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THE OCCURRENCE OF TWO SIGNIFICANTLY DISTINCT RACES OF ENDAMOEBA HISTOLYTICA*

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Some 20 years ago a number of papers were published reporting detailed measurements of cysts of *Endamoeba histolytica*. A number of investigators had reported variation in cyst size earlier, but in 1917 Wenyon and O'Connor (1) first definitely stated that this species was composed of races distinguishable by the size of their cysts. Dobell and Jepps (2) independently came to the same conclusion from a study of 200 patients among whom 125 had cysts with diameters above ten microns and 75 had cysts below this size. Two of the cases showed both large and small cysts. The implication of this paper is that there are two races, one with large cysts 10–15 microns, and the other with small cysts 7–9 microns. In 1918 these same authors (3) reported a beautifully precise experimental investigation of the cyst size, carefully measuring 500 cysts from each of seven patients. They concluded that they had demonstrated at least five races, and that this probably represented only a minimum number. We shall carefully examine this conclusion below. The same year Smith (4) reported the measurements of 1000 cysts from 30 patients and found distributions in size that led him to doubt the existence of more than two or possibly three strains. In 1919 Dobell (5) reiterated his earlier results and cast aspersions on the method used by Smith, who answered in the same year (6) with further experimental results and decided that there was no reason to modify his earlier conclusions. Since 1919 there appear to be no further reports dealing specifically with cyst measurements as a basis for distinguishing races or strains of this species.

In the current literature one finds casual mention of different strains with the usual division being into "large" and "small" races. In 1923 Boeck (7) in describing this organism said, "probably three size races; a small race six microns to nine microns in diameter; a medium sized race 10 to 12 microns in diameter; and a large race 13 to 15 microns in diameter." He gives no evidence for this tripartite division. Statements in current standard textbooks are even less specific. Hegner (8) says, "Different size races are indicated by differences in size of the cyst." Chandler (9) has essentially the same statement, while Craig and Faust (10) say that "Dobell and others have shown that there are distinct strains or races of this parasite which produce large and small cysts. . . ." Craig (11) in his standard work on amebiasis mentions the division into large

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[1] Statistical analysis by the junior author.

and small races and refers to Dobell and Jepps' division into five races. Other reports in the literature either do not mention cyst size at all or very roughly classify cysts into "large" and "small" races.

In summary, the current situation seems to be a recognition of the fact of variation in cyst size, uncritical acceptance of Dobell and Jepps' hypothesis that the species has many races, with the paradoxical situation that most authors speak only of two races, one large and one small. Withal, there is an apparent tendency to disregard size differences in most experimental work on amebiasis.

It is the purpose of this paper to demonstrate that there are only two significantly distinct races of this species and to point out that these differ not only in respect to cyst diameters, but also in some other aspects.

I. RACIAL EVIDENCE PRESENTED BY DOBELL AND JEPPS

The approach to this problem must begin with a critical examination of the work of Dobell and Jepps (3) which, because of the precision of experimental technique and the certainty with which the authors express their conclusions, has become the classic study on cyst sizes.

These investigators measured 500 cysts from each of seven different patients. For two of these patients, H8 and H7, unimodal curves were obtained, the modes of cyst diameters falling at 6.0 and 7.5 microns respectively. For two more, also having unimodal curves, E42 and B1, the diameters had modes at 12.0 and 13.5 microns respectively. On the basis of these modes and without any consideration of the dispersion of the curves, i.e., without further statistical analysis, the authors concluded that they had demonstrated four races. The remaining three cases differed from the first four in that the distribution curves were bimodal rather than unimodal. The mode for one of these cases was 6.0 microns and for the other two the modes were 7.5 microns, i.e., identical to those obtained for case H8 and H7. This identity of modes was taken as additional supportive evidence for two of the races. The other modes of the bimodal cases did not correspond to any of the previously found modes, but were identical among themselves at 10.5 microns. This identity was taken as a demonstration of still another race. Thus by finding five distinct modes the authors reached their conclusion that at least five distinct races had been demonstrated.

An analysis of their data, however, reveals certain serious objections. One of the most important of these is their sole consideration of modes, for the mode does not consider the range or dispersion of measurements around it, and it follows that two distributions having closely approximated modes need not necessarily have the same dispersion. The result, as we will presently show, is that certain strains which Dobell and Jepps considered identical were in fact not alike due to differences in their means and dispersions.

