50 ratio of phosphate buffer and glutamate is not jeopardized by the somewhat lower than "normal" rate of respiration obtained with 100% phosphate, since experimental results are based on randomized complete block designs and the higher respiration rate was observed in 5 out of 7 cases. The nature of this synergestic effect of glutamate and phosphate is not clear, mainly because so little is known of the metabolism of fowl sperm.

The sharper decrease in respiration rate after dilution of fowl semen with Tyrode diluent may be related to the distinctive action of the chloride ions on sperm(1). Koefoed-Johnsen and Mann(9), observed that surfactants caused inhibition of fructolysis, respiration and motility. The chloride ion probably does not cause immediate disruption of the normal sperm morphology but eventually may act as surfactants do. Whether sperm stored in Tyrode diluent respond with a higher respiration rate to succinate than do phosphate diluted stored sperm should be investigated. Such a phenomenon was observed by Koefoed-Johnsen and Mann(9) after treatment of mammalian semen with surfactants.

The inhibition of respiratory activity by glutamate is, however, not attributable to replacement of chloride, for glutamate also inhibited respiration rate when compared to a phosphate diluent. Other data from our laboratory did not give evidence that phosphate causes damage to fowl sperm(1).

Glycine, which is known to act as a chelating agent(9), and glutamate may have a similar effect on respiratory activity partly because of the shared characteristic of acting as a chelating agent. In view of the fact that tetracycline hydrochloride was added to all diluents and in view of its chelating effect (10) it seems that further investigation is necessary.

Summary. Glutamate, which can be catabolized by fowl semen, has an inhibitory effect on respiratory rate and in some experiments on rate of fructolysis. This effect may be explained partly by the catabolism of the glutamate and partly by its chelating effect. Glycine, which acts similarly to glutamate, is not catabolized by fowl semen.

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Effect of Hypophysectomy on Adrenocortical Response to Bilateral Carotid Constriction. (26482)

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Gann, Mills and Bartter(1) have demonstrated that bilateral constriction of the common carotid artery causes an increase in aldosterone secretion in normal dogs. Considerable evidence has accumulated that ACTH affects aldosterone secretion(2,3). This fact makes it difficult to decide whether increased aldosterone secretion in any given experiment is due to increased secretion of ACTH or to a proposed nonpituitary aldos-

terone-stimulating hormone(4), when the experiment has been performed in animals with intact pituitaries. The present experiments were done in order to determine whether the response to carotid constriction is dependent on increased secretion of ACTH. Some of the results have been reported previously in abstract form(5).

Materials and methods. The right lumboadrenal veins of 11 male mongrel dogs weighing 12.0-15.8 kg were cannulated by the method of Hume and Nelson(6) under pentobarbital anesthesia. Transbuccal hypophysectomy was then performed in 7 of the dogs. Fifty-five minutes after operation 2 hypophysectomized dogs were given 5 milliunits (mU) ACTH (Upjohn) intravenously, followed by a constant infusion of 30 mU of ACTH per hour. This amount of ACTH was found in previous experiments to maintain 17-hydroxycorticoid output at 50-75% of maximum(7). Ninety-five minutes after hypophysectomy, adrenal cannulation, or both, the common carotid arteries were constricted in each of the 11 dogs by tying a ligature around the artery and an 18-gauge hypodermic needle, after which the needle was withdrawn.

Adrenal venous blood specimens were collected from the 2 dogs receiving ACTH before the constant infusion was started, and from all dogs 60 and 90 minutes after hypophysectomy and/or cannulation. Specimens were collected from 2 hypophysectomized dogs 15 minutes after carotid constriction, from the remaining 9 dogs 30 minutes after constriction, and from all 11 dogs 60 minutes after constriction. The adrenal venous blood specimens were centrifuged promptly and the plasma frozen for subsequent analysis of aldosterone content by the double isotope derivative technic of Kliman and Peterson(8), and 17-hydroxycorticoids by the Silber-Porter method(9). Femoral and lingual blood pressures were monitored throughout the experiment with Statham strain gauges and a Grass Model 4 polygraph. Gross examination of the sella and hypothalamus at autopsy showed all hypophysectomies to be complete.

