thesized from UDPG but since phosphorylase is at a high concentration, the polysaccharide is broken down and cannot be identified. With this reservation, on the basis of the present observations, it may be suggested that in the uterus of the rabbit and rat, glycogen synthesis occurs from G-1-P by the action of phosphorylase and not through the UDPG pathway.

Summary. Phosphorylase activity was observed in the uterus of the rabbit and rat. In the rabbit uterus enzymatic activity was confined to the myometrium while in the rat phosphorylase activity was present in the luminal epithelium as well as the myometrium. In both animals enzymatic activity was highest following estrogen stimulation. In the uterine sections of the castrate and hormone treated animals it was not possible to demonstrate histochemically UDPG-glycogen transferase. The results seem to indicate that in the uterus of the rabbit and rat only one polysaccharide forming pathway is present and perhaps the polysaccharide is synthesized from G-1-P and not from UDPG through the enzyme UDPG-glycogen transferase.

1. Stetten, D., Stetten, M., *Physiol. Rev.*, 1960, v40, 505.
2. Takeuchi, T., Kuriaki, H., *J. Histochem. Cytochem.*, 1955, v3, 153.
3. Takeuchi, T., Glenner, G., *ibid.*, 1961, v9, 304.
4. Bo, W. J., *ibid.*, 1961, v9, 430.
5. ———, *ibid.*, 1959, v7, 403.
6. Hess, R., Pearse, A. G. E., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 569.

Received June 12, 1962. P.S.E.B.M., 1962, v111.

Release of Antidiuretic Hormone Due to Common Carotid Occlusion and Its Relation with Vagus Nerve.* (27741)

SHUNICHI USAMI, BRANKO PERIC† AND SHU CHIEN
Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City

In recent years there has been an increase in interest in the regulation of blood volume (1, 2, 3). Because of its action on water output through the kidneys, the antidiuretic hormone (ADH) has received particular attention. The homeostatic role of this hormone in blood volume regulation has been established by the finding that the circulating ADH concentration increases after the reduction of blood volume by hemorrhage(4). Although the posthemorrhage increase in circulating ADH concentration may partially be explained by a reduced rate of ADH inactivation(5), there is a definite and marked increase in rate of ADH release(6). In a series of experiments, Gauer, Henry and their co-workers(7) have demonstrated the role of left atrium volume receptors and vagus nerves in regulating the release of ADH. However, the extent to which other receptors in the vascular system can influence ADH release has not been systematically studied. In circulatory physiology, the occlusion of common carotid arteries is a commonly used maneuver to simulate systemic hypotension in eliciting sympathetico-adrenal activity. The possibility that such maneuver may also lead to ADH release was studied in the experiments reported here.

Methods. Healthy mongrel dogs apparently in normal hydration were used in these experiments. Food was withheld from the animals for about 18 hours prior to experiment. Free access to water was allowed. After the dogs had been anesthetized by intravenous injection of sodium pentobarbital (33 mg/kg), the common carotid arteries were exposed low in the neck. A femoral artery was cannulated for measurement of arterial pressure with the use of Sanborn pressure transducer and recorder. The degree of anesthesia was kept at a relatively constant level by intermittent injection of small quantities of sodium pentobarbital

* Aided by research grants from Nat. Inst. Health, U.S.P.H.S.

† International Postdoctoral Fellow, Nat. Inst. Health, U.S.P.H.S.

TABLE I. Effects of Common Carotid Occlusion on Plasma ADH Concentration of 5 Normal Dogs.

	ADH conc. (μU/ml)	Heart rate (per min.)	Arterial pressure (mm Hg) Systolic / Diastolic
Control (mean $\pm$ S.E.$_m$)	87 ($\pm$ 10.4)	176 ($\pm$ 13.1)	179 ($\pm$ 11.7) / 122 ($\pm$ 16.5)
Occlusion* (mean $\pm$ S.E.$_m$)	81 ($\pm$ 11.5)	195 ($\pm$ 11.4)	243 ($\pm$ 12.3) / 157 ($\pm$ 15.5)
Change	−6	+19	+64 /+35
P value (t test)†	$>$ .2	$<$ .01	$<$ .001 $<$ .001

* Values obtained at 2 minutes after beginning occlusion.
† Applied to changes observed in individual dogs.

