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HYPERGLYCAEMIA PRODUCED BY THE POLYAMINES SPERMINE AND SPERMIDINE

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1 Intravenous injection of rabbits with the polyamines spermine (10-30 mg/kg) and spermidine (50-90 mg/kg) produced hyperglycaemia.

2 Given by intraventricular injection, spermine and spermidine gave rise to hyperglycaemia in doses 250-500 times smaller than those effective intravenously.

3 Intraventricular injections of the polyamines also produced hyperglycaemia in rats. Adrenal demedullation abolished the response.

4 In the rabbit, the hyperglycaemia resulting from intraventricular injection of polyamines was abolished in reserpine-treated animals. Anaesthesia had no effect on the response unless accompanied by anoxia, when the response was potentiated.

5 Intracisternal injections of the polyamines in rabbits were less effective than intraventricular injections in that doses about four times larger were required to elicit an equivalent response.

Introduction

Many years ago, Evans, Vennesland & Schneider (1939) showed that spermine produces hyperglycaemia in rabbits when doses of 15-25 mg/kg are given by the intramuscular route. More recently, intramuscular injections of spermine have also been shown to cause hyperglycaemia in man (Risetti & Mancini, 1954). It has been established that the hyperglycaemia produced by barbitone, leptazol and morphine is of central origin (Stewart & Rogoff, 1922; Hasselblatt & Sproull, 1961). Since it is now clear that spermine and the related compound spermidine are present in the brain in high concentration (Shaw & Pateman, 1973), a further investigation of the hyperglycaemia produced by those compounds seems appropriate.

The present paper shows that doses of these polyamines much smaller than those effective intravenously produce hyperglycaemia when injected into the cerebral ventricles. The effects of anaesthesia, reserpine and adrenal demedullation on the hyperglycaemia produced in response to intraventricular injection are described and in addition the effectiveness of intraventricular and intracisternal injection is compared.

Methods

Most experiments were done on New Zealand White or Sandy Lop rabbits weighing between 2 and 3 kg. To facilitate intraventricular injection, a metal cannula (David Kopf, California, USA) was implanted so that its tip lay in the left lateral ventricle. The cannula was screwed into a hole in the skull drilled at a point 3.5 mm lateral to the bregma with the aid of a Kopf stereotaxic frame and cannula insertion kit. The cannula was implanted in the vertical plane with its tip 9 mm below the skull surface. Dental cement (Durelon, ESPE GmbH) was used to secure the cannula to the skull.

For intracisternal injections, the Kopf cannula was used as a guide cannula. The injection tube of the cannula was replaced with 20 gauge stainless steel tubing which projected 14.5 mm below the threaded portion of the cannula. The guide cannula was screwed into a small perspex holder (Fig. 1) and with the animal held securely in the stereotaxic frame a midline incision was made in the skin overlying the supraoccipital process. The overlying tissue was then scraped away, care being taken not to sever the levator muscles, and the cannula and holder lowered into place so that the cannula tip rested just above the dura overlying the cisterna magna. The holder was secured to the skull with the aid of two 2 mm x 5 mm Vitallium mandibular screws (Howmedica UK Ltd) and the skin sutured.

Female Wistar rats weighing 200 g were fitted with Yeda brain injection guide cannulae (Yeda, Rehovot, Israel). The threaded portion of the cannula was shortened by half. With the animal in the stereotaxic frame, a 1.5 mm diameter hole was

