of the extracts contain similar or identical antigens in addition to the major specific antigens. Each cross reaction might represent a unique antigen common to the two interacting insect extracts or there might be an antigen common to all of the extracts. For each cross reacting pair one expects inhibition by extracts of each member of the pair involved. When extracts other than those of the cross reacting pair also inhibit this is evidence for a common antigen in all the inhibitory extracts. By this criterion there appears to be an antigen common to mayfly, fly, roach and cricket; one common to fly, roach and cricket; one common to fly and roach, and another to cricket and roach. It cannot be determined from these data whether these are single or multiple antigens. There is evidence then for cross-reacting antigens of wide and of limited distribution among the insects studied which cannot be correlated with insect taxonomy. The original source of these antigens is probably the insects themselves but could also be due to fortuitous factors such as common bacterial contaminants.

Gel diffusion studies were initiated in part to confirm and amplify the findings in hemagglutination. Although apparent confirmation of extensive cross reactions was obtained, normal rabbit serum produced the same patterns as anti-insect rabbit serum. Gel diffusion, therefore, can neither confirm nor negate the results obtained by hemagglutination.

This study introduces at least two interesting possibilities. One is that the observed bands of identity with normal serum are artefacts and therefore due warning should be given those using the reported methods. The second possibility is that the bands are specific reactions between heterogenetic components of the insect extracts with some natural antibody present in rabbit serum. One type of natural antibody that might be involved is the isoagglutinin. If this is the responsible agent then one would have to conclude that the extracts contained materials related to mammalian blood group substances. Investigation of certain aspects of this problem is being made.

Summary. 1. Extracts of mayfly, fly, cockroach, cricket and silk have been investigated by hemagglutination and agar precipitation technics. 2. Evidence has been obtained for the presence of specific antigens in each extract as well as antigens common to one or more of the other extracts. 3. The minimum number of antigens in each extract has been determined by agar diffusion. 4. The agar diffusion and hemagglutination data provide information helpful in purification studies.

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Cartesian Diver Technics for Embryonic Chick Hearts.* (23726)

JAY A. SMITH
Department of Physiology and Pharmacology, Chicago Medical School, Chicago 12, Ill.

The embryonic chick heart is ideal for certain metabolic studies because it is readily available, inexpensive, small enough to permit ready diffusion of dissolved gases to and from all parts, yet large enough to be handled easily.

Such hearts, however, are too small for successful use in the conventional Warburg apparatus and too large for use in the cartesian diver as previously described(1,2,3). But, by adopting a feature of the Warburg, namely

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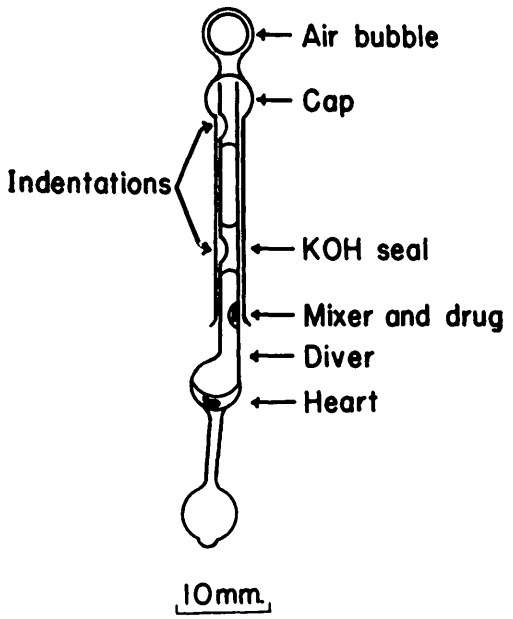

FIG. 1. A cartesian diver suitable for work with the embryonic chick heart.

shaking, to the cartesian diver, and by perfecting convenient methods for reducing loss of gas from the diver, accurate measurement of oxygen consumption by the embryonic chick heart becomes possible. In addition, by perfecting a method for mixing a drug with the solution containing the heart during the course of an experiment, observations before and after addition of the drug become practical.

