sera increases in the migration velocity of alpha lipoprotein also were observed. Incubation of 10 normal and 9 hyperlipemic sera with the alcohol-soluble fraction of the phosphatide complex (which consists of ⅔ lecithin and ⅓ cephalin) produced little or no change in the migration velocities of the serum lipoproteins. Incubation of these sera with the alcohol-insoluble fraction, (which consists of ⅔ lipositol and ⅓ cephalin) produced an increase in the migration velocities similar to that noted with the whole phosphatide complex. Therefore, lipositol may be the compound responsible for the change observed. Determination of free fatty acids after incubation with either fraction failed to disclose any differential liberation of free fatty acids by the two fractions. The mechanism by which the alcohol-insoluble lipositol fraction increases the migration velocity of beta lipoprotein remains unexplained.

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Method for Study of Electrocardiogram of Early Chick Embryo within the Shell. (22652)

ABEL A. LAZZARINI, JR.* AND J. WELDON BELLVILLE.†
(Introduced by L. Corsan Reid.)
Deparments of Surgery and Surgery (Anesthesiology), New York Hospital.

The electric activity of isolated heart muscle of the chick embryo as an organ, or as a portion in tissue culture, has been studied by a number of investigators(1,2,4,5,6,7,10,11). Lagen and Sampson(8) employed this preparation, and Friedman and Bine(3) dissected the embryonic duck heart to study electrocardiographic changes in evaluating drugs. Today, isolated muscle from a number of animals is used in physiologic and pharmacologic studies for standardization of drug action upon the myocardium. All these technics involve considerable trauma and the use of artificial

* Present address: New York University Post-Graduate Medical School.

† Present address: Memorial Hospital, New York City.

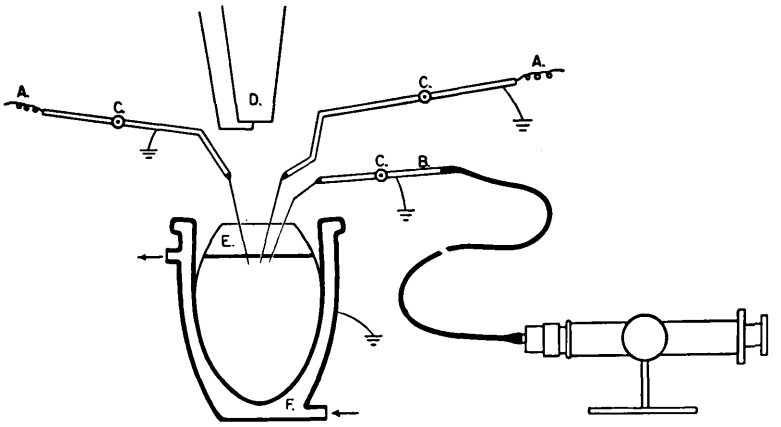

FIG. 1. Schematic drawing of the equipment. A—Connection from microelectrodes to recording apparatus; B—Insulated needle holder with ground connection; C—Universal ball type joint for needle holder; D—Dissecting microscope objectives; E—Egg air chamber; F—Egg holder with water jacket.

media with loss of spatial orientation of the fibers.

It is the purpose of this paper to describe a simple technic for obtaining electrocardiograms of early chick embryos without removal from the shell. Observations on the effect of drugs or of changes in temperature in such preparations can easily be made. The entire embryo remains in its natural environment, thus more physiological recordings can be expected.

Method. Medium sized Rhode Island eggs were incubated from 3 to 18 days at 103°F with daily rotations. From these, well developed embryos 3 to 16 days old were chosen. The eggs were mounted, air chamber on top, in a metal egg holder with a circulating water jacket (Fig. 1). The shell overlying the air sac was first sterilized with a 1% benzolkalium solution‡ and then removed, creating a window about 2 cm square. The mounted egg was placed under a dissecting microscope so that the tips of the recording electrodes were under direct vision. With incident light heart action was observed through the air sac membrane. Each electrode consisted of hypodermic stainless steel needles, one inch long, gauge #26, cut off at the hub and soldered to shielded copper wire. This was mounted in a special needle holder with ball joint (Fig. 1) to facilitate application to the selected area. The needles were insulated with varnish except for 3 mm at the tip.

