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ADRENALINE RELEASE DURING INSULIN HYPOGLYCAEMIA IN THE RABBIT

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Since the work of Cannon, McIver & Bliss (1924), it has been generally accepted that during insulin hypoglycaemia adrenaline is reflexly released from the adrenal glands when blood sugar falls below a certain critical level and that the effect of this release is to restore low blood sugar towards normal levels. Recently, however, other views have been put forward. Thus, Dunér (1953, 1954) finds that an inverse proportion exists between the level of blood sugar and adrenaline secretion; a rise of blood sugar decreases whereas a fall increases the output of adrenaline from the glands. According to Weil-Malherbe & Bone (1954) the blood concentration of adrenaline falls after insulin has been given. Further, the observations of Simeone & Vavoudes (1948) and others suggest that adrenaline, in man at least, is not responsible for the restoration of the normal level of circulating glucose after insulin hypoglycaemia. Our observations on the rabbit, now reported, bring new evidence to show that 'adrenaline' is released and to support the hypothesis of a critical level. They show also that while 'adrenaline' release usually mitigates the fall of blood sugar it is not the only factor involved in restoring the normal level of blood glucose after insulin hypoglycaemia.

METHODS

Details of the methods have already been published (Grant, 1935; Armin & Grant, 1953, 1955, 1957*a*, *b*). Briefly the indicator for the presence of adrenaline or other constrictor substance in the circulating blood of the rabbit receiving insulin is vasoconstriction in its denervated or sympathectomized ear. The constriction is detected and its degree assessed both by naked eye examination of the depilated ear (transmitted daylight) and by ear temperature (copper-constantan thermocouple). The concentration of the constrictor substance in the blood is assayed by injecting 0·1 ml. blood or plasma into the central artery of the denervated ear of another rabbit and comparing the constrictor effect with that of adrenaline in known concentration. Special precautions are taken to prevent the release of 5-hydroxytryptamine during the separation of plasma (Armin & Grant, 1957*a*). Heparin is the anticoagulant used. The rate of release of the constrictor substance

into the circulation is assayed by determining a few days later the rate of adrenaline infusion required to reproduce the constriction brought about by the insulin injection.

Rabbits with half or three-quarter lop ears are used. One ear is denervated at least a week beforehand by excising the superior cervical ganglion and portions of the great and posterior auricular nerves. Since the rabbit is apt to eat the distal part of an insensitive ear if this is long enough to come within reach of its mouth, in animals with long ears the sensory supply is left intact and the sympathetic supply removed by excising both superior cervical and stellate ganglia. Sympathectomized vessels are as sensitive to constrictor substances as are those denervated (Armin, Grant, Thompson & Tickner, 1953). In what follows, the term 'denervated' vessels includes also those deprived of their sympathetic supply alone.

The rabbits are fed on pellets (Diet S.G. 1, E. Dixon and Co.; protein carbohydrate ratio 1:3 approximately) with the addition of 6–8 oz. (0·17–0·23 kg) cabbage daily and with water always available. Observations are almost always begun between 9 and 10 a.m. and last 2–3 hr. Food is withdrawn 16–18 hr previously.

Conscious animals are restrained by the box previously described (Armin & Grant, 1957*b*). Light anaesthesia and prolonged quietude are attained by the subcutaneous injection of phenobarbital sodium 1–1·25 ml./kg 10% (w/v) followed about 1 hr later by the intravenous injection of pentobarbital sodium 0·25 ml./kg. Any dissection required for the insertion of catheters is carried out under local procaine anaesthesia. In the conscious animal the vessels used for catheterization are the central artery and marginal vein of the ear; in the anaesthetized animal, usually the femoral artery and vein, sometimes the carotid artery and the jugular vein. When required, arterial blood pressure and pulse are measured by mercury or capacitance manometer (Armin & Grant, 1957*a*) and respiration rate by means of a waistcoat (Armin & Grant, 1951).

The insulin (AB insulin, British Drug Houses or Parke, Davis) is injected intravenously in doses of either 0·5, 1 or 3 units per rabbit, the volume for injection being made up to 0·5 or 1 ml. with 0·9% NaCl solution.

Blood glucose is estimated by a modification (Wright, 1957) of the method described by King (1951). Arterial blood samples of 0·2 ml. are taken as described by Armin & Grant (1957*b*) at 5 min intervals throughout the observation, insulin being injected between the third and fourth samples. It is estimated that the blood glucose levels determined by this method are correct to the nearest 2 mg/100 ml. (S.D. = 2·2 mg/100 ml.).

To prevent the release of adrenaline from the adrenal glands several procedures are adopted. In some animals both glands are denervated by excising portions of the nerves going to them, care being taken not to interfere with the blood supply to the glands. In others, the right gland is denervated and the left excised. In others again, both glands are excised at a one-stage operation (Armin & Grant, 1955). Animals deprived of both glands are given 10 mg cortisone acetate intramuscularly at operation and subsequently either 0·5 mg DOCA or 1·25 mg cortisone acetate, daily or every other day; they are examined after death for completeness of adrenal removal.

All observations are carried out on a table the temperature of which can be controlled; heating is adjusted to keep rectal temperature nearly constant. Since denervated ear temperature in the undisturbed conscious or anaesthetized animal remains parallel to, and from 2 to 4° C below, rectal temperature, rectal temperature is not usually shown in the figures and ear temperature is adjusted a few tenths of a degree higher or lower to allow for the departure of rectal temperature from the initial level. Further to simplify the figures, ear temperature, though measured every minute, is charted for only every 5 min except where the beginning of a rise or fall requires to be noted.

RESULTS

Level of arterial blood glucose in control animals

Under our conditions the mean value of the initial level of arterial blood glucose in the conscious fasting animal is 88 mg/100 ml. (S.D. = 10 mg/100 ml.);

individual values range from 72 to 119 mg/100 ml. Under light anaesthesia, the mean value is 83 mg/100 ml. (S.D. = 11 mg/100 ml.) and the range from 68 to 108. In control observations on both conscious and anaesthetized animals blood glucose usually remains about its initial level for the next 2 hr. If, however, the initial values are near the upper or lower limits of the range, they tend to fall or rise towards the mean value.

Hypoglycaemic response to insulin

Blood glucose concentration begins to fall within 5 min after the insulin injection. The rate and degree of fall vary considerably, even in the same animal on successive occasions. Usually, however, independently of the dose, the fall continues steeply for 20–30 min but is checked when the level has fallen to between 40 and 60 mg. After the fall caused by 0·5 u. insulin, blood glucose then rises gradually, often with one or more fluctuations, towards the initial level, which is regained after about 2 hr. After 3 u. the level may or may not fall about 10 mg lower than with the smaller dose, but it remains low longer and the initial level is not reached until after 2 hr.

General effects of insulin administration

Except for vasoconstriction in the denervated ear, which is described below, we have detected few signs of disturbance after the insulin injection. In the conscious animal a moderate tachycardia (e.g. pulse rate rising from 250 to 300 beats/min) develops with the onset of the constriction, but there is little or no rise of mean blood pressure. In the anaesthetized animal both pulse rate and blood pressure may remain unchanged or blood pressure may fall 10–20 mm Hg with the fall of blood sugar, and rise again later as blood sugar recovers. The pulse rate rises and falls with the blood pressure. The normal ear vessels remain constricted or dilated according to whether body temperature is relatively low or high, or dilate or constrict as the rabbit becomes warmer or cooler (Grant, 1935). No convulsive attacks occurred in the animals with intact adrenal glands; an occasional rabbit has become either unduly restless or sleepy about half to one hour after the injection of insulin, the time when blood sugar is at its lowest.

