

γ globulin percentage remained essentially unchanged. The total circulating albumin decreased markedly, the γ globulin tended to decrease slightly, whereas the α globulins changed but little or increased. The maintenance of the circulating α globulins in the depleted state, even though the albumin has been markedly reduced, suggests that the replacement of α globulin in the dog is much more efficient than that of albumin.

The plasma A/G ratios determined by Howe's method were always smaller than those obtained by electrophoresis. Plasma from one normal and one depleted dog was separated into albumin and globulin fractions by Howe's technic. Electrophoretic analyses of these fractions showed that the albumin fraction of the normal dog contained 23% contaminating globulin whereas the globulin fraction contained 6% albumin. The albumin fraction of the depleted dog contained 39% contaminating globulin and the

globulin fraction 3% albumin. Thus, incomplete precipitation of the globulins by the Howe method accounted for the higher A/G ratio. The decreased ratios by both methods reflected the relatively greater reduction in the albumin fraction in the depleted state.

Summary and Conclusions. Electrophoretic analyses of plasma showed that protein depletion in dogs resulted in marked decrease in circulating albumin, and slight decrease in γ globulin. In contrast to these decreases the α globulin content remained essentially unchanged or increased. The α globulins, however, always increased in concentration, which was in part due to the accompanying fall of plasma volume. The Howe method for the determination of albumin: globulin ratios resulted in higher ratios than those obtained by electrophoretic analyses, because of the contamination of globulins in the "albumin" fraction.

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The Antigenicity of *Shigella paradysenteriae* (Flexner) in Saline-in-Mineral-Oil Emulsion.*

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Several reports have recently demonstrated that the immune response to antigens may be markedly prolonged, and often elevated by the suspension of these antigens in the aqueous phase of a saline-in-mineral-oil emulsion.¹⁻³ Killed acid-fast bacilli have been included also in the oil emulsion in a

number of these instances,^{3,4} but Freund has demonstrated in rabbits that in some instances at least, this was not a requisite.² The Henles have recently obtained extremely favorable results in human subjects injected with influenza virus in saline-in-mineral-oil emulsion.⁵

In the search for a method of improving the response to *Shigella paradysenteriae* vaccines, the oil emulsion technic was investigated in mice, rabbits, and humans. Conditions were chosen for the experimental animals that

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¹ Freund, J., and McDermott, K., *PROC. SOC. EXP. BIOL. AND MED.*, 1942, **49**, 548.

² Freund, J., and Bonanto, M. V., *J. Immunol.*, 1944, **48**, 325.

³ Friedewald, W. F., *J. Exp. Med.*, 1944, **80**, 477.

⁴ Freund, J., and Walter, A. W., *PROC. SOC. EXP. BIOL. AND MED.*, 1944, **56**, 47.

⁵ Henle, W., and Henle, G., *PROC. SOC. EXP. BIOL. AND MED.*, 1945, **59**, 179.

TABLE I.
Agglutinin Titer in Mice After Receiving 20 μ g of *S. paradysesterae* Vaccine in Saline, or in Saline-in-oil Emulsion.

Menstruum	Agglutination titer after (weeks)						
	1	2	6	10	12	14	18
Saline	40	20	4	4	8	4	5
Saline-in-oil emulsion	40	10	16	256	256	256	160

could be applied to man. Since it was felt that the inclusion of acid-fast bacilli in the adjuvant mixture would be contra-indicated for human use, because of the danger of tuberculin hypersensitivity reactions,⁴ almost all of the observations were made without them.

The oil emulsion vaccines were made essentially according to the technic of Freund.¹ Falba,[†] a stabilizing agent derived from lanolin, was mixed by electric stirrer with a saline suspension of the alcohol-killed cells of *S. paradysesterae* until a smooth, pasty emulsion was formed. The mineral oil[‡] was then added and stirred in until the emulsion was well dispersed. The proportion of the constituents, antigen in saline: falba: mineral oil was 1:1:4, respectively. All the vaccines were administered subcutaneously. The technics of the agglutination and mouse protective tests are described in detail in a previous publication.⁶

Results in Mice. A series of 35 inbred white Swiss mice were given a single injection of 20 μ g (in 0.15 ml) of cells of a strain of *Shigella paradysesterae* ("V368D")[§] in saline. A similar group was injected with the same dose

of the identical vaccine suspended in 0.15 ml of oil emulsion. At appropriate intervals, groups of 4-5 mice were exsanguinated from the heart, and the blood pooled. The sera thus obtained were titrated for their antibody content by agglutination test. Table I represents the course of the titers in the two groups over a period of 22 weeks.

As can be seen from these data, the peak titer of the saline menstruum group was reached in one week, and dropped to low levels quite rapidly. The peak titer in the oil emulsion group (about 6 times higher than in the controls) was reached by 10 weeks, and only gradually fell after 14 weeks. It may be noted here that normal mouse serum will practically never agglutinate this strain of *S. paradysesterae*. It has been shown that peak titers of mouse antiserum for this organism, after injection of large doses of antigen, range from 1:640 to 1:1280. Several other series in mice have confirmed the validity of the above findings.

Three small experiments have also been undertaken in which 2 strains of *Mycobacterium phlei*, and one strain of *M. butyricum*^{||} were included in the oil phase of the emulsion vaccine described above. Agglutinin levels determined up to 6 or 8 weeks showed no definitely significant increase ascribable to the presence of the acid-fast bacilli.

