

Influence of Age upon Response to Meningococcal Endotoxin in Rabbits. (21239)

RICHARD T. SMITH* AND LEWIS THOMAS,

*From the Heart Hospital Research Laboratories and Department of Pediatrics,
University of Minnesota Medical School, Minneapolis.*

In previously reported studies on the mechanism of the generalized Schwartzman reaction in this laboratory (1-6), young albino rabbits, weighing between 1 and 1.5 kilos each, were routinely employed in the majority of experiments. Recently, in the course of an investigation involving larger rabbits, a paradoxical difference was noted in the vulnerability of young and mature animals to the lethal effect of a single injection of meningococcal endotoxin. Doses of endotoxin which produced little or no evidence of systemic intoxication in the young rabbits were observed to be lethal within a few hours after injection in older animals of the same stock. In view of the increased susceptibility of older rabbits to a single injection, a comparison was made of the reaction to 2 doses of endotoxin, spaced 24 hours apart. It was found that the incidence of the generalized Schwartzman reaction, as indicated by the incidence of bilateral cortical necrosis of the kidneys, was much lower in the older group of animals.

Materials and Methods. Hybrid albino rabbits of both sexes were obtained from a single breeder, housed in air-conditioned and humidity-controlled quarters, and maintained on a diet of Purina rabbit pellets and water. Rabbits of 2 age ranges were used: the group designated "large" in the text to follow were between 10 and 12 weeks of age, and weighed 2.0 to 3.0 kilos, and the "small" animals were 4 to 7 weeks old and weighed 0.5 to 1.0 kilo.

* This work was performed during tenure of Fellowships with the Helen Hay Whitney Foundation and the National Research Council.

This work was done under the sponsorship of the Commission on Acute Respiratory Diseases, Armed Forces Epidemiological Board, supported in part by the Office of the Surgeon General, Department of the Army, and aided by grants from the National Institutes of Health, Public Health Service, and the Minnesota Heart Assn.

TABLE I. Influence of Age on Mortality in Rabbits following a Single Intravenous Injection of Meningococcal Endotoxin.

Avg age (wk)	Avg wt (k)	Mortality Dilution of endotoxin*					Totals
		1-20	1-40	1-80	1-160	1-320	
11	2.42	5/8†	5/8	3/8	4/8	3/8	20/40
4.5	.62	0/8	1/8	0/8	0/8	0/8	1/40

* 2 cc of indicated dilution of endotoxin inj. intravenously.

† Numerator—No. dead within 18 hr. Denominator—No. in group.

The endotoxin was derived from a strain of meningococcus (44B) obtained from Dr. Gregory Schwartzman, Mount Sinai Hospital, New York. The method of preparing the material has been described (1). Various dilutions of endotoxin were made in sterile physiological saline, and injected intravenously in a volume of 2.0 cc; no adjustment of dosage for the body weight was made. Colloidal iron in the form of saccharate of iron oxide ("Pro-ferrin", supplied by Sharpe and Dohme, Inc.) was employed in some of the experiments in order to enhance the lethal effect of endotoxin, as described in a previous report (5). The material was injected intravenously in a dosage of 2.0 cc per kilo, 2 hours before the injection of endotoxin.

Results. Lethal Effect of Single Injection of Endotoxin. A titration of meningococcal endotoxin was performed in 40 large and 40 small rabbits, employing 5 different dilutions of endotoxin. A summary of the results is shown in Table I. Within 2 to 3 hours after the injection, all of the large animals became obviously ill, with weakness, labored respirations, and varying degrees of prostration, and within 18 hours 20 of the 40 were dead. In contrast, although some of the small animals were relatively inactive for several hours after the injection, few showed evidences of severe intoxication and only one died. When the dose of endotoxin is estimated in terms of

body weight, the data indicate that the small rabbits tolerated at least 50 times the amount of endotoxin which was lethal for the large animals. The majority of deaths in the large rabbits occurred within 12 hours after injection, and after this many of the surviving animals began to recover from the shock-like condition produced by the endotoxin. By the end of 24 hours the survivors appeared to be completely well, and no additional deaths occurred after this time. It is evident that a factor of individual susceptibility, in addition to the age of the rabbits, was of importance in determining the outcome. It will be noted in Table I that although each of the doses of endotoxin was lethal for large rabbits, there was no dose which caused death in all animals. Moreover, the dosage could not be correlated with the proportion of animals killed; 2-fold or greater increases in endotoxin did not cause a proportional increase in mortality.

