Determination of Regional Blood Flow in Gastrointestinal Tract of Intact Animals. (26671)

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Lack of an *in vivo* method for measuring regional blood flow in the gastrointestinal tract has limited knowledge of gut physiology relative to this important parameter. As a consequence, the relationship of circulatory disturbances to local function and local pathology in the intact animal and man is relatively unexplored. It has been difficult to obtain precise information relating gut circulation to general circulatory dynamics and homeostasis. This preliminary report describes a radioisotopic technic devised to study regional circulation in the gut as applied to the descending colon.

Method. After a 48 hour fast, mongrel dogs were anesthetized with pentobarbital. The colon was washed with warm tap water until clean. A specially designed thin-wall glass (30 mg/cm^2) Geiger-Muller tube with a 5-inch anode length is encased in a closely machined aluminum sleeve 0.008 inch thick. A rounded tip for easy passage and conical shaped tailpiece to seal and ground the lead wires are cast in Epon.* The tube is passed a measured distance into the colon per rectum and its position is fixed by clamping the lead cable to the dog's tail.

Tube dimensions are 6 inches overall length and ¾ inch in diameter. A 4-foot rubber-covered wire cable extends from the tailpiece. One milliliter (containing 40 μc) of a rapidly cleared colloid incorporating a pure beta emitting radioisotope ($CrP^{32}O_4$) (1) is injected rapidly into a jugular vein. The blood dilution curve of the radioisotope is continuously monitored by the colon-probe connected to a counting rate meter (Nuclear-Chicago), set to the 0.5 second time constant. Counting rate is continuously registered on a recorder (Brown) with a frequency response of 0.3 second full scale. Cardiac output is simultaneously determined from the same bolus by means of a femoral artery dilution curve obtained by drawing blood through a polyethylene helix surrounding an externally shielded Geiger-Muller tube. The areas under the cardiac output curves are obtained by planometric integration. The counting rate of the tube is calibrated by aspiration of a known concentration of $CrP^{32}O_4$ through the helix surrounding the tube. Cardiac output (l/min) is then the quotient of injected radioactivity divided by the area under the extrapolated curve.

Results. Fig. 1 shows the results of occluding 3 small arteries supplying the region of the colon around the probe. The figures at the top show the isotope dilution curve in the femoral artery (cardiac output) on the left and the usual normal colon curve on the right before arterial occlusion (arrow at extreme lower right). The 2 arterial dilution curves (cardiac output) are quite similar, while the colon curve following arterial occlusion has changed considerably from the control curve. The initial peak has disappeared. The curve maximum appears seconds later than the initial peak and falls away slowly. This reduced and delayed curve probably represents limited blood flow from non-occluded collaterals, since lead foil shielding of the serosal surface of the moni-

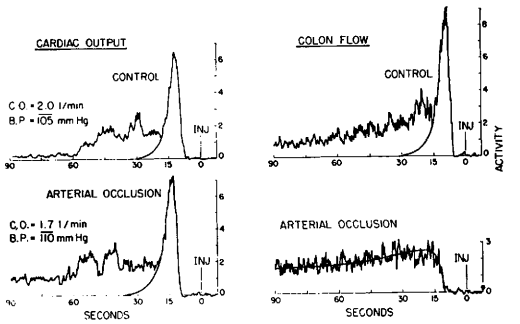

FIG. 1. Effect of local arterial occlusion on colon blood flow. Ordinate of cardiac output curve is concentration in arterial blood, cpm × 10^3, in sample volume in helix. Ordinate of colon flow curve is activity, cpm × 10^3, of blood in region of the colon probe.

* Epon is Shell Chemical Co. epoxy resin (824) used with curing agent V.

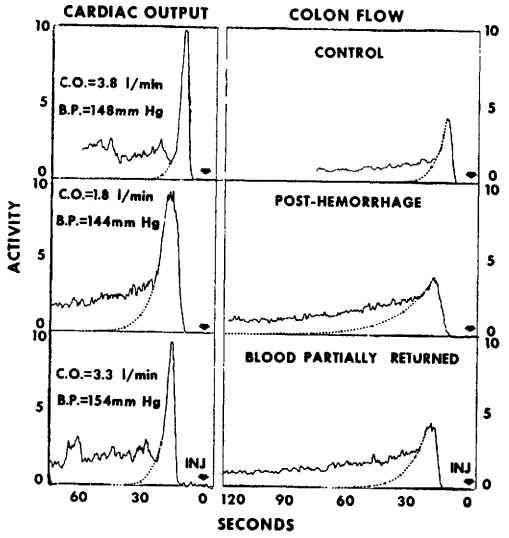

FIG. 2. Effect of hemorrhage on colon blood flow. Ordinate of cardiac output curve is concentration in arterial blood, cpm $\times 10^3$, in sample volume in helix. Ordinate of colon flow curve is activity, cpm $\times 10^3$, of blood in region of the colon probe.

tored colonic segment did not affect the colon dilution curve.