‡ Winthrop Laboratories, 1450 Broadway, New York.

To obtain maximum deflection in recordings from chick embryos 5 days old, electrodes were usually applied to the right wing and to the anterior wall of the chest. In older embryos, satisfactory recordings were obtained from the right wing, the left thigh or left wing. The needles were inserted deeply into the muscle and left in position during the period of observation.

When the embryos became quite active, as they are after 8 days of incubation, movement that interfered with the recordings was controlled by injection of 3:1000 mg curare or similar drugs.

Recordings were made on a Grass III D electroencephalograph by using EKG filter setting. The gain was adjusted to obtain a tracing of at least 7 mm deflection for the R wave. Following each recording, the instrument was calibrated with a standard value for the gain setting used. The recorder paper speed was 30 or 60 mm per second. When control tracings had been made, the drug to be tested was injected into an allantoic vein with a Pyrex glass microneedle, made in a De Fonbrune microforge and connected to an 0.01 cc tuberculin syringe. Electrocardiographic changes were recorded at various intervals. Throughout each test, a constant or

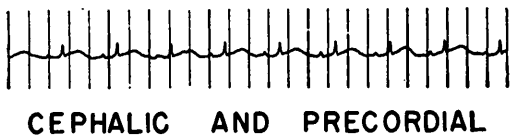

FIG. 2. R wave standard: 40 μvolts. Distance between vertical lines: 1/10 sec.

a variable temperature was maintained by means of the circulating water jacket to study temperature influence on heart action. All drug solutions were preheated before injection to the same temperature as that of the water jacket.

Results. Examples of the recordings obtained by this method from embryos at a constant temperature of 103°F are shown in Fig. 2, 3, and 4. Fig. 2 represents electrocardiograms of a chicken embryo 3 days old. At this age the P wave, the QRS complex and T wave are differentiated. The control tracing had a pulse rate of 240 beats per minute. Fig. 3 illustrates the control tracing in a 6-day-old chick embryo, and the same preparation 5 minutes after an injection of 0.1 cc of 1:10000 Ouabain (2.5 mg/cc) with heart block. Fig. 4 shows the control tracing from a 16-day-old chicken embryo. The control heart rate was 345 beats per minute. Sixty seconds after the injection of 0.2 cc of 1:1 billion Isuprel, the rate was 400 beats per minute.

Discussion. By maintaining the embryo in its natural environment, physiological conditions are maintained during recording of the EKG. The dissecting microscope permits correlation of direct heart observation with EKG changes. According to Lagen and Sampson(8) there is no nerve supply to the

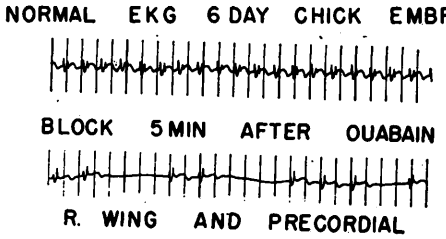

FIG. 3. R waves standard: 40 μvolts. Distance between vertical lines: 1/5 sec.