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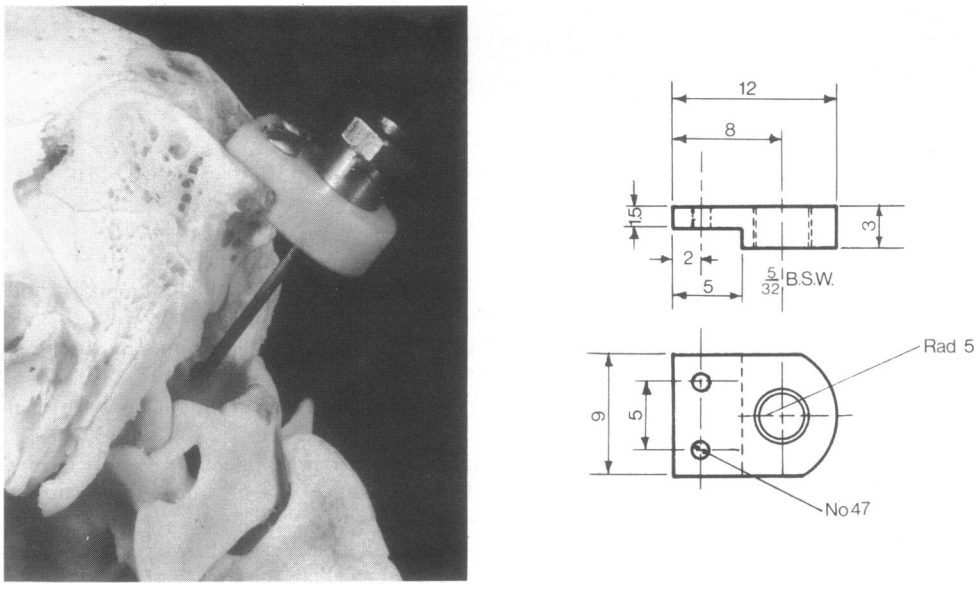

Fig. 1 Cisternal guide cannula and holder. Dimensions are in mm unless otherwise stated.

drilled in the skull at a point 2.5 mm lateral and 1 mm posterior to the bregma. The hole was tapped with a 2.0 mm diameter 0.4 mm pitch thread and the guide cannula screwed into place and fixed with Durelon. The target site was the left lateral ventricle. Adrenal demedullation in rats was carried out as described by Evans (1936).

All surgery was performed under antiseptic conditions with the animals anaesthetized with pentobarbitone sodium (30 mg/kg). The adrenal demedullations were carried out three days before ventricular cannulation. All the cannulated animals were allowed to recover for at least a week before they were injected. In both rats and rabbits food and fluid intake was reduced for two days following the operation. By the fourth day, normal feeding and drinking was re-established and the body weight had returned to the preoperative level. On the day of injection, all the animals were allowed free access to food and water overnight. Food was removed 3 h before injection. Intraventricular injections in rabbits were made in a volume of 200 μl. For intracisternal injections, the dura was punctured with a 25 gauge needle inserted down the guide cannula and about 100 μl cerebrospinal fluid withdrawn into the syringe containing the injection solution. This fluid, together with 100 μl of injection solution, was then injected. Intraventricular injections in rats were made in a volume of 20 μl with a 27 gauge needle which had been shortened so as to penetrate 4 mm below the skull surface.

Spermidine trihydrochloride and spermine tetrahydrochloride (Sigma, London) were freshly dissolved in sterile pyrogen-free 0.9% w/v NaCl solution (saline) before use. Doses of drugs throughout the text refer to the base.

Blood samples from rabbits were obtained by puncturing the marginal ear vein. Samples from rats were obtained by decapitating the animal. In each instance, the sample volume was 100 μl. Blood glucose content was measured by the GOD–Perid method (Werner, Rey & Wielinger, 1970). The method is specific for glucose, reproducible and linear for blood glucose concentrations up to 500 mg/100 ml. The presence of spermidine or spermine in the reaction mixture, in an amount equivalent to a blood polyamine content of 11 mg/ml, did not interfere with the determination.

Results

Intravenous injection

The finding of Evans *et al.* (1939) that spermine produces hyperglycaemia was confirmed. A dose of 10 mg/kg increased blood glucose content 16 mg/100 ml above the resting value whereas a 20 mg/kg dose produced an increase of 99 mg/100 ml and 30 mg/kg produced a rise of 114 mg/100 ml. In each instance the maximum level was recorded 30 min after the injection and

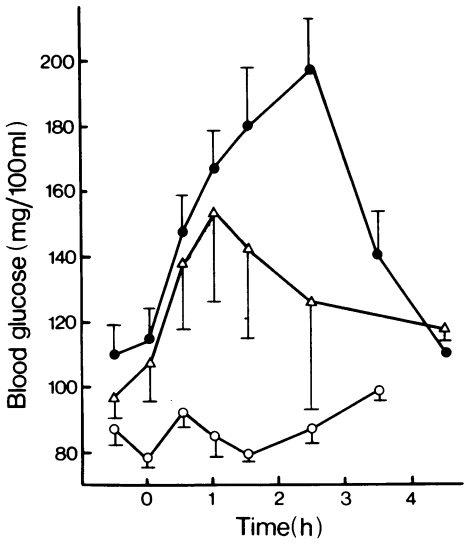

Fig. 2 Effect of i.v. injections of spermidine on rabbit blood glucose content. At zero time the animals were given spermidine 50 mg/kg (○); 70 mg/kg (△); or 90 mg/kg (●). Each value is the mean blood glucose content of four animals. Vertical bars indicate s.e. mean.

