when incubated with 4+ sera (40 percent) was higher than given by ova separated by methods based on sedimentation and decantation (20 percent or less).

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10. We are grateful to Mrs. A. R. de Sala for her cooperation and technical assistance, and to Drs. J. V. Rivera, K. Rivera, M. Cancio and J. Oliver-Gonzalez for their valuable suggestions and helpful criticism.

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Plague Toxin: Its Effect in vitro and in vivo

Abstract. *The murine toxin of* Pasteurella pestis *inhibited the respiration of heart mitochondria from the rat and the mouse but had little or no effect on the respiration of mitochondria from the rabbit, chimpanzee, dog, and monkey. Alterations occurred in the S-T segments of the electrocardiograms recorded from rats injected with ¼ to 10 LD_{50} of toxin, but not in those from rats dying of hemorrhagic shock, hypoxia, intoxication with glucose, or* Escherichia coli *endotoxin. No abnormalities were observed in electrocardiograms from rabbits injected with large amounts of toxin.*

Purified murine toxin from *Pasteurella pestis* is lethal to mice and rats, but not to rabbits (*1*). It has also been found that the respiration (in terms of oxygen consumption) of mitochondria obtained from the heart of the toxin-sensitive rat is inhibited by the toxin, whereas heart mitochondria from the toxin-resistant rabbit are unaffected. It was our purpose to study further the specificity of the effect of the toxin on animals other than the rat, mouse and rabbit, and to correlate the observations in vitro with a number of experiments in vivo based on electrocardiographic measurements of intoxicated and unaffected animals.

Pasteurella pestis, strain TJW, was grown under conditions described previously (*2*). The toxin, which was prepared and purified as before (*2*), had an intraperitoneal LD_{50} for albino Swiss mice of 45 to 56 μg toxin/kg body weight (calculated according to the method of Reed and Muench) (*3*).

Heart mitochondria were isolated according to the method of Cleland and Slater (*4*), and oxygen consumption was measured by means of a sensitive polarographic method, according to the procedure outlined by Packer *et al.* (*1*). Protein concentration was determined by a modified Lowry method described by Oyama and Eagle (*5*).

The rats were anesthetized with nembutal when electrocardiographic measurements were made. Blood pressure was measured with a mercury manometer by cannulating an exposed femoral artery. Needle electrodes were inserted into the four legs of the rats and connected to an upright Sanborn electrocardiograph. After the blood pressure stabilized following the surgical procedures, a standard three-lead electrocardiogram was recorded as a baseline. Toxin was then inoculated (¼ LD_{50} to 10 LD_{50}) and electrocardiographic tracings were obtained, as well as hematocrit and blood pressure measurements.

Hemorrhagic shock was induced in the animals by severing the femoral artery; they were intoxicated with glucose by injecting intraperitoneally 5 to 10 ml of a 50-percent glucose solution; hypoxia was brought about by placing a plastic bag over the head of the animal.

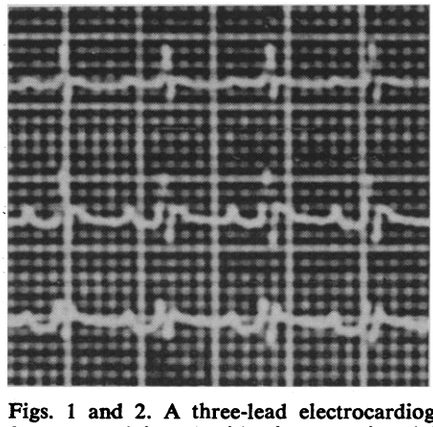
The chimpanzee, dog, and monkey remained completely unaffected when injected with amounts of toxin that represented 50 to 100 times the mouse LD_{50} on a kg body weight basis. Experiments in vitro have shown that the respiration of the heart mitochondria of these animals was likewise unaffected, as shown in Table 1. With α-ketoglutarate as substrate, significant inhibition was apparent only with mitochondria from the rat and mouse heart and not with those obtained from the monkey, dog, rabbit, or chimpanzee.

Table 1. Effect of plague toxin on the respiration of heart mitochondria from the rat, mouse, monkey, dog, rabbit, and chimpanzee. The reaction mixture in the cuvette (1.0 ml) consisted of mitochondrial protein (1.69 mg for the rat and mouse, 1.39 mg for the other animals); 0.32*M* sucrose, 0.01*M* KCl, 0.001*M* disodium salt of ethylenediaminetetraacetic acid, 0.02*M* phosphate buffer; the *p*H was 7.5, and the temperature, 26°C. Plague toxin was incubated with the reaction mixture for 3 minutes before testing respiration; the control was incubated without toxin. Respiration was allowed to continue for 90 to 120 seconds and then α-ketoglutarate was added to give a final 10 m*M* concentration.

Source of mitochondria	O_2 consumed (μmole/lit. sec)		Inhibition (%)
	No toxin	Toxin (2.5 mg)	
Rat	0.84	0.53	37
Mouse	0.60	0.44	26
Monkey	0.70	0.70	0
Dog	0.64	0.62	4
Rabbit	0.43	0.41	5
Chimpanzee	0.48	0.52	0

Following these experiments, attempts were made to correlate the changes in vitro with the results of the experiments in vivo. It was assumed that since mitochondria from the rat heart were susceptible to the action of the murine toxin of *Pasteurella pestis* alterations in the myocardial physiology of the rat might be expected which could possibly be detected by electrocardiographic measurements. The results are shown in Figs. 1 and 2. Alterations in the S-T segment of the electrocardiogram occurred within 60 minutes after the injection of ¼ to 10 LD_{50} of toxin, and prior to changes in hematocrit or blood pressure measurements. It was interesting to note that in surviving animals that received ¼ LD_{50} of toxin, the initial changes in the electrocardiograms were no longer evident after 24 to 48 hours, or after the animal had completely recovered.

