that most CPZ effects are related to cell-surface alterations[20], thus providing an explanation for the observed inhibition of concanavalin A-induced lymphocyte blastogenesis[21] and generation of cytotoxic lymphocytes in mixed lymphocyte culture[22] by CPZ. This latter drug and haloperidol were postulated to decrease DNA synthesis in cultured lymphocytes by the same mechanism[23]. Furthermore, the CPZ-induced decrease of calcium movements[24] may explain the observed depression of ear thickness at 4 h since previous workers have demonstrated the involvement of mast cell degranulation in the early events of contact hypersensitivity to picryl chloride in the mouse[24]. A decrease of the nonspecific irritative properties of picryl chloride is probably not involved, as suggested by the comparison of nonsensitized, nontreated and CPZ-treated mice. While antihistaminic properties deserve attention with respect to delayed hypersensitivity reactions[25] depressant effects of HPD throw more emphasis on the role of stress[26].

Our data suggest that major tranquillizers are able to depress in vivo cell-mediated immunity, the consequences of which deserve further investigation with respect to psychiatric diseases and their pharmacological management.

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Enzyme-linked immunosorbent assay to detect anti-sea nettle venom antibodies

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Summary. A micro enzyme-linked immunosorbent assay has been developed to detect serum antibodies to sea nettle venom. This sensitive assay is useful for monitoring specific immunological response in envenomated patients or in immunized animals.

The sea nettle *(Chrysaora quinquecirrha)* injects a nematocyst venom into the skin of bathers who contact its long tentacles. The sting of this coelenterate results in painful cutaneous lesions, local muscle cramps and corneal ulcerations[3]. Both repeatedly stung individuals and challenged animals possessed elevated levels of anti-sea nettle venom serum antibodies[4,5].

During the last decade, investigations in this laboratory on the sea nettle have revealed the presence of degradative enzymes and pharmacologically active components within its nematocyst venom[3]. In this paper the details of a sensitive enzyme-linked immunosorbent assay (ELISA) are reported.

Materials and methods. All inorganic and organic chemicals were reagent grade quality or better. Alkaline phosphatase (type VII), p-nitrophenylphosphate and bovine serum albumin (BSA, RIA grade) were obtained from Sigma Chemical Co. (St. Louis, MO). Goat anti-human IgG and IgM were products of Miles Laboratories, Inc. (Elkhart, IN). Goat anti-guinea-pig IgG (heavy and light chains) were obtained from Cappel Laboratories, Inc. (Cochranville, PA). Guinea-pigs were supplied by Rockland Farms (Gilbertville, PA).

Preparation of the antigen. Tentacles were removed from live *C. quinquecirpha* and nematocyst venom (SU) (25 mg protein/ml; 1 mouse i.v. $LD_{50}=0.06$ ml) was prepared as previously described[6,7]. For immunization of guinea-pigs, the crude venom, free of other tissue components, was coupled with starch as follows: 10 g of soluble starch (in 10 ml of 2 M sodium carbonate) and 4 g of cyanogen bromide (in 4 ml of acetonitrile) were allowed to react for 3 min in an ice bath. The reaction was stopped with 20 ml of cold coupling buffer (0.1 M sodium bicarbonate, 1 M sodium chloride, pH 8.5). The mixture was then shaken for 5 min and the slurry filtered on an Amicon filter (XM 50) to remove buffer and solvents. The activated starch 'cake' was resuspended in an equal volume of coupling buffer and 0.6 ml of SU was added. After stirring overnight (16 h) at

Venom specific IgG and IgM levels in the sera of a control and a jellyfish envenomated patient

Days following envenomation to sera collection	Antibody titer* IgG	IgM
2	100	400
30	200	150
365	3200	100
1095	50	10
Control (never stung)	10	10

* Antibody titers were determined by the ELISA technique described in 'materials and method' using anti-human IgG and IgM labelled with alkaline phosphatase as conjugate.

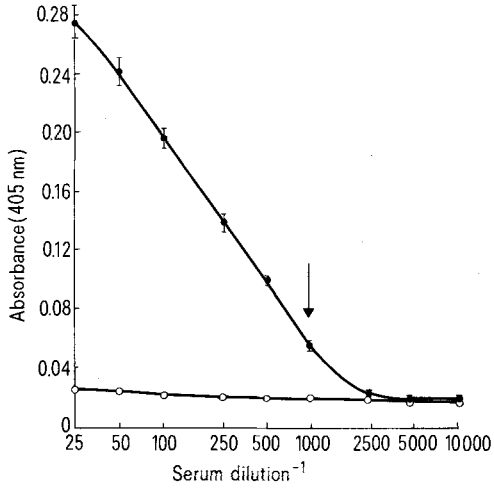

Figure 1. ELISA titration of guinea-pig antiserum produced against sea nettle venom. Titrations were performed as described in 'materials and methods'. Symbols are defined as follows: ●, guinea-pig anti sea nettle venom antiserum; ○, control guinea-pig (no sea nettle conjugate) serum; ┬, ELISA titer. The brackets indicate standard deviation.

