ject each assay to rigid statistical analysis. This is not possible with the majority of assays for "insulin-like" activity. In the case of the adipose tissue methods Beigelman(3) and Martin *et al.*(2) employ systems which compare response of one epididymal fat body to insulin and the other to the diluted or undiluted serum. This gives no information as to whether responses obtained in a given experiment are qualitatively similar. Serial dilutions of standard and unknown are necessary together with a measurement of the variability of the response. In the experimental design herein presented these factors have been taken into consideration and an assay method presented which is reproducible and accurate. A multiple dose level assay can be conducted on 3-4 ml of serum and a relative potency determined with confidence limits.

After this work was essentially completed Humbel(4) reported a method similar in design. Using animals fasted for 24 hours he found that the utilizable assay range under his conditions was 10-1000 microunits/ml in contrast to 15-240 microunits/ml reported here. From his data the assay slope was estimated to be 1.5-2.0. In contrast the authors analyzed 6 consecutive assays (non-fasted animals) conducted just prior to submission of this manuscript and found an average slope of 11.2 ± 1.2. A comparison of the responsiveness of epididymal fat bodies from 24 hour fasted and non-fasted animals was also conducted. Fig. 2 shows the marked difference in the slopes of the responses. The steeper slope increases the precision of the assay materially. The estimated lambda value $\left(\lambda = \frac{s}{b}\right)$ using Humbel's data was 0.3 or greater, whereas the present method consistently gave lambda values less than 0.15.

In addition to the length of fasting there were other differences: (a) the useful body weight range (110-135 *vs.* 120-160 g), (b) crystalline bovine serum albumin instead of gelatin in the incubation medium, (c) 150 mg % of glucose *vs.* 200 mg %, and (d) number of animals used for each assay (6 *vs.* 4). The use of 6 animals permits the potency determination of 3 samples per assay.

Summary. A highly reproducible and precise method for determination of "insulin-like" activity using glucose uptake by the epididymal fat body has been presented. It permits the assay of "insulin-like" activity in as little as 3 ml of serum with a calculation of relative potency with confidence limits as well as a test for assay validity.

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Properdin Titers in Sera from Germfree Rats.* (26190)

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Pillemer *et al.*(1) showed that normal human sera and other mammalian sera contain a protein, properdin, which in conjunction with complement and Mg^{++} constitutes a natural defense mechanism of the blood. They were of the opinion that properdin was a

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TABLE I. Properdin Titers in Sera from Germfree, Ex-germfree and Conventional Rats.

Germfree status		Ex-germfree status		Conventional rats		
		14 days	42 days			
Animal No.	Sample I	Sample II	Sample III	Animal No.	Sample II	Sample III
1	<1	2	8	10	2	4–8
2	<1	8	4	11	4	4–8
3	1	4	8	12	16	16–32
4	1–2	1–2	8	13	16	8–16
5	2	8	8	14	16	4–8
6	4	8	16	15	16	16
7	4	8	16	16	16–32	16
8	4	8–16	8–16	17	32	32
9	4	8–16	8	18	32	8

substance differing from antibodies. Pillemer(2) briefly mentioned that properdin was present in sera from germfree rats.

In this paper the properdin titers of germfree rats are compared with those of normal rats of the same strain. The properdin titers of the germfree animals were also studied after the animals had been transferred to the animal room and contaminated with the microbial flora from conventional rats.

Material and methods. The germfree rats were reared according to Gustafsson(3,4). Animals from 5 different litters of the 9th and 11th generations of the inbred germfree strain were used. Nine germfree animals of both sexes and aged 125 days were compared with 9 conventional rats aged 125-140 days. All animals had been reared on the autoclaved diet D_7(4) on raised screens. This diet is not antigen-free, since it contains casein and a number of dead bacteria. About 1.5 ml of blood was sampled from germfree animals when still in the germfree apparatus. They were afterwards transferred to the animal room and given an enema of 1 ml of a suspension of cecal contents from a conventional animal. After 14 days and 42 days new blood samples were collected from these previously germfree animals and from control animals.

