tisera is calculated to extend from 0.80 to 1.41. Since β-globulin in normal serum is found to be only 0.77, which is outside the fiducial limits, it may be inferred that the apparent increase in β-globulins, although slight, is statistically significant.

The 5% fiducial limits for γ-globulin component are 0.59 and 1.38. Since γ-globulin in the normal serum is well below the lower limit it is clear that the increase in γ-globulin is significant.

Antitoxin titers are not correlated with γ-globulin content. This tends to confirm the impression that the alterations in serum composition result from a non-specific response to the stimulus of Freund's adjuvant.

Summary. 1) Guinea pigs injected with diphtheria toxoid and Freund's complete adjuvant developed antibodies which neutralized the toxin in guinea-pig skin tests and formed visible precipitates in flocculation tests. Precipitation of the antigen-antibody complex was inhibited in antigen excess only, indicating that the guinea-pig antitoxin resembled that produced in the rabbit rather than the horse. 2) The γ-globulin content of pooled antisera, as determined by zone electrophoresis on paper strips, was significantly higher than that of normal guinea-pig serum.

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Received May 4, 1960. P.S.E.B.M., 1960, v104.

Comparative Hemolytic Complement Activities of Germfree and Conventional Guinea Pig Serum. (25883)

WALTER L. NEWTON, ROBERT M. PENNINGTON AND JACOB E. LIEBERMAN
(Introduced by K. Habel)
National Institutes of Health, Bethesda, Md.

Fresh serum from normal guinea pigs is an excellent source of complement. It was of interest to determine whether serum of germfree guinea pigs also contained complement, and to compare its hemolytic activity with that of serum from conventional animals. Such studies could reveal whether contact with bacteria and parasites that normally contaminate the conventional animal is an important factor in determining serum complement level. As far as known, data on complement activity in germfree animals are limited to the following recent observations: Sera from 3 germfree rats each contained "50 units/ml" whereas sera from 3 conventional animals contained "65-80 units/ml"(1). A pool of sera from 6 germfree guinea pigs showed the same hemolytic complement activity as a pool from conventional animals of same age and strain(2).

Materials and methods. Germfree guinea pigs were obtained by Caesarean section of conventional pregnant animals from the NIH colony, and were maintained in Reyniers Germfree System units according to procedures and sterility checks described previously (3,4). Periodically, samples of fresh feces, food, bedding, etc., were examined microscopically and inoculated into various media. Failure to demonstrate presence of viable organisms constituted the basis for calling the animals "germfree." The diet consisted essentially of autoclaved mixture of oatmeal, commercial guinea pig pellets, kale, and carrots, to which vitamins were added(5). Each unit contained litters from 3 females, usually 9-10 guinea pigs. At 1, 2, and 3 weeks of age, 3 germfree (GF) guinea pigs, one from each litter, were removed, anesthetized immediately, and bled from the heart. Serum was separated from the clot by centrifugation and

stored at −70°C to minimize any possible loss of complement activity(6). Two groups of conventional animals obtained from same colony at age 2-4 days, were used as controls and maintained in animal room. One group (CV), was fed usual nonsterile stock diet of kale, carrots, and commercial guinea pig pellets. As a control for diet, the other group (CVD) was maintained on the same autoclaved ration given the germfree animals. Aside from containing living micro-organisms, controls also differed from germfree animals in that they were delivered normally and had suckled for a short period before being weaned. However, these variables have been difficult to control because it has not been possible to keep Caesarean-born, non-suckled guinea pigs alive for appreciable periods with any consistency outside the germfree system. Sera from 81 animals (9 each of GF, CV and CVD, at 1, 2, and 3 weeks of age) were collected over 3 months and tested simultaneously for complement activity, one month after last specimen was collected. Later, a second series, consisting of sera from 27 animals approximately 3 months old, was also examined to compare complement activity in more mature germfree and conventional animals. A modification of a technic(7) for determining 50% hemolytic complement activity, or K value, was used. This modification embodied use of spectrophotometer and increased the accuracy of determining the 50% hemolytic unit of complement.

TABLE I. Comparative Hemolytic Complement Activity of Germfree and Conventional Guinea Pig Serum.

Age in wk	Avg* amounts (ml) of 1:100 serum required to lyse 50% of sensitized sheep RBC's in 2% suspension = K values		
	Germfree (GF)	Conventional (CV)	Conventional on germfree diet (CVD)
1	1.40 (1.07-1.70)	1.26 (1.10-1.52)	1.77 (1.23-2.20)
2	1.06 (.90-1.18)	1.06 (.92-1.12)	1.15 (.90-2.10)
3	1.08 (.85-1.28)	.93 (.80-1.05)	1.24 (.68-1.65)
12	.64 (.49-.96)	.68 (.60-1.05)	.66 (.48-.83)

* Each avg based upon results with sera from at least 7, usually 9, animals. Figures in parentheses indicate range of values.