Methods. Hearts from chicken eggs incubated 5 days were used; at this age the hearts are of maximum size permitting excellent diffusion of gas to all parts, are not dependent upon a coronary circulation, and have a dry weight of about 200 μg. The hearts were removed from the embryos and the blood vessels cut from them with glass tubing pulled to a very fine point. The hearts were introduced into the mouth of the diver by simple hand-held pipets and were shaken into the bulb of the diver. All excess fluid was removed by suction, and then a measured quantity, usually 10.0 mm^3, of Ringer or Locke solution was added. In general, the cartesian diver technics previously described(1,2,3) were employed. The temperature of the bath was 37.25 $\pm$ 0.002°C. This limited variation in temperature was attained by operating the heating current at a voltage only a trifle more (when "on") and a trifle less (when "off") than that required to maintain the temperature of the bath. The suspension fluid was that of Holter(3). The manometer was filled with Brodie's solution(4) adjusted to a P_o of 10133. Changes in the manometric pressure required to suspend the diver stationarily were corrected for changes in atmospheric pressure read from a sensitive anaeroid barometer (Wallace and Tiernan, Belleville, N. J., Model FA139). The diver and heart were viewed through a microscope at a magnification of 40x. Properties of a diver suitable for work with the embryonic chick heart are given in Table I and Fig. 1. The indentations in the neck (Fig. 1) aid in

TABLE I. Properties of a Cartesian Diver Suitable for Embryonic Chick Heart.

	Wt in air, mg	Wt in suspension fluid, mg*	Specific gravity	Vol, mm^3
Cap	285.2	4.8	1.353	210.8
Mixer	5.4	2.2	2.245	2.4
Fluids: Ringer, etc.	10.1		1.005	10.0
Drug droplet	1.0		1.005	1.0
5% KOH	10.4		1.038	10.0
Total fluids	21.5			21.0
Heart (estimated)	1.3		1.06	1.2
Diver (No. 136)	420.6		2.23	188.6
Vol of gas in diver when suspended stationarily			127.7 mm^3	
Diver constant (when P_o is 10133 and temp. 37.3°C)			1 mm = 1.11 $\times$ 10^{-2} mm^3	

* By method of Zeuthen(9).

placing the KOH seal and in filling the upper portion of the neck with suspension fluid so that the initial suspension can be accomplished at approximately atmospheric pressure. The tail of the diver is bent to allow it to lie flat against the wall of the diver holder during mixing (see below). To hold the seals and the droplet of drug (see below) in place in the neck of the diver, the interior was coated with silicone (DC-200, Dow Corning Corp., Midland, Mich., 5% in chloroform), and the divers were baked at 360°C for one hour(5,6,7). In addition the necks were dried with cotton swabs and with a small hot wire after introduction of the heart. To facilitate agitation of the fluids during shaking and to ensure mixing of the entire droplet of drug (see below), the silicone was then etched from the bulb of the diver and from the lowest millimeter of the neck of the diver with 30% sodium hydroxide solution. The diver constant was calculated as previously described(1,2,3) taking into consideration the weight and volume of the diver, of the cap, of the mixer, of the fluids, and of the heart, the latter perforce by estimation (Table I). At the close of each experiment each heart was weighed on a quartz fiber balance somewhat like that of Lowry(8). The balance had a capacity of 1200 μg and a sensitivity of 1.5 μg. Oxygen consumption could then be expressed as cubic millimeters per milligram of dry heart.

Caps. The wide neck of the divers and the temperature at which the experiments were conducted introduced the hazard of excessive loss of gas from the diver. Plugs and various types of seals were tried and found inconvenient to use. Caps (Fig. 1) were found easy to use, aided in keeping the diver erect, and reduced the inadvertent loss of gas virtually to zero. The caps were constructed from thin walled glass tubing selected to fit snugly yet move freely over the neck of the diver. A bubble of air was sealed into the top of the cap giving it a very high center of buoyancy. The weight of the cap was adjusted carefully by fusing in or removing glass so that the cap would sink very slowly when filled with and immersed in suspension fluid. Each cap was weighed in air and then weighed in suspension fluid by the method of Zeuthen (9). Knowing these weights and the specific gravity of the suspension fluid, calculation of the volume of the cap is possible.