Lethal effects of endotoxin following an injection of colloidal iron saccharate. Reticulo-endothelial "blockading" agents such as thorotrast, trypan blue(2), colloidal carbon, and colloidal iron saccharate(5), were previously demonstrated to enhance to a remarkable degree the lethal effect of endotoxin, an effect which was interpreted to indicate that the reticuloendothelial system may be involved in natural protection against endotoxin. It seemed important to determine whether the lethal effect of endotoxin was similarly influenced by these materials, in rabbits of different ages. Groups of large and small rabbits were given an injection of colloidal saccharate of iron oxide in a dose of 2 cc/kilo. Two hours later the animals were injected with 2 cc of different dilutions of meningococcal

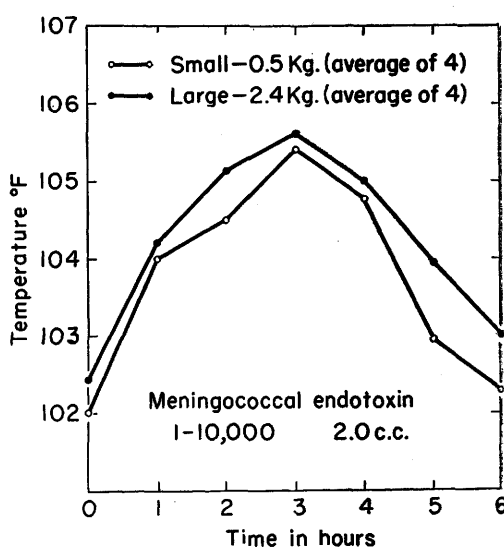


FIG. 1. Temperature response of large and small rabbits given 2 cc, 1-10000 meningococcal endotoxin intravenously. Each point represents avg in four rabbits.

endotoxin. The results are shown in Table II. It will be seen that death was produced in both groups by small doses of endotoxin. The vulnerability of the small animals was now equal to, or slightly greater than that of the larger group, indicating that enhancing effect of colloidal iron is greater in the young rabbits.

Influence of age on pyrogenic effect of endotoxin. An intravenous injection of gram-negative bacterial endotoxin produces in rabbits an abrupt temperature elevation, rising to a maximum in one to 3 hours, and subsiding over a 6- to 8-hour period. Groups of small and large rabbits were given intravenous injections of various amounts of meningococcal endotoxin, and the rectal temperature was determined hourly for the ensuing 6-hour period. The result of an illustrative experiment, in which 4 large and small rabbits received 2 cc of 1-10000 dilution of endotoxin, are shown in Fig. 1. Similar results were obtained with other dilutions of endotoxin. In contrast with the striking difference in the lethal effect of endotoxin for large and small rabbits, no significant differences in the febrile response could be demonstrated.

Influence of age on incidence of generalized Shwartzman reaction. The generalized Shwartzman reaction, characterized by bi-

TABLE II. Influence of Age on Mortality in Rabbits Injected with Endotoxin following an Injection of Colloidal Iron Saccharate.

Avg age (wk)	Avg wt (k)	Mortality				
		Dilution of endotoxin*				Total
		1-320	1-640	1-1280	1-2560	
11	2.60	3/4†	2/4	2/4	0/4	7/16
5	.59	3/4	3/4	4/4	1/4	11/16

* Rabbits were given colloidal iron saccharate intravenously, 2 cc/kilo, and 6 hr later, 2 cc of the dilution of endotoxin indicated.

† Numerator—No. dead within 18 hr. Denominator—No. in group.

TABLE III. Influence of Age on the Incidence of the Generalized Shwartzman Reaction.

Batch of endotoxin*	Size of rabbits†	No. in group	No. of deaths‡		No. with renal cortical necrosis
			After 1st inj.	After 2nd inj.	
A	Large	39	20	11	3 (7.7%)
	Small	38	1	2	26 (68.4%)
B	Large	29	8	7	0 —
	Small	28	1	2	8 (28.5%)

* See text.

† Large rabbits 11 wk old, average 2.5 kilo. Small rabbits were 5 wk old, average 0.6 kilo.

‡ Two intravenous injections of endotoxin were given, 24 hr apart. No. of rabbits dying within 18 hr after each inj. are recorded.

lateral cortical necrosis of the kidneys, is produced in rabbits by the administration of 2 intravenous injections of endotoxin spaced 18-24 hours apart. In previous studies of the reaction conducted in this laboratory, young rabbits have been used in most experiments. In view of the increased vulnerability to a single injection of endotoxin encountered in older rabbits, it was of interest to determine whether susceptibility to the generalized Shwartzman reaction was comparably influenced by age. Groups of large and small rabbits were given 2 intravenous injections of endotoxin, spaced 24 hours apart. Two batches of endotoxin, differing in potency, were employed. Batch A was known to produce bilateral renal cortical necrosis in 70% of small rabbits; the incidence with batch B was approximately 30%. All surviving rabbits were sacrificed 24 hours after the second injection, and the incidence of the generalized Shwartzman reaction was determined by gross examination of the kidneys. The results, summarized in Table III, indicate that the incidence of the generalized Shwartzman reaction is much lower in large rabbits, despite the increased vulnerability to the lethal effect of endotoxin in these animals. The low incidence with batch A was partly attributable to the fact that the majority of large rabbits died within a few hours after the first or second dose of endotoxin, before sufficient time had elapsed for the development of renal cortical necrosis. However, the results with batch B, which permitted survival in half of the group but caused renal necrosis in none, suggest an actual decrease in susceptibility to the reaction in older rabbits.

Discussion. It has been demonstrated that immature rabbits, weighing less than one kilo,

are much less vulnerable to the primary lethal effect of meningococcal endotoxin than larger, more mature animals. Doses greater than 50 times the amount which killed large rabbits were tolerated with little or no evidence of illness by the small animals. The difference in susceptibility was eliminated by the intravenous administration of colloidal iron prior to endotoxin, which caused a greater degree of enhancement of the lethal effect in the small rabbits.