Effect of insulin on the denervated ear vessels

The constriction is like that due to adrenaline in that it affects vessels of all calibres. Its course and degree are variable and unpredictable in different rabbits and even in the same rabbit in response to the same dose of insulin on successive occasions. The injection of insulin does not provoke any immediate change in the denervated vessels but they almost always begin to contract about 15 min later (range, 5–25 min). Usually constriction progresses rapidly to reach a moderate degree, ear temperature falling steeply to 5 or 10° C below

its initial level (Fig. 1). Constriction may be more gradual and the fall of ear temperature slower (Fig. 3). Sometimes constriction is only slight, ear temperature falling no more than 1 or 2° C; rarely it fails entirely. Exceptionally, on the other hand, constriction becomes gross; the ear pales greatly, as it does after a large haemorrhage, while its temperature falls to near that of the room (Fig. 2).

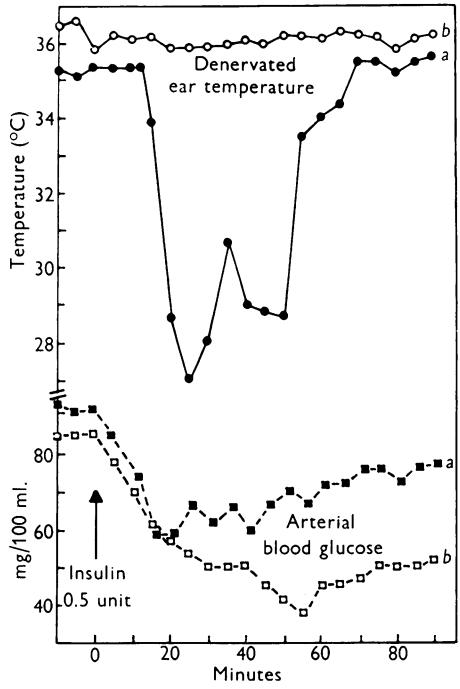

Fig. 1. Rabbit, 2·6 kg, conscious; 0·5 u. insulin. Denervated ear temperature and arterial blood glucose (*a*) before and (*b*) 8 days after excision of left adrenal gland and denervation of right.

Constriction may persist unchanged until the insulin effect begins to pass off, that is, about an hour after 0·5 u. and several hours after 3 u. Often, however, the constriction is interrupted by one or more periods of rapid and more or less complete relaxation (Figs. 1, 3). The first begins between 10 and 20 min after the onset of constriction and lasts 5 or 10 min, during which time ear temperature rises steeply and may reach its initial level before falling again as constriction returns. The final relaxation may be rapid or gradual and interrupted.

Constriction may be associated with a fall of mean blood pressure but this fall does not seem to be responsible for either the constriction or the fall of temperature. Thus, both occur although blood pressure does not fall; they may persist although blood pressure rises, and they may pass off although blood pressure remains low. Figure 7 shows a blood-pressure fall of at least 20 mm Hg

without material lowering of ear temperature. Again, blood transferred from the rabbit receiving insulin to the denervated ear of another rabbit does not fail to cause constriction when the denervated vessels of the donor rabbit are constricted. Moreover, transfers show that the rate and degree of ear cooling in

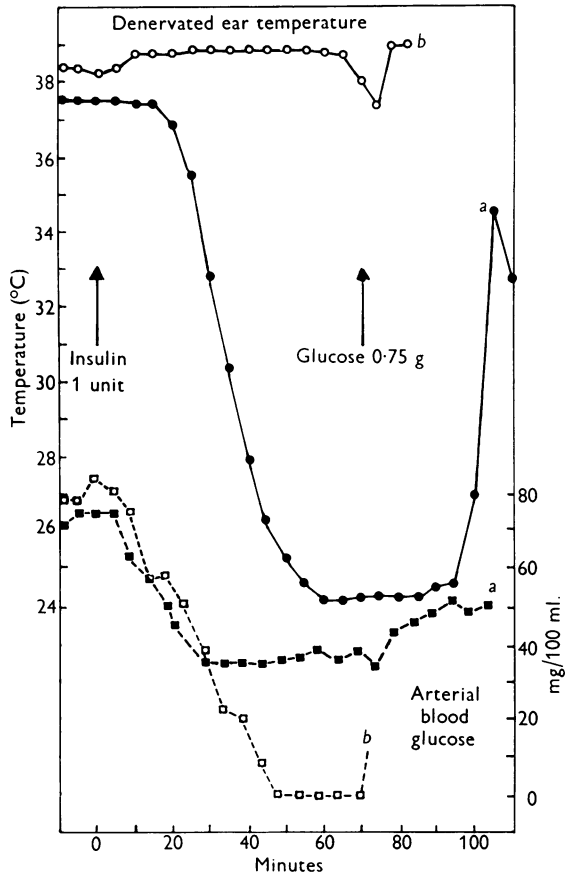

Fig. 2. Rabbit, 2·3 kg, anaesthetized; 1 u. insulin. Denervated ear temperature and arterial blood glucose (*a*) before and (*b*) 5 days after excision of left adrenal gland and denervation of right. On second occasion periodic respiration developed and 3 min later 0·75 g glucose was injected I.V.

the donor animal indicate the rate of change of blood constrictor activity. Thus, a gradual fall of ear temperature is associated with a gradually increasing constrictor activity in the transferred blood, and a rapid fall with a rapid increase. A steady level of reduced temperature is associated with a maintained degree of constrictor activity. So also a more or less rapid warming of the ear indicates more or less rapid decrease of constrictor activity. The time of temperature change follows closely the change of constrictor activity; the delay between an

increase or reduction as shown by transfers and the corresponding changes of ear temperature is no more than about 1 min. The adrenaline equivalent of the ear constriction during the insulin reaction is usually about 0·1 μg/l. but it is sometimes nearer 1·0 μg/l. The rapid intravenous injection of sufficient glucose to restore arterial glucose to normal is followed by a rapid return of ear temperature and of blood constrictor activity to initial levels. Control observations show that glucose given during an adrenaline infusion does not alter the constrictor activity of the blood or raise ear temperature.

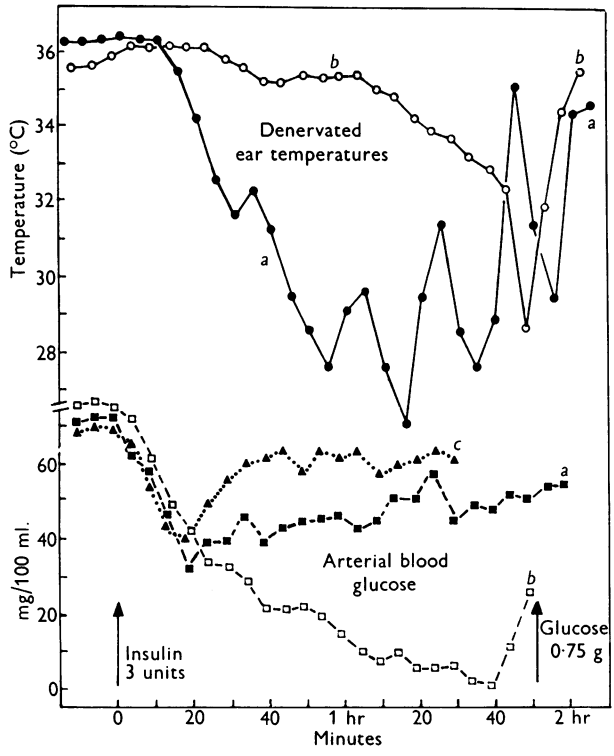

Fig. 3. Rabbit, 2·1 kg, anaesthetized; 3 u. insulin. Denervated ear temperature and arterial blood glucose (*a*) before and (*b*) 4 days after excision of left adrenal gland and denervation of right; periodic respiration developed and glucose injected I.V. (*c*) 13 days after operation with addition of adrenaline adjusted to match the ear temperature of (*a*) for 1½ hr after insulin. Rate of infusion ranged from 0·06 to 0·27 μg/kg/min; total infusion 32 μg.

