A calibration of the pipettes under conditions of use was accomplished using potentiometric titration of the acid delivery from each pipette. A calibrated 10-ml microburette was used for the titrations, the capillary tip dipping into the solution being titrated. The sulfuric acid delivered by the micropipettes was 13.54 *N*; titrant alkali was 0.018 *N*. The titration ratio between acid and alkali was determined for 1.00-ml samples of the diluted acid after accurate 1 : 100 dilution, using Normax pipette and volumetric flask. Replicate titers to pH 5.3[1] were 7.361, 7.358, and 7.360 ml, yielding an average of 7.360 ml ± 0.003 SE. Considering the dilution factor, the average titer thus represents a 10.00 µl delivery of the 13.54 *N* acid. The micropipette volume may be calculated from the relation: Average volume = (average titer in ml/7.360 ml) × 10.00 µl. The chief source of error in this calibration method is the error of dilution of the concentrated acid.

TABLE 1
COMPARATIVE CALIBRATION OF 10-MICROLITER PIPETTES

Pipette No.	Mercury content		Acid delivery	
	Replicates	Av ± SE*	Replicates	Av ± SE*
1	3	9.968 ± .006 µl	2	9.961 ± .009 µl
2	4	9.858 ± .020	2	9.841 ± .000

* Standard error = $\sqrt{\frac{\Sigma(d^2)}{N(N-1)}}$

For comparison, the pipettes were also calibrated with mercury by the method of Kirk (*1*). The results given in Table 1 show that the two methods agree to better than 0.1% and demonstrate an equality between content and delivery for silicone-treated micropipettes.

Complete delivery of the concentrated acid from the treated pipettes is indicated by the close agreement among replicate titers of single deliveries (without rinses), and by the agreement obtained between mercury content and acid delivery. In order to confirm complete delivery, the second pipette was tested for "holdup" of acid by rinsing with several portions of the titration mixture after the end point of pH 5.3 was reached. This caused a shift of less than 0.03 pH unit below the end point value. A retention of 10^{-5} µl of 13.54 *N* acid on the inner surface of the pipette would result in lowering the pH of the unbuffered titration mixture by more than 0.3 pH unit. Therefore, the "holdup" of acid by this pipette was less than 10^{-6} µl.

The acid delivery method of calibration may perhaps be preferred by workers who have used neither method previously. It has the advantage of simulating conditions of pipette use by the operator, thus providing a check of the operator's technique at the time of calibration. Presumably the potentiometric titration of acid delivery could be replaced by titration using the double indicator of Hawes and Skavinski (*4*) without great loss of accuracy for the calibration procedure.

Several precautions should be noted with regard to the use of a silicone on pipettes. The coating becomes imperfectly water-repellent unless carefully stored completely dry or completely wet so that occasional repetition of the silicone treatment is necessary. Unless it is subsequently demonstrated that the technique of silicone removal and recoating does not lead to changed pipette volumes, calibration after each cycle is necessary.

Silicone coating of microliter pipettes of the self-adjusting type (Microchemical Specialties No. 282A–283B) provides even greater convenience in their use. Certain of these pipettes, before treating with silicone, will hold several microliters of drainage liquid between the upper capillary and the bulb, which is difficult to recover by rinsing. After siliconing, such pipettes drain completely so that no visible trapping of the drainage volume occurs.

It is apparent from these results that water-repellent coating, such as that provided by Desicote, may introduce an era of accurate and convenient volume measurement for microliter chemistry as well as for macro-analysis. Such a coating provides equality between content and delivery for these 10-µl pipettes, and presumably for larger micropipettes as well (*3*).

[1] The end point was arbitrarily taken as pH 5.3 to minimize carbon dioxide absorption during the titration.

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Propagation of a Strain of *Endamoeba histolytica* in Tissue-bearing Culture

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It is not known whether *Endamoeba histolytica* can invade host tissues by virtue of its own invasive properties or whether in some way certain bacteria lend assistance in the invasion of the tissues and, perhaps, contribute to the production of the characteristic lesions seen in patients with amebiasis. The experiments to be reported here were undertaken with the thought that if *E. histolytica* could be established in cultures containing animal tissues of various kinds, free of bacteria, it would be possible to study (*a*) the mode of entry of the amebae into the tissues, (*b*) the

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effects of the amebae on these tissues, (*c*) tissue specificity of the amebae, if any, (*d*) the influence of certain bacteria on the ability of the amebae to invade and damage the tissues, and (*e*) the effects of therapeutic agents on the amebae.