resting levels were again noted within 90 min at 10 and 20 mg/kg and within 120 min at 30 mg/kg. Spermidine also produced hyperglycaemia in rabbits when it was injected intravenously, though it was less potent than spermine. Blood glucose concentrations achieved with 50-90 mg/kg of spermidine are shown in Figure 2. Animals given these high doses of spermine and spermidine became sedated. Respiration was slow and laboured and the highest doses of spermidine produced gasping. Muscle tone was reduced. Sometimes, after 90 mg/kg spermidine, but not at lower doses, the hind limbs were paralysed, the knee-jerk reflex absent and sphincter control lost.

Intraventricular injection

Polyamines injected into the cerebral ventricles gave rise to hyperglycaemia in doses very much smaller than those effective by intravenous injection. The time course of the hyperglycaemia produced by spermidine or spermine is shown in Figure 3. When given by this route, the two polyamines appear to be about equipotent. Doses of the order of 100-150 μg raised the blood glucose content by about 20 mg/100 ml over 30-60 min whereas, if 1 mg was given, an intense hyperglycaemia lasting up to 4 h resulted. An injection of 200 μl saline was without effect (Figure 3).

Within a few minutes of the injection of 250 μg of spermine or spermidine, the respiration became rapid and shallow. There was sedation, though the animal remained responsive to external stimuli. These effects persisted for some 2 hours. With higher doses, clonic convulsions sometimes followed. Spermine given in a dose of 1 mg almost always produced convulsions whereas the same dose of spermidine did so only infrequently. Convulsions were generally not observed until several hours after sedation, tachypnoea and hyperglycaemia had disappeared. Injections of the polyamines invariably produced anorexia and adipsia lasting 24 h or more. The hyperglycaemic response was either greatly reduced or absent if the injection of spermine or spermidine was repeated on the following day. If the animal was allowed to recover for a week and to re-establish its normal feeding pattern, the response was reproducible.

Effect of reserpine The hyperglycaemia produced by 500 μg of spermidine was abolished in two rabbits which had been pretreated for a week with reserpine (1 mg/kg) given daily by intraperitoneal injection. In two more animals pretreated in the same way, 500 μg of spermine also failed to produce hyperglycaemia. However, polyamine injection invariably produced a much more pronounced sedation in reserpine-treated than in untreated animals. The daily dose of reserpine produced few signs of sedation but following the injection of 500 μg of polyamine, the animal became extremely depressed and there was ptosis, drooping of the head and loss of muscle tone. In an animal given reserpine (2.5 mg/kg) daily for five days, an intraventricular injection of 500 μg spermidine was lethal.

Effect of pentobarbitone sodium When 250 μg of spermidine or spermine was given to rabbits (2 animals for each polyamine) which had been anaesthetized 30 min earlier with an intravenous injection of pentobarbitone sodium (30 mg/kg), the resulting hyperglycaemia was at least as intense as that seen in the conscious animal. Anaesthesia was maintained throughout the experiment by giving a further 10 mg/kg pentobarbitone sodium by the intraperitoneal route 1 h after injection of polyamine. It was noticed that the polyamine tended to increase the depth of anaesthesia. In a fifth animal that became anoxic, presumably because of respiratory depression arising out of this effect, a blood glucose concentration of more

