A Method for Collection of Intermittent Samples of Adrenal Vein Blood. (24367)

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Collection of adrenal vein blood is widely used for the study of adrenal secretory activity as well as other details of adrenal physiology. The methods generally employed consist of ligating the left adrenal vein and cannulating the ipsilateral adrenolumbar vein. The technic has been developed by Hume and Nelson for large laboratory animals(1) and has been modified by Singer and Stack-Dunne(2) for the rat. The method of Wada *et al.*(3) utilizing the exteriorized adrenal offers many advantages although technically more difficult than the above procedures. In experiments on catechol secretion from the intact undisturbed adrenal gland (to be reported separately), a method for collecting intermittent samples of adrenal vein blood over long periods of time was desired. Moreover, as catechol secretion was being measured from the innervated gland, a procedure was needed which avoided tension, manipulation or disturbance to the adrenal during collection periods or when the blood was being returned to the animal.

Methods. The following procedure has been found quite suitable for collection of intermittent adrenal vein samples from cats and with minor modifications from rats but should be equally suitable for other animals as well. The left kidney is first isolated and the renal vein cleared and occluded near the inflow into the vena cava. The renal pedicle is then ligated and the kidney either removed or left *in situ*. The renal vein is next cannulated with proper sized polyethylene tubing. After isolating and placing loose ligatures around the left adrenal and adrenolumbar vein, the latter ligature is tied and the vein cannulated with the same, or slightly smaller, sized polyethylene tubing as placed in the renal vein. Both tubes are pushed through the body wall and joined by means of a small flexible piece of tubing. The adrenal vein ligature is then tied, the renal vein opened and the abdomen closed. The entire venous effluent from the adrenal is now being returned to the animal's body by means of the "adreno-renal shunt." When it is desired to collect the adrenal effluent, the shunt is disconnected and the blood allowed to drip into appropriate containers. The renal vein may be clamped or maintained slightly elevated to prevent backflow during this time. Following the collection period, the "shunt" is reconnected. Thus, at no time is the adrenal manipulated or disturbed. In a series of dogs, cats and rats, flow through the shunt has been maintained for periods up to 6 hours with periodic interruptions for sample collections. Longer periods of time present no difficulty and the method may also be adapted for chronic experiments. Preparation of the "adreno-renal shunt" is shown diagramatically in Fig. 1. Flow in the "shunt" can be easily measured by injecting a tiny bubble of air into one portion of the tube and timing its progress through a previously calibrated section. The flow during the shunt period was found to decrease only very slightly when compared to the flow obtained by direct measurement during the collection periods. Heparin is used to control clotting.

Discussion. The above technic appears to offer certain advantages over procedures now in general use. The loose ligature generally placed about the adrenal vein and which is occluded during collection periods in other methods is often difficult to control with accuracy. In addition, it is difficult to know with certainty with the abdomen closed that this ligature has been sufficiently tightened or loosened according to the experimental needs. Furthermore, in experiments involving normally innervated adrenals, it is not always easy to avoid undue tension upon the adrenal when manipulating this ligature. This dis-

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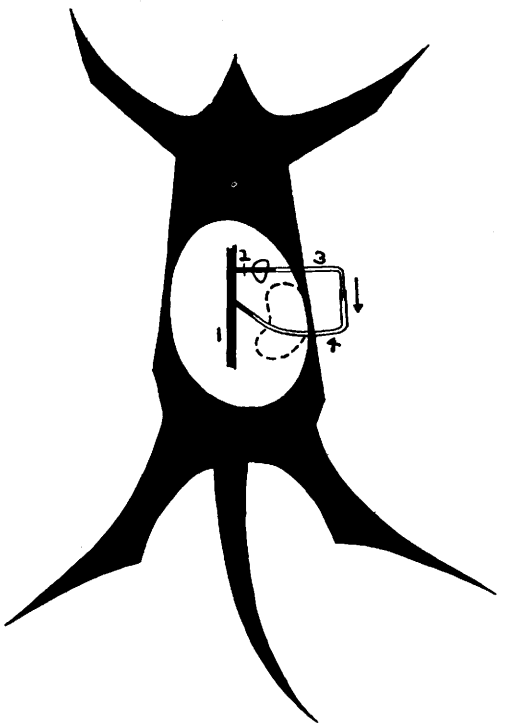

FIG. 1. Schematic illustration of "adrenal-renal shunt". Shown are inferior vena cava (1), ligated adrenal vein (2), cannulated adrenal lumbar vein (3), cannulated renal vein (4) and outline of kidney. Arrow indicates direction of blood flow.

turbance may interfere with adrenal hemeodynamics or influence the secretory activity of the adrenal. The "adreno-renal shunt" also has the advantage that measurements of flow rate can be easily obtained during the shunt period. It is not necessary to utilize the renal vein to return blood to the animal. Other abdominal veins may also serve this purpose.

Summary. A method is described for collecting intermittent samples of adrenal venous blood. This method is applicable to rats, dogs, cats and other laboratory animals. It has the advantage of involving no manipulation, tension or interference with adrenal hemodynamics during periods of collection or when the adrenal effluent is being returned to the animal through a cannula placed in an ipsilateral abdominal vein.

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1. Hume, D. M., Nelson, D. H., Surgical Forum—Clinical Congress of Am. Coll. of Surgeons. 1954, v5, W. B. Saunders Co., Philadelphia, 1955, p568.

2. Singer, B., Stack-Dunne, M. D., *J. Endocrin.*, 1955, v12, 130.

3. Wada, M., Seo, M., Abe, K., *Tohoku J. Exp. Med.*, 1935, v26, 381.

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Temperature-dependence of Dinitrocresol Stimulation of Aerobic and Anaerobic Lactate Production in Ascites Tumor Cells. (24368)

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Stimulation by 4,6-dinitro-o-cresol (DNC) of glucose use and lactate production in Ehrlich carcinoma and Crocker sarcoma 180 ascites tumor cells has been described(1). Further studies of 2 features of this stimulation are here reported. These are: (1) stimulation by DNC occurs under anaerobic as well as aerobic conditions and, (2) stimulated aerobic rate exceeds stimulated anaerobic rate at certain concentrations of DNC. The earlier experiments were carried out at 25°. In the present studies, similar experiments were carried out at 15° and 37° as well as 25°; it has been found, especially with the Ehrlich carcinoma, that degree of stimulation by DNC is markedly temperature-dependent and that it has a different value aerobically than it has anaerobically. Thus, the ratios of stimulated aerobic rates to anaerobic rates also vary with temperature.

Methods. Ehrlich carcinoma ascites tumor cells were maintained, harvested, and washed free of red and white blood cells as previously described(1) except that a solution of 0.15 M

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