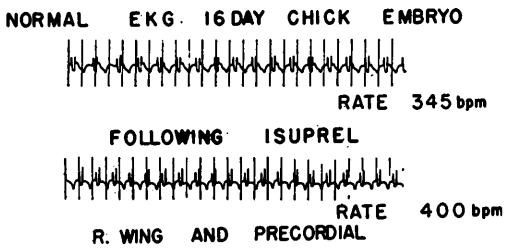

FIG. 4. R wave standard: 100 μvolts. Distance between vertical lines: 6/10 sec.

heart of the chicken embryo until the 6th day. Therefore, 3- and 4-day-old embryos provide a denervated heart preparation which may be valuable in the study of drug action when it is desirable to differentiate direct effect on the heart muscle. The use of chicken embryos within the shell to assay drugs presents definite advantages over methods currently employed, such as low cost, easy preparation, uniformity in quality, and the possibility of evaluating minute quantities of cardiac drugs. This testing procedure is also a suitable device for studying the effects of temperature changes upon the heart, and the action of drugs under hypothermia, since the time required for cooling and rewarming the entire chicken embryo is considerably less than that needed for larger animals.

Summary. A method for the recording of electrocardiograms of the chick embryo within the shell after 3 to 16 days of incubation is described. Early results on evaluation of drug action upon the myocardium at different temperatures suggest, because of the simplicity and low cost, considerable advantages over present technics.

We wish to express our acknowledgement to Dr. J. S. Lieberman who contributed to the design of the electronic equipment.

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Some Observations on Nature of Refractoriness to Histamine Liberators.* (22653)

M. B. SLOMKA AND ANDRES GOTH.
Department of Pharmacology, University of Texas, Southwestern Medical School, Dallas.

The ability of a variety of agents including compound 48/80† to liberate histamine *in vivo* and *in vitro* has been well established. A phenomenon of acute tolerance development has been described for many of these agents and its mechanism is commonly ascribed to depletion of available body histamine stores by previous administration of the histamine liberator(1). The experiments here reported suggest the existence of an alternate mechanism of production of a refractory state to these histamine liberators.

Methods. Mongrel dogs were anesthetized with 33 mg/kg sodium pentobarbital. Arterial blood pressure was recorded from a common carotid artery and injections were made into a femoral vein. Serial blood samples were withdrawn in heparinized syringes from the other femoral vein in those experiments in which estimations of plasma histamine were made. Plasma histamine levels were determined by direct addition of the plasma to segments of guinea pig ileum suspended in atropinized Tyrode's solution, the contraction height being compared with those produced by known amounts of histamine. Pharmacologic identification of the stimulating agent as histamine was established by the inhibitory effect of Benadryl (final concentration $1:1x10^6$).

Two methods of administration of compound 48/80 were employed: (A) 200 μg/kg compound 48/80 was administered as a single rapid injection. (B) A series of graded doses beginning with 1.5 μg/kg and increasing to 200 μg/kg compound 48/80 was administered by slow injections spaced 10 to 15 minutes apart (cumulative dose of 596 μg/kg over a 3- to 5-hour period).

Dogs thus treated were challenged with 200 μg/kg compound 48/80 injected rapidly, and in several instances by 0.1 ml/kg of 10% Tween 20.

Results. The precipitous and prolonged hypotensive effect of a rapid injection of 200 μg kg compound 48/80 in the anesthetized dog (Fig. 1) qualitatively agrees with that

TABLE I. Plasma Histamine Concentrations before and after Administration of Compound 48/80.

Pre-treatment	No. of dogs	Plasma histamine conc. (μg/ml) Control	Min. after 200 μg per kg compound 48/80: 5	15-30
None	3	.13 ± .04*	.57 ± .10	.40 ± .05
596 g/kg, compound 48/80 †‡	4	Not detectable§	Not detectable	—

* Mean ± stand. dev.
† This amount of compound 48/80 was administered over a period of 3-5 hr in a series of graded doses ranging from 1.5-200 μg/kg.
‡ No histamine was detectable in plasma samples obtained every 30 min. during this period.
§ Sensitivity of the histamine assay method is estimated to be .05 μg histamine.

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† Compound 48/80 is a mixture of the dimers, trimers, and tetramers resulting from the condensation of N-methylhomoanisylamine and formaldehyde. Compound 48/80 was kindly made available to us by Dr. E. J. de Beer, The Wellcome Research Laboratories, Tuckahoe, N. Y.