significantly from that of group I ($t = 2.6227$; $2P < 0.025$). Significant differences were also demonstrable on day 5.5 ($t = 3.0818$; $2P < 0.02$) and on day 6 ($t = 3.0066$; $2P < 0.02$) (Figure 1). No adjuvant activity was demonstrable when the simultaneous injection of SRBC and ET was given mice pretreated by 20 µg *S. marcescens* endotoxin daily for 9 days (group III). In the spleens of mice treated alone by an intravenous injection of 100 µg ET from *S. marcescens* (group IV) only a short-term multiplication of the relative numbers of direct PFC was detectable (Figure 1). The adjuvant effectiveness of ET from *S. marcescens* was observed again in the second series of experiments (Figure 2). But the development of both direct and indirect PFC was found to be significantly suppressed when 4×10^8 SRBC and 100 µg ET from *S. marcescens* were simultaneously injected into mice pretreated with relatively large amounts of ET from either *S. marcescens* (group III) or *S. typhi* (group IV) (Figure 2). Serological investigations resulted in similar findings.

The data presented here give evidence that pretreatment of mice with a constant dose of 20 µg ET per day during a period of 9 days inhibits the adjuvant activity of ET, whereas the normal hemolysin response was not found to be altered. A significantly diminished primary immune response was found, however, when pretreatment was performed with larger amounts of ET (Figure 2). In general it is accepted that the uptake of particulate antigens by macrophages is a necessary prerequisite for the initiation of the primary immune response[12]. Lipopolysaccharides persist for a long period in the body and may cause toxic damage of cells in blood and tissues[1]. Thus it could be suggested that the repeated administration of relatively large amounts of ET effects an exhaustion of the reticulo-endothelial system (RES) meaning that the immunosuppressive effect induced by ET would be mainly due to an injury of the afferent limb of the immune apparatus. This concept is supported by the observation that RES 'blockade' induced by injection of carbon effects a significant suppression of the development of hemolysin-producing spleen cells[12]. At first sight it appears to be less probable that the immunosuppressive effect of ET is mainly due to a loss of progenitor immunocytes. But it may be recalled that there are some signs for the pluripotential character of progenitor immunocytes, thus possibly being able to differentiate along some path other than the immunological one[13,14]. Taking such considerations into account, it is conceivable that the repeated injection of increasing amounts of bacterial lipopolysaccharides can lead to reduction in progenitor immunocytes, since the injection of ET is regularly followed by an enormous increase in white blood cells[1,6].

Zusammenfassung. Der Adjuvanseffekt von bakteriellem Endotoxin gegenüber Schaferythrocyten war nicht nachweisbar, wenn Mäuse vor der primären antigenen Stimulierung 9 Tage lang eine tägliche Injektion von 20 µg Endotoxin erhielten. Andererseits wurde unter diesen Bedingungen eine Primärreaktion gefunden, wie sie nach alleiniger Injektion des Erythrocytenantigens zur Ausbildung gelangt. Die Zahlen an 19S- und 7S-Antikörper bildenden Zellen sowie die Serumantikörpertiter waren jedoch signifikant vermindert, wenn vor Applikation der immunisierenden Injektion höhere Dosen von homologem oder heterologem Endotoxin gegeben worden waren.

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Binding of Aflatoxins B_1 and G_1 to Human Serum Proteins

Recently RAO et al.[1], using fluorescence technics, reported that aflatoxin B but not G was bound with plasma albumin when crude aflatoxins were incubated with human plasma. We have used ^{14}C-labelled pure aflatoxins to re-examine this problem, and have found that aflatoxin B_1 is bound mainly with γ-globulin, whereas aflatoxin G_1 is bound mainly with albumin.

Materials and methods. Pure aflatoxins B_1 and G_1 labelled with ^{14}C, specific activity 5.8 and 2.7 µC/µmole respectively, were prepared according to ADYE and MATELES[2]. Both 'normal clinical chemistry control serum' (Hyland, Division Travenol Laboratories, Los Angeles, Calif.) and serum collected from healthy individuals were used.

