production and breakdown of hemoglobin. Abnormal serum iron values may be caused by severe blood losses and certain pathological conditions.

No explanation is apparent for the increased mean serum iron value of 122 μg % for the 50 to 59 year group as compared with 113 μg % in the preceding and 110 μg % in the following decade. The mean value for the women in the first half of this decade was the same as that for women in the last half. Therefore this higher value cannot be related to age within the decade.

Approximately one-third of the women between the ages of 40 and 52 years were going through the menopause. Their mean serum iron value was 9 μg % lower than women in this age range who had not reached the menopause, and 10 μg % lower than those who had passed the menopause. The difference, however, was not significant.

The nutritional and medical significance of the decrease of serum iron with advancing age in healthy women is not apparent. The process of aging may modify those physiological factors which influence the level of serum iron in such a way as to cause this decrease.

Summary. (1) The serum iron and hemoglobin values were determined for 275 healthy women ranging in age from 17 to 86 years. Mean values for all women were 116 ± 33 μg % and 13.2 ± 0.9 g %, respectively. (2) Analysis of variance indicated a difference of serum iron levels among different age groups which was significant at the 1% level. The regression coefficient of serum iron on age was —.343, indicating a decrease of serum iron with advancing age. This decrease was not accompanied by a decrease in hemoglobin values, nor was there a correlation between serum iron and whether the menopause had been reached or passed. (3) There is no apparent nutritional or medical significance to the decrease of serum iron levels with age in healthy women.

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Pressure Pulse Recording from an Isolated Mammalian Heart Preparation Based on a New Principle.* (18891)

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Certain problems of cardiodynamics can be studied profitably by the use of an improved isolated mammalian heart preparation. The mode of relaxation of the ventricle, for example, may hold further information concerning the control of cardiac output, for it is increasingly apparent that the remarkable property of the heart is not only that it can pump so much, but also that it can fill so efficiently. A study of the relaxation process of the ventricle is difficult, however, when large volumes of blood are being pumped by the heart.

In order to study the relaxation process adequately an isolated heart preparation should possess the following attributes: (a) generate ventricular pressure pulses of normal contour, (b) possess easy control of atrial and ventricular systolic and diastolic pressures, (c) pump no blood, *i.e.*, have a fixed ventricular volume, and (d) not deteriorate over a period of many hours. The preparation to be described possesses all these characteristics to a satisfactory extent.

The preparation. Heyman and Kochman (1) apparently introduced the idea of using a donor dog to supply the blood and pressure head for the perfusion of the coronary arteries of the isolated heart. Although their

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1. Heyman, J. F., and Kochman, M., *Arch. Pharm. et de Therap.*, 1904, v13, 379.

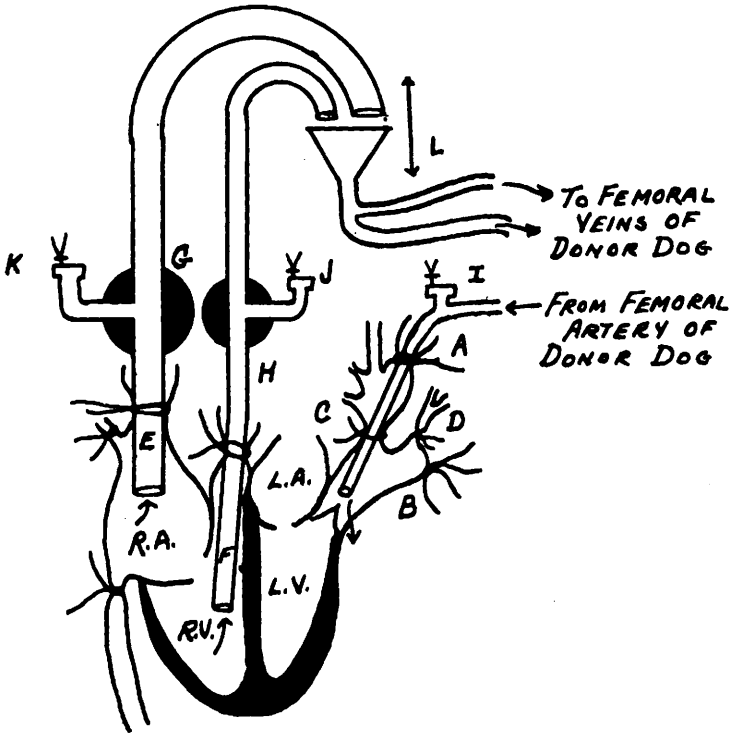

FIG. 1. Schema used to perfuse the dog heart *in situ*. Explanation in text.

method has not been used extensively it has an advantage over the use of mechanical perfusion systems, namely, that the presence of the intact lungs, liver and kidney of the donor dog can maintain a more normal blood chemistry.

