on the ovaries has been doubted by Cranston on the grounds that estrone injection immediately reestablishes cyclicity in *Lithospermum*-fed animals. It is possible that the *Lithospermum* effect is a threshold one of just sufficient magnitude to disrupt cyclicity, but insufficient to produce irreversible changes in the tissues involved in estrous cycles. Perhaps the use of a more potent concentrate of the active material would clarify this matter. There is the possibility, too, that the factor interacts with circulating gonadotrophins, although we have neither positive nor negative evidence of such action.

Summary. (1) The anti-estrous factor of *Lithospermum* appears to reside in the neutral water fraction. (2) The anti-estrous factor is most abundant in the flowers and seeds of the dried plant, and almost wholly absent from the stems. (3) Interference with estrus is stopped almost immediately, regardless of the length of treatment, when *Lithospermum* is withdrawn and animals returned to normal diet. (4) The C_3H strain is more sensitive to *Lithospermum* than is the Rockland Swiss strain. (5) The action of *Lithospermum* is unlike that of thiouracil. (6) The factor induces no observable changes in the anterior pituitary, thyroid, suprarenals, or pancreas; some atresia is observed in the ovaries, and some atrophy of the uteri.

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In vivo Observations on the Distensibility of the Femoral Venous System.*

ROBERT S. ALEXANDER. (Introduced by Carl J. Wiggers.)

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

The marked engorgement of a vein when its central end is occluded tends to create the impression that veins are highly distensible structures. This illusion is probably created by the filling of partially collapsed veins[1] rather than by a true distension of the venous walls, since studies of segments of large veins[2,3] have revealed a relative lack of distensibility over the physiological range of venous pressures. The question arises as to whether observations on the large veins suited to direct distensibility measurements are necessarily typical of the venous system as a whole. Hochrein and Singer, for example, found the femoral vein considerably more distensible than the inferior vena cava.[2] Should the smaller veins and venules be highly distensible, alterations in venous pressure would be of considerable significance in altering the partition of the circulating blood volume between the venous system and other channels of the circulatory system.

Clark[4] attempted an estimate of the distensibility of the entire venous system of the forearm *in vivo* by employing a plethysmographic method. Her results were difficult to interpret, however, because of the inability to differentiate changes in venous volume from concomitant changes in tissue fluid volume. A more direct approach to this problem in the experimental animal is suggested by the simple observation that if the venous outflow from an organ is watched at the time the outflow pressure is suddenly lowered, the fall in pressure will be observed to produce a transient gush of excess blood. This excess blood obviously represents a volume passively flushed out of the system by

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1 Ryder, H. W., Molle, W. E., and Ferris, E. B., Jr., *J. Clin. Invest.*, 1944, **23**, 333; Duomarco, J., Rimini, R., and Predari, F. N., *Rev. Argentina Cardiol.*, 1946, **12**, 333.

2 Hochrein, M., and Singer, B., *Arch. Exp. Path. Pharmacol.*, 1927, **125**, 301.

3 Green, H. D., *Medical Physics*, Otto Glasser, Editor, Year Book Publishers, Chicago, 1944, 213.

4 Clark, J. H., *Am. J. Physiol.*, 1933, **105**, 418.

virtue of the reduction in the intravascular distending pressure. In a rigid system such a gush would be absent; in a highly distensible system it should represent a considerable volume. Although it is difficult to delimit the precise anatomical locus from which this excess blood arises, it must be predominantly from the venous system since a pressure change at the point of venous outflow from an organ could have very little effect on the pressures on the arterial side of the circuit. The present study represents an attempt to quantitate this phenomenon in the venous bed of the hind leg of the dog.

Methods. This analysis demanded an accurate method for measuring venous outflow. The simplest procedure is to open the appropriate vein and collect the outflow in a graduated cylinder for an accurately measured time interval. To be adaptable to the present study this method had to be made continuous so that the successive changes in rate of outflow produced by a change in the outflow pressure could be determined. This may be accomplished by determining the increase in volume in the collecting receiver gravimetrically. In preference to the rather cumbersome and frequently inaccurate mechanical devices that have been used for this purpose, we have developed a "strain gauge flowmeter" to obtain a continuous record of the increase in weight of the collecting vessel. This affords a small compact unit which may be placed directly under the receiving vessel and connected to the recording system by light flexible wires of any desired length. The resulting record is a quantitatively accurate graph of the cumulative venous outflow; any excess or deficit in this outflow produced by a change in outflow pressure may be measured directly on the original record.

