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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various approaches have failed, however, to confirm such direct hepatic action of alloxan (12-14). Recently we have carried out a series of experiments with perfusion of the isolated liver of the bull frog and have been able to demonstrate that alloxan significantly inhibits both the spontaneous and the adrenalin induced glycogenolysis.

Material and methods. The liver preparation for perfusion was set up according to the original description by Froehlich and Pollak (15) and the modification as to rate of flow and to pressure by Geiger and Loewi(16). Bull frogs—rana catesbeiana—of 200-250 g weight, were used and kept in hibernating condition at 4°C. The animals were killed by decapitation without use of an anesthetic. The liver was removed while preserving the abdominal vein and the venous sinuses; it was placed on a wire gauze plate on a tripod under which a small collecting beaker was placed. The perfusion system consisted of 2 funnels connected by rubber tubings with a 3-way stop cock, the third rubber tubing being mounted with a No. 26 needle. This needle was inserted into the abdominal vein and secured by ligation. The perfusate passed through this system and the liver with constant pressure so that 5-7 drops per minute were collected from the venous sinuses. Amphibian Ringer solution(17) (NaCl 0.66, KCl 0.015, $CaCl_2$ 0.015, Na HCO_3-Ph 7.8, aqu. dest. ad 100) and amphibian Ringer-Glucose solution which contained 200 mg% glucose were used as perfusates and placed into either of the 2 funnels. Where oxygenated solutions were employed saturation was maintained by bubbling O_2 through the perfusate in the funnel. When the effects of alloxan or adrenalin were studied, the material was injected directly into the rubber tubing near the abdominal vein. The preparation of the livers for perfusion was completed within 5 minutes. The perfusion experiments were carried out at room temperature over periods of 3 hours, during which time the livers appeared healthy and maintained almost normal color and consistency. The organs were weighed before and after the experiment; a gain of about 10% of the original wet weight was noted in most experiments, due to retention of perfusate.

TABLE I. Effect of Perfusion with Amphibious Ringer Solutions without Oxygen, with Oxygen and with the Addition of 200 mg % Glucose on Glycogenolysis of the Surviving Liver of the Bull Frog.

Perfusate	Time in min. 10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220
Amphibious Ringer sol.	22	10	0	0	0	18	27	32	26	41	25	28	29	31	20	—	—	—	—	—	—	—
	34	9	6	0	0	0	29	27	36	48	40	41	36	32	34	38	36	32	—	—	—	—
Oxygenated amphibious Ringer sol.	23	14	0	0	3	0	6	0	0	2	3	0	0	0	0	0	0	0	—	—	—	—
	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	—	—	—	—
Oxygenated amphibious Ringer sol.—After 40' Ringer-glucose sol.	29	0	0	0	* 146	202	206	200	200	197	203	203	194	194	194	200	200	201	203	204	206	200
	16	14	0	0	119	200	200	206	210	212	212	212	202	204	198	196	196	198	200	200	194	197

* After 40 min. the perfusate was changed to oxygenated amphibious Ringer-glucose sol.
Each figure represents the glucose concentration (mg %) of the perfusate collected from the liver during periods of 10 minutes.

TABLE II. Effect of Alloxan upon Glycogenolysis of Isolated Liver of the Bull Frog Perfused with Ringer Solution without Oxygen.

Time in min.																	Wet wt of liver (g)	
10	20	30	40	50	60	70	80	90	100 *	110	120	130	140	150	160	170	180	Before	After
27	6	0	0	0	18	20	21	23	31	21	20	12	10	12	6	0	—	15.0	15.3
11	0	0	2	0	12	24	27	26	9	13	7	10	0	0	0	0	—	19	19
31	16	6	0	0	0	19	28	38	18	9	0	0	0	0	0	0	—	18.6	19

* Indicates time when 10 mg alloxan were inj. into perfusate.
Each figure represents the glucose concentration (mg %) of the perfusate collected from the liver during periods of 10 minutes.

An initial period of perfusion with amphibious Ringer solution served to wash out any free blood and blood glucose. After 20′ to 30′ the fluid usually returned water-clear and sugar-free; then the specific experiment was started. The perfusate was collected at 10′ or 20′ intervals. The glucose determinations were done with the Nelson modification of the Folin-Wu Micro method(18).

Results. A) In a first series of experiments the spontaneous glycogenolysis of the isolated and perfused liver was studied, using as perfusates a) non-oxygenated, b) oxygenated amphibious Ringer solution and c) oxygenated amphibious Ringer-Glucose solution.