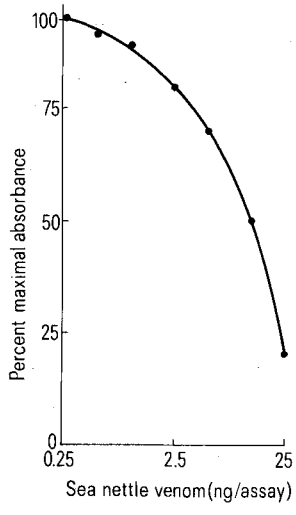

Figure 2. Competitive assay to detect sensitivity of the ELISA. 25 µl of 1:200 dilution of hyperimmune guinea-pig sera (same as in fig. 1) was incubated with 25 µl of different dilutions of the sea nettle venom on a polystyrene plate that was previously treated with the venom (25 µg/well). The concentrations causing 50% inhibition in absorbance was 12.5 ng.

4 °C, the reaction mixture was again filtered and the amount of venom bound to activated starch was determined by subtracting the amount of protein in the filtrate from the total amount of protein. The venom-starch conjugate was resuspended in saline to give a final concentration of 10 mg protein per ml.

Immunization of guinea-pigs. 3 guinea-pigs were immunized against crude sea nettle venom by 8 weekly s.c. injections of 0.45 ml of the antigen. The guinea-pigs were bled via cardiac puncture and their sera were tested by ELISA to detect anti-sea nettle venom antibody. Serum of a guinea-pig which did not receive any sea nettle venom injections was included as a control.

ELISA. Wells of polystyrene microtiter plates (Limbro No. 76-301-05) were coated with BSA and glutaraldehyde as described by Saunders[8]. Sea nettle venom was diluted in 0.1 M phosphate buffered saline (PBS), pH 7.4 and 50-µl aliquots containing 25 µg venom were added to each well. The plates were air dried under a current of forced air at room temperature. The plates were washed 3 times with 200 µl of PBS containing 0.05% Tween 20 (PBS-Tween) before use. For antibody titration, serial dilutions of antiserum were prepared in 0.01 M phosphate buffer (pH 7.4) and 50-µl aliquots were added to microplate wells that were previously treated with the venom. The plates were incubated for 2 h at 37 °C, washed 3 times in PBS-Tween, and 50 µl of 1:100 dilution of the conjugate (alkaline phosphatase labelled anti-species-immunoglobulin prepared by the method of Voller et al.[9] was added to each well. The plates were again incubated for 1 h at 37 °C, washed 3 times in PBS-Tween, and reacted for 15 min with 100 µl of p-nitrophenylphosphate substrate solution[9]. The reaction was terminated by the addition of 25 µl of 3 M sodium hydroxide. Absorbance at 405 nm was determined in a Titertek Multiskan Microplate Reader (Flow Laboratories, Inc.). The ELISA titer was defined as the serum dilution which gave a higher absorbance than the control serum in the 1st dilution.

To determine the sensitivity of the ELISA, 25 µl of SU diluted in 0.1 M PBS was added to a microplate to which 25 µg sea nettle venom per well had been air dried as described above. This was followed by immediate addition of 25 µl of 1:200 dilution of hyperimmune guinea-pig sera. The plate was incubated, washed and treated with the conjugate as described above.

Envenomated human sera. During the summer of 1977, a healthy 8-year-old white boy was stung by a sea nettle in the Chesapeake Bay. His sera was collected 2, 30, 365 and 1095 days after envenomation and tested by ELISA for levels of anti-sea nettle venom IgG and IgM antibodies. A healthy, white adult male, who had never been stung by a sea nettle, served as the control.

Results and discussion. Antibodies specific to sea nettle venom were obtained from all 3 guinea-pigs that were immunized with the starch-venom conjugate. A typical ELISA antibody titration curve is shown in figure 1. Only a low degree of non-specific binding was observed with the control guinea-pig sera suggesting that the antibody bound the venom in a highly specific manner.

Figure 2 shows the sensitivity of this ELISA to the sea nettle venom. Competitive assays, as performed by co-incubation of different venom dilutions and hyperimmune guinea-pig sera on venom coated plates, showed that 12.5 ng of the venom caused 50% inhibition of antibody binding to the plate.