This relationship between ear temperature and constrictor activity of the blood provoked by the injection of insulin is the same as that existing between ear temperature and the constrictor activity of the blood caused by the intravenous infusion of adrenaline. A change in the rate of infusion is followed within a minute by a change of constrictor activity and of ear temperature. Moreover, when in the same rabbit on a subsequent occasion the adrenaline

infusion is adjusted to give approximately the same temperature change as that previously caused by insulin, then the constrictor activity of the blood is much the same on the two occasions.

Rate of release of constrictor substance in response to insulin

It seems, therefore, that an adrenaline-like substance (or substances) is released into the blood stream during the insulin reaction and that we may justifiably use the results of adrenaline infusion to assay its rate of release. Figure 5 shows the temperature-lowering effect on the denervated ear of different rates of adrenaline infusion in a series of 40 rabbits. The effect varies considerably. Thus, while an infusion of 0·2μg/kg/min usually causes a fall of between 6 and 7° C, the fall may be no more than 3·5 or as much as 12° C. This is in keeping with the considerable variation in constrictor response provoked in different animals by injection of adrenaline or other constrictor substance into the artery of the denervated ear. In the individual rabbit, however, the responses on successive occasions are much the same. We have not detected any significant difference between the effects of infusions given to conscious or anaesthetized animals or between rabbits with adrenal glands intact, denervated or lacking. The rate of infusion usually required to cause a just appreciable fall of temperature (about 0·5° C) is about 0·006μg/kg/min. An infusion of 0·8μg/kg/min causes gross constriction with blanching of the ear and brings ear temperature near to that of the room; any further increase in the rate causes little or no further cooling. On this basis, Fig. 2 indicates the release of constrictor substance beginning about 15 min after insulin injection, rapidly rising to a sustained level and followed at about 1½ hr by a rapid return towards the resting level. The temperature fluctuations of Fig. 3 mean successive fluctuations in the rate of release.

We may say, then, that denervated ear temperature can be used to follow from minute to minute the release of constrictor substance into the circulation. The fall of ear temperature from its initial level indicates to within about 1 min the detectable beginning of increased release of constrictor. The rate of fall of ear temperature indicates the rate at which the release is increased, and a steady level of temperature connotes a steady rate of release. A rise of temperature denotes a decrease in output, and a return of ear temperature to its initial level means that the rate of release has returned to its resting level. We cannot by our methods measure the resting level but it must be small. Evidence for this is the negligible constrictor activity of resting blood on transfer, less than 0·01μg adrenaline/l., and the very small rate of adrenaline infusion, 0·006μg/kg/min, required to cause a just appreciable fall of ear temperature.

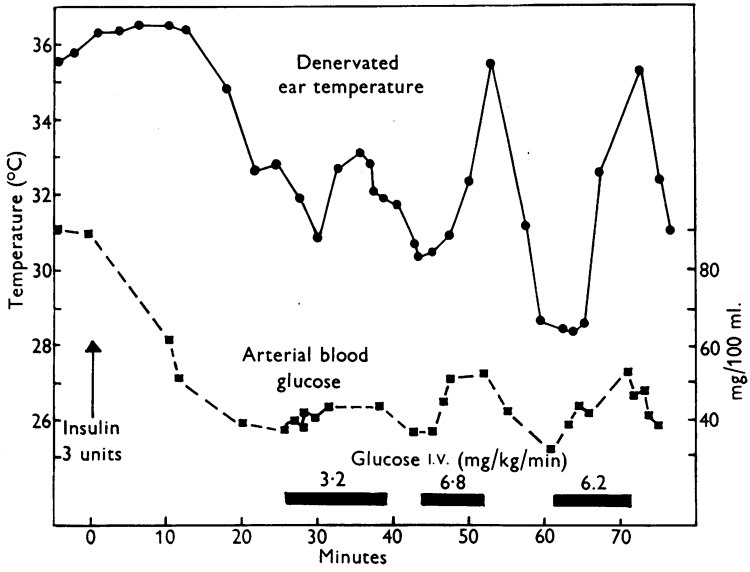

Fig. 4. Rabbit, 2·4 kg, conscious; 3 u. insulin and intermittent glucose infusion I.V. at rate of 3·2 6·8 and 6·2 mg/kg/min. Denervated ear temperature and arterial blood glucose.

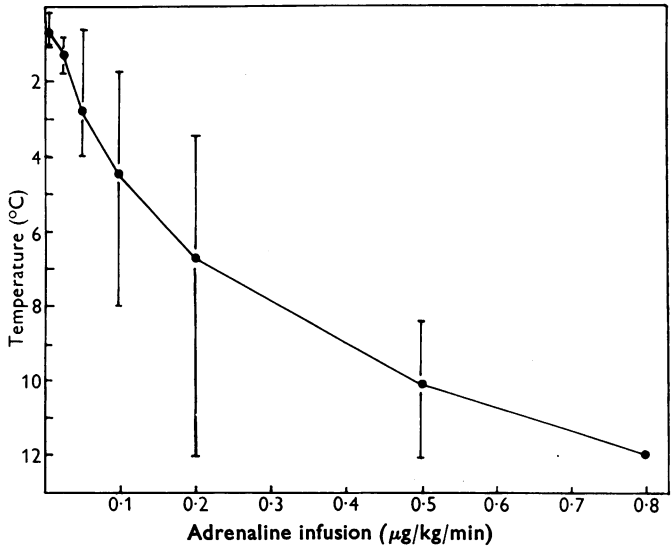

Fig. 5. Range and mean value of fall of denervated ear temperature caused by various rates of adrenaline I.V. infusion. Summary of observations on 40 rabbits.

The level of blood glucose and constrictor substance release

Constriction of the denervated vessels does not begin until blood glucose level falls below a critical level of about 70 mg/100 ml.; the actual level varies in different animals. Thus, Figs. 1 and 4 show that in two conscious rabbits ear temperature begins to fall when blood sugar has reached about 70 mg (Fig. 1) and about 50 mg/100 ml. (Fig. 4). In two anaesthetized rabbits the levels are about 55 in Figs. 2 and 3. The fall of temperature continues with the fall of blood sugar and if this goes below about 40 mg, ear temperature comes to lie near that of the room, e.g. Fig. 2. When blood sugar returns to within 10 mg below the level at which constriction began, ear temperature begins to rise, and Figs. 1 and 3 show that fluctuations in the level of blood sugar within this 10 mg zone are associated with more or less corresponding fluctuations of ear temperature. When, towards the end of the insulin reaction, blood sugar rises to about the critical level, the constriction comes to an end and ear temperature remains at about its initial level; Fig. 1 exemplifies this. On occasion, on the one hand constrictor substance may be released at a higher level of blood glucose, for example between 80 and 85 mg: on the other hand, release may not take place even though blood glucose falls to a low level, for example to below 30 mg/100 ml.