A second objection is that races intermediate in size to Dobell and Jepps' five races were not apt to be detected by a study of modes. This is due to the peculiar circumstances inherent in the technique in measuring cysts of *E.*

histolytica. An ocular micrometer is used and the diameter of the cyst is obtained by estimating its size to the closest linear division on the scale. This estimate is then multiplied by a factor in order to obtain a value in microns. The result in practice is that differences less than approximately one micron are not detected and thus intergradations of measurements artificially accumulate at one micron intervals.

It is evident from the above considerations that any analysis by consideration of modes is inherently defective. One can readily see that both the modes and the coincidence of modes obtained by Dobell and Jepps were largely artificial values. As a consequence the question arises whether or not large numbers of intermediate sized strains might not actually exist, especially if more than seven cases were examined. That this is so can be demonstrated from Dobell and Jepps' own data by a method which allows a more precise analysis, namely

TABLE 1

Calculated means and standard deviations of cyst measurements made by Dobell and Jepps

	CASE	NUMBER OF CYSTS	MEAN	STANDARD DEVIATION	MODE
			μ		μ
E130	Small	242	6.35	.47	6.0
H8	All small	500	6.55	.80	6.0
H11	Small	38	6.97	.64	7.5
E79	Small	254	7.23	.92	7.5
H7	All small	500	7.51	.87	7.5
E130	Large	262	10.57	1.28	10.5
H11	Large	465	10.70	1.35	10.5
E79	Large	270	10.86	1.47	10.5
E42	All large	500	12.05	1.05	12.0
B1	All large	500	13.55	1.39	13.5

by comparing means[2] instead of modes, and by considering the dispersion of measurements, i.e., the standard deviations. Very fortunately the authors have presented the raw data for their measurements and these values may be calculated. The data are presented in table 1.

First, one notes that the dispersion of measurements about the mean values, as represented by the standard deviations, is relatively small. This small dispersion reveals in these particular data a marked tendency towards an artificial accumulation of cases at one value or mode, and emphasizes the faultiness of modal analysis. However, it is more important to note that Dobell and Jepps' modes and means agree closely in only four of the ten instances (H7, E42, B1, and E130); the remainder have differences from 0.2 to 0.5 microns, i.e., sufficiently large that they cannot be neglected. It is, therefore, obvious that whereas

[2] Dobell and Jepps apparently do not consider the difference between means and modes important. In his 1919 book, Dobell (5) in summarizing the findings consistently calls figures means which actually are modes. By so doing he gives his conclusions a precision not at all commensurate with the statistical precision of his data.

their modes seemed to indicate certain strains to be identical, a study of means and standard deviations shows that they differ. The mean size of samples of small cysts, for example, were: 6.35, 6.55, 6.97, 7.23 and 7.51 microns, indicating even in these five cases two intermediate sizes between those designated as separate races by Dobell and Jepps. The differences between the means of each possible pair of these samples are significant except for H11 (6.97) which is not significantly different from any other sample. The conclusion, therefore, is that certain of the "races" considered identical by Dobell and Jepps are unlike and that their data indicate the existence of even more than five size-strains.

Probably the most important fact is the great overlapping of what these investigators call distinct races. A glance at their five curves shows that large numbers of cyst measurements of any one of their races are common to a large proportion of those of another race or even of two other races. In other words, some of the so-called distinct races are actually more like than unlike, when one considers the large number of cysts common to several distributions.

As a consequence it seems very doubtful whether minor strain variations in size may be appropriately designated by the term "race." No proof exists that each of these strains has the attribute of size constancy, nor that they differ in any other respect than that of size. We will presently show that when large numbers of individuals are studied, numerous strains are found which might better be considered components of a single race showing minor variations in size similar to those commonly observed in measurements of a biological nature.

This brings us to a consideration of other facts that indicate that there are, rather, only two races of cysts produced by *E. histolytica*; that, in contrast, these two races are remarkably distinct so far as the range of sizes that is found in each; that there is no evidence to show that there is size mutation between one and another; and finally, that accumulating data indicate that these two races have fundamental differences besides size.

