between subjects is also encountered in clinical electrocardiography.

Conclusion. A technic is described, and representative tracings shown, for taking serial electrocardiograms in anesthetized dogs. This technic gives virtually no contour changes in serial electrocardiograms and is therefore suitable for chronic experiments.

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Properties of Diphtheria Antitoxins Produced in Guinea Pigs with Use of Freund's Adjuvant.* (25882)

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In an earlier investigation of the toxins of several strains of *Corynebacterium diphtheriae*(1) we prepared antisera by inoculating guinea pigs with diphtheria toxoid incorporated in Freund's complete adjuvant. Antitoxins produced in this way were used in the conventional flocculation test(2) as well as in gel diffusion and immuno-electrophoresis experiments. The present report is concerned with the nature of the antibody and with protein composition of the sera as determined by zone electrophoresis on paper strips.

Materials and methods. Toxoids were prepared, as described previously(1), by incubating 1% solutions of dialyzed, lyophilized culture filtrates with 0.5% formalin for 6 weeks at 36°C. Three strains of *C. diphtheriae* were used to produce the toxins: the conventional PW 8 (type *intermedius*) and 2 strains of type *gravis*. For preparation of antisera, 6 groups of 500 g guinea pigs were selected. Each animal received 10 mg of toxoid (1 ml) mixed with 1 ml of Freund's complete adjuvant(3) containing *Mycobacterium butyricum* injected intramuscularly in the back of the neck. A 1-ml dose of the toxoid alone was inoculated into each animal at the same site one week later. Two weeks after second injection, the guinea pigs were bled, and the serum of each was titrated for antitoxin content by the guinea-pig skin test(4). Sera from animals that had received the same antigen and had approximately the same skin-test titer were pooled. Pooled serum from 20 uninoculated guinea pigs served as a control. The pooled antisera were re-titrated by skin test using Nat. Inst. of Health reference toxin #2562. L_f values of sera were obtained using flocculating toxin #8030, containing 60 L_f units per mg. Standard flocculating serum #F-6 was used as positive control. To obtain precipitation curves, various mixtures of PW-8 toxin and guinea-pig antitoxin were incubated for 1 hour at 37°C followed by 18 hours at 4°C, then measured for turbidity in a Fisher Nefluorophotometer. The protein composition of pooled antisera and of pooled normal guinea-pig serum was determined by paper-strip zone electrophoresis, using a Spinco Model R apparatus. Five or more replicate samples (0.006 ml) of each serum were allowed to migrate for 16 hours in Veronal buffer, pH 8.6, ionic strength 0.10, with a constant current of 2.5 ma per strip. The patterns were developed by staining with bromphenol blue and were scanned by an integrating densitometer. Protein nitrogen content of each serum was determined by a micro-Kjeldahl method and this value, multiplied by the factor 6.25, was taken as total

* The authors are indebted to Jacob E. Lieberman for statistical analysis of electrophoretic data and to Robert Blereau for nephelometric measurements.

TABLE I. Diphtheria Antitoxin Content of Guinea-Pig Sera as Determined by Skin Tests and Flocculation Reactions in 10 Serum Pools.

No. of guinea pigs in pool	Antigen used to immunize	Units specific antitoxin/ml (skin test)	L_f units antitoxin/ml
17	PW8	150	150
18	*(intermedius)*	200	200
33	*gravis* G 1	10	25
6		30	31
9	*gravis* G 2	20	
9		5	
10		5	31
10		25	25
15		5	
5		10	10
F6 (control)			240

protein content. The absolute concentration of each electrophoretic component was then computed and expressed as g per 100 ml of serum.

Results. The antitoxin content, determined by skin tests and flocculation reactions, is shown in Table I for each of the 10 serum pools. The PW8 preparation elicited a greater antibody response than either of the 2 *gravis* type toxoids. This was undoubtedly due to a greater amount of toxoid in the formalinized culture filtrate, for the PW8 strain is a copious toxin producer.

There was close agreement between skin-test end points and L_f values in 5 of the 8 serum pools upon which both tests were performed. In 2 of the pools (3 and 7) L_f values were considerably higher than skin test titers.

Precipitation curves for the reaction of PW-8 toxin with homologous guinea-pig antiserum (Fig. 1), indicate that there is inhibition in the region of antigen excess only. Thus the precipitins produced in the guinea pig in these experiments are of the "rabbit type"(5).

From the summary of electrophoresis data in Table II it may be seen that γ-globulin, and to some extent β-globulin is more plentiful in immune sera than in normal. Furthermore, the increase in γ-globulin has been at the expense of albumin and α-globulin fractions since total protein is essentially unchanged.

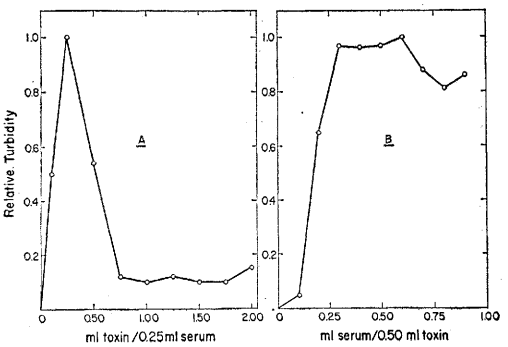

FIG. 1. Precipitation curves for reaction between diphtheria toxin (PW8) and homologous antiserum produced in guinea pigs. (A) Antibody held constant, antigen concentration varied. (B) Antigen held constant, antibody concentration varied.

If it is assumed that the alteration in protein composition is a non-specific response to the adjuvant,† and effects of the toxoids are ignored, then the 6 observations may be treated as replicate experiments which afford an estimate of mean values and standard deviations of protein components in the antisera. The estimates of standard deviation were made with 5 degrees of freedom, and the corresponding "t value" is 2.571.

The fiducial range for β-globulin in the an-

TABLE II. Protein Composition of Pooled Antisera Produced in Guinea Pigs by Inoculation with Diphtheria Toxoids and Freund's Adjuvant.

Inoculum		No. of animals	Albumin	α-glob.	β-glob.	γ-glob.	Total
			g/100 ml				
None		20	2.49	2.35	.77	.35	5.96
PW8 toxoid and Freund's adjuvant		10	2.20	1.78	1.06	.96	6.00
		10	1.93	2.19	1.15	1.29	6.56
G 1	*Idem*	10	2.06	2.23	1.25	.90	6.44
G 2	"	11	2.05	1.86	1.08	.98	5.97
		9	2.02	1.83	1.22	.89	5.96

† In a single animal injected with Freund's adjuvant alone, a similar elevation in γ-globulin was observed.

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

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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