Chick embryo tissues were used in the experiments because they were readily available and tests could be made using embryos of specified ages. Since *E. histolytica* propagates only under anaerobic conditions by all known cultural techniques, it was desirable to use a tissue capable of existing and probably proliferating at low oxidation-reduction potentials. Accordingly, chick embryos of 4–5 days' incubation at 39° C were used. The source of eggs for these experiments was a hatchery with pullorum-free, heavy breed chickens.

Two methods were used. The first was as follows: The embryo was removed from the shell, washed with Hank's balanced salt solution, and cut into three or four segments 2–4 mm in diameter. The cut segments were transferred to a 16 × 150 mm culture tube containing 2 ml of tissue culture nutrient fluid made up as follows:

Hank's balanced salt solution	22.5 ml
Normal horse serum	25.0 "
Chick embryo extract (EE_{50})	1.5 "
Penicillin G (2500 u)	1.0 "
Total	50.0 "

Approximately 3000 *E. histolytica* trophozoites (200 strain[3]), washed with balanced salt solution and suspended in chick embryo extract (0.2 ml EE_{50}), were then inoculated into the tissue culture. After inoculation, the cotton-plugged cultures were placed in a Brewer anaerobic jar, and the air was replaced with hydrogen. At the end of 48 hr incubation at 37° C, the cultures were removed from the jar and transplants made by transferring small portions of the blocks of tissue and a small amount of the fluid (0.1–0.15 ml) to freshly prepared tubes made up as before. At the same time, small portions of the blocks of tissue were placed on a slide and examined for amebae. Bits of tissue were also inoculated into S-F medium (*1*) as a further check on the presence of amebae. To test for bacterial sterility, approximately 1.0 ml of the culture was transferred to a Wassermann tube and treated with penicillinase. This material was inoculated into a shake tube containing 15 ml of anaerobic agar (BBL[4] thioglycollate agar #146) and examined for bacterial colonies after 72 hr at 37° C. The remaining tissue blocks were fixed in Bouin's fluid for sectioning and staining.

The second method was a modification of the first. The chick embryos were ground as thoroughly as possible in a mortar without abrasive, and the macerated tissues suspended in sufficient tissue culture nutrient fluid to make a 10–20% suspension. Varying quantities of the suspension were added to a series of four culture tubes (16 × 150 mm), 1.0 ml to the first tube, 0.5 ml to the second, 0.25 ml to the third, and 0.1 ml to the fourth. Sufficient nutrient fluid was then added to each tube to make the total volume 2.0 ml. These tubes were inoculated with *E. histolytica,* strain 200, in the same manner as described above, and placed in the Brewer anaerobic jar. At the end of 48 hr of incubation at 37° C, transplants were made by transferring 0.25 ml to new cultures. Transfers were also made to S-F medium and to thioglycollate agar shake tubes. As a further check on the bacterial sterility, inoculations were made into fluid thioglycollate dextrose medium, on blood agar plates, into fluid thioglycollate medium enriched with 25% normal horse serum, and on Sabouraud's agar slants.

The results obtained using the first method with tissue blocks are summarized in Table 1.

TABLE 1

RESULTS OF ATTEMPTS TO PROPAGATE *E. histolytica* (STRAIN 200) IN TISSUE-BEARING CULTURES CONTAINING BLOCKS OF TISSUE FROM 4- TO 5-DAY CHICK EMBRYOS

Transplant	Amebae		Bacterial culture (No. colonies)
	Direct examination	Culture in S-F medium	
1	+	+	20
2	+	+	20
3	±	+	0
4	±	0	0
5	+	+	0
6	0	0	0

As shown in Table 1, five successful 48-hr transplants of the 200 strain of *E. histolytica* were accomplished, as evidenced by the presence of amebae in the tissues, either on direct examination or in subcultures into S-F medium, or both. None was present on the sixth transplant, and the series was discontinued after two more transplants not shown in the table. Attempts to repeat the above experiments under aerobic conditions have failed.

Direct examination of the tissues under the microscope revealed that there were amebae present in the tissue blocks but not in the surrounding fluids. Confirmation of the presence of the amebae within the blocks was obtained by examining sections of these tissues stained with hematoxylin and eosin after Bouin's fixation. Study of these sections revealed typical *E. histolytica* trophozoites lying between the tissue cells. There was little evidence of cellular reaction or change around the trophozoites, and the cells of the chick tissue appeared to be in good condition throughout the blocks. The cytoplasm of the amebae appeared quite clear and homogenous. Rare cytoplasmic inclusions were seen in the trophozoites. These might have been nuclear remnants of phagocytized tissue cells.

