cells, the demonstrated phagocytosis of a third particulate substance by mast cells suggests that uptake of substances from the tissue environment may be a process with broad significance with respect to mast cell function.

1. Padawer, J. and Fruhman, G. J., J. Cell Biol. **35**, 181A (1967).

2. Padawer, J. and Fruhman, G. J., Experientia **24**, 471 (1968).

3. Padawer, J., Anat. Record **160**, 404 (1968).

4. Michels, N. A., *in* "Handbook of Hematology" (H. Downey, ed.), 1, p. 232. P. B. Hoeber, New York (1938).

5. Selye, H., "The Mast Cells." Butterworths, London and Washington, D.C. (1965).

6. Tsuda, S., Virchows Arch. Pathol. Anat. Physiol. **247**, 123 (1923).

7. Nagayo, M., Centralbl. allgem. Pathol. U. Pathol. Anat. **43**, 289 (1928).

8. Kiyono, K "Die Vitale Karminspeicherung." Fischer, Jena (1914).

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Assay of Spleen Cells Forming Antibodies to O-Antigens of Salmonellae (33456)

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Quantitative assays of antibody production by single spleen cells in agar have been carried out with red blood cells (1), red blood cells coated with bacterial antigens (2), and with gram-negative bacteria (3) as antigens. The latter are lysed in the presence of antibody and complement (4–6) and sterile plaques are produced around bacteriolysin-producing spleen cells. The experiments described here were carried out to examine (i) whether a direct assay without the use of antigen-coated red blood cells can also be used for testing the immune response to typhoid and paratyphoid vaccines, (ii) whether there exist differences between the response to living or heat-killed microorganisms, and (iii) whether antibody production is primarily limited to species-specific antigens of the microorganisms or comprises, from the beginning of the immune response, species-specific *and* genus-specific antigens.

Materials and Methods. Salmonella typhosa, strain 0901 with the antigenic formula IX, XII and *Salmonella paratyphi A* with the antigenic formula I, II, XII, were kindly provided by Dr. L. S. Baron, Department of Bacterial Immunology, WRAIR, Washington, D. C. Bacteria grown 20 hr at 37° on the surface of nutrient agar plates were used as antigens for immunization and for the assays as well. Forty mice were divided in 4 groups, each of which was inoculated intraperitoneally on the same day with 2×10^8 microorganisms as follows: (i) *S. typhosa,* living, (ii) *S. typhosa,* heat-killed at 65° for 1 hr, (iii) *S. paratyphi A,* living, and (iv) *S. paratyphi A,* heat-killed at 65° for 1 hr.

Assays for the detection of the number of spleen cells producing O-antibodies were carried out as follows: 2 ml of melted 1.4% Noble's agar (Difco) were mixed in small test tubes in a water bath at 50° with 2 ml of sterile, double-strength Eagle's minimal essential medium (MEM), and 0.2 ml of a 0.5% solution of diethylaminoethyl-dextran. The spleens were removed under sterile conditions at 4°, a suspension of single cells was prepared and cells from individual spleens were suspended in 2 ml of Hank's balanced salt solution. If the presence of large numbers of antibody-producing cells was suspect-

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TABLE I. The Appearance of Antibody-Producing Cells in Murine Spleens after Intraperitoneal Injection of Four Different Salmonella Suspensions (containing 2×10^8 microorganisms).

Interval between inoculation and test (days)	Test organisms	Antibody-producing cells in spleens of mice immunized with:			
		S. typhosa (living)	*S. typhosa* (65°, 1 hr)	*S. paratyphi A* (living)	*S. paratyphi A* (65°, 1 hr)
1	*S. typhosa*	0	0	0	0
	S. paratyphi A	0	0	0	0
3	*S. typhosa*	0	0	170	10
	S. paratyphi A	0	0	20	10
4	*S. typhosa*	3200	11,200	12,800	10,400
	S. paratyphi A	1680	2600	4800	6400
7	*S. typhosa*	21,720	15,420	21,280	15,360
	S. paratyphi A	12,000	14,240	7600	8200
9	*S. typhosa*	0	0	0	0
	S. paratyphi A	10	10	10	20

