cinolone) after sensitization with dihydrotachysterol (DHT) either directly by gavage or indirectly by nursing from DHT treated mothers. Milk from DHT treated mothers was capable of sensitizing neonates for thymic calcification only if nursing was permitted within the 24-hour period that the maternal DHT sensitization occurred.

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On the Nature of Tolerance of Endotoxin.* (29921)

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Tolerance of endotoxin has been found to be related to the pinocytic and phagocytic capacity of the reticulo-endothelial system (1-4). Recently the plasma of the tolerant animal has been found to contain a transferable factor that suppresses pyrogenicity and increases the rate of phagocytosis in the recipient(5-7). Such data, though doubtless of considerable significance, do not sufficiently explain what it is that constitutes increased tolerance.

We have reported the presence in normal spleen of a protein with an esterase-like behavior(8) which is capable of rapidly degrading endotoxin(9), as indicated by tests: *a,* for toxicity of endotoxin for the 10-day-old chick embryo, and for the pertussis-treated mouse(10), and *b,* for the capacity of endotoxin to fix complement on exposure to specific antibody.† That this protein can alter the endotoxin molecule was also demonstrated by its ability to block 2 phage receptor sites on the endotoxin molecule,‡ and by production of multiple precipitation bands, instead of a single one, on Ouchterlony plates. The validity of these tests for assessing the presence or absence of potency in this protein fraction of spleen was affirmed by the finding that the results by more than one of the above 5 methods on any given test sample, though not quantitatively comparable, have nearly always been in the same direction.

The endotoxin-detoxifying protein appears to be an important feature of the host's defense mechanisms for the following reasons:

Normal rabbits, after a brief exposure to traumatic shock, lose their defense against bacterial endotoxin, so that they succumb to as little as several gammas instead of several milligrams of a potent endotoxin(12). In such rabbits the detoxifying fraction of spleen, which is always potent in normal rabbits, has lost its potency.

A degree and duration of traumatic shock that is usually lethal in normal rabbits is not lethal in rabbits with increased tolerance of endotoxin(13). This increased resistance to traumatic shock disappears when tolerance of endotoxin disappears.

Such data led to the study reported below, which was undertaken to determine how far

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‡ We are indebted to Drs. Gabriel Michael and Fred Rosen, Harvard Medical School, for this unpublished observation.

the potency of the endotoxin-detoxifying protein correlates with the animal's state of tolerance.

Experimental. New Zealand white rabbits were rendered tolerant by a series of 7 serially doubled doses of *E. coli* 0111:B4 endotoxin (Difco) injected intravenously at 48-hour intervals in a period of 14 days, starting with an initial dose of 25 γ. No endotoxin was administered thereafter, except to rabbits surviving more than 30 days, when a second series of injections was started, giving 50 γ every 48 hours up to 3 doses.

The presence of an induced increase in tolerance of endotoxin was affirmed by observing in random samples from non-tolerant and the tolerant series: 1. That non-tolerant rabbits exposed to hemorrhagic shock for 2 hours failed to survive an injection of 50 γ of endotoxin given 4 hours later (4 experiments), whereas tolerant rabbits challenged the same way survived (4 experiments). 2. That tolerant animals survived 6 hours of hemorrhagic shock (3 of 4 experiments), whereas non-tolerant rabbits did not (4 of 5 experiments). 3. The blood of more than half of a series of non-tolerant rabbits in 6-hour shock yielded a positive test for endotoxin(14), whereas the blood of all of 8 tolerant rabbits after 6 hours of shock was negative. 4. Non-tolerant rabbits in 6-hour shock exhibited severe and persistent granulocytopenia, but tolerant rabbits did not develop granulocytopenia.