These observations on the spontaneous changes of blood sugar during the insulin reaction are borne out by others in which the fall of blood sugar is modified by infusions of glucose. Figure 4 illustrates a series of seven experiments, all with the same general result. In this example constriction began when blood sugar fell to about 50 mg, and continued as it fell lower. With each of the three infusions ear temperature began to rise when blood sugar reached about 40 mg. The first infusion raised blood sugar to only about 43 mg/100 ml.; the rise of ear temperature was slight and transient. With the second and third infusions, blood sugar was raised to just over 50 mg/100 ml. and on each occasion ear temperature rose rapidly to near the initial level. As blood sugar again fell below 50 mg/100 ml. ear temperature also fell. In six of these experiments the critical range seemed to be between 40 and 50 and in the remaining one between 50 and 60 mg/100 ml. At any time constrictor-substance release can be abruptly reduced to resting level by the intravenous injection of an adequate dose of glucose.

The release of constrictor substance usually occurs during the steep fall in blood sugar, and since constrictor substance is adrenaline-like it might well be responsible for the check to the fall and the subsequent slow rise. According to Laurence & Stacey (1952) hexamethonium (a ganglion-blocking agent which inhibits release of adrenaline) given along with insulin intensifies the hypoglycaemic effect in rabbits. We find, similarly, that hexamethonium, given during the course of insulin hypoglycaemia when the denervated ear vessels are

constricted and the fall of blood sugar has been checked, relaxes the vessels and provokes a renewed steep fall of blood sugar, for example from 50 to 20 mg/100 ml. This fall of blood sugar can be prevented by adrenaline infusion. Hexamethonium bromide in doses up to 20 mg/kg (not preceded by insulin), though relaxing normal ear vessels if these are constricted by sympathetic nerve activity, does not relax denervated ear vessels constricted by adrenaline infusion and does not alter blood sugar.

However, instances occur in which the steep fall of blood sugar after insulin injection is checked before constrictor substance is released. For example, in one anaesthetized rabbit given an infusion of insulin at the rate of 0·12 u./kg/hr, blood sugar fell steeply from 95 to about 55 mg/100 ml., at which level the fall was checked 5 min before the ear temperature began to fall. Other instances occur in which there is no indication of release although blood sugar falls below the critical level. For example, in one anaesthetized rabbit given 3 u. insulin blood sugar fell in 20 min from 70 to 40 mg/100 ml. and remained at that level for half an hour without a fall in the temperature of the denervated ear. These instances indicate that, on occasion at least, some factor other than release of constrictor substance controls the fall of blood sugar.

Effects of adrenal denervation and adrenalectomy

After bilateral adrenal denervation or excision of one gland and denervation of the other the animals remain well. Fasting blood sugar is not significantly lower than in the intact rabbit. These operations usually intensify both the degree and duration of the hypoglycaemia. So long as blood glucose remains above about 30 mg/100 ml., the denervated ear vessels do not become constricted (Fig. 1). Often, however, after 3 u., and sometimes after only 0·5 u. insulin, blood sugar falls to very low levels and may become unmeasurable. The rate of fall remains about the same as in the intact animal. Constriction of the denervated vessels, considerably delayed as compared with that in the intact animal, usually begins about half an hour after insulin injection, when blood sugar has fallen to between 20 and 30 mg, but may be postponed for an hour until blood glucose is below about 10 mg/100 ml. The constriction is often gradual for a time but may be rapid from the start (Fig. 7).

In the conscious animal convulsive struggles are liable to develop when blood glucose is below about 10 mg/100 ml. These render further detailed observation hardly feasible; moreover, struggling, by itself, suffices to provoke ear constriction (Grant, 1935). The injection of glucose immediately quietens the animal and relieves the constriction. In the anaesthetized animal, convulsive movements remain in abeyance, but after a period of gradual increase in rate of respiration (not due to a rising body temperature) periodic respiration of the Cheyne-Stokes type develops. During the periods of apnoea lasting for ½–2 min

the blood becomes deeply cyanosed, blood pressure falls and the pulse slows. With returning respirations, the blood quickly becomes arterial in colour and blood pressure and pulse rate rise. The ear constriction persists and intensifies (Fig. 7). During the constriction blood sugar may or may not rise slightly and transiently (Fig. 7). In only one instance (illustrated in Fig. 3) have we seen a

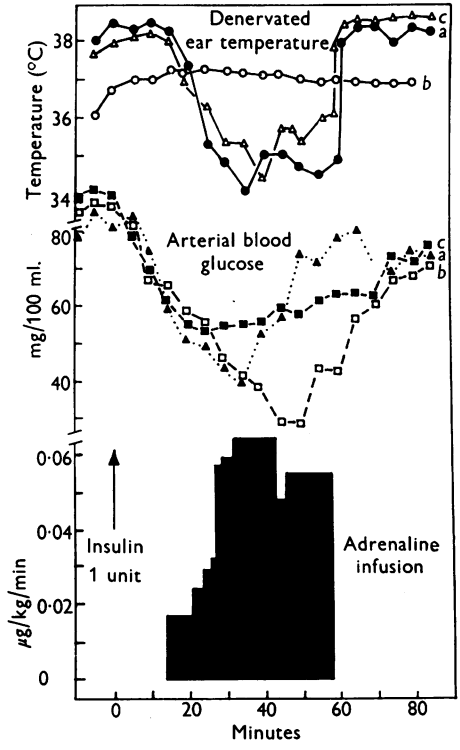

Fig. 6. Rabbit, 2·4 kg, anaesthetized; 1 u. insulin. Denervated ear temperature and arterial blood glucose. (*a*) Intact animal; (*b*) 26 days after excision of left and denervation of right adrenal gland; (*c*) 15 days after (*b*) with addition of adrenaline infusion to match the changes of ear temperature as in (*a*). Infusion rates ranged from about 0·02 to 0·06 μg/kg/min; total infusion 5·5 μg.

greater rise, and then periodic respiration did not occur. Administration of oxygen through a mask quickly abolishes the cyanosis and the periodic respiration. The blood continues a bright arterial colour though apnoea persists. Blood sugar remains low and the constriction of the ear vessels continues until the reaction is brought to an end, either by death or by the intravenous injection of glucose. With the cooling of the ear, constrictor activity of the blood, as shown by transfers to the denervated ear of another rabbit, increases and reaches a maximum, commonly about 0·1 μg/l. Further, when the constriction has been allowed to persist for some time, then in contrast to what happens in

the intact animal, constrictor activity and ear temperature are slower in returning to their initial levels after the injection of glucose.

These effects of adrenal denervation can be prevented by the infusion of adrenaline at a rate sufficient to reproduce the ear-temperature curve of the intact animal (see Figs. 3 and 6). Undue hypoglycaemia, convulsions and periodic respirations do not develop and the blood-sugar curve remains like that of the intact animal.

