corresponding areas on the electrophoretic strips are shown in Figure 2B.

The X-band was dialyzable and unstainable by bromphenol blue; therefore it is unlikely to be a protein complex. Furthermore, a collection of about 40 X-bands was pooled and extracted with normal saline. The extract was then tested by thin-layer chromatography[5]. The result showed that the unknown substance retained its aflatoxin B_1 character.

Whether or not the substances contained in these bands are involved in the in vivo transport mechanism for these mycotoxins remains to be studied[6].

Zusammenfassung. In vitro Experimente mit ^{14}C-markiertem, gereinigtem Aflatoxin zur Untersuchung der Bindung von Aflatoxin B_1 und G_1 an verschiedene Serumproteine ergaben, dass Aflatoxin B_1 hauptsächlich mit γ-Globulin, G_1 dagegen vorwiegend mit Albumin bindet.

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Effect of Vagal Denervation on Insulin Release after Oral and Intravenous Glucose

Vagal stimulation has been reported by several authors to elicit an increased release of immunoreactive insulin (IRI)[1–3], although negative findings have also been described[4]. In view of the rich autonomic innervation of the pancreatic islets[5–10], it appears possible that the insulin release, resulting from vagal stimulation, is caused by a direct effect on the β-cells. On the other hand, it is well established that denervation of the pancreas does not incapacitate the β-cells, which are still capable of producing and releasing insulin and of maintaining a normal blood sugar level[11,12]. Although so far the effects of vagal denervation on the mechanism of insulin release have not been extensively studied, some observations have been documented. FROHMANN et al.[3] reported that in the dog only a portion of the releasable insulin was under vagal control and that glucose-mediated insulin release was unaffected by vagotomy. It was concluded that the vagus has little if any effect on insulin secretion in response to glucose loading. However, NELSON et al.[4] found a decreased elimination rate of glucose after vagotomy in 11 out of 13 dogs. MILLER[12], on the other hand, recently observed that intragastric infusion of glucose to fasted, vagally denervated monkeys resulted in higher and earlier peaks in the blood sugar and IRI curves. No difference was observed between vagotomized and control monkeys after intraduodenal glucose infusion.

In the present study, the mechanism of insulin release was studied by assay of IRI in plasma after giving oral or intravenous glucose to control and vagally denervated rats (male Wistar rats, weighing 100–150 g). In the experimental group (18 rats) both vagal trunks were cut just below the diaphragm. At the same time a pyloroplasty was performed in order to prevent gastric dilation. Pyloroplasty alone was performed in the control group (18 rats). The animals were left to recuperate from the operation for a minimum of 2 weeks. Before receiving oral or intravenous glucose, the rats were housed in wire mesh cages and deprived of food but not water for 24 h. Glucose was given through an oro-gastric tube (1.5 g/kg body weight in a 10% solution) or injected as a single injection into a tail vein (1.5 g/kg body weight, given in a volume of 0.5 ml/100 g body weight). Serial blood samples (250 µl) were taken by the orbital bleeding

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Table I. Oral glucose load

Time (min)	Glucose mg/100ml		Immunoreactive insulin µU/ml	
	Pyloroplasty	Vagotomy + pyloroplasty	Pyloroplasty	Vagotomy + pyloroplasty
0	52 ± 2 (18)	43 ± 4 (18)	13 ± 5 (18)	17 ± 5 (18)
8	142 ± 7 (9)	115 ± 5 (10) [a]	62 ± 14 (9)	65 ± 14 (10)
15	126 ± 5 (18)	156 ± 6 (18) [b]	70 ± 11 (18)	65 ± 13 (18)
30	112 ± 6 (18)	157 ± 11 (17) [b]	34 ± 5 (17)	41 ± 8 (17)
60	91 ± 6 (18)	78 ± 5 (18)	25 ± 4 (18)	28 ± 2 (18)
120	70 ± 2 (9)	60 ± 5 (8)	27 ± 3 (9)	21 ± 10 (8)

Mean ± S.E.M. (*n*). [a] $0.01 > P > 0.001$. [b] $P < 0.001$.

