node, submaxillary gland, thymus, liver, and plasma. The changes in lysozyme activity in some of the organs are probably associated with transplantation of granulopoietic elements. Biochemical significance of lysozyme is not known, but it may reflect immunological activity. In some of our lymphatic tissue homogenates examined microscopically not all cells were disrupted, so that the experimental procedure of freezing and thawing the homogenate, then rehomogenizing, was carried out. This increased the enzyme activity yield in nearly every organ studied.

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1. Suu, V. T., Congdon, C. C., Kretchmar, A. L., PROC. SOC. EXP. BIOL. AND MED., 1963, v113, 481.
2. Kretchmar, A. L., Congdon, C. C., *Am. J. Physiol.,* 1961, v200, 102.
3. Congdon, C. C., Kretchmar, A. L., *Exp. Mol. Path.,* 1963, v2, 277.
4. Kretchmar, A. L., Congdon, C. C., *Transplantation,* 1963, v1, 298.
5. Litwack, G., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 764.
6. Brendler, H., *Science,* 1951, v114, 61.
7. Shugar, D., *Biochim. Biophys. Acta,* 1952, v8, 302.
8. Myrvik, Q. N., Weiser, R. S., *J. Immunol.,* 1955, v74, 9.
9. Cohn, Z. A., Hirsch, J. G., *J. Exp. Med.,* 1960, v112, 983.
10. Brumfitt, W., Glynn, A. A., *Brit. J. Exp. Path.,* 1961, v42, 408.
11. Archer, G. T., Hirsch, J. G., *J. Exp. Med.,* 1963, v118, 277.
12. Fleming, A., *Proc. Roy. Soc., London,* 1922, v93, 306.
13. Hiatt, R. B., Engle, C., Flood, C., Karush, A., *J. Clin. Invest.,* 1952, v31, 721.
14. Congdon, C. C., Urso, I. S., *Am. J. Path.,* 1957, v33, 749.
15. Perri, G. C., Faulk, Margrit, Mellors, Jane, Stock, C. C., *Nature,* 1962, v193, 649.
16. Cappuccino, J. G., Reilly, H. C., Winston, S., *Cancer Res.,* 1962, v22, 850.
17. Speece, A. J., *J. Histochem. Cytochem.,* submitted for publication.
18. Litwack, G., *J. Biol. Chem.,* 1958, v231, 175.

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Production of 7S and 19S Antibodies to the Somatic Antigens of *Salmonella typhosa* in Rabbits.* (29050)

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Numerous reports indicate that antibodies to most antigens appear in both the 19S and 7S globulins. These two molecular species of antibodies have been demonstrated in rabbits immunized with erythrocytes(1,2), bovine γ-globulin(3), human serum albumin(4), hemocyanin(4), diphtheria toxoid(4) and bacteriophage(5). Chickens produced both kinds of antibody to bovine serum albumin(6) and to bacteriophage(7). In frogs and goldfish both rapidly and slowly sedimenting antibodies were found(7). In human serum, antibodies to diphtheria toxoid(8) and *Brucella suis*(8) and certain antibodies to erythrocytes(9,10) have been found in both 19S and 7S globulins. Although both 7S and 19S antibodies to *Salmonella* flagella antigens were demonstrated in human serum(11) and in rabbit serum(4), antibodies to the somatic antigens of *S. typhosa* appeared only in the 19S fraction of human serum after immunization with TAB vaccine(11) and only in the 19S fraction of rabbit serum after foot pad inoculation(4). Since the implication that antibodies to lipopolysaccharide antigens may be entirely macroglobulins is a matter of considerable significance, we have studied further by cellulose chromatography, mercaptoethanol sensitivity and ultracentrifugation the O agglutinins in the serum of rabbits hyperimmunized with *S. typhosa*. Evidence that 7S as well