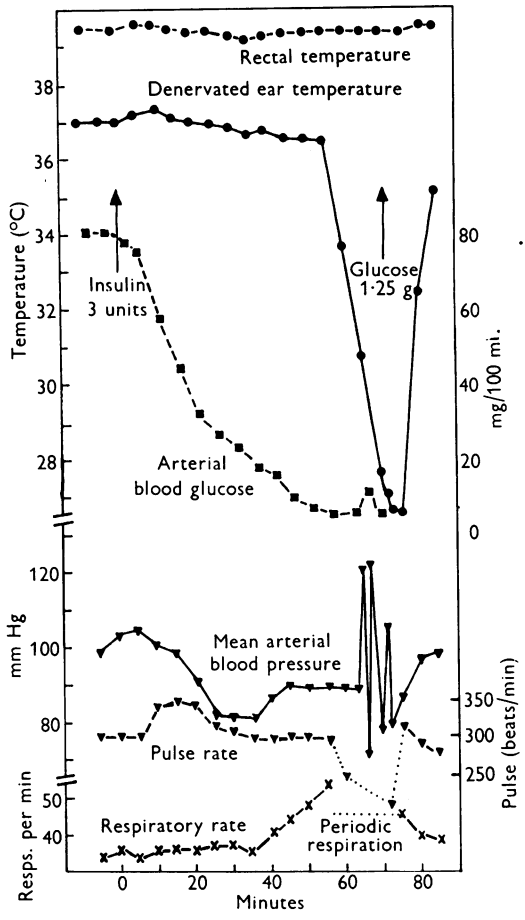

Fig. 7. Rabbit, 1·6 kg, anaesthetized; 3 u. insulin 2 days after bilateral adrenal denervation. Rectal and sympathectomized ear temperature and arterial blood glucose; mean blood pressure, pulse and respiration rates. Periodic respiration developed; 1·25 g glucose injected I.V.

Sometimes, however, after adrenal denervation, insulin causes no greater or longer-lasting fall of blood sugar than in the intact animal, although no constriction occurs in the denervated ear to indicate release of constrictor substance. In such instances infusion of adrenaline at a rate sufficient to reproduce

the ear vasoconstriction of the intact animal makes but little difference to the blood-sugar curve.

In the rabbit deprived of both adrenal glands the hypoglycaemic effect of insulin depends on the treatment given after operation. When the animal is maintained on DOCA, fasting blood sugar tends to be a little lower and insulin to provoke a greater and longer-lasting fall of blood sugar than in the intact state. In the cortisone-treated animal, on the other hand, fasting blood sugar tends to be a little higher and the hypoglycaemic effect of insulin to be reduced. These points are shown in Fig. 8, which illustrates the effects of DOCA

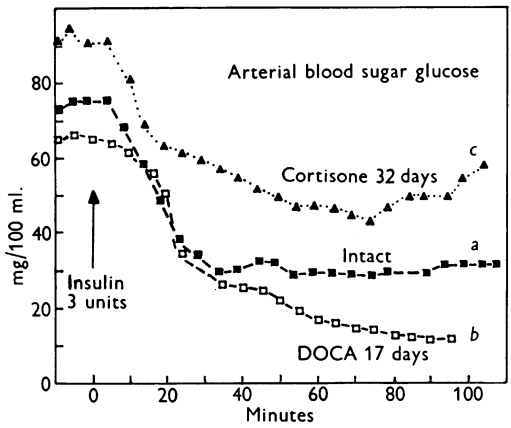

Fig. 8. Rabbit, 2·1 kg, anaesthetized; 3 u. insulin. Arterial blood glucose. (*a*) Intact animal before bilateral adrenalectomy after operation; maintained on cortisone until 9th day when similar blood glucose curve obtained; DOCA substituted until 17th day when curve (*b*) obtained. Cortisone then replaced and curve (*c*) obtained on 32nd day.

and cortisone in the same animal. Three days before the operation of bilateral adrenalectomy, 3 u. insulin provoked the hypoglycaemia of curve *a* which was accompanied by a strong vasoconstriction of the denervated ear, causing a cooling like that of Fig. 3. For 8 days after operation the rabbit was maintained on cortisone. On the 9th day insulin provoked a hypoglycaemia much like that in the intact animal, but with quicker recovery and without causing constriction in the denervated ear. DOCA was then substituted for cortisone, and on the 17th day after operation insulin caused the hypoglycaemia shown in curve *b*; this provoked periodic respiration and constriction in the denervated ear like that of Fig. 7 and necessitated the injection of glucose for recovery. DOCA was then stopped and cortisone again given. Curve *c* shows the effect of insulin on blood sugar at 32 days after operation. The relatively mild hypoglycaemia was not accompanied by vasoconstriction in the denervated ear.

Responses of normal ear vessels

One further point remains to be noted. It has been seen that in the intact animal the normal ear vessels show no particular change associated with the hypoglycaemia. But in the rabbit lacking adrenal glands, or with these glands denervated, the normal ear vessels respond to the hypoglycaemia by

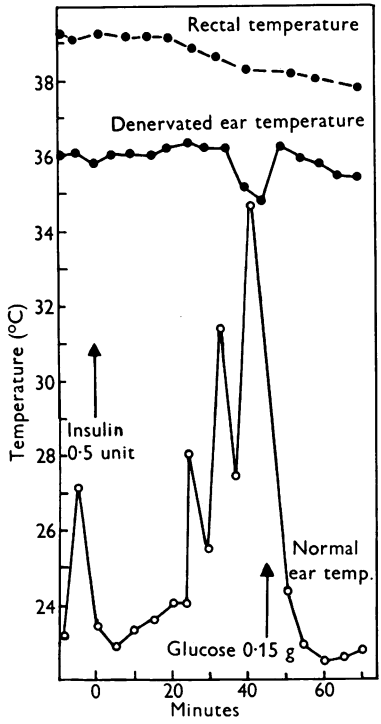

Fig. 9. Rabbit, 1·5 kg, 0·5 u. insulin 30 days after excision of left adrenal gland and denervation of right. Temperature of rectum, denervated and normal ears. Convulsion at 42 min; 0·15 g glucose injected I.V. at 45 min.

vasodilatation, as is exemplified by Fig. 9. In this instance, soon after denervated ear temperature began to fall a convulsion developed; glucose was injected and the reaction brought to an end. Blood sugar was not measured but must have fallen to about 10 mg/100 ml. The dilatation of the normal ear vessels began before the constriction in the denervated ear and took place although rectal temperature fell. With the injection of glucose, the normal ear vessels again became constricted while the denervated vessels relaxed.

The nature of the constrictor substance

The foregoing observations suggest that, in the intact animal, adrenaline from the adrenal glands is largely responsible for the constrictor activity

appearing in the circulating blood during hypoglycaemia. The adrenaline equivalent of this activity as assayed on the denervated ear of another rabbit ranges from 0·1 to 1·0μg/l. On three occasions on which the adrenaline equivalent of blood and the plasma separated from it was about 1·0μg/l. we tested the plasma also on the rat's uterus sensitized to adrenaline by dibenyline (Holzbauer & Vogt, 1954). The lowest concentration of adrenaline causing a distinct inhibition of the carbachol contraction of the uterus was 0·1μg/l. in two and 1·0μg/l. in the third. However, in none did the plasma cause either inhibition or augmentation of the carbachol contraction; but when adrenaline was added to the plasma to give these concentrations then the mixtures caused inhibitions like those of adrenaline alone. Because of the difficulties in detecting adrenaline in low concentration in plasma we do not regard this failure as necessarily indicating that the substance is not adrenaline. We have similarly failed with the rat's uterus to provide further information about the nature of the substance released into the blood stream in the adrenal denervated or adrenalectomized animal.