Table II. Intravenous glucose load

Time (min)	Glucose mg/100 ml		Immunoreactive insulin μU/ml	
	Pyloroplasty	Vagotomy + pyloroplasty	Pyloroplasty	Vagotomy + pyloroplasty
0	40 ± 2 (18)	43 ± 3 (18)	4 ± 2 (18)	4 ± 1 (18)
3	303 ± 24 (16)	322 ± 24 (16)	178 ± 19 (16)	92 ± 13 (17)*
7	254 ± 21 (15)	278 ± 23 (17)	123 ± 19 (15)	101 ± 18 (17)
15	181 ± 17 (15)	207 ± 20 (17)	97 ± 27 (16)	55 ± 11 (17)

Mean ± S.E.M. (*n*). * $P < 0.001$.

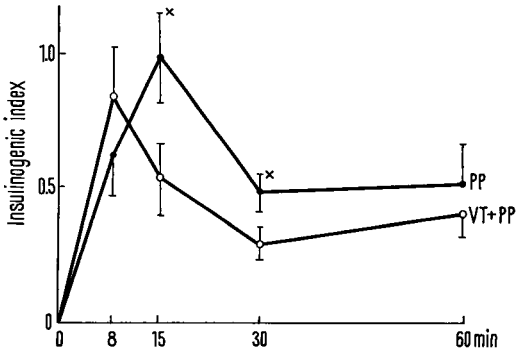

Fig. 1. Insulinogenic index after oral glucose load in rats with vagotomy and pyloroplasty (VT + PP) and pyloroplasty alone (PP). *, $0.05 > P > 0.01$.

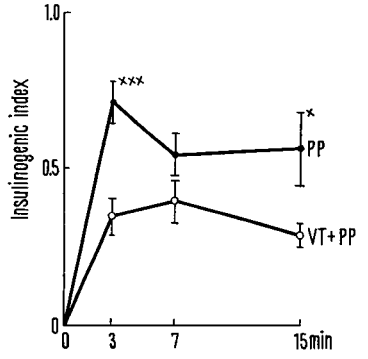

Fig. 2. Insulinogenic index after intravenous glucose load in rats with vagotomy and pyloroplasty (VT + PP) and pyloroplasty alone (PP). *, $0.05 > P > 0.01$; ***, $P < 0.001$.

technique, using commercially available constriction pipettes as previously described for mice[13]. No anaesthesia was used during the experiments. The sampling procedure requires no more than 10 sec and the rats were allowed to move around freely in their cages between samplings. Blood glucose was determined enzymatically by the method of MARKS[14]. Plasma insulin was determined by radioimmunoassay[15].

There was no difference between vagotomized and control rats in the fasting blood glucose or IRI levels. Oral glucose load resulted in a transient increase in the blood glucose level in the control rats. In the vagotomized rats the increase in the blood glucose level was slower and longerlasting (Table I). There was no difference in the absolute IRI levels between control and vagotomized rats during the experiment. However, if the insulinogenic index (Δ IRI/Δ glucose) was calculated according to SELTZER et al.[16], there was a moderate but statistically significant difference ($P < 0.05$) between vagotomized and control rats at 15 and 30 min after administration of glucose (Figure 1). The slow and more protracted elevation of the blood glucose level in the vagotomized group may be explained to some extent by disturbances in gastric emptying mechanisms[17]. The decreased insulinogenic index at 15 and 30 min, however, suggests an impaired glucose-mediated insulin secretory capacity, which may be masked at the 8 min value by the influence of insulinotropic gut hormones[18]. After intravenous glucose load, the increase in the plasma IRI was much less in the vagotomized rats ($P < 0.001$) than in the control rats (Table II). Accordingly, the insulinogenic index at 3 min after injection was markedly different ($P < 0.001$) in the 2 groups (Figure 2).

In conclusion, the finding of a higher insulinogenic index after oral glucose load in both control and vagotomized animals, as compared to the intravenous groups, supports the concept of an entero-insular axis as an important factor in insulin release[18,19]. The results also indicate that this mechanism is still operative after vagotomy, while the direct glucose-mediated insulin release is significantly impaired[20].

Zusammenfassung. Perorale Glykosebelastung ergab bei vagotomierten Ratten Erhöhung des Blutglykosespiegels. Nach intravenöser Glykosezufuhr wurden niedrigere Werte von immunoreaktivem Insulin im Serum gefunden. Ebenso war der insulinogene Index bedeutend niedriger sowohl nach peroraler als auch nach intravenöser Glykosezufuhr. Vagotomie dürfte somit die glykosebedingte Insulinfreisetzung reduzieren.

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