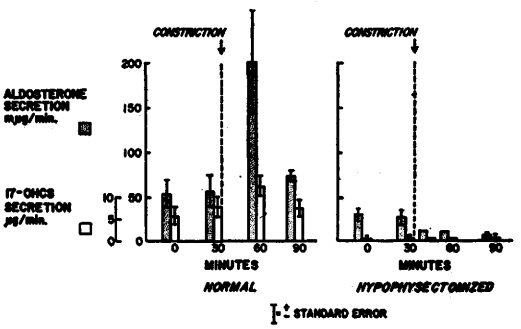

FIG. 1. Effect of carotid artery constriction on adrenal secretion in dogs.

Results. Mean aldosterone outputs 60 and 90 minutes after cannulation in the nonhypophysectomized dogs were 56 ± 18 (mean ± standard error of the mean) and 57 ± 22 mμg/minute, with a rise to 202 ± 75 mμg/minute 30 minutes after carotid constriction, and to 73 ± 8 mμg/minute 60 minutes after constriction. However, the magnitude of the rise varied considerably in individual animals, and the mean value 30 minutes after constriction is not significantly different from the pre-constriction control. Mean control 17-hydroxycorticoid output values in these stressed dogs were 5.7 ± 2.6 and 7.8 ± 3.5 mμg/minute, rising to 12.3 ± 3.1 and 7.4 ± 2.5 mμg/minute 30 and 60 minutes after carotid constriction (Fig. 1). In the 5 untreated hypophysectomized dogs, control aldosterone output values were 30 ± 7.8 and 26 ± 8.5 mμg/minute. Fifteen minutes after carotid constriction, the mean output (2 dogs) was 11 mμg/minute. Thirty minutes after constriction, it was 10 ± 2.1 (3 dogs), and 60 minutes after constriction it was 6 ± 1.8 mμg/minute. The corresponding values for 17-hydroxycorticoid output were 0.5 ± 0.7 and 0.7 and 0.9 μg/minute in the control period and 0.9, 0.7 and 0.6 ± 0.8 μg/minute after constriction.

The aldosterone and 17-hydroxycorticoid output values for the 2 hypophysectomized dogs which were treated with ACTH are shown in Table I. Seventeen-hydroxycorticoid output was increased during the infusion, but no consistent change occurred in aldosterone or 17-hydroxycorticoid output after carotid constriction.

Tying the carotid artery around an 18-

TABLE I. Effect of Carotid Constriction on Adrenocortical Secretion in Hypophysectomized Dogs Infused with ACTH.

	Control	30 mU ACTH/hr				
	Time after hypophysectomy (min.)					
	45	60	90	95	125	155
Aldosterone (mμg/min.)						
Dog 1	27	30	80	Carotod con-	50	80
" 2	60	80	82	striction	25	57
17 OHCS (μg/min.)						
Dog 1	.2	4.2	4.8		3.8	4.0
" 2	.2	5.3	4.5		5.2	7.2

gauge needle caused a 30-60 mm Hg fall in lingual arterial pressure, and a decrease in lingual pulse pressure from a mean of 43 to a mean of 5 mm Hg. The systemic pressure rose sharply after constriction, then fell gradually to its preconstriction level in 1 hour.

Discussion. The present data show clearly that although a rise in aldosterone secretion may follow bilateral constriction of the common carotid arteries in the normal dog, it does not occur in the hypophysectomized animal. It therefore appears that carotid constriction leads to increased secretion of ACTH, which is responsible for any increase in aldosterone secretion. The rise in 17-hydroxycorticoid secretion that occurred in the normal dogs after carotid constriction supports this conclusion.

