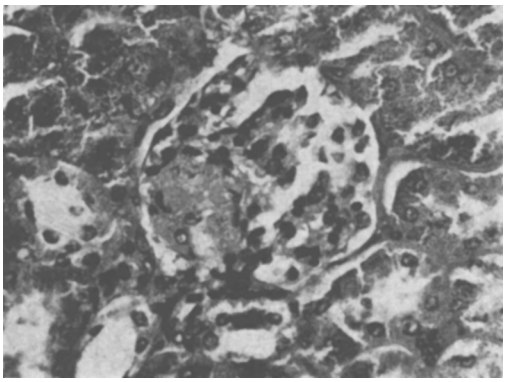

FIG. 3.
Rabbit No. 349 A.
Note the eccentric globoid blomerular lesion. The cells of the proximal convoluted tubules are laden with droplet material of protein origin.

beta-globulinemia and hypoalbuminemia in alloxan treated rabbits and in diabetic humans. This supports the statement of Blackman *et al.*, that hyaline coagula seen in the kidney, not identifiable as fibrin consist chiefly of precipitated globulins.(13)

Summary. (1) Homologous gamma globulin injected intraperitoneally elevated the level of serum globulin and depressed the albumin without inducing significant albuminuria.

(2) Characteristic glomerular and tubular changes occurred in the animals in which protracted hyperglobulinemia was produced.

(3) Serum protein levels reverted to normal when injections were discontinued.

(4) Glomerulonephritis was not present.

(5) The resemblance of the glomerular lesions to the nodular lesion of intercapillary glomerulosclerosis is noteworthy.

13. Blackman, S. S., Jr., Barker, W. H., Buell, M. V., and Davis, B. D., *J. Clin. Invest.*, 1944, v23, 163.

Received January 20, 1950. P.S.E.B.M., 1950, v73.

Effect of Single Inocula of *Endamoeba histolytica* Trophozoites on Guinea Pigs.*† 17683)

ELVIO H. SADUN, G. M. CARRERA, IRIS M. KRUPP, AND DOROTHY S. ALLAIN.
(Introduced by E. C. Faust.)
From the Department of Tropical Medicine and Public Health, Tulane University, New Orleans.

Guinea pigs are very susceptible to infections with *E. histolytica* of human origin. Carrera and Faust(1) reported that 33 out of 34 guinea pigs inoculated into the terminal ileum with doses of 200,000 or more amebae developed typical amebic lesions. Taylor and coworkers(2) found an infectivity rate of 58%, which could be increased up to 100% with certain dietary modifications. In order to study further certain aspects of this host-parasite relationship the following experiments were carried out.

Materials and Methods. A total of 84 guinea pigs of both sexes and of approximately the same weight and age were kept in separate cages through the experiments and were fed mixed Rabbit and Guinea Pig Diet. The operative procedure was the same as that described by Carrera and Faust(1) except that the inoculum was injected directly into the cecal pouch. Aseptic technic was used throughout the operation. A clone which had been isolated some months previously from our strain no. 22 was maintained, together with the bacteria associated with the parent strain, by serial passage every 48 hours in Balamuth's medium(3). The trophozoites used for inoculation were collected by pooling the content of several flasks which had been

* Supported by a grant from the Division of Research Grants and Fellowships, National Institutes of Health, Bethesda, Md.

† Thanks are extended to Dr. Huldah Bancroft, Professor of Biostatistics in our Department, for checking our statistical interpretations.

1. Carrera, G. M., and Faust, E. C., *Am. J. Trop. Med.*, 1949, v29, 647.

2. Taylor, D. J., Greenberg, J., and Coatney, G. R., *J. Parasit.*, 1949, v35, 24.

3. Balamuth, W., *Am. J. Clin. Path.*, 1946, v16, 380.

TABLE I.
Effect of Graded Numbers of *E. histolytica* on Production of Intestinal Lesions in Guinea Pigs.