ed, further 10-fold dilutions of the original spleen cell suspension were made in Hank's solution. The bacteria were washed off from the agar surface and suspended in physiological saline solution yielding suspensions with an optical density of 170 in a Klett photometer (red filter). A sample (0.2 ml) of the spleen cell suspension was placed in the center of a plastic petri dish (100 mm in diameter) and nearby was placed 0.4 ml of the bacterial suspension. Onto these separate drops, the content of a test tube containing MEM agar (previously kept in a 50° water bath) was poured and thoroughly mixed by careful movements of the petri dish until a homogenous distribution of the bacteria and the spleen cells was obtained. After hardening, the mixtures were incubated for 1 hr at 37°. Subsequently 1.5 ml of a 1:4 dilution of guinea pig complement in acetate barbiturate buffer were spread over the surface of the plates, which were kept at 4° for 1 hr in order to permit diffusion of complement in the absence of bacterial growth. The plates were then incubated at 37° for 18 hr. In the presence of antibody-forming spleen cells, round, clear and transparent plaques appeared in the greyish, opaque agar in which an uniformly dispersed bacterial growth had taken place.

Results. The results of experiments summarized in Table I show that *S. typhosa*, strain 0901 was more sensitive to the bactericidal serum action and yielded higher number of sterile plaques than the simultaneously examined strain of *S. paratyphi A*. Antibody-producing cells appeared in the spleens of animals immunized with *S. paratyphi A* 1 day earlier than in the spleens of animals immunized with *S. typhosa*, and on day 4 after the injection of the antigens the number of antibody-forming cells appeared to be higher in spleens from animals immunized with living *S. paratyphi A* than in spleens from animals immunized with living *S. typhosa*. On day 7 after the inoculation no significant differences in the plaque counts obtained with different spleens were observed, however, the higher sensitivity of *S. typhosa*, strain 0901, was maintained throughout the experiment.

Simultaneously with the plaques assays we examined the number of viable microorganisms present in the spleens of animals that had been immunized with living bacteria. Table II shows that, in spite of antibody production on day 3 after immunization, over 10^6 bacteria were still present in the spleen; this bacterial count decreased slowly and on day 9 when the number of antibody-producing cells had receded, more than 10^4 microorganisms were still present in the spleens.

When immunized animals received a boos-

TABLE II. The Persistence of Living Salmonellae in Spleens of Mice during a Period in Which Antibody-Producing Cells Appear.[a]

No. of inoculation	Strain / Day:	Bacterial counts on different days after inoculation					
		1	2 (× 10⁴)	3	4 (× 10⁵)	7	9 (× 10⁴)
1	*S. typhosa*	8.0 × 10⁷		1.8 × 10⁶	6.3	1.2 × 10⁵	8.0
	S. paratyphi A			1.3 × 10⁶	4.2	8.5 × 10⁴	2.0
2	*S. typhosa*	3.0 × 10⁶	1.8	1.6 × 10³		6.0 × 10¹	0
	S. paratyphi A	3.5 × 10⁶	2.5	5.6 × 10³		0	

[a] Inoculum: 2 × 10⁸ bacteria.

ter dose of 2 × 10⁸ bacteria 2 weeks after the first injection the regression of the viable count in the spleen was much quicker and reached the zero point within 7 days (Table II).

Agglutinin titers for *S. paratyphi A* and *S. typhi* in nonvaccinated mice varied between 1:2 and 1:5. Slight increases in these titers were observed on day 7 post vaccination with titers of 1:10 for *S. typhi* and 1:5 to 1:10 for *S. paratyphi A*. Significant rises were observed on day 8 postvacination with titers for both strains varying from 1:20 to 1:50. On day 10 postvaccination sera were obtained with titers of 1:100 for the homologous and 1:20 to 1:50 for the heterologous strain. The regression of the viable bacterial counts in the spleens may be ascribed to the presence of circulating antibodies which had been produced as a result of the primary inoculation.

Cross-absorption experiments were carried out with sera taken on day 10 postvaccination. They showed that *S. paratyphi A* immune serum lost its agglutinins completely by absorption with *S. typhi*, while the titer of *S. typhi* immune serum absorbed with *S. paratyphi A* was only reduced for *S. typhi* but not lost. Accordingly, it was assumed that immunization with *S. paratyphi A* resulted in the exclusive production of anti-XII agglutinins, whereas immunization with *S. typhi* resulted mainly in the formation of anti-XII agglutinins and also in the formation of a small amount of specific anti-IX antibodies. Another explanation for the results of the cross-absorption experiments could be that in the absorbed *S. typhi* immune serum the highly sensitive strain 0901 still indicated some residual antibodies, while the less sensitive *S. paratyphi A* strain was not agglutinated.

