Effect of metabolic poisons on anaphylactic shock

SIR,—The mechanism responsible for the release of histamine in anaphylaxis and the effect of various metabolic poisons on this process *in vitro* have received a good deal of attention (Moussatché & Prouvost-Danon, 1958; Rothschild, 1961; Chakravarty, 1962). The anaphylactic release of histamine can be prevented by metabolism inhibitors and by anoxia; the inhibition of metabolism may be counteracted by glucose. This would strongly suggest that the anaphylactic release of histamine is an energy requiring process, where the adenosine triphosphate (ATP) level in the tissue plays a decisive role.

As demonstrated earlier, dextran-induced anaphylactic oedema (Görög & Szporny, 1965a), as well as ultraviolet-induced erythema (Görög & Szporny, 1964) are effectively inhibited in guinea-pigs by some metabolic poisons.

The protective effect against anaphylactic shock of non-steroid anti-inflammatory agents, like phenylbutazone or salicylate, and some water-soluble glucocorticoids (Görög & Szporny, 1965b) is established and we now report the influence of some metabolic poisons on anaphylactic shock *in vivo*, and relate this to the action of the poisons on tissue metabolism.

Guinea-pigs of 300 to 400 g were sensitised by two intraperitoneal injections of 0·05 ml 5% ovalbumin solution at 24 hr intervals (Herberts, 1955). 21 to 30 days after sensitisation, the animals were made to inhale 5% albumin aerosol. The point when an animal exhibited signs of dyspnoea, by lying on its side and turning its head to right and left, was taken as the preconvulsion time. At this point, the animals were resuscitated by the inhalation of oxygen (Smith, 1961). Animals with shorter preconvulsion times than 100 sec, were used one week later. Each dose was given to six animals. The quotient of treatment time/control time indicates the inhibition. The preconvulsion time $\times 10$ of the daily untreated control group was regarded as the limit. The substances were administered intraperitoneally 30 min before the albumin aerosol. The inhibitory action on glucolysis was measured in homogenates of rat kidney (LePage, 1947). After incubation for 40 min at 37°, the amount of the lactic acid produced was measured spectrophotometrically (Barker & Summerson, 1948).

Of the investigated substances (Table 1), arsenate and maleate failed to avert shock. Upon comparing effective with toxic doses, the dose ratios were no worse than those in the case of shock inhibition by several established anti-inflammatory agents.

The facts in Table 2 illustrate *in vitro* inhibition of glucolysis. *In vivo*, 2-deoxyglucose accumulating within the cell as 2-deoxyglucose-6-phosphate, inhibits the glucolysis (Sols & Crane, 1954) and therefore the inhibitory effect cannot be proved by our homogenate technique. The first step in glucolysis, the phosphorylation of glucose, is inhibited by glyceraldehyde; hence to investigate this effect, fructose-1,6-diphosphate had to be omitted from the system, and glucose alone served as substrate. Under such conditions, the activity of the system was much weaker than in the presence of fructose-1,6-diphosphate. Our findings confirm the observation that malonate in high concentration inhibits glucolysis (Fawaz & Fawaz, 1962). Some parallelism can be noted in concentrations inhibiting shock and those inhibiting glucolysis *in vitro*. The protection against shock afforded by 2,4-dinitrophenol, is particularly marked at small doses; amounts approaching toxic doses have diminished action.

These experiments indicate that anaphylactic reaction can be successfully prevented *in vivo* by non-toxic doses of substances which inhibit metabolism.

TABLE 1. EFFECT OF METABOLIC POISONS ON ANAPHYLACTIC SHOCK IN GROUPS OF SIX GUINEA-PIGS

Substances	Dose mg/kg	Ratio†	Toxicity in rats intraperitoneally
Sodium monoiodo-acetate	20 10	8·8 5·4	MLD = 30–50
Sodium fluoride ..	5 4 2·5	8·7 4·4 1·1	**MLD = 28–35
Sodium malonate ..	300 200	7·0 6·0	LD50 = 1100*
2-Deoxyglucose ..	500	10·0	LD50 = 2000
DL-Glyceraldehyde ..	1000 500	10·0 8·6	LD50 = 2000
2,4-Dinitrophenol ..	20 10 5 2·5	5·6 7·1 10·0 3·9	LD50 = 20
Sodium arsenate ..	10	1·2	**MLD = 34·7–44·6
Sodium maleate ..	300	1·6	LD50 = 600

* The toxicity of malonate differs from the published value, i.e., LD50 = 2500. (*Handbook of Toxicology*, Editor, Spector, W., 1956. London: Saunders Co.).
** Data from: *Handbook of Toxicology*.
† Control preconvulsion time: 78·2 sec (30 animals).

