can be expected to benefit most from Premarin.

Summary. It is hypothesized that mental impairment decreases in patients with atherosclerosis treated with Premarin. One hundred and one patients were studied, 51 who had had cerebral thrombosis and 50 who had recovered from an acute myocardial infarction. The Rorschach test was first administered when the patients began either Premarin or placebo therapy, and was repeated after 6 to 16 months of treatment. The tests were assessed for indications of impairment in thinking, and each patient received a score denoting amount of increase or decrease in impairment after treatment. There was considerable overlap in scores between Premarin and placebo patients, and gains were relatively small for individual patients. Nevertheless, improved mental functioning was found in both cerebral and cardiac patients treated with Premarin in comparison with those on placebo, and a positive association was observed between Premarin therapy and decreased mental impairment.

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Isolating the Total Coronary Artery Inflow for Administering Pharmacologic Agents: New Method Without Coronary Dissection.* (28467)

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One means of studying the pharmacology and physiology of the heart is to administer pharmacologic agents to anesthetized open-chest dogs while directly measuring cardiac performance. Drugs injected intravenously affect the entire cardiovascular system and its regulating mechanisms, while those injected into a single coronary artery affect only the part of the heart supplied by that particular vessel. Cannulating both coronary arteries to inject drugs into the total coronary artery inflow is difficult, especially when direct measurement of left ventricular output is also desired. The method herein reported is a relatively simple technique for injecting pharmacologic agents into the iso-

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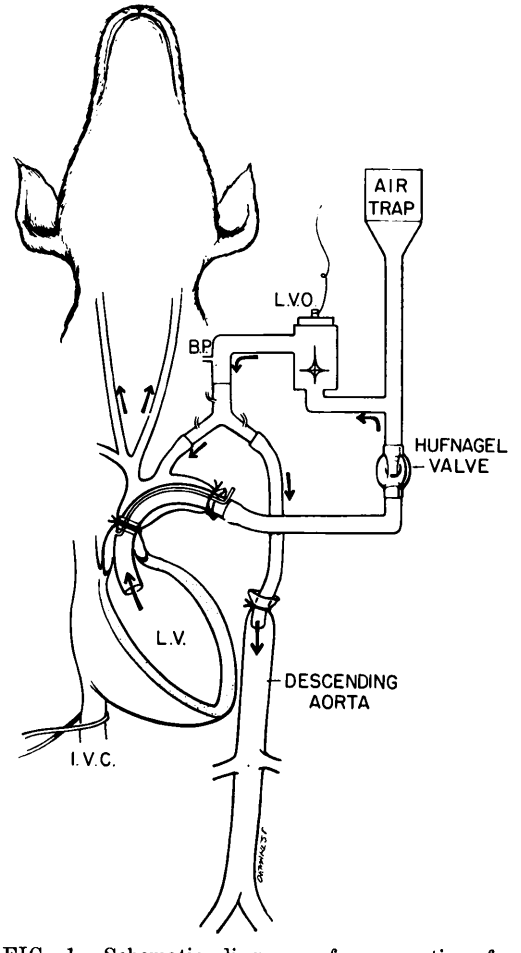

FIG. 1. Schematic diagram of preparation for isolating total coronary artery inflow, injecting pharmacologic agents, and measuring left ventricular outflow (see text for description). L.V.O. = left ventricular output; B.P. = side arm for recording arterial blood pressure; L.V. = left ventricle; I.V.C. = inferior vena cava.

lated total coronary artery inflow; no coronary dissection is required and left ventricular output is measured without further cannulations.

Methods. Dogs weighing between 15 and 25 kg were premedicated with morphine sulfate, 1 mg/kg, intramuscularly, and were anesthetized 30 minutes later with 1% chloralose in polyethylene glycol,† 100 mg/kg, intravenously. The left chest was opened and artificial respiration started. The left subclavian artery was dissected free near its origin. The descending thoracic aorta was freed for a length of 6-8 cm by ligating 2 or 3 pairs of intercostal arteries. The inferior vena cava was looped so that it might be temporarily occluded. The pericardium was incised and the heart suspended in a pericardial cradle. Umbilical tape was passed around the ascending aorta proximal to the origin of the brachiocephalic artery. Anticoagulation was effected with 10 mg/kg heparin, intravenously.