The potency of the detoxifying protein was determined as follows:

Rabbits were killed by exsanguination at various intervals following the first or second series of injections of endotoxin. Some had been exposed to 6 hours of hemorrhagic shock before they were killed. The serum was frozen at once and later assayed for toxicity and for titer of antibody to the endotoxin. The spleens were removed aseptically immediately after exsanguination. They were weighed, and processed at once or put into the deep freeze and processed later, as follows:

The entire spleen was homogenized in a Potter-Elvehjem ground glass homogenizer in cold (4°C) saline at a concentration of 0.5 g per ml. The homogenate was centrifuged at 20,000 $\times$ g for 30 minutes. The supernatant was decanted and brought to 30% $(NH_4)_2SO_4$ saturation by addition of the solid salt. After stirring the resulting suspension for 15 minutes, it was spun at 10,000 RPM for 20 minutes. This supernatant was discarded, and the residue (the 30% ammonium sulphate precipitate or "30F" fraction) was resuspended in half the original volume of saline. The detoxifying capacity of this suspension was determined by exposing 1 ml to 10 γ, or multiples thereof, of *E. coli* 0111:B4 endotoxin (LD_{80} in chick embryo = 1 γ) for 30 minutes at 37°C, after which 0.1 ml of the reaction mixture was injected into each of 6 10-day-old chick embryos. The survival of at least 4 of the 6 chick embryos was taken to signify detoxification.

The detoxifying potency of the splenic extract was also tested by the various methods referred to above against equal numbers of lethal doses of the endotoxins of *A. aerogenes* and of *Coli B.*, to which the rabbits had not been exposed.

The serum antibody-titer of all rabbits was determined by the complement fixation technic(11) on bloods taken from time to time in order to see whether there is any correlation during the period of fluctuating resistance to endotoxin between quantitative change in antibody and the potency of the detoxifying protein.

Results. 1. Spleens of tolerant rabbits were larger and some 50% heavier at the time of maximum tolerance than spleens of normal rabbits. When tolerance had returned to normal by the thirtieth day, the size and weight of the spleen had reverted to that of the normal spleen. A series of 3 doses of endotoxin in the next 6 days increased the size and weight of the spleen.

2. *Potency of splenic extract* (Fig. 1). One ml of the 30F fraction of spleen from non-tolerant rabbits, prepared as described, detoxified 3-5 times the LD_{80} of endotoxin in the chick embryo. The same preparation from animals on the first day after maximum tolerance was reached inactivated 10-20 times the LD_{80}, on the tenth day afterward 5 to 10 times the LD_{80}, and on the thirtieth day afterwards 3 to 5 times the LD_{80}. Six hours

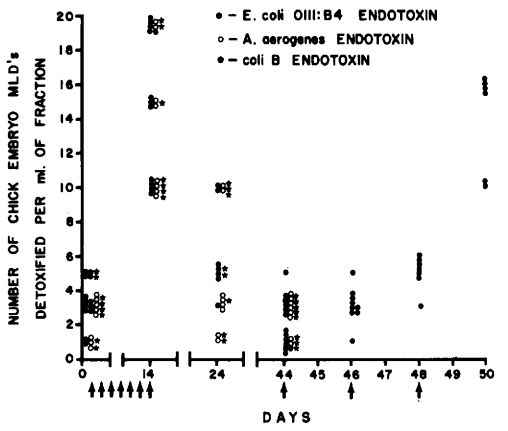

FIG. 1. Detoxifying potency of fraction of spleens from rabbits made tolerant of *E. coli* 0111:B4 endotoxin. Each arrow below abscissa indicates administration of endotoxin.

of hemorrhagic shock at these corresponding periods reduced the range of these detoxifying values to 0, 1-3, 0-5, and 0 respectively.

The fraction of spleen from rabbits given 3 doses of endotoxin at 48-hour intervals after tolerance had returned to normal showed restoration to near maximum potency, *i.e.*, 10-15 LD_{80} ml on the sixth day.

These shifts in potency were the same for all 3 endotoxins when assayed by the chick embryo and at least one of the other tests referred to above.

3. *Serum antibody titer of tolerant rabbits.* The rise in antibody titer varied considerably from rabbit to rabbit. On the day of maximum tolerance the average antibody titer was 17 times normal. Twenty days later, when the potency of the splenic enzyme had reverted to normal, the antibody titer was still at this level. With each successive dose of endotoxin this titer rose promptly and steeply (Fig. 2). The same was true of the potency of the splenic enzyme although this rise was of a different order of magnitude.