II. EVIDENCE FOR THE EXISTENCE OF TWO RACES OF *E. histolytica*

During the past three years two of us (JJS and EGH), working independently, have measured *E. histolytica* cysts from a total of 283 individuals. One of us (JJS) measured 1659 cysts from 215 cases. These cases were largely carriers although a few presented symptoms referable to their infections. None were convalescent dysenterics. The measurements were obtained from specimens fixed in modified Schaudinn's fluid (saturated corrosive sublimate in water, two parts; absolute alcohol, one part; glacial acetic acid, 5 per cent), stained with iron hematoxylin and mounted in Canada balsam. Measurements of 1336 cysts (EGH) from 68 individuals were of living cysts in normal saline or aqueous smears. These cases were detected in routine stool examinations from hospital wards and included carriers, cases with varying severity of symptoms, and a few convalescent dysenterics.

As living cysts are larger than wet-fixed, stained cysts, a correction is necessary to bring all measurements to a common basis. In order to obtain this cor-

rection factor, each of us, working independently, compared measurements obtained from wet-fixed, stained smears with those of living cysts. Within the range of measurements in which the vast majority of cysts fall, namely, 7–14 microns, an average difference of one micron was observed, i.e., wet-fixed, stained cysts mounted in balsam were found to measure approximately one micron less than living cysts.

These results, it may be noted, are similar to the findings of Dobell and Jepps (3) who in a carefully performed investigation observed that living cysts in saline, or cysts stained with iodine, measured approximately one micron more than stained cysts mounted in balsam. This one micron difference they say, represents an average and their work indicates that smaller cysts, measuring 4.5 microns in stained preparations, actually measured only 5 microns as living, whereas stained cysts of 18 microns measured 20 microns when living. The vast majority of cysts, however, are intermediate in size, and consequently an average correction of one micron is satisfactory.

Having established the necessary corrections due to variations resulting from different techniques we can, by applying these, compare our own measurements, some of which were on living specimens and others from cysts stained and mounted. Similarly the results obtained by Dobell and Jepps (3) (cysts stained and mounted in balsam) and A. Malins Smith (6) (measurements in iodine) can be considered on a comparable basis to the results obtained in our own work. Corrections have been made so that all data is on the common basis of measurements obtained from stained specimens, but in one of the figures, to be presented, we have also included corresponding values for both living and stained specimens as both have considerable practical usefulness.

In figure 1 we have graphically shown the average diameter and range of measurements obtained from 62 individuals from each of which ten or more cysts were measured.[3] We have also included measurements from Dobell and Jepps' (see table 1) 7 cases, and those reported by A. Malins Smith (6) in 30 cases, mostly convalescent dysenterics. Each horizontal line represents an individual case showing the mean and the range of measurements, except those examined by Smith who gives data for the means only. The curve AB passes through the mean of each of the individual cases which have been arranged according to ascending values of the means. The exact values for these means together with the number of cysts measured in each case are shown in table 2.

A striking demonstration afforded by the figure and the table is the existence of two series of means divided by a distinct gap between 7.74 and 9.85 microns. The adjacent means within each of these series show no significant differences. On the other hand each individual mean of one series is significantly different from any given mean of the other series.

Taking the nine micron line as a dividing point, the series of cases with the smaller cysts shows only eight cases with cysts greater than nine microns and

[3] The justification for using cases with so few cyst measurements is that the standard deviations of the means are of the order 0.5 to 0.1. Even with the larger values the means are ten times greater than their standard errors indicating definite statistical validity.