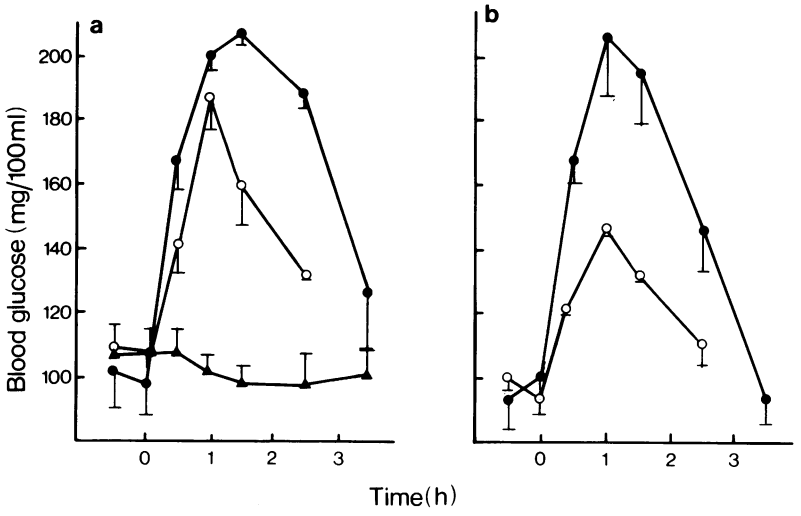

Fig. 3 Hyperglycaemia produced in rabbits by intraventricular injection of polyamines. At zero time 250 μg (○) or 500 μg (●) of spermine (a) or spermidine (b) or 200 μl saline (▲) was injected into the left lateral ventricle. Each value is the mean blood glucose content of four animals. Vertical bars indicate s.e. mean.

than 400 mg/100 ml was noted following the injection of 250 μg spermine. Pentobarbitone anaesthesia did not itself cause any significant change in blood glucose concentration.

Intraventricular injection in rats The polyamines also produced hyperglycaemia when they were given by intraventricular injection to rats (Table 1), though the response was less reproducible than that seen in rabbits. With doses of 100 μg or more, the animals became sedated and showed ptosis, piloerection and hypothermia. Spontaneous locomotor activity was greatly reduced.

The polyamines were also given to groups of rats which had been subjected to adrenal demedullation. The blood glucose concentration of the animals was measured 1 h after injection when hyperglycaemia is maximal. No hyperglycaemia was produced (Table 2), though the behavioural effects were the same as those seen in intact animals.

Intracisternal injection

The intracisternal injection of rabbits with doses of polyamines sufficient to give rise to pronounced hyperglycaemia by intraventricular injection resulted in only a small increase in blood glucose content. An increase of 20 mg/100 ml was produced by 500 μg spermine whereas 500 μg spermidine caused an increase of 11 mg/100 ml. At these doses the animals showed no sedation or tachypnoea. When the dose of spermine or spermidine was increased to 2 mg, the animals became hyperexcitable and clonic convulsions were often observed. These convulsions which began within a few minutes of injection and

Table 1 Blood glucose in rats after intraventricular injection of polyamines

Agent injected	*Min after injection*			
	0	*30*	*60*	*120*
Spermine (250 μg)	89 ± 14	71 ± 6	122 ± 13	103 ± 16
Spermidine (250 μg)	58 ± 5	74 ± 5	134 ± 46	88 ± 14
Saline (20 μl)	71 ± 3	79 ± 4	77 ± 5	81 ± 5

Each value represents the mean concentration (mg/100 ml with s.d.) for 4 animals.

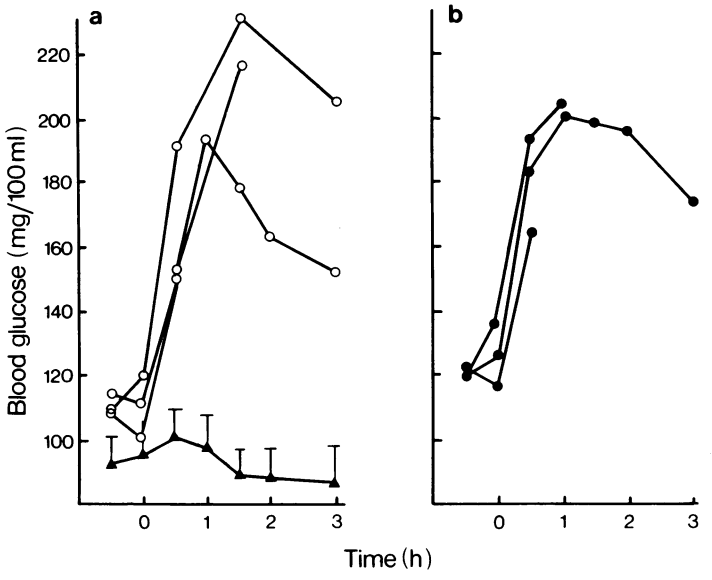

Fig. 4 Hyperglycaemia produced by intracisternal injection of polyamines in rabbits. At zero time 2 mg spermidine (●); 2 mg spermine (○) or 100 μl saline (▲) was injected into the cisterna magna. For polyamine-injected animals, individual values are plotted. Cessation of the plot indicates the death of the animal. For saline-injected animals, the mean value from three animals and s.e. mean is indicated.