DISCUSSION

Denervated structures in various animals have been used by other workers to indicate 'adrenaline' release during insulin hypoglycaemia; for example the cat's heart (Cannon *et al.* 1924), the rabbit's pupil (Abé, 1924) and ear (Freeman, Smithwick & White, 1934) and the human limb (Freeman *et al.* 1934; Allwood, Ginsburg & Paton, 1957). We have shown, however, that the rabbit's ear, deprived of its sympathetic or both sensory and sympathetic nerves, is particularly suitable for this purpose, since not only the calibre of its vessels but also its skin temperature respond quickly to changes in the constrictor activity of the circulating blood. We have previously shown that reduced mean blood pressure plays little part in the constriction of the ear vessels associated with haemorrhage (Armin & Grant, 1955). So also in hypoglycaemia not only the calibre of the vessels but also the skin temperature seem but little affected by the changes in mean blood pressure that may occur under our conditions. Continuous recording of ear temperature thus provides a relatively simple means of following changes in this activity from minute to minute in the conscious or anaesthetized animal. The cooling of the ear is graded to the degree of constrictor activity in the blood, whether this is due to a substance released within the body or introduced from without. Checks are provided by the direct naked-eye examination of the ear vessels by transmitted light and by transfers of blood samples to the denervated ear of another rabbit.

The results of our observations agree, for the most part, with those of other workers and there seems to be no doubt that the hypoglycaemia caused by the injection of insulin into various animals stimulates the reflex release of an

adrenaline-like substance from the adrenal glands. We have not identified the substance, but in the intact animal it is probably mainly adrenaline. Gaddum & Holzbauer (1957) remark there is evidence of various kinds that the secretion of adrenaline, but not that of noradrenaline, is increased by hypoglycaemia. But we have shown that the adrenal glands are not the sole source of adrenaline-like activity in the blood during the insulin reaction, just as we have previously shown to be the case after a struggle, after haemorrhage or the administration of a pyrogen (Grant, 1935; Armin & Grant, 1955, 1957*b*). The substance responsible for this activity in the animal with denervated adrenals or without these glands is unknown. It can hardly be adrenaline: it might be noradrenaline entering the blood stream as a spill-over from widespread sympathetic-nerve activity. It seems to be released when blood glucose falls below about 40 mg/100 ml. But at the time when it is released the normal ear vessels, at least, show no indication of sympathetic-nerve activity, for they relax as the denervated vessels are constricted.

Other workers, like us, have assayed the 'adrenaline' output during insulin hypoglycaemia by determining the adrenaline infusion required to elicit the same changes in the test object used. Thus, Houssay, Lewis & Molinelli (1924) find that transfusion of adrenal-vein blood from a dog receiving insulin causes hyperglycaemia in another dog. To reproduce this hyperglycaemia by adrenaline requires an infusion of 5μg/kg/min. Abé (1924) estimates that less is required, usually over 0·5 and nearer 1·0μg/kg/min, to match the dilatation of the denervated pupil in the rabbit caused by apparently large doses of insulin. In man a urinary output of adrenaline like that caused by 0·1 u. insulin/kg requires an adrenaline infusion of the order of 0·15μg/kg/min (Elmadjian, Lamson, Freeman, Neri & Varjabedian, 1956). Our own findings are more in accord with the last than with the earlier results. The adrenaline infusion required to match the vasoconstriction caused in the denervated ear of the rabbit by doses of insulin of up to 3 u. per rabbit is often about 0·2μg/kg/min, rising to about 0·5μg for an unusually strong constriction, but is always under 1·0μg/kg/min. By the biological testing of adrenal-vein blood, Dunér (1954) estimates that 10 u. insulin cause in the anaesthetized cat an increase of adrenaline (but not of noradrenaline) secretion of up to tenfold or more over the resting level. The maximum adrenaline output in any of his experiments is 0·12μg/kg/min from one gland.

The effect of insulin on the 'adrenaline' concentration in the peripheral blood has also been estimated by a number of workers. Weil-Malherbe & Bone (1954), using a chemical method, are alone in finding that the injection of insulin entails an immediate lowering of the adrenaline concentration which precedes the onset of hypoglycaemia. By biological testing of plasma extracts, Holzbauer & Vogt (1954) find that in the undisturbed conscious dog the adrenaline content is always below 0·25μg/l. and sometimes less than a quarter of this.

After administration of insulin (0·2–2·1 u./kg) the adrenaline concentration ranged from 0·25 to 6·4μg/l. and no noradrenaline was detected. In one man the resting value was below 0·06μg/l. and after insulin (0·24 u./kg) rose to 1·8μg/l. In one man also after insulin, Armin & Grant (1957) found that the adrenaline equivalent of plasma ultrafiltrates rose from the resting level of less than 0·01μg/l. to between 0·1 and 1·0μg/l. These values are the same as those now found for the rabbit's blood.

The relation that has been seen to exist between ear temperature and blood sugar provides strong evidence to support the hypothesis of Canonn *et al.* (1924) and against that of Dunér (1954). Dunér does little more than remark that an inverse proportion exists between the level of blood glucose and the amount of adrenaline secreted from the suprarenal glands; hyperglycaemia decreases but hypoglycaemia increases secretion. He does not discuss the view of Cannon and his colleagues (Cannon *et al.* 1924). According to this, adrenaline output is not increased until blood sugar has fallen in the conscious cat to a critical level of 80–70 mg/100 ml. (60–110 mg under chloralose anaesthesia); with a continued fall of blood sugar the heart rate becomes faster, indicating a greater output of adrenaline (Cannon, 1929). Houssay *et al.* (1924) find that in dogs under chloralose anaesthesia extra adrenaline secretion does not occur until blood sugar has fallen to 50 mg/100 ml., while La Barre & Houssa (1932) state that it occurs when blood sugar falls below 75 mg and is much accentuated when blood sugar falls below 50 mg/100 ml. (They say 50 mg/l. but this seems a misprint.) Our own findings are that adrenaline-like activity in the blood is not increased until blood sugar has fallen below a critical level which varies for different rabbits, is usually from about 70 to 50 mg/100 ml. and is apparently the same for the animal either when conscious or lightly under the influence of phenobarbitone and pentobarbitone. Below this critical level there is a range of about 10 mg/100 ml. within which, as blood sugar is falling or rising, adrenaline activity is increasing or declining. Below this range, constrictor activity remains steadily and considerably increased, whether blood sugar is rising or falling. But a rise of blood sugar from below into this range is accompanied by a decrease of constrictor activity and a rise of blood sugar to the critical level and above is accompanied by a return of adrenaline-like activity to its resting level.

With the relatively small doses of insulin that we have used, blood sugar, after an initial steep fall, often remains within the critical range for some time and shows small fluctuations. These are associated with corresponding and considerable fluctuations in constrictor activity. We have found no reference in previous papers to such fluctuations in 'adrenaline' output during the insulin reaction, but Figs. 2 and 5 of Cannon *et al.* (1924) show variations in the increased heart rate, and Figs. 3 and 4 of Freeman *et al.* (1934) also show variations in the temperatures of rabbit's ear and human finger that might be

due to variations in 'adrenaline' output, though the data are insufficient to determine the point.