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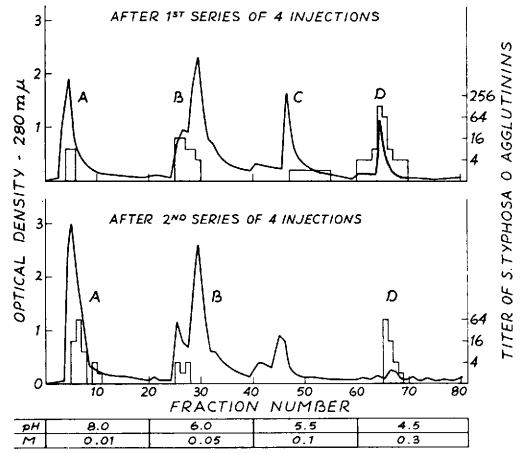

FIG. 1. Distribution of O agglutinins in fractions of rabbit anti-*Salmonella typhosa* serum eluted from DEAE cellulose. Rabbit 2, Table I.

as 19S antibodies are formed under these conditions is presented.

Materials and methods. The vaccine used was prepared from a smooth, motile strain of *S. typhosa* isolated from a patient in 1948. The method was similar to that described by Roschka(12) and successfully used by Edwards(13). Bacteria grown on agar plates were suspended directly in absolute ethanol, heated at 56°C for 4 hours and at 37°C overnight. The ethanol was replaced with acetone and the suspension again left at 37°C overnight. After washing the bacteria twice with acetone, the acetone was decanted and the bacteria were dried and weighed. Rabbits, 4 to 5 lb., were immunized by intravenous or by toe pad injection. For the latter the inoculum was divided among several toes of both hind feet. The antigen used in agglutination tests was prepared from the same strain of *S. typhosa* by suspending the 24-hour growth on agar plates directly in 95% ethanol(14). After heating for 4 hours at 56°C, the suspension was centrifuged and the bacteria were resuspended in saline containing 30% ethanol. For use, this heavy stock suspension was diluted in saline to give 80% light transmittance on a Coleman model 9 Nepho-colorimeter using a 10 mm cuvette. Agglutinin titers were determined by adding 0.5 ml of this suspension to each 0.5 ml of a series of doubled dilutions of serum or of serum fraction. Tests were incubated at 50°C overnight. Titers were recorded as the reciprocal of the highest final dilution of serum or fraction that showed definite macroscopic clumping when viewed with a strong light against a dark background. Five ml portions of serum were chromatographed on DEAE cellulose as previously described(15) eluting twenty 10 ml fractions with each of 4 phosphate solutions with decreasing pH and increasing molarity (Fig. 1). Ultracentrifugation at 35,000 rpm for 15 hours in a sucrose gradient was done as described by Edelman *et al*(16). Treatment with 0.1 M 2-mercaptoethanol was according to Grubb and Swahn (17).

Results. Table I summarizes the results obtained with 4 rabbits inoculated intravenously and 3 rabbits that received the vaccine by toe pad injection. Rabbits 13, 14, 15 and 16 are strictly comparable since all 4 received the same amount of vaccine and were injected and bled according to the same schedule. The amount of antigen injected and time of injection is shown in relation to the O agglutinin titers at various times. The maximum titers observed in the two groups of rabbits show no advantage for either method of inoculation. In those rabbits that received the vaccine intravenously, there was a tendency for maximum agglutinin levels to be reached promptly with a subsequent decline in titer in spite of continued antigenic stimulation. More time was usually required to reach maximum antibody levels in the rabbits injected in the toe pads.

All serum samples were chromatographed on DEAE cellulose. Two typical chromatograms obtained on the first 2 serum samples from rabbit 2 are shown in Fig. 1. The O agglutinin titer of each fraction was determined. By taking the sum of all the titers for individual 10 ml fractions as representing the total amount of agglutinin recovered from the column, it was then possible to estimate, as a percentage of the total, the amount of agglutinin eluted by each of the phosphate solutions designated respectively as A, B, C and D.