Results in rabbits. Groups of 3 rabbits each were injected with 2 doses of *S. paradysesterae* Flexner "V368D" alcohol killed bacteria 3 days apart (total 50 μ g). The antigen was either suspended in saline, in a mineral-oil emulsion, or adsorbed onto alum. The volume of each dose was 0.15 ml for each menstruum. Sample bleedings were taken

[†] Falba is the trade name of a lanolin-like substance prepared by Pfaltz and Bauer, Inc., New York, N.Y.

[‡] The brand of mineral oil used was Atreol No. 9, a light mineral oil, U.S.P., prepared by the Atlantic Refining Co.⁷

⁶ Smolens, J., Halbert, S. P., Mudd, S., Doak, B. W., and Gonzalez, L., *J. Immunol.*, in press.

⁷ Halbert, S. P., Smolens, J., and Mudd, S., *J. Immunol.*, 1945, **51**, 39.

[§] This strain was originally received as a V strain. However, it has been typed by Dr. A. J. Weil using both absorbed serums and "pattern agglutination" technic.⁸ He designates the primary antigens as III(IV).

⁸ Weil, A. J., Black, J., and Farsetta, K., *J. Immunol.*, 1944, **49**, 321, 341.

^{||} The strains of *M. phlei* were kindly supplied by Dr. J. Freund and Dr. H. E. Morton, and the strain of *M. butyricum* by Dr. W. Friedewald.

from the ear veins prior to immunization, and at appropriate intervals thereafter, the serum from each group being pooled. The fold increase in mouse protective titers is represented in Fig. 1.

In a total of 4 such experiments, with varied dosage, or with soluble antigen⁶ replacing the killed bacteria, 3 showed essentially the same sort of pattern. That is, the elevation of antibody titer was markedly prolonged by the oil emulsion menstruum and the peak titers were somewhat higher than the saline control groups. In no instance did

the alum menstruum bring about maintenance of high antibody levels.

Summary and Conclusions. Investigations have been carried out with a saline-in-mineral-oil emulsion vaccine containing dysentery antigen. The results in mice and rabbits reveal a striking improvement in the antibody response with this menstruum, especially as far as prolongation of high titers is concerned. There was often a definite increase in the peak titers as well. These results have been obtained in the absence of acid-fast bacilli.

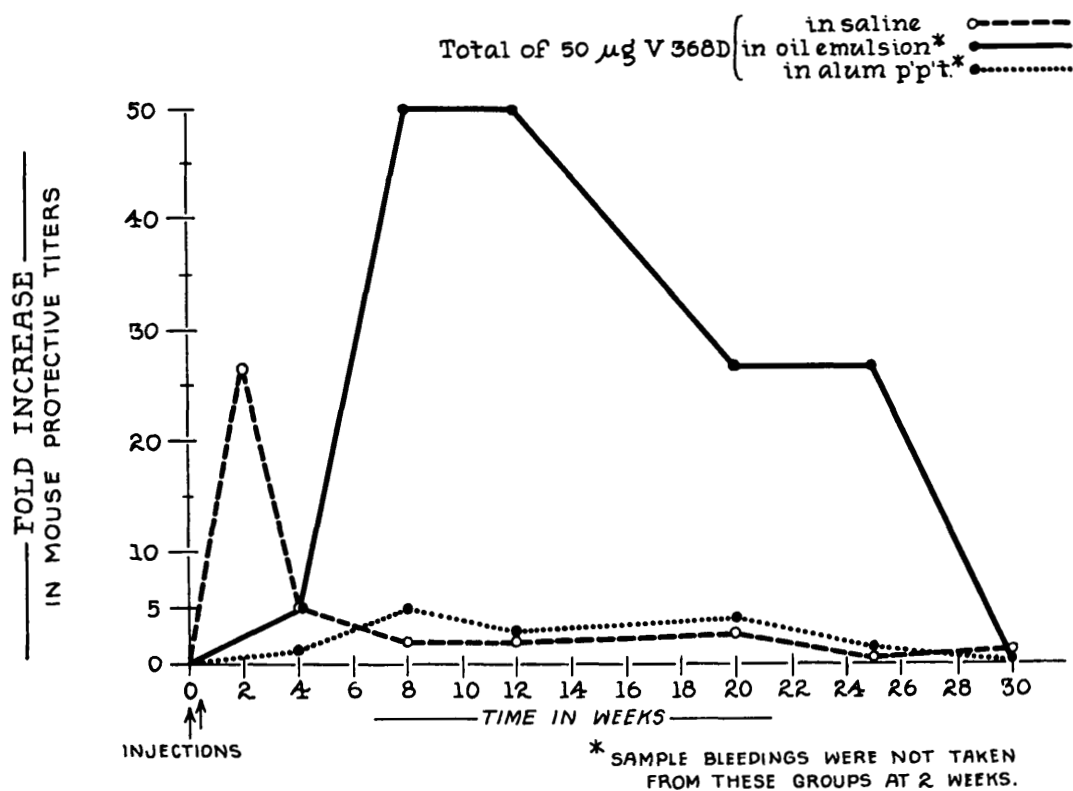


FIG. 1.
Comparative Antigenicity for Rabbits of *S. paradysenteriae* Killed Bacteria.