sacrificed at 1 hour, 24, 48, 72 and 96 hours and at 2 and 3 weeks after injection. Dilutions of the homogenized organs obtained at autopsy were injected into 7 day embryonated eggs, and after proper incubation the periembryonic fluids from these eggs were tested for the presence of mumps virus by the C.F. test.

The results of the first test of mouse organs and tissues removed one hour after injection of mumps virus are shown in Table I. It is observed that spleen, lung and liver contained considerable virus. A trace of virus was found in the brain, while none was found in the testes. Although further negative results are not tabulated here, we found the virus neither in the pancreas, heart muscle, nor blood.

In these experiments, the mice which received intranasal virus got only one-twentieth the amount of virus given intraperitoneally. This intranasal dose however was sufficient to give rise to demonstrable titers of virus in the liver and lung one hour after injection.

Twenty-four hours after injection of mumps virus into mice we found, at autopsy, only traces of virus in the spleen, lung, liver and brain. As before, no virus was found in the testes, pancreas, heart muscle, and blood.

No mumps virus was found in the organs and tissues heretofore examined in mice sacrificed at 2, 3, and 4 days, and at 2 and 3 weeks after injection of virus. Negative results after these longer periods of time indicate that inapparent infection does not occur.

Summary. Mumps virus administered to mice intraperitoneally or intranasally appears within an hour in the lungs, liver, spleen, and to a lesser extent in the brain (in the latter case after intraperitoneal injection only.) Sojourn of viable virus in organs where found is short. Testes, pancreas, heart muscle, and blood were found not to contain virus at any time. Mice used in these tests of mumps virus did not exhibit symptoms of toxicity at all comparable to mice injected at different times in the past with influenza virus, although these 2 viruses are somewhat similar otherwise.

Received August 19, 1949. P.S.E.B.M., 1949, **72**.

Production of Acute Experimental Circulatory Failure by Graded Pulmonary Artery Constriction.* (17358)

MATTHEW N. LEVY† AND ROBERT M. BERNE‡ (Introduced by Carl J. Wiggers.)

From Department of Physiology, Western Reserve University Medical School, Cleveland, Ohio.

Because of the practical and technical limitations of studying congestive heart failure in human subjects, numerous attempts have been made to produce heart failure in animals employing many different methods. Various drugs have been administered, including chloroform, ethyl alcohol, potassium chloride, chloral hydrate, and diphtheria toxin,[1] posterior pituitary extracts,[2] and quinidine,[3] in an effort to depress the myocardium. In addition, irritants have been injected into the pericardium[4] and myocardium;[5] the ventricular wall has been severely cauterized,[6] the coronary arteries ligated,[7,8] and the myocardial capillaries plugged with foreign sub-

* This research was supported by a grant from the Life Insurance Research Foundation.

† U. S. Public Health Service Research Fellow.

‡ Sarah Welt Fellow.

1 Fahr, G., and Buehler, M. S., *Am. Heart J.*, 1943, **25**, 211.

2 Nolasco, J. B., and Kohrman, R., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 108.

3 Reiss, R. A., and DiPalma, J. R., *Am. J. Physiol.*, 1948, **155**, 336.

4 Armstrong, T. G., *Quart. J. Exp. Physiol.*, 1940, **30**, 263.

5 Luisada, A., *Medicine*, 1940, **19**, 475.

6 Starr, I., Jeffers, W. A., and Meade, R. H., *Am. Heart J.*, 1943, **26**, 291.

7 Orias, O., *Am. J. Physiol.*, 1932, **100**, 629.

8 Gross, L., Mendlowitz, M., and Schauer, G., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 446.

stances.[9] In general, there have been two serious drawbacks to these methods: 1) the difficulty in controlling the severity of the experimental procedures, and 2) concomitant undesirable side effects, such as induction of arrhythmias and toxic actions on other organs of the body.

Since a reduced cardiac output—variously occasioned—is probably the initiating factor leading to the typical signs of congestive heart failure, the prerequisite for any technic attempting to produce experimental circulatory failure is a reduction of the output of the heart without lowering the blood pressure to shock levels. Therefore, we have employed a graded pulmonary artery stenosis as an experimental approach to the problem. This procedure possesses the following virtues: the cardiac output may be reduced significantly without concurrent marked changes in arterial pressure; a strain is placed on the right ventricle; any desired gradation of severity may be applied rapidly and easily; and finally, the procedure is readily reversible.