It seems that the method described above enables us to study early reactions of the host to living and killed vaccines, not only in terms of quantitative assays on one of the final products of the immune response—the serum antibody, but also in terms of much earlier cellular events, including the fate of living bacteria in antibody-producing organs and the interaction between antibody-producing cells and living bacteria. This method therefore should make it possible to study very early stages of the immune response under modified conditions such as in the presence of agents that stimulate or depress antibody responses.

Summary. The appearance and kinetics of antibody-producing spleen cells was demonstrated after use of *S. typhosa*, strain 0901, and *S. paratyphi A* as immunizing antigens and as indicators in a bacteriolytic (or bacteriostatic) plaque assay in agar. The results showed that in the very beginning of the immune response antibody-producing cells appeared which were able to produce mainly antibodies against the XII antigens which are present in both *S. typhosa* and *S. paratyphi A*. The number of plaques was dependent on the sensitivity of the strain employed for the assay; the highly sensitive strain 0901 produced higher numbers of plaques in the presence of homologous antibodies and also in the presence of anti-*S paratyphi A* antibodies than the *S. paratyphi A* strain. No significant differences were found in the response to living and to heat-killed bacteria.

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1. Jerne, N. K. and Nordin, A. A., Science 140, 405 (1963).

2. Canellas, I. and Intravartola, A., Bacteriol. Proc. 1965, Adstr. No. M84, p. 53,

3. Schwartz, S. A. and Braun, W., Science 149, 209 (1965).

4. Rowley, D. and Wardlaw, A. C., J. Gen. Microbiol. 18, 529 (1958).

5. Michael, J. G. and Braun, W., Proc. Soc. Exptl. Biol. Med. 102, 486 (1959).

6. Michael, J. G. and Braun, W., J. Bacteriol. 87, 1067 (1964).

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Dose Study of ^{32}P Incorporation into the Nucleic Acids of Rat Lymphoid Tissues* (33457)

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Radiophosphorus (^{32}P) is widely used to study DNA synthesis in proliferating tissues. Although the toxicity of large doses, especially in lymphoid tissues, is well known (1–3), studies are lacking which demonstrate that the smaller doses usually employed in tracer studies do not also modify the rate of DNA synthesis. Because a number of studies have demonstrated the toxicity of tracer doses of thymidine-^{3}H (4–8), the effect of small doses of ^{32}p on DNA synthesis has been restudied in the lymphoid tissues of rats. The argument of this study is that if ^{32}P does not depress DNA synthesis then the dose incorporation curve of ^{32}P into DNA should be linear and, furthermore, this curve extrapolated to 0 dose should give 0 incorporation.

Materials and Methods. Female, albino, Sprague-Dawley rats weighing 178–207 g were given food and water *ad libitum*. Carrier free ^{32}p (Na_2HPO_4) in 0.45–1.00 ml of 0.9% sterile NaCl was injected intraperitoneally after lightly anesthetizing the rats with ether. At intervals of 4 and 48 hr after injection groups of 5–6 rats at each dosage level were killed with ether. Pooled lymph nodes (axillary, brachial, and mediastinal) and approximately 200 mg of thymus from each rat were placed in tubes of ice-cold (4°) 5% CCl_3COOH and immediately homogenized. At the highest dose of 9.5 μCi/g of body weight there was gross atrophy of the thymus and the lymph nodes at 48 hr and this necessitated the pooling of the lymph nodes into only two samples for the specific activity measurements. The acid soluble phosphate (ASP) fraction, the ribonucleic acid (RNA) fraction, and the deoxyribonucleic acid (DNA) fraction were subsequently isolated (9). The amount of phosphorus in an aliquot of each fraction was measured by the colorimetric method of Fiske and Subbarow (10). The radioactivity of a 1-ml aliquot of each sample was estimated in a Packard Tri-Carb liquid scintillation counter using 9 ml of absolute alcohol as a solvent and 10 ml of scintillation mixture (2.5 diphenyloxazole, 4 g and 1,4-bis-2-(phenyloxazolyl)-benzene, 100 mg in 1 liter of toluene). The specific activity of each sample was calculated as the counts per minute per microgram of phosphorus.

Results. There was linear incorporation into DNA, RNA, and the ASP fraction of doses of ^{32}P ranging from 0.191 to 1.91 μCi/g of body weight in the lymph nodes (Table I) and in the thymus (Table II) at both 4 and 48 hr after injection. This contrasted with the nonlinear dose-incorporation curves observed for tritiated thymidine in a variety of tissues (8). All of the regression lines calculated from the doses 0.191 to 1.91 μCi/g of body weight (Table III) had intercepts at 0 dose which did not differ significantly from 0

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