is present in liver at any one time. Rats without spontaneous glycosuria (Exp. 2, Fig. 2) excreted up to 3 g of glucose during a 24-hour period. If this temporary exacerbation of diabetes is due to an effect of epinephrine upon liver, it must be described in terms of changes in turn-over rather than as a single discharge of glucose from liver. The maximum glycosuria usually occurred on the 2nd day after beginning the continuous injection of epinephrine.

The possibility that epinephrine stimulates the adrenal cortices to secrete diabetogenic amounts of cortical hormones can be considered. Large doses of epinephrine can stimulate the adrenal cortices via the increased release of corticotropin in acute experiments(5), but there is no satisfactory evidence known to the authors that a true state of hypercorticalism can be induced by epinephrine.

The largest dose of epinephrine used in this study, 1000 μg per day, is near the maximum that can be tolerated by the rat. When the dose was doubled, as in Exp. 2, some of the rats died. In preliminary studies it was found that either a dose of 500 or 1000 μg of epinephrine per rat per day would cause death in some animals unless the animals were first adapted to a smaller dose.

Summary. Epinephrine was administered to normal and to partially depancreatized force-fed rats by continuous subcutaneous injection for periods of 3 and 4 weeks. Simultaneously the control animals were injected with saline. Increased amounts of glucose were excreted by both normal and partially depancreatized rats during the injection of epinephrine. The peak response occurred on the second day after starting the injection of epinephrine or increasing the dose. All of the animals showed adaptation to epinephrine so that the peak response was not sustained.

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Enzymatic Evidence for Intrinsic Oxytocic Activity of the Pressor-Antidiuretic Hormone. (20559)

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Until recently there has been uncertainty as to whether the oxytocic activity of vasopressin preparations was due to contamination of the preparations with oxytocin or to an intrinsic oxytocic activity of the pressor-antidiuretic hormone. Evidence for the latter explanation was afforded by the work of Popenoe, Pierce, du Vigneaud, and van Dyke (1). They found that the most highly purified arginine-vasopressin prepared in this laboratory possessed approximately 600 pressor units per mg by the arterial blood pressure method in cats and an oxytocic activity of 80-90 units per mg by the method of Coon(2), based on the lowering of the blood pressure of the fowl, and 30 units per mg by the rat uterus method.† On the other hand, a highly purified oxytocin preparation(3) possessing

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† Coon(2) found that when the pressor-oxytocic ratio of a pituitary solution was above 2.5 the oxytocic assay value obtained by the depressor method was higher than that obtained by the guinea pig uterine method.

an oxytocic potency of 500 units per mg had no effect on the arterial blood pressure of cats and less than 0.5 unit per mg of antidiuretic activity in the *diabetes insipidus* dog(1).

Analytical studies indicated that the vasopressin preparation mentioned could not have been contaminated with sufficient oxytocin to account for the oxytocic activity. Previously it had been shown that oxytocin was a polypeptide containing leucine, isoleucine, tyrosine, proline, glutamic acid, aspartic acid, glycine, cystine, and ammonia. Analysis of this vasopressin preparation by starch column chromatography(4) yielded phenylalanine, tyrosine, proline, glutamic acid, aspartic acid, glycine, arginine, cystine, and ammonia, but no detectable leucine or isoleucine. It was therefore concluded that vasopressin possessed intrinsic oxytocic activity. Additional support of this conclusion has come from zone electrophoresis studies reported by Taylor, du Vigneaud, and Kunkel(5). After the establishment of the isoelectric point and the mobility of oxytocin and vasopressin it became obvious that if purified oxytocin and vasopressin were mixed together, one ought to obtain 2 oxytocin peaks and one vasopressin peak with one of the oxytocin peaks coinciding with the pressor peak if the pressor material actually possessed oxytocic activity. The prediction was borne out by such a study. A third line of evidence for the intrinsic oxytocic activity of vasopressin is presented in this paper. The enzyme, trypsin, had been shown by Croxatto and Croxatto(6) and by preliminary studies on highly purified material in this laboratory‡ to destroy the pressor activity of vasopressin preparations but not the oxytocic activity of oxytocin preparations. A study of the effect of trypsin on the oxytocic and pressor activity of an arginine-vasopressin preparation free of leucine and isoleucine and of a lysine-vasopressin preparation was therefore undertaken. Control studies were made of the enzyme action on oxytocin.

