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THE RELATIONSHIP OF THE BLOOD SUGAR LEVEL TO THE SEVERITY OF ANAPHYLACTIC SHOCK

BY

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Procedures used to increase the susceptibility of rats and mice to anaphylactic shock have included the operation of hypophysectomy (Molomut, 1939) or adrenalectomy (Weiser, Golub & Hamre, 1941), the injection of *Bordetella pertussis* vaccine at the time of sensitization (Malkiel & Hargis, 1952; Sanyal & West, 1958a), and the injection of insulin immediately before the challenging dose of antigen (Sanyal, Spencer & West, 1959; Adamkiewicz, Sacra & Ventura, 1964). These procedures have a common factor in that hypoglycaemia is present when the specific antigen is injected (Parfentjev & Schleyer, 1949; Sanyal, 1960), and attempts have now been made to analyse the mechanisms involved by which alterations in the blood sugar level modify the severity of anaphylactic shock. A preliminary note has already been published (Dhar & Sanyal, 1963).

METHODS

Groups of 8 Wistar albino rats (body weight 100–150 g) and albino mice (body weight 20–25 g) were sensitized by an intraperitoneal injection of horse serum (1 ml.) and challenged by an intravenous dose of the same amount of antigen 12 days later (Sanyal & West, 1958a). In some experiments, *Bordetella pertussis* vaccine (0.25 ml. of 80×10^9 organisms/ml.) was injected with the sensitizing dose of antigen. Rabbits were also sensitized and challenged with horse serum, but egg-white (50% v/v in saline) was used as the antigen in guinea-pigs, the intraperitoneal route being used for sensitization and an intravenous dose or aerosol (4% antigen in saline) for the challenge 21 days later (Sanyal & West, 1958b).

Experimental diabetes

Food was withheld from rats and mice for 24 hr (although free access to water was allowed) and then they were injected with alloxan to produce diabetes. The dose in rats was 300 mg/kg subcutaneously and in mice it was 100 mg/kg intravenously. Rabbits developed diabetes when they were injected intravenously with two doses of alloxan (100 mg/kg) 4 days apart. Attempts to produce experimental diabetes in guinea-pigs (Sitaramyya & Subba Rao, 1961) were unsuccessful.

Glucose hyperglycaemia

Sensitized rats were injected intraperitoneally with 5 ml. of a 25% (w/v) solution of glucose in water to produce hyperglycaemia (Adamkiewicz & Adamkiewicz, 1960); control animals were injected with 5 ml. of a 8.5% (w/v) solution of sodium chloride in water (a solution which is equimolar with that of 25% glucose). Injections of these hypertonic solutions usually produced death in 48 hr with

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subdural haemorrhages (Selye, 1952), but, when sensitized rats were challenged with antigen immediately after the injections, those dying by about 2 hr showed haemorrhagic lesions in the small intestine, symptoms indicative of anaphylactic shock. Mice were injected with 1 ml., and guinea-pigs with 10 ml., of the 25% solution of glucose to produce hyperglycaemia.

Hypoglycaemia

Insulin (5 i.u./kg) was injected intraperitoneally to produce hypoglycaemia in rats and mice 30 min before challenge with the specific antigen. Guinea-pigs and rabbits were injected similarly with 10 i.u./kg.

Blood sugar

The blood sugar levels were determined at different times using the modified Folin Wu method (King & Wootton, 1956); the mean values are expressed as mg/100 ml. ± S.E.

RESULTS

Anaphylaxis in the rat

Treatment with *B. pertussis* vaccine significantly reduced the blood sugar level both of untreated rats and of rats injected with the antigen (Table 1). Anaphylactic shock in rats injected with the vaccine at the time of sensitization was so severe that all 8 animals died within 2 hr with typical haemorrhagic lesions in the intestines (Sanyal & West, 1958a). This result contrasts markedly with that of mild shock found in 8 rats when there was no vaccine present. A lowered blood sugar concentration may thus contribute to the severity of the shock.

