Increase of Haptoglobin Concentration in Mouse Serum by Endotoxin and by a Serum Factor

One of the characteristics of bacterial endotoxins is their ability to increase the level of some enzymes in different organs of animals[1–5]. As described previously[6], and also shown here, endotoxin was found to increase the level of haptoglobin in mouse serum. This is a very sensitive process, and it was therefore chosen for the detection of very small amounts of endotoxin.

In this paper, we show that mouse serum, after treatment with rivanol, was found to have the same ability to increase haptoglobin and interact with γ-globulin, similarly to the bacterial endotoxin.

Materials and methods. For the treatment of mouse sera with rivanol, the sera were collected from 2–4 month old C57Bl male mice, unless otherwise indicated. The mice were bled from the eye, and the pooled blood of 150–250 animals was centrifuged. The serum obtained was mixed with an equal volume of 1% rivanol (2-ethoxy-6:9-diaminoacridine lactate) solution. The mixture was left in ice for 5 min, and then centrifuged at 30,000g for the same period of time. Removal of the rivanol was by passing the clear supernatant obtained through a column of potato starch[7]. This final solution, free of rivanol – referred to below as rivanol-treated serum – was used in most of the experiments.

Rivanol-treated sera were prepared similarly also from human (4 healthy donors), bovine, sheep, rabbit, hamster (inbred LSH/SS and random-bred GH strains), and rat (inbred Lewis and random-bred CR/RAR strains) sera.

The effect of rivanol-treated sera and endotoxin on haptoglobin concentration was studied in inbred, 33–41-day-old female C57Bl/6/Jax mice. The mice were injected i.v. with 0.25 ml of rivanol-treated serum or endotoxin, and in the control group with 0.01M phosphate buffer, pH 7.4. The endotoxin (lipopolysaccharide B of *S. typhimurium*) was purchased from Difco Laboratories.

The effect of human 7S γ-globulin on the haptoglobin concentration in mice was also studied. This protein was purchased from Mann Research Laboratories, as a 100% pure preparation.

Each experimental group was composed of 6–10 mice. The mice were bled from the eye 24 h after injection, the blood of each animal being collected in a separate tube. The blood samples were centrifuged in a cooled centrifuge, and the sera used for the estimation of haptoglobin according to the method of TARUKOSKI[8]. Human hemoglobin was used for the estimation of haptoglobin, and the results are expressed as hemoglobin binding capacity HbBC per 100 ml of serum.

In some experiments, the haptoglobin was examined on polyacrylamide electrophoreograms[9,10].

Results. Figure 1 shows that injection of different dilutions of rivanol-treated serum of C57Bl mice increased significantly the haptoglobin concentration in the serum of the recipient mice. Heating for 10 min at 100 °C did not decrease the activity of the rivanol-treated serum. Serum without rivanol treatment had no effect on the haptoglobin concentration.

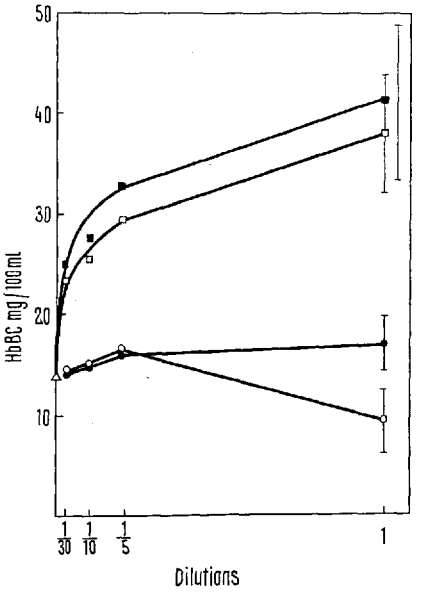

Fig. 1. The effect of rivanol-treated serum on the level of haptoglobin in mice. Untreated serum (○); heated serum (●); rivanol-treated serum (□); heated rivanol-treated serum (■). Mean and 95% confidence interval; 6 mice per group.

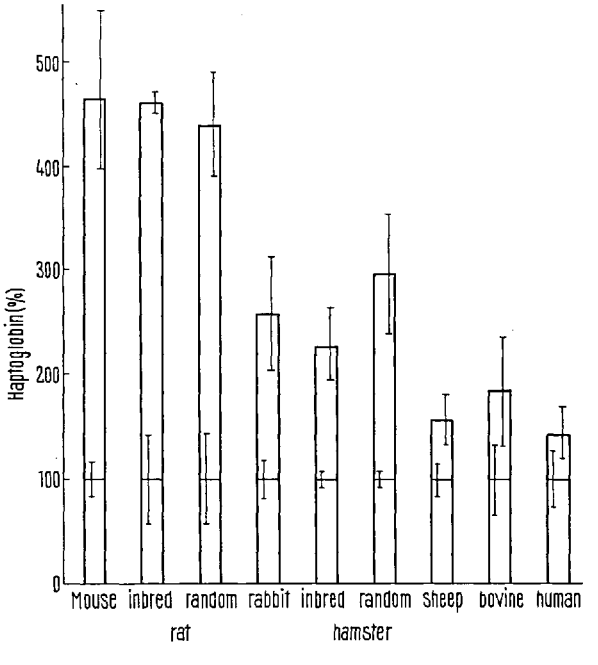

Fig. 2. The effect of rivanol-treated sera from different species on haptoglobin content in mice. The results are expressed as percent of change, the control groups representing 100%. Mean and 95% confidence interval; 6 mice per group.

