with ^{51}Cr by incubation for 24 h with $Na_2{}^{51}CrO_4$ (1 μCi/10^6 cells/ml).

Mitotic activation of fresh blood lymphocytes (10^6/ml in 20% pooled human serum gelatin/Eagles MEM) incubated with X-irradiated LCL cells (added to give a final concentration of 10^5 cells/ml soon after X-irradiation) was assessed by the incorporation of thymidine-[methyl-^{3}H] 0.5 μCi; 150 mCi/mmole) into the trichloroacetic acid-insoluble fraction over the interval day 6–7. Details of the methods are to be found in refs. 4 and 12. Suitable controls lacking X-irradiated LCL cells or fresh lymphocytes were treated identically.

The cytotoxic capacity of the activated blood lymphocytes was assessed by the chromium release technique. Control cultures contained donor red cells and either blood lymphocytes of the donor or the X-irradiated LCL cells used as stimulant but not both[5]. In each experiment the response of blood lymphocytes from an unrelated donor as well as from the original LCL donor were compared. As a further control (to cover any possible change induced by X-irradiation) X-irradiated blood lymphocytes were cultured with autochthonous or allogeneic blood lymphocytes.

Stimulation (enhanced ^{3}H-thymidine incorporation) induced by autochthonous LCL cells occurred with the lymphocytes from all six patients studied. The activated lymphocytes were cytotoxic to ^{51}Cr-labelled autochthonous and allogeneic cells. The results of two typical experiments are shown in Tables I and II. The stimulation and cytotoxicity of the lymphocytes in the autochthonous situation was not a result of changes induced by the X-irradiation since X-irradiated blood lymphocytes did not stimulate autochthonous lymphocytes nor was it due to foetal calf serum antigens in the culture fluid since human serum was used as the serum supplement in all experiments. Moreover, GREEN and SELL[9] have reported that foetal calf serum antigens were not important in their autochthonous stimulations.

It may be concluded that neo-antigens on the LCL cells initiate a lymphocyte stimulation, in vitro, resulting in the death of the LCL cells. The fact that allogenic as well as autochthonous LCL cells can be killed by lymphocytes stimulated with autochthonous LCL cells suggests that either the killing phase is of limited specificity or that there are cross reacting antigens shared by the various LCL cells. These points are currently under investigation.

If it is accepted that 'deviant' lymphocytes, similar to LCL cells, may arise in vivo as a result of viral transformation or other cause, the in vitro phenomena described must closely mimic an immunological surveillance mechanism operative in the host.

Résumé. Les petits lymphocytes du sang, après avoir été mis en contact pendant plusieurs jours avec les cellules lymphoïdes d'une lignée autochthone, peuvent tuer les cellules de cette lignée et d'autres lignées. Ces investigations peuvent apporter de nouvelles données sur l'opération de contrôle immunologique.

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Immunization against Cobra Venom

Active immunization of experimental animals against snake venoms is hampered by two features: first, the toxicity precludes application of high initial doses; second, the main toxic principles, against which it is imperative to obtain high antibody titres, are poor antigens because of their low molecular weight[1].

By cross-linking cobra venom with glutardialdehyde we have been able to circumvent both these difficulties. The toxicity was profoundly reduced, whereas good antibody formation against the toxins was observed. It seems possible to use this procedure for the routine immunization of antiserum-yielding animals, and it may even be adaptable to the active immunization of humans.

Naja naja venom was treated in the following manner. A 2% toxin solution in 0.1 *M* phosphate buffer, pH 6.8, was prepared and 1% glutardialdehyde solution (0.01 ml/mg venom) was added under constant stirring. The mixture was allowed to react for 2 h at room temperature. After emulsifying with an equal volume of complete Freund's adjuvant, the preparations were immediately injected i.m. or s.c. into rabbits.

By the treatment with glutardialdehyde, the toxins were largely inactivated. Whereas all of 3 injected rabbits (3 kg) died after 2 mg genuine venom (in Freund's adjuvant), in the course of a few hours, all of 10 animals survived 20 mg cross-linked venom, without symptoms of adverse effects. Two rabbits died after 40 mg.

Two rabbits (3 kg) received 10 mg cross-linked venom. They were boosted 3 times with the same and twice with the double amount. After $3^1/_2$ months venous blood was drawn from the ear vessels and the serum checked by agar-immune electrophoresis. Both antisera developed several precipitation lines against the components of whole cobra venom, among them the basic toxins (Figure, upper trough), as well as against purified preparations of direct lytic factor (DLF[2]) and phospholipase A.

The toxin-neutralizing potency of 1 of the 2 antisera was investigated in mice. The LD_{50} of the genuine venom

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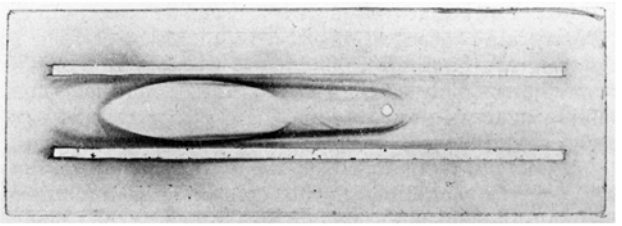

Immune electrophoresis of *Naja naja* venom (30 µg, in the well). Troughs: 2 specimen of rabbit anti-*Naja-naja* serum, raised with glutardialdehyde-treated venom. Cathode to the left.

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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