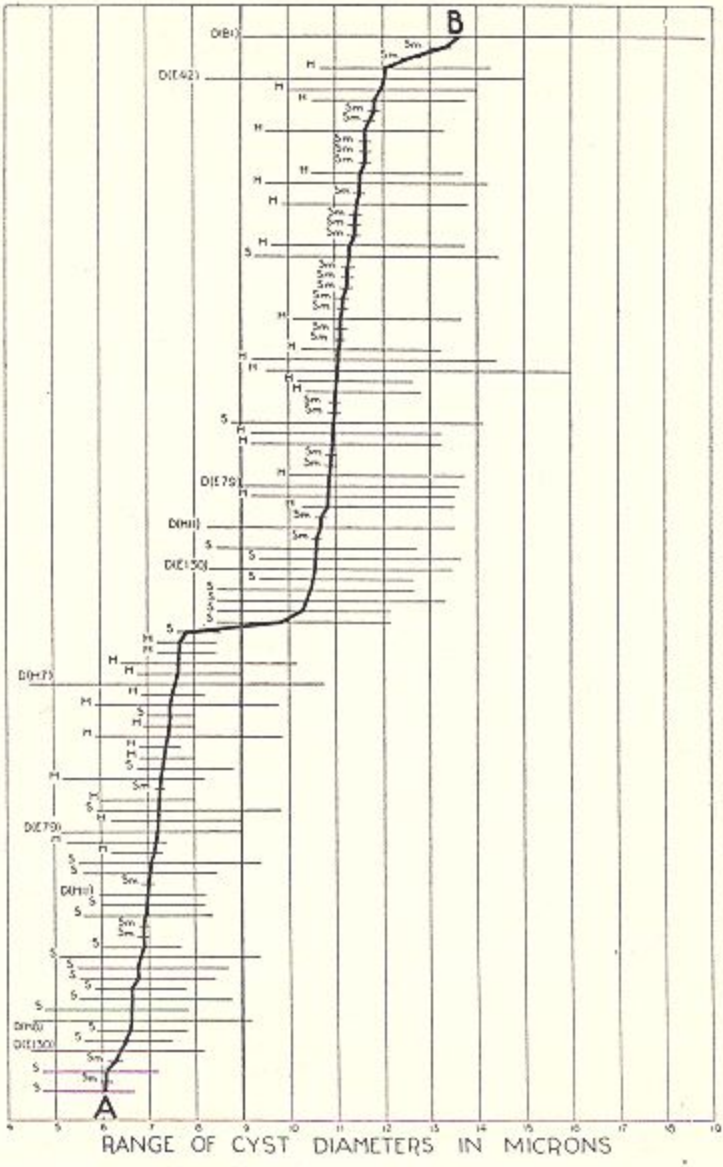

FIG. 1. The horizontal lines show the range of cyst diameters (measurements on the basis of wet-fixed, stained specimens) obtained by independent workers in each of 99 individuals harboring *E. histolytica*. The line AB passes through the mean diameter of each strain. Note that the 9 micron line divides all strains into two series, a larger with average diameters from 10–14 microns, and a smaller from 6–8 microns. The mean and range of sizes are largely similar within each series; the strains belonging to different series are markedly dissimilar. (D, Dobell and Jepps; S, Sapero; H, Hakansson; Sm, A. Malins Smith (mean diameters only).

the series with the larger cysts shows only nine cases with any cysts measuring less than nine microns. This overlap in 17 cases may appear considerable but is brought about by only a very small number of cysts at the extremes of

TABLE 2

Mean diameters of cysts from individual cases tabulated according to ascending values of the means

SMALL RACE			LARGE RACE		
Mean	Number of cysts	Author*	Mean	Number of cysts	Author*
μ			μ		
6.04	18	S	9.85	70	S
6.10	43	Sm	10.33	30	S
6.10	15	S	10.39	100	S
6.30	31	Sm	10.49	19	S
6.35	242	E130	10.50	21	S
6.45	18	S	10.57	262	E130
6.53	11	S	10.58	101	S
6.55	500	H8	10.59	16	S
6.56	11	S	10.60	15	Sm
6.57	11	S	10.69	465	H11
6.60	10	S	10.70	27	Sm
6.75	16	S	10.81	20	H
6.77	19	S	10.83	20	H
6.84	35	S	10.86	270	E79
6.89	15	S	10.87	50	H
6.90	27	Sm	10.90	33	Sm
6.90	37	Sm	10.90	19	Sm
6.95	17	S	10.94	28	H
6.97	12	S	10.96	25	H
6.97	38	H11	10.99	31	S
7.00	43	Sm	11.00	24	Sm
7.02	15	S	11.00	22	Sm
7.05	75	S	11.03	21	H
7.06	16	H	11.05	25	H
7.19	15	H	11.06	14	H
7.23	254	E79	11.07	15	H
7.23	21	H	11.07	55	H
7.26	13	S	11.10	39	Sm
7.29	15	H	11.10	50	Sm
7.30	131	Sm	11.11	18	H
7.30	25	H	11.20	27	Sm
7.32	15	S	11.20	39	Sm
7.38	28	H	11.30	28	Sm
7.39	15	H	11.30	50	Sm
7.43	51	H	11.30	50	Sm
7.46	13	H	11.30	16	S
7.48	12	S	11.32	25	H
7.48	20	H	11.40	30	Sm
7.51	16	H	11.40	29	Sm
7.51	500	H7	11.40	29	Sm
7.56	16	H	11.48	28	H
7.58	55	H	11.50	28	Sm
7.61	29	H	11.53	20	H
7.62	29	H	11.54	16	H

