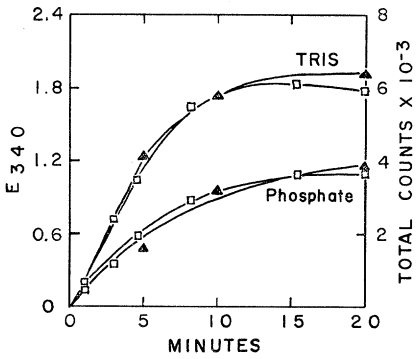

Fig. 2. Inhibition of the shunt in phosphate buffer as measured by TPN reduction and $C^{14}O_2$ formation from glucose-1-C^{14}. For the TPN reduction, reactions were carried out in duplicate vessels either in 0.05*M* Tris buffer, *p*H 7.4, plus 0.09*M* KCl, or in 0.05*M* potassium phosphate buffer, *p*H 7.4. Experimental vessels contained 0.5 μmole of G-6-P, 1.3 μmole of TPN^+, 5.0 μmole of ATP, and 10 μmole of $MgCl_2$ in a final volume of 3.0 ml. In control vessels either G-6-P or TPN^+ was omitted. The reaction was started by addition of 0.2 ml of an enzyme extract prepared as described in the text. The increase in optical density at 340 mμ was followed in a Beckman model DU spectrophotometer at room temperature. For the $C^{14}O_2$ production experimental vessels contained 0.5 μmole of glucose-1-C^{14} instead of 0.5 μmole of G-6-P; otherwise the reactants were the same. Open squares represent optical density at 340 mμ. Solid triangles indicate $C^{14}O_2$ production.

shunt. In intact ascites cells, for example, very little glucose is oxidized by way of this pathway (*9, 10*), most of it proceeding via glycolysis. We have found, however, that with extracts of these cells, the addition of an excess of TPN^+ or of a TPNH electron acceptor allows essentially all of the glucose to follow the shunt route. Methylene blue (*9, 10*) and pyruvate (*10*) have been found to stimulate the direct oxidation of glucose in intact ascites cells, presumably by acting as electron acceptors for TPNH. We have also found that oxidized glutathione will stimulate the shunt pathway in intact cells. These findings certainly implicate the availability of TPN^+ as an important factor in determining how much glucose is metabolized by way of the shunt pathway.

Under our experimental conditions the P_i concentration of the medium has been shown to influence the pathway of glucose breakdown in tissue extracts. It should be noted that the level of phosphate used to produce this effect is about four to ten times the average intracellular P_i concentration of intact Ehrlich ascites tumor cells (*11*). It is not surprising, however, to find that a P_i level greater than physiological is required to alter the pathways. Generally, in experiments designed to test cofactor requirements in cell-free systems, it is necessary to add a higher concentration of cofactor than is actually present in the intact cell. In order to test the effect observed in these studies under more physiological conditions we are at present attempting to devise means of varying the intracellular P_i concentration. It is clear, however, that no matter what the physiological effect of P_i turns out to be, in attempts to compare the relative contributions of the glycolytic and shunt pathways in glucose metabolism (at least in cell free systems), the P_i concentration of the medium should be carefully controlled (*12*).

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2. The following abbreviations are used in this text: P_i, inorganic orthophosphate; G-6-P, glucose-6-phosphate; TPN^+, oxidized triphosphopyridine nucleotide; TPNH, reduced triphosphopyridine nucleotide; Tris,tris-hydroxymethyl-aminomethane; ATP, adenosine triphosphate.
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5. The maximum amount of G-6-P that proceeds by way of the shunt was calculated assuming that 2 μmole of TPNH was formed per micromole of G-6-P utilized.
6. The glycolysis medium consisted of 5 μmole of ATP, 2 μmole of ADP, 10 μmole of $MgCl_2$, 1.4 μmole of DPN^+, 80 μmole of nicotinamide, and 20 μmole of $KHCO_3$ per 3.0 ml of incubation mixture.
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12. A preliminary report of this work was presented at the 132nd meeting of the American Chemical Society. This work was supported in part by a grant from the National Science Foundation (NSF G-2145).

* Public Health Service predoctoral research fellow of the National Cancer Institute.

