antibody responses in children and adults might have been a function of the particular serotypes involved rather than of the age of the subjects, such an explanation is unlikely in view of what is known about serologic responses of children and adults in general. Further study of adenovirus type 3 infections, which occur commonly in both children and adults, should resolve this question.

The CF antibody response appeared to be a quite useful indication of adenovirus infection in adults but was of much less value among children. The latter were more apt to have pre-existing CF antibody, but this factor alone was not responsible for the relatively low rate of significant responses. Thus, of 9 adults with pre-existing CF titers of 8 or 16 all showed significant rises. Of 12 children with the same pre-existing CF titers only 5 (42%) showed significant responses.

Although much remains to be learned about adenovirus HI antibodies such as the time of their first appearance, duration of their persistence, and relationship of heterotypic responses to prior adenovirus infections, the data presently available indicate that they should prove to be useful tools in clinical and epidemiologic studies of adenovirus infections in man.

Summary. Paired sera from 36 children naturally infected with adenovirus types 1, 2, 3, or 5 and from 27 adults naturally infected with adenovirus types 4 or 7 were studied for homotypic and heterotypic hemagglutination-inhibition (HI) antibodies and for complement-fixing (CF) antibody. Homotypic HI responses were observed in most instances. Heterotypic HI responses were relatively uncommon among the children but occurred in adults with about the same frequency as has been reported for heterotypic neutralizing antibody responses in the same type of population. Significant CF antibody responses were detected in most of the adults but in less than one-half of the children.

The author is indebted to Dr. Joseph A. Bell and his associates for supp'ying sera from infected children and to Dr. Robert M. Chanock and his associates for supplying sera from infected adults. Mrs. Janet Hovis, Mr. Horace Turner, and Mrs. Myra Wormald furnished valuable technical assistance.

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Received Aug. 4, 1961. P.S.E.B.M., 1961, v108.

Effect of Immune Serum on Hemorrhagic Shock and on Metabolism of Endotoxemia by the R.E. System.* (26971)

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The irreversible phase of traumatic shock has been attributed to a loss of the R.E. system's capacity to take up and destroy endotoxin, which is being continuously absorbed into the circulation from the intestine(1). If this is a correct hypothesis, serum containing antibodies to endotoxin might be useful to prevent development of the irreversible phase, because such serum antibodies facilitate uptake of endotoxin by R.E. cells(2).† In a normal R.E. cell uptake of enodtoxin is

* Aided by a grant from Nat. Inst. Health, Bethesda, Md., and by a contract with the Office of the Surgeon General, Dept. of the Army.

† This proposal was suggested to us by P. Grabar of Institute Pasteur, Paris.

followed by immediate breakdown(3). In the injured R.E. cell uptake, though greatly reduced, is still appreciable; but significant detoxification cannot be detected(3). From this evidence, one would not expect that serum antibodies would be helpful. Nevertheless, we considered it desirable to investigate the question by direct experiment.

The data reported below demonstrate that serum antibody improves the capacity of the injured R.E. cell to take up endotoxin, but does not restore its capacity to detoxify endotoxin or improve the animal's capacity to tolerate hemorrhagic shock.

Methods and materials. Preparation of immune serum. Serum antibody was produced in 4 ways: 1. Three ml of a heat-killed 18 hour broth culture of *E. coli* $0111B_4$ was injected intraperitoneally 3 times a week until the serum agglutination titre reached 1:10,000.‡ This usually required 8 weeks. The animals were rested for 4-5 days and the blood harvested by heart puncture. Injections were then resumed until the next bleed. This routine was maintained with regular checks to determine that the antibody level was still maintained. Sick animals or those showing a drop in titre were discarded.

2. The cells of a heat killed broth culture of the same *E. coli* $0111B_4$ were collected, washed 3 times, and suspended in saline to a density of 1500 million/ml. One ml of this suspension was injected intravenously 3 times a week. After a high agglutination titre was reached, the serum was harvested as described.

3. Endotoxin of the same *E. coli* $0111B_4$, suspended in saline, was injected intravenously 3 times a week, beginning with a first dose of 0.1 mg followed by graded increases up to 1 mg. After a high agglutination titre was reached (3-6 weeks) the serum was harvested as described.

4. Plasma from rabbits dying of irreversible hemorrhagic shock was injected at regular intervals into the ear vein of normal rabbits. The recipient's serum was harvested when a satisfactory agglutination titre was achieved.