These antibody distributions for all sera

TABLE I. Distribution of O Agglutinin in Chromatographic Fractions of Sera of Rabbits Immunized with Acetone Dried *Salmonella typhosa*.

Rabbit	Amt of antigen injected and day of injection	Total mg antigen injected	Day of bleeding	Titer	% of O agglutinin in fractions* A	B	C	D
	Rabbits injected intravenously							
R 2	.25, .5, 1.0 and 2.0 mg on days 0, 4, 8 and 12	3.75	18	5,120	5	24	4	67
	2, 4, 8 and 13 mg on days 22, 25, 30 and 35	30.75	42	5,120	50	5	0	45
	16, 30, 30 and 30 mg on days 45, 49, 53 and 57	136.75	64	1,280	66	3	0	31
R 112	.01, .04, .16, .75 and 3.0 mg on days 0, 3, 5, 7 and 10	3.96	17	10,240	23	3	2	72
R 13	.02, .1 and .5 mg on days 0, 10 and 18	.62	29	40,960	4	3	9	84
	2.5 and 12.5 mg on days 29 and 42	15.62	49	2,560	10	1	0	89
R 14	.02, .1 and .5 mg on days 0, 10 and 18	.62	29	10,240	5	2	9	84
	2.5 and 12.5 mg on days 29 and 42	15.62	49	5,120	13	18	4	65
	Rabbits injected in toe pads							
R 15	.02, .1 and .5 mg on days 0, 10 and 18	.62	29	2,560	3	1	0	96
	2.5 and 12.5 mg on days 29 and 42	15.62	49	5,120	2	1	0	97
R 16	.02, .1 and .5 mg on days 0, 10 and 18	.62	29	5,120	6	1	0	93
	2.5 and 12.5 mg on days 29 and 42	15.62	49	20,480	6	2	0	92
R 111	.5, .5 and .5 mg on days 0, 12 and 19	1.5	26	10,240	1	2	3	94
	.5 mg on day 34	2.0	41	5,120	2	1	10	87

* Fractions A, B, C and D were eluted with 0.01, 0.05, 0.1 and 0.3 M phosphate respectively.

are shown in Table I. Rabbits inoculated intravenously eventually developed sera in which from 10 to 66% of the agglutinins appeared in fraction A. The relative amount of agglutinin in fraction A increased as immunization was continued in rabbits 2, 13 and 14. In the sera of rabbits immunized by toe pad injection from 1 to 6% of the agglutinins appeared in fraction A suggesting that the route of injection may influence the types of antibody produced. In most instances, relatively small amounts of agglutinin were detectable in fractions B and C.

The agglutinins in fraction A, eluted with 0.01 M phosphate were presumed to consist almost entirely of 7S γ-globulin while the agglutinins eluted in fraction D with 0.3 M phosphate should consist largely of macroglobulin(18-20). To support this assumption fractions were examined for stability in mercaptoethanol (ME) and for sedimentation in a sucrose gradient. Pools, designated as fractions A, B, C and D, were made from the 10 ml fractions containing detectable agglutinins in each of the 4 sections of the chromatograms. Each pool was concentrated by dialysis against polyethylene glycol(21) followed by dialysis against saline. The eluate from each section was thus reduced to about one-tenth its original volume. The effect of ME on the agglutinins in fractions A, B, C and D from the serum obtained from rabbit 2 on the eighteenth day is shown in Table II. The titer of fraction A was unchanged while the agglutinins in fraction D were almost completely destroyed. The agglutinins present in fractions B and C were also at least partially destroyed by ME. The globulins in these fractions are undoubtedly mixtures and presumably include the β_{2A}-(22) or γ_{1A}-(20) globulin.

Table III shows the distribution of agglutinins at various levels after sucrose gradient centrifugation of chromatographic fractions A

TABLE II. Effect of Mercaptoethanol on O Agglutinins in Various Chromatographic Fractions.

