stimulant effects of LSD; 2) anticonvulsant activity against electrically and chemically induced seizures; and 3) analgesic activity. In the anesthetized dog and cat, blood pressure and respiration were affected only by lethal doses of the drug. Large doses of amphenidone did not alter blood pressure responses to acetylcholine, epinephrine, norepinephrine or serotonin. Contractions of nictitating membrane in response to electrical or chemical stimulation were not altered by amphenidone. Normal functioning of isolated heart was not affected by addition of massive doses of the drug to perfusion fluid, nor, in the intact dog, was the ECG (lead 2) changed.

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Standardization of Electrocardiographic Recording in Repeated Experiments on Supine Anesthetized Dogs.* (25881)

C. NEWTON,† A. ELLIS AND S. ZAREMSKI (Introduced by L. N. Katz)
Cardiovascular Dept., Medical Research Institute, Michael Reese Hospital and Medical Center, Chicago, Ill.

There is great difficulty in obtaining reasonably constant electrocardiographic contour in repeated experiments upon dogs because the dog's heart is so mobile. This has been shown in this department(1) and also by Harris and Hussey(2) and Gross and Benison (3). The lateral position is no more successful as shown by Peterson, *et al.*(4), and the data of Lalich, *et al.*,(5). The former group(4) found better reproducibility in the supine position. This is the position we employed, taking not only limb leads but chest leads as well. Our study showed that the position employed minimized or eliminated the instability of serial records as seen in previous studies.

Materials and methods. A total of 32 electrocardiograms were taken on 12 healthy mongrel dogs weighing from 12 to 20 kg. One dog had 10 serial records and most had 3. The records were generally taken 3 days apart. The dogs were anesthetized with Pentothal Sodium, 20 mg/kilo, and placed supine on a V-shaped wooden table and tied down by means of lateral traction on the limbs. The electrode areas were shaved and coated with conducting jelly (Sanborn Redox). German silver plate electrodes were strapped on the 4 limbs just above the paws. Previous trials had shown that other limb positions or

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† Postdoctorate Research Fellow, Nat. Heart Inst.

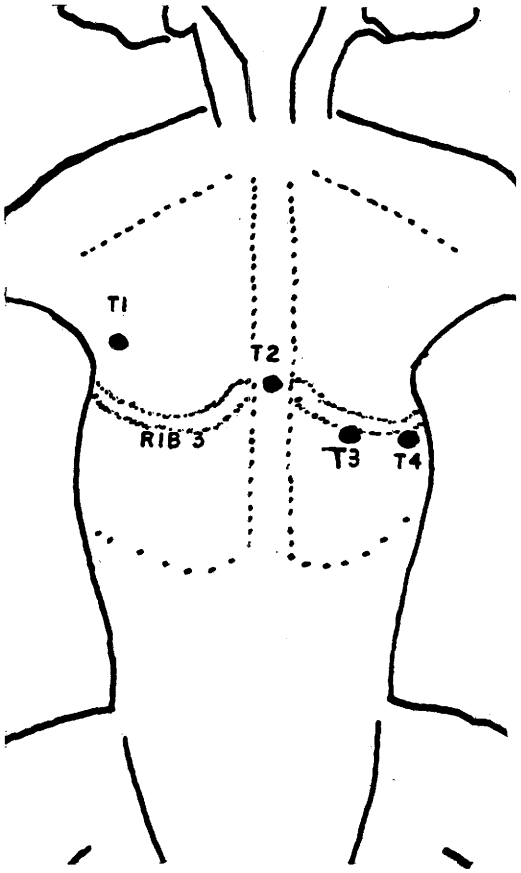

FIG. 1. Sketch of supine dog indicating position of sternum and third rib and location of chest positions for electrodes.

subcutaneous needle electrodes gave almost identical tracings. Suction electrodes, one inch in diameter, were used on the precordium. After preliminary testing of the various contour patterns over the precordium, 4 chest positions were selected (Fig. 1) because they had good anatomical landmarks and were representative of the chief contour types encountered. However, it is obvious that one type blended into another when the precordial electrode roamed over the chest. These precordial leads are not comparable to the human "V" leads in anatomical position or contour and therefore are labeled "T" leads. T_1 is in the 2nd right intercostal space at anterior axillary line. T_2, 3rd rib at midsternum. T_3, 4th left intercostal space midway between sternum and left anterior axillary line. T_4, 4th left intercostal space at anterior axillary line.

Results. Although there were variations in electrocardiographic contour between dogs, each dog's electrocardiogram was characteristic and essentially constant in successive tracings, (Fig. 2). This consistency was seen in all dogs studied. This body position and combination of leads is therefore suitable in evaluating electrocardiographic contour alterations in chronic experiments without concern over changes due to simple shifts in position of the dog's mobile heart.

As there are variations in contours encountered in different dogs (Fig. 3), each dog must serve as its own control. Such variation

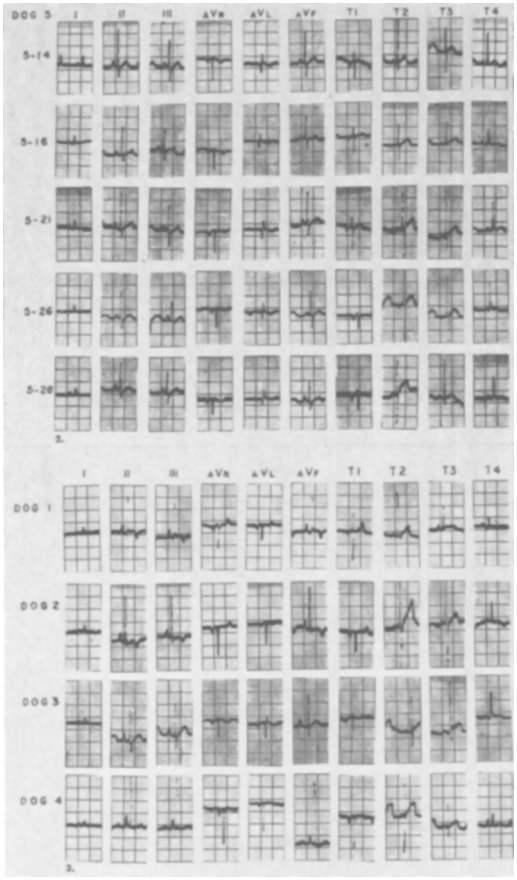

FIG. 2. Limb and chest leads of dog 5 taken on different days to show the fair degree of constancy in contour in the several leads. Discussed in text.

FIG. 3. Limb and chest leads in dogs 1 to 4 to show amount of variation seen among several dogs with leads employed. Discussed in text.

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)

SARA E. BRANHAM, C. W. HIATT AND DONALD B. RIGGS
Division of Biologics Standards, Nat. Inst. Health, Bethesda, Md.

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

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