(usually about 4 mg/kg/hr) into the peritoneal cavity. In some dogs, the vagus nerves were sectioned bilaterally in the neck. The carotid occlusion experiments were started at least one hour after all operative procedures had been completed. The occlusion of common carotid arteries was done by applying serrefine clamps to the arteries low in the neck. The period of occlusion varied from 2 to 10 minutes in different experiments.

Assay of antidiuretic hormone. Blood samples were obtained from the femoral artery or the external jugular vein for ADH assay. The samples were immediately centrifuged and the plasma separated. Antidiuretic activity of the plasma was determined in rats by a bioassay technic(8). Female Sherman rats, weighing from 190 to 260 g, were anesthetized with intraperitoneal injection of Inactin‡ (6 mg/100 g). Under local Xylocain infiltration, the neck was exposed and the trachea cannulated. The stomach was intubated for administration of the hydrating solution (1.5% ethanol and 0.05% NaCl in water). An external jugular vein was catheterized with a fine polyethylene tubing (PE-10) for injection of samples and standards. The urinary bladder was catheterized suprapubically with most of the dead space ligated. After the intubation of the stomach, the hydrating solution was given in 2 or 3 aliquots totalling 8 ml per 100 g. Degree of hydration was maintained at a constant level by placing the rat on a balance. A loss of rat weight by 0.1 g could, through mercury contacts, trigger open a solenoid valve§ through which an appropriate amount of hydrating solution would enter the stomach of the rat. The urine drops were registered on a Sanborn Recorder. After a steady rate of urine flow had been obtained, a known amount of a sample or a standard was injected through the jugular catheter and followed by 0.1 to 0.2 ml of 0.9% NaCl. The standard used was the USP posterior pitutary standard (USP Reference Standards, New York City). Starting at 2 minutes after injection, the number of urine drops was counted for 10 minutes and compared with that obtained in a 10-minute control period immediately before injection. A standard curve was constructed relating the percent of change in urine flow to the logarithm of 2 different doses of standards. Using this curve, the concentration of ADH in the plasma samples was estimated from the percent change in urine flow following their injection. The time interval between successive injections into a given rat was at least 45 minutes.

Results. Normal dogs. In 5 normal dogs with vagi intact, the plasma ADH concentration averaged 87 μU/ml (S.E.$_m$ 10.4). During the bilateral occlusion of common carotid arteries, arterial pressure and heart rate increased. The plasma ADH concentration of samples taken during the period of occlusion showed no significant difference from the control values obtained before occlusion. In all 5 normal dogs studied, the ADH concentrations at 2 minutes after beginning the occlusion averaged 93% of the control (Table I) and they showed no statistically significant difference from the control values (Student t test $P > 0.2$).

Vagotomized dogs. In the 9 vagotomized dogs the plasma ADH concentration averaged 108 μU/ml (S.E.$_m$ 22.1). During the bilateral occlusion of common carotid arteries, arterial pressure and heart rate increased to compar-

‡ Promonta, Hamburg, Germany. Available from Henley Co., New York City.

§ Skinner solenoid valve. Available from Nielson Hydraulic Equipment Co., Pelham Manor, N. Y.

TABLE II. Effects of Common Carotid Occlusion on Plasma ADH Concentration of 9 Vagotomized Dogs.

