Induced Immunological Unresponsiveness to Staphylococcus Toxoid in Rabbits.*† (25179)

ROBERT K. LINDORFER AND PADMANABHA SUBRAMANYAM
(Introduced by B. S. Pomeroy)
Division of Vet. Bacteriology and Public Health, College of Vet. Medicine, University of Minnesota, St. Paul

Many investigators(1-6) demonstrated, by serological methods, that a state similar or identical to actively acquired tolerance(7) can be induced in different animals using a variety of antigens. To our knowledge no reports have appeared which relate actively acquired tolerance to animal's susceptibility to disease. Since many infectious diseases are known to be transmitted *in utero, in ovo,* or neonatally, it is possible that this state could play an important role in the pathogenesis of these diseases. The work reported herein demonstrated that resistance of rabbits to dermonecrotic toxin is related to their neonatal exposure to staphylococcus toxoid.

Methods. Staphylococcus toxin and toxoid. Staphylococcus aureus Wood 46 was used for production of toxin by the method of Leonard and Holm(8) except that the toxin was produced by growing the organism in an atmosphere of 75% CO_2 and 25% oxygen. Toxin was converted to toxoid by addition of formalin to final concentration of 0.3%. The process of toxin-toxoid conversion was followed by the hemolysin test and by dermonecrotic effects in rabbit skin. *Injection of rabbits with antigen.* Neonatal exposure of rabbits to toxoid was accomplished by injecting half the animals in each litter intraperitoneally with staphylococcus toxoid, on each of the first 5 days of life. The dosage was: 1st day 0.1 ml, 2nd day 0.2 ml, 3rd day 0.3 ml, 4th day 0.4 ml, 5th day 0.5 ml. Hyperimmunization of rabbits at 62 days of age was accomplished by injecting 0.5 ml of toxoid intraperitoneally on alternate days for a total of 5 injections. *Hemolysin inhibition titration.* This titration was done on serum samples obtained on the 61st and 92nd day of life. The unit of hemolysin was established by incubating 1 ml of 2% suspension of rabbit red blood cells with 1 ml of serial 2-fold dilutions of toxin. The tubes were incubated 1 hour at 37°C, then one hour at room temperature. The highest dilution giving complete hemolysis was taken as the unit. Two units of hemolysin were used for hemolysin inhibition test in which 1 ml of a 2% suspension of rabbit cells was incubated with a mixture of 2 full units of hemolysin contained in 0.5 ml, and 0.5 ml of a serial 2-fold dilution of serum. *Dermonecrotic effects of toxin.* On the 92nd day of life 0.1 ml of serial 2-fold dilutions of toxin were injected intradermally into rabbits (undiluted, 1/5, 1/25, 1/125). Four days after injection the diameter of resulting lesions was measured and total area of necrosis was computed from these measurements.

Results. The results of first experiment are compiled in Table I. At 61 days of age, the day before hyperimmunization was begun, no animals in Group A and B showed an antihemolysin titer. This and the fact that animals of Group B were also negative at 92 days of age, would indicate that rabbits injected with staphylococcus toxoid during the first 5 days of life were incapable of producing antihemolysins. On the other hand, results obtained with Group C indicated that the rabbit between 2 and 3 months of age was capable of responding by producing antibodies to staphylococcus toxoid. The most critical comparison is that between Groups A and C. Animals in Group C which received toxoid only after reaching 62 days of age showed a relatively high antihemolysin titer and a small area of necrosis, while those in Group A which had received both neonatal and hyperimmunization injections of toxoid, produced less than 1/5 the antihemolysin, and the necrotic area in these animals was almost twice as large as

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TABLE I. Effects in Rabbits of Acquired Tolerance on Antihemolysin Production and Resistance to Staphylococcus Toxin.

Group	No. of rabbits	Neonatal inj. 1-5 days	Hyperimm. inj. 62-70 days	Avg antihemolysin titer 61 days	Avg antihemolysin titer 92 days	Necrosis* in cm², 96 days
A	11	Yes	Yes	Neg	1/14	3.5
B	8	"	No	"	Neg	13.6
C	5	No	Yes	"	1/74	2.1
D	6	"	No	"	Neg	17.6

* Avg area of necrosis.

TABLE II. Effects in Rabbits of Acquired Tolerance on Antihemolysin Production and Resistance to Staphylococcus Toxin.