TABLE 2—*Concluded*

SMALL RACE			LARGE RACE		
Mean	Number of cysts	Author*	Mean	Number of cysts	Authors*
μ			μ		
7.74	13	S	11.60	50	Sm
			11.60	20	Sm
			11.60	37	Sm
			11.63	20	H
			11.70	50	Sm
			11.80	11	Sm
			11.84	23	H
			11.95	19	H
			12.05	500	E42
			12.08	14	H
			12.60	49	Sm
			13.30	50	Sm
			13.55	500	B1

* S, Sapero; H, Hakansson; Sm, Smith; the others from Dobell and Jepps are shown by their designations.

dispersion so that actually the overlap is only slight. In contrast, it is to be noted that within each series, the vast majority of cyst measurements of the various cases overlap markedly leaving no indication of distinct distributions.

In summary, the findings made from a study of individual strains in 99 cases show: (1) the existence of numerous strains with means intermediate to, and beyond those found by Dobell and Jepps; (2) that the means of individual strains are fairly evenly distributed rather than tending to group at the particular values which would correspond to those for the five races postulated by Dobell and Jepps; (3) that the differences in the sizes of individual strains are of a very minor degree as is best shown by the great overlapping of the individual strains. The suggestion is that these individual strains of *E. histolytica* show the minor variations frequently exhibited by living organisms, and that therefore their designation as races is not justified. In contrast, however, the grouping of individual strains into two series, a large and a small, is one that is remarkably distinct and suggests the existence of two races of *E. histolytica* distinguishable by cyst size.

In addition to this comparison of mean sizes of cysts in individual hosts, which we have just carried out, there is another method of analysis, namely, the determination of cyst size distribution of individual cysts without regard to the sample or host from which the cysts came. To do this we have pooled all data and from these constructed curves. This method is particularly useful, now that we have studied individual strains, for modes will appear only if any component distributions do not considerably overlap and as we are seeking distinct and not overlapping distributions with minor variations, the method affords another check on the bi-racial hypothesis. If but two distinct races exist as the above data have indicated, a bimodal curve should be obtained.

The results are shown in figure 2, where four curves have been plotted. Curve A is from Dobell and Jepps' data, curves B and C are from our own original data and a fourth, curve D, represents measurements reported by A. Malins Smith.

Curve A shows the distribution of cyst measurements from 7 cases with a total of 3500 measurements. Curve B was similarly constructed from measurements of cysts from 215 cases. The number of measurements averaged 8 per case with a total of 1659 cysts measured. Curve C comprises 1336 cyst measurements from 68 individuals, an average of 19 measurements per case. Curve D is replotted to a comparable scale from a curve of 1000 cyst measurements made by A. M. Smith. These were from 30 patients with 10 to 50 cysts measured in each case. The curves have been constructed on the basis of values for stained specimens mounted in balsam.

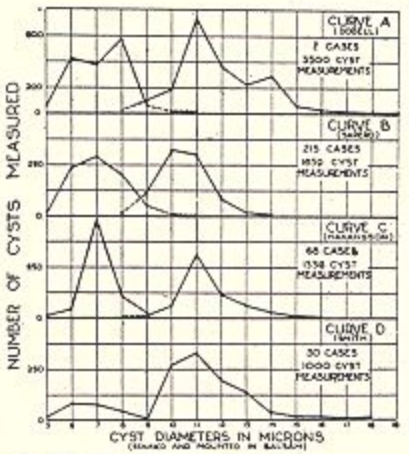

FIG. 2. THE DISTRIBUTION OF E. HISTOLYTICA CYST MEASUREMENTS (VALUES ON BASIS OF WET-FIXED, STAINED CYSTS) AS SHOWN BY THE DATA OF INDEPENDENT WORKERS

The curves of all are in essential agreement, each presenting two distinct and largely similar distributions for the cysts of this organism.