Individual 0.5 ml portions of serum were incubated with the ^{14}C-toxins (23.6 µg of B_1 and 15.8 µg of G_1) for 2 h at 37 °C, and 5 µl aliquots then subjected to paper electrophoresis as previously described[1]. Another 5 µl aliquot was mixed with BRAY's solution[3] and counted in a liquid scintillation counter. The remaining material in the tube was dialyzed through cellophane against 0.01 *M* phosphate buffer (pH 7) at 4 °C for 24 h. The undialyzed material was subjected to paper electrophoresis and liquid scintillation counting as above.

For toxin detections the electrophoretic strips were placed in contact with Sakura X-ray film in cassette, and the film developed after 60 days exposure. Unstained electrophoretograms were viewed under UV and the bands compared with those seen on stained strips. The electrophoretograms were dissected into 4 portions as shown in Figure 2B. The paper sections were counted in a liquid scintillation counter according to GEIGER and WRIGHT[4].

Results and discussion. Experiments were first carried out with plasma samples exactly by the procedure of RAO et al.[1] except that pure aflatoxin B_1 or G_1 was used. Since the results were different from those of RAO et al. the procedures used by these investigators were modified by using serum samples instead of plasma, pure

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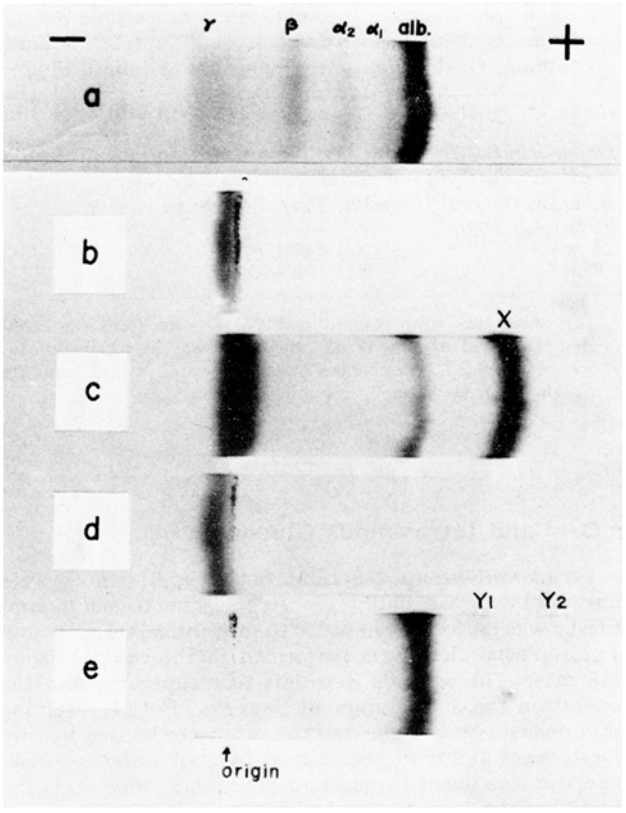


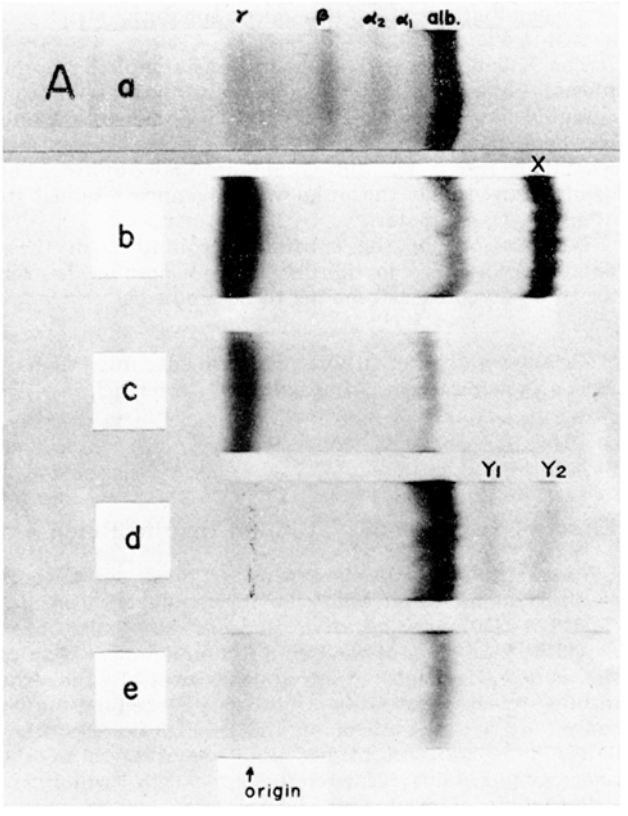
Fig. 1. Electrophoretic patterns of aflatoxins and aflatoxins incubated with normal human serum. a) Serum pattern stained with bromphenol blue; others are autoradiograms: b) aflatoxin B_1; c) aflatoxin B_1 + serum; d) aflatoxin G_1; e) aflatoxin G_1 + serum.