We employ a gauge manufactured by the Statham Laboratories, Los Angeles, California. This is a self-contained Wheatstone bridge which is accurately balanced to null current when there is no stress applied to the pin actuating the sensitive resistance elements of the bridge. Although designed primarily for the measurement of minute displacements, these gauges may be used for the direct measurement of stresses in certain ranges. A gauge with a full range of eight ounces is well suited to measuring blood volumes of the order of 100 cc. The output of the gauge is sufficient to directly actuate optically recording galvanometers having periods somewhat less than 0.1 second.

In our application of this device, the outflow receiver (a beaker of 100 cc to 250 cc capacity) is placed on a small aluminum platform 8 x 10 cm. This platform is suspended on 3 points, 2 of which are steel pins pivoting on tapered bearings, and the third is the actuating pin of the strain gauge. The actual displacement of the platform over the full range of the gauge is so slight that frictional resistance in the suspension is of little consequence. To correct for variations in the mechanical advantage of the suspension due to alterations in the precise position of the beaker on the platform, we followed the practice of calibrating each record. This was accomplished by withdrawing an accurately measured volume (usually 20 cc) after each flow measurement and recording the resultant galvanometer deflection before the receiver had been moved from its recording position. Tests of the flow meter revealed almost complete linearity between galvanometer deflection and the volume (weight) in the collecting vessel, the deviation from linearity being 2.5% for the maximum range of the apparatus. By calibrating in the mid-range of each determination, this was reduced to an error of about $\pm 1\%$ and was disregarded.

The dogs were anesthetized with Na barbital (300 mg/kg) and heparinized. In preparing the hind leg, collateral circulation was excluded by severing all connections as high on the hip as possible except for the femoral artery, the femoral vein, the sciatic nerve, and the femur. The sciatic nerve was left intact and carefully protected with saline-soaked cotton to preserve some degree of vasomotor control. The femur was left intact to permit immobilization of the preparation without danger of kinking the blood vessels; collateral flow through the femur itself is negligible.[5] A cannula inserted dis-

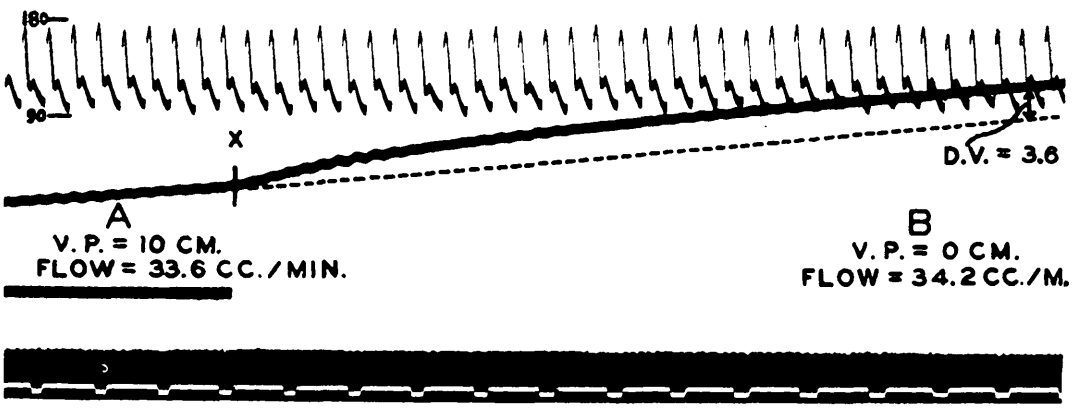

FIG. 1.
Record illustrating method of calculating distensibility volume (D.V.) from flow-meter tracing. At point "X" the venous outflow pressure was lowered from 10 cm to 0 cm. Time signal below indicates 1-second intervals.

