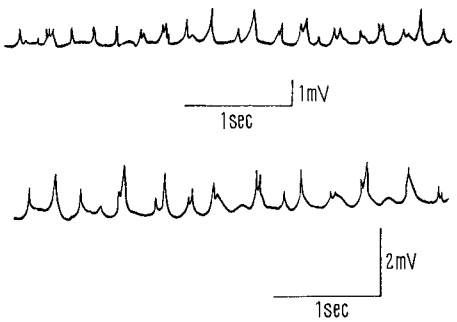
Effect of Tetrodotoxin on *Ascaris* Somatic Muscle

It has been shown in certain nerve and muscle tissues that tetrodotoxin selectively depresses sodium conductance rise during action potential spikes[1]. Certain preparations, notably the taenia coli of the guinea-pig, that show spontaneous spikes can, however, be treated with large concentrations of tetrodotoxin without showing measurable changes in spike height[2], a result favourable to the conclusion that in these preparations calcium conductance and not sodium conductance is involved in the spike. It was therefore decided to see whether tetrodotoxin had any effect on the potential spikes that can be recorded from the non-myofibrillar bellies of *Ascaris* somatic muscle cells.

Several *Ascaris* prevulval preparations[3] that were showing reliable spikes in a suitable saline were treated with tetrodotoxin up to a concentration of 5×10^{-3} g/l. In no case was the spike height or frequency significantly changed, and neither were they changed when the muscle was washed again with pure saline. In some experiments the toxin was left in contact with the preparation for 2 h. The effectiveness of the toxin was checked post-experimentally on frog sciatic nerve.

Negative results from experiments of this type must be interpreted with some caution as there are several membranes around the muscle cell belly visible electron microscopically[4] that could interfere with the passage of the toxin to the site of action. However, the insensitivity to tetrodotoxin would tend to confirm that in respect of the ionic mechanism of the spike the *Ascaris* somatic muscle cell is more akin to smooth muscle with calcium-conductance spike than to skeletal muscle with sodium-conductance spike. A sensitivity to Mn^{++} ions at 5 m*M* concentration and the high value of ^{22}Na loss from loaded muscles (up to 130 picomoles cm^{-2} sec^{-1}) in vivo[4] as well as the time-course of the spikes lend weight to this suggestion.



Typical records showing muscle cell potential spikes before (upper) and after (lower) of tetrodotoxin to the bathing saline. The concentration in this experiment was 5×10^{4} g/l, one tenth of that used in the most stringent experiment.

Résumé. La tetrodotoxine ne produit aucun effet sur les pointes de voltage qu'on enregistre dans les muscles somatiques de l'*Ascaris*. Ce fait s'accorde avec l'hypothèse que les ions autres que ceux du sodium sont les porteurs de charge pendant les pointes de voltage.

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Enhancing Effect of Insulin on Endotoxin Lethality

When bacterial endotoxins are injected into experimental animals an initial, transient hyperglycemia is produced, followed by a prompt fall in blood sugar to hypoglycemic levels. Although several mechanisms have been suggested to account for the toxicity of lipopolysaccharide endotoxins, the effect of these agents on carbohydrate metabolism has received scant attention. BERRY et al.[1] reported that the survival of experimental animals receiving a lethal dose of endotoxin and a therapeutic injection of cortisone appeared to be related to carbohydrate metabolism. SHANDS et al.[2] have suggested that in mice rendered hyperreactive to endotoxin by BCG injection, or zymosan, altered glucose metabolism may be involved in the observed toxic manifestations. In in vitro metabolic studies endotoxin has been shown to exert an insulin-like action on cellular glycolytic processes[3]. Since the induction of hypoglycemia can augment the susceptibility of animals to many inflammatory and hypersensitivity reactions, as well as to certain physical stresses[4-6], we felt that it would be of interest to determine the effect of insulin on the susceptibility of mice to bacterial endotoxin.

Six groups of 15 g female CFW mice, 8–10 mice per group, were used in this experiment. 3 groups were injected i.p. with 0.5 IU of Iletin insulin. 10 min later 1 of these groups was challenged i.p. with 200 µg of *Salmonella typhosa* 0901 endotoxin, another with 400 µg endotoxin, and the third group received no associated injection. The remaining 3 groups received endotoxin only (200, 400 and 800 µg). Deaths were tabulated at 18 h. From the results shown in the Table it is apparent

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that the preliminary injection of insulin greatly increased the susceptibility of mice to endotoxin shock.