The results obtained with the second method, using macerated chick embryo tissue, are shown in Table 2.

[3] The 200 strain of *E. histolytica* was obtained from M. C. McCowen, of the Lilly Research Laboratories. It was obtained by Mr. McCowen from C. W. Rees, of the National Institutes of Health.

[4] Baltimore Biological Laboratories.

Table 2 reveals that 10 successful 48-hr transplants of the 200 strain of *E. histolytica* were made in medium containing either 1.0 or 0.5 ml of macerated chick embryo tissue. These transplants are being continued, and all evidence indicates that the amebae can be maintained in this manner indefinitely. Direct examination of these transplants at the time of transfer revealed the presence of numerous amebae, counts indicating 3000–6000 trophozoites/ml. The transplants in the two series with 0.5 and 1.0 ml of tissue suspension routinely contained similar numbers of amebae. The series of transplants in which 0.25 ml of the tissue suspension was used had similar numbers of amebae present in the cultures for the first 6 transplants. There were but few amebae present in the seventh transplant and none in the eighth, as confirmed by the negative culture to S-F medium. The series of transplants in which 0.1 ml of the macerated tissue suspension was used did not contain amebae after the sixth transplant.

There were large numbers of isolated tissue cells along with numerous bits of tissue in which the cells had not been completely broken apart by the grinding process. Many of these cells were presumably viable, as evidenced by the finding of pulsating heart muscle on one occasion. Whether any tissue proliferation was occurring in these cultures was not determined. No positive subcultures for bacteria or fungi were obtained by any method used.

The results shown in Table 2 would appear to indicate that the 200 strain of *E. histolytica* can be transplanted anaerobically for an indefinite period in the presence of chick embryo tissue in tissue culture nutrient fluid. There is little doubt that the amebae propagated in the substrate. Since on each transplant 0.25 ml of the culture was inoculated into 2 ml of new culture, constituting a one-in-nine dilution, the 10 transplants represented a dilution of the original inoculum of more than 1 in 10^9. Failure to subculture bacteria from any of these transplants by any technique so far employed has indicated that the cultures were bacterially sterile, but further evidence is needed.

Failure to maintain the amebae beyond 5 transplants in large blocks of chick embryo tissues, as indicated in Table 1, in contrast to the results with macerated tissues may possibly be explained on the basis of the observation that where blocks were used the amebae were present only within the blocks; hence, on transfer of portions of the block, the amebae may not have been able to leave the block easily and enter the new ones.

The significance of finding trophozoites in the tissue blocks is not at present known. The failure to observe marked changes in the tissue cells around the amebae was interpreted as indicating that the amebae were not gaining entry into the tissues by means of some lytic ferment. This would support the hypothesis that entry of the amebae under these circumstances might be purely mechanical. Further investigation of this is now in progress.

In more recent experiments it has been found that suspensions of brain, liver, heart, or thigh muscle tissues of 10-day chick embryos also support excellent propagation of the 200 strain of *E. histolytica*. It has also been found that the suspensions of 4- to 10-day chick embryo tissues support propagation of the amebae when incubated aerobically, provided the tissue concentration is high.

A more complete report of the above findings and an extension of the observations on the entry of the amebae into various types of tissue will be forthcoming in the near future.

TABLE 2

RESULTS OF TRANSPLANTS OF THE 200 STRAIN OF *E. histolytica* IN TISSUE-BEARING CULTURES CONTAINING VARYING AMOUNTS OF MACERATED 4- TO 5-DAY CHICK EMBRYO TISSUES

Tissue suspension 10–20% (ml)	Nutrient fluid (ml)	Direct microscopic examination for amebae*										Subculture to S-F medium†									
		Transplant										Transplant									
		1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
1.0	1.0	++	++	++	++	++	++	++	++	++	++	+	+	+	+	+	+	+	+	+	+
0.5	1.5	++	++	++	++	++	++	++	++	++	++	+	+	+	+	+	+	+	+	+	+
0.25	1.75	++	++	++	++	++	++	+	±	±	0	+	+	+	+	+	+	+	0	0	0
0.1	1.9	+	+	+	+	+	±	±	±	0	0	+	+	+	+	+	+	0	0	0	0

* Readings: ++ = 3–5 amebae per low power field; + = 1–3 amebae per low power field. These examinations were done by placing a drop of the culture on a slide and applying a cover slip.

† The finding of *E. histolytica* in the S-F medium subcultures after 48 hr incubation at 37° C was considered positive, and no attempt made at quantitation.

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