ment membrane. In more advanced lesions, gamma globulin was also seen in many of the swollen endothelial cells as well as in the precipitated protein within the capillary lumen and in Bowman's space.

In animals which survived 7½ months, and which had not received DOCA for the last 3½ months, no active vascular lesions were found. In only a few of the thickened vessel walls was gamma globulin still present. In the kidney focal glomerulo-fibrosis and cystic dilatation of the tubules without gamma globulin were noted.

Discussion. Localization of specific fluorescence in the vessel wall suggests binding of rat gamma globulin in the damaged smooth muscle. Control experiments with anti-rat plasma rabbit serum from which anti-rat gamma globulin had been removed yielded no specific fluorescence in the necrotic vessels. This would suggest that rat gamma globulin is selectively deposited in the wall of the damaged vessel and that this is not the result of simple diffusion of plasma constituents from the circulating blood into the tissues.

The observation by others of fibrin in vascular lesions of human peri-arteritis nodosa (12) and of serum albumin along with gamma globulin in vascular lesions of experimental serum sickness(3) should caution against hasty conclusions as to the significance of these findings.

In the kidney on the other hand, increased amount of gamma globulin in the more damaged glomeruli may merely reflect altered permeability secondary to severe capillary damage.

Summary. Experimental necrotizing arteritis was produced in female Sprague-Dawley rats by administration of large doses of DOCA and NaCl after unilateral nephrectomy. Presence of RGG in damaged vessel walls was demonstrated by fluorescence antibody technic of Coons. Gamma globulin is deposited in smooth muscle of damaged small arteries, especially in early phases of vascular damage, suggesting that antibody may not be required for this process.

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Microfluorimetric Evidence of Antigenic Difference Between *Entamoeba histolytica* and *Entamoeba hartmanni*. (25186)

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The complex of amebae known as *Entamoeba histolytica* includes: a large hematophagous form (20-40 μ diameter) associated with acute amebic dysentery; a medium sized form (10-20 μ) generally found in asymptomatic carriers; and a small organism (5-11 μ) generally regarded as never associated with disease(1,2). Burrows(3) recently reviewed the literature dealing with so-called "small race" and, in addition, contributed new morphological data comparing small and medium sized amebae. He concluded that the "small race" was properly a different species, and that its original designation of *Entamoeba*

hartmanni (Prowazek, 1912) was correct. This distinction is of practical, as well as academic, importance since 30 to 50% of human infections assigned to *E. histolytica* are probably due to *E. hartmanni*(1). Asymptomatic infection with the former calls for specific therapy due to its potential pathogenicity, whereas the latter may be ignored. Conventional serologic evidence for the relationship of the 2 forms is meager but, in general, supports the hypothesis of antigenic distinction between the 2 forms(3). Using an immunocytochemical technic, we demonstrated previously that fluorescein-labeled antisera to *E. histolytica* and *Entamoeba coli* stained the homologous species brightly, but stained *E. hartmanni* poorly, providing direct microscopic evidence of antigenic differences among the 3 amebae(4). Staining results were evaluated visually and no objective quantitation of fluorescence was made. The present communication reports use of a microfluorimeter to measure quantitatively the fluorescence of amebae stained with fluorescent antibody, and shows that the results obtained offer quantitative evidence of species distinction between *E. hartmanni* and *E. histolytica*. Complete reports dealing with all phases of this long-term project will be reported.

Materials and methods. Antiserum to strain 22 of *E. histolytica* was prepared in rabbits by inoculating them with amebae growing in culture with an unidentified anaerobic, spore-forming bacterial species. The globulin portion of the serum was labeled with fluorescein (5). Four strains of *E. histolytica* (K-9, M-18, 22 and Huff), and one each of *E. hartmanni* (335), *E. coli* (W28), and a free-living ameba, *Entamoeba moshkovski,* were studied. Ameba smears were prepared by suspending saline-washed organisms from single tubes of 48 hour cultures in 0.15 M acetate buffer, pH 4.7, and drying drops of suspension on to slides. Each smear contained from 1000 to 4000 amebae. Preparations were exposed to 0.1 ml of anti-*E. histolytica* labeled conjugate, and duplicate preparations were exposed to the same volume of normal, labeled rabbit globulin. Prior to this, both solutions had been adjusted by fluorimetric means to the same content of protein-bound fluorescein. After one hour incubation, smears were washed, dried, mounted in glycerol, and examined. Fluorescence intensity measurements were taken of single, entire amebae with the background blocked off by a variable iris diaphragm in the focal plane of the microfluorimeter(6). At the same time, the diameter of each ameba was ascertained, since the diaphragm had previously been calibrated in terms of the microscopic field circumscribed at any setting. A "brightness/unit area" figure was computed for each organism in terms of amperes of phototube current/square μ of ameba. Average brightness of 50 amebae compared staining reactions of the different strains. Since amebae prepared as described above tended to stain to varying degrees even with normal, liver-absorbed conjugate(5), staining results were evaluated in terms of ratio of reaction with anti-*E. histolytica* conjugate to that with normal conjugate. Specificity of staining with 2 of the *E. histolytica* strains was demonstrated in separate experiments by inhibition test of Coons and Kaplan (5) as modified by the author(7).