Procedures for isolating the total coronary artery inflow and recording the left ventricular output are illustrated in Fig. 1. The central end of the left subclavian artery was cannulated. The inferior vena cava was occluded while the descending thoracic aorta was clamped, cannulated distally, and severed. The left ventricular output was temporarily carried from the aortic arch to the head and to the right upper extremity *via* the brachiocephalic artery, and to the rest of the body *via* the left subclavian artery and tubing connecting it to the descending thoracic aorta. A specially designed double lumen cannula was inserted through the central end of the descending thoracic aorta and passed retrograde into the left ventricle. The cannula was then connected to the external circuit. By tying the previously placed umbilical tape, the entire left ventricular output was directed through the large lumen of the cannula, a Hufnagel valve(1) replacing the function of the aortic valve, and thence through a Shipley-Wilson rotameter. The left ventricular output was returned to the arterial circulation *via* the distal end of the descending thoracic aorta and the central end of the left subclavian artery. Arterial blood thus flowed retrograde through the left subclavian artery, around the outside of the cannula within the aortic arch, and out the brachiocephalic artery. The patency of this latter system was tested by clamping the tubing leading to the left subclavian artery and observing a typical carotid artery occlusion response.

As a result of these cannulations and ligations, the coronary ostia were isolated in the segment of aorta between the aortic valve and the umbilical tape. The coronary arteries

† Trade name: Carbowax 200, a Union Carbide Chemical Co. product.

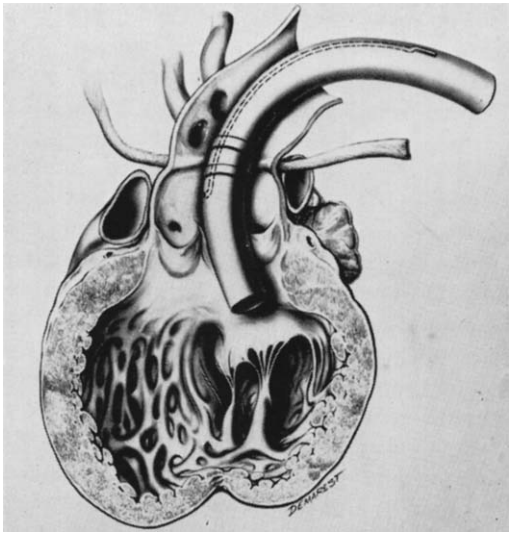

FIG. 2. Drawing made post-mortem with cannula *in situ* after opening left ventricle and aorta. The small inner tube of the cannula is indicated by broken lines where it lies within the large lumen of cannula.

were supplied by blood passing through the aortic valve around the outside of the cannula during systole. The aortic valve leaflets closed around the cannula during diastole, since pressure tracings from the isolated segment showed no evidence of aortic insufficiency. The small inner tube of the cannula ended in this isolated aortic segment, and injections through this tube mixed only with the total coronary artery inflow.

Fig. 2 is a drawing of the cannula *in situ*. The cannula is ½ inch metal tubing, curved 160° of the circumference of a circle having a radius of 2½ inches. Near its distal end there is a small tube (internal diameter 0.026 in.), which enters the large lumen and extends within it to a point 1⅜ inches from the left ventricular end of the cannula where it emerges from the greater curvature. The two ridges on the outside of the cannula are palpable through the wall of the aorta, and when the umbilical tape is tied between them, the small inner tube ends ⅜ inch proximal to the tape. Also visible in the drawing are the aortic valves, coronary ostia, orifices of the brachiocephalic and left subclavian arteries, the umbilical tape, and the mark on the wall of the aorta made by the pressure of the tape.