This description of the relation between blood sugar and adrenaline-like activity in the blood recalls that previously described by Grant (1935) for the relation between body temperature and vasomotor tone in the rabbit's normal ear. It suggests the existence of some central structure which is stimulated to activity when blood sugar falls below a certain critical level and which thus initiates the reflex release of 'adrenaline'. Dunér's (1953) observations suggest that a structure sensitive to changes in the blood-glucose concentration exists in the hypothalamus. It is to be remembered that in the experimental animal the actual concentration of glucose to which such a structure is likely to respond will probably be closer to plasma glucose than to blood glucose. It is known that in species other than the anthropoids (Somogyi, 1933, Peters & Van Slyke, 1946) the blood corpuscles contain but little sugar and therefore plasma sugar is considerably higher than blood sugar. According to our own observations plasma sugar in the rabbit is approximately 1·5 times that of whole blood.

While there is thus much evidence to show that the level of blood glucose determines 'adrenaline' release, there is less to show that in the intact animal the released substance modifies blood sugar. The check to the fall and the subsequent rise of blood sugar could be said to result not from 'adrenaline' release but from the operation of the other factor or factors which, as has been seen, can on occasion restore blood sugar. It is true that the general result of animal experiment is to show that the hypoglycaemic effect of insulin is potentiated when adrenaline release is prevented (*a*) *by denervation or demedullation of the adrenal glands* in cats (Cannon *et al.* 1924; Britton, Geiling & Calvery, 1928; Schlossberg, Sawyer & Bixby, 1933; Berg & Zucker, 1937) rabbits (Freeman *et al.* 1934) and dogs (Houssay *et al.* 1924; Crandall & Cherry, 1939); (*b*) *by adrenalectomy* in cats (Cannon *et al.* 1924) dogs (Crandall & Cherry, 1939) and rabbits (Sundberg, 1923): only Stewart & Rogoff (1923) found no difference in the effects of insulin in normal and adrenalectomized rabbits, but they used doses of insulin convulsant for normal animals—in these experiments cortisone was not given; Crandall & Cherry (1939) gave occasional doses of cortical extract to their dogs; Sundberg (1923) mentions that some of his rabbits had cortical transplants; (*c*) *after hexamethonium* in dogs (Schachter, 1951) and in rabbits (Laurence & Stacey, 1952). It has therefore been generally accepted in the past that adrenaline release is responsible for, or at least assists in, restoring low blood sugar towards normal levels, although it is recognized that recovery from insulin hypoglycaemia does not require the presence of the adrenal medulla (Peters & Van Slyke, 1946).

But it might be said of adrenal denervation and adrenalectomy, as French & Kilpatrick (1955) say of hexamethonium, that these operative procedures do not specifically prevent adrenaline release and may thus deprive the body of

some other mechanism for restoring blood sugar. However, it has been shown that if the adrenaline secretion prevented by all three measures is replaced by adrenaline infusion in approximately the amount released by the intact animal, then the blood-sugar curves are restored to normal. The fact that the blood-sugar curves after insulin are normal in the adrenalectomized but cortisone-maintained animal can be taken to mean that the factor or factors other than adrenaline which tend to restore blood sugar require sufficient cortisone for their operation. It may be that the maintenance dose of cortisone which we have given is actually more than is required to replace the lost normal amount. It seems reasonable to suggest that ordinarily the level of cortisone secretion in the rabbit is low and adrenaline is required to counteract the hypoglycaemic effect of insulin. On occasion, for some reason or other, cortisone secretion is increased and then blood sugar recovers after the administration of insulin without or with less than the normal secretion of adrenaline.

In man it seems that the insulin–blood-sugar curves are normal not only after adrenalectomy with cortisone maintenance (Bergenstal, Huggins & Dao, 1955; Ginsburg & Paton, 1956) but also after adrenal denervation (Simeone & Vavoudes, 1948; French & Kilpatrick, 1955). The effects of hexamethonium are doubtful, since the doses hitherto used have been too small to be adequate (Billington, Paton, Reynolds & Sherlock, 1954; Di Salvo, Bloom, Brust, Ferguson & Ferris, 1956). But it is known that adrenaline is released (Holzbauer & Vogt, 1954) and it is difficult to believe that this does not influence blood sugar. It is known also, that sufferers from Addison's disease are very sensitive to the action of insulin (Fraser, Allbright & Smith, 1941). However, there is at present no evidence to show that in man adrenaline release during insulin hypoglycaemia is closely related to a critical level of blood sugar.

One further point requires discussion. According to Allwood *et al.* (1957) the blood vessels of the normal human hand show a variable response during the insulin reaction, apparently not related to the hypoglycaemia; in the hand deprived of its sympathetic nerves the vessels contract. Ginsburg & Paton (1956) have shown that in the adrenalectomized human subject the normally innervated vessels dilate conspicuously. The same reactions occur in the rabbit's ear; that is, (*a*) no special reaction for the normal ear vessels in the intact rabbit, (*b*) constriction of the sympathectomized or denervated ear vessels in the intact rabbit and (*c*) dilatation of the normal ear vessels when the blood sugar falls below about 30 mg/100 ml. in the rabbit with adrenal glands denervated or lacking. The dilatation is not due to a rise of body temperature and promptly subsides when blood sugar is raised by the injection of glucose. According to Rössel & Osswald (1953–54) in intact conscious rabbits with body temperature held at 37° C and given large doses of insulin (30 u. subcutaneously) the constricted normal ear vessels begin to dilate when the blood sugar falls to 35 mg/100 ml., and when convulsions begin the dilatation is great. Constric-

tion returns when blood sugar rises to 60–70 mg/100 ml. after the injection of glucose. They explain the vasodilatation as the result of increased acetylcholine production due to a great reduction of the inhibiting influence of glucose on the activity of cholineacetylase. In our rabbits the circulating blood at the time exerts a constrictor effect on the denervated ear vessels. The normal ear vessels are much less sensitive to circulating constrictor substances and we attribute their dilatation to a central inhibition of sympathetic nerve activity.

SUMMARY

1. The denervated ear vessels have been used to study the release of an adrenaline-like substance ('adrenaline') into the circulation during insulin hypoglycaemia in the rabbit.

2. 'Adrenaline' is not released until blood sugar has fallen to a certain critical level and ceases when blood sugar again rises to that level.

3. Depending on the level of blood sugar attained, 'adrenaline' release may be persistent or intermittent during the insulin reaction.

4. The adrenal glands are not the sole source of the 'adrenaline'.

5. The general conclusion is reached that while 'adrenaline' usually mitigates the extent of the fall of the blood sugar, other factors are responsible for the restoration to normal levels.

6. The findings are discussed in relation to previous animal experiments and to recent observations by other workers on man.

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REFERENCES

ABÉ, Y. (1924). Das Verhalten der Adrenalinsekretion bei der Insulinvergiftung. *Arch. exp. Path. Pharmak.* **103**, 78–83.

ALLWOOD, M. J., GINSBURG, J. & PATON, A. (1957). The effect of insulin hypoglycaemia on blood flow in intact and sympathectomized extremities in man. *J. Physiol.* **139**, 97–107.

ARMIN, J. & GRANT, R. T. (1951). Observations on gross pulmonary fat embolism in man and the rabbit. *Clin. Sci.* **10**, 441–469.

