TABLE I. Sensitization by Thallium to Dihydrotachysterol Overdosage.

Treatment	Mean final body wt (g)	Calcification (scale 0-3)			
		Cutaneous	Renal	Aortal	Cardiac
Thallium-Ac	122 ± 1.47	0	0	0	0
DHT	104 ± 3.75	1.8 ± .42	0	.3 ± .15	.6 ± .33
Thallium-Ac + DHT	79 ± 3.12	3.0 ± 0	2.8 ± .15	1.1 ± .20	1.3 ± .41
Restraint + DHT	95 ± 2.10	0	0	0	0

sections of specimens fixed in alcohol-formol. The means of these readings, as well as the mean final body weights, are listed in Table I (with standard errors).

Results. Cutaneous lesions were absent in rats treated with thallium alone and moderate in those receiving DHT alone, while combined treatment with both these agents resulted in maximal cutaneous calcinosis in all animals (Fig. 1). No renal lesions were detectable in rats treated with thallium acetate or DHT, alone; but rats given both these compounds exhibited a clearly demarcated zone of intense calcification in the corticomedullary junction zone (Fig. 2).

Thallium acetate also failed to cause calcification in aorta or heart, however, a slight degree of calcium deposition was detectable in these organs, following treatment with DHT alone. The intensity of the aortal calcification was significantly ($P<0.01$) aggravated by concurrent treatment with thallium acetate; but in the heart, this aggravation was not statistically significant ($P = 0.2$).

The sensitization to DHT afforded by thallium acetate could not be ascribed to the stressor effect of the latter, because our earlier findings showed that pretreatment with stress produced nonspecific cross-resistance against DHT-intoxication(5). This was confirmed under the conditions of the present experimental series by the fact that, far from aggravating the syndrome of DHT-intoxication, 24 hours of restraint actually prevented it.

Summary. A single dose of thallium acetate, which in itself produces no detectable organ lesions in the rat, causes severe nephrocalcinosis strictly limited to the corticomedullary junction line if an otherwise nonnephrotoxic dose of dihydrotachysterol (DHT) is administered simultaneously. The DHT-induced calcification in aorta and in traumatized skin regions is greatly aggravated by concurrent treatment with thallium acetate. This sensitizing effect of thallium acetate cannot be ascribed to its stressor action, since under comparable circumstances, exposure to stress (restraint) actually prevents the manifestations of DHT-intoxication.

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Preliminary Report on a Hemagglutination Test for Entamoebae.* (26355)

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Previous serologic tests for amebiasis have made use of antigens prepared from amoebae grown symbiotically in cultures with mixed or single bacteria from the intestinal tract of man or animals. Such antigens have shown

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inconsistent results(1,3,4,5,6,7,8,10,11,13,15). It therefore seemed desirable to determine the specificity of antigens prepared from entamoebae grown in association with symbionts that do not occur in the intestinal tract. *Trypanosoma cruzi* reported by Phillips(12) to support growth of *E. histolytica* was first selected for such study.

Methods. 1. Preparation of antisera in rabbits. Two amoebae, *E. invadens* and *E. histolytica*, (DKB strain) were selected for preliminary study. The former was chosen because it is morphologically similar to *E. histolytica*, produces pathologic changes in reptiles, and may be grown in axenic culture. It was thought that *E. invadens* might possibly produce an antigen suitable for use in human amebiasis tests. As *E. invadens* did not grow profusely in either Miller's(9) or Stoll's(14) medium in our hands, both of the amoebae were adapted to monobacterial cultures of *Aerobacter aerogenes*. Amoebae of each species were washed as free as possible from the bacteria, suspended in saline, counted and injected into rabbits subcutaneously and intraperitoneally by appropriate schedules for production of homologous antisera. Serum samples were collected from the rabbits before immunization and at intervals until a substantial titer of antibodies was attained.

2. Preparation of antigen for serologic tests. Antigens for serologic tests were produced by growing each species, *i.e.*, *E. invadens*, and *E. histolytica* (DKB strain) with *T. cruzi* in Phillips' medium previously obtained through the courtesy of Dr. Wm. Balamuth. Growths of cultures were harvested and centrifuged at 1000 rpm for 10 minutes in an International #2 centrifuge, and the sediments washed twice in 85% NaCl. Two distinct layers of the sediment were obtained. The upper fluffy white layer consisting mostly of trypanosomes was removed and the gray mucoid sediment that remained, contained 11,500,000 trophozoites of amoebae per milliliter when examined in a Neubauer counting chamber. No cysts were seen. The amoebae were suspended in 10 ml of chilled, distilled water, and ruptured by vigorous passage through a 20 gauge needle. This preparation was allowed to stand in the refrigerator over night, the extract then being restored to isotonicity by adding 10 ml of 1.7% NaCl, and clarified by high speed centrifugation in the cold. The resulting water-clear solution provided a stock antigen at a dilution of 1:20. By using amoebic antigen from cultures grown with *Trypanosoma cruzi* no cross reactions were expected with the *Aerobacter aerogenes* or its medium that had previously been injected into rabbits for production of antisera.

