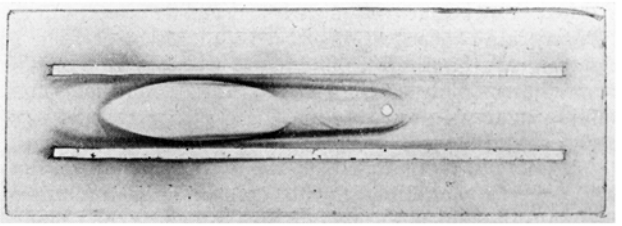

Immune electrophoresis of *Naja naja* venom (30 µg, in the well). Troughs: 2 specimen of rabbit anti-*Naja-naja* serum, raised with glutardialdehyde-treated venom. Cathode to the left.

at i.p. injection was 0.24 µg/g mouse, determined in groups of 6 mice (25–30 g) per dose. Samples of active venom solution were incubated with increasing amounts of antiserum for 60 min at 37 °C .The toxicity of these mixtures was again assayed in mice (25–30 g). 0.005 ml antiserum was sufficient entirely to detoxify one LD_{50}, i.e. 1 ml neutralized about 200 LD_{50} of venom = 1400 µg.

A group of 5 rabbits (2.5 kg) was immunized by 2 initial doses of 20 mg each of cross-linked venom applied on 2 successive days. One animal died after the second injection. The rabbits were boosted 3 times with the same dose of 20 mg. After an immunization period of 2 months, the antisera of these animals developed precipitation lines in agar immune electrophoresis against the components of whole cobra venom (Figure, lower trough) as well as against purified DLF and phospholipase A. After an additional booster dose of 20 mg 1 ml antiserum of 1 rabbit neutralized 167 LD_{50}.

These experiments demonstrate that cobra venom is largely detoxified by the treatment with glutardialdehyde. Nevertheless immunogenicity is preserved or even increased in the case of the low molecular weight components, as shown by antibody production against DLF and protection from lethal effects.

The treatment of antigen for immunization used here should be applicable to other venoms with the same success. This has been suggested also by HABEEB and HIRAMOTO[3].

Zusammenfassung. Durch Behandlung mit Glutardialdehyd wird *Naja-naja*-Gift weitgehend entgiftet. Einzeldosen bis zu 20 mg pro Kaninchen können ohne Nebenwirkungen injiziert werden. Durch Immunisierung von Kaninchen wird eine gute Antikörperbildung gegen *Naja-naja*-Gift – und vor allem auch gegen die niedermolekularen Toxine – erzielt.

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The Effect of Several Diuretics on Adenosine-Diphosphate Induced Platelet Aggregation in vitro

The formation of blood platelets aggregates on an injured intimal surface is now generally accepted to be the initial phase in the hemostatic response and thrombosis formation[1]. Furthermore, GAARDER et al.[2] have suggested that the release of intrinsic platelet adenosine diphosphate (ADP) plays a major role in the formation of platelet aggregates.

Because sulfhydryl inhibitors such as N-ethyl maleimide and *p*-chloromercuribenzoic acid (PCMB) can prevent ADP-induced platelet agglutination[3], MUSTARD and PACKHAM[4] have suggested that the platelet surface contains sulfhydryl groups which are involved in the platelet aggregation reaction. Several authors[5–7] have reported that the diuretics meralluride (Dilurgen, Mercardan, Mercuhydrin, Mercuretin), mersalyl (Salyrgan, Salurin, Mersalin, Mercusal) and ethacrynic acid (Edecil, Hydromedin, Edecrin, Endecril) inhibit the activity of a number of enzymes by binding to their sulfhydryl groups. The possibility therefore exists that these diuretics, by virtue of their thiol group binding activity, would also inhibit platelet aggregation. The purpose of this investigation was a) to study the effect of these drugs on in vitro platelet aggregation induced by ADP and b) to compare the effects on platelets obtained from guinea-pig and rat.

Materials and methods. Blood was collected from female rats and guinea-pigs by cardiac puncture into siliconized glass tubes containing 3.8% sodium citrate in a final ratio of 9:1 (blood/citrate). Platelet-rich-plasma (PRP) was obtained by centrifugation at 31 × *g* for 5 min at room temperature and platelet counts were determined on a Coulter Counter (Model B) according to the method of BULL et al.[8]. PRP was diluted accordingly with modified Tyrode's solution (pH 7.4) consisting of 9 g NaCl, 0.2 g KCl, 1 g $NaHCO_3$, 1 g dextrose and 0.05 g NaH_2PO_4 per liter of solution.

PRP was ppreincubated at room temerature in the presence of the test drug. Selection of this temperature was based upon the recent report that platelet viability and integrity is best maintained when stored at 22 °C[9].