TABLE 1

EFFECT OF *BORDETELLA PERTUSSIS* VACCINE ON THE MORTALITY OF RATS AND MICE AS A RESULT OF ANAPHYLACTIC SHOCK 12 DAYS AFTER SENSITIZATION. THE BLOOD SUGAR VALUES (MG/100 ML. ± S.E.) OF THE RATS BEFORE CHALLENGE ARE ALSO SHOWN
Insulin (5 i.u./kg) was used as a control challenging agent in one series of experiments

Bordetella pertussis vaccine	Sensitizing agent	Blood sugar (rats)	Challenging agent	Mortality (out of 8 animals)			
				Rats		Mice	
				2 hr	24 hr	2 hr	24 hr
Absent	None	97 ± 6·8	Horse serum	0	0	0	0
Present	None	66 ± 16·4	Horse serum	0	0	0	0
Absent	Horse serum	98 ± 4·6	Horse serum	0	0	4	4
Present	Horse serum	68 ± 10·4	Horse serum	8	8	8	8
Present	Horse serum	67 ± 11·1	Insulin	0	0	0	0

Whereas there were no deaths in the groups of rats after anaphylactic shock when no vaccine was present, all 8 animals died within 2 hr after insulin had been injected 30 min before challenge (Table 2); blood sugar levels were of the same order as those found when the vaccine (but no insulin) was injected with the sensitizing dose, and they were significantly lower than the control values 98 ± 4.6 mg/100 ml.). Typical haemorrhagic lesions in the intestines were found at necropsy in all of these animals (Sanyal, *et al.*, 1959).

In experimental diabetes produced 4 days before challenge, the mean blood sugar level at the time of challenge was 283 ± 32.6 mg/100 ml. and only mild shock developed, although the rats had received the vaccine 12 days previously; there were no deaths within

TABLE 2

EFFECT OF DIFFERENT PRETREATMENTS ON THE MORTALITY OF RATS AND MICE AS A RESULT OF ANAPHYLACTIC SHOCK 12 DAYS AFTER SENSITIZATION WITH HORSE SERUM. THE BLOOD SUGAR VALUES (MG/100 ML.±S.E.) OF THE RATS BEFORE CHALLENGE ARE ALSO SHOWN

Bordetella pertussis vaccine	Pre-treatment	Blood sugar (rats)	Mortality (out of 8 animals)			
			Rats		Mice	
			2 hr	24 hr	2 hr	24 hr
Absent	None	98± 4·6	0	0	4	4
Absent	Insulin	57±11·0	8	8	8	8
Present	None	68±10·4	8	8	8	8
Present	Alloxan	283±32·6	0	2	1	7
Present	Glucose	409±31·0	1	8	8	8
Present	Alloxan +Insulin	101± 6·0	0	4	0	6

2 hr but 2 out of 8 animals died overnight (Table 2). Another group of 8 similarly sensitized rats with alloxan diabetes were injected with insulin 30 min before challenge and, although their blood sugar levels were no different from the control values, 4 of them died within 24 hr. Seven rats of a further group of 8 receiving the large doses of glucose survived more than 12 hr but all died by 24 hr.

Anaphylaxis in the mouse

Results in the mice were similar in pattern to those in the rats. In the absence of *Bordetella pertussis* vaccine, only half of the mice died in 2 hr after anaphylactic shock whereas all died in 2 hr when insulin was injected just before challenge (Table 2). When the vaccine was present but no insulin was used, all the mice died also in 2 hr (Table 1). In experimental diabetes (confirmed by positive urine tests for sugar), one mouse in a group of 8 died within 2 hr and 6 more died within the 24 hr. Thus, alloxan diabetes in the mouse did not provide complete protection but significantly increased survival time. Insulin had little effect on the action of alloxan (Table 2).

