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Tracing the changes in capillary permeability during rat anaphylaxis

SIR,—When rats die after anaphylactic shock, there is always haemoconcentration and gross haemorrhage in the small intestine with occasional damage to the lungs and heart (Dawson, Starr & West, 1966). It was of interest therefore to examine the distribution of the specific antigen used for challenge after it has been suitably labelled with radioactive iodine, and to trace its localization in target organs in the rat.

Groups of male Sprague-Dawley rats, 120–150 g, were sensitized by an intraperitoneal injection of horse serum (0·5 ml) mixed with *Bordetella pertussis* vaccine (0·25 ml of 80,000 $\times$ 10^6 organisms per ml). Twelve days later, they were injected intravenously, under light ether anaesthesia, with 2 ml of the solution of labelled horse serum (equivalent to 1 ml original serum) and killed 3 hr later. The peritoneal cavity of each animal was washed for 2 min with 0·5 ml 0·9% saline and the fluid was then removed. Different tissues were dissected, cleaned and weighed. Radioactivity in the saline washing and in the tissues was counted in a Packard Tricarb liquid scintillation counter. The phosphor consisted of naphthalene, PPO, dimethyl POPOP, xylene, 1,4-dioxane, and ethanol. Counting efficiency was 38·0 $\pm$ 0·23%. The degree of diffusion of the labelled serum from the circulation into the peritoneal cavity was taken as a measure of the change in capillary permeability occurring in anaphylaxis.

To prepare the iodine-labelled horse serum, carrier-free sodium iodide solution in 0·9% saline (29 ml containing 9·47 μc ^{131}I) was added slowly, with continuous stirring, to an equal volume of horse serum at pH 7·5. Hydrogen peroxide (1·0 ml, 100 vols) was then added to release nascent iodine, and the reaction was continued in a shaking incubator at 37° for 1·5 hr (McFarlane, 1956). The mixture was dialysed against 2 litre quantities of ice-cold distilled water for up to 72 hr until 0·5 ml aliquots of the dialysate showed no radioactivity. With this method, iodination of the serum protein is minimized, and the physical and chemical characters of the horse serum are retained.

The results show that diffusion of ^{131}I-labelled horse serum into the peritoneal cavity of rats after anaphylactic shock is about 4 times greater in sensitized animals (average net activity of peritoneal washings, 75 $\pm$ 10 counts/min) than in control non-sensitized animals (activity, 18 $\pm$ 6 counts/min) given the same dose (2 ml) of labelled antigen. Thus, capillary permeability is greatly increased in animals undergoing anaphylactic shock and radioactive antigen passes through the intestinal vasculature into the cavity of the peritoneum. However, this was the only difference found as the radioactivity in the heart, small intestine, thymus, liver, spleen, lung, kidney and brain of sensitized rats after challenge was not significantly different from that of non-sensitized animals similarly challenged.

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Antagonistic action of vitamin D_2 on noradrenaline-induced lipolysis *in vitro*

SIR,—Results obtained with prostaglandin E_1 and digitoxin suggested that a possible role was played by calcium in the lipolysis induced by noradrenaline (Fassina & Contessa, 1966, 1967). The variations of the calcium concentration in the incubation medium of epididymal fat modified the stimulating action of noradrenaline on lipolysis. We therefore investigated the influence on the same lipolytic process of vitamin D_2, a drug known to have a specific effect on calcium metabolism.

Vitamin D_2 acts as an inhibitor of lipolysis *in vitro* (Table 1). The drug antagonizes the noradrenaline-induced lipolysis starting from a concentration of 10^{-6}M, as indicated by the decreased concentration of free fatty acids as well as of glycerol in the incubation medium of rat epididymal fat. The basal lipolysis was not significantly affected.

TABLE 1. INHIBITION BY VITAMIN D_2 OF NORADRENALINE-INDUCED LIPOLYSIS *in vitro*

Drugs in the incubation medium Molar conc.	△ FFA* μ-equiv./g/150 min	△ Glycerol* μmoles/g/150 min
Noradrenaline $0{\cdot}2 \times 10^{-5}$	20·86 ± 0·77	9·31 ± 0·70
Noradrenaline ,, + Vit. $D_2 10^{-6}$	15·03 ± 1·19	5·25 ± 0·88
Noradrenaline ,, + Vit. $D_2 10^{-4}$	10·54 ± 1·31	3·34 ± 0·40
Noradrenaline ,, + Vit. $D_2 10^{-3}$	5·25 ± 0·35	2·10 ± 0·20

Rat epididymal fat (100 ± 10 mg) was incubated in 2 ml of Krebs–Ringer bicarbonate buffer containing 2·5% bovine albumin, at 37° for 150 min, in a metabolic shaker. Free fatty acids (FFA) (Dole, 1956) and glycerol (Korn, 1955) were titrated in the incubation medium. Drugs were added to the medium before introducing the fat. Vitamin D_2 was dissolved in ethanol. The same volume of ethanol (0·05 ml) was added in all assays.

* FFA and glycerol absolute increase over control (fat incubated without drugs). Each value represents the mean ± s.e. of six experiments.

Vitamin D_2 dissolved in ethanol was inactivated by an exposure to ultraviolet light for 16 hr. As indicated by Cima, Levorato & Mantovan (1967), after this period the chromatographic spot of vitamin D_2 almost completely disappeared, but the inactivated drug still inhibited noradrenaline-induced lipolysis. Consequently, the effect of vitamin D_2 on lipolysis does not seem to be connected with the specific vitamin action.

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