference was probably insignificant. The matter was not pursued further because of the relative inaccuracy of this method of assay and also because of the difficulty of securing an adequate sample of blood for testing (25 ml.) without at the same time provoking adrenaline release, as shown by vasoconstriction in the sympathectomized ear.

DISCUSSION

In view of previous work by others (for references see Wright, 1960) and especially that of Moloney and his co-workers (Moloney & Coval, 1955; Moloney & Goldsmith, 1957; Moloney & Aprile, 1959) it seems reasonable to conclude that the transient hyperglycaemia provoked in the rat, cat and rabbit by the injection of guinea-pig anti-insulin serum is due to the neutralization of their endogenously secreted hormone by insulin antibodies which are not species-specific. Although we have failed to demonstrate the disappearance of insulin from the blood, one of us (Wright, 1959*a*) has shown that, *in vitro*, serum from some of the insulin-treated guinea-pigs used in the present experiments will abolish the stimulant effect of various insulins upon glucose consumption by the isolated rat diaphragm, but not that of synthetic hypoglycaemic agents such as synthalin and phenethyldiguanide. Further, it is clear that in normal animals the hyperglycaemia is not due to the introduction of a foreign protein or to release of endogenous adrenaline. In the normal cat, for example, a general reaction was produced by both normal and anti-insulin serum, but hyperglycaemia followed injection only of the latter. In the normal rabbit and rat neither normal nor anti-insulin serum provoked a general reaction and no vasoconstriction suggesting adrenaline release was observed in the sympathectomized ear of the rabbit. In both normal and adrenalectomized rabbits anti-insulin serum, but not normal serum, induced a conspicuous hyperglycaemia.

A feature of interest from the immunological point of view is that the intensity of the hyperglycaemic response in rabbits is not diminished by repeated injections of anti-insulin serum; thus in Fig. 3 the response to the 5th dose (curve *B*) does not differ significantly from that to the first (curve *A*). The response to the first injection could be due entirely to the neutralization of endogenous insulin by the antibody, but later responses

could be partly due to foreign-protein reactions which are apparent in the sensitized animal. In Fig. 4 it is seen, however, that the injection of normal serum into the same sensitized animal referred to in Fig. 3 produces a foreign-protein reaction but only very mild hyperglycaemia, whilst anti-insulin serum injected soon after evokes no foreign-protein reaction but hyperglycaemia which, though still conspicuous, is less than that found in previous or subsequent experiments in which anti-insulin serum alone was injected. In the normal rat repeated injections of anti-insulin serum resulted in an increase in the hyperglycaemic response and control serum produced marked hyperglycaemia associated with signs of a non-specific foreign-protein reaction in sensitized animals. The implications of these observations are not known and this matter has not been pursued further. It does, however, seem certain that endogenous release of adrenaline plays no significant part in the hyperglycaemic response induced in rabbits by anti-insulin serum, whether it is given for the first time or to an already sensitized animal.

The failure of the anti-insulin serum to provoke a hyperglycaemia in the guinea-pig was not unexpected. Moloney & Coval (1955) have shown that serum from guinea-pigs treated with bovine or pig insulins will neutralize the actions in mice of bovine, pig, sheep and rabbit insulins, but not that of insulin from the guinea-pig itself. Such insulin-treated guinea-pigs remain normoglycaemic and tolerate convulsive doses of pig insulin. They concluded, therefore, that the guinea-pig secretes insulin which is immunologically distinct from the hormones secreted by pigs, sheep, rabbits and cattle. Harris, Sanger & Naughton (1956) have found that bovine, pig, sheep and horse insulins differ slightly from one another in chemical composition, whilst minor immunological differences have been demonstrated by Berson & Yalow (1959). If, as seems likely, chemical structure is related to immunological behaviour, then the structure of guinea-pig insulin must differ markedly from that of any of the insulins mentioned above. Indeed, Goldsmith & Moloney (1957) have already shown that guinea-pig insulin possesses at least one physical property which distinguishes it from bovine insulin. It would be of interest, therefore, to extend the range of animals injected with guinea-pig anti-insulin serum; those which fail to become hyperglycaemic might also secrete insulin which differs in composition from those which have been investigated so far.