3. Hemagglutination test. A modification of Boyden's(2) original tanned cell hemagglutination procedure, developed by one of the authors, (W.P.L.), for toxoplasmosis work was employed. This involved (a) substitution of human O cells for sheep cells, and (b) tanning the cells at concentrations of tannic acid ranging from 1:80,000 to 1:120,000 at a temperature of 2°C. The antigen was attached to the tanned cells at a pH of 6.4 and a temperature of 37°C.

Four controls were used in the test: (i) serum collected from the rabbits before injection with antigen, (ii) antiserum from rabbits injected with antigen prepared from *A. aerogenes* only, (iii) tanned human O cells with no antigen and (iv) human O cells with *T. cruzi* antigen.

The antigen prepared from *E. invadens* titered to an optimum dilution of 1:40 and the antigen from *E. histolytica* to 1:280.

Results and discussion. 1. The reciprocal reactions of these preliminary hemagglutination tests are shown in Table I.

2. At this point during the investigation a serum from a man with amebic liver abscess was obtained and tested both with the antigen of *E. histolytica*, DKB, and the antigen of *E. invadens*. With the antigen of *E. histolytica* an antibody titer of 1:512,000 was

TABLE I. Homologous and Heterologous Reactions of *E. invadens* and *E. histolytica*, DKB, with Antisera Produced in Rabbits.

	Antisera	
Antigens	*E. invadens*	*E. histolytica*
E. invadens	2048	32
E. histolytica	128	2048

TABLE II. Hemagglutination Test Studies in Amebiasis.

Sera from persons with	No. sera tested	No. positive	% pos.
1. No suspected amebiasis	30	1*	2.0
2. Three or more stools negative for *E. histolytica*	18	0	
3. Diarrhea or dysentery. *E. hist.* trophs. in stools	37	36	
4. Chronic G.E. symptoms. *E. hist.* in stools	13	13	98.0
5. Amebic liver abscess	12	12	
6. No current clinical amebiasis *E. hist.* in stools Probably "carriers"	34	23	68.0†

* Had traveled in Mexico.

† Most "carriers" were from tropical areas.

obtained while with the antigen prepared from *E. invadens* an antibody titer of only 1:256 was obtained. It was therefore evident that the common antigenic relationship existing between *E. histolytica,* DKB, and *E. invadens* as judged by the current hemagglutination test is slight and that further studies to determine its applicability to human amebiasis should be made with antigen prepared from *E. histolytica.*

3. Preliminary application of hemagglutination test to human sera. Sera tested to date have been procured from Los Angeles, and from abroad through the courtesy of Dr. Lei Kian Joe of Djakarta, Indonesia; Dr. Molina Pasquel, Inst. de Salubridad Y Enfermedades Tropicales, Mexico City; Dr. T. C. Backhouse, School of Public Health and Tropical Med., Sydney, Australia; and Dr. Elsdon-Dew, The Amoebiasis Research Unit, Durban, South Africa. Groupings of the sera tested, number of sera from each group and number and percentage positive are recorded in Table II.

The following comments may be made. a. Only one, or 2% of 48 control sera from persons without clinical amebiasis or whose stools are negative for *E. histolytica* was positive by the hemagglutination test. This person had previously traveled in the tropics.

b. Sixty-one or 98% of the sera from 62 persons with clinical amebiasis showing either a current history of dysentery or diarrhea, chronic intestinal amebiasis or amebic liver abscess, yielded a positive hemagglutination test. The one negative serum was drawn 6 days after onset with dysentery which was the shortest period after onset of any case reported in our series. (See Note(†), Table III).

c. Sera from persons who came to the hospital with complaints other than amebiasis but whose stools were positive for *E. histolytica* showed 68% to have a positive hemagglutination test. Most of these individuals were from Indonesia. Judging from the report of Magath and Meleney(8) "carriers" in the United States show a low percentage of posi-

TABLE III. Correlation of Hemagglutination and Complement Fixation Tests in Amebiasis.

*Reciprocals	Complement fixation titers*									Totals	A.C.‡
512,000								1	1	2	1
128,000						1	3		2	6	1
32,000						1	1	2	1	5	0
8,000	2	2	1	3	1	1	2			12	4
2,048	2	1	3	1	2	2	2			13	5
512	3	3	2	1	1					10	4
128	7			1						8	3
32	4	1								5	0
8	2									2	0
Neg.	46			1			1†			48	8
	Neg.	8	16	32	64	128	256	512	1024	111	26

† Taken 6 days after onset of disease.

‡ Many of the sera tested had been forwarded by mail from overseas.

tive complement fixation tests for amebiasis and it is quite possible that when sera from additional "carriers" living in temperate zones are studied, a lower percentage of positive hemagglutination tests will also be evident. This would indicate that *E. histolytica* is at times present in the human bowel without sufficient tissue invasion to stimulate antibody production.

d. Of special interest is a 38-year-old woman, continuously resident either in Michigan or California, who exhibited a mass in the colon thought to be malignant. A partial colectomy was performed. The removed mass was determined by the pathologist to be an ameboma. The serum from this patient taken a few days after surgery showed a hemagglutination titer of 1:32,000.

e. Three individuals with amebic liver abscess showed a 4-fold drop in hemagglutination test titer in 8 months after therapy and one person with chronic amebiasis showed a drop in titer from 1:128 to negative 2 months after treatment.