Fig. 1 shows the schema currently used. Blood from the femoral artery of the donor dog enters the ascending aorta via a cannula (A) introduced through a common carotid artery. The aorta is tightly ligatured at the arch (B), as are all head vessels (C) and the left subclavian artery (D). The only pathway for escape of the perfusion blood is through the coronary artery orifices of the isolated heart or through the orifices of the vasa vasorum vessels in a short segment of the ascending aorta and aortic arch.

The coronary venous blood is returned to the donor dog either through a large cannula (E) placed in the superior vena cava, the inferior vena cava and the azygos vein being ligated, or through a cannula (F) placed in the right ventricle by retrograde passage past the pulmonic valve. Manipulation of stopcocks (G) and (H) determines through which channel the coronary venous blood shall return to the donor dog and the manner in which the right ventricle shall contract, *i.e.*, "isometrically" or "isotonically." Optically recording manometers measure the perfusion pressure (I), right ventricular pressure (J), and right atrial pressure (K). The height of the opening of the outflow tubes (L) in relation to each other controls the systolic and diastolic pressure when stopcocks G and H are open.

The consecutive steps in making the preparations are as follows: Two dogs of average size are anesthetized with morphine sulfate and sodium barbital. The chest of the dog whose heart is to be isolated is opened widely by a midsternal incision under adequate artificial respiration. Loose ligatures of strong cotton cord are placed around vessels indicated in Fig. 1. The ligatures passed around the azygos vein and left subclavian artery are tied immediately.

The donor dog is prepared next. Both femoral veins and one femoral artery are exposed with a minimum of trauma. Short metal cannulas of large diameter are tied into one femoral vein and into the femoral artery. The donor dog is then heparinized (4 mg heparin/kg, immediately, and 5 mg each half hour thereafter). A saline filled piece of Tygon tubing 60 cm long and 5 mm I.D. (internal diameter) tipped with a straight brass cannula 15 cm long and 3 mm I.D. is connected to the metal cannula previously placed in the femoral artery. A 100 mm laboratory funnel serves as the receptacle for the blood returning from the recipient heart. A *Y* tube is attached to the funnel and each arm of the tube is fitted with about 40 cm of 5 mm I.D. tubing. After filling with saline, one tube is attached to the metal cannula previously placed in the femoral vein. The other tube is attached to a No. 14 urethral catheter which is introduced well into the inferior vena cava via the other femoral vein. The height of the funnel is adjusted in accordance with the donor dog's venous pressure so that there is the least practical volume of saline in the funnel. The donor dog's animal board is placed on a level about 30 cm below that of the recipient dog's board to facilitate the return of blood to the donor.

The recipient dog is now heparinized in the same manner as the donor dog. The brass perfusion cannula connected to the tubing leading from the donor dog's femoral artery is introduced into the ascending aorta of the recipient dog via a common carotid near its origin and tied in tightly. It is essential that the perfusion line must be free from air bubbles. A large cannula 10 cm long and 1 cm I.D. equipped with a 3-way stopcock and about 40 cm of large tubing (1 cm I.D) is introduced into the right atrium via the superior vena cava and tied in tightly. This cannula and tubing is also filled with saline, but the presence of air bubbles is immaterial. Pinch clamps are used to guard this tubing and the perfusion tubing from emptying during manipulation. A cannula 15 cm long and 4 mm I.D. connected with a large 3-way stopcock and 40 cm of Tygon tubing 5 mm I.D. is filled with saline and the end opposite the cannula fixed in position over the receiving funnel (as is the end of the tubing attached to the cannula in the superior vena cava). This completes the preliminary preparations.