Previous workers who successfully produced experimental pulmonic stenosis were interested in its relation to pulmonary embolism[10] or in the cardiac dynamics of such a lesion,[11] and only incidental attention was directed to the effects on the peripheral circulation. Furthermore, there is only a narrow range of constriction which will give changes simulating clinical heart failure. If the constriction is too mild, right atrial and aortic pressures remain unaltered, and the only observable result is some increase in the right intraventricular pressure during systole. On the other hand, if the degree of constriction exceeds the optimum, an uncompensated drop in cardiac output occurs with a decrease in arterial blood pressure to shock levels.

Since we plan to utilize this technique in the future to determine the primary effects of reduction of cardiac output upon renal function, it seemed advisable to supplement our knowledge of the dynamic cardiovascular changes which are produced by increasing the resistance to right ventricular ejection. In addition, an attempt was made to elucidate the mechanism by which the central venous pressure becomes elevated during this procedure.

Methods. Mongrel dogs weighing between 8 and 16 kg were anesthetized with morphine followed by intravenous injection of sodium barbital (ca. 200 mg/kg). The chest was opened through the midline and respiration was maintained artificially. The pulmonary artery was dissected free near its origin, and was constricted by means of a strong cord noose attached to a screw device, permitting fine gradations of circular constriction. In each of the 13 experiments which were successfully performed, right atrial and aortic pressures were optically registered by means of modified Gregg manometers. In 5 of these experiments, right intraventricular pressures were also recorded, and in 5 others the pressures in the pulmonary artery distal to the point of constriction were recorded. A calibration was made at the end of each record in relation to a fixed base-line.

Results. No alterations in right atrial or aortic pressures could be detected until the pulmonary artery was constricted to a certain critical degree, estimated by previous workers[10] to be about 60% of occlusion. Pressure pulses registered from the aorta and right atrium during progressive, gradual constriction of the pulmonary artery are presented in Fig. 1 and 2. For reasons outlined by Wiggers,[12] right atrial pressures were measured at 3 points in all curves:

1. A point—the maximum point attained during atrial systole;

2. Z point—just prior to closure of the A-V valves; and

3. V point—just prior to opening of the A-V valves. These three points are indicated on the right atrial curve of record A in Fig. 1.

Using these criteria, records B and C of

[9] Roos, A., and Smith, J. R., *Am. J. Physiol.*, 1948, **153**, 558.

[10] Gibbon, J. H., Jr., Hopkinson, M., and Churchill, E. D., *J. Clin. Invest.*, 1932, **11**, 543.

[11] Fineberg, M. H., and Wiggers, C. J., *Am. Heart J.*, 1936, **11**, 255.

[12] Wiggers, C. J., *Physiology in Health and Disease*, 5th edition, Lea & Febiger, Philadelphia, 1949.

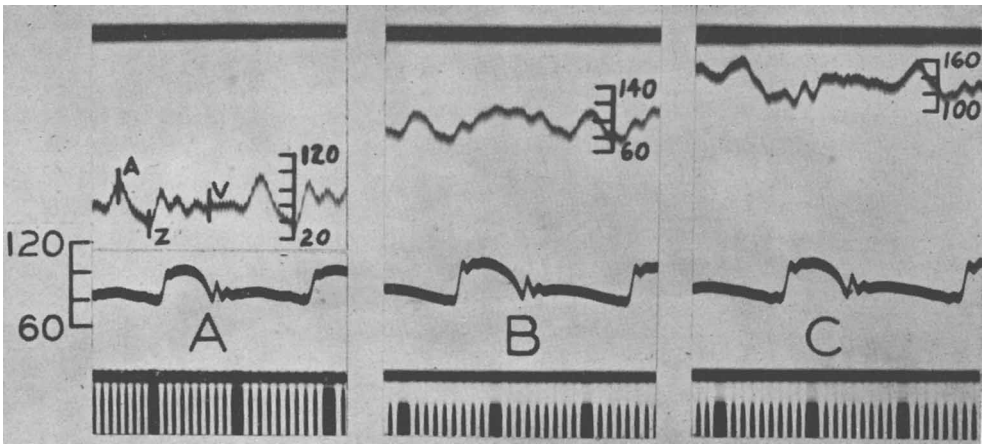

FIG. 1.
Right atrial (upper) and aortic (lower) pressure curves in experimental pulmonic stenosis. Record A—control; record B—minimal constriction beyond the critical degree; record C—slight additional constriction.

