application of 50 μg or i.v. injection of 250 μg/100 g body weight of endotoxin from *E. coli*, the blood flow begins to slow down. The granulated periendothelial cells and the tissue mast cells are partially degranulated. A narrowing of the vessel lumen results from an increase in the volume of the endothelial cells and pericytes. At the same time the plasticity of the circulating blood cells changes. Adherence to the wall of the granulocytes precedes the formation of microthrombi. A dose-dependent blockade develops to varying degrees in the venules, capillaries and much later in the arterioles. An increase in the permeability of the vessels follows, which leads to microbleedings.

All these alterations of the microcirculation observed after the injection of endotoxin – in the same dose as described above – are prevented in guinea-pigs and hamsters by pretreatment with endotoxoid-2. The animals received by i.v. route 150 μg/100 g body weight of endotoxoid-2. Twenty-four hours later vitalmicroscopic observation of the guinea-pig mesenterium and the hamster cheek pouch showed that local as well as i.v. application of endotoxin from *E. coli* did not lead to any disturbance of the microcirculation.

It is of interest that a glucofuranosid derivative (Glyvenol®, CIBA Aktiengesellschaft, Basel) which was fed 2 h before the application of endotoxin protects the microcirculation of these animals in the same way (URBASCHEK, VERSTEYL and GÖTTE[6]).

Zusammenfassung. Die Endotoxin-bedingten Störungen an der Mikrozirkulation – geprüft an der Hamsterbackentasche und dem Meerschweinchenmesenterium – bleiben aus, wenn den Tieren 24 h zuvor detoxifiziertes Endotoxin verabreicht wurde.

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Induction of Antibodies Against Pure Proteins in *Xenopus laevis* Daud.

While many authors have long ago proved the formation of antibodies of the agglutinin and phagus-inhibiting type in Amphibia, both Anura[1,2] and Urodela[3], attempts to obtain from these animals antibodies of the precipitine-type against pure proteins always failed[4]. The proteins used as antigens were serum albumins or serum globulins of mammals, that is proteins with low molecular weight.

On this basis the author has used as an antigen the hemocyanine (Hc) from a Gasteropod, the *Viviparus ater* Cristofori et Jan, which is a protein with high molecular weight (6,760,000)[5], easily obtainable in the pure state.

Materials and methods. Three adult females of the clawed frog, *Xenopus laevis* Daud. were injected into the lymphatic sac, with 3 injections of 0.3 ml of pure Hc + 0.3 ml of adjuvant (complete Freund adjuvant Difco), each at a distance of a week after the previous one.

The hemocyanine, extracted from 10 specimens of *Viviparus* was purified by dialysis and ultracentrifugation[6]; its protein concentration, calculated with the biuret method, was 3.2 mg/ml.

Throughout the whole experiment the xenopus were kept at a constant temperature of about 25 °C and fed with minced ox-liver. Twenty days after the last injection 2 animals were killed and their blood drawn directly from the heart; from a third xenopus a small amount of blood was drawn from the marginal vein of the interdigital membrane, and afterwards it was injected with 0.5 mg (in 0.2 ml) of cortisone[7]. The animal was completely bled 12 h after the injection. The maximum dilution at which the sera are still active was calculated with the ring-test for all of them; this test was performed also on the serum after the cortisone treatment. The antigenic reaction was also tested with the method of the double diffusion on 1% agar microplates in 0.85 NaCl, against Hc of *Viviparus* and against Hc of *Potamobius fluviatilis* L. In order to study to which fraction of the globulins the antibody activity was correlated, the antiserum was analysed by electrophoresis on agar for 2 h, in veronal buffer (pH 8.4 i.s. 0.05) and then antibodies were looked for, placing the antigenic Hc on the migration plate.

Finally one of the 3 antisera was dialyzed for 12 h at room temperature against 2-mercaptoethanol (2-ME) 0.1 M[8], which neutralizes 19 s γ-globulins.

Results. The sera of the 3 xenopus after the third injection of Hc, gave a positive reaction against Hc of *Viviparus* (Figure 1), while no arc of precipitate was obtained when the antiserum was tested against Hc of *Potamobius* (Figure 2).

As was proved by the immunoelectrophoretic analysis (Figure 3), the antibody activity is correlated to the γ-globulins fraction.

With the ring-test method it was shown that the 3 antisera are effective up to a dilution of 1:30, while the serum of *Xenopus* treated with cortisone was active up to a dilution of 1:120. It was also observed (Figure 4) that

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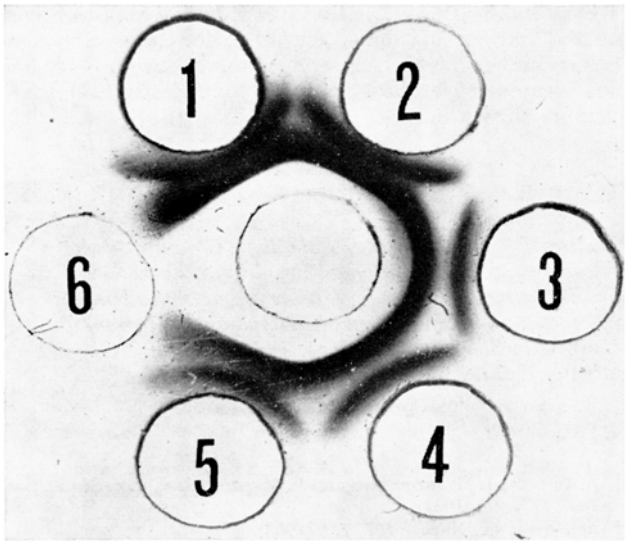

Fig. 1. Immunodiffusion on agar microplate. 1, 5 *X. laevis* sera anti-hemocyanine (Hc); 6 serum of control *X. laevis*; in the central well Hc of *V. ater*. (Amidoschwartz staining).