Fraction*	Treatment	Titer
A	Untreated	16
	ME	16
B	Untreated	64
	ME	8
C	Untreated	32
	ME	0†
D	Untreated	256
	ME	4

* Serum from rabbit 2 after 18 days.
† No agglutination in a 1:4 dilution.

and D from 2 rabbits. The titers were influenced by the manner in which the levels were sampled after centrifugation but in both instances the agglutinins of fraction A were concentrated in the middle area while those of fraction D were concentrated near the bottom of the tube.

Discussion. There have been a number of reports indicating that at least the major portion of the antibodies for the somatic antigens of the typhoid bacillus is present in the 19S γ-globulin. Enders(23) found nearly all of the O antibody in the Cohn fraction III-1 of pooled human plasma while Deutsch *et al*(24) reported that the γ_1 fraction of human plasma contained the major portion of the typhoid O agglutinins. Studying the destruction of agglutinins by sulphydryl compounds Grubb and Swahn(17) found that human anti-salmonella IX agglutinin was destroyed by ME. Typhoid O agglutinins in human sera resulting from TAB immunization were found by Lospalluto *et al*(11) to be exclusively 19S by chromatography and sensitivity to ME. Using

TABLE III. O Agglutinin Titers of Chromatographic Fractions After Centrifugation in a Sucrose Gradient.

Sucrose gradient fraction	Chromatographic fraction A R.2*	A R.112†	D R.2*	D R.112†
1 (top)	0‡	0	0	32
2	0	0	0	32
3	64	64	0	32
4	16	0	0	256
5 (bottom)	8	0	64	256

* Sucrose gradient fractions removed from top.
† Sucrose gradient fractions removed from bottom.
‡ No agglutination in a 1:4 dilution.

paper electrophoresis, Laurell(25) achieved partial separation of the H and O anti-*Salmonella typhimurium* agglutinins in the γ- and β_2-globulins respectively. Laurell(25) also obtained a similar separation of anti-typhoid H and O agglutinins in rabbit immune sera although there was some overlapping. A single rabbit anti-salmonella IX serum was treated with ME by Grubb and Swahn(17) thereby reducing the titer from 160 to 80. Failure to destroy completely the agglutinins in this rabbit serum by this procedure suggests that the serum may have contained both 7S and 19S antibody. Bauer and Stavitsky(4) also examined the typhoid O agglutinins in the sera of immunized rabbits and characterized these agglutinins as γ_1 by electrophoresis and as heavy globulins by ultracentrifugation. Until the present report there has apparently been no conclusive demonstration of 7S antibodies for *Salmonella* somatic antigens.

There can be little doubt that the rabbits used in this study produced 7S agglutinins for the somatic antigens of *S. typhosa* as shown by elution from DEAE cellulose, resistance to ME and sucrose gradient centrifugation. Thus it appears that rabbits produce both 7S and 19S antibodies to this lipopolysaccharide as well as to other antigens(1-5). The change from the early appearance of 19S to the later production of 7S, however, takes place more slowly and less completely in the case of the somatic agglutinins. Sera from only one of our rabbits showed a predominance of 7S and this occurred only after very large amounts of antigen had been injected intravenously. Several factors may have contributed to the failure of other investigators to find 7S somatic agglutinins in human sera. The total amount of antibody in pooled normal plasma or in human serum after immunization is certainly not as great as the amount in the serum of hyperimmunized rabbits and the 7S agglutinins may not have been detectable by the methods used. The route of inoculation is probably a factor also since rabbits injected intravenously produced somewhat higher proportions of 7S than did the rabbits receiving toe pad inoculation.

Summary. Seven rabbits immunized with acetone dried *Salmonella typhosa* produced both 19S and 7S agglutinins for the typhoid somatic antigens although the 19S antibodies predominated in most serum samples even after repeated injections of vaccine. The results suggest that the route of injection and amount of antigen injected may influence the relative amounts of 19S and 7S antibodies appearing in the serum.