4. Complement fixation test. The same antigen for *E. histolytica* used in the hemagglutination test described above was employed in the complement fixation test performed in accordance with the standard procedure recommended by the Virus Laboratory, Calif. State Dept. of Public Health. It has been used at a dilution of 1:320, based on net weight of the amoebae, and was not anticomplementary when tested in a dilution of 1:80. Table III shows data obtained from 136 sera tested, 111 of which were satisfactory by both tests. As many of these sera had been shipped from overseas without refrigeration and had been stored for long periods of time, an unusually high number were anticomplementary and could not be included in this comparison. From the 111 that were satisfactory, it will be observed that a. 46 of 48 sera testing negative by the hemagglutination test were also negative by the complement fixation test. b. Of 7 sera positive with titers of 1:8 and 1:32 by the hemagglutination test only 1 gave a positive complement fixation test. c. Of 42 sera positive by the hemagglutination test at titers between 1:128 and 1:8000, 14 gave negative complement fixation tests. The remaining 28 or 67% were positive at titers ranging from 1:8 to 1:256. d. All hemagglutination tests positive to titers of 1:32,000 or above were positive by the complement fixation test. Such complement fixation test titers ranged from 1:128 to 1:1024.

It will be observed that the antigen prepared from *E. histolytica* DKB works equally well in the hemagglutination test and the complement fixation test, moreover there is no evidence of cross reaction with the *T. cruzi* controls, all of which were negative. In areas where Chagas disease occurs it is possible that positive control sera from persons infected with *T. cruzi* may be encountered.

The antigen showed no evidence of being anticomplementary when used in the complement fixation test, thus avoiding a problem often encountered by previous workers. As it is probable that this same antigen may be used successfully in other serologic tests for amebiasis, experiments to determine its chemical composition are planned.

Summary. Antigens prepared from *E. invadens* and *E. histolytica,* DKB strain grown in monomicrobial culture with *T. cruzi* in Phillips' medium have demonstrated high sensitivity and been used successfully in reciprocal hemagglutination tests with specific antisera prepared in rabbits. The antigen prepared from *E. histolytica* has also been used in both the hemagglutination test and the complement fixation test employing sera from individuals with and without amebiasis. In the hemagglutination test 98% of the former were positive while only 2% of the latter sera were positive. Sera from "carriers" most of whom lived in the tropics, gave 68% positive correlation.

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Suppression of Virus-Induced Corneal Toxicity in Rabbits by Pretreatment with Nitrogen Mustard.* (26356)

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When a large amount of Newcastle disease virus (NDV) is inoculated into the anterior chamber of the rabbit eye, there results a toxic reaction characterized by corneal opacity and characteristic microscopic changes of corneal endothelium(1,2). No evidence of increase in infectious virus is associated with the above reactions. Since an extensive infiltration of leucocytes, particularly polymorphonuclear leucocytes (PMN), into ocular tissues as well as aqueous humor, is a constant feature, it was considered possible that the infiltrating cells might play an essential role in the pathogenesis of the corneal reaction. Because of the known suppressive effect of nitrogen mustard on PMN *in vivo,* this agent was employed in an effort to study the above possibility.

Materials and methods. Virus: The L-Kan 1948 strain of NDV, a heat resistant strain(3) obtained from Dr. H. Rubin, was used in all experiments. It was cultivated in 10-day-old embryonated eggs by inoculating 10^4 plaque forming units (PFU) of stock virus into the allantoic cavity. The allantoic fluid harvested at 48 hours was pooled and centrifuged at 3000 rpm for 5 minutes to eliminate cell debris. It was dispensed into glass tubes in a volume of 1 ml per tube and tightly sealed and stored at –35°C until used. The titer of the virus in this allantoic fluid was 2×10^9 PFU with a hemagglutinin titer of 1280 units per ml. NDV infectivity was determined by using the plaque count technic on cells derived from chick embryo (4).

Rabbit. Normal healthy male albino rabbits weighing between 4 and 4.5 lb were used.

Nitrogen mustard (HN_2). Mustargen hydrochloride (Methyl-bis [beta-chloroethyl] -amine HCl) (Merck, Sharp and Dohme, Philadelphia, Pa.) was used. It was dissolved in sterile double-distilled water just before injection into rabbits.

Intraocular injection of virus. Rabbits were anesthetized with intravenous sodium Nembutal. Two minutes prior to intraocular injection of virus, a drop of Pontocaine HCl (Winthrop Labs, N. Y.) was topically applied to the cornea. The technic for injection of the inoculum into the anterior chamber of the eye has been described(2).

Preparation of corneal endothelium for microscopic examination. Corneal endothelium was stained with a solution of silver nitrate and an entire sheet of stained endothelium was separated from the cornea and mounted on a slide according to methods described previously(2).

Methods of scoring corneal lesions. 1) *Endothelial rosettes.* Microscopic lesions on the silver stained endothelium of the cornea, referred to as endothelial rosettes, were counted by examining the entire endothelium

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