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Endotoxin Tolerance Induced by Detoxified Endotoxin (Endotoxoid) (32765)

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Beeson (1) first described the induction of endotoxin tolerance by pretreatment with extremely low doses of endotoxin. Since then several workers have produced tolerance towards endotoxin by repeated injections of toxic preparations given over several days. However the toxicity of these substances precludes the clinical application of these findings.

Using detoxified endotoxins (endotoxoid), Freedman *et al.* (3) have induced in animals resistance to infection. Further, Noll and Braude (7) have succeeded in inducing pyrogen tolerance in rabbits, and in increasing the resistance of mice to lethal challenge doses of endotoxins, by immunizing the animals with detoxified endotoxins.

Chemically detoxified endotoxins of the type used in the experiments to be described were obtained from *Serratia marcescens* bovine-type endotoxin by Nowotny (8). These endotoxoids were almost nontoxic while retaining several useful properties of the parent endotoxin (4,9).

The question now raised is, whether protection against the lethal action of endotoxins in general can be induced by pretreatment with this endotoxoid in particular. The present paper describes the induction of endotoxin tolerance in guinea pigs (Pirbright) by treatment with endotoxoid-2 (endotoxin partially deacylated with potassium methylate).

Experiments. Section A. Ninety guinea pigs received by the intravenous route 150 μg/100 gm of body weight of endotoxoid-2. Six animals died within 1 day. Twenty-four hours after injection the 84 survivors were divided into three groups (see Table I) each group being then injected with toxic endotoxin from either *E. coli 0111, Proteus vulgaris,* or *Brucella melitensis.* The three control groups of guinea pigs received intravenously only the corresponding toxic endotoxin. The endotoxins were prepared by the trichloroacetic acid method (2) as described by Urbaschek (11).

Section B. The Ouchterlony (10) gel diffusion method, the very sensitive passive hemagglutination technique (6) and the complement fixation technique of Kolmer (5) were employed to study the serological status of the following groups of animals: (i) Ten guinea pigs were injected intravenously with endotoxoid 150 μg/100 gm body weight. These guinea pigs were bled twenty four hours after the endotoxoid injection. (ii) Ten guinea pigs received the same endotoxoid dose and 24 hours later were challenged with a lethal dose of *E. coli* endotoxin. Twenty-four hours after the challenge the animals were bled. 3. Ten guinea pigs and four rabbits were immunized with toxic *E. coli* endotoxin. The immunized animals were bled on the seventh day after the last injection. As antigen *E. coli* endotoxin and endotoxoid were used.

Results. In Section A experiments, endotoxin from *E. coli, P. vulgaris,* and *B. melitensis* caused a high mortality, when injected

TABLE I. Survival in Endotoxin-Pretreated Guinea Pigs after Injection of Different Endotoxins.

Injection (μg/100 gm iv)	Endotoxin-pretreated (150 μg/100 gm i.v.)		Controls	
	Dead/total	Survival (%)	Dead/total	Survival (%)
ET *E. coli* 0111				
300	3/50	94	38/50	24
ET *P. vulgaris*				
400	2/24	91.6	21/24	12.5
ET *B. melitensis*				
600	0/10	100	4/10	60

intravenously into guinea pigs (see Table I). Within 24 hours of a single injection of endotoxoid the animals were protected against the lethal action of these endotoxins (see Table I). The observation period was 72 hours and death never occurred later than 24 hours after the injection of endotoxin.

In Section B experiments, neither the guinea pigs from group 1 nor the guinea pigs from group 2 showed circulating antibodies detectable with either gel diffusion, passive hemagglutination or complement fixation tests. The animals of group 3 were immunized with *E. coli* endotoxin to obtain control sera for the serological techniques. These sera all gave a positive reaction with the *E. coli* endotoxin used as antigen and a negative reaction with the endotoxoid employed as antigen.

