activity of an equimolar amount of nicotinic acid. This activity may be due to impurities or slight conversion during sterilization. Glenn *et al.*[4] have reported nikethamide to be inactive for this organism. Dorfman *et al.*[8] have found it to be slightly active for *dysentery bacilli*, while Knight[9] and Landy[10] have found it inactive for *Staph. aureus*.

[8] Dorfman, A., Koser, S. A., Reames, H. R., Swingle, K. F., and Saunders, F. J., *J. Inf. Dis.*, 1938, **65**, 163.

[9] Knight, B. C. J. G., *Biochem. J.*, 1938, **32**, 1241.

[10] Landy, M., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 504.

The compound was autoclaved for one hour with various concentrations of NaOH, HCl, and H_2SO_4 at 15 pounds and the resulting mixtures were assayed with *L. arabinosus*. The results summarized in Fig. 1 show that NaOH is most effective, while HCl is somewhat more potent than H_2SO_4 in converting nikethamide to an active compound. Nikethamide is less easily converted to active material than is the "precursor" found in wheat bran, since autoclaving with 0.1 N NaOH, 1 N HCl, or 1 N H_2SO_4 seems to bring about 100% conversion of the latter substance.

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Mechanism of the Diabetogenic Action of Alloxan.*

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Alloxan has been found to produce sustained diabetes mellitus in rats, rabbits, and dogs (Shaw-Dunn and McLetchie,[1] Goldner and Gomori,[2,3] Bailey and Bailey[4]). This diabetes is due to degeneration of the beta cells in the islets of Langerhans (Shaw-Dunn, Sheehan and McLetchie[5]) and consequently to the decrease of insulin production (Goldner and Gomori[6]).

Prior to the establishment of the diabetic syndrome the blood sugar of the rabbit shows a characteristic biphasic reaction, which was first described by Jacobs.[7] An immediate hyperglycemia which reaches its peak within 2 or 3 hours is followed by a severe, often fatal, hypoglycemia, which after a duration of 15 to 20 hours yields to the ultimate hyperglycemia and glycosuria. The hypoglycemic phase is explained by Shaw-Dunn and coworkers[5] as the effect of large amounts of insulin, which are released suddenly from the degenerating beta cells. The phenomenon of the initial hyperglycemia raises the question whether alloxan inhibits insulin before it destroys the site of insulin production. It seemed, therefore, of interest to investigate the question whether insulin is able to prevent the initial rise of the blood sugar after alloxan poisoning and also whether insulin can protect the beta cells against alloxan injury. The latter problem seemed of special interest, since it has been found that insulin treatment and the maintenance of a normal blood sugar level will protect animals against the diabetogenic effect of anterior pituitary

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[1] Shaw-Dunn, J. S., and McLetchie, N. G. B., *Lancet*, 1943, **2**, 384.

[2] Goldner, M. G., and Gomori, G., *Endocrinology*, 1943, **33**, 297.

[3] Gomori, G., and Goldner, M. G., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 287.

[4] Bailey, C. C., and Bailey, O. T., *J. A. M. A.*, 1943, **122**, 1165.

[5] Shaw-Dunn, J. S., Sheehan, H. L., and McLetchie, N. G. B., *Lancet*, 1943, **1**, 484.

[6] Goldner and Gomori, to be published in *Endocrinology*.

[7] Jacobs, H. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 407.

TABLE I.

Blood Sugar Curves in 10 Rabbits After Intravenous Injection of Alloxan, of Alloxan Plus Insulin, and of Insulin. Rabbits 80, 2, and 16 died in hypoglycemic convulsions after 6, 2 and 5 hours, respectively.

Rabbit No.	Alloxan, mg/kg	Insulin, unit/kg	Application	Blood sugar level: Fasting	1	2	3	4	5	6	24	48
					Hours after injection							
1	**200**	**0**	i.v.	108	251	327	—	80	—	28	111	—
62	200	0	i.v.	102	338	378	—	57	—	40	53	460
80	150	0	i.v.	123	305	306	272	—	—	32	—	—
2	200	1	i.v.	82	40	32	—	—	—	—	—	—
16	200	1	into	115	83	61	85	—	37	—	—	—
4	150	1	different veins	107	70	53	75	—	—	55	113	279*
38	200	1	i.v.	111	79	69	69	60	—	—	438	—
18	200	1	mixture	117	65	64	93	84	—	43	—	507
52	150	1		122	139	141	135	—	—	45	—	532
37	0	1	i.v.	121	30	36	39	55	90	115	—	—

*After 72 hours.

extract (Campbell, Haist, Ham, and Best[8]).