Platelet aggregation was studied at 37 °C by a modification of the turbidometric method of BORN[10] in which the changes in optical density (at 600 nm) of PRP are monitored during aggregation using a Beckman DBG spectrophotometer connected to a recorder. Continous agitation of the cuvette contents was provided by a mixer mounted directly above the cuvettes on a removable sample com-

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Test compound[a]	Effect on platelet aggregation induced by	
	$9.1 \times 10^{-7} M$ ADP in the rat	$1.8 \times 10^{-7} M$ ADP in the guinea-pig
Ethacrynic Acid	O[b]	O
Meralluride	I[c]	I
Mersalyl	I	I
p-Chloromercuribenzoic acid	I	I
Methylmercuric chloride	S[d]	S

[a] Platelet rich plasma was preincubated for 20 min in the presence of $7 \times 10^{-3} M$ ethacrynic acid, $2 \times 10^{-3} M$ meralluride, $1 \times 10^{-3} M$ mersalyl, $2.5 \times 10^{-4} M$ *p*-chloromercuribenzoic acid and $1 \times 10^{-4} M$ methylmercuric chloride. Spectrophotometer determinations were carried out at a platelet dilution corresponding to 300,000 platelets/mm³ for rat and 150,000 platelets/mm³ for guinea-pig. [b] O, no effect. [c] I, complete inhibition. [d] S, caused aggregation in the absence of ADP.

partment cover. A description of this device is in press[11]; briefly it consists of 4 replaceable glass rods, 2 per cuvette, positioned so that the light beam can pass between them, a solenoid which oscilates the glass rods at 60 c/sec and a powerstat which controls the amplitude of the oscillations.

Compounds were obtained from the following sources: Adenosine diphosphate and *p*-chloromercuribenzoic acid (Mann), mersalyl (Sigma), ethacrynic acid (Merck Sharp and Dohme), meralluride (Lakeside Lab.) and methylmercuric chloride (Alpha Inorganics).

Results and discussion. The Table shows the effect of the test compounds on ADP-induced platelet aggregation in both rat and guinea-pig. PCMB and methylmercuric chloride were included as reference compounds because their reactions with sulfhydryl groups and effects on platelet aggregation have been previously investigated in other species[4]. The presence of meralluride and mersalyl resulted in complete inhibition of ADP-induced aggregation, while ethacrynic acid, the other diuretic possessing sulhydryl binding activity, had no effect. The stimulatory effect observed with $1 \times 10^{-4} M$ and $1 \times 10^{-3} M$ (not illustrated) methylmercuric chloride is of interest in view of the report of ROBINSON et al.[12] that complete inhibition of canine platelet aggregation was observed with methylmercuric nitrate concentrations of $6.3 \times 10^{-5} M$ or greater. It thus appears that inhibition of platelet aggregation cannot be predicted solely on the basis of organic molecules containing mercury or possessing the capacity to bind sulfhydryl groups.

Commercial preparations of the organic mercurial diuretics, mersalyl and meralluride, contain large amounts of theophylline added to prevent breakdown of the organomercuric complex. This compound has been shown to inhibit the c-AMP phosphodiesterase enzyme[13], thus effectively maintaining high levels of c-AMP in the platelets. Since high platelet levels of c-AMP are associated with inhibition of aggregation[14], the possibility must be considered that the presence of theophylline in these preparations would potentiate the platelet aggregate inhibiting effect of these mercurial diuretics. Experiments are now in progress to determine whether these diuretics also affect the synthesizing enzyme (adenyl cyclase) or the degrading enzyme (c-AMP phosphodiesterase) involved in the regulation of c-AMP levels in platelets.

Résumé. L'agrégation des plaquettes, induite par l'ADP exogène, fut observée par turbidimétrie en mesurant les variations de la densité optique. Le méralluride, le méersalyl et le PCMB préincubés avec les plaquettes de rat et de cobaye préviennent l'agrégation, tandis que l'acide éthacrynique n'a aucun effet. Par contre, le chlorire méthylique de mercure la provoque en l'absence d'ADP.

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Inhibition of Lymphocyte Transformation by Guanidinosuccinic Acid, a Surplus Metabolite in Uremia

When patients with renal failure become uremic, cellular immunity is suppressed and homograft rejection is delayed[1,2]. This alteration in immunity probably is related to an abnormality of lymphocyte function. Although washed lymphocytes from uremic guinea-pigs transform normally under stimulation from phytohemagglutinin (PHA)[3], several investigators have observed that blood plasma from uremic patients inhibits the transformation of lymphocytes to lymphoblasts in vitro[4,5]. Recently a dialyzable uremic toxin has been suggested to explain inhibition of PHA-stimulated lymphocyte transformation[6]. Because the lymphocyte plays an important role in cellular immunity, we are presently attempting to identify dialyzable uremic toxins which might alter immunity by inhibiting lymphocyte transformation.

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