Shaking. It was soon found that oxygen did not dissolve into the fluids fast enough to meet the needs of the heart in the diver. This was remedied by shaking the divers á la Warburg. To this end the diver holder was mounted on a movable frame bearing a small motor with an eccentric weight on its shaft. The speed of the motor was controlled and kept constant with a rheostat and a constant voltage transformer. The divers were shaken at about 15 oscillations per second through an excursion of about 3 mm. Shaking with greater violence occasionally broke the seals in the diver.

Experiments next established that as the intensity of shaking was increased from least up to that usually used (attained at 80 volts) there was an increase in oxygen uptake; when the intensity was increased still more, there was no further increase in oxygen uptake. This indicates that the heart can be supplied with all the oxygen it can consume. In other exploratory experiments it was established that the oxygen uptake of hearts from embryos incubated 6 days was very appreciably more than that of hearts from embryos incubated 5 days. Since the experiments were done with 5-day hearts, it is apparent that much more oxygen was available than usually required. Vertical currents in the suspension fluid would introduce errors in the apparent pressure required to suspend a diver stationarily. Creation of such vertical currents by shaking was prevented by filling the diver holder and a few centimeters of its stem with suspension fluid. Shaking was stopped during observation on the rate and beat of the heart and during the final critical immobilization of the diver during each pressure measurement.

Mixing. In almost all experiments it was desirable to measure oxygen consumption and observe the rate and beat of the heart before and after a drug; to this end a method was sought in which a drug could be mixed with the fluid surrounding the heart followed by immediate resumption of accurate measure-

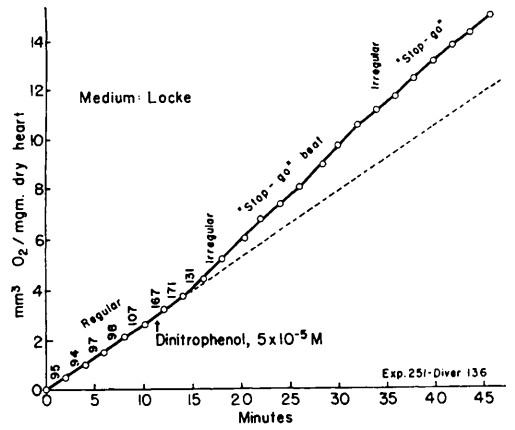

FIG. 2. Oxygen consumption and heart rate and beat before and after dinitrophenol.

ment and observation. Methods employing pressures for mixing(6,7,10,11) were found to be unsatisfactory for experiments with chick hearts: the altered pressures produced an apparent uptake or release of gas, probably by altering the quantity of gas dissolved in the fluids. The following method was found to be satisfactory. A droplet, usually 1.0 mm^3, of fluid containing the drug was deposited within the neck of the diver (Fig. 1), and a glass coated iron "mixer" was pulled into it with the aid of a small magnet. After the control period, the mixer was maneuvered from outside the diver holder by means of a powerful, hand-held, permanent magnet so that the mixer was pulled slowly into the bulb of the diver, towing the droplet containing the drug with it. In this fashion mixing could be accomplished without disturbing the contents of the diver in any undesirable way. In nearly all experiments all of the droplet was mixed successfully, but in case of failure, it was practical to estimate the portion of the droplet that had been actually mixed. The construction of the mixer was quite similar to Claff's "magnetic flea"(12).

Results. The oxygen consumption of an embryonic chick heart before and after a representative drug is shown in Fig. 2. Above the curve is also shown the heart rate and certain characteristics of the heart beat. The figure shows that when dinitrophenol was added to the Locke solution bathing the heart the oxygen consumption increased appreciably and the heart assumed a characteristic type of arrhythmia. This increase in oxygen consumption induced by dinitrophenol is comparable to that described by Lee(13).

In other experiments sodium iodoacetate was added to Locke solution bathing the heart; the oxygen consumption decreased, and the heart eventually stopped beating entirely. Such a decrease in oxygen consumption induced by iodoacetate is also comparable to that described by Lee(13).