tally into the femoral vein was connected by rubber tubing to an outflow orifice. A shunt circuit was provided to permit normal venous drainage of the leg into an external jugular vein between flow determinations as well as to permit return of the blood collected in the flow meter after each observation. The outflow orifice was a short glass tube mounted on a rigid vertical rod to which was attached a centimeter scale. The vertical rod was well lubricated and provided with suitable stops so that the outflow tube could be rapidly shifted from one hydrostatic pressure level to another with a minimum of mechanical jarring. To allow freedom of movement of this outflow tube, the connections with the vein represented a total length of 50 cm. The significant resistance to blood flow which might be introduced by connections of this length was avoided by making them of as large a bore as practical, the rubber tubing having a bore of 6 mm, and cannulae and connecting links having a bore of 4 mm. The outflow pressure as determined by the hydrostatic level of the outflow orifice was read directly in centimeters of blood, zero pressure reference being the approximate level of the vena cava.

The standard recording practice was to allow the flow to equilibrate at the initial outflow pressure and then record the rate of flow for a period of 10 seconds. The outflow pressure was then quickly shifted to the second level while the recording continued and in general a period of about 20 seconds allowed for equilibration of the flow at this new level. To maintain arterial pressure constant, a compensating reservoir was connected with a carotid artery and elevated to a hydrostatic level of 110 mm Hg. Most dogs of reasonable size would fill the dead space of the reservoir with about 200 cc of blood in equilibrating at this pressure, so that adequate blood was available to compensate for that collected in the flow meter and which was temporarily lost from the circulation. As a check on the arterial pressure perfusing the preparation, optical tracings of the pressure recorded from the opposite femoral artery were superimposed on the flow record.

—*Experimental Results.* The nature of the experimental recordings is illustrated in Fig. 1. The moment of pressure lowering (X) is followed by a transient steep rise which shortly equilibrates at a rate of flow (B) slightly in excess of the initial flow (A). In measuring the records, the assumption was made that at the moment the outflow pressure was lowered the arterial inflow changed to a value equal to the venous flow achieved after final equilibration at B. This assumption is not strictly correct because there will be a

[5] Green, H. D., Lewis, R. N., Nickerson, N. D., and Heller, A. L., *Am. J. Physiol.*, 1944, **141**, 518.

slight lag before pressure-flow relationships equilibrate at the new outflow-pressure level. Analysis of the outflow records with the thought of deriving a correction factor for this error, however, revealed that with changes in outflow pressure of only 10 cm H_2O the error was too small to warrant such an arbitrary correction. The gush of excess blood accompanying the sudden lowering of outflow pressure or what may be termed the "distensibility volume" (DV) may then be measured directly on the graph. For illustrative purposes this measurement is constructed graphically in Fig. 1; in actual practice the records were measured in reference to the time signal marks to correct for any variations in speed of the recording paper.

It is obvious that a comparable measurement may be obtained by the inverse procedure of suddenly elevating the outflow pressure, in which case there would be a momentary deficit in the outflow representing the volume of blood retained by the distension of the system at the higher pressure. Alternate determinations by the two procedures have yielded values in close quantitative agreement. We have selected the first method for routine observations merely because of the subjective satisfaction of seeing the distensibility volume as a positive increment.

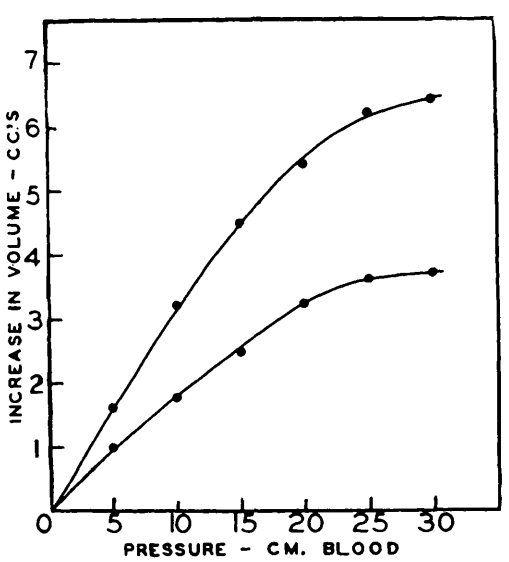

FIG. 2.