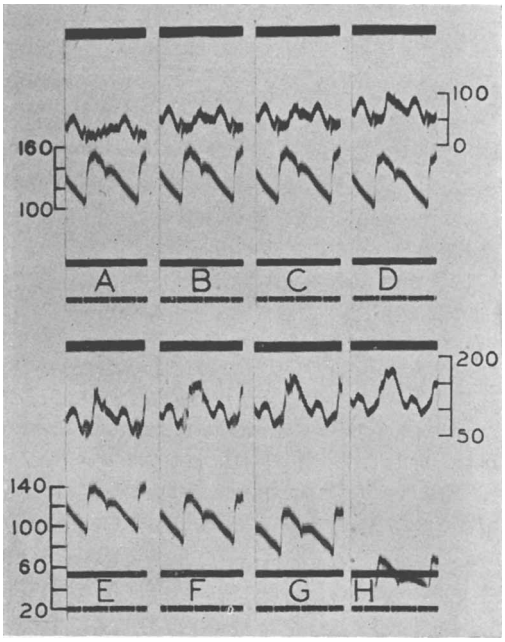

FIG. 2.
Right atrial (upper) and aortic (lower) pressure curves during control (record A) and progressively more severe pulmonic stenosis (records B through H). Discussion in text.

Fig. 1 reveal that a two-stage mild constriction of the pulmonary artery caused a relatively large elevation of the right atrial pressure as compared to control record A, with no significant alteration in the aortic pressure. This rise in right atrial pressure is present at all points measured, but is most pronounced at the V point, amounting to an increment of 46 mm water in B, and of 83 mm water in C. The aortic systolic pressure remained within 5 mm Hg and the diastolic within 1 mm Hg of the control levels, the larger pulse pressure being due to a slight slowing of the heart. Such records show that lesions causing increased resistance to right ventricular discharge can effect an increase in central venous pressure without alteration of the arterial pressure and without graphic evidence that left ventricular discharge has been decreased.

We next studied an entire series of progressively severe, graded constrictions of the pulmonary artery in order to ascertain whether immediately recognizable criteria could be established which enabled one to determine that a significant reduction in cardiac output had been produced without lowering the arterial pressure to shock levels. The effects of such a sequence of graded constrictions are presented in Fig. 2.

Minimal constriction of the pulmonary artery beyond the critical point has elicited a 35 mm water elevation of right atrial pressure at the V point in record B and a further increase to 40 mm water in C, as compared to control record A, with no significant alteration in the aortic pressure. The first significant decrease in the aortic pressure is

present in record D, falling to 149/102 mm Hg, as compared to the control value of 155/110 mm Hg in record A. This slight drop in aortic pressure is accompanied by an additional rise in right atrial pressure of 55 mm water above control. This then is the first definite indication that the output of the left ventricle has been diminished.

With further compression of the pulmonary artery, the changes in right atrial and aortic pressures become more pronounced, as observed in records E through H. Even in record G, the aortic pressure is still within normal limits (116/80 mm Hg), while the central venous pressure has risen to 123 mm water above control levels, measured at the V point. Very slight additional constriction then produced a fall in aortic pressure to shock levels (record H). Throughout the entire series of records, no significant change in heart rate is present.

The circulatory conditions represented in records D to G simulate the cardinal features of clinical congestive heart failure, namely elevation of central venous pressure, reduction of cardiac output, and an arterial pressure within normal limits. We have been able to maintain these conditions for four hours or more, requiring only minimal adjustments of the clamp. Therefore, we shall attempt to reproduce these stages during our anticipated studies on the effects of reduced cardiac output upon renal function. The conditions existing in record H would, of course, be unsatisfactory since the arterial pressure is within shock levels. Stages B and C also would be unsatisfactory, since minute changes in cardiac output which might be present would probably be within the limits of error of any method for measuring the cardiac output (*e.g.* Fick principle).