4 August 1958

On the Role of the Vagus in the Control of Aldosterone Secretion

Abstract. Aldosterone in adrenal venous blood of dogs is increased by constriction and decreased by release of constriction of the inferior vena cava. Section of the cervical vagi did not affect the rise of aldosterone but prevented its fall. The receptors for stimuli to increases of aldosterone secretion may not be the same as those for stimuli to decreases.

Although there is evidence that the control of aldosterone secretion depends in part upon a function of intravascular volume (*1*), no mechanism has been proposed whereby stimuli from volume changes can be transmitted to the adrenal cortex. In view of the known modification of vagal impulses by changes in intrathoracic blood volume (*2*), we sought to determine whether the vagus plays a role in the control of aldosterone secretion.

Acute experiments were carried out in normal dogs anesthetized with Nembutal. Cannulas were placed in the lumbo-adrenal vein (*3*), and blood from the adrenal was collected intermittently for determination of aldosterone. To avoid stimulation of aldosterone secretion by blood loss per se, blood was infused at the rate at which it was lost. Radioactive (C^{14}) cortisone was added to the plasma, which was then extracted with dichloromethane. The dried extract was chromatographed on the following three systems: (i) chloroform:hexane:formamide:water (90:10:5:5); (ii) toluene; methanol:water (10:7:3); (iii) toluene: ethyl acetate:methanol:water (9:1:5:5). The cortisone-plus-aldosterone zone of (i) and (ii) was eluted for transfer to the next system. Aldosterone was determined by measuring soda fluorescence on paper in a fluorimeter (*4*) and corrected for losses as indicated by the C^{14} count on (iii). The mean recovery was 53 ± 10 percent (standard deviation). No other adrenal steroid which produces soda fluorescence has been found to run with aldosterone after passage through these three systems.

Aldosterone secretion was stimulated by constriction of the inferior vena cava above the diaphragm (*5*) by means of an inflatable cuff so as to raise the femoral venous pressure by 10 cm of water. The hemodynamic effects of caval constriction were estimated, in all experiments, from continuous tracings of brachial arterial, right atrial, and femoral venous pressures on a Sanborn 150 eight-channel recorder and from intermittent determinations of hematocrit.

In some experiments the vagi were sectioned; this was done at the level of the thyroid cartilage.

Figure 1*a* shows the results of a "control" experiment, in which constriction was applied twice and released once. By 60 minutes after caval constriction, aldosterone secretion had risen, and by 90 minutes after release of the constriction it had fallen. The temporal relationships were found to be highly reproducible, and these time intervals were used in all subsequent experiments where rises or falls of aldosterone secretion were under investigation.

Table 1*A* shows the effects of caval constriction on 13 occasions; Table 1*C*, the effect of release of constriction on five occasions. Effects of constriction in increasing, and of release of constriction in decreasing, aldosterone secretion are highly significant ($p < .001$ for both effects).

After vagal section, caval constriction was as effective in increasing aldosterone secretion as it was when the vagi were

Table 1. Effect of caval constriction and vagal section on aldosterone secretion (μg/hr). The mean, plus or minus standard deviation, is given at the bottom of each column.

A. Vagi intact		*B.* Vagi cut		*C.* Vagi intact		*D.* Vagi cut			*E.* Vagal section alone			
							Release			Time after section (min)		
Contr.	Constr.	Contr.	Constr.	Constr.	Release	Constr.	90 min	150 min	Contr.	60	150	210
1.8	8.0	6.9	15.1	8.0	3.3	4.3*	7.4		5.0	5.7	2.8	4.2
3.3	13.6	0.4	10.3	7.6	2.3	10.4	3.4	8.3	9.0	9.3	10.0	11.6
3.1	7.6	2.7	11.9	11.4	3.4	11.9	9.3		3.6	3.0	2.3	2.3
2.5	7.2	5.0	14.0	6.0	4.6	14.0	20.0		0.4	5.3		
6.9	11.4	4.1	9.6	20.0	13.7	9.5*	7.7	10.9	2.7	6.3		
3.4	7.9	3.0	11.0			9.6	7.9		5.0	3.3		
2.6	4.3					11.0	9.5		4.1	7.4		
2.5	7.2								3.0	4.9		
1.2	4.3											
3.0	10.4											
5.8	9.5											
1.3	6.0											
5.3	20.0											
3.28 ± 1.73	9.03 ± 4.22	3.68 ± 2.32	11.98 ± 2.16	10.6 ± 5.43	5.5 ± 4.64	10.10 ± 2.79	9.31 ± 4.74		4.10 ± 2.47	5.65 ± 2.05		
($p < .001$)		($p < .001$)		($p < .001$)		($p > .5$)			($p > .5$)			

* Vagi sectioned immediately before release.

intact. Figure 1*b* shows an experiment in which caval constriction was performed after vagal section. Table 1*B* gives the results of six such experiments. The effect of constriction in increasing aldosterone secretion is again highly significant ($p < .001$).