	ADH conc. (μU/ml)	Heart rate (per min.)	Arterial pressure (mm Hg) Systolic / Diastolic
Control (mean ± S.E.$_m$)	108 (± 22.1)	170 (± 8.7)	154 (± 8.2) / 99 (± 5.2)
Occlusion* (mean ± S.E.$_m$)	285 (± 57.2)	185 (± 9.8)	213 (± 13.5) / 148 (± 8.2)
Change	+177	+15	+59 /+49
P value (t test)†	< .001	< .02	< .001 < .001

* Values obtained at 2 minutes after beginning occlusion.
† Applied to changes observed in individual dogs.

able degrees as in the normal dogs. The plasma ADH concentration increased markedly during carotid occlusion and the rise could be detected as early as 15 seconds after beginning the clamping (Fig. 1). The ADH concentration usually reached a peak at one or 2 minutes after beginning the occlusion of the common carotid arteries (Fig. 1). The plasma ADH concentrations at 2 minutes after beginning the occlusion averaged 264% of the control (Table II) and the rise was significant at the 99% level ($P < 0.001$).

In some experiments, before and after carotid occlusion, simultaneous samples were obtained from the external jugular vein and the femoral artery. The simultaneous ADH concentrations of plasma obtained from these 2 sites showed no significant difference. Hence the results obtained from these 2 sampling sites have been grouped together.

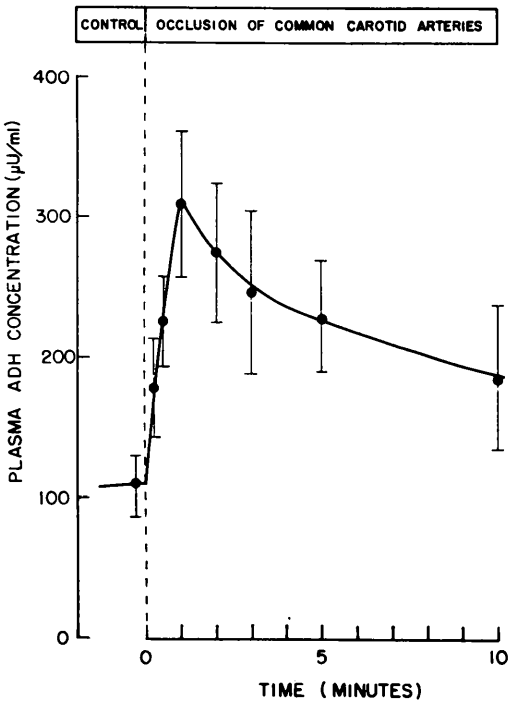

FIG. 1. A composite plot showing effect of bilateral common carotid occlusion on plasma ADH concentration of 9 vagotomized dogs. Vertical bars represent stand. errors of mean.

Atropinized dogs. In 4 dogs with vagi intact, atropine sulfate (0.1 mg/kg or 1 mg/kg) was given ½ hour before the occlusion experiments. The pre-occlusion plasma ADH concentrations averaged 89 μU/ml (S.E.$_m$ 11.9) in these atropinized animals. During the bilateral occlusion of common carotid arteries, arterial pressure increased, but plasma ADH concentration did not (Table III). In these atropinized dogs, the vagus nerves were then sectioned in the neck. Electrical stimulation of the peripheral end of either vagus for 15 seconds with currents supplied from a square-wave stimulator (Grass S-4) caused no decrease in heart rate. The stimulation parameters were as follows: frequency 30/sec., pulse duration 1 msec. and strength 5 to 10 volts. In such atropinized, vagotomized dogs, bilateral common carotid occlusion resulted in a marked increase in plasma ADH concentration.

Discussion. These experiments showed that occlusion of common carotid arteries caused elevation of plasma ADH concentration only if the vagus nerves had been previously sectioned. Within 2 minutes after beginning the occlusion, the plasma ADH concentration became as high as 286% of the control. The degree and speed of the elevation of plasma ADH concentration obviously cannot be explained on the basis of a reduced rate of ADH inactivation, but rather points to an increase in ADH release.

The necessity of removing the vagi to obtain an elevated circulating ADH during caro-

TABLE III. Effects of Common Carotid Occlusion on Plasma ADH Concentration of 4 Atropinized Dogs.