Plot of the data obtained from 2 of the dogs in the series showing the increase in volume of the venous bed of the hind leg produced by increases in the outflow pressure in the femoral vein.

In this fashion the distensibility volumes for 10 cm pressure changes in overlapping intervals for the range of 0 to 30 cm of blood have been determined. Simple addition of these individual values permitted plotting the data in the conventional volume-pressure manner. Two representative curves are shown in Fig. 2. It should be especially noted that the volumes given are those *in excess* of the volume in the system at zero pressure. It is unfortunate that this initial volume cannot be assessed by the present method.

Discussion. As an alternative to distensibility measurements on isolated segments of veins, the present method has a major advantage and an important limitation. Its advantage resides in the fact that it reveals the over-all effect of a change in the central venous pressure on the volume of blood pooled in the peripheral venous channels of the organ under study, and thus the values may be transferred directly to the interpretation of such changes in the circulatory dynamics of the intact animal. In contrast to direct observations on isolated veins, however, these data are of meager assistance in describing the precise physical properties of the system. This latter problem involves far more than the failure to define the exact anatomical limits of the distensible system under study. The pressure change produced at the central end of the femoral vein will not be paralleled by an equal pressure change throughout the femoral venous bed any more than it will produce an equal change in the femoral arterial pressure. On the contrary, it will represent an alteration in the normal venous pressure gradient, the change in this gradient being maximal at the outflow cannula and decreasing progressively as one traces the venous circuit back to the capillaries. It seems highly doubtful that a relatively small change in central venous pressure would significantly alter the pressure in the arterial portion of the capillaries if the arterial pressure head remains constant, a

relationship which justifies our use of the present method to study venous distensibility. However it also leaves in doubt a knowledge of the precise pressure changes produced in those segments of the venous bed which are yielding the distensibility volume when the central venous pressure is lowered. In addition it must be recognized that measurements of venous distensibility *in situ* are not determined solely by the properties of the vein walls, but may be influenced to some extent by the tension in extra-vascular supporting structures.[1]

The results obtained as illustrated in Fig. 2 reveal a volume-pressure relationship that exhibits the same trend as has been found typical of other blood vessels. The relationship is roughly linear over the pressure range of 0 to 20 cm of blood, the range which may be regarded as the range of normal physiological variation. Above this pressure the system appears to be approaching its elastic limit as the curves show a significant flattening in the 20 to 30 cm range. The actual magnitude of the distensibility volumes, moreover, is in accord with inferences derived from measurements on large veins which indicate a relatively low distensibility for the venous system. For a pressure change of 0 to 20 cm we observed an average distensibility volume of only 4.7 cc for the entire venous system in the hind leg of the dog as compared with an actual blood flow that averaged 31.3 cc min. at an outflow pressure of 0 cm. As central venous pressure rises above this normal range the rigidity of the veins becomes even greater. This indicates that the venous system of the hind leg serves fairly efficiently as a network of pipes returning the blood from the periphery to the heart.

A complete evaluation of these distensibility characteristics would require information as to the total venous volume of the hind leg. We are not satisfied, however, that valid estimates of the average venous volume of the hind legs of mongrel dogs can be made with information or methods that are at present available. It should also be emphasized that the distensibility measurements reported here deal strictly with passive changes in venous volume without any consideration of changes in the volume of the venous bed which might be produced by venomotor action. There is ample evidence that active changes in the vein walls can occur,[6] but there is no conclusive evidence to demonstrate their significance *in vivo*.

Summary. The gush of excess blood that appears in the venous outflow from an organ when the outflow pressure is suddenly lowered has been selected as an index of the distensibility of the venous system of that organ. With the aid of a new type of flow meter to quantitate venous outflow, this phenomenon has been studied in innervated hind legs of anesthetized dogs. The results indicate a relatively low distensibility in the venous system of the hind leg of the dog; the volume-pressure data demonstrating a fairly linear relationship in the venous pressure range of 0 to 20 cm of blood, with a trend toward even less distensibility at higher pressures.

[6] Franklin, K. J., *A Monograph on Veins*, Charles C. Thomas, Springfield, Ill., 1937.