Group	No. of rabbits	Neonatal inj. 1-5 days	Hyperimm. inj. 62-70 days	Avg antihemolysin titer 61 days	Avg antihemolysin titer 92 days	Necrosis* in cm², 96 days	Dead/Inj.
A	10	Yes	Yes	Neg	1/2.4	61	4/10
B	6	"	No	"	Neg	94	4/6
C	6	No	Yes	"	1/24	32	0/6
D	5	"	No	"	Neg	71	2/5

* Avg area of necrosis.

that in Group C.

The second experiment, Table II, confirms in every respect results presented in Table I. Some animals in this experiment died as a result of utilizing a toxin of greater potency. Although the number of animals was small, there is good correlation between size of necrotic areas produced and survival of the animal.

Exps. 1 and 2 show distinct differences not only in size of necrotic lesions produced but also in the character of the lesions which developed in the respective groups. In Group A, which had received neonatal and hyperimmunization injections, necrotic lesions were large, and the diffuse edge of the lesion although visible was relatively indefinite. Contrariwise, in Group C which had received only hyperimmunization injections, lesions were smaller, discrete and surrounded by vivid erythematous ring. These lesions healed faster than those in Group A.

Exp. 3 (Table III), was designed to determine whether Arthus type of sensitization was responsible for some of the necrotic reaction which occurred upon injection of toxin. The procedure used was the same as that employed previously except that toxoid instead of toxin was used to challenge the rabbits. This experiment confirms the serological results of the first 2 experiments and demonstrates clearly that the Arthus reaction was not a factor in development of necrosis.

Discussion. This work is to our knowledge the first demonstration that the state of acquired tolerance is related to actual resistance to infectious disease. If these findings are confirmed and it is established that acquired tolerance is a general immunological phenomenon, it should enable us better to understand the pathogenesis of several important infectious diseases transmitted *in utero, in ovo* or neonatally.

Although actively acquired tolerance and immunological paralysis(9) may bring about the same result, namely, "block" the ability of

TABLE III. Effects in Rabbits of Acquired Tolerance on Antihemolysin Production and Arthus Response to Staphylococcus Toxoid.

Group	No. of rabbits	Neonatal inj. 1-5 days	Hyperimm. inj. 62-70 days	Avg antihemolysin titer 61 days	Avg antihemolysin titer 92 days	Arthus response 96 days
A	8	Yes	Yes	Neg	1/50	Neg
B	6	"	No	"	Neg	"
C	5	No	Yes	"	1/150	"
D	5	"	No	"	Neg	"

the individual to form detectable antibodies, some differences seem to exist between these 2 phenomena. Relatively large doses of polysaccharide antigens which tend to linger in the system for considerable periods of time(9,10, 11) have usually been used to induce immunologic paralysis, whereas the antigens used thus far to demonstrate actively acquired tolerance were proteins. Where studies have been made, proteins disappear from the system in a relatively short time(12,13,14). In addition, whereas immunological tolerance can be induced at any age, actively acquired tolerance could only be induced in individuals before birth or shortly thereafter. Future studies will probably clarify the relationship of these 2 phenomena.

Actively acquired tolerance seems to bear a superficial resemblance to enhancement of mouse mammary tumor transplants induced by certain tissue factors(15,16,17,18). However, the fact that adult mice have been used in studying cancer enhancement phenomenon would tend to discount such a similarity. Further work needs to be done for an accurate assessment of any similarity.

Summary. 1. Rabbits exposed to staphylococcal toxoid early in life have a reduced capacity to respond to the same toxoid in later life as evidenced by development of low antihemolysin titers. 2. Resistance to dermonecrotic effects of staphylococcal toxin is also reduced by early exposure of rabbits to the toxoid. 3. The Arthus response does not play a role in development of lesions under conditions of these experiments. 4. The possible role of acquired tolerance and similar phenomena in infectious diseases is also discussed.

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Inhibition of Human Salivary and Prostatic Acid Phosphatase and Yeast Enolase by Low Fluoride Concentrations.* (25180)

Q. T. SMITH, W. D. ARMSTRONG AND LEON SINGER
Dept. of Physiological Chemistry, Medical School, University of Minnesota, Minneapolis

The inhibitory effect, *in vitro,* of fluoride on enzymes including enolase and acid phosphatase from several sources is well known. However, little attention has been paid to quantitation of effects which might be observed in the range of possible physiological concentrations of fluoride. The present investigation was designed to study(1) the characteristics of inhibition of acid phosphatases of saliva and prostatic tissue and of enolase by low

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