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).

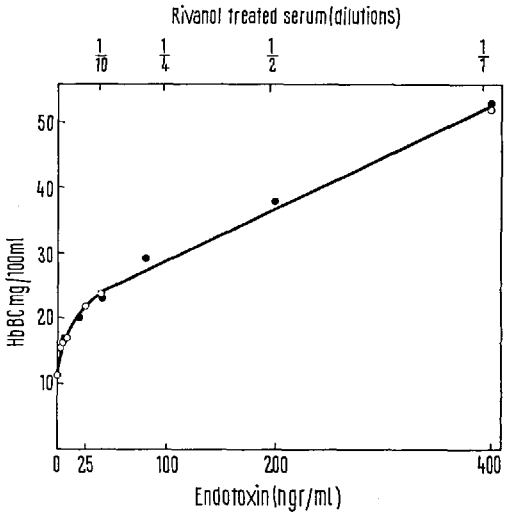

Fig. 3. Comparison of the activity of rivanol-treated serum with that of endotoxin on elevation of haptoglobin concentration in sera of mice. Endotoxin (○); rivanol-treated serum (●).

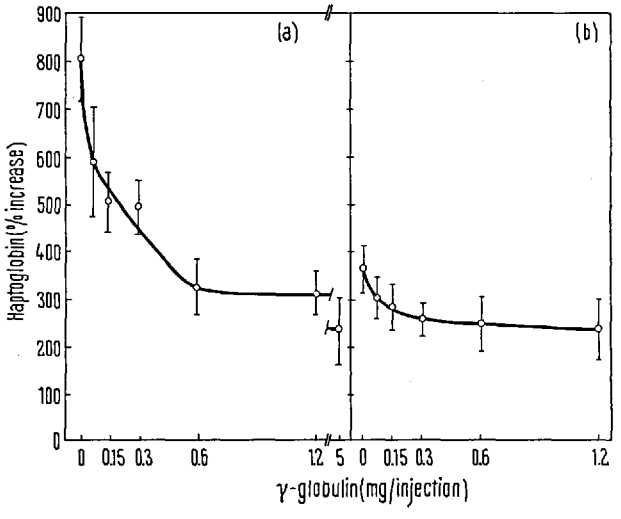

Fig. 4. The inhibitory effect of human 7S γ-globulin on the activity of endotoxin and of rivanol-treated serum. Different concentrations of γ-globulin were mixed with endotoxin – 0.1 μg/injection (a) or with rivanol-treated AKR mouse serum at a ratio of 1:1 (b), and injected after standing for 15 min 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.

Ahuva Knyszynski and M. Burger

Department of Experimental Biology, Weizmann Institute of Science, P.O. Box 26, Rehovot (Israel), 21 January 1971.

[11] J. Hořejši and R. Smetana, Acta med. scand. *155*, 65 (1956).
[12] R. C. Skarnes and L. C. Chedid, *Bacterial Endotoxins* (Quinn and Boden Company, Inc., Rahway, New Jersey 1964), p. 575.

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

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[3]. All antigenic preparations were made according to the method described by KESSEL et al.[4]. 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 (4×10^5/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.

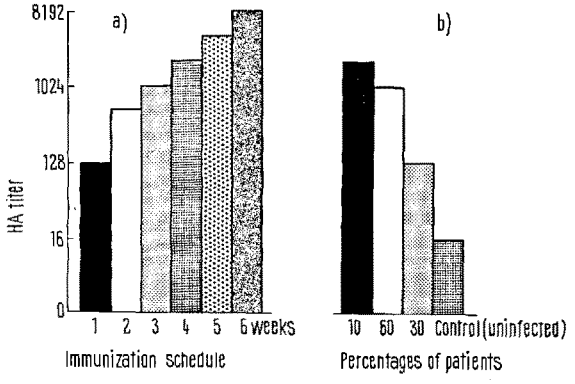

Fig. 1. Comparison of HA titers. a) Rabbit immunization schedule in weeks with HA reactivity of the antiserum. b) Percentages of HA reactivity of the human sera.