The blood specimens were allowed to stand at room temperature for 2 hours and then centrifuged. The serum was afterwards drawn off and frozen at –60°C.

Properdin was determined according to the zymosan assay of Pillemer *et al.*(5) The reagents necessary (RP and R3) were prepared from human, pooled sera according to instructions given by Pillemer *et al.*(5). Before use these reagents were tested with 5 normal human sera which have been used as our standard sera for this purpose for the last 2 years. All samples were studied on the same day to exclude variations due to zymosan and sensitized sheep erythrocytes. The results were expressed in units described by Laurell(6).

All rat sera were diluted ¼. When properdin titers were found to be less than 4 units sera were also studied undiluted. No test was performed with the modified zymosan-assay(6) because the amounts of serum obtained from blood samples were not large enough to permit both tests.

Results. Table I shows that properdin titers of germfree animals were originally significantly lower than those of conventional animals, and that after exposure of the animals to a conventional environment (sample II and III) the titers rose to about the same levels as those of conventional animals.

Discussion. Using electrophoretic as well as immunologic technics Gustafsson & Laurell(7,8) found significantly lower concentrations of gamma- and beta-globulins in germfree rats than in control groups of normal rats, while the other serum fractions were within normal limits. Gamma-globulin values of sera from ex-germfree animals gradually rose but did not reach normal level until about 2 months after the animals had left the germfree environment. No changes were found in albumin and alpha-globulin levels 14 days after exposure of the germfree animals to a conventional environment. The germfree rats might therefore be regarded as healthy, suggesting that the normal flora of micro-organisms are an important stimulant

of gamma-globulin production.

The germfree rats studied with respect to serum properdin content belonged to the same strain and were reared under the same conditions as those of the series of Gustafsson & Laurell(7,8). The germfree rats in the present investigation may therefore be regarded as hypo-gamma-globulinemic. The plasma homeostasis of properdin, like that of the gamma-globulins, seems to be regulated by the normal bacterial environment. In this respect, therefore, properdin falls in the same category as the gamma- and beta-globulins.

Pillemer *et al.*(1) were of the opinion that properdin was a substance not related to antibodies. On the other hand evidence is accumulating(9,10,11,12,13) that properdin may not be a single, unique substance, but represents a group of normal antibodies cross-reacting with antigens present *i.a.* in zymosan.

Pillemer(2) showed that sera from germfree rats possessed properdin when tested in the zymosan assay. Pillemer's data have been published more in detail by Wagner (14). Properdin values of these germfree animals were on the same level as those of the conventional rats. This might *per se* suggest that properdin is not an antibody provided, however, that the animals were not stimulated by antigens. That this might be the case in the referred investigation is suggested by the fact that the animals showed a considerable titer of antibodies against *M. epidermidis*.

Osawa & Muschel(13) reported that the sera of 6-week-old germfree guinea pigs were comparable in bactericidal activity against *Sh. dysenteriae* and *S. typhosa* strain 0901 to that of the sera of conventional animals. They pointed out that these findings were not conclusive, since "the germfree animals are not free of antigenic stimulation nor of passively transmitted maternal antibody."

Neither could the possibility be excluded in our series that antigenic stimulation of the germfree animals by diet or by dead bacteria in the food occurs. However, based on the significantly decreased gamma- and beta-globulin levels in the sera of the presently studied strain of germfree rats it might be assumed that antigenic stimulation of the germfree animals—both with respect to intensity and to number of different antigens encountered—is much weaker than that of conventional animals. This might explain the low properdin values obtained in the zymosan assay with sera from germfree animals, as possibly only a few of the globulins or antibodies crossreacting with the antigens of zymosan may have been formed or stimulated to increase in titer. Under the influence of a normal bacterial flora the properdin of the ex-germfree animals gradually approach normal levels like the gamma-beta globulins.

Summary. 1. The properdin content of sera from germfree rats is lower than that of conventional animals. 2. Properdin titers of germfree animals gradually reach normal values after exposure to a conventional environment. 3. It is concluded that properdin level, like gamma-globulin level, is influenced by the normal bacterial flora.

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