aflatoxins to facilitate the interpretation of results, ^{14}C labelling to increase the sensitivity of aflatoxin detection, and neutral phosphate buffer during dialysis instead of alkaline barbitone buffer to avoid deterioration of aflatoxins in aqueous solution[5].

Representative experiments demonstrating the electrophoretic patterns of aflatoxins and serum-bound aflatoxins are shown in Figure 1. Both aflatoxin B_1 and G_1 were retained at the origin with a very slight migration toward the cathode. Both were bound with albumin. After complete dialysis (Figures 2A and B), aflatoxin B_1 was shown to be bound mainly with γ-globulin fraction while aflatoxin G_1 bound mainly with albumin.

With aflatoxin B_1, a strong band (X) appeared about 1.8 cm away from the albumin toward the anode (Figure 1, strip c). The X-band could easily be seen under UV as a blue fluorescent band on the electrophoretic strip. With aflatoxin G_1, 2 very faint bands (Y_1 and Y_2) appeared beyond albumin (Figure 1, strip e). These weak bands usually could not be detected by UV.

After the aflatoxin-serum mixtures were dialyzed for 24 h, both X-band and Y_1 and Y_2 bands disappeared (Figure 2A). The results indicated that aflatoxins bound in these bands were dialyzable. The radioactivities of the



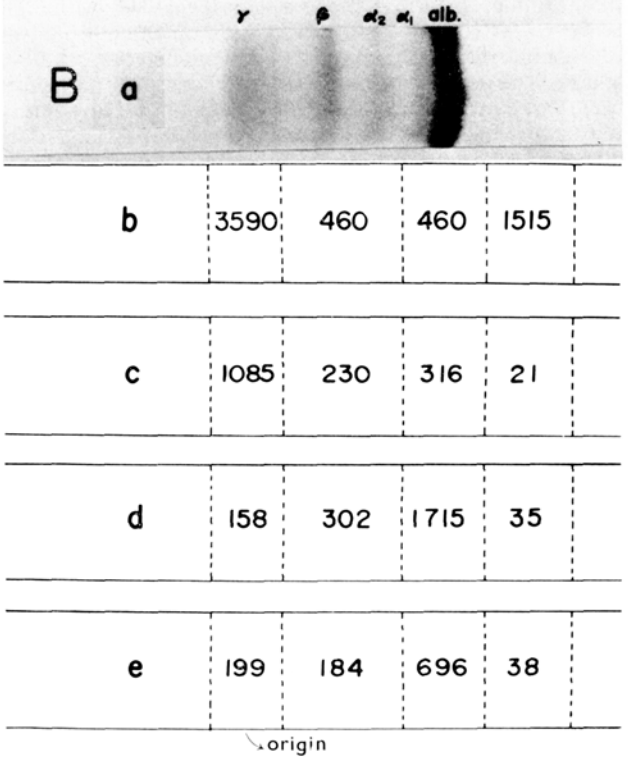


Fig. 2. Electrophoretic patterns of aflatoxins incubated with normal human serum before and after the mixtures were dialyzed for 24 h in 0.01 *M* phosphate buffer (pH 7) at 4 °C. A) Autoradiograms. B) Radioactivity, in dpm, of the corresponding areas of electrophoretograms. Each value represents the average from 3 samples. a) Serum pattern stained with bromphenol blue; b) aflatoxin B_1 + serum, without dialysis; c) aflatoxin B_1 + serum, with dialysis; d) aflatoxin G_1 + serum, without dialysis; e) aflatoxin G_1 + serum, with dialysis.

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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] $P < 0.001$.