	ADH conc. (μU/ml)	Heart rate (per min.)	Arterial pressure (mm Hg) Systolic / Diastolic
Control (mean ± S.E.$_m$)	89 (± 11.9)	179 (± 23.2)	166 (± 23.7) / 116 (± 23.2)
Occlusion* (mean ± S.E.$_m$)	92 (± 19.8)	189 (± 30.2)	212 (± 34.2) / 142 (± 30.2)
Change	+3	+10	+46 /+26
P value (t test)†	> .7	> .3	< .01 < .02

* Values obtained at 2 minutes after beginning occlusion.
† Applied to changes observed in individual dogs.

tid occlusion agrees with report of Share and Levy(9), who used a purification procedure(10) before antidiuretic assay. In our experiments, although such purification procedure was not used, there was indication that the elevated plasma catecholamine concentration during carotid occlusion did not interfere with the bioassay of ADH. In a vagotomized dog, plasma obtained before and during carotid occlusion was analyzed fluorometrically(11) for concentration of catecholamines. Control values were 0.023 μg/l of epinephrine and 0.65 μg/l of norepinephrine. At 2 minutes after beginning occlusion, epinephrine and norepinephrine concentrations rose to 0.073 and 1.81 μg/l respectively. During bioassay of ADH, usually only 0.1 ml of the "during occlusion" plasma was injected. Hence the amounts of catecholamine introduced into the rats were about 7.3×10^{-6} μg of epinephrine and 1.81×10^{-4} μg of norepinephrine. Injection of pure catecholamines into the rats used for ADH assay showed that as much as 0.04 μg of epinephrine or norepinephrine did not show any antidiuretic activity.

In contrast to the findings of our experiments and those of Share and Levy(9) Perlmutt(12) indicated that in dogs with intact vagi circulating ADH could increase after carotid occlusion, provided that certain degree of hydration was maintained. Although the hydration of dogs in Perlmutt's experiments(12) may explain the difference in results, it must be pointed out that he did not assay the ADH concentration. The urine flow of the dog subjected to carotid occlusion was used as an index of ADH concentration. In view of the known sympathetico-adrenal activity caused by common carotid occlusion (13), it is difficult to rule out the influence of factors other than ADH on the urine flow. Since the renal delay time is not always constant(14,15), renal clearance data obtained in a short, unsteady period (*e.g.*, during 5 minutes of carotid occlusion) cannot be easily interpreted. Hence Perlmutt's statement that the glomerular filtration rate and renal plasma flow increased during carotid occlusion is debatable. The renal plasma flow under these conditions is especially difficult to ascertain and to interpret, since (a) the extraction ratio of p-aminohippuric acid may change and (b) intrarenal distribution of plasma flow may vary even if the total renal flow should remain constant.

In cutting the vagus nerves, both the afferent and efferent fibers were sectioned. To investigate whether it was the afferent or the efferent fibers that must be removed for release of ADH to occur on carotid occlusion, atropine was used in the last group of experiments. After administration of atropine, which blocks the efferent vagal activity at the neuroeffector junction, carotid occlusion, as in normal dogs, did not result in ADH release. Hence the removal of the efferent fibers in vagi was not sufficient. That the elimination of afferent fibers was responsible for the effects seen after vagotomy is further supported by the finding that carotid occlusion caused ADH release in these atropinized dogs if the vagi had also been sectioned. These findings also demonstrated that atropine did not block the response of the hypothalamico-hypophyseal system to carotid occlusion.