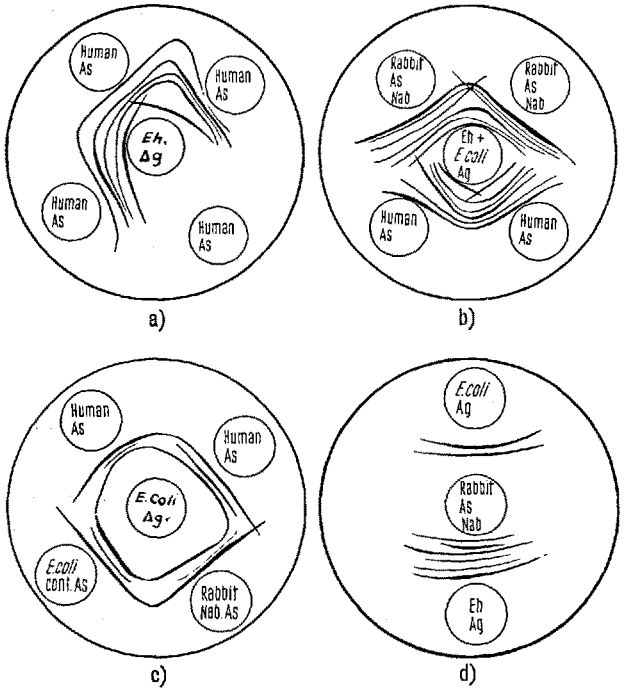

Fig. 2. Diagrammatic representation of some of the typical reactions on agar-gel diffusion. As, antiserum; Nab, nonabsorbed; Ag, antigen; Eh, *Entamoeba histolytica*; *E. coli*, *Escherichia coli*.

The absorption of *E. coli* antibodies from the rabbit antiserum was carried out by the method described previously[5]. 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.[5], 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.

Antigen-antibody reactions were studied by the OUCHTERLONY[6] 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* antigen showed specific sensitivity in the detection of antibodies from the human sera samples. Titers at high and low level were easily detectable. The highest titers 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 titers 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 titers in the range of 128. The non-infected controls showed titers 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* antigen 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.

[1] M. N. LUNDE and L. S. DIAMOND, Am. J. trop. Med. Hyg. *18*, 1 (1969).
[2] Z. ALI KHAN and E. MEEROVITCH, Am. J. trop. Med. Hyg. *17*, 528 (1968).
[3] W. D. BOECK and J. DRBOHLAV, Am. J. Hyg. *14*, 540 (1925).
[4] J. F. KESSEL, W. P. LEWIS, C. M. PASQUEL and J. A. TURNER, Am. J. trop. Med. Hyg. *14*, 540 (1965).
[5] M. GOLDMAN and W. A. SIDDIQUI, Expl. Parasit. *17*, 326 (1965).
[6] O. OUCHTERLONY, Acta path. microbiol. scand. *32*, 231 (1953).

The results of the present study have clearly established that the antigen-antibody system in *E. histolytica* is specific and can be routinely used for serological diagnosis of amebiasis. The present authors were not able to detect more than 5 well defined bands, though numerous other workers have reported upto 10 precipitin bands by the OUCHTERLONY method[7-9]. Various reactions of non-identity and some inexplicable bands and spurs were also detected. These perhaps represent the cross reactions of various *E. histolytica* strains which are distributed in nature and are responsible for amebiasis symptom complex. The results are quite suggestive that certain antigenic components of *E. histolytica* are responsible for specific immunological activities, and, as such, indirect hemagglutination and immunodiffusion tests can be successfully employed for the detection and demonstration of antibodies in clinical and subclinical symptoms of *E. histolytica* infections. The above view is also supported by many similar studies on serodiagnostic techniques for amebiasis[7,8,10,11].

Zusammenfassung. Immunologischer Nachweis von Amöbiasis gelingt auch durch indirekte Hämagglutination und durch Gel-Diffusion.

S. AHMAD and M. D. MATHUR

Department of Microbiology, Medical College, Aligarh Muslim University, Aligarh (U.P., India), 22 July 1970.

[7] S. E. MADDISON and R. ELSDON-DEV, Expl. Parasit. *11*, 90 (1961).
[8] F. O. ATCHLEY, A. H. AUERNHEIMER and M. A. WASLEY, J. Parasit. *49*, 313 (1963).
[9] B. TALIS, M. LAHAV and S. BEN-EFRAIM, Bull. Res. Coun. Israel *10E*, 130 (1963).
[10] S. E. MADDISON, I. G. KAGAN and R. ELSDON-DEW, Am. J. trop. Med. Hyg. *17*, 540 (1968).
[11] With financial assistance awarded to the senior author under the scheme of the individual research grants from Muslim University, Aligarh.