Group No.	No. of animals	No. of amebae in inoculum	Results at necropsy within 20 days from time of inoculation: No. of animals in group: Without lesions	No. of animals in group: With lesions	Accumulative total: Without lesions	Accumulative total: With lesions	% infectivity
I	11	200,000 destroyed by freezing	11	0	46	0	0
II	7	100	7	0	35	0	0
III	8	500	8	0	28	0	0
IV	8	1000	7	1	20	1	5
V	8	5000	4	4	13	5	28
VI	9	10,000	5	4	9	9	50
VII	13	100,000	2	11	4	20	83
VIII	9	500,000	1	8	2	28	93
IX	7	2,500,000	0	7	1	35	97
X	4	5,000,000	1	3	1	38	97

Fifty per cent infective dosage = 10,000 amebae.

inoculated with a known number of trophozoites 48 hours before. The pooled material was then centrifuged for approximately 5 minutes at a speed of 1800 rpm and the supernate removed. Centrifugation at the above speed up to 15 minutes has been found to produce no harmful effect on the viability and multiplication of the amebae. The number of trophozoites per ml of medium was calculated after counting a representative sample in a hemocytometer chamber. Dilutions with the supernate were then made to obtain the desired number of amebae per ml. The volume of inoculum was always the same; *viz.*, 1 ml for the guinea pigs that received less than 100,000 amebae each, and 2 ml for those that received 100,000 or more. The animals were weighed on the day of inoculation and again every 5 days up to the time of necropsy 20 days later. They were sacrificed by exsanguination under nembutal anesthesia. Immediately thereafter the body weight was recorded, the abdominal cavity was opened and the intestines were carefully examined for any gross evidence of ulceration. A sample of the cecum was promptly fixed in Bouin's fluid for histologic examination. Fecal films made from the cecal content were then examined under the microscope for the presence of *E. histolytica*. When guinea pigs were found dead they were inspected for gross evidence of intestinal ulceration and the cecum was examined for the presence of amebae. Whenever the guinea pig had died within a few hours, histologic examination of the cecum was carried out as above.

A few animals that died as a result of anesthesia or of other causes unrelated to amebiasis are not reported. Eighty-four guinea pigs included in this series belonged to 10 groups. Seven each received 100 amebae (Group II), 8 received 500 (Group III), 8 received 1,000 (Group IV), 8 received 5,000 (Group V), 9 received 10,000 (Group VI), 13 received 100,000 (Group VII), 9 received 500,000 (Group VIII), 7 received 2,500,000 (Group IX) and 4 received 5,000,000 (Group X). It was planned that if none of the animals of Groups II and III became infected they were to serve as controls.

In order to test whether the lesions which might be produced were derived from concentrates of bacteria and/or from products of decomposed amebae, about 50 ml of medium containing 100,000 active amebae per ml were dispensed into 2 tubes. The contents of one tube were immersed in a bath of alcohol and dry ice which had attained a temperature of approximately –70°C and were rapidly twirled to insure quick freezing. After 5 minutes the tube was placed in the incubator at 37°C and thawed. Samples from this tube were then examined by direct micro-

TABLE II.
Effect of Graded Numbers of *E. histolytica* on the Survival of Guinea Pigs.

Group No.	No. of amebae in the inoculum	Results within 20 days from time of inoculation					
		No. of animals in group		Accumulative total		% mortality	Avg from inoc. to death, in days
		Alive	Dead	Alive	Dead		
I	200,000 destroyed by freezing	11	0	53	0	0	—
II	100	7	0	42	0	0	—
III	500	8	0	35	0	0	—
IV	1000	8	0	27	0	0	—
V	5000	4	4	19	4	17	10.7
VI	10,000	5	4	15	8	34	10.7
VII	100,000	5	8	10	16	61	10.4
VIII	500,000	3	6	5	22	81	9.2
IX	2,500,000	1	6	2	28	93	6.5
X	5,000,000	1	3	1	31	97	8.0

$$\text{Proportional distance} = \frac{50 - 34}{61 - 34} = 0.59.$$

$$LD_{50} = 10{,}000 + (90{,}000 \times 0.59) = 63{,}000 \text{ amebae.}$$

scopic examination and by culture and found positive for bacteria and negative for living amebae. Two ml of the medium thus treated were then inoculated intracecally into each of 11 guinea pigs (Group I). One ml of the contents of the tube that had not been immersed in the freezing bath was inoculated into each of 4 guinea pigs, which became infected.

