20 min periods during stimulation at a frequency of 20/sec, the same authors found a reduced rate of ACh liberation the first $^1/_2$ h after cutting. After that time, the ACh liberation returned to approximately the precutting value. These temporary effects can probably be avoided when recordings are made at later stages after cutting (Figures 1 and 2). On the other hand, HUBBARD and WILLIS[3] demonstrated that presynaptic hyperpolarization, though increasing the absolute EPP amplitudes, did not substantially counteract the progressive decline of these amplitudes in curarized preparations. This seems to rule out hyperpolarization as an explanation of the differences between the tetanic trains here presented (Figure 1 B and C, and Figure 2 B) and those seen during later stages of curarization.

If the records of the tetanic EPP trains presented above give a correct picture of the normal course of transmitter release during tetanization, the mechanisms involved in this release at the mammalian neuro-muscular junction seem to be more persistent than generally assumed.

Zusammenfassung. Am isolierten Phrenicus-Zwerchfellpräparat der Ratte wurden, nach transversaler Durchschneidung der Muskelfasern auf jeder Seite der Endplattenregion, Endplatten-Potentiale, ohne Zusatz von modifizierenden Substanzen, intrazellulär registriert. Während kurzdauernder tetanischer Nervenreizung erwies sich der Abfall der EPP-Amplituden als unerheblich und die Freisetzung des neuro-muskulären Überträgers Acetylcholin bei Säugetieren während des Tetanus, entgegen der gewöhnlichen Auffassung, als offenbar anhaltender.

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Intestinal Transport of Glucose and Sodium: Changes in Alloxan Diabetes and Effects of Insulin

SOLS[1] has reported that insulin stimulated glucose absorption from intestinal loops, but it has been suggested that changes in blood sugar levels may have been responsible for the effect[2,3]. Insulin in vitro failed to increase galactose uptake by rings of hamster intestine[4]. In view of these conflicting reports, it seemed of interest to study effects of insulin on glucose transport in vitro by everted segments of rat intestine, a preparation free from the influences of changes in blood composition and flow. Several reports indicate that glucose absorption is increased in alloxan-diabetes[5–7], but results concerning effects of insulin again conflict. Recently, it has been shown that intimate relationships exist between intestinal transport of Na and glucose[8–10], but there appear to be no reports on effects of insulin and the alloxan-diabetic state on Na absorption. Therefore, glucose and Na transport in alloxan-diabetes and the effects of insulin in normal and diabetic rats were studied.

Male Holtzman rats weighing about 300 g were used. All were fasted for 18 h prior to sacrifice. One 15 cm segment of upper jejunum and a second of lower jejunum and upper ileum were taken from each rat and incubated for 90 min in a Dubnoff shaker. The buffer was a Krebs-bicarbonate with glucose 3 mg/ml. Details of segment preparation and incubation appear elsewhere[11]. Alloxan was given by tail vein at a dose of 40 mg/kg after a 24 h fast, blood sugar determined one week later and rats with values above 300 mg% considered diabetic. All were sacrificed 13 or 14 days after alloxan. Insulin was administered in divided doses, half as PZI 18 h before sacrifice and the rest as Lente Iletin 1 h before sacrifice. Glucose in the serosal (absorbed) fluid was determined by the SOMOGYI method[12] and Na by flame photometry. Segments were dried to constant weight and absorption in μg/mg tissue dry weight determined.