The influence of age on the response of animals to various effects of bacterial products has been the subject of several studies. Zahl, Hutner, and Cooper (7), studying the effects of *Shigella paradysenteriae* endotoxin in mice, showed that when the lethal dose of endotoxin was calculated on the basis of body weight, the young mice appeared to be more resistant than the older ones. However, there was no significant difference in the absolute dose of endotoxin causing death, disregarding body weight, in the 2 groups.

The effects of various products of gram-positive organisms have also been investigated in animals of different ages. Burky (8) found that young rabbits were more resistant to the lethal effect of staphylococcal filtrates than older ones. Parish and Okell (9) injected hemolytic streptococcal filtrate ("scarlet fever toxin") intravenously into rabbits of age groups comparable to those used in the present experiments. The young animals tolerated amounts of the material which were lethal to the older ones.

The difference between the response of large and small rabbits would appear to be based upon one of 2 factors. Either the small animals are in possession of a mechanism for defense which becomes less effective during

maturation, or the young are lacking in a vulnerable or "sensitive" mechanism which appears during maturation. The observation concerning the effect of colloidal iron on the reaction to endotoxin is compatible with the first interpretation. It has been suggested on the basis of other studies(2,6,12) that the reticuloendothelial system is concerned in the protection of normal animals against endotoxin. An intravenous injection of materials known to be taken up by the reticuloendothelial cells, such as thorotrast, trypan blue, colloidal carbon, or colloidal iron, creates temporarily a condition of markedly enhanced vulnerability to the lethal effect of very small doses of endotoxin. If the special resistance of young rabbits were the result of a relatively greater activity of the reticuloendothelial system in these animals, it might be expected that interference with the functioning of this system would eliminate the resistance. It was found that an injection of colloidal iron prior to toxin enhanced the vulnerability of both large and small rabbits, and at the same time eliminated the difference between the 2 groups by a relatively greater effect on the small animals. It seems possible that the resistance of younger rabbits may be related to a function of the reticuloendothelial system, but in the absence of more definitive information concerning the actual effect of colloidal iron on this system, the matter must remain speculative.

That there may be dissociation between the various physiological effects of endotoxin has been suggested by other studies(10). The results of the present study tend to support the idea that one response to endotoxin may be independent of another. Despite the considerable differences in the lethal effect in the 2 age groups, no demonstrable differences in the febrile reaction to small amounts of endotoxin was encountered.

As to the generalized Shwartzman reaction, produced by 2 successive intravenous doses of endotoxin, the results obtained also indicate that more than one mechanism is involved in the effects of endotoxin. The small rabbits, relatively invulnerable to the primary lethal effect of a single injection of endotoxin, showed a high incidence of renal cortical

necrosis after 2 injections, while the older animals, which were much more easily killed by one dose, were relatively insusceptible to the reaction.

For practical purposes, the low incidence of the generalized Shwartzman reaction in mature rabbits indicates that such animals are not suitable experimental subjects for investigation of this reaction.

Summary. A comparison was made of the response to meningococcal endotoxin in young (4-7 weeks) and more mature (10-12 weeks) rabbits, with the following results: 1. Young rabbits were relatively unaffected by a single intravenous injection of endotoxin, in doses more than 50 times the amount which caused prostration and death in older animals. 2. The febrile reaction to small doses of endotoxin was not significantly different in the 2 groups of rabbits. 3. An intravenous injection of colloidal iron saccharate enhanced the lethal effect of endotoxin in all rabbits, and eliminated the difference in susceptibility between young and older animals. 4. The generalized Shwartzman reaction, with bilateral cortical necrosis of the kidneys, occurred in a high proportion of young rabbits after 2 intravenous injections of endotoxin. This reaction could not be elicited in more than 10% of the older rabbits.

1. Thomas, L., and Good, R. A., *J. Exp. Med.*, 1952, v96, 605.
2. Good, R. A., and Thomas, L., *ibid.*, 1952, v96, 625.
3. Thomas, L., Denny, F. W., Jr., and Floyd, J., *ibid.*, 1953, v97, 751.
4. Good, R. A., and Thomas, L., *ibid.*, 1953, v97, 871.
5. Smith, R. T., Thomas, L., and Good, R. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1953, v82, 712.
6. Thomas, L., and Good, R. A., *J. Exp. Med.*, 1952, v95, 409.
7. Zahl, P. A., Hutner, S. H., and Cooper, F. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1943, v54, 137.
8. Burky, E. L., *J. Allergy*, 1932, v3, 438.
9. Parish, J. J., and Okell, C. C., *J. Path. and Bact.*, 1930, v33, 527.
10. Thomas, L., *Ann. Rev. Physiol.*, 1954, v16, 467.
11. ———, *Ann. N. Y. Acad. Sci.*, 1953, v56, 799.
12. Beeson, P. B., *J. Exp. Med.*, 1947, v86, 39.

Received July 14, 1954. P.S.E.B.M., 1954, v86.