Similar changes did not occur in rats dying from hemorrhagic shock, hypoxia, or intoxication with glucose or *Escherichia coli* endotoxin. Electrocardiograms from the rabbit, which is resistant to murine toxin, did not show any abnormality, even when the toxin



Figs. 1 and 2. A three-lead electrocardiogram recorded from a normal rat (left) and from a rat injected with plague toxin (right).

was administered in amounts up to 50 mg, equivalent to 1000 LD_{50}/kg body weight.

Thus the observations reported earlier (*1*) are now extended to include the chimpanzee, dog, and monkey. It is evident that the action of the murine toxin from *Pasteurella pestis* on mitochondria varies with respect to the species from which the mitochondria are isolated, and that this variation is correlated with the susceptibility and resistance of the animals to the action of the toxin. Our present results also support our earlier hypothesis (*1*) that the susceptibility of animals to the action of the murine toxin of *Pasteurella pestis* is related to the ability of the toxin to inhibit the respiration of heart mitochondria.

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Chemically Induced Epileptiform Seizures in the Cat

Abstract. *Long-term epileptiform seizures were induced in the cat by a single stimulation of the basolateral components of the amygdala with cholinergic agents. These electro-physiological changes were accompanied by persisting alterations of the animal's "emotional responsiveness." It is evident that electroencephalographic seizure activity and overt behavior can be dissociated in this species.*

The role of the amygdaloid complex in "emotional" behavior has been studied theoretically (*1*) as well as experimentally (*2*) by many workers in the past 25 years. Marked changes in "emotional responsiveness" have been observed following complete or partial destruction of the amygdaloid complex, and motor as well as electrophysiological seizures have been induced by electrical stimulation of this portion of the brain. We have now studied the specific effects of stimulating the amygdaloid complex with various cholinergic substances.

Chemical stimulation was applied directly to the basolateral components of the amygdaloid complex of cats. This was accomplished by means of double-walled cannulas insulated with Teflon, which permit repeated applications of minute amounts (0.1 to 2.0 μg) of chemicals in crystalline form to a precisely defined locus in the brain, and allow electroencephalographic recordings to be made from the site of chemical stimulation. The cannulas were implanted stereotaxically into the amygdaloid complex of both hemispheres; details of the chemical stimulation procedure have been described elsewhere (*3*). In addition, recording electrodes were implanted bilaterally into the corticomedial amygdala, postero-ventral hippocampus, ventromedial and lateral hypothalamus, midbrain reticular formation, caudate nucleus, and occipital and motor cortex. A subcutaneous silver wire, or a skull screw, was used as the indifferent electrode. Following recuperation, various neurohumoral agents, as well as control substances, were applied to the basolateral region of the amygdala. The site of stimulation has been verified histologically in five animals.

Within approximately 4 minutes of applying 1 μg acetylcholine chloride (*4*) bilaterally to the basolateral portion of the amygdala, marked electrophysiological seizure activity was recorded from the site of chemical stimulation as well as from the corticomedial aspects of the amygdaloid complex. The high-amplitude, low-frequency, spike activity gradually spread to hypothalamic and hippocampal leads and finally dominated the electrical pattern of all areas of the brain from which electroencephalographic recordings were obtained.

Overt motor seizures appeared within 10 to 15 minutes of chemical stimulation, and were accompanied by an increase in the frequency of spike discharges from all leads. For a period of 10 to 15 minutes, the animals salivated profusely and showed electrophysiological as well as overt motor disturbances which appeared strikingly similar to those seen during epileptic attacks in clinical patients. After the motor seizures the animals appeared exhausted, but were hypersensitive to any form of sensory stimulation. Normal handling was impossible, and the animals attacked other cats as well as the experimenter without apparent provocation. These attack responses were well coordinated and directed, but much more vicious than those normally seen in the cat.

Several epileptiform seizures lasting 2 to 5 minutes were observed during the 24-hour period following cholinergic stimulation. The overt motor disturbances disappeared in the course of the next 24 to 48 hours but the electrical activity of the brain (particularly of the amygdala and hippocampus) remained highly abnormal for many days. Bursts of high-amplitude, low-frequency spikes were recorded as late as 10 to 15 days after a single stimulation with acetylcholine chloride.

Essentially the same effects were obtained when choline chloride carbamate (carbachol) (*5*) was applied to the amygdaloid complex, but the electrophysiological as well as behavioral changes appeared to be permanent (see Fig. 1). Very intense motor seizures appeared within minutes of the bilateral application of carbachol and persisted with variable intensity for several hours. Continuous spike discharges were recorded from all leads throughout this period. The uninterrupted motor seizures subsided within 2 to 3 hours of applying carbachol, but the electrical activity of all brain areas continued to show pronounced disturbances in the form of high-amplitude spike discharges. Brief motor seizures (lasting 2 to 4 minutes) occurred at 20- to 30-minute intervals throughout the next 24 hours. The frequency of these motor disturbances decreased gradually over the following weeks to approximately one or two per day, but complete recovery was not observed during a 5-month observation period.

Immediately after stimulation with carbachol, pronounced affective changes became apparent which continued essentially unabated throughout the observation period. Cats which were formerly tame and friendly became extremely vicious and hypersensitive. They remained completely refractory to normal handling and attacked other cats as well as people without provocation or apparent concern for their own safety. Repeated attempts to make them more docile failed to produce any noticeable effects. Following the initial