For the study of the capacity of serum antibody to facilitate *in vivo* uptake and destruction of endotoxin, the following experiments were carried out. Dogs were exposed to severe hemorrhagic shock for 2 hours, and were then transfused. One hour later, when systolic blood pressure ranged between 80-130mm/Hg, the peritoneal cavity was opened under pentobarbital anesthesia with sterile precautions, and 2 mg of the *E. coli* endotoxin from *E. coli* $0111B_4$ (labelled with P_{32}) in 2 ml of saline was injected during a period of 5 minutes into a cannulated marginal artery of the spleen. Normal or immune serum (18 ml) was injected into the same artery either some minutes before the endotoxin, or with the endotoxin. In some instances the serum and endotoxin were mixed and held at 37°C for 15 minutes before injection. Ten minutes after the injection the spleen was removed and homogenized in normal saline in a sterile endotoxin-free Waring blendor. The splenic homogenate was held at 4°C until examined for toxicity, but P_{32} content was determined at once, and compared with total P_{32} injected, to determine (approximately) the percent uptake of endotoxin(3). Toxicity of the homogenate was determined by the chick embryo method of Smith and Thomas (4). In our experience with this test, if 4 or more of 6 embryos tested are killed, the test substance contains endotoxin; but if 2 or less are killed there is no measurable amount of endotoxin present.

To test the capacity of serum antibodies to prevent development of irreversibility in hemorrhagic shock, the standard 6 hour hemorrhagic shock experiment was carried out in 2 groups of adult albino rabbits:—those with an intestinal flora rich in coliform bacteria, and those with an intestinal flora poor in coliform bacteria. The latter received 2-3 g

TABLE I. Effect of Immune Serum on Survival Rate of Rabbits Exposed to Hemorrhagic Shock for 6 Hours.

Type of intestinal flora	No. exp.	Immune serum	Survival rate in %
Coliform rich	14	—	7
" "	11	+	9
Coliform-poor plus *E. coli* by gavage	6	—	0
idem	13	+	0

‡ By the Widal tube dilution technic.

TABLE II. Effect of Immune Sera on Uptake and Detoxification of Endotoxin by Injured Spleen.

No.	% Uptake by spleen		Toxicity of homogenized spleen	
	Normal serum	Immune serum	Normal serum	Immune serum
	A. Infusion of Serum 10 min. Before Endotoxin			
1	14	33(1:640)*	+ (5/6)**	— (1/6)**
2	20	13(1:2560)	+ (5/6)	+ (5/6)
3	19	37(1:2560)	+ (6/6)	? (3/6)
4	4	35(1:2560)	+ (6/6)	+ (6/6)
5	5	14(1:1280)	? (3/6)	? (3/6)
6	4	17(1:640)	+ (6/6)	+ (6/6)
7	15	11.5(1:10,000)	+ (5/6)	+ (6/6)
Avg.	11.5%	22.9%		
	B. Infusion of Mixture of Immune Serum and Endotoxin			
1	33	31(1:10,000)	+ (6/6)	+ (6/6)
2	12	54(1:10,000)	+ (6/6)	+ (6/6)
3	10	47(1:10,000)	+ (6/6)	— (1/6)
4	14	48(1:10,000)	+ (6/6)	? (3/6)
5	—	20(1:10,000)	—	+ (6/6)
6	—	39(1:10,000)	—	+ (6/6)
7	—	20(1:10,000)	—	+ (4/6)
8	—	40(1:10,000)	—	+ (6/6)
Avg.	17.2%	37.4%		

* Figures in parentheses show agglutination titre of immune sera.
** Figures in parentheses show mortality of embryos tested for presence of endotoxin; 4 or more per 6 tested is considered positive; 3 of 6 is borderline; and less than 3 is considered negative.

(wet wt) of *E. coli* $0111B_4$ by gavage 3-4 hours before shock was started. Twenty to 30 ml of immune serum was given intravenously 18 hours, 2 hours, 30, 15 or 5 minutes before starting the hemorrhage, or 15 to 30 minutes after starting the hemorrhage. Because none of the immune sera were specific for the endotoxins produced by the normal intestinal flora of the rabbit, it was expected that their potential effectiveness would best be evoked in the coliform-poor rabbits gavaged with the *E. coli* $0111B_4$.

Results (Tables 1 and 2). Table 1 shows the effect of immune serum on survival rate after exposure to 6 hours of hemorrhagic shock. Of the series of 44 rabbits, approximately half received no serum or normal serum, the remainder immune serum, in most instances within 30 minutes prior to shock. The immune serum produced no change in survival rate or the pathology at death.

Table 2 shows the effect of various types of immune serum on uptake and detoxification of endotoxin by the injured spleen. The difference in uptake between shocked spleens pretreated with normal and immune sera is enough to indicate a favorable effect of the serum antibodies on uptake of endotoxin.

The degree of detoxification achieved by the shocked spleens was assayed in terms of percent of surviving chick embryos. The effect of immune serum so determined does not indicate a significant improvement in anti-endotoxic action of the R.E. cell.

Summary and conclusion. Serum containing antibody specific for an endotoxin improves uptake of the endotoxin by the damaged R.E. system. But breakdown of the endotoxin taken up by this system is not thereby improved. Such immune sera given to rabbits in severe hemorrhagic shock for the purpose of improving the clearance of circulating endotoxin do not lower the mortality rate.

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Received Aug. 11, 1961. P.S.E.B.M., 1961, v108.