Table I represents the results of 2 experiments each. It can be seen that during an initial period of not more than 30′ decreasing amounts of glucose are obtained. During this time the perfusate is blood tinged and the glucose undoubtedly originates from the blood which is washed out. Afterwards the perfusate returns sugar-free for various periods of time. If non-oxygenated perfusion fluid is used (Exp. 1 and 2) glucose reappears in the perfusate about 30′ later and reaches a relatively stable level which is maintained for 2 to 3 hours. With the vascular tree now free of blood, this glucose must originate from the liver parenchyma and is evidence of spontaneous glycogenolysis. If O_2 is added to the perfusate the collected fluid remains practically free of glucose for the duration of the experiment, *i.e.*, 3 hours, indicating that with oxygen supply spontaneous glycogenolysis does not occur (Exp. 3 and 4). A glucose-Ringer solution saturated with O_2 returns from the liver with essentially unaltered sugar content (200 mg %) indicating that under these conditions neither glycogenolysis nor glycogenesis occurs unless both processes proceed at identical rates so as not to be noticeable from determinations of sugar-intake and output (Exp. 5 and 6).

B) The second group of experiments was undertaken to test the effect of a standard dose of alloxan (10 mg) on the spontaneous glycogenolysis of the surviving frog liver perfused with non-oxygenated amphibious Ringer solution. The liver was perfused for a period of 90 minutes before alloxan was injected. This initial period served not only to wash out any extraneous blood but also permitted the onset of spontaneous glycogenolysis.

Table II demonstrates that after the administration of alloxan the glucose content of the perfusate begins to decrease gradually until it finally disappears entirely. This inhibition of the spontaneous glycogenolysis is

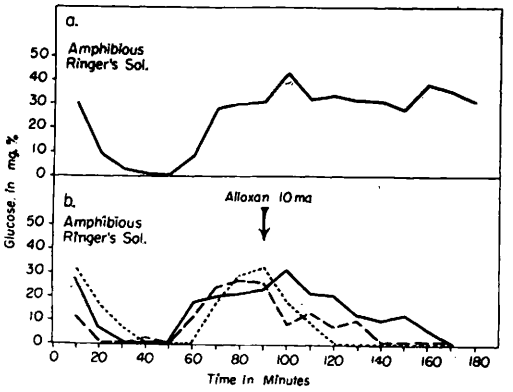

FIG. 1. a) Spontaneous glycogenolysis of isolated liver of bull frog perfused with amphibious Ringer solution without oxygen. b) Effect of alloxan on spontaneous glycogenolysis of isolated liver of bull frog perfused with amphibious Ringer solution without oxygen.

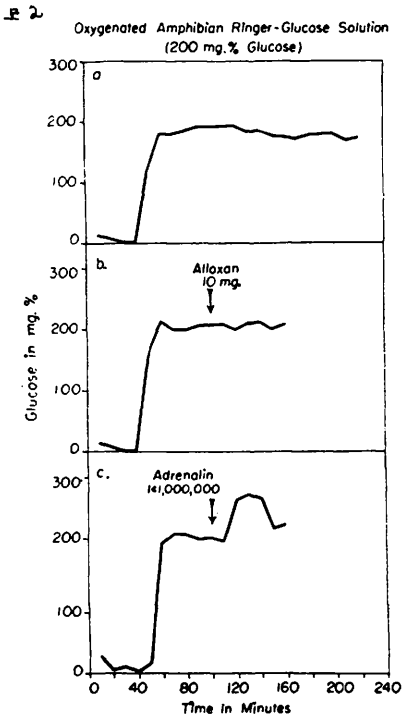

FIG. 2. Effect of adrenalin (1:1000000) and alloxan (10 mg) on isolated liver of bull frog perfused with oxygenated amphibious Ringer-glucose solution (200 mg %). During first 40 min. the liver is perfused with oxygenated Ringer solution without glucose.

noticed as early as 10′ after alloxan administration and lasts for more than one hour. Its reversibility cannot be deduced from the experiments presented since these were terminated while the inhibition persisted. Fig. 1 demonstrates graphically the spontaneous glycogenolysis of the perfused liver (a) and its inhibition by alloxan (b).

If alloxan is added to an oxygenated perfusate which, as shown, delays spontaneous glycogenolysis no evidence of its effect can be obtained. This is demonstrated in the following experiment (Fig. 2) where an oxygenated glucose containing perfusate was used. Fig. 2 shows that the amount of glucose recovered from the returning perfusion fluid was the same without (a) and with (b) alloxan administration. Fig. 2c shows in comparison the increase of the glucose return after adrenalin injection.