Fig. 2 illustrates the effect of hemorrhage (which reduces gut blood flow) on the shape of the colon curve. The upper curves are normal control curves before hemorrhage. The curves in the middle were obtained after removing 250 ml of blood (15 ml/kg). The ascending limb of the colon curve is slowed, peak is delayed and slightly decreased in height; descending limb shows delayed washout of the isotope. The cardiac output has fallen from 3.8 l/min to 1.8 l/min. The lower curves were obtained after replacing one-half (125 ml) of the blood. They demonstrate a partial return toward the control curves. After all the blood was returned (not shown) the curves do not differ from the top control curves.

Fig. 3 shows an experiment to study the effect of local venous occlusion. After moderate hemorrhage (to reduce colon blood flow), umbilical tapes were placed around 3 veins draining the region of the colon around the probe. The uppermost colon flow curve was obtained after hemorrhage (8 ml/kg) and placement of umbilical tapes around the veins without occlusion. The post hemorrhage control curve has slower than normal ascending and descending limbs and a

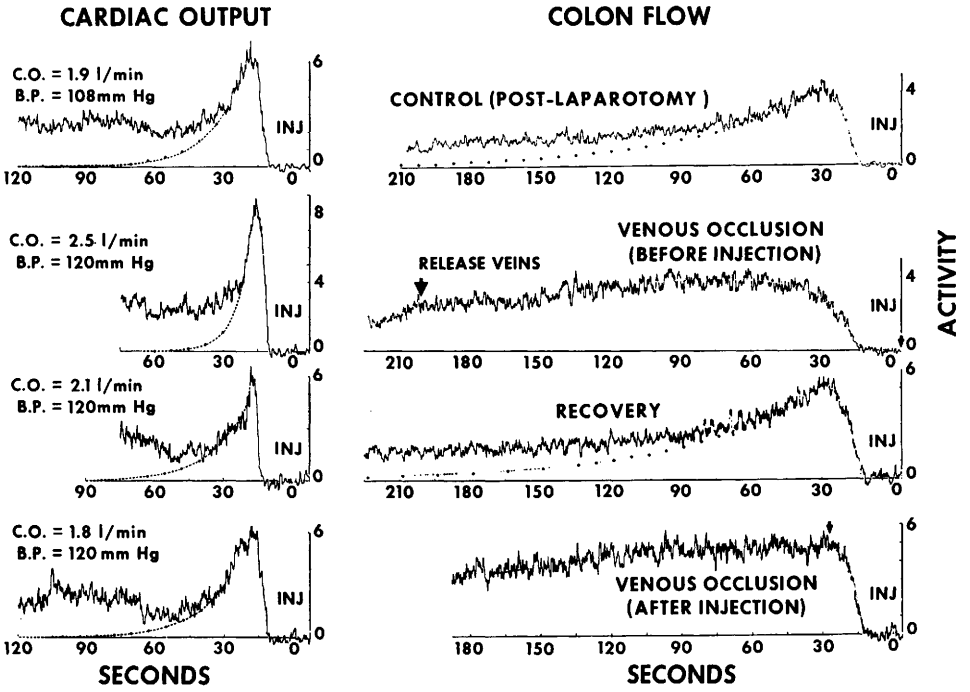

FIG. 3. Effect of local venous occlusion on colon blood flow. Ordinate of cardiac output curve is concentration in arterial blood, cpm $\times 10^3$, in sample volume in helix. Ordinate of colon flow curve is activity, cpm $\times 10^3$, of blood in region of the colon probe.

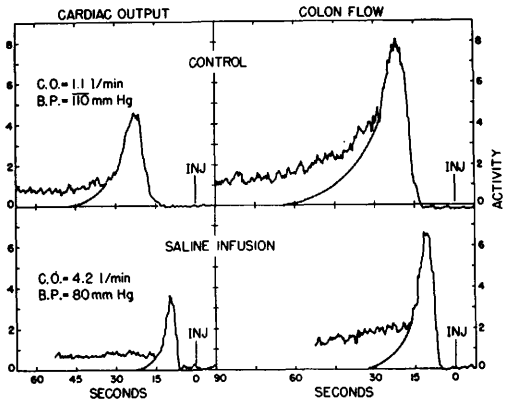

FIG. 4. Effect of saline infusion on colon blood flow. Ordinate of cardiac output curve is concentration in arterial blood, cpm $\times 10^3$, in sample volume in helix. Ordinate of colon flow curve is activity, cpm $\times 10^3$, of blood in region of colon probe.

rounded peak resembling the middle curve in Fig. 2. The second colon curve was obtained when the injection of the isotope was made 5 minutes after the veins were occluded. Circulation time is unchanged, the ascending limb of the curve is slowed still further, the peak is delayed, plateau prolonged, and washout delayed until the veins are released after which there is a sharp increase in the washout slope (2nd curve, release veins). Cardiac output was slightly increased. The third colon curve was obtained 25 minutes after the veins had been released (recovery) and shows a return to control curve pattern. The bottom colon curve was obtained by injecting the bolus of isotope and occluding the veins (arrow) after the curve had reached its peak. From this curve it is clear that blood containing the isotope entered the colon region without difficulty but sudden venous occlusion traps the blood and retards its exit.