Discussion. Detoxified endotoxin produced from *S. marcescens* endotoxin is able to induce nonspecific tolerance towards the lethal action of endotoxins from *E. coli, B. melitensis* and *P. vulgaris*. It should be noted that the tolerance is developed within 24 hours of a single injection of endotoxoid.

Using a toxic preparation of *S. marcescens* endotoxin Wharton and Creech (16) could demonstrate, that tolerance towards the lethal action of endotoxin was established in mice 1 day after a single injection. This tolerance was transient reaching its maximum in 3–5 days.

According to our concept of endotoxin shock, endotoxin tolerance prevents, among other manifestations of endotoxin, the action of the mediators released and potentiated by endotoxins (12–15).

Tolerant guinea pig sera, as was expected, do not contain antibodies against endotoxin at levels detectable with the techniques used. The experiments exclude, therefore, the possibility of endotoxoid induced tolerance being due to the stimulation of antibodies.

Immunoelectrophoretic comparisons of normal guinea pig sera with tolerant guinea pig sera (as obtained in group 2) showed an increase in the lipoprotein fraction of the alpha-1 region.[1]

Summary. Nonspecific tolerance towards the lethal action of endotoxins from different gram-negative bacteria can be induced within 24 hours by a single injection of *S. marcescens* endotoxoid. This endotoxin tolerance is not based on circulating antibodies. By means of the gel diffusion method, the passive hemagglutination technique, and the complement fixation technique, it could be demonstrated, that sera of tolerant guinea pigs do not contain antibodies against endotoxins or against the tolerance-inducing endotoxoid.

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Oxidative Assimilation by *Serratia marcescens** (32766)

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This study, preliminary to one on endogenous respiration and energy of maintenance, summarizes observations on oxidative assimilation by *Serratia marcescens*. The influence of age of the cells and of cell concentration is considered. Glycerol was employed in most of the tests as the exogenous substrate to avoid any direct polymerization of substrates such as sugar to endogenous reserves.

Materials and Methods. The general methods employed were the same as previously described (1,2). *Serratia marcescens* Z-4 was grown for 16 hours at 30°C nutrient agar (Difco) or on this medium containing 1% glucose. The cells were harvested, washed, and suspended in a basal salt solution (2) to avoid any specific ion effects. In the aging experiments the washed suspensions were shaken in stoppered flasks on a wrist-action shaker at 30°C and samples were removed at daily intervals. Total cell ^{14}C was determined in samples of cells killed with $HgCl_2$ (0.2 ml of 5% $HgCl_2$ per 2 ml of suspension), firmly bound ^{14}C in cells from 2 ml of suspension acidified with 0.2 ml of 10% H_2SO_4. Glycerol was determined colorimetrically (3).

Results. A Qo_2 of 60 was observed following the addition of 8 μmoles (204mμC) of glycerol-U-^{14}C to 6 mg dry weight of freshly harvested cells of *S. marcescens* suspended in 2 ml of basal salt solution. The rate of O_2 consumption (or $^{14}CO_2$ production) decreased abruptly after the time (break-point) that approximately 300 μl or about one half of the theoretical amount (627 μl) of O_2 required for complete combustion had been consumed. The Qo_2 continued to decrease and approached the value (15) observed for the endogenous control. No difference was noted between the behaviors observed with cells grown on nutrient or on glucose agar.

Results of quantitative tests for glycerol in supernatant fluids from nonacidified suspensions indicated that less than 10% of the initial glycerol remained in solution while ^{14}C determinations indicated 22% residual ^{14}C. A portion of this residual ^{14}C probably is in matter relatively resistant to oxidation since the ^{14}C content of the supernatants decreased very slowly after the break-point, a behavior noted with other organisms and substrates (4).

The O_2 consumption values to the break-point, uncorrected for endogenous respiration, suggested about 50% assimilation of the glycerol while the data for total cellular ^{14}C indicated that only about 36% of the initial ^{14}C was present in the cells. One fifth (9 mμc) of the intracellular ^{14}C at the break-point was present in the pool fraction, i.e., material released on acidification of the suspension. The ^{14}C distribution data lead to a more accurate

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