The statistical constants of the four curves are given in table 3. Neglecting for the moment the broken line extensions in three of the curves, an analysis reveals the following: (1) all curves show two distinct and largely separate distributions; (2) curves B, C, and D are essentially bimodal, with the modal points nearly coinciding; (3) the low points of the curves (or the points of cross over), fall within a narrow range of approximately 8.95 to 9.1 microns; and (4) the differences between the means of the two distributions are very large. From these considerations it is apparent that the independently derived data of several workers are in essential agreement, demonstrating in each case the existence of but two distinct size distributions for the cysts of *E. histolytica*.

The apparent exceptions, or dissimilarities appear only in the curve of Dobell and Jepps. Here there are four peaks or modes in the curve, but there at once appears a very likely explanation for these modes which are not present in the curves of other workers, namely, that curve A is derived from data on but 7

cases. These modes are the result of large numbers of measurements on single cases, and can be considered significant only if they persist when larger numbers of cases are examined. That they do not persist upon the inclusion of larger numbers of cases is evident from the other curves. Actually, the curve constructed from Dobell and Jepps' data agrees well in all other respects and we are of the opinion that larger sampling would have led to perfect agreement.

TABLE 3

Statistical constants of curves in figure 2

CURVE	NUMBER CYSTS	MEAN	STANDARD DEVIATION	STANDARD ERROR OF THE MEAN
		μ		
Small				
A (Dobell)*	1534	6.95	.95	.024
B (Sapero)	703	7.05	.92	.034
C (Hakansson)	525	7.17	.62	.027
D (Smith)	194	6.68	.82	.059
Large				
A (Dobell)	1997	11.31	1.63	.036
B (Sapero)	744	10.52	1.09	.040
C (Hakansson)	504	11.45	1.32	.059
D (Smith)	810	11.58	1.28	.044
Mixed (small half)				
A (Dobell)†	534	6.81	.87	.037
B (Sapero)	107	7.13	1.18	.114
C (Hakansson)	194	7.67	1.08	.077
D‡				
Mixed (large half)				
A (Dobell)†	997	10.71	.98	.031
B (Sapero)	134	10.41	.86	.074
C (Hakansson)	133	11.47	1.17	.101
D‡				

* These figures include data from all seven of Dobell's cases.

† These figures are for Dobell's bimodal cases only.

‡ Smith does not distinguish between uni-modal and bimodal cases.

Having demonstrated the existence of a large and small race of this organism, we were interested in determining the overlap between the two races as shown by the data of various workers. This was possible in curves A, B, and C, and has been indicated by the dotted line extensions interpolated between the two size distributions. In practice one observes that nearly all measurements in a given case fall say, at 7 microns, but on occasion a few cysts measuring 10, 11, or 12 microns are found. Conversely, a few cysts measuring 7 or 8 microns occasionally are found in a stool in which all the other values fall around 12 microns. One cannot definitely say in these cases whether these cysts represent

"giants" or "dwarfs" of a single race or the existence of very scanty numbers belonging to a second race. The same situation occurs where two races are present in a single host, there being often a few intermediate sized cysts which cannot be definitely assigned to either distribution. These dotted lines, then, demonstrate the overlap as encountered in practice. It is apparent that the overlap is remarkably small, and therefore, the distributions of the large and small groups are very distinct.

If we now combine all the measurements included in the four curves of figure 2 into a single curve, the curve characteristics appear as shown in figure 3 with curve constants presented in table 4. It will be noted in the figure that

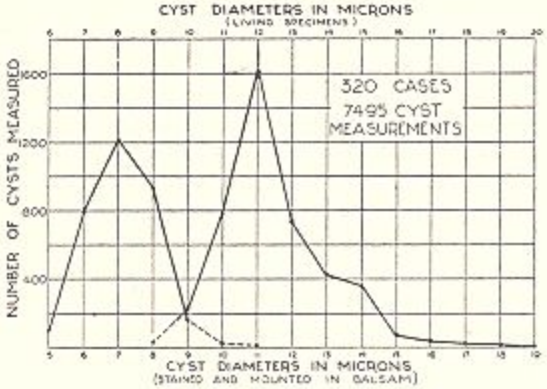

FIG. 3. THE DISTRIBUTION OF CYST MEASUREMENTS OF E. HISTOLYTICA FROM THE COMBINED DATA OF VARIOUS WORKERS

Two distinct distributions indicate the existence of a large-sized and small-sized race of the organism.