The heart is isolated by quickly performing the following steps in sequence: (1) Tie off the brachiocephalic artery, (2) Tie off the inferior vena cava, (3) Remove pinch clamp from the outflow tubing leading from the right atrium, (4) Tie off the aorta tightly, (5) Remove pinch clamp from the perfusion line, (6) Insert and secure cannula into right ventricle via pulmonary artery, (7) Remove the pinch clamp from the tubing draining the right ventricle. If the above steps have been properly done there is no cardiac standstill or arrhythmia. The coronary venous blood will return in good quantity (25-100 cc/min.) either by way of the right atrium or right ventricle, depending upon the arrangement of the height of the respective outflow tubes and the 3-way stopcocks.

Since the blood cools considerably during its passage through the external circuit, it is advisable to supply heat to the donor dog. Gauze or nylon filters have been used in the receiving funnel, but they probably are unnecessary with adequate heparinization.

Pressure pulse contours. The pressure pulses obtained from this preparation are satisfactory as to contours and pressures. The cardiac chambers may be arranged to beat against either an open or closed outflow system. Segments A, B, C, and D, Fig. 2, show ventricular and atrial pressure pulses recorded from the same animal in various combinations.

Control of pressures. The ease with which intra-cavitary pressures are controlled varies with the combination of opened and closed outflow systems used. When both the atrial and ventricular outflow systems are open the diastolic pressure of both atrium and ventricle can be adjusted independently by varying the relation between the height of the opening of the outflow tubing. When one outflow system is closed the diastolic pressure in that chamber will follow the changes in the dias-

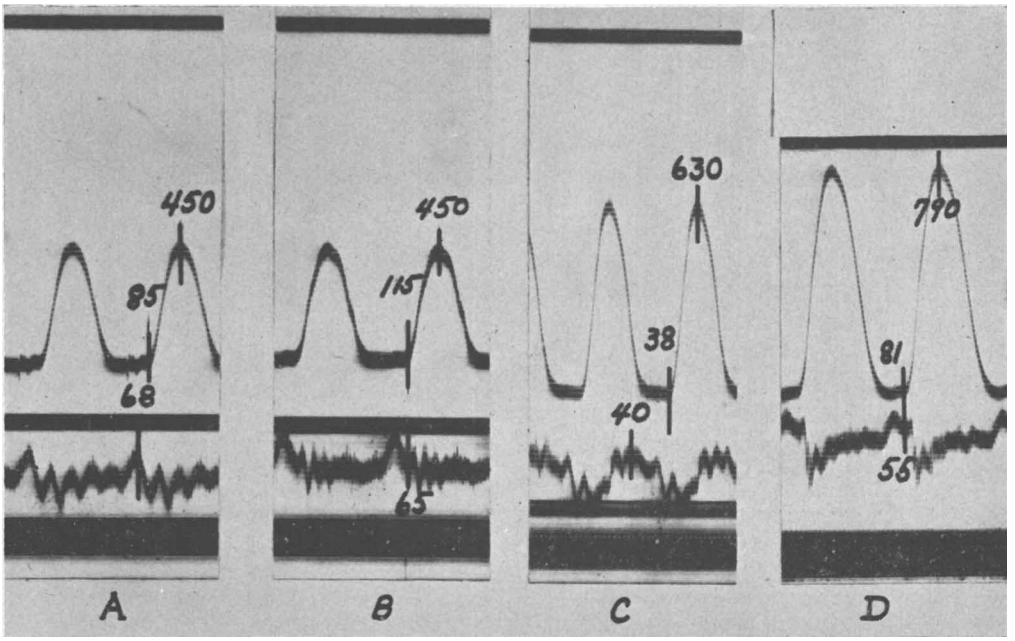

FIG. 2. Right ventricular curves (top) and right atrial curves (bottom) recorded from isolated hearts. A, both ventricle and atrium contracting "isotonically;" B, right atrium contracting "isometrically," and right ventricle "isotonically;" C, right atrium contracting "isotonically" and right ventricle "isometrically;" D, both right ventricle and atrium contracting "isometrically." Calibration in mm saline. Discussion in text. (Note: the terms "isometrically" and "isotonically" are used only to indicate the relative amount of myocardial fiber shortening.)

tolic pressure of the open system. With both outflow systems closed, the pressure in both chambers will increase until failure ensues.

Ventricular isometric contraction. It was hoped that the ventricle could be arranged so that it contained a fixed (and adjustable) volume of blood throughout the cardiac cycle, *i.e.*, a true isometric ventricle. Visual impression indicates that this is impossible in the dog's right ventricle since there is apparent diminution of the size of the ventricle during systole when the right ventricular overflow system is completely closed. This occurs at all pressure levels. At moderate to high pressure levels (above ca 150 saline diastolic) tricuspid regurgitation supervenes, judged by the development of a palpable thrill. Whether or not other functional avenues of escape of blood from the ventricle (Thebesian veins?) exist must be considered. However, the changes in volume of the ventricle during a cycle apparently are slight.

