Action of Bacterial Toxins on Respiration of Chicken Erythrocytes.* (17514)

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In the first paper of this series (Hunter and Larsh)(1) a brief statement of the general problem which is being investigated in this laboratory was presented. The present experiments were designed to demonstrate what effects, if any, a number of bacterial exotoxins have on the functioning of cells as indicated by changes in respiration.

The toxins were obtained from the Lilly and Lederle Laboratories§ with the exception of *B. cereus*. This organism was grown in Bacto proteose peptone No. 3 broth and incubated at 37.5°C for 36-60 hours. The fluid suspensions were filtered through Seitz or Berkefeld filters and stored at 4°C with the relative humidity controlled at 95-100%.

A suspension of erythrocytes was obtained by centrifuging heparinized chicken blood. Equal volumes of cells and toxin, cells and heated toxin, cells and formalized toxin, and cells and preservatives (phenyl mercuric acetate or merthiolate) were mixed. Respiration measurements were made at 37°C, using a Warburg apparatus. One or 2 cc of the various cell mixtures were added to each Warburg vessel which contained 0.3 cc of 20% KOH in the center well. The time which elapsed from the mixing of the suspensions until the first reading was made varied from 15 minutes to an hour. Readings were started after a 10-minute period for temperature equilibration and were continued for varying periods of time up to 25½ hours. Sterile technics were used throughout and tests for contamination using agar slants and fluid thioglycollate were made at the end of each experiment.

The results are summarized in Table I, which also includes information concerning the relative strength of most of the toxins. It can readily be seen that formalized toxins are of no value as controls since formalin markedly inhibits the respiration of these cells. Except for the case of tetanal and streptococcal toxins, the heated toxins give essentially the same values as the Ringer Locke controls. Since the toxins were not highly purified, the conclusions which follow are tentative and may well have to be modified when experiments using purified toxins are available.

Summary. 1. Chicken erythrocytes were exposed to various bacterial toxins for several hours and the rate of oxygen consumption was measured at 37°C.

2. Toxins from *Clostridium perfringens* appeared to increase the rate of oxygen consumption initially even though they hemolyzed the cells. After several hours a decrease was observed.

3. Toxins from *Clostridium septicum* had little effect on the rate of oxygen consumption.

4. Toxins from *Clostridium tetani* apparently increased the rate of oxygen consumption for a period of more than 24 hours.

5. Toxins from *Bacillus cereus* had little effect, but slight acceleration of oxygen consumption was noted in most of the experiments.

6. Toxins from *Micrococcus pyogenes,* Var. *aureus* increased the rate of oxygen consumption during the first hour. This was followed by hemolysis of the cells and almost complete inhibition of respiration.

7. Toxins from *Corynebacterium diphtheriae*

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1. Hunter, F. R., and Larsh, Howard W., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 281.

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TABLE I.
Rate of Respiration of Chicken Erythrocytes in the Presence of Various Bacterial Exotoxins.

Cells exposed to equal volumes of:	μ L O_2/10^9 cells/hr												Remarks
	Ringer Locke			Heated toxin (75-82°C, 75 min.)			Formalized toxin (0.02 M formaldehyde 2 wk at 37°C + 2 mo. at 6°C)			Toxin or preservative			
	1st hr	2nd hr	3-5th hr	1st hr	2nd hr	3-5th hr	1st hr	2nd hr	3-5th hr	1st hr	2nd hr	3-5th hr	
1:20,000 phenyl mercuric acetate	9	10								9	11		No effect
1:10,000 merthiolate	9	10								9	10		" "
Cl. perfringens—strength unknown	9	9	8*	11	11		3	3	2*	14	7	0*	Initial acceleration, partial inhibition; complete inhibition‡
Cl. septicum—160 MLD for mouse/cc	11	11		11	11		<1	<1		8	9		Little or no effect
Cl. tetani—300,000 MLD for guinea pig/cc	10	10	10†	13	14	13†	6	5		13	14	13†	Possibly slight acceleration
B. cereus—strength unknown	9	9		11	10		1			15	15		" " "
M. aureus—15,000 dermal necrotic doses/cc	11	10	10	10	9	9	6		4	18	4	0	Initial acceleration, marked inhibition; complete inhibition‡
C. diphtheriae—800 MLD for guinea pig/cc	12	12	12	10	8		5		2–3	20	7	4–3	Initial acceleration, partial inhibition, marked inhibition
Str. pyogenes—strength unknown	11	11		3	3		<1	2		3	<1		Marked inhibition, almost complete inhibition

* 6–7 hr. † 3–4 hr. ‡ Possibly result of hemolysis.

increased the rate of oxygen consumption slightly during the first hour. This was followed by a gradual decrease in rate until after 11 hours, then there was complete inhibition.

8. Toxins from *Streptococcus pyogenes* markedly inhibited the rate of oxygen consumption although there was no hemolysis.

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Studies on Proteinuria in the Rat. (17515)

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Most investigations of protein excretion in the urine have assumed that protein in quantity is an abnormal constituent of the urine. It has furthermore been commonly assumed that mammalian kidneys extract from plasma a protein-free fluid in each species. The existence of a normal proteinuria in the Slonaker strain of white rats has been described qualitatively by Addis.(1,2) Protein excretion has been studied by Smetana(3) by injecting dye-labelled foreign protein into the blood of several mammals and observed deposition of dyed particles within the lining cells of the proximal convoluted tubules. Oliver(4,5) used similar technics both *in vivo* and in perfused isolated kidneys, with similar results. Dock(6) injected Evans blue intraperitoneally in rats and subsequently observed dye deposition in the renal tubules. The present studies again demonstrate a normal proteinuria, both albuminuria and globulinuria, under a variety of environmental conditions, in the albino rat. The passage of plasma protein through the rat kidney is studied by labelling the native plasma protein with Evans blue, and examining, in fixed tissues, the disposition of the Evans blue-protein complex.

Procedures. A. *Protein determinations.* Urine was collected from rats, weighing from 180 to 250 g each, while in individual metal metabolism cages. During periods of urine collection, none of which was longer than 24 hours, no food was present in the cage. The animals studied included 14 male animals of the Wistar strain, on "Rockland Rat Diet", and located in a constant temperature room at 27° C; 12 animals, 8 males and 4 females, of the Sprague-Dawley-Holtzman strain, at ordinary room temperature, that had been on a diet of fortified powdered milk for a month; and 8 male rats of the Sprague-Dawley-Holtzman strain that had been subjected to a constant air temperature of 4° C for 3 months.

a) The urine was collected individually, centrifuged clear, and tested for protein content by heating and adding acetic acid. The precipitate so obtained was spun down washed and tested by the biuret test, xanthoproteic test, charring, and Millon test.

b) Qualitative protein fractionations of 72-hour urine specimens from 9 normal rats were performed. Urine was saturated with ammonium sulfate and a precipitate was interpreted as albumin. Globulin was precipitated by half saturation with ammonium sulfate. In both instances the precipitates were tested with protein reagents as above.

c) Quantitative protein measurements were made on 24-hour pooled urine from 5 normal male rats. Total protein was determined by precipitating with trichloracetic acid, centrifuging, dialyzing against distilled water at 5° C till chloride-free, and drying to con-

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