The estimates of insulin-neutralizing activity are admittedly only approximate but they have proved a useful guide for further work. They are higher than those obtained with similarly treated guinea-pigs by Moloney & Goldsmith (1957), whose titres generally ranged from 0·23 to 1·04 u. insulin/ml. serum and were only occasionally higher. The guinea-pigs from which all but one of the present batches (No. 16204) were ob-

tained yielded consistently more active serum than that of other guinea-pigs treated in the same way. It does appear, however, that the guinea-pig is capable of producing more potent anti-insulin serum than other animals. As such it proved very useful for our purposes, but since it is a small animal whose reaction to insulin as an antigen is very variable (Moloney & Goldsmith, 1957) it is not convenient for the production of large amounts of consistently potent serum.

The uniform rapid rise in blood-sugar concentration that begins so quickly after the injection of anti-insulin serum points to a possible explanation for the hyperglycaemia. The initial mean rate of increase in blood-glucose concentration in fed rabbits (3·04 mg/100 ml./min) is not significantly different from that found in a much larger group of fed rats ($3{\cdot}16 \pm 0{\cdot}11$ mg/100 ml./min); the sugar contents of bloods examined by the present method and by the specific glucose oxidase method were identical. From this the rate at which glucose enters the extracellular space may be calculated by assuming that this space is equivalent to 20 % of the total body weight (Gamble, 1947) and that the concentration of glucose in the plasma, and hence in the extracellular space, is in the rabbit 1·5 times (Armin & Grant, 1959) and in the rat 1·3 times (personal observations in ten normal rats) that of the whole-blood concentration. On this basis glucose enters the extracellular space of the rabbit at a rate of 0·91 mg/min/100 g body weight, which is slightly greater than that in the rat (0·82 mg/min/100 g body weight). In the fasting rat the rate of glucose accumulation (0·36 mg/min/100 g body weight) is slower. In fed animals glucose enters the blood stream from the intestines, and the difference between the rates of glucose accumulation in fed and fasting rats may therefore represent the rate of glucose absorption from the gut (0·46 mg/min/100 g body weight). The liver is the major, if not the only, other source of glucose in the blood and it is still very doubtful whether insulin exerts any effect upon its metabolism. On the other hand, insulin does affect metabolism in extra-hepatic tissues, of which muscle and fat are the most important (see Stadie, 1954). It seems reasonable to conclude, therefore, that the glucose accumulating in the fasting injected rat represents the glucose normally consumed by extra-hepatic tissues under the influence of endogenously secreted insulin. If so, this rate is about a quarter of the total glucose turnover rate of normal rats, namely 1·67 mg/min/100 g body weight according to Feller, Strisower & Chaikoff (1950). The latter rate, however, includes the glucose consumption of the liver and also of the brain, which is not influenced by insulin. This hypothesis provides a working explanation for the hyperglycaemic effect of anti-insulin serum, but further work is required for proof.

SUMMARY

1. Serum from guinea-pigs treated with repeated subcutaneous injections of bovine insulin was injected intravascularly into rabbits, rats, a cat and a guinea-pig.

2. The transient hyperglycaemia induced in all animals except the guinea-pig is thought to be due to neutralization by insulin antibodies in the serum of endogenously secreted insulin; it is not due to non-specific foreign-protein reaction.

3. The degree and duration of hyperglycaemia produced in the rat is related to the dose of intravenously injected anti-insulin serum; a rough method of assay of insulin-neutralizing potency is described.

4. The rapid initial rate of increase in blood-sugar concentration observed in fasting rats probably reflects the normal rate of glucose consumption by insulin-sensitive tissues.

We wish to acknowledge the generous help of Messrs Boots Pure Drug Co. Ltd., who provided the recrystallized bovine insulin and especially of Mr K. L. Smith and Mr V. J. Birkinshaw who assayed the anti-insulin potency of serum Batch Y. We also wish to thank the Medical Research Council for a grant and Miss Sheila Haizelden and Mr Kenneth Kilbourn for technical assistance.

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