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).

Since a similar effect to that of the rivanol-treated mouse serum on haptoglobin concentration was obtained by endotoxin, 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 denatured by rivanol. The selective action of rivanol on proteins was shown by Hořejší and Smetanā. It is possible that the factor represents a complex of endotoxin and γ-globulin, which is formed during the process of degradation of endotoxin in the organism.

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 C57BI 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. 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
made because of its colonic ecological relationship with *E. histolytica*.

**Materials and methods.** Preparation of antigen: Monobacterial amebic cultures were grown in Boeck and Drbohlav’s egg-slant medium. All antigenic preparations were made according to the method described by Kessel et al. Control antigen from penicillin-inhibited *E. coli* was also similarly treated.

Preparation of antiserum: 4 rabbits were immunized with monoxenic *E. histolytica* antigen. One control rabbit was also immunized with *E. coli* antigen. During the entire course of immunization each test animal received a total volume of 5 ml of cells (4×10⁷/ml) in 4 injections. 3 weeks after the last injection all the animals were bled to death and the serum was obtained. The antiserum was merthiolated (1/10,000) and stored at −20°C.

Antigen-antibody reactions were studied by the Ouchterlony plate method. Antigen (1/10) dilution was placed in the central well and was allowed to diffuse and react with the antisera in the peripheral wells. All the 125 human sera samples were tested for the detection of antibodies. Human sera from the non-infected controls, along with the absorbed and nonabsorbed rabbit sera, were also similarly treated.

*Results.* The *E. histolytica* antiserum showed specific sensitivity in the detection of antibodies from the human sera samples. Titters at high and low level were easily detectable. The highest titters were obtained with rabbit anti-*E. histolytica* serum. Figure 1, a and b shows the comparative data on hemagglutination reactivity of the rabbit and human immune sera. The rabbit immune serum gave a fairly high HA reactivity (8192) at the end of the 6th week of the inoculation schedule and this remained more or less constant till the end (9 weeks). The highest titters obtained in the human sera samples were around (2048) in about 10% of cases. The HA titer of 1024 was obtained in 60% of human sera samples. The remaining 30% showed titters in the range of 128. The non-infected controls showed titters between 8 and 16.

Figure 2, a, b, c and d shows the diagrammatic representation of some of the characteristic results on ID tests. In the majority of human sera samples, 4–5 clear cut bands were detectable (Figure 2a). The nonabsorbed rabbit antiserum gave certain precipitin bands which were to some extent identical with human sera samples in some cases (Figure 2b). The *E. coli* antiserum was responsible for 2 broad bands and 1 narrow arc in the control rabbit anti-*E. coli* serum, in the nonabsorbed anti-*E. histolytica* serum and also in 2 sera samples from the patients (Figure 2c). Figure 2d illustrates the separation of precipitin bands in the nonabsorbed rabbit serum against *E. coli* and *E. histolytica* antigens. The antibodies were detectable by the ID tests in all the HA positive sera. The entire spectrum of precipitin bands observed in the ID tests ranged from somewhat unresolved lines to clear cut bands showing reactions of partial to complete identity.

*Discussion.* The results indicate that *E. histolytica* essentially has a multiple antigen system. At least 4–5 different antigenic components or determinants are involved in eliciting the immune responses in amebiasis.

The absorption of *E. coli* antibodies from the rabbit antiserum was carried out by the method described previously. The antiserum was also exposed to the growth medium for the absorption of antibodies against any of its possible components. Indirect hemagglutination test as described by Kessel et al. was performed in microtiter plates on all the sera. Human type 0 Rh-positive red blood cells were treated with (1/120,000) tannic acid solution and sensitized with serial dilutions (1/5 to 1/80) of antigen. All the sera and appropriate controls were then tested for antibodies with 2% suspension of sensitized cells.

Ouchterlony plate method. Antigen (1/10) dilution was placed in the central well and was allowed to diffuse and react with the antisera in the peripheral wells. All the 125 human sera samples were tested for the detection of antibodies. Human sera from the non-infected controls, along with the absorbed and nonabsorbed rabbit sera, were also similarly treated.

Results. The *E. histolytica* antiserum showed specific sensitivity in the detection of antibodies from the human sera samples. Titters at high and low level were easily detectable. The highest titters were obtained with rabbit anti-*E. histolytica* serum. Figure 1, a and b shows the comparative data on hemagglutination reactivity of the rabbit and human immune sera. The rabbit immune serum gave a fairly high HA reactivity (8192) at the end of the 6th week of the inoculation schedule and this remained more or less constant till the end (9 weeks). The highest titters obtained in the human sera samples were around (2048) in about 10% of cases. The HA titer of 1024 was obtained in 60% of human sera samples. The remaining 30% showed titters in the range of 128. The non-infected controls showed titters between 8 and 16.

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