Table 2 Blood glucose in normal and demedullated rats at 1 h after an intraventricular injection of polyamine

Agent injected	*Normal*	*Demedullated*
Spermine (250 μg)	201 ± 89	92 ± 17*
Spermidine (250 μg)	135 ± 35	99 ± 12*
Saline (20 μl)	104 ± 2	82 ± 4

Each value is the mean (mg/100 ml with s.d.) for 5 animals.
* $P < 0.05$.

recurred at frequent intervals for about an hour, proved lethal in most animals although some survived. Tachypnoea also started within a few minutes of injection and persisted for some 90 min in animals that survived the convulsions. In addition, a pronounced hyperglycaemic effect was observed (Figure 4).

Discussion

The intravenous injection of polyamine in rabbits produced hyperglycaemia only when very high doses were administered. The behavioural effects associated with these high doses are similar to those seen previously in mice and rats (Shaw, 1972) and probably arise partly as a result of a direct central action and partly from neuromuscular and ganglion blockade and histamine release. However, spermine and spermidine give rise to hyperglycaemia when injected into the cerebral ventricles in doses 250-500 times smaller than those required by intravenous injection. This is a much greater differential than that seen with morphine (Borison, Fishburn, Bhide & McCarthy, 1962) and almost certainly indicates that the central nervous system is the primary site of action. The existence of a blood-brain barrier to the polyamines (Kremzner, Barrett & Terrano, 1970) explains the relative inefficiency of intravenous injections. The experiments carried out on rats indicate that release of adrenaline from the adrenal medulla is the mechanism by which hyperglycaemia occurs since the response is absent in the demedullated animal. The possibility that smaller doses of polyamines would produce hyperglycaemia in rats because of lower brain size was not borne out; with the same dose as that given to rabbits a smaller rise in blood glucose concentration occurred. This finding might be interpreted as further evidence for the involvement

of adrenaline release since rabbits are particularly susceptible to the metabolic effects of adrenaline (Cori, Cori & Buchwald, 1930). It proved impossible to remove the adrenal medullae of rabbits since the tissue was extremely friable and the medulla could not be removed by squeezing the gland as in the rat. However, the observation that the response was abolished in the reserpine-treated animal supports our hypothesis. There is nothing unique about this mode of action. It is probably responsible for the hyperglycaemia produced by morphine (Stewart & Rogoff, 1922), barbitone, leptazol (Hasselblatt & Sproull, 1961) and salicylate (Gaitonde, Joglekar & Shaligram, 1967).

Whereas the hyperglycaemia which follows an intraventricular injection of morphine is suppressed by pentobarbitone anaesthesia (Feldberg & Shaligram, 1972), that produced by the polyamines is not. The observation that hyperglycaemia produced by spermine was potentiated when the anaesthetized animal became anoxic is predictable. Asphyxia itself produces hyperglycaemia, though the response is primarily dependent on the adequacy of the liver glycogen store and is largely independent of the integrity of the adrenals (Stewart & Rogoff, 1922). It is, therefore, important to consider both the possibility of anoxia being present and the state of the liver glycogen store when changes in blood glucose content are to be measured. Indeed Stewart & Rogoff (1922) used special diets to ensure that the animals' liver glycogen stores were full during their experiments. In our experiments, in an attempt to maintain a balance between hyperglycaemia produced by recent food consumption and depletion of glycogen stores by starvation, food was removed 3 h before polyamine injection. Experiments (unpublished) carried out in our laboratories indicate that 4 h starvation produces a significant reduction in rat liver glycogen content, total depletion occurring within 18 hours. In view of the action of intravenous injections of high doses of polyamines on respiration, it is likely that asphyxia may have made some contribution to the hyperglycaemia observed during the present investigation. On the other hand, asphyxia is not implicated in the sequence of events following intraventricular injection since the effect on respiration is quite different. The rapid development of tolerance to daily intraventricular injections of polyamines seen in the present investigation almost certainly mirrors depletion of glycogen stores since the animals ate very little over the three day period.