Results. The data shown in Table I were examined by an analysis of variance. It is obvious that there was no difference in hemolytic complement activity among 12-week-old animals. It is quite apparent that presence or absence of living micro-organisms is not a factor in determining complement levels.

Among younger animals, differences occurred between some groups. However, data were inconsistent in this regard. For example, average values of all 3 groups of 1-week-old animals differed significantly from each other ($P<.01$). Yet sera from the 3 groups of 2-week-old animals showed essentially the same activity. With the 3-week-old animals there were, again, significant differences among the groups ($P<.05$). It is difficult to interpret this variation, particularly since all sera of younger animals were tested at the same time with a single lot of reagents. However, the presence of living micro-organisms did not appear to affect complement levels. In fact, differences were usually larger between conventional animals on the 2 types of diet than between germfree and conventional animals.

All 1-week-old animals showed weaker complement activity than 3 adult animals tested concurrently. The latter, used as controls for test procedure and not shown in the Table, had K values ranging from 0.76 to 0.79, whereas values obtained with the youngest animals ranged from 1.07 to more than 2.20. Some complement levels were so low that K values had to be determined by extrapolation. This tended to reduce the accuracy of higher values and may have been a factor contributing to the variation encountered with younger animals. As a group, sera from 1-week-old animals had complement activity averaging only ⅓ to ½ of 12-week-old animals. We have not seen any published reference to this interesting age effect.

Summary. Hemolytic complement levels of more than 100 1-, 2-, 3-, and 12-week-old germfree and conventional guinea pigs were compared. There was no consistent significant difference between complement levels of

germfree and conventional animals, especially among older animals. All groups of 12-week-old animals had serum complement levels 2 to 3 times those of 1-week-old animals.

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Received May 4, 1960. P.S.E.B.M., 1960, v104.

Role of Inhibitors in Hemagglutination Behavior of Mumps Virus.* (25884)

KARI CANTELL AND KARI PENTTINEN (Introduced by Thomas Francis, Jr.)
State Serum Inst., Helsinki, Finland

Mumps virus agglutinates red cells (rbc) of several animal species. However, it has been reported that the rbc of some species (1,2,3,4) are not agglutinable by mumps virus. Special attention has been paid to the inability of some mumps strains to agglutinate human rbc(5,6,7). According to Sohier *et al.* (7), 9 mumps virus strains fell into 2 groups as regards their hemagglutination (HA) behavior. The first group (Enders type), including 3 of the strains, agglutinated equally well fowl and human erythrocytes, the second group of 6 strains (Habel type) agglutinated human rbc only feebly or not at all. Sensitivity of mumps virus to HA inhibitors present in tissues and fluids of chick embryos is known(8,9). The role of these inhibitors in determining ability of some strains of mumps virus to agglutinate human rbc is the object of this study. It will be shown that all the studied virus strains (also Habel types) agglutinated human erythrocytes almost as well as fowl cells after elimination of inhibitors from the virus fluids.

Materials and methods. The following strains of mumps virus were employed in this study; number of passages undergone in this laboratory in the allantoic (all.) or amniotic (amn.) cavities of the embryonate egg are given in parentheses: Enders strain (68-149 all.), Habel strain (1-12 amn., 1-82 all.) obtained from Dr. V. J. Cabasso(6), KS strain (1-8 amn., 1-32 all.) isolated in this laboratory(10), Chopin strain (1-4 amn.) supplied by Dr. R. Sohier(7). For HA tests blood was taken only from fowls whose rbc were found to give uniformly high titers with mumps virus. Human erythrocytes were always taken from the same person of O blood group. Red cells washed 3 times were stored as a 10% suspension and used at a concentration of 0.5% in the assay procedure. Titrations were carried out at room temperature (18-20°C) on plastic plates by the pattern method(11) and read after 2 hours. Amniotic and allantoic fluids containing virus were treated by various procedures to free them of inhibitor activity. Some were dialyzed for 24 hours against phosphate buffered saline (pH 7.4) at 4°C; others were diluted 5-fold and sonicated for 30 minutes at ice temperature (20 KC. MSE Mullard) while some were merely incubated 2 days at 35°C. Enzymatic treatment was effected by incubating at 37°C for 18 hours a mixture of 2 parts of cholera filtrate (Philips-Roxane) and one part of fluid containing virus. In all HA titrations of the virus preparations treated with cholera filtrate, sodium citrate was added to the reagents to give a final concentration of one per cent. To remove inhibitors by differential centrifugation, one ml of virus fluid and 9 ml of buffered saline were combined and centri-

* Supported by grant from Sigrid Juselius Fn.