Results. Relationship between size of inoculum and infection. As indicated in Table I, twenty days after inoculation, there was no evidence of infection in the controls that received the residue from 200,000 amebae treated by rapid freezing (Group I). In the groups inoculated with living amebae the number of infected animals was proportionately greater as the size of the inoculum was increased.

The ED_{50} for guinea pigs under these experimental conditions was 10,000 amebae. Animals were regarded as infected only when they presented typical gross or microscopic lesions in which trophozoites of *E. histolytica* were seen; and in every case in which a diagnosis of amebic infection was made by gross examination of the lesions and by finding amebae in the cecal content microscopic examination revealed typical ulcers with amebae. *Vice versa*, in every case in which no lesions were observed after a careful gross examination and no amebae were found in the cecal content, no lesions were observed by microscopic examination. The amebae observed in the cecal content of animals found infected at necropsy 20 days after infection were often few in number, rounded up and moribund, while those observed in guinea pigs that died as a result of the infection several days after inoculation were abundant, very active, and not uncommonly were seen with ingested red blood cells. In the great majority of animals the infection overwhelmed the natural resistance of the guinea pigs and caused their death. A perusal of Table II shows that the number of animals which died as a result of the infection was proportionately greater as the size of the inoculum was increased. The LD_{50} was found to fall between 10,000 and 100,000 amebae (Groups VI and VII). By interpolation of the proportional distance it was found to be approximately 63,000. Death occurred in all cases between 5 and 17 days after inoculation. On the average, death occurred 9.3 days after inoculation and the greatest number of guinea pigs died on the eighth day. In general, the size of the inoculum was found to have an inverse relationship to the average time between inoculation and death. While not significant from one size of

TABLE III.
Effect of Body Weight of Guinea Pigs on Their Susceptibility to Infection with *E. histolytica.*

Group No.	No. of amebae in inoculum	Animals that became infected		Animals that resisted infection	
		No.	Mean wt	No.	Mean wt
IV	1000	1	195	7	256
V	5000	4	205	4	313
VI	10,000	4	230	5	244
VII	100,000	11	193	2	163
VIII	500,000	8	219	1	265
IX	2,500,000	7	204	0	—
X	5,000,000	3	183	1	190
Totals		38		20	
Avg			205		252

inoculum to another, the average time from infection to death of the guinea pigs that received 5,000 amebae each (Group V) and those that received 2,500,000 amebae each (Group IX) was found to be significant. The values obtained using the *t* test(4) are:

$$t = 3.94;\ 0.01 > p > 0.001.$$

The apparent rise in the average time for the group receiving an inoculum of 5,000,000 amebae may be due to the fact that only 4 animals were inoculated.

The relationship between body weight and infection. Although in the course of these experiments guinea pigs of approximately the same size and age were chosen, some observations were possible on the relationship between body weight and infection. First, infection with *E. histolytica* produced a rapid loss of weight of the experimental animals. The mean weight at the time of inoculation of the 31 guinea pigs that died was 202 g, while at the time of death, on the average 9.3 days later, it was 151 g. Also, the weight at time of inoculation seemed to have an inverse correlation with the degree of susceptibility of the guinea pigs to infection with *E. histolytica.* Table III shows that in every group but one (Group VII) the average weight at the time of inoculation of the guinea pigs which became infected, was considerably lower than that of the guinea pigs which resisted the infection. On the average, the mean weight of 38 guinea pigs which later became infected, was 205 g at the time of inoculation, while that of the 20 guinea pigs that did not become infected was 252 g.

The difference in weight at the time of inoculation of the guinea pigs which later became infected and those that were not found infected (Groups IV through X) was tested by comparing the difference in weight to the standard error of the difference. The values obtained are:

$$\frac{\text{actual diff.}}{\text{S.E. diff.}} = \frac{47}{15} = 3.15$$

For this value P = 0.002. The difference is therefore regarded as significant.

