

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 method⁷⁻⁹. 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-DEV, *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 establishment of parasitosis as a result of an immunity response¹.

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 movement².

Immunodepressive substances like corticosteroids and antilymphocytic sera³⁻⁴ 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 cells⁷, 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 marked⁸.

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 STEEL⁹.

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
Hydrocortisone acetate	0	0	9	73	47	27	23	32	17	16	38
	0	6	27	10	66	37	26	73	28	38	20
	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).

Table II. Statistical analysis

Days after infection	5	7	10	13	18	20	24	26	28	31
Control vs adriamycin	C = A	C = A	C = A	C = A	C < A ^a	C < A ^b	C < A ^b	C < A ^b	C < A ^b	C < A ^b
Control vs hydrocortisone	C = I	C = I	C = I	C < I ^b	C < I ^b	C = I	C < I ^b	C < I ^b	C < I ^b	C < I ^b
Hydrocortisone vs adriamycin	I = A	I = A	I = A	I = A	I > A ^a	I = A	I > A ^a	I = A	I = A	I = A

C, number of worms of control-group; A, number of worms of adriamycin-treated group; I, number of worms of hydrocortisone acetate-treated group. ^a $P \leq 0.05$; ^b $P \leq 0.01$.

treated with hydrocortisone, the number of helminths which had reached the intestine was statistically superior to that of the controls. In addition, the time of stay of the parasites in the host was prolonged. In fact, beginning from the 18th day, the number of intestinal parasites observed was statistically superior to that of the controls. 3. Adriamycin did not modify the degree of parasitosis with respect to the controls but significantly prolonged the duration of infection.

In fact, the number of intestinal parasites in the animals treated with adriamycin, beginning from the 18th day, was statistically superior to that of the controls.

Conclusions. The data reported demonstrate interference of adriamycin and hydrocortisone in the course of infection due to *N. brasiliensis* in the mouse with partly analogous and partly different aspects. Hydrocortisone with its anti-inflammatory and immunodepressive action probably acted both on the tissue type defence mechanisms^{10,11}, which limited the extent of parasitosis, and on the specific immunity mechanisms of self-cure¹ (cellular hypersensitivity reaction and humoral antibody reactions).

Adriamycin did not demonstrate any activity on the invasion phenomena, while it played a significant role in the self-cure.

It is not likely that adriamycin had an indirect action capable of favouring persistence of the parasites in the intestine. In fact, some signs of subtoxicity of the antibiotic in the intestinal epithelium (de-epithelization)

could favour rather than inhibit expulsion of the parasites, making the implantation site unsuitable.

Therefore, it can be supposed that adriamycin acted on the essential mechanisms of self-cure by depressing the hypersensitivity phenomena or by inhibiting antibody formation. The latter possibility was enhanced by the findings mentioned⁷ on the primary humoral response of the mouse to sheep red blood cells.

Up to now, the presence of specific antihelminthic antibodies in the animals treated with adriamycin has not been investigated. Experiments will be carried out in this direction in order to confirm this hypothesis.

Riassunto. È stata messa in evidenza l'azione immunodepressiva dell'adriamicina dell'infezione del topo con *Nippostrongylus brasiliensis*. L'antibiotico prolunga il periodo di permanenza dei parassiti nell'ospite, mentre non modifica la fase invasiva della parassitosi.

C. DELLA BRUNA and A. SANFILIPPO

Farmitalia, Istituto Ricerche di Base, Via dei Gracchi 35, I-20146 Milano (Italy), 28 December 1970.

¹⁰ W. H. TALIAFERRO and M. P. SARLES, *J. infect. Dis.* 64, 157 (1939).

¹¹ L. THOMAS, *Ann. N.Y. Acad. Sci.* 56, 799 (1953).

Élévation du taux de la corticostérone plasmatique au cours de la surrénalite expérimentale de la souris

La production d'une surrénalite expérimentale chez la souris a déjà été démontrée¹, elle est caractérisée par des lésions inflammatoires discrètes et localisées de l'organe cible comprenant en particulier des infiltrations lymphocytaires. Le maximum de ces lésions est observé entre la 2^e et 3^e semaine qui suit l'immunisation, parallèlement il est parfois possible de mettre en évidence un titre faible en anticorps circulants spécifiques de la surrénale. Chez le rat la répétition des immunisations peut aboutir à des lésions surrénaliennes importantes² entraînant une diminution de poids de la glande et du taux de la corticostérone plasmatique³. Ces dernières constatations correspondent bien aux observations cliniques d'insuffisance de la surrénale. Dans le travail présent il est apparu que chez la souris, après une seule immunisation avec de la surrénale hétérologue, on obtenait une élévation de la corticostérone plasmatique semblant la conséquence de l'aggression immunologique.

Matériel et techniques. Des souris CF₁ âgées de 3 à 4 mois ont été réparties en trois groupes: Le groupe A comprenant 7 souris, a servi de témoin, le groupe B comprenant 4 séries de 10 souris immunisées de façon identique à l'aide d'un mélange constitué par un volume d'adjuvant de Freund complet et d'un volume d'une suspension de tissu surrénalien de lapin dans du tampon isotonique pH 7,2.

Chaque souris a reçu par voie sous-cutanée en une seule fois mais en plusieurs points 0,3 ml du mélange: 0,1 ml dans la patte droite, 0,1 ml dans le flanc droit et 0,1 ml dans le flanc gauche. La quantité de surrénale de lapin ainsi injectée à chaque souris correspond à 30 mg de tissu.

Le groupe C était constitué par 2 séries de 10 souris ayant reçu respectivement: Série C 1: une immunisation avec un extrait de foie de lapin dans l'adjuvant de Freund complet. Les conditions de l'immunisation étant ana-