Fig. 4 shows an experiment in which 1 liter of saline was infused after the control curves were obtained. After saline infusion the cardiac output increased from 1.1 liter per minute to 4.2 liter per minute. Circulation time to the colon is shorter and the changes in the colon curve are similar to those seen in the arterial dilution curve. Both ascending and descending limbs of the colon curve are steeper than those in control curve and probably represent increased blood flow to the colon.

Discussion. The range of the P^{32} beta particle in tissue is obtained by centering the colon probe in a series of calibrating cylinders of increasing radius and filling each cylinder with an aliquot of P^{32} solution. It is found that 73% of the maximal counting rate is the result of the radioactivity present within 1 mm of the outer surface of the probe, 94% of maximal counting rate (infinite volume) lies within 4 mm of the probe surface. Because the range of the beta particle in tissue is very short (P^{32} half thickness 0.8 mm) the volume of blood monitored by the colon probe is virtually limited to the segment of colon surrounding its anode length. Incorporation of the beta particle into a colloid retains the isotope intravascularly until removed by the reticuloendothelial system.

The cardiac output as represented by the arterial dilution curve is the input function. The simultaneously determined colon flow curve is the resultant of the input function of isotope concentration (cardiac output) modified by local vascular factors in the region of the colon monitored by the probe. The colon curves represent several aspects of regional circulation: (a) circulation and transit times, (b) fraction of cardiac output perfusing the monitored region, (c) rate of local blood turnover.

As yet blood flow has not been quantitated and interpretation of the regional colon flow curves is based upon experiments which alter regional flow in a predictable way such as local arterial or venous occlusion and hemorrhage which decrease flow, and saline infusion which increases regional colon flow above normal. In a number of experiments simultaneous timed collections of the regional venous outflow verified directly the changes in regional blood flow.

While at present the procedure is qualitative, the curve shapes are nevertheless a sensitive index of directional change in blood flow in the region under study, where the animal serves as its own control. This procedure is being used to study the circulatory parameters of physiological, pharmacological

and pathological interest in the colon.

Summary. A technic is described for estimating regional blood flow in the gastrointestinal tract. This method utilizes the short range of the beta particle in tissue to limit the blood volume monitored by a special Geiger-Muller tube placed in the intestinal lumen.

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Corticotropin Releasing Activity of Synthetic Lysine Vasopressin.* (26672)

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Our attempts to isolate a corticotropin releasing factor (CRF) from extracts of porcine posterior pituitary have been complicated by the facts that CRF is apparently quite difficult to separate from vasopressin, and that vasopressin itself has been shown to possess corticotropin (ACTH) releasing activity. When doing CRF bio-assays on fractions containing vasopressin it is necessary to take into account any "CRF" activity due to the vasopressin present. In this paper we report our values for ACTH-releasing activity of synthetic lysine vasopressin, using the same *in vivo* CRF bio-assay(1) used in our isolation work.

Materials and methods. Synthetic lysine vasopressin was kindly provided by Dr. V. du Vigneaud, Dept. of Biochemistry, Cornell Univ. Medical College. The sample used in these experiments (JM V 68/16) was reported to contain 260 International Units (U)/mg. Two assays of this sample *vs* USP Posterior Lobe Reference Standard in dibenamine treated rat by method of Dekanski(2) gave us 258 (95% fiducial limits: 228-291) and 267 (95% fiducial limits: 256-279) U/mg, respectively; the mean of these 2 assays, 262 U/mg, was taken as the pressor activity of the sample. The entire solid sample as received was dissolved in deionized water for pressor assay, the solution then divided into aliquots and each of these lyophilized and stored in deep freeze; assays for CRF could then be carried out on a solid sample freshly diluted in physiological saline.

For CRF assays, 150-170 g male rats from Holtzman Co., Madison, Wisc. were used. Only modification of the *in vivo* assay procedure of Guillemin(1) was that morphine was injected 35 min after nembutal injection instead of 25 min. Uninjected rats and rats injected with 0.3 ml physiological saline instead of vasopressin were introducd randomly throughout each assay as controls. Release of ACTH was determined by measuring concentration of free corticosteroids in 2 ml of plasma by the method of Silber as modified by Guillemin(3). In rats, corticosterone (cpd B) is the principal steroid determined.

In several experiments, male Holtzman rats of the same weight range were hypophysectomized and 24 hrs later injected with the vasopressin sample; these rats were not treated with nembutal-morphine but were anesthetized with ether before injection of sample. After 15 min, blood was drawn and plasma corticosteroids were determined as in the standard CRF assay.

Results are shown in Table I. Controls in each case include all values from both uninjected and saline injected rats, since statistical analysis of the two types of control values showed no significant difference between them. Data for the 3 dose levels of vasopressin giving significant increases of

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