Immunodepressive Activity of Adriamycin in Experimental Infection of the Mouse with *Nippostrongylus brasiliensis*

Immunity phenomena in helminthiasis are caused by complex mechanisms, some of which are characteristic of this type of disease. An aspect of particular interest in intestinal helminthiasis is the spontaneous expulsion of the parasites by the host (self-cure) which occurs some time after establishement of parasitosis as a result of an immunity response[1].

A good model for study of self-cure is the infection caused by *Nippostrongylus brasiliensis* in the rat. This infection is characterized by the penetration of the larvae through the skin, migration in the blood circulation to the heart and then to the lungs, and eventually to the intestines where maturation of the parasites takes place.

After a certain period the parasites are spontaneously expelled with an exponential type movement[2].

Immunodepressive substances like corticosteroids and antilymphocytic sera[2-4] interfere in this phenomenon by significantly prolonging the duration of parasitosis which, therefore, constitutes a valid parameter for estimating the immunodepressive action of drugs.

With this experiment we wished to investigate whether adriamycin, an antitumour anthracycline antibiotic[5,6] which had shown immunodepressive activity on the primary antibody response of the mouse to sheep red blood cells[7], interferes in the host-parasite relationship in experimental infection of the mouse with *N. brasiliensis*.

The mouse is an abnormal host for *N. brasiliensis*. Nevertheless, the infection takes a course similar to that observed in the normal host, although the immunity reactions are more marked[8].

Material and methods. 3 groups of 60 Cobs Swiss albino mice were infected s.c. with 300 larvae of *N. brasiliensis* per mouse and then treated i.p. with adriamycin (1st group) with a daily dose of 1 mg/kg for the entire duration of the experiment and with hydrocortisone acetate (2nd group) i.m. with a dose of 25 mg/kg on alternate days. The 3rd group, which was not subjected to any treatment, served as control.

The results were statistically analyzed with the non-parametric method of Krauskal-Wallis and multiple comparisons were conducted by means of the method proposed by STEEL[9].

Results. The data obtained are reported in Table I and the results of the statistical analysis in Table II. It can be observed that: 1. In the infested animals the number of intestinal parasites reached a maximum value at the 7th day and then gradually decreased. 2. In the animals

Table I. Number of worms per mouse (5 mice per group)

Days after infection	3	5	7	10	13	18	20	24	26	28	31
Control	0	9	18	52	7	7	4	0	0	5	1
	0	0	8	8	7	1	2	1	1	0	3
	0	7	36	7	4	1	2	4	0	5	2
	0	20	16	2	10	2	5	4	3	3	1
	0	2	20	10	0	3	1	2	3	3	1
Adriamycin	0	6	11	35	5	12	17	5	10	10	27
	0	19	10	14	19	10	13	21	19	13	14
	0	22	32	7	42	8	12	11	25	32	9
	0	18	7	6	8	34	17	19	6	8	96
	0	2	40	8	23	7	6	7	6	11	10
Hydro-	0	0	9	73	47	27	23	32	17	16	38
cortisone	0	6	27	10	66	37	26	73	28	38	20
acetate	0	1	12	56	11	51	0	31	18	18	14
	0	17	89	43	69	22	79	27	12	76	57
	0	11	63	22	19	45	46	17	65	14	19

[1] W. MULLIGAN, in *Immunity to Parasites* (Ed. A. E. R. TAYLOR; Blackwell Scientific Publications, Oxford and Edinburgh 1968), p. 51.
[2] N. C. G. SHARP and W. F. H. JARRETT, Nature, Lond. *218*, 1161 (1968).
[3] C. J. PARKER, Expl Parasit. *11*, 380 (1961).
[4] L. RETHY and G. TOTH, Nature, Lond. *218*, 1055 (1968).
[5] F. ARCAMONE, C. CASSINELLI, C. FANTINI, A. GREIN, P. OREZZI, G. POL and C. SPALLA, Biotech. Bioeng. *11*, 1101 (1969).
[6] F. ARCAMONE, G. FRANCESCHI and S. PENCO, Tetrahedron Lett. *13*, 1007 (1969).
[7] A. M. ISETTA, C. INTINI and M. SOLDATI, Experientia *27*, 202 (1971).
[8] D. A. PORTER, Am. J. Hyg. *22*, 444 (1935).
[9] G. D. STEEL, Biometrics *17*, 539 (1961).