Left ventricular output, coronary sinus outflow, arterial blood pressure, myocardial contractile force, and left atrial pressure were continuously recorded on a Grass Polygraph. Coronary sinus outflow was monitored with a second rotameter by a method previously reported(2) and served as an index of left coronary artery flow(3). Arterial blood pressure was measured with a Statham pressure transducer, P23AC, attached to the side arm marked B.P. in Fig. 1. Myocardial contractile force was measured with a Walton Strain gauge sutured to the right ventricle, and heart rate was counted directly from this tracing. Electronically damped left atrial pressure was recorded with a Statham pressure transducer, P23BC, attached to a polyethylene tube inserted through the left auricle. The preparation permits other measurements, such as myocardial PO_2, coronary A-V differences, and pressures within other chambers of the heart.

In examples of the method subsequently described, 50 μg nitroglycerin and 3 μg epinephrine were injected separately through the small inner tube. The dead space of the small inner tube plus appropriate connecting tubing and a three-way stopcock measured 0.25 ml. Each drug was injected in a volume of 0.5 ml and was washed in with an additional 0.5 ml saline. An injection of 1 ml of saline caused no change in any of the measurements.

Results and discussion. Fig. 3 is a record-

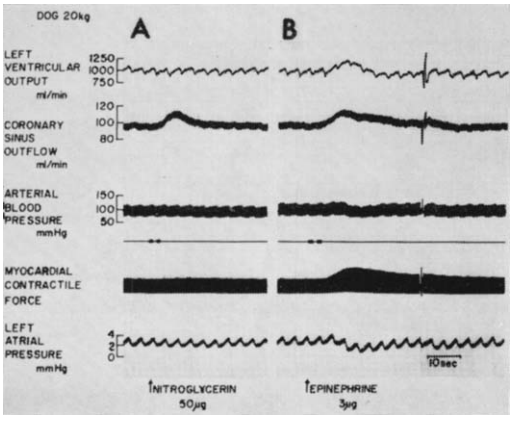

FIG. 3. Effect of single injections of nitroglycerin 50 μg (A) and epinephrine 3 μg (B) into total coronary artery inflow.

ing of single injections of pharmacologic agents into the total coronary artery inflow. Immediately following the injection of 50 μg nitroglycerin (Fig. 3A), coronary sinus outflow increased for 12 to 14 seconds, while left ventricular output, arterial blood pressure, myocardial contractile force, left atrial pressure, and heart rate remained unchanged. Injection of 3 μg epinephrine (Fig. 3B) caused a simultaneous increase in coronary sinus outflow and myocardial contractile force associated with a moderate increase in left ventricular output, a slight biphasic response in arterial blood pressure, a slight fall in left atrial pressure, and an increase in heart rate from 180 to 195 beats/min; all measurements returned to pre-injection levels by the end of 60 seconds. In other experiments, proportionally similar actions were observed with pre-injection left ventricular outputs as high as 1750 ml/min and heart rates as low as 110 beats/min.

Pressure recordings from the isolated aortic segment were taken through the small inner tube of the cannula and left intraventricular pressure was measured simultaneously. Systolic pressures were the same. The pulse pressure did not exceed 30 mm Hg. The high diastolic pressure in the isolated aortic segment is explained by rapid filling through the wide aortic ring near the peak of intraventricular pressure, and by great compliance of the isolated aortic segment maintaining such high diastolic pressure despite the run-off of coronary artery inflow.

Coronary sinus outflow was measured before and after the umbilical tape was tied. Isolating the aortic segment had no apparent effect on coronary blood flow, since coronary sinus outflow did not change. It appears that the small change in mean coronary perfusion pressure was accommodated by auto-regulation(4). The volume of the isolated aortic segment also did not seem to limit coronary artery inflow. When measured post-mortem with the cannula *in situ* by ligating the aortic ring and the coronary arteries, it varied from 3 to 7 ml depending upon the cross-sectional area of the aorta and the placement of the umbilical tape. *In vivo,* almost 3-fold increases in coronary sinus outflow were observed in some experiments with coronary dilating agents.