In control experiments Locke solution was mixed with Locke solution bathing the heart; this treatment did not alter the rate of oxygen consumption nor the heart rate and beat.

Results of experiments with ouabain have been presented previously(14).

Discussion. Actual experiments indicate that there is no change in oxygen consumption when no change might reasonably be expected (control), there is an increased consumption when an increase might be expected (dinitrophenol), and that there is a decreased consumption when a decrease might be expected (iodoacetate).

Fig. 2 shows that the actual points of measurement of oxygen consumption lie within a narrow band and form a smooth curve; this indicates that shaking did not introduce errors in these measurements. Fig. 2 also shows that mixing did not distort measurement of oxygen consumption.

These methods should also be applicable to studies of tissues whose size is roughly intermediate between those that can be studied by Warburg technics and those that can be studied with the conventional cartesian diver. In addition, the use of oil seals to limit evaporation of fluids and to reduce loss of mobile gases, such as carbon dioxide, should make possible measurement of many phenomena other than oxygen consumption.

Summary and conclusion. Methods are described adapting the cartesian diver for experimentation with the embryonic chick heart. It is concluded that such methods indeed measure oxygen consumption of embryonic chick hearts with accuracy.

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Potentiation of Smooth Surface Caries by Sodium Dehydroacetate Variously Administered to the White Rat. (23727)

I. ZIPKIN AND F. J. MCCLURE (Introduced by O. Mickelsen)
Nat. Inst. of Dental Research, N.I.H., P.H.S., U. S. Dept. of Health, Bethesda, Md.

In a previous study(1) dehydroacetic acid (DHA) markedly potentiated the severity of smooth surface caries in the rat when incorporated in a cariogenic diet at the 0.1% level. To extend this finding and to evaluate the possible extra-oral systemic effect of the compound on caries, DHA was administered by intubation and intraperitoneal injection as well as in the diet and drinking fluid.

Methods. The water soluble sodium salt DHA-S* was used throughout but the dosage levels are given in terms of DHA† to conform with the study previously reported(1). Litter-mated weanling, female, Sprague-Dawley rats individually housed in screen-bottomed cages received the cariogenic diet of McClure and Folk containing an autoclaved skim milk powder(2) for 60 days. The experimental design of the study is shown in Table I. Twenty-four litters of rats were used in Exp. A and B, and 40 litters in Exp. C. The diets were fed *ad libitum* in Exp. A and B. In Exp. C fresh diet was offered daily on a paired feeding basis. Distilled water intake was *ad libitum*. The DHA drinking fluids in Experiment A varied between 0.25, 0.50, and 0.75 mg/ml, and the volumes were controlled in order to equilibrate fluid DHA intake with dietary DHA. The diet and weight gain were measured routinely. The solutions used for intubation and intraperitoneal injection contained 10.0 mg DHA/ml (12.4 mg DHA-S/ml) and were given in 2 divided doses daily except on week ends when half amounts were administered. The mean daily DHA intake by these extra-oral routes in Exp. B and C is shown in Table I. Since 0.10% DHA in the diet had markedly potentiated caries in 3 previous trials(1) and Exp. A, no dietary DHA control was included in Exp. B. To further substantiate the caries potentiating effect of parenterally administered DHA, the regimen of injection used in Exp. B was followed in Exp. C. Amounts of DHA varying from 0.60 to 1.0 ml/day were injected and control rats were injected with equal volumes of distilled water. In Exp. B and C, the rats were kept on the cariogenic diet for one week before intubation and injection of DHA, since weanling rats did not tolerate DHA by these routes of administration. After 60 days on experiment, the rats were sacrificed and the molar teeth were scored for smooth surface

* Obtained from Gaines Chemical Works, Carlstadt, N. J. Calculated for $C_8H_7NaH_2O$: C, 46.16; H, 4.36. Found: C, 46.20; H, 4.44.

† Molecular weights of sodium salt monohydrate and free acid are 208.1 and 168.1 respectively, so that an 0.124% solution of the former is equivalent to 0.10% solution of the free acid DHA.