In agreement with the earlier work, the oxytocic activity of oxytocin was not destroyed by trypsin but the pressor activity of vasopressin was destroyed. However, the present work shows that the oxytocic activity of arginine-vasopressin in contrast to the oxytocic activity of oxytocin is destroyed by trypsin. Thus additional, convincing proof that the oxytocic activity of vasopressin is not due to oxytocin and that vasopressin has intrinsic oxytocic activity is offered. The study of lysine-vasopressin gave parallel results. Undoubtedly the basic amino acids present in the vasopressin preparations make these hormones susceptible to an enzymatic attack to which oxytocin is resistant.

Experimental. Pressor and oxytocic activities reported in this study were based on assays standardized by reference to the U.S.P. Posterior Pituitary Standard. Oxytocic assays were performed according to the chicken depressor method of Coon(2). Pressor assays were obtained by measurements of the arterial blood pressure response of cats anesthetized with sodium phenobarbital. Arginine-vasopressin was prepared from bovine posterior powder by the method already described(7). It possessed 390 units per mg of pressor activity and 54 units per mg of oxytocic activity by the chicken depressor assay. The preparation was free of leucine and isoleucine.

Forty-five mg of the arginine-vasopressin preparation and 1.8 mg of trypsin§ were dissolved in 5 ml of water and adjusted to pH 7 with $NaHCO_3$. The mixture was incubated at 38°C for 5.5 hours. Assays before and after incubation showed that there was an almost complete loss of pressor and of oxytocic activity. It was apparent that the enzymatic degradation of the vasopressin preparation had resulted in a destruction of both pharmacological properties. Control studies of the effect of trypsin on oxytocin demonstrated that the oxytocic activity was not destroyed and thereby substantiated the above conclusion.

The effect of trypsin on lysine-vasopressin which had been prepared from hog glands(8) was also investigated. The initial activity of

‡ Taylor, S. P., and du Vigneaud, V., unpublished data.

§ Crystalline trypsin containing approximately 50% Mg SO_4 was obtained from the Worthington Biochemical Laboratory, Freehold, N. J.

the preparation was found to possess approximately 175 pressor units per mg, 30 oxytocic units per mg by the chicken depressor method, and 9 oxytocic units per mg by the rat uterus method. After the incubation with trypsin there was a destruction of the oxytocic as well as the pressor activities of the lysine-vasopressin.

Summary. 1. It has been shown that trypsin had no effect on the activity of oxytocin but destroyed both the pressor and oxytocic activities of arginine-vasopressin. These conclusions offer enzymatic evidence, in addition to the evidence already obtained from analytical and electrophoretic studies, for the inherent oxytocic activity of arginine-vasopressin. 2. It has also been demonstrated that a lysine-vasopressin preparation possessed oxytocic activity and that this activity was destroyed with the pressor activity by trypsin.

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Effect of Alloxan on Isolated Liver of the Bull Frog.* (20560)

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The mechanism of the alloxan-induced initial fluctuations of the blood sugar is not yet clearly understood. Two alternate hypotheses have been offered to explain the alloxan hypoglycemia. A pancreatic origin seemed to be implied mainly by the observations that the hypoglycemia is a) preceded by histological evidence of Beta cell degeneration(1), and b) absent in animals who were previously depancreatized or made diabetic by alloxan or whose pancreatic blood supply is occluded temporarily while alloxan is injected intravenously (2,3). It appeared from this evidence that the transitory hypoglycemia was the result of endogenous insulin leaking from the disintegrating beta cells. This interpretation was challenged, however, when it was found that a) alloxan hypoglycemia could still be induced in freshly depancreatized dogs at least in some instances(4-6), and b) the insulin content of the pancreatic islets does not decrease until several hours after the onset of the hypoglycemic phase(6). Therefore, an extrapancreatic —probably hepatic-origin of the alloxan hypoglycemia was postulated(7,8). A direct effect of alloxan on the liver of the frog was demonstrated by Houssay and Gershman(9) in perfusion experiments where alloxan was found to inhibit glycogenolysis. Decrease of the spontaneous glycogenolysis was also shown by Kepinov(10) in the isolated and perfused rat liver. Weber(11) presented evidence that alloxan diabetic rabbits are able to store more glycogen in the liver after glucose feeding than normal animals. Other investigators using

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