1. Stelos, P., *J. Inf. Dis.,* 1958, v102, 103.
2. Pike, R. M., Schulze, M. L., *Immunology,* 1961, v4, 425.
3. Rosenblatt, E., Johnson, A. G., PROC. SOC. EXP. BIOL. AND MED., 1963, v113, 156.
4. Bauer, D. C., Stavitsky, A. B., *Proc. Nat. Acad. Sci. U. S.,* 1961, v47, 1667.
5. Bauer, D. C., Mathies, M. J., Stavitsky, A. B., *J. Exp. Med.,* 1963, v117, 889.
6. Benedict, A. A., Brown, R. J., Hersh, R., PROC. SOC. EXP. BIOL. AND MED., 1963, v113, 136.
7. Uhr, J. W., Finkelstein, M. S., Franklin, E. C., *ibid.,* 1962, v111, 13.
8. Heremans, J. F., Vaerman, J. P., Vaerman, C., *J. Immunol.,* 1963, v91, 11.
9. Abelson, N. M., Rawson, A. J., *ibid.,* 1959, v82, 435.
10. Fahey, J. L., Morrison, E. G., *J. Lab. and Clin. Med.,* 1960, v55, 912.
11. Lospalluto, J., Miller, W., Jr., Dorward, B., Fink, C. W., *J. Clin. Invest.,* 1962, v41, 1415.
12. Roschka, R., *Klin. Med.,* 1950, v5, 88.
13. Edwards, P. R., *Pub. Health Rep.,* 1951, v66, 837.
14. White, P. B., *Med. Res. Council G. Brit.,* Spec. Rep. Ser. No. 103, 1926.
15. Pike, R. M., Schulze, M. L., *J. Immunol.,* 1960, v85, 523.
16. Edelman, G. M., Kunkel, H. G., Franklin, E. C., *J. Exp. Med.,* 1958, v108, 105.
17. Grubb, R., Swahn, B., *Acta Path. et Microbiol. Scand.,* 1958, v43, 305.
18. Lospalluto, J., Ziff, M., *J. Exp. Med.,* 1959, v110, 169.
19. Stanworth, D. R., *Nature,* 1960, v188, 156.
20. Vaerman, J. P., Heremans, J. F., Vaerman, C., *J. Immunol.,* 1963, v91, 7.
21. Kohn, J., *Nature,* 1959, v183, 1055.
22. Rockey, J. H., Kunkel, H. G., PROC. SOC. EXP. BIOL. AND MED., 1962, v110, 101.
23. Enders, J. F., *J. Clin. Invest.,* 1944, v23, 510.
24. Deutsch, H. F., Alberty, R. A., Gosting, L. J., *J. Biol. Chem.,* 1946, v165, 21.
25. Laurell, A. B., *Acta Path. et Microbiol. Scand.,* 1955, Suppl. 103.

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Hypoglycemia Following Endotoxin Administration in Animals with Liver Damage. (29051)

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Previous studies in this laboratory(1,2) have demonstrated that guinea pigs pretreated with a sublethal dose of carbon tetrachloride (CCl_4) are remarkably susceptible to the lethal action of bacterial endotoxin. Endotoxin susceptibility is greatest when hepatic necrosis is maximal (about 48 hours after CCl_4 administration). It was also observed that CCl_4-treated animals became prostrate and died sooner than normal animals given an endotoxin dose of comparable lethality. These findings suggested that a different type of response to endotoxin might occur in these animals with severe liver damage. This study documents the occurrence of progressive and profound hypoglycemia in CCl_4-treated guinea pigs given a lethal dose of endotoxin, a phenomenon which was not seen in normal animals.

Materials and methods. Female Hartley strain of guinea pigs weighing between 300 and 400 g were used. Endotoxin of *E. coli* 026-B6 (Boivin type) was obtained from Difco Laboratories. The intravenous route was used for all endotoxin injections. CCl_4 treatment consisted of the subcutaneous injection of 0.15 ml 48 hours before endotoxin challenge. This dosage caused severe centrilobular hepatic necrosis but was not lethal(2). The animals were either allowed Purina rabbit