Whereas vagal section did not prevent a rise in aldosterone secretion following caval constriction, it did prevent a fall in aldosterone secretion following release of constriction. Figure 1*b* shows an experiment in which release of constriction was performed after vagal section. Table 1*D* gives the results of seven such experiments. There was no significant effect on aldosterone secretion ($p > .5$). The return to control levels of femoral venous pressure and of brachial arterial and right atrial mean and pulse pressures, and of the hematocrit, showed that release of constriction was as effective in reversing the hemodynamic effects of caval constriction in these experiments as it was in those in which the vagi were intact.

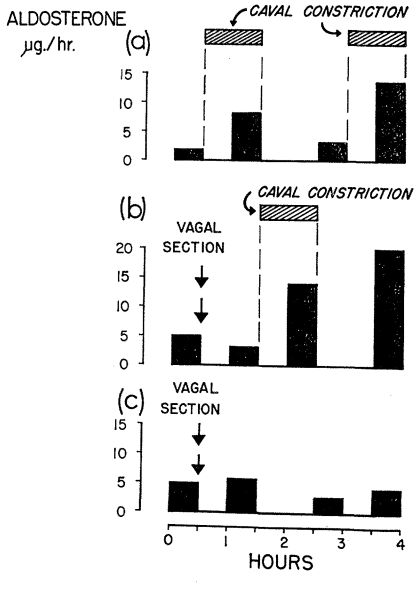

Fig. 1. Aldosterone from adrenal vein blood of dogs: (*a*) during constriction and release of inferior vena cava; (*b*) during constriction and release of inferior vena cava after vagal section; (*c*) following vagal section alone.

In two experiments (Table 1*D*) the constriction was applied with the vagi intact, and the vagi were sectioned immediately prior to release of constriction. A fall in aldosterone was prevented, as in the other experiments in this group. In two experiments, after 150 minutes there was still no tendency for the aldosterone to fall (Table 1*D*).

Vagal section alone (without caval constriction) was without consistent effect on aldosterone secretion, as shown by the results of the experiments reported in Table 1*E*.

These experiments confirm a report that caval constriction above the diaphragm raises aldosterone secretion (*5*) and show that this phenomenon is readily reproducible within 1 hour. The increase of aldosterone secretion so produced *does not* depend upon the integrity of the vagus nerve.

These experiments demonstrate, furthermore, that release of caval constriction lowers aldosterone secretion to control levels, and they show that this phenomenon is readily reproducible within 90 minutes. The effect on aldosterone secretion of release of caval constriction, as estimated from these experiments, *does* depend upon the integrity of the vagus nerve.

The findings cannot be explained by assuming a constant stimulus to increased aldosterone secretion, with ultimate control depending upon inhibitory vagal impulses. If this were the case, vagal section alone should have led to increases of aldosterone secretion.

The tracings of hemodynamic events and hematocrit values showed return to control conditions within 1 hour after release of caval constriction, whether the vagi were sectioned or not. If these parameters reflect the effective stimuli to aldosterone secretion, then it appears that the pathways mediating stimuli which lead to increases of aldosterone secretion may be different from those mediating stimuli which lead to decreases. The latter are dependent upon the integrity of the vagus; nothing is known about the former.

These experiments throw no direct light on the possible role of the diencephalon in mediating control of aldosterone secretion (*6*). They do supply data consistent with such a hypothesis in providing a pathway whereby "volume" stimuli may reach the central nervous system.

The results suggest that the concept of a single "volume receptor" for body-fluid volume (*7*) is an oversimplification. The stimuli that signalize expansion of effective body-fluid volume may well depend upon receptors other than those that mediate the stimuli which signalize contraction of effective volume.

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1 August 1958