TABLE 2. EFFECT OF METABOLIC POISONS ON GLUCOLYTIC ACTIVITY OF RAT KIDNEY *in vitro*

Substances	Final conc. M	Lactic acid μM produced/hr/mg nitrogen		Inhibition %
		Control	With substances	
Sodium monoiodo-acetate	1×10^{-4} 5×10^{-4}	13·80	7·40* 2·15	46 84
Sodium fluoride ..	1×10^{-4} 5×10^{-4}	14·20	6·25 1·32	56 91
Sodium malonate ..	2×10^{-2} 6×10^{-2}	11·70	8·60 3·20	26 73
DL-Glyceraldehyde ..	1×10^{-3} 5×10^{-3}	4·58	3·10 0·25	32 94
Sodium maleate ..	2×10^{-2}	13·80	13·10	5
Sodium arsenate ..	1×10^{-2}	14·50	14·25	2
2,4-Dinitrophenol ..	1×10^{-3}	13·65	12·90	5

* = Every value is the average of two parallels.

Thus the mechanism of anti-inflammatory action shows a close relationship to the mechanism responsible for the inhibition of anaphylactic shock. In both effects the inhibition of the ATP-generating system is involved. On the other hand, the difference between the two effects indicates that 2,4-dinitrophenol exerts no inhibitory action on experimental inflammation, with the exception of the anaphylactic dextran oedema in which its inhibitory effect is pronounced (Stenger, 1959).

Chemical Works of Gedeon Richter, Ltd,
Pharmacological Laboratory,
Budapest, Hungary
September 13, 1965

P. GÖRÖG
L. SZPORNY

References

Barker, S. B. & Summerson, W. H. (1941). *J. biol. Chem.*, **138**, 535–554.
Chakravarty, N. (1962). *Nature, Lond.*, **194**, 1182–1184.
Fawaz, E. N. & Fawaz, G. (1962). *Biochem. J.*, **83**, 438–445.
Görög, P. & Szporny, L. (1964). *J. Pharm. Pharmacol.*, **16**, 635–636.
Görög, P. & Szporny, L. (1965a). *Acta Physiol. Hung.*, **26**, 263–267.
Görög, P. & Szporny, L. (1965b). *J. Pharm. Pharmacol.*, **17**, 250–251.
Herberts, G. (1955). *Acta Soc. med. upsal.*, **60**, 246–269.
LePage, G. A. (1948). *J. biol. Chem.*, **176**, 1009–1020.
Moussatché, H. & Prouvost-Danon, A. (1958). *Experientia*, **14**, 414–415.
Rothschild, A. M. (1961). *Ibid.*, **17**, 555–556.
Sols, A. & Crane, R. K. (1954). *J. biol. Chem.*, **210**, 581–595.
Stenger, E. G. (1959). *Arch. Int. Pharmacodyn.*, **120**, 39–47.

An improved method for obtaining twitch responses to acetylcholine in the rat diaphragm

SIR,—Retrograde injection into the venous drainage of the right hemidiaphragm was originally developed by Burgen, Dickens & Zatman (1949) as a method for obtaining twitch responses to added acetylcholine. The method has more recently been described in detail by Paterson (1965) in studies on the pharmacology of denervated and innervated rat muscle.

In our quantitative investigations we experienced some difficulty in obtaining repeatable results using the preparation as described. Since the response of this preparation to retrograde injection is dependent not only upon the dose of drug, but also on the speed of delivery and volume of the injection, we have devised a method of mechanically delivering drug solutions to the diaphragm. The preparation was made as described by Paterson (1965) with the exception that the dead space of the injection cannula was reduced to 0·07 ml by using fine bore polythene tubing (Arnold Howell Ltd., ARH/3, hand drawn to 1 mm o.d.) as the cannula, and tied as low down into the thoracic vena cava as possible to minimise "ballooning" from the pressure of injection. After filling the cannula

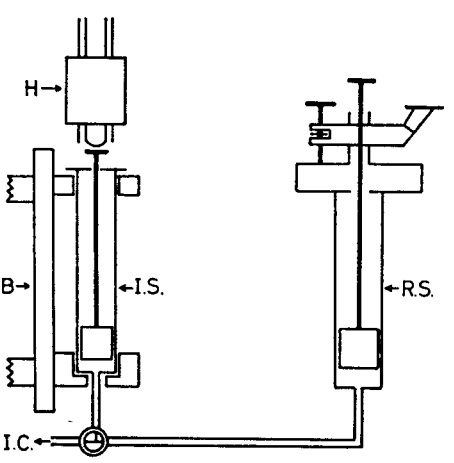

FIG. 1. Diagram of apparatus for the mechanical retrograde intravenous injection of acetylcholine into the isolated rat diaphragm. B. Brass holder for injection syringe. H. Knee jerk hammer. I.C. Injection cannula. I.S. Injection syringe. R.S. Reservoir syringe. T. Three way tap.