By keeping the ventricular volume relatively fixed during the entire cardiac cycle it has been possible to demonstrate that ventricular relaxation can occur up to the onset of the next systole. Fig. 3, A and B, shows two segments taken from a record in which the heart rate slowed slightly when the systolic pressure increased and systole shortened as the result of injection of 40 μg of epinephrine. The early diastolic portion of the relaxation curve became steeper and the remainder of the diastolic portion of the curve continued to decline until the onset of the next systole. Atrial pressure was less than ventricular throughout diastole (although rising slightly) until late in the atrial systolic phase. Thus, ventricular filling from the atrium must have been negligible. The zero level for both atrium and ventricle was the same.

Viability. As long as an adequate perfusion pressure is maintained these isolated hearts show no tendency to fail. Many experiments have been terminated at the end of 4 or 5 hours with the isolated heart showing a considerable amount of reserve (as tested by increasing the diastolic pressure to abnormal

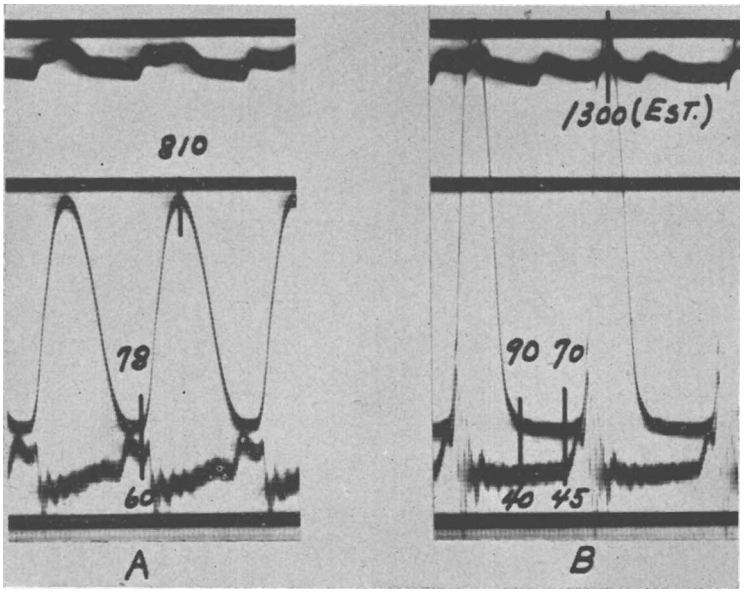

FIG. 3. Right ventricular curves (middle) and right atrial curves (lower) recorded from the isolated heart before (segment A), and after (segment B) an exceedingly small dose of epinephrine. (The top curve is perfusion pressure.) Discussed in text.

levels) without deterioration of the pressure pulses.

Failure of the preparation, when it occurs, is the result of an unexplained default of the donor dog. Preliminary findings indicate that the condition resembles that of irreversible hemorrhagic shock, but the condition is not accounted for on the basis of external blood loss.

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Spectrophotometric Determination of Tromexan in Plasma and Serum.* (18892)

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The ethyl ester of bis 3,3′ (4-hydroxy coumarinyl) acetate, Tromexan, is available for therapeutic use as an anticoagulant. It is presumed to compete with vit. K in hepatic synthesis of prothrombin. The anticoagulant activity is gauged by the decrement in plasma prothrombin.

The absorption, distribution, destruction, and excretion of Tromexan were studied in part by Pulver and von Kaulla(1). These authors used an indirect colorimetric method of analysis. It is the purpose of this paper to present an accurate and rapid method for the determination of Tromexan levels.

The test. Tromexan is extracted by 2,2, 4-trimethylpentane from plasma or serum which has been acidified with hydrochloric acid. The optical density of the resultant solution of Tromexan is determined at a wave length of 310 mμ in a Beckman spectrophotometer. *Reagents.* (1) Concentrated hydrochloric acid (C.P.). (2) 2,2,4-trimethylpen-

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1. Pulver, R., and von Kaulla, K. N., *Schweiz. med. Wchnschr.*, 1948, v78, 956.