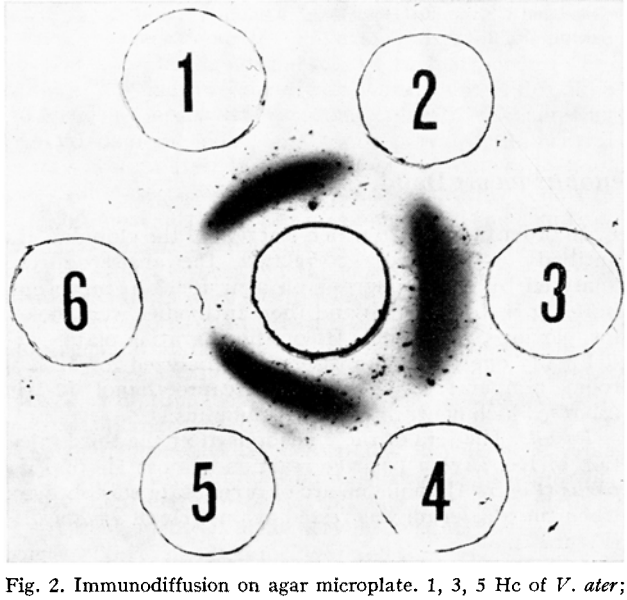

Fig. 2. Immunodiffusion on agar microplate. 1, 3, 5 Hc of *V. ater*; 2, 4, 6 Hc of *P. fluviatilis*; in the central well anti-Hc serum of *X. laevis*. (Amidoschwartz staining).

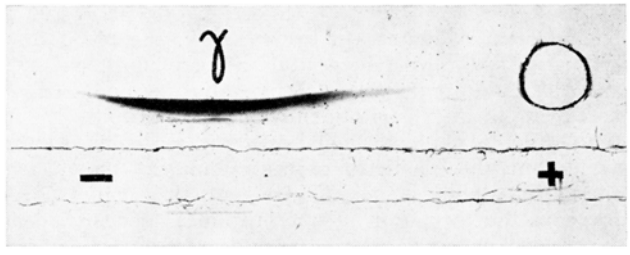

Fig. 3. Immunoelectrophoresis. Anti-Hc serum of *X. laevis* against Hc of *V. ater*. (Amidoschwartz staining).

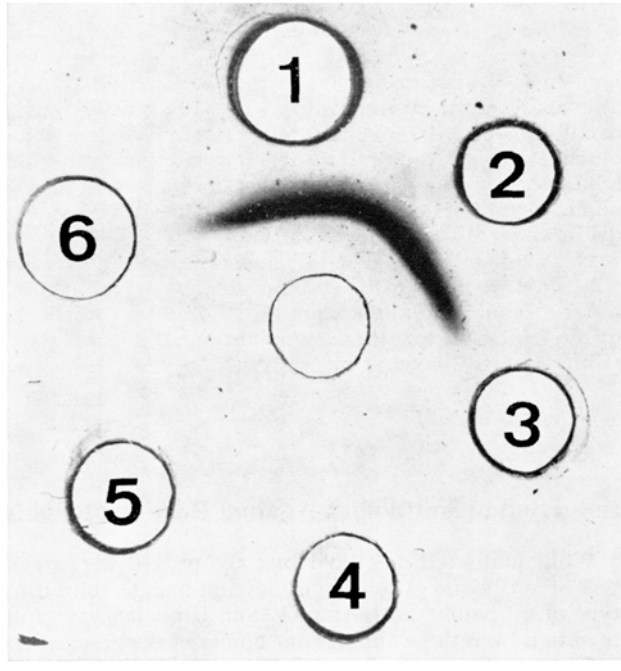

Fig. 4. Immunodiffusion on agar microplate. 1, 2 Anti-Hc serum of *X. laevis*; 3, 4, 5 anti-Hc xenopus serum treated with 2-mercapto-ethanol; 6 control xenopus serum; in the central well hemocyanine of *V. ater*. (Amidoschwartz staining).

the antibodies are mercaptoethanol sensitive; in fact, after treating the serum with 2-ME, no precipitate arcs were observed on the microplates of immunodiffusion.

From these results the following can be concluded: (1) *Xenopus* is able to give precipitine-type antibodies against pure proteins, provided the latter have a sufficiently high molecular weight; (2) these antibodies are highly specific and mercaptoethanol sensitive; (3) in *Xenopus* antibody production is stimulated by cortisone.

It must be remembered that, in Amphibia, the antibody production is correlated to the temperature at which the animal is kept during the immunization treatment[8,9].

It is still to be proved up to which molecular weight of the antigen the animals used in the experiment can give detectable immunological reaction. Further research with hemocyanines of different molecular weight will answer this question. We still need clarification also as to the nature of the antibodies obtained, and particularly whether they can be compared to the γ-*M* (19s γ-globulins) of higher animals, as the 2-ME sensitivity might suggest.

Ultracentrifugation and separation of the sera, as performed on Sephadex column, will supply further information on the subject.

Riassunto. Tre *Xenopus laevis* Daud., mantenuti a temperatura costante di 25 °C, sono stati immunizzati con una proteina pura ad alto peso molecolare, l'emocianina di *Viviparus ater* Cristofori et Jan. Tutti gli animali hanno risposto con produzione di anticorpi altamente specifici e mercaptoetanolo sensibili. Si è potuto inoltre confermare che l'anticorpopoiesi in *Xenopus* dipende dalla temperatura alla quale l'animale è tenuto durante il trattamento e che è stimolata dall'azione del cortisone.

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