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Similar results were obtained with the rivanol-treated sera of other strains of mice (i.e. C3H/eB, SWR, BALB/c, ICR, SJL/J and AKR). In addition to the rivanol-treated mouse serum, similarly treated rat serum had an intense effect, that of rabbit and hamster had a lesser effect, while the rivanol-treated sera of sheep, bovine and humans showed marginal activity (Figure 2).

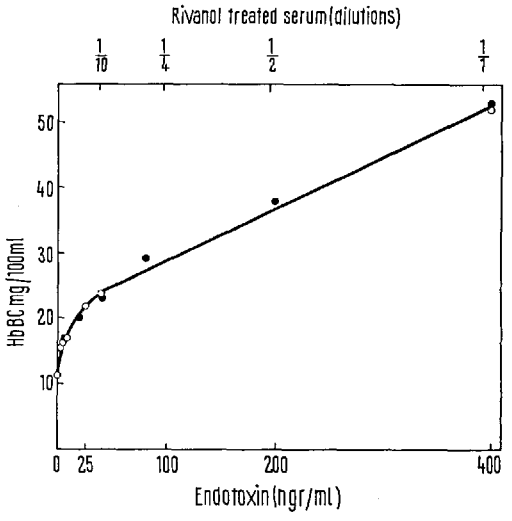

Fig. 3. Comparison of the activity of rivanol-treated serum with that of endotoxin on elevation of haptoglobin concentration in sera of mice. Endotoxin (○); rivanol-treated serum (●).

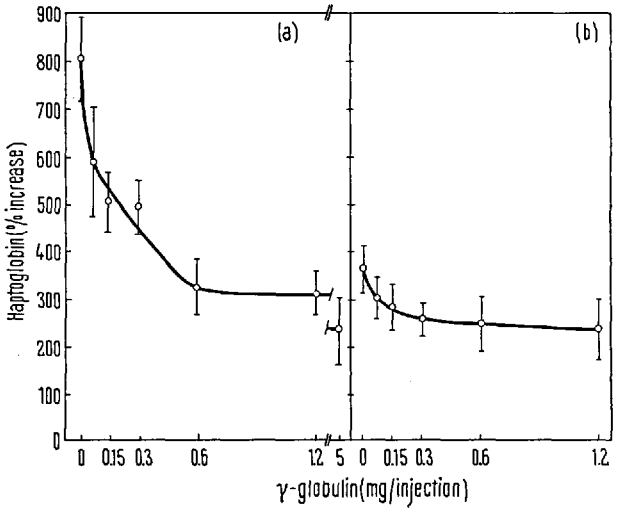

Fig. 4. The inhibitory effect of human 7S γ-globulin on the activity of endotoxin and of rivanol-treated serum. Different concentrations of γ-globulin were mixed with endotoxin – 0.1 μg/injection (a) or with rivanol-treated AKR mouse serum at a ratio of 1:1 (b), and injected after standing for 15 min in the cold. Mean and 95% confidence interval; 6–10 mice per group.

Since a similar effect to that of the rivanol-treated mouse serum on haptoglobin concentration was obtained by endotoxin[6], we studied the similarity of these 2 materials. It is shown (Figure 3) that there was an identical response to different amounts of rivanol-treated serum, and to endotoxin. This experiment also showed that undiluted rivanol-treated serum had the same effect as 0.4 μg/ml endotoxin.

The bacterial endotoxins are strong antigens; it was therefore of interest to verify whether 7S γ-globulin isolated from human serum interferes with endotoxin in its enhancing effect on haptoglobin concentration in mouse serum. Figure 4a shows that γ-globulin, injected together with 0.1 μg endotoxin, reduced the effect of the latter. The γ-globulin also reduced the effect of rivanol-treated serum (Figure 4b), indicating a further similarity between these 2 materials.

Attempts further to characterize the factor present in rivanol-treated sera revealed that it could be precipitated by a saturation of 80% ammonium sulphate, and when fractionated with ethanol, the highest activity was recovered in the supernatant after 50% (v/v) saturation by ethanol. The factor was also found to be stable at pH 1.

Discussion. The results described here suggest the presence of a latent factor, similar or identical to at least one of the bacterial endotoxins. The latency of the factor could be assumed to be due to a binding of the factor to a protein, which is split and denaturated by rivanol. The selective action of rivanol on proteins was shown by HOŘEJŠI and SMETANA[11]. It is possible that the factor represents a complex of endotoxin and α-globulin, which is formed during the process of degradation of endotoxin in the organism[12].

Résumé. L'injection de sérums provenant de différentes souches de souris, ainsi que de rats, de hamsters et de lapins, respectivement traités au Rivanol, de même que l'injection d'endotoxine bactérienne à des souris de souche C57Bl a pour effet d'augmenter la concentration d'haptoglobine dans le sérum de ces dernières. La fraction purifiée de 7S γ-globulines sériques humaines réduit cet effet.

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Immune Responses in Amebiasis

Several workers have investigated the antigen-antibody reactions in *Entamoeba histolytica*[1,2]. In the present study, an attempt has been made to illustrate the antigen-antibody reactions in *E. histolytica* by indirect hemagglutination (HA) and immunodiffusion (ID) methods. The above techniques were employed for the detection of specific antibodies in the immune rabbit serum and in the sera of 125 patients with symptomatic and asymptomatic infections of *E. histolytica*. Monobacterial cultures of *E. histolytica* were grown with penicillin-inhibited resting cell suspensions of *Escherichia coli*. The choice of *E. coli* as the bacterial associate was