This ELISA test was also suitable for titration of venom specific IgG and IgM antibodies in an envenomated patient. The patient had high IgM for a brief period followed by high IgG that persisted for about a year after envenomation (table).

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N-Nitrosospermidine: the principal nitrosation product of spermidine[1]

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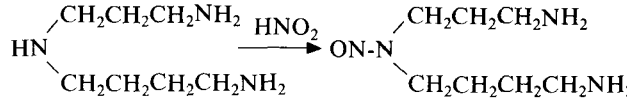
Summary. N-(4-Aminobutyl)-N-(3-aminopropyl)nitrosamine (N-nitrosospermidine) was identified as a major product in the reaction between spermidine and nitrous acid. N-Nitrosospermidine was not significantly mutagenic in the Ames assay.

The carcinogenic N-nitroso compounds[2] have not only been detected in foods, but demonstrated to be formed from nitrite and N-alkyl compounds in vitro with human gastric juice, as well as in vivo in animals[3]. A large number of N-alkyl compounds, which yield N-nitroso compounds on reaction with nitrous acid, are known to be present in foodstuffs[4]. Among them, polyamines, spermidine and spermine, have secondary amino groups, and are normal constituents of various kinds of foods[5]. The investigation of nitrosation products of polyamines has been limited to the identification of nitrosamines produced by the reaction of polyamines with a large excess of nitrous acid[6]; however, the studies on the reaction of polyamines with a limited amount of nitrous acid seems important for the evaluation of the possible biohazards caused by the N-nitroso compounds derived from polyamines. In this paper, the results of identification and mutagenicity studies on a major N-nitroso compound formed by the reaction of spermidine with a limited amount of nitrite are presented.

At pH 3.5, 50 mM [tetramethylene-1,4-^{14}C]-spermidine (New England Nuclear, Boston, Mass.) was reacted with 65 mM sodium nitrite for 16 h at room temperature. The major N-nitroso compound formed by the reaction was isolated from the reaction mixture by ion-exchange chromatography (SP-Sephadex, linear gradient elution with pyridinium acetate buffer, pH 5.0, from 0.1 M to 1.5 M), followed by cellulose column chromatography (isopropylalcohol - acetic acid - water, 4:1:1). The product, a diamino compound as judged by the elution position in the ion-exchange chromatography, was homogeneous in thin-layer chromatography (table 1). The overall yield of the N-nitroso compound was 22.5%, which was calculated from the radioactivity recovered in the purified product.

The absorption spectrum of the product [$\lambda_{max}^{H_2O}$ nm (ε): 232 (7100), 343 (87)] agreed closely with those of dialkylnitrosamines[7]. Removal of the nitroso group from the nitroso compound by treatment with hydrogen bromide in glacial acetic acid[8] yielded spermidine as the sole product (table 1). From these results, the structure of the nitroso compound was identified as N-(4-aminobutyl)-N-(3-aminopropyl)nitrosamine (N-nitrosospermidine).



Spermidine N-Nitrosospermidine

The content of N-nitrosospermidine in the reaction mixture described above was found to be 27% of the starting material by use of the reversed isotope dilution method. Since 40% of the spermidine used for the reaction was recovered unchanged from the reaction mixture, the yield of N-nitrosospermidine corrected for unchanged spermidine was 45%. Therefore, N-nitrosospermidine is the major product of the initial reaction between spermidine and nitrous acid.

At pH 3-4, 100 mM spermidine and 120 mM sodium nitrite were allowed to react for 16 h at room temperature. A 200-µl aliquot of the reaction mixture, which was free from nitrite, was assayed for bacterial mutagenicity as described by T. Yahagi et al.[9] using *Salmonella typhimurium* TA1535, TA1537, TA98 and TA100 as the tester strains. In the absence of 9000×g supernatant of rat liver homogenate

Table 1. Thin-layer chromatography of spermidine and its derivatives

	R_f-values i-PrOH–conc. HCl–H_2O (8:3:2)	i-PrOH–acetic acid–H_2O (4:1:1)	n-BuOH–conc. HCl–H_2O (7:2:1)
N-Nitrosospermidine	0.43	0.25	0.19
N-Nitrosospermidine treated with HBr	0.23	0.08	0.08
Spermidine	0.23	0.07	0.09

Samples were chromatographed on thin-layer plates of microcrystalline cellulose (Avicell SF, Funakoshi Pharm. Co. Ltd., Tokyo). Radioactive spots were located by autoradiography and amine spots by ninhydrin spray.