C) We now proceeded to investigate the effect of alloxan on the adrenalin-induced glycogenolysis. The following experiments were carried out: a) adrenalin 1:1,000,000 was injected into the abdominal vein 60′ after perfusion with oxygenated amphibious Ringer solution had been started. This resulted in the appearance of a significant amount of glucose in the returning perfusate for a period of 40′ to 60′. Afterwards the perfusate became again sugar-free. b) The administration of the same dose of adrenalin was followed after 20′ by an injection of 10 mg alloxan (1 cc of 1% solution in Ringer). The adrenalin glycogenolysis was not affected by this subsequent administration of alloxan. c) The same doses of adrenalin and alloxan were injected simultaneously. Glucose failed to appear in the returning perfusate. d) Alloxan administration preceded the injection of adrenalin. This experiment was car-

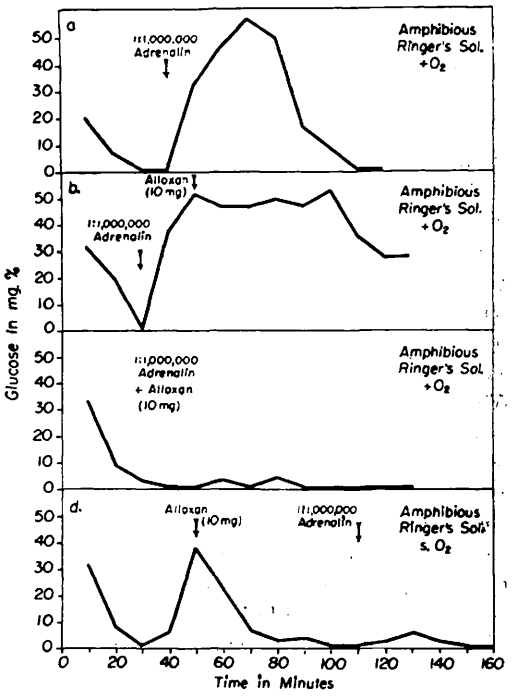

FIG. 3. a-c) Effect of adrenalin (1:1000000) and alloxan (10 mg) upon glycogenolysis of isolated liver of bull frog perfused with oxygenated Ringer solution.

a) Adrenalin-glycogenolysis. b) Alloxan administered 20′ after adrenalin does not affect adrenalin glycogenolysis. c) Alloxan administered simultaneously with adrenalin prevents adrenalin glycogenolysis. d) Alloxan inhibits spontaneous glycogenolysis and prevents adrenalin glycogenolysis in isolated liver of bull frog perfused with amphibian Ringer solution without oxygen.

ried out with non-oxygenated Ringer solution in order to demonstrate the alloxan inhibition of the spontaneous glycogenolysis. Adrenalin failed to reinduce glycogenolysis. Representative curves of these 4 types of experiments are shown in Fig. 3.

Discussion. Our finding that alloxan inhibits the spontaneous glycogenolysis as well as the adrenalin induced glycogenolysis of the perfused frog liver is in full agreement with the observations of Houssay and Gershman (9). The failure of Brunfeldt and Iversen (13) to obtain similar results rests probably in their technic. They apparently perfused under too great pressure and observed "rapidly occurring changes in the appearance of the liver and violent edematization." The difference between our findings and those of Kepinov(10) is a quantitative one only. In his experiments the inhibition of the spontaneous glycogenolysis was only partial and far shorter lasting than in ours. It is most likely that this can be explained as a species difference since he worked with the liver of a warm blooded animal.

The demonstration of an extrapancreatic effect of alloxan on carbohydrate metabolism is undoubtedly of significance for its diabetogenic action and particularly for the mechanism of the initial fluctuation of the blood sugar. It tends to weaken the evidence for the pancreatic origin of alloxan hypoglycemia. Whether the inhibition of hepatic glycogenolysis is its sole or a contributing cause will have to wait for further investigation. From the presented evidence the alloxan effect upon the liver appears to be an immediate one; the secondary hypoglycemic phase, on the other hand, becomes manifest not before 3 or 4 hours after alloxan administration. This time interval may be required until changes in liver metabolism reflect themselves in the periphery; yet, during this same period one usually observes a transitory hyperglycemic phase which has been considered by some to be due to adrenal stimulation(3,19). The finding that alloxan inhibits adrenalin glycogenolysis, however, tends to eliminate this mechanism as its cause.

Thus the acceptance of an hepatic origin of the alloxan hypoglycemia, far from solving the problem, opens again the question of the origin of the initial hyperglycemia.

Summary. 1. The technic for perfusion of the liver of the bull frog is described. 2. Alloxan inhibits the spontaneous glycogenolysis of the perfused liver. 3. Adrenalin glycogenolysis is inhibited by alloxan if given previously or simultaneously. 4. The significance of these findings for the alloxan-induced blood sugar fluctuations is discussed.

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