It is possible that hypophysectomy abolishes the responsiveness of the adrenal to a nonpituitary aldosterone-stimulating factor. Since the administration of ACTH causes prompt rises in the secretion of both 17-hydroxycorticoids and aldosterone(10) up to 6 hours after hypophysectomy, loss of responsiveness probably does not play a role in these studies. To rule out the possibility that some circulating ACTH is necessary for adrenal responsiveness, 2 hypophysectomized dogs were given ACTH by constant infusion while the adrenocortical response to carotid constriction was measured. The dose of ACTH chosen produced and maintained a 4-fold rise in 17-hydroxycorticoid secretion, but subsequent carotid constriction produced no change in the secretion of these hormones or aldosterone. Thus increased aldosterone secretion observed in normal dogs seems to be due to increased secretion of a pituitary factor, and this factor is probably ACTH.

Summary. To determine whether the increase in aldosterone secretion following constriction of the carotid arteries was due to release of pituitary ACTH, 4 normal dogs, 5 acutely hypophysectomized dogs and 2 acutely hypophysectomized dogs given ACTH by constant infusion were subjected to constriction of both common carotid arteries. A variable rise in aldosterone secretion occurred in dogs with intact pituitaries after carotid constriction, with an associated increase in 17-hydroxycorticoid secretion. In hypophysectomized dogs and in ACTH-infused hypophysectomized dogs, this rise failed to occur. The data support the conclusion that the increase in aldosterone secretion following carotid constriction is due to pituitary release of ACTH.

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Bile Duct Obstruction and Regeneration of the Liver.* (26483)

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It was recently observed in this laboratory that ligation of the common bile duct of the rat stimulated proliferation of liver cells in the absence of liver cell necrosis(1). The present work was carried out to study further the effect of biliary obstruction on hepatic regeneration, and to compare regeneration due to biliary obstruction with that following partial hepatectomy. Incorporation of tritiated thymidine (H^3-thymidine) into deoxyribonucleic acid (DNA) was quantitated in autoradiographs, and mitoses were counted in histologic sections to detect and quantitate regeneration(2).

Higgins and Anderson(3) performed partial hepatectomies after bile duct ligation in rats: Wet and dry weights were correlated with the histologic appearance and with hepatic mitoses; it was found that weight was restored as well or better than in normal rats subjected to partial hepatectomy. On the basis of gross and histologic observations, however, they interpreted restoration of weight in obstructed liver to be due to engorgement with bile and blood, and they concluded that regeneration was impaired after bile duct ligation. Cameron(4) studied survival of slices of normal and obstructed liver placed as autotransplants in the omentum of rats. He found that in liver from animals with bile duct obstruction liver cells did not survive as long as from normal rats. He concluded that bile duct ligation inhibited regeneration. Ferguson, Rogers and Vars(5) studied total liver weight, protein content, and mitotic activity and found that in rats that had undergone bile duct ligation at the time of partial hepatectomy, regeneration was as great or greater than regeneration in partially hepatectomized normal liver. Weinbren(6) studied rat liver subjected to partial hepatectomy after bile duct ligation. He compared gain in weight of remaining liver lobes, and found no difference between obstructed and normal liver; he concluded that bile duct ligation did not inhibit regeneration.

Materials and methods. Forty male rats of the Sprague-Dawley strain,‡ separated by litter-mates into 24-hour and 48-hour experimental groups were used. Animals were obtained at 21 days of age, and were maintained in this laboratory until 119 to 124 days of age, at which time they weighed 315 to 415 g. They were housed individually in an air-conditioned room, and were fed laboratory chow and tap-water *ad libitum.* Animals were divided into groups as follows: 12 underwent ligation of the common bile duct midway between liver and duodenum, followed immediately by partial hepatectomy of approximately 50% of the liver(7). Twelve rats were subjected to partial hepatectomy alone; 8 to ligation of the bile ducts alone, and 8 to a sham operation consisting of

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