A more detailed perusal of Fig. 2 reveals additional information which is not directly pertinent, but which is of some importance. It is evident in the right atrial curves registered during relatively severe stenosis of the pulmonary artery (records E through H) that a steep rise in slope occurs during ventricular systole, reaching a peak near the end of ventricular systole. These curves simulate closely those described by Little during experimental tricuspid regurgitation,[13] and were recorded at a time when the central venous pressure was very high and the right ventricle was markedly dilated. Preliminary tests utilizing the injection of a small amount of concentrated dye (T1824) into the right ventricle tended to confirm the presence of tricuspid insufficiency. Within 3 to 5 seconds after injection during severe pulmonic stenosis, the concentration of dye in the right auricle and right ventricle were almost identical, and the amount of dye recovered from both sites was very large.

To further supplement our knowledge of the altered circulatory dynamics of pulmonic stenosis, pressures were recorded in several experiments from the pulmonary artery distal to the point of constriction during progressive compression of the pulmonary artery. As illustrated in Fig. 3, slight constriction beyond the critical point elicited a vibratory irregularity representative of a systolic murmur, as seen in the pulmonary artery curves of records B and C. In record B, the aortic pressure was slightly depressed, although quite frequently a pulmonic murmur was observed before any alteration occurred in the aortic or right atrial pressures. Furthermore, in some experiments the pulmonary artery and aortic pressures were slightly elevated when constriction just sufficient to produce a murmur was applied. In the pulmonary artery curves of records C and D, a sharp peak is present at the very beginning of systole. This is analogous to the anacrotic incisura described by Katz, Ralli, and Cheer in experimental aortic stenosis.[14] More severe constriction eventually resulted in a marked fall of systolic, diastolic, and pulse pressures in the pulmonary artery, as seen in record D. With release of constriction, these pressure changes were reversed, often with a transitory elevation of pulmonary artery pressures above control levels.

Right intraventricular pressures were re-

[13] Little, R. C., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 602.

[14] Katz, L. N., Ralli, E. P., and Cheer, S., *J. Clin. Invest.*, 1928, **5**, 205.

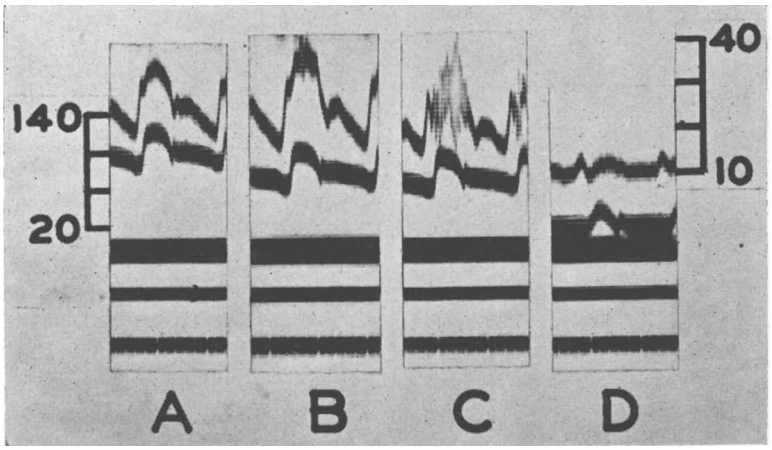

FIG. 3.
Pulmonary artery (upper) and aortic (lower) pressure curves during the control period (record A) and during gradually progressive pulmonic stenosis (records B through D).

corded in several experiments during experimental pulmonic stenosis, and the findings were in accord with those reported by Fineberg and Wiggers.[11] To repeat these findings briefly, degrees of occlusion below about 60% elevated the systolic but not the diastolic pressures (initial tensions), while aortic and right atrial pressures were unaffected. This systolic pressure rise is believed to be a passive phenomenon, due solely to the increase in resistance, with no alteration in the force of ventricular contraction. Further degrees of occlusion eventually produced an elevation of initial tension as well as of systolic pressure. However, very soon after the rise in initial tension appeared, the systolic pressure began to diminish as the constriction progressed. This was considered by Fineberg and Wiggers to be evidence of myocardial failure, due to tremendous stretching of the myocardium, perhaps combined with diminished coronary flow, the result of decreased aortic pressure.