Eighteen hours after the administration of either insulin or up to 800 μg of endotoxin there were no deaths. Significant mortality was found, however, among mice pretreated with insulin and challenged with either 200 or 400 μg of endotoxin (70% and 90% mortality, respectively).

Insulin is capable of heightening the sensitivity of experimental animals to the vasoactive amines, histamine and serotonin[7], to immediate and delayed-type hypersensitivity states[4], as well as to anaphylactoid agents such as dextran and peptone[4,6]. The present experiment suggests that insulin-induced hypoglycemia can also exacerbate endotoxin shock in mice. This result is consistent with the thesis that there is a reciprocal relationship between the glycemic state of a host and its susceptibility to a wide variety of stressor agents[4-6]. If the enhanced susceptibility of insulin-pretreated animals to endotoxin shock is a result of the compounding of the hypoglycemic effects of both of these agents, one might expect that glucose would exert a protective effect against endotoxin shock. Recently, it has been demonstrated that glucose is capable of prolonging the survival time of adrenalectomized rats challenged with endotoxin[8]. We are currently investigating this phenomenon in intact animals.

Effect of insulin on susceptibility of CFW mice to *Salmonella typhosa* endotoxin

Sensitizing agent	Dose IU	Challenge agent[a]	Dose μg	Dead/ total[b]
–	–	Endotoxin	200	0/10
–	–	Endotoxin	400	0/9
–	–	Endotoxin	800	0/10
Insulin	0.5	–	–	0/8
Insulin	0.5	Endotoxin	200	7/10
Insulin	0.5	Endotoxin	400	9/10

[a] Challenge agent injected 10 min after sensitizing agent. All injections i.p. [b] Deaths tabulated 18 h after challenge.

Résumé. Nous avons constaté que les souris traitées à l'insuline possèdent une sensibilité élevée à l'endotoxine de bactéries à gram négatif. Ce résultat concorde avec l'hypothèse d'une relation inverse entre la quantité de glucose dans le sang et la sensibilité d'un hôte à une grande variété de «stressors».

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Ethanol Blood Levels Following Acute i.v. Administration in Mice

The rate of metabolism of ethanol in vivo is generally accepted as being linear and independent of blood concentration. The principal rate controlling factors are thought to be the amount of liver alcohol dehydrogenase and the availability of NAD[1]. In the present study the rate of disappearance of blood alcohol has been measured after a single i.v. injection and has been found to be both concentration dependent and, at high concentrations, nonlinear.

Materials and methods. Adult, male, DBA/2 mice, *20–25* g, were injected with ethanol in physiological saline into the tail vein. Doses of 0.33, 0.67 and 2.67 g/kg were used. The duration of the injection was 1 min and blood samples were withdrawn at 5, 20, 35 and 50 min after the injection was complete. Samples were taken from the retro-orbital sinus directly into a 50 μl disposable micropipette and the ethanol concentration determined by the method previously described[2].

Results. The results are shown in the Figure. At the lowest dose level (0.33 g/kg) the blood ethanol concentration decreases at a rate of 17.3 μg/ml/min during the first period and falls to less than half this rate during the second interval. For the 0.67 g/kg dose the rate during the first 2 periods is constant and equal to the initial rate at the lower dose level. In the final 15 min period the rate decreases slightly but not significantly. After the 2.67 g/kg dose the initial decline of blood alcohol is more than twice that seen after the lower doses ($P < 0.002$). During the second interval the rate is somewhat greater than that after the smaller doses but in the final 15 min is very significantly smaller ($P < 0.01$).

Discussion. The results obtained here with the 2 lower doses (0.33 and 0.67 g/kg) conform with the idea that the rate of ethanol metabolism is linear and independent of concentration provided that the concentration exceeds that required to saturate the available alcohol dehydrogenase. Below this concentration blood alcohol levels are known to fall exponentially[3] and this explains the fall off in rate seen here at the lowest dose. The results obtained with the highest dose (2.67 g/kg) do not, however, fit this concept. The total metabolism over the whole 50 min period is substantially the same as that seen after the lower doses but this is made up of a very rapid initial rate balanced by a small or negligable final rate. The large increase in rate in the initial period

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