The precise location of the central site at which polyamines act has not yet been determined. It seems, however, that it can best be reached by injection into the ventricular system. In early experiments before correct co-ordinates for injection into the lateral ventricle had been established, some injections were inadvertently made into cerebral tissue. No hyperglycaemia ensued. The relative inefficiency of intracisternal injections, which required doses some four times larger than those injected into the ventricles for an equivalent hyperglycaemic effect, may also provide a clue to the site. It has recently been shown that morphine, which is also less effective by intracisternal than intraventricular injection (Borison *et al.*, 1962), exerts its action on the ventral surface of the brain stem (Feldberg & Gupta, 1973); intracisternal injection is less effective because in animals which have no foramen Magendie, the brain stem is less easily reached from the cisterna magna than from the ventricular system. Since rabbits do not have a foramen Magendie (Blake, 1900; Schaltenbrand & Putnam, 1927), a similar argument can be applied to the action of the polyamines.

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References

BLAKE, J.A. (1900). The roof and lateral recesses of the fourth ventricle considered morphologically and embryologically. *J. Comp. Neurol.*, **10**, 79-108.

BORISON, H.L., FISHBURN, B.R., BHIDE, N.K. & McCARTHY, L.E. (1962). Morphine-induced hyperglycaemia in the cat. *J. Pharmac. exp. Ther.*, **62**, 88-105.

CORI, C.F., CORI, G.T. & BUCHWALD, K.W. (1930). The mechanism of epinephrine action. VI Changes in blood sugar, lactic acid and blood pressure during continuous intravenous infusion of epinephrine. *Am. J. Physiol.*, **93**, 273-283.

EVANS, E.A., VENNESLAND, E. & SCHNEIDER, J.J. (1939). Effect of spermine on tissue oxidations. *Proc. Soc. exp. Biol. Med.*, **41**, 467-470.

EVANS, G. (1936). The adrenal cortex and endogenous carbohydrate formation. *Am. J. Physiol.*, **114**, 297-308.

FELDBERG, W. & GUPTA, K.P. (1973). Morphine hyperglycaemia: the site of action. *J. Physiol., Lond.*, **224** 85-86P.

FELDBERG, W. & SHALIGRAM, S.V. (1972). The hyperglycaemic effect of morphine. *Br. J. Pharmac.*, **46**, 602-618.

GAITONDE, B.B., JOGLEKAR, S.N. & SHALIGRAM, S.V. (1967). The hyperglycaemic action of sodium

salicylate. *Br. J. Pharmac. Chemother.*, **30**, 554-560.

HASSELBLATT, A. & SPROULL, D.H. (1961). Hyperglycaemia induced by drugs. *J. Physiol., Lond.*, **157**, 124-136.

KREMZNER, L.T., BARRETT, R.E. & TERRANO, M.J. (1970). Polyamine metabolism in the central and peripheral nervous system. *Ann. N.Y. Acad. Sci.*, **171**, 735-748.

RISETTI, U. & MANCINI, G. (1954). The Tokuoka reaction for spermine. *Acta neurol.*, **9**, 391-395.

SCHALTENBRAND, G. & PUTNAM, T. (1927). Untersuchungen zum Kreislauf der Liquor cerebrospinalis mit Hilfe intravenose Fluorescineinspritzungen. *Dt. Z. Nervheilk.*, **96**, 123-132.

SHAW, G.G. (1972). Some pharmacological properties of the polyamines spermine and spermidine–a reappraisal. *Arch. int. Pharmacodyn. Ther.*, **198**, 36-48.

SHAW, G.G. & PATEMAN, A.J. (1973). The regional distribution of the polyamines spermidine and spermine in brain. *J. Neurochem.*, **20**, 1225-1230.

STEWART, G.N. & ROGOFF, M. (1922). Morphine hyperglycaemia and the adrenals. *Am. J. Physiol.*, **62**, 93-112.

WERNER, W., REY, H.G. & WIELINGER, H. (1970). Properties of a new chromogen for the determination of glucose in blood according to the GOD/POD (glucose oxidase–peroxidase) method. *Z. analyt. Chem.*, **252**, 224-228.

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