The afferent fibers in the vagi seem to carry both stimulatory and inhibitory influences on ADH release(3). Since the plasma ADH concentration has been found to increase after vagotomy(9), at least during the first 30 minutes, the over-all influence of vagi seems to be inhibitory to ADH release. In our experi-

ments, plasma ADH concentration one hour after vagotomy averaged slightly higher than that found in normal dogs, but the difference was not significant statistically ($P>0.5$). The absence of ADH release in dogs with vagi intact can be explained on the basis of the inhibitory influence on ADH release carried by some vagal afferents. Such inhibitory influence may be tonic and keeps the hypothalamico-hypophyseal system in a low state of excitability. Or alternatively, the inhibitory influence in vagal afferents may be operative in a phasic manner. For example, it may be triggered by the rise in systemic arterial pressure occurring during carotid occlusion. When the inhibitory influence, be it tonic or phasic, in afferent vagi is removed by vagotomy, occlusion of common carotid arteries resulted in a release of ADH.

Summary. In bilaterally vagotomized dogs, occlusion of common carotid arteries caused an increase in plasma ADH concentration, which averaged 264% of control at 2 minutes after beginning the occlusion. This increase in ADH was not observed in normal dogs with vagi intact or in dogs with efferent vagi blocked by atropine. It is concluded that the afferent vagal fibers exert some inhibitory influence on the supraoptico-hypophyseal system and that carotid occlusion results in ADH release when the vagal inhibition is removed.

The authors wish to thank Dr. Wilbur H. Sawyer for his advice on the technic for bioassay of ADH and Dr. William W. Walcott for his help in building the equipment for bioassay.

1. Gregersen, M. I., Rawson, R. A., *Physiol. Rev.*, 1959, v39, 307.
2. Reeve, E. B., Allen, T. H., Roberts, J. E., *Ann. Rev. Physiol.*, 1960, v22, 349.
3. Farrell, G., Taylor, A. N., *ibid.*, 1962, v24, 471.
4. Rydin, H., Verney, E. B., *Quart. J. Exp. Physiol.*, 1938, v27, 343.
5. Usami, S., Chien, S., *The Physiologist*, 1961, v4, 125.
6. Weinstein, H., Berne, R. M., Sachs, H., *Endocrinology*, 1960, v66, 712.
7. Gauer, O. H., Henry, J. P., Sieker, H. O., *Progress in Cardiovascular Diseases*, 1961, v4, 1.
8. Sawyer, W. H., *Methods in Medical Research*, 1961, v9, 210.
9. Share, L., Levy, M. N., *The Physiologist*, 1961, v4, 107.
10. Share, L., *Endocrinology*, 1961, v69, 925.
11. Cohen, G., Goldenberg, M., *J. Neurochem.*, 1957, v2, 58.
12. Perlmutt, J. H., *Fed. Proc.*, 1962, v21, 207.
13. Heymans, C., Neil, E., *Reflexogenic Areas of the Cardiovascular System*. Little, Brown & Co., Boston, 1958.
14. Berger, E. Y., Dunning, M. F., Steele, J. M., Jackenthal, R., Brodie, B. B., *Am. J. Physiol.*, 1950, v162, 318.
15. Morales, P. A., Crowder, C. H., Fishman, A. P., Maxwell, M. H., Gomez, D. M., *ibid.*, 1950, v163, 454.

Received June 21, 1962. P.S.E.B.M., 1962, v111.

The Reflex Nature of Release of Antidiuretic Hormone upon Common Carotid Occlusion in Vagotomized Dogs.* (27742)

SHU CHIEN, BRANKO PERIC† AND SHUNICHI USAMI
Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City

In experiments reported in an accompanying paper(1), it was found that bilateral occlusion of common carotid arteries in vagotomized dogs elicited the release of antidiuretic hormone (ADH). The present experiments are designed to investigate whether such ADH release is due to a reflex originating from receptors in the common carotid arteries and their bifurcations or to the effect of reduced cerebral blood flow on the hypothalamico-hypophyseal system.

Methods. Healthy mongrel dogs apparently in normal hydration were used in these experiments. Food was withheld from the ani-

* Aided by research grants from Nat. Inst. Health, U.S.P.H.S.

† International Postdoctoral Fellow, Nat. Inst. Health, U.S.P.H.S.