Histopathologic studies. These were limited to the cecum, because as previously reported(1) the cecum is always involved whenever amebic lesions occur in the guinea pig. Five representative sections per animal were studied. Prompt fixation of the tissues in Bouin's fluid, and staining of the sections with hematoxylin and eosin allowed the amebae to be readily distinguished. The architecture of the amebic lesions in the present series, was found to be similar in all repects to that previously reported(1) *viz.*, extensive superficial areas of invasion of the mucosal epithelium, and narrower, deeper lesions with an occasional perforation of the whole thickness of the intestinal wall. Whenever the amebae were found in association with large bacterial colonies, there was intense local cellular exudation and necrosis. But at times amebae were seen colonizing the subepithelial tissues, without evidence of much host-tissue reaction and without apparent concomitant bacterial invasion. The presence of *Balan-*

4. Fisher, R. A., *Statistical Methods,* 10th Ed., 1948, 122.

tidium caviae in some of the infected animals as well as in some of the controls did not appreciably alter the character of the normal mucosa. Whenever this ciliate was seen in the neighborhood of an amebic lesion, it did not appear to be in the advancing border of the lesion but was situated in the necrotic menstruum filling the crater of the ulcer. In rare instances it was seen in the subepithelial spaces. In the control animals and those reported as negative no ulcers of any kind were observed in the cecal mucosa; on the other hand, all the animals found to be positive by gross examination and by direct smear of the cecal feces, were found on histologic examination to have typical amebic lesions.

Summary. Guinea pigs were inoculated intracecally with graded single doses of from 100 to 5,000,000 amebae. None of the animals that were given less than 1,000 amebae developed infection. The 50% infective dose under the experimental conditions was 10,000 amebae. The mean weight of the guinea pigs that later became infected was significantly smaller than that of those which resisted infection. Thirty-one out of 50 guinea pigs that received single inocula of from 5,000 to 5,000,000 amebae died as a result of the infection. The LD_{50} under the experimental conditions was 63,000 amebae. Death occurred in all cases between 5 and 17 days after inoculation, on the average 9.3 days after inoculation, and the greatest number of guinea pigs died on the eighth day. In general, the size of the inoculum was found to have an inverse relationship to the average time between inoculation and death. Histopathologic studies confirmed necropsy examination. All animals found to be positive by gross examination and by direct smears of the cecal content were found to have typical amebic lesions, while in none of the controls and of those reported as negative were ulcers of any kind observed by histologic sections.

Received January 20, 1950. P.S.E.B.M., 1950, v73.

Relationship Between Physiological Disposition and Adrenolytic Action of 2-(N-p'-tolyl-N-m'hydroxy-phenyl) - aminomethyl) - imidazoline hydrochloride (C-7337).* (17684)

PASSIE S. JONES, HARRY A. WALKER AND ARTHUR P. RICHARDSON.
From the Department of Pharmacology, Emory University School of Medicine, Emory University, Ga.

The relationship of physiological disposition to pharmacological action of adrenolytic agents has not been widely studied because of the lack of sensitive chemical tests for those agents which have been most extensively used up to the present time. The discovery of 2-(N-p'-tolyl-N-m'hydroxyphenyl-aminomethyl)-imidazoline hydrochloride (C-7337) has for the first time made such investigations possible since this compound may readily be determined in biological fluids and is a potent adrenolytic agent of low toxicity(1-5).

Chemical methods. A number of possible reactions involving the free phenolic group of C-7337 was investigated. The most satisfactory was found to be a modification of the 4-amino-antipyrine color reaction(6). The

* The authors wish to thank Dr. F. F. Yonkman of the Ciba Pharmaceutical Products, Inc., for the generous supply of C-7337.

1. Herrold, E. A., Cameron, A., Earl, A., Roth, F., Smith, N., Sorenson, E., and Craver, B. N., *Fed. Proc.*, 1949, v8, 302.

2. Warren, M. R., Woodbury, R. A., and Trapold, J. H., *Fed. Proc.*, 1949, v8, 343.

3. Grimson, K. S., Chittum, J. R., and Longino, F. N., *Fed. Proc.*, 1949, v8, 61.

4. Meier, R., Yonkman, F. F., Craver, B. N., and Gross, F., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 70.

5. Walker, H. A., Wilson, S., Heymans, C., and Richardson, A. P., *Arch. int. Pharmacodyn.*, 1950, in press.