Co., Inc., Milwaukee, Wisc., USA; methionine-CD_3, epinephrine-α-D_2-β-D_1, norepinephrine-α-D_2-β-D_1 and dopamine-β-D_2 were obtained from Merck, Sharp and Dohme, Kirkland, Quebec, Canada. OV-17, 3% on Anakrom Q, 90–100 mesh, from Analabs, North Haven, Conn., USA; trifluoracetic anhydride and pentafluoropropionic anhydride from Pierce Chemicals, Rockford, Ill., USA. Epinine-N-CD_3 was generously donated by Jansen Farmaceutica (Beerse, Belgium) and α-methyl dopamine was the gift of Dr J. Daly, NIAMD, NIH.

Results and discussion. Epinine in the superior cervical ganglia of rats was detected and identified by GC-MS. The mass spectra of the PFP derivatives of epinine and epinine-CD_3 are shown in figure 1. The amounts of epinine and other amines present in the homogenates of superior cervical ganglia were determined from the relative peak heights of the unlabelled compounds and the added deuterated standards at appropriate retention times for the gas chromatographic columns and m/e in the mass spectra. A typical tracing from the GC-MS recording, after injection of an aliquot of the derivatives derived from the homogenate of sympathetic ganglia to which deuterated standards had been added, is shown in figure 2.

Norepinephrine is the major catecholamine present in sympathetic ganglia (figure 3). The amount of norepinephrine present progressively increases with age as the ganglia and the animal grow in size. Dopamine levels are highest at birth, decline during the next few days, and then increase slightly as the ganglia become larger. The amounts of the N-methylated derivatives of these catecholamines are considerably lower. Epinephrine levels are highest at birth but its levels are maintained only slightly below those found at birth for at least 30 days. Thus the relative amount of epinephrine compared to norepinephrine and ganglia size decreases with age, from 10% of norepinephrine at birth to less than 1% at 30 days of age.

Epinine, the N-methylated derivative of dopamine, is present in very small amounts. Its level is highest at birth, at which time its levels are about half those of epinephrine, and rapidly declines during the first 4 days of life. The amount of epinephrine present is usually less than one-tenth those of dopamine (figure 3). 20 min after i.p. injection of methionine labelled with deuterium on the methyl group (CD_3), labelled epinephrine and epinine are found in the superior cervical ganglia. The relative enrichment of epinephrine with CD_3 approaches that of methionine found in the carcass, but that of epinine is only 4% of epinephrine (table).

The results of these experiments establish that epinine is present in the superior cervical ganglia and can be formed in vivo. The amounts of epinine present, however, are very small, and even at their highest are less than half those of epinephrine. The relatively low enrichment with CD_3 after injection of methionine-CD_3 indicates that the rate of epinine formation in the ganglia is very slow compared to that of epinephrine and that formation of epinephrine from epinine is at most a minor pathway.

Relative enrichment with CD_3 of epinine and epinephrine after administration of methionine-CD_3

	Relative enrichment
Methionine (carcass)	26 ± 3%
Epinephrine (SCG)	19 ± 4.8
Epinine (SCG)	0.74 ± 0.12

Newborn rats received 10 μg/g methionine-CD_3 i.p. The rats were killed after 20 min. The relative enrichments with CD_3 of the indicated compounds were determined by GC-MS.

In vitro binding of citrinin to serum protein[1]

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Summary. In vitro study shows that the mycotoxin citrinin binds to human serum albumin.

The binding of toxins to plasma proteins and subsequent biological activity has been observed earlier[3]. Such observations have been made with the food-borne mycotoxins, aflatoxin and ochratoxin[4–6]. The present communication is a report on the binding of the mycotoxin citrinin to human serum protein.

Material and method. Pure crystalline citrinin (isolated from cultures of Aspergillus candidus or Penicillium citrinum) was used in this study. Human serum was centrifuged out from pooled blood samples of healthy adult volunteers and used fresh. 3 different preparations were then obtained: a) 1 ml of serum was dialysed at 4–7 °C overnight against 1 l of barbitone-sodium barbitone buffer (ionic strength –0.05; pH 8.6) and the dialysed serum was ready for electrophoresis; b) 0.2 ml of chloroform solution of citrinin (200 μg) was taken in a small beaker and dried. To the dry toxin, 1 ml of serum was added and incubated at 37 °C for 2 h with intermittent gentle agitation. This was then dialysed and kept ready for electrophoresis; c) a solution of citrinin was prepared in barbitone buffer (200 μg/ml) just before electrophoretic run. Paper electrophoresis was carried out on a strip (10 × 30 cm) of Whatman No. 3 paper. 10 μl each of a), b) and c) was spotted separately but alongside and placed for electrophoretic run in barbitone buffer at 180 V and 5 mA for $3^1/_2$ h at room temperature (29–31 °C).