Discussion. As previously reported by Gibbon, Hopkinson, and Churchill,[10] progressive compression of the pulmonary artery beyond about 60% of occlusion produces a gradual elevation of central venous pressures and a fall in aortic pressures. With carefully graded constriction, we were usually able to reach a point where a moderate elevation of right atrial pressure could be elicited with no significant alteration in the aortic pressure. With more severe degrees of occlusion, aortic pressures dropped, the right ventricle became markedly dilated, and curves were often obtained which were characteristic of tricuspid regurgitation. In attempting to explain the elevation of central venous pressures during this procedure, the marked venous distention observed during severe pulmonic stenosis indicates that the principal factor is an increase in the quantity of blood within the central veins. It is frequently stressed by

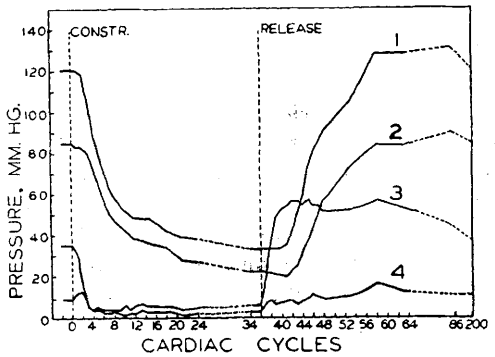

FIG. 4.
Plot showing the effects in successive beats of an abrupt occlusion and subsequent release of the main pulmonary artery on aortic systolic (1) and diastolic (2) and on pulmonary systolic (3) and diastolic (4) pressures, the latter two recorded distal to the occluding clamp. Discussion in text.

present-day investigators that it is dynamically impossible to produce an elevation of the venous pressure combined with a reduction in the cardiac output unless the blood volume is augmented. To prove this concept, many involved and complicated experimental procedures and physical models have been contrived.[6,15,16] Simple and decisive expedients, such as were used in this research, have largely been ignored. Our results demonstrate unequivocally that an increase in total blood volume is not an obligatory factor in causing an elevation of the central venous pressure, since extreme pressure variations can be produced within a few seconds, and can be maintained for many hours. The increase in the volume of blood contained within the central veins during our experiments is independent of any changes in total blood volume, and must be due to redistribution of the blood already present within the circulatory system.

Sufficient reduction of the lumen of the pulmonary artery acts primarily to diminish the output of the right ventricle. Initially, therefore, following constriction of the pulmonary artery there is a brief period in which the venous return to the heart exceeds the amount of blood pumped away by the right ventricle, resulting in a progressive increase in central venous pressure. Due to the decreased cardiac output, however, the venous return gradually diminishes, until it again becomes equal to the reduced cardiac output. The right atrial pressure then becomes restabilized, but at a higher level of pressure. It is within the relatively brief period during which there is an inequality between the cardiac output and venous return that a redistribution in the circulating blood volume has occurred. The problem resolves itself into an attempt to determine the source of the blood which contributes to the venous return over and above the suddenly diminished output of the right ventricle during this short period.

Fig. 4 presents graphic proof that a shift of blood does occur between the pulmonic and systemic circulations during pulmonic stenosis. Following a sudden drastic occlusion of the pulmonary artery, the systolic and diastolic pressures in the pulmonary artery distal to the point of constriction dropped abruptly, and the pulse pressure was reduced almost immediately to minute values. However, systolic, diastolic, and pulse pressures in the aorta diminished very gradually over the course of about 30 beats. Thus, while the entry of blood into the pulmonary circuit was markedly reduced, blood still was extracted from the pulmonary vascular bed at almost the normal rate for a few cardiac cycles, and at a slowly diminishing rate for many more. When the constriction was suddenly released, the pulmonary arterial pressures rose almost immediately to super-normal values, while the aortic pressures recovered much more gradually, indicating a refilling of the depleted pulmonary vessels to normal. Thus, our data favor the concept that the lungs serve an auxiliary function as a rather efficient blood reservoir interposed between the right and left ventricles. In addition to a shift of blood from the pulmonic to the systemic circuit, it is very possible that a transfer of blood occurs within the systemic circuit itself. This may take place from the arteries, capillaries, and small veins toward the central veins, or blood might be released from the various blood reservoirs located on the systemic side of the circulation, notably the spleen in the dog. This possibility deserves further study.