Results. Pertinent data are summarized in Table I. Average diameters of 25 living ame-

TABLE I. Relative Uptake of Fluorescent Globulin by Seven Strains of Amebae.

Ameba strain	Avg diameter of 25 living amebae	Avg brightness of 50 amebae exposed to labeled E.h. antiglobulin	Avg brightness of 50 amebae exposed to labeled Normal globulin	Ratio of antiglobulin : normal globulin
E. histolytica K-9	18.0 ± 1.6*	4.11 ± .36	1.23 ± .28	3.3
" M-18	15.8 ± 1.6	4.05 ± .40	1.15 ± .12	3.5
" 22	15.0 ± 1.4	3.10 ± .22	.81 ± .14	3.8
" Huff	not done†	3.01 ± .32	1.46 ± .18	2.1
E. hartmanni 335	11.4 ± 1.3	1.31 ± .14	2.79 ± .34	.46
E. coli W28	23.8 ± 1.6	1.97 ± .22	2.95 ± .41	.67
E. moshkovski	15.7 ± .78	.81 ± .07	.95 ± .08	.85

* Confidence intervals given represent twice stand. errors of means.

† Avg diameter of stained amebae of this strain was the same as that of strains M-18 and 22, indicating that living amebae were probably about 15 μ in size.

bae of each strain (except Huff) are presented to show significant size differences between *E. hartmanni* and the 4 strains of *E. histolytica*. Absolute brightness levels of *E. histolytica* strains were considerably higher than those found for other amebae. However, because of the varying uptake of normal conjugate, differences between strains are made more evident by comparing the ratios of brightnesses resulting from reaction with the 2 conjugates. It is clear from these ratios that the 4 strains of *E. histolytica* took up considerably more fluorescent antiglobulin than normal globulin, from 2.10 to 3.8 times as much. On the other hand, the 3 other amebae took up more of the normal conjugate than of the anti-*E. histolytica* conjugate. Of the 4 strains of *E. histolytica*, Huff was significantly different from the other 3 strains, whereas the latter differed from each other insignificantly.

Discussion. The magnitude and consistency of the differences shown between 4 known *E. histolytica* strains and 2 large, non-*histolytica* species, *E. coli* and *E. moshkovski*, are a strong indication that true antigenic differences are being measured by this technic. The fact that the small ameba, *E. hartmanni*, falls definitely within the non-*histolytica* type of reaction is evidence that it, too, is antigenically distinct from *E. histolytica*.

The difference between *Eh*-Huff and the 3 other strains of *E. histolytica* correlates well with invasiveness of the strains in hosts from which they were originally obtained. *Eh* K-9, M-18 and 22 were all isolated from patients with active amebic dysentery or disseminated amebiasis. *Eh*-Huff was the only strain derived from a non-symptomatic individual who carried the infection without apparent ill effects for several years(8). Thus, there is indication here of a relationship between invasiveness and antigenic character. The bacterial associates of these strains and *E. hartmanni* were non-defined but probably similar, since about 2 weeks before the experiment was performed, an emulsion from the same stool specimen was added to them to stimulate growth of the amebae.

It is obviously necessary to extend these observations before making a final commitment, but it appears that use of "*Entamoeba hartmanni*" to designate the so-called "small race-*histolytica*" may be considered to have a serologic basis in addition to the other bases described in the literature. Furthermore, the microfluorimetric approach used in this work may make it possible to recognize different serotypes within the same species, thus contributing to a better understanding of amebiasis.

Summary. Fluorescence of 7 ameba strains was measured with a microfluorimeter following their exposure to fluorescent anti-E *histolytica* and fluorescent normal globulin. Four strains of *E. histolytica* were similar to each other, but were markedly different from *E. coli*, *E. moshkovski* and so-called "small race *E. histolytica*" = *E. hartmanni*. In the *E. histolytica* group, 3 originally invasive strains differed significantly from one non-invasive strain.

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