Effects of insulin in normal animals appear in the Table. The term 'absorption' means net transfer from mucosal (outer) fluid to serosal fluid. The hormone caused a marked increase in glucose absorption and a small de-

Effects of insulin and alloxan diabetes on Na and glucose transport

Group	No. of segments	Glucose absorption (μg/mg/h)	Serosal fluid glucose concentration (mg/ml)	Na absorption (μg/mg/h)	Fluid absorption (mg/mg/h)
Normal	11	48 ± 6	7.4 ± 0.6	11.8 ± 1.1	4.3 ± 0.3
Normal insulin 0.75 μ	11	80 ± 5^a	11.3 ± 0.4^a	9.0 ± 1.1^a	4.2 ± 0.4
Normal	6	45 ± 8	7.9 ± 1.5	9.8 ± 2.3	3.5 ± 0.8
Diabetic	6	96 ± 5^a	10.8 ± 0.6^a	19.0 ± 2.1^a	7.0 ± 0.7^a
Diabetic	6	96 ± 8	9.6 ± 0.6	19.1 ± 1.5	7.9 ± 0.6
Diabetic insulin 0.75 μ	8	96 ± 9	11.4 ± 0.7^a	13.7 ± 1.1^a	6.2 ± 0.5^a
Diabetic	6	80 ± 6	11.1 ± 0.3	12.5 ± 1.6	5.2 ± 0.5
Diabetic insulin 2.50 μ	8	76 ± 7	12.5 ± 0.5^a	9.5 ± 0.6^a	4.0 ± 0.3^a

Mean $\pm$ S.E. $^a P < 0.05$.

[1] A. SOLS, S. VIDAL, and L. LARRALDS, Nature *161*, 932 (1948).
[2] A. SOLS, Rev. Espan. Fisiol. *7*, 1 (1951).
[3] M. LOURAU, Exper. *15*, 193 (1959).
[4] R. K. CRANE, Physiol. Rev. *40*, 789 (1960).
[5] F. PAULS and D. R. DRURY, Am. J. Physiol. *137*, 242 (1942).
[6] L. LASZT and H. VOGEL, Nature *157*, 551 (1946).
[7] R. K. CRANE, Biochem biophys. Res. Comm. *4*, 436 (1961).
[8] T. Z. CZÁKY and M. THALE, J. Physiol. (London) *151*, 59 (1960).
[9] I. BIHLER and R. K. CRANE, Biochim. biophys. Acta *59*, 78 (1962).
[10] S. G. SCHULTZ and R. Zalusky, J. gen. Physiol. *47*, 1043 (1964).
[11] K. A. AULSEBROOK, Endocrinology *68*, 1063 (1961).
[12] M. SOMOGYI, J. biol. Chem. *160*, 61 (1945).

crease in Na absorption. The high final serosal fluid glucose concentrations seen in the insulin treated rats indicate a marked increase in the sugar concentrating activity of the mucosal cells, a process which has also been taken to be a measure of sugar transport[13]. Since water accompanies Na movement from mucosal fluid to serosal fluid, the small intestine does not concentrate Na, and absorption is generally taken as the measure of Na transport[13]. Segments from alloxan-diabetic rats transported more glucose than non-diabetics, confirming the results of previous studies. In addition, Na transport was approximately doubled in the diabetic animals. The final series of experiments was concerned with the effect of insulin in alloxan-diabetes. Given first at the same dose level employed in normal rats (0.75 U/rat) the hormone depressed Na absorption and increased serosal fluid glucose concentration as was observed in normal animals, but failed to change glucose absorption. The same results were observed in a second group in which insulin was given in larger amounts (2.5 U/rat). The failure of insulin to increase sugar absorption in alloxan-diabetes may be related to decreased fluid absorption, an effect of the hormone seen in diabetic but not in normal rats.

The increases in sugar transport seen in both alloxan-diabetic and insulin treated normal rats appears contradictory. However, the increased glucose and Na transport in alloxan diabetes may have been the result of mucosal cell metabolic changes compensatory to the renal losses of glucose and Na characteristic of the diabetic state.

SCHULTZ has recently proposed a model system suggesting linked entry of Na and actively transported sugars into the mucosal cell in which Na and the sugar share a common carrier[10]. The complex dissociates inside the cell and Na is removed by a 'pump' located at the serosal surface of the cell. Sodium removal favors dissociation of the common carrier and the sugar probably moves out of the cell down its concentration gradient. Changes in alloxan-diabetes appear to fit this hypothesis of linked Na and glucose entry, since absorption of both is increased. However, it appears that insulin increased glucose transport by some other pathway, since Na absorption is decreased by the hormone. The decreased Na absorption produced by insulin may be accountable to depression of dissociation of the Na-carrier-glucose complex by high intracellular levels of sugar accumulated under the influence of the hormone, reducing the amount of Na available to the serosal 'pump'[14].