Summary and conclusions. An experimental attempt was made to simulate acutely the cardinal circulatory changes of congestive heart failure for the purpose of creating a preparation in which the incipient effects on renal function could later be studied.

Optically recorded pressure pulses from the aorta, pulmonary artery, and right atrium are presented, and show that judicious compression of the pulmonary artery can reduce the output of the left ventricle significantly without incurring a drastic fall of arterial pressure. These dynamic studies also revealed that such graded pulmonary artery constriction results in (a) a reduction of pulmonary sys-

[15] Starr, I., and Rawson, A. J., *Am. J. Med. Sc.*, 1940, **199**, 27.

[16] Starr, I., *Am. J. Med. Sc.*, 1940, **199**, 40.

tolic and diastolic pressures distal to the constriction, and the development of a systolic murmur, (b) a rise in maximal and initial tensions in the right ventricle, and (c) an elevation of right atrial pressures. In some instances, relative tricuspid insufficiency developed.

The elevation of central venous pressure is obviously associated with translocation of a large volume of blood to the venous side of the circulation. Evidence is presented that a part of this transfer occurs from the pulmonary vascular bed, although it is admitted that a shift may also occur within the systemic circuit itself toward the central veins.

Received August 19, 1949. P.S.E.B.M., 1949, **72.**

Cultivation of Poliomyelitis Virus in Cultures of Human Foreskin and Embryonic Tissues.* (17359)

THOMAS H. WELLER,† FREDERICK C. ROBBINS,‡ AND JOHN F. ENDERS.

From the Research Division of Infectious Diseases, Children's Hospital, the Department of Tropical Public Health, Harvard School of Public Health, and the Departments of Bacteriology and Pediatrics, Harvard Medical School, Boston, Mass.

Recently, the propagation *in vitro* of the Lansing strain of poliomyelitis virus in human embryonic tissues was reported and evidence presented that this virus is capable of multiplying in cells other than those of nervous origin.[1] These experiments have been continued and this agent now has been carried for a total period of 224 days through 13 serial cultures in which the tissue consisted of mixed human embryonic skin and muscle. This strain has also been maintained for 173 days in two lines of 11 serial cultures each and composed respectively of human embryonic intestine and brain.

Additional experiments described here in a preliminary manner are reported. Two objectives were in mind: (a) to determine whether the Lansing strain was capable of multiplying in completely differentiated non-nervous tissue as well as in embryonic tissue; (b) to determine whether the Brunhilde strain of poliomyelitis virus—a strain immunologically distinct from the Lansing group[2] and not adaptable to rodents—could, like the Lansing strain, be cultivated in non-nervous human embryonic tissues.

Propagation of the Lansing strain in human foreskin tissue. As a source of completely differentiated non-nervous tissue fragments of human foreskin were employed. The use of this tissue was suggested by the report of Blank, Coriell, and Scott,[3] who explanted it on the chorioallantoic membrane according to the method of Goodpasture. The material was derived from patients between 4 and 11 years of age. Each prepuce was sufficient for the preparation of at least 8 cultures and before mincing was washed 2 or 3 times in nutrient fluid[4] containing 50 units each of streptomycin and penicillin per ml. The fluid phase of the cultures, which contained the same concentration of antibiotics was removed and replaced at intervals of 4 days. The original set of cultures was inoculated with 0.1 cc of a 10% suspension of pooled mouse brains infected with the Lansing strain. As inoculum for subcultures 0.1 ml of the pooled

* Aided by a grant from the National Foundation for Poliomyelitis, Inc.

† Work done while a post doctorate Fellow of the United States Public Health Service.

‡ Senior Fellow in Virus Diseases of the National Research Council.

1 Enders, J. F., Weller, T. H., and Robbins, F. C., *Science*, 1949, **109**, 85.

2 Bodian, D., Morgan, I. M., and Howe, H. A., *Am. J. Hyg.*, 1949, **49**, 234.

3 Blank, H., Coriell, L. L., and Scott, T. F. McNair, PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 341.

4 Weller, T. H., and Enders, J. F., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 124.