Discussion. The foregoing experiments confirm a previous report of the presence of an endotoxin-detoxifying principle in spleen that is not found elsewhere, except in considerably lower potency, gram for gram, in liver, and in still lower potency, gram for gram, in lung. It is not detectable in serum, kidney, striated muscle, heart muscle or gut.

Serum detoxifying factors have been demonstrated *in vitro* by a number of investigators(15-19). The criteria employed to demonstrate this activity vary. They include pyrogenicity, tumor necrotizing effect, antigenicity, release of phosphate, and reduced lethality. In some instances artificial conditions such as ionic control were required to effect detoxification. What significance any of these systems have in the host's defense mechanism has not been demonstrated.

The kinetics of the *in vitro* interaction of our preparation from spleen with endotoxin resemble those of the interaction of the normal spleen perfused *in vivo* with endotoxin (20). They differ from those observed in the *in vitro* interaction of serum with endotoxin.

Since degree of tolerance to endotoxin varies with amount or potency of the detoxifying protein, and not with the curve of the antibody titer, we consider the degree of tolerance to endotoxin to be a measure of an adaptive enzyme response(21). This is a nonspecific response since it applies to other endotoxins as well as to the one administered to increase tolerance.

This interpretation of our data does not deny the role of immune substances in blood in effecting transmural transfer and subsequent intracellular exposure of the endotoxin to the detoxifying protein(4). We have observed normal titers of complement(22,23) and high titers of antibody in shock up to the time of death. The poorer clearance of endotoxin in shock would, therefore, appear

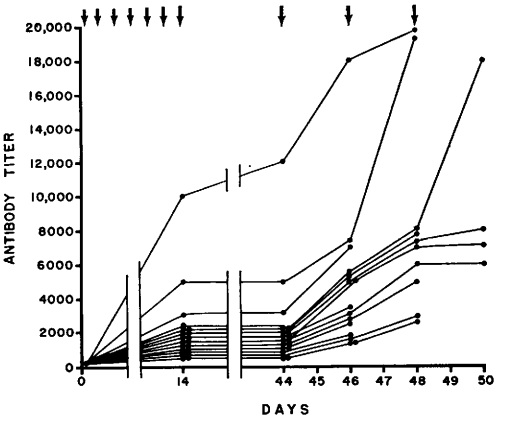

FIG. 2. Change in antibody titer in rabbits made tolerant of *E. coli* 0111:B4 endotoxin. Each arrow represents administration of endotoxin.

to be better understood in terms of failure of intracellular retention rather than failure of delivery to the site of the detoxifying protein. We, therefore, believe that while these immune substances are an essential part of the defense mechanism, the host's ability to defend itself against endotoxin depends ultimately on the intracellular detoxifying principle.

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Renal Vascular Response to Hypothermia.* (29922)

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Past investigations(1-8,10) have been concerned with the response of the isolated or intact kidney to hypothermia. Results have varied, and interpretations are difficult due to complicating effects of increased blood viscosity and elevated extravascular pressure when blood temperature is decreased(7,10). Previous reports show that renal blood flow may be little affected(1), increase(4) or decrease(2-8,10) as a result of total body cooling. Levy(7) has attempted to separate viscosity influences from active renal vascular responses; however, his calculations were dependent on indirect procedures. The present study is devised to determine if renal vasodilation or vasoconstriction occur in response to hypothermia in the isolated denervated dog kidney. The complicating interfering components of increased blood viscosity and tissue pressure elicited by the hypothermic state have been avoided by the experimental design.

Methods. Experiments were carried out on a total of 22 dog-perfused kidneys. Five preparations served as controls in which body temperature was not altered. Dogs were intravenously anesthetized with sodium pentobarbital 30 mg/kg, and utilized as follows: One dog served as the experimental animal in each study and was subsequently immersed in the prone position in a large water bath

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