Anaphylaxis in the guinea-pig

The blood sugar levels of guinea-pigs sensitized with egg-white (110 ± 11.0 mg/100 ml.) were similar to those of control animals (120 ± 5.8 mg/100 ml.). Five died within 15 min and a further one out of 8 animals died within 2 hr as a result of anaphylactic shock when challenged intravenously with egg-white. Another group of 8 sensitized guinea-pigs were injected with insulin before challenge and had blood sugar levels of 38 ± 14 mg/100 ml. ; all died in anaphylactic shock within 15 min. In a further group of 8 sensitized animals, injections of glucose before challenge (to raise the blood sugar level to 460 ± 80 mg/100 ml.) failed to modify the severity of the anaphylactic shock and all died with typical asphyxia. When the challenge was made by the inhalation route, all 8 sensitized guinea-pigs died within 4 min when insulin was injected before placing the animals in the aerosol chamber, whereas only 4 out of 8 guinea-pigs died when insulin was not used.

Anaphylaxis in the rabbit

When insulin was injected before challenge (to lower the blood sugar by over 40%), the intensity of the anaphylactic shock was unaltered and 4 out of 8 rabbits died within

30 min, just as they did in the control groups (blood sugar 98 ± 16 mg/100 ml.). However, in experimental diabetes (blood sugar values 214 ± 74 mg/100 ml.), the anaphylactic shock was mild and none of the rabbits died within 24 hr.

DISCUSSION

The results show that, when the blood sugar level in rats and mice is markedly reduced from the control values, anaphylactic shock is so severe that all the animals die. Lowering of the blood sugar has been achieved in the present work by injecting either *B. pertussis* vaccine at the time of sensitization or insulin 30 min before the challenging dose of antigen. Whereas hypoglycaemia aggravates anaphylactic shock, hyperglycaemia offers protection, at least to some rats and mice. There are quantitative differences between the two species but the pattern is similar. For example, when alloxan diabetes was induced, only 2 out of 8 of the rats died after anaphylactic shock whereas 7 out of 8 of the diabetic mice died. In both species, all 8 animals in the control groups died. Death is delayed in all cases when diabetes is present or when hyperglycaemia has been temporarily produced by injections of glucose. It is possible that the quantitative difference between rats and mice under these hyperglycaemic conditions is related to the fact that mice are more susceptible to anaphylactic shock than rats ; when animals were sensitized without *B. pertussis* vaccine, 4 out of 8 mice died, whereas no rats died as a result of anaphylactic shock.

In the guinea-pig, hypoglycaemia also aggravates anaphylactic shock, but hyperglycaemia (produced by injections of glucose) does not modify the severity of the shock. In the rabbit, although hypoglycaemia does not increase the intensity of anaphylactic shock, hyperglycaemia gives protection to the animals so that only mild shock results. Thus there is a considerable species variation in the relationship between blood sugar levels and intensity of anaphylactic shock. Furthermore, results *in vivo* do not always agree with those obtained *in vitro*. For example, Chakravarty (1962) showed that glucose does not inhibit the anaphylactic release of histamine from minced guinea-pig lungs, and histamine is involved in anaphylactic shock in this species and may be the main mediator participating in the reaction.

Clinical asthma rarely occurs in the diabetic patient and in many instances asthmatic symptoms improve with the onset of the diabetic state (Swern, 1931 ; Van Ufford, 1952). On the other hand, some nocturnal attacks of asthma have been associated with hypoglycaemia (Abrahamson, 1941). Thus, adrenaline, one of the established remedies for the treatment of asthma and anaphylactic shock, may act through its effect in mobilizing blood sugar as well as through its dilator action on the smooth muscle of the respiratory tract and its pressor action on the blood vessels.

SUMMARY

1. In rats and mice, the severity of anaphylactic shock is altered by changes in the concentration of glucose in the blood. When hypoglycaemia is induced by *Bordetella pertussis* vaccine or insulin, shock is potentiated whereas in hyperglycaemia induced by injections of alloxan or glucose shock is delayed and severity is decreased.

2. In guinea-pigs, hypoglycaemia aggravates anaphylactic shock but hyperglycaemia fails to produce protection.

3. In rabbits, hypoglycaemia does not modify anaphylactic shock whereas hyperglycaemia greatly reduces the severity of the shock.

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