The special virtue of the method is its relative simplicity; it involves cannulation of only the descending thoracic aorta and left sub-clavian artery. The need for dissecting and cannulating both left and right coronary arteries is obviated, and left ventricular output can be measured without additional cannulations. Its limitations are inherent; it is an acute experiment in anesthetized open-chest animals; the size of the circulatory system of the experimental animal must be sufficient to carry the 200 ml added space of the instrumentation illustrated in Fig. 1; total coronary artery inflow cannot be recorded as a direct measurement and is not included in left ventricular output.

The method may be used in a variety of ways. A continuous infusion can be given through the small inner tube(5). Dose response curves to specific pharmacologic agents may be obtained(6), related compounds compared, and new agents screened and evaluated. The actions of a single drug may be studied by intra-coronary, intravenous, and intra-arterial injection. Inserted into the "Y" connecting tube are small tubes, which allow injection into the arterial circulation (Fig. 1). This arrangement also permits evaluation of the peripheral contribution to prolonged actions observed after a single intra-coronary injection. Moreover, the actions of a drug on the rest of the cardiovascular system can be studied by intravenous or intra-arterial injection while a specific blocking agent is simultaneously administered by intra-coronary route.

The preparation can be modified for study of the aortic arch receptors by isolating the second segment of aorta between the umbilical tape and the ligature distal to the origin of the left subclavian artery (Fig. 1). For this purpose, the common carotid arteries must be cannulated distally and perfused directly from the outflow tubing of the left ventricular output rotameter. With the head circulation secured, the aortic arch segment may be isolated by ligating the right subclavian artery distal to the point where it is crossed by the right vagus nerve, and clamp-

ing the branch of the inverted "Y" tube that is shown perfusing the central end of the left subclavian artery in Fig. 1. Utilizing the central ends of the common carotid and left subclavian arteries, the isolated aortic arch around the outside of the cannula can be perfused and its pressure recorded. Many experiments requiring such a preparation were suggested by Aviado and Schmidt(7).

Summary. In anesthetized open-chest dogs, a relatively simple method is described for isolating total coronary artery inflow without coronary dissection by using a specially designed double lumen cannula. The small lumen is used for injecting pharmacologic agents into the coronary artery inflow; the large lumen permits direct measurement of left ventricular output. The method can be modified for studying the aortic arch receptors.

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Critical Point Preparations of Intact Cells for Electron Microscopy.* (28468)

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The use of intact cells for study with the electron microscope has been limited by the structural distortion produced by drying and the thickness of the unsectioned cell which blocks penetration by the electron beam. The desirability under certain circumstances of observations on the intact cell, however, is obvious. The area of cell surface is vastly greater than in cell sections and, although 3 dimensional reconstructions of cells can be made from serial thin sections, the overall relationship of some of the major cell components could be determined more readily in the whole cell. Finally, possible variations in structural dimensions may not be apparent in sections but may be perfectly obvious in the intact cell if the structures in question are visible and identifiable. The present report demonstrates the application to tissue cells of the critical point method of Anderson previously applied to viruses and bacteria(1,2). The critical point of a fluid, as defined by Ramsey and quoted by Anderson is "that point at which the liquid, owing to expansion, and the gas, owing to compression, acquire the same specific gravity and consequently mix with each other." Essentially, then, by this method liquid (water) can be removed from a cell at the critical temperature with the surface tension at zero and drying without distortion or flattening is possible.

Materials and methods. In the method used by Anderson and followed here, water is replaced by alcohol, this replaced by amyl acetate and the latter replaced by liquid CO_2 whose critical temperature of 31.1 C is more convenient than the very high critical temperature of water. Chicken fibroblasts were grown on coated electron microscope grids as described previously(3,4). After the cells had attached to the supporting film, the grid mounts were removed, washed and fixed with veronal-buffered osmium for one minute. The fixed preparations were then re-washed in acetate-veronal buffer of Michaelis and passed

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