Experimental Procedure. Three rabbits out of a series of 6 of the same size and breed were treated simultaneously with a diabetogenic dose of alloxan and with insulin, the 2 substances being injected into 2 different veins, the remaining 3 animals with the same doses of a mixture of alloxan and insulin, kept at room temperature for 60 minutes prior to intravenous injection. A freshly prepared 5% solution of alloxan (Eastman) was used and 150 and 200 mg per kg were given. The insulin dose was 1 unit per kg, a solution of insulin zinc crystals (Eli Lilly and Company) being used. Insulin sensitivity curves and blood sugar curves of rabbits of the same stock injected with the same doses of alloxan alone served as controls. The animals were fasted for 20 hours prior to the experiment. The blood sugar level was determined before the injection and at hourly intervals afterwards. The Miller-Van Slyke[9] micromethod for blood sugar estimation was used.

Results. The results are shown in Table I. The blood sugar curves of rabbits 1, 62, and 80 demonstrate the typical response to a diabetogenic dose of alloxan. The blood sugar level rises immediately after injection, reaches an extreme peak of 300-400 mg % after 2 hours, and falls to a convulsive level soon afterwards. If no glucose injections are given at this time only a few animals will recover to develop hyperglycemia and glycosuria. Rabbit 62 shows such a spontaneous recovery. Rabbit 80 died in hypoglycemic convulsions and rabbit 1 was killed 24 hours after injection. These 3 rabbits are representative of 24 animals which received alloxan injections and followed similar courses. However, the early blood sugar changes were not followed in all of them. At present we have in our series 3 animals with sustained diabetes of more than two months' duration.

The blood sugar curve of rabbit 37 demonstrates normal insulin sensitivity.

The blood sugar curves of the 6 animals which had received injections of alloxan and insulin have in common the fact that they do not show the initial alloxan hyperglycemia. A very slight elevation of the blood sugar is seen in the curve of rabbit 52, negligible if compared with the increase of 200% in the control animals 1, 62, or 80. All other animals show an early hypoglycemic response, which in rabbit 2 was as severe as if insulin alone had been given. This rabbit died in hypoglycemic convulsions. Rabbits 16, 4, 38, and 18 show a moderate fall of the blood sugar within 2 hours after injection. After 3 or 4 hours the blood sugar rises somewhat and seems to be returning to the fasting level. These 4 animals, however, show a second

[8] Campbell, J., Haist, R. E., Ham, A. W., and Best, C. H., *Am. J. Physiol.*, 1940, **129**, 328.

[9] Miller, B. F., and Van Slyke, D. D., *J. Biol. Chem.*, 1936, **114**, 583.

hypoglycemic reaction during the fifth or sixth hour after injection. Rabbit 16 died in this phase in spite of glucose injections. Rabbits 4, 38, 18, and 52 recovered and developed diabetes on the second or third day.

A biopsy from the pancreas of rabbit 52 was taken 5 days after development of diabetes and showed the characteristic changes found after alloxan injection. The beta cells were shrunken or had disappeared completely; the alpha cells appeared normal and seemed to have increased in numbers.

It can be concluded from these observations that alloxan does not inhibit insulin and that the initial alloxan hyperglycemia of the rabbit can be prevented by insulin. Insulin, however, does not protect the islet cells against the injurious effect of alloxan, nor does it prevent the development of alloxan diabetes. The first 4 hours of the blood sugar curves of the 6 rabbits 2, 16, 4, 38, 18, and 52 can be explained as a combination of the hypoglycemic effect of insulin and the hyperglycemic effect of alloxan. In all instances the insulin effect was predominant, since the alloxan hyperglycemia did not appear. In a single instance, however, insulin hypoglycemia was as severe as it would have been if no alloxan had been given simultaneously. The second period of the curves does not differ from the blood sugar curves after alloxan alone. During this period the effect of the injected insulin was diminishing while the hypoglycemic phase of alloxan poisoning had just set in.

Comment. The mechanism of alloxan poisoning is unknown. The experiments here presented give further evidence for the assumption that alloxan affects the beta cells directly and does not act through the medium of disturbed blood sugar regulation. The initial hyperglycemia can be prevented by insulin, the following hypoglycemia by glucose injections, and yet the beta cells will degenerate and diabetes will develop. This is in contrast to the mechanism of pituitary diabetes. Here, the early hyperglycemia, provoked by injections of anterior pituitary extract, is indispensable to the establishment of diabetes, and the maintenance of a normal blood sugar level by whatever means (insulin, phlorhizin, or fasting) prevents the exhaustion of the islet cells and the development of the diabetes. In alloxan poisoning the diabetogenic process will take its course regardless of whether the initial hyperglycemia (which is an accompanying symptom rather than a causal factor) is prevented or not. This has been shown by us also in experiments on dogs, where neither simultaneous and prolonged treatment with insulin nor phlorhizin injections will protect the beta cells against alloxan poisoning.[6] It seems, therefore, that alloxan exerts its effect by direct damage to the beta cells. It does not inhibit insulin, nor does it cause exhaustion of the islet tissue by overstrain.