ARMIN, J. & GRANT, R. T. (1953). The artery of the denervated rabbit's ear as a sensitive pharmacological test object. *J. Physiol.* **121**, 593–602.

ARMIN, J. & GRANT, R. T. (1955). Vasoconstrictor activity in the rabbit's blood and plasma. *J. Physiol.* **128**, 511–540.

ARMIN, J. & GRANT, R. T. (1957*a*). Further observations on vasoconstrictor substances in blood of rabbit and man. *J. Physiol.* **138**, 401–416.

ARMIN, J. & GRANT, R. T. (1957*b*). The vasoconstriction caused by a pyrogen. *J. Physiol.* **138**, 417–433.

ARMIN, J., GRANT, R. T., THOMPSON, R. H. S. & TICKNER, A. (1953). An explanation for the heightened vascular reactivity of the denervated rabbit's ear. *J. Physiol.* **121**, 603–622.

BERG, B. N. & ZUCKER, T. F. (1937). Blood sugar recovery from insulin hypoglycaemia after section of the splanchnic nerves. *Amer. J. Physiol.* **120**, 435–439.

BERGENSTAL, D. M., HUGGINS, C. & DAO, T. L-Y. (1955). Metabolic effects of adrenalectomy in man. In *CIBA Foundation Colloquia on Endocrinology*, **8**, 415–437.

BILLINGTON, B. P., PATON, A., REYNOLDS, T. B. & SHERLOCK, S. (1954). The effect of hexamethonium bromide on the circulatory and metabolic response to insulin hypoglycaemia in man. *J. Lab. clin. Med.* **43**, 880–887.

BRITTON, S. W., GEILING, E. M. K. & CALVERY, H. O. (1928). Medulliadrenal secretion and carbohydrate metabolism. *Amer. J. Physiol.* **84**, 141–156.

CANNON, W. B. (1929). Organisation for physiological homeostasis. *Physiol. Rev.* **9**, 399–431.

CANNON, W. B., MCIVER, M. A. & BLISS, S. W. (1924). A sympathetic and adrenal mechanism for mobilizing sugar in hypoglycaemia. *Amer. J. Physiol.* **69**, 46–66.

CRANDALL, L. A. & CHERRY, I. S. (1939). The effects of insulin and glycine on hepatic glucose output in normal, hypophysectomised, adrenal denervated and adrenalectomised dogs. *Amer. J. Physiol.* **125**, 658–673.

DI SALVO, R. J., BLOOM, W. L, BRUST, A. A., FERGUSON, R. W. & FERRIS, E. B. (1956). A comparison of the metabolic and circulatory effects of epinephrine, nor-epinephrine and insulin hypoglycaemia with observations on the influence of autonomic blocking agents. *J. clin. Invest.* **35**, 568–577.

DUNÉR, H. (1953). The influence of the blood glucose level on the secretion of adrenaline and noradrenaline from the suprarenal. *Acta physiol. scand.* Suppl. 102.

DUNÉR, H. (1954). The effect of insulin hypoglycaemia on the secretion of adrenaline and noradrenaline from the suprarenal of cat. *Acta physiol. scand.* **32**, 63–68.

ELMADJIAN, F., LAMSON, E. T., FREEMAN, H., NERI, R. & VARJABEDIAN, L. (1956). Excretion of epinephrine and norepinephrine after administration of insulin and methacholine. *J. clin. Endocrin.* **16**, 876–886.

FRASER, R., ALLBRIGHT, F. & SMITH, P. M. (1941). The value of the glucose tolerance test, the insulin tolerance test, and the glucose-insulin tolerance test in the diagnosis of endocrinologic disorders of glucose metabolism. *J. clin. Endocrin.* **1**, 297–306.

FREEMAN, N. E., SMITHWICK, R. H. & WHITE, J. C. (1934). Adrenal secretion in man. The reactions of the blood vessels of the human extremity, sensitized by sympathectomy, to adrenaline and to adrenaline secretion resulting from insulin hypoglycaemia. *Amer. J. Physiol.* **107**, 529–534.

FRENCH, E. B. & KILPATRICK, R. (1955). The role of adrenaline in hypoglycaemic reactions in man. *Clin. Sci.* **14**, 639–651.

GADDUM, J. H. & HOLZBAUER, M. (1957). Adrenaline and noradrenaline. *Vitam. & Horm.* **15**, 151–203.

GINSBURG, J. & PATON, A. (1956). Effects of insulin after adrenalectomy. *Lancet*, **271**, 491–494.

GRANT, R. T. (1935). Further observations on the vessels and nerves of the rabbit's ear, with special reference to the effects of denervation. *Clin. Sci.* **2**, 1–33.

HOLZBAUER, M. & VOGT, M. (1954). The concentration of adrenaline in the peripheral blood during insulin hypoglycaemia. *Brit. J. Pharmacol.* **9**, 249–252.

HOUSSAY, B. A., LEWIS, J. T. & MOLINELLI, E. A. (1924). Rôle de la sécrétion d'adrénaline pendant l'hypoglycémie produite par l'insuline. *C.R. Soc. Biol., Paris*, **91**, 1011–1013.

KING, E. J. (1951). *Micro-methods in Medical Biochemistry*. 2nd ed. London. Churchill.

LA BARRE, J. & HOUSSA, P. (1932). À propos des variations de l'adrenlinémie au cours de l'hypoglycémie insulinique. *C.R. Soc. Biol., Paris*, **109**, 967–969.

LAURENCE, R. D. & STACEY, R. S. (1952). Effect of hexamethonium on the response to insulin in animals and man. *Brit. J. Pharmacol.* **7**, 255–260.

PETERS, J. P. & VAN SLYKE, D. D. (1946). *Quantitative Clinical Chemistry*. 2nd ed. Vol. 1. London: Ballière, Tyndall and Cox.

RÖSSEL, W. & OSSWALD, H. (1953–54). Das Verhalten der Ohrgefässe des Kaninchens in der Hypoglykämie. *Z. ges. exp. Med.* **122**, 465–469.

SCHACHTER, M. (1951). Hexamethonium and insulin hypoglycaemia. *J. Physiol.* **115**, 206–209.

SCHLOSSBERG, T., SAWYER, M. E. MCK. & BIXBY, E. M. (1933). Studies of homeostasis in normal sympathectomised and ergotamised animals. 3. The effect of insulin. *Amer. J. Physiol.* **104**, 190–194.

SIMEONE, F. A. & VAVOUDES, H. (1948). The early effect of lumbodorsal sympathectomy upon the response to insulin in man. *Surgery*, **24**, 326–341.

SOMOGYI, M. (1933). The distribution of sugar and rate of glycolysis in the blood of some mammals. *J. biol. Chem.* **103**, 665–670.

STEWART, G. N. & ROGOFF, J. M. (1923). The action of insulin on adrenalectomised rabbits. *Amer. J. Physiol.* **65**, 342–345.

SUNDBERG, C. G. (1923). Sur l'action de l'insuline après l'extirpation des capsules surrénales. *C.R. Soc. Biol., Paris*, **89**, 807–810.

WEIL-MALHERBE, H. & BONE, A. D. (1954). The effect of insulin on the levels of adrenaline and noradrenaline in human blood. *J. Endocrin.* **11**, 285–297.

WRIGHT, P. H. (1957). Plasma-insulin estimation by the rat diaphragm method. *Lancet*, **273**, 621–624.