Zusammenfassung. Die Natrium- und Glucoseabsorption in Dünndarmsegmenten alloxandiabetischer Ratten war erhöht. Durch Insulininjektionen wurde bei diesen Tieren die Natriumabsorption herabgesetzt, während bei normalen Ratten Insulin die Glucoseabsorption erhöhte und die Natriumabsorption erniedrigte.

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[13] T. H. WILSON, *Intestinal Absorption* (W. B. Saunders Co., Philadelphia 1962).

[14] I thank Miss V. WEBB for technical assistance. Supported by NIH Grant AM-05025.

Potentiation of Bradykinin and Eledoisin by BPF (Bradykinin Potentiating Factor) from *Bothrops jararaca* Venom

As shown in previous papers from this laboratory[1-3], a factor called BPF (bradykinin potentiating factor), extracted from the venom of *Bothrops jararaca*, was capable of increasing some of the pharmacological activities of bradykinin on the guinea-pig ileum, rat uterus and rabbit duodenum, as well as the hypotensive effects of the polypeptides in cats and dogs. BPF has no effect upon contractions elicited by histamine and acetylcholine, and little effect on the actions of angiotensin and oxitocin on the smooth muscles of the guinea-pig ileum and the rat uterus respectively.

In this paper an analysis of the effects of BPF was extended to the polypeptide eledoisin, since it has been shown that this substance is more resistant to inactivation by plasma enzymes and considerably more active than bradykinin on the guinea-pig ileum and blood pressure of the cat and the dog[4-7].

(a) *Experiments on the guinea-pig ileum.* The deductions as to the potentiation of the agonists by BPF were taken after plotting the reciprocals of the effects (1/y) against the reciprocals of the doses (1/x) according to the technique described previously[8,9]. BPF produced a definite potentiating effect upon bradykinin (BRS 640, Sandoz) and kallidin (KL 698, Sandoz) responses without any apparent action on eledoisin (ELD 950, Sandoz) contractions. Figure 1 gives a view of the experimental data recorded from two typical experiments. We can see that bradykinin and kallidin lines tend to displace towards eledoisin lines during the treatment of the guinea-pig ileum with BPF. It must be noticed that, when the concentration of BPF reached 5 μg/ml, the sensitivity of the isolated preparation to bradykinin and eledoisin was almost the same. Concentrations of BPF up to 10 μg/ml did not consistently change the pattern of responses obtained with 5 μg/ml.

(b) *Arterial blood pressure experiments.* These experiments were performed on the arterial blood pressure of the dog and cat recorded from a common carotid artery by means of a mercury manometer. BPF potentiates the

[1] S. H. FERREIRA and M. ROCHA E SILVA, Ciência e Cultura *15*, 276 (1963).

[2] S. H. FERREIRA, Brit. J. Pharmacol., *24*, 163 (1965).

[3] S. H. FERREIRA, Thesis Fac. Med. Ribeirão Prêto (1964).

[4] F. OLMSTEDT and I. H. PAGE, Am. J. Physiol. *203*, 951 (1962).

[5] E. STÖRMER and B. BERDE, J. Pharm. exp. Therap. *140*, 349 (1963).

[6] V. ERSPAMER and A. GLAESSER, Brit. J. Pharmacol. *20*, 516 (1963).

[7] F. SICUTERI, F. FANCIULLACCI, G. FRANCHI, and S. MICHELACCI, Exper. *19*, 44 (1963).

[8] M. ROCHA E SILVA, Arch. int. Pharmacodyn. *118*, 74 (1959).

[9] M. ROCHA E SILVA and J. GARCIA LEME, Med. exp. *8*, 287 (1963).