1 Part of Ph. D. thesis, University of Madras, 1973.

2 Acknowledgment is due to Dr E. R. B. Shanmugasundaram (University of Madras) and Dr J. Jayaraman (Madurai University) for kind guidance.

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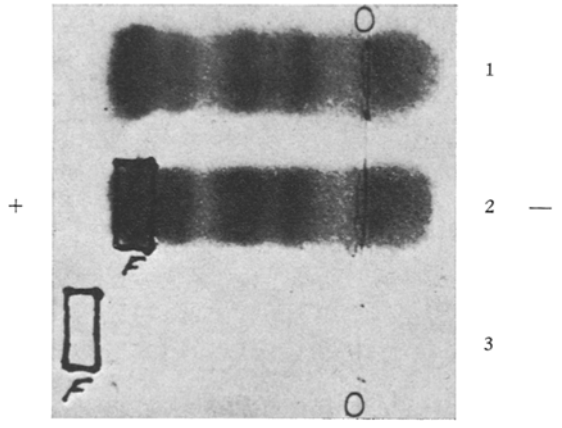
After the run, the paper was air dried and moistened with 5% acetic acid. The wet strip was then viewed under UV light for green-yellow fluorescence (characteristic of citrinin). The fluorescing portions were marked F. The strip was then stained for proteins overnight using bromophenol blue. 5% acetic acid was employed for destaining.



1, Human serum (dialyzed); *2*, Human serum–citrinin mixture (dialyzed); *3*, Citrinin; *F*, Fluorescence portions (marked before staining).

Results and discussion. From the figure it can be seen that a fluorescing portion was present in the electrophoretic path of human serum-citrinin mixture, and this corresponded with the albumin band, when stained. The only other fluorescence marked was that of pure citrinin (run alongside) and this had moved towards the anode faster than the adjacent albumin fraction of serum. These observations suggest that a) citrinin is capable of binding to at least one of the human serum proteins-albumin, and b) the binding of citrinin to albumin is fairly stable because the complex was not affected by dialysis. In a separate experiment it was shown that undialyzed human serum-citrinin mixture exhibited 2 fluorescent spots, one corresponding to free citrinin and the other to that of albumin portion. All the above observations could be noted again when agar gel was employed for electrophoresis. Moreover, studies with ^{14}C-citrinin confirmed the binding of citrinin to serum albumin.

It is felt that the observed in vitro binding of citrinin to serum protein fraction may help in understanding the possible transport mechanism of toxic citrinin. Similar binding studies with cellular components will throw light on the possible mode of action of citrinin with reference to its toxicity in animals and plants[7–9].

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Hemoglobin binding capacity of heat incubated sera of different haptoglobin subtypes

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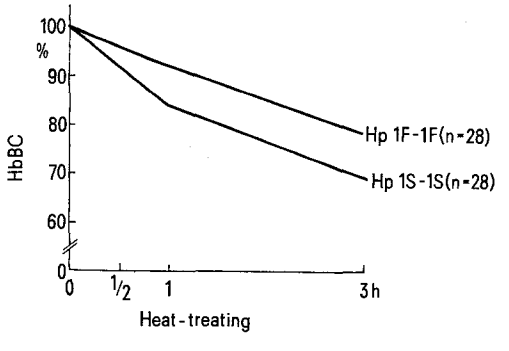
Summary. Sera of haptoglobin subtypes Hp 1F-1F and Hp 1S-1S incubated at 56 °C show a different degree of reduction in the hemoglobin binding capacity. The difference is small but significant.

Earlier it was demonstrated that in serum incubated at 56 °C the hemoglobin binding capacity (HbBC) is reduced and the extent of reduction varies with the Hp phenotype in the order of decreasing Hp 1-1 < Hp 2-1 < Hp 2-2[1]. The duration of the heat incubation also influences the extent of reduction in the HbBC[2]. Agar-gel electrophoretic investigations proved that a smaller quantity of Hp-Hb complexes are formed in heat-treated serum and this quantity depends on the phenotype[3]. On the basis of examinations of complexes formed with sera of the phenotype Hp 1-1, one may conclude that even within these phenotypes there is an additional variation among the individual sera[4]. These findings suggested different heat-stability for sera of different Hp subtypes.



Change of the HbBC mean value of sera heat-treated at 56 °C as compared in percent to their not-treated controls.

Materials and methods. 28 sera of Hp 1F-1F and Hp 1S-1S subtypes were analyzed. Subtypifying was carried out according Pastewka et al.[5]. Photometric measurement of the peroxidase activity of the complex formed: the sera were divided into 4 parts. One remained untreated, this represented the native control sample. The other 3 were kept for half, 1 and 3 h respectively in water bath at 56 °C. The placing of samples into the water bath was carried out in such a manner that heat-incubation of all samples was finished at the same time. Following this,

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