TABLE 4

Constants of total distribution curves shown in figure 3

CYSTS	NUMBER	MEAN	STANDARD DEVIATION	STANDARD ERROR OF THE MEAN	PER CENT OVERLAP
		μ			
Small.....	3192	7.09	.937	.016	4.9
Large.....	4322	11.48	1.504	.023	5.5

values both for wet-fixed, stained specimens and living cysts have been included as both are of practical usefulness. In this curve it is evident that only two distinct and prominent modes remain, the additional modes present in Dobell and Jepps' curve having disappeared. In addition to only two distinct and prominent modes each component curve is essentially a normal distribution curve. The difference between the means of these two curves, which is 4.39 microns, has a standard error of 0.028, giving a critical ratio of over 150, representing certainty that the difference is not due to chance sampling. Not only are the means of the two distributions significantly different but there is a strikingly small overlap between them.

The point of beginning overlap for values of stained, wet-fixed mounted specimens is at 9 microns; for living specimens it is at 10 microns. The smaller cysts have only 4.9 per cent at or above this value; the large cysts, 5.5 per cent at or below.

We may summarize the results of our second method of analysis by saying that it also has demonstrated the existence of only two races of *E. histolytica*, and further, that the hypothesis that other races exist is not tenable unless there exist other sizes not encountered in the rather large series analyzed herein.

Some interpretations of practical interest may be derived from figure 3. Of practical significance is the ease with which any individual cyst may be properly allotted to either the small or large race. For example, the probability of a single cyst measuring less than 8 microns, belonging to the large race, is so remote that with practical certainty it can be classed as a small race. Likewise cysts larger than 10 microns can be safely called large race. Individual cyst measurements between 8 and 10 microns may obviously belong to either race; yet the measurement of as few as eight or nine other cysts will in a vast majority of cases establish the proper race. Bearing these points in mind, we may state as a general practical rule that the 10 micron line for measurements on living cysts of *E. histolytica* may be used to divide the organism into the large or small race. Similarly, when cyst measurements are made on wet-fixed, stained specimens mounted in balsam, the 9 micron line divides the large from the small race.

Apparently strains having mean diameters of 10 microns do occur, but these must be exceedingly rare. We have not encountered any in the several hundred samples involved in this analysis, but Dobell and Jepps (3) found one such strain in 202 *E. histolytica* infections, and Wenyon and O'Connor's (1) case "Kettlewell" is another example. Whatever the significance of these intermediate sized strains is, their occurrence appears to be so rare that they do not constitute an important problem.

III. FURTHER DATA AND CONSIDERATIONS PERTAINING TO RACES OF *E. histolytica*

Occurrence of large and small races

Since undertaking the above investigation we have classified all strains encountered according to race. The results are shown in table 5. It will be noted that the small race occurred in well over half the cases, and as these percentages represent routine stool examinations from general hospital wards, the importance of clarifying the role of the smaller strains of *E. histolytica* is particularly emphasized. Persons harboring both races simultaneously were found in 6.3 per cent of the cases. In such stools almost all the individual cysts could be readily differentiated as belonging to either the large or small race, but a few cysts occasionally were found straddling the ten micron line and leaving one in doubt as to which race they belonged.

Racial constancy

In order to designate races of *E. histolytica* according to size, there should be evidence to show that size is an inherent characteristic, i.e., not determined by

the environment of the organism. In our own experience changing environment has not led to a transmutation of the large to a small race, or the converse, nor so far as we can ascertain, has any such change been observed by other workers. Probably the most convincing evidence that the size of the two races is not determined by environment, is the co-existence, as noted in 6.3 per cent of our cases, of both races in the same host. Further evidence in this regard was afforded one of us (EGH) in repeated examinations of a large series of stools from various patients in a Panama asylum. These cases were studied over a period of many months during clinical periods of remission and relapse; yet in no case harboring a large race was there evidence that the size had changed to that of the small race, or the converse.

There is, on the other hand, evidence that minor variations in the average cyst diameter of a given strain may appear from time to time coincident with environmental changes, but it is important to note that these variations are always within the ranges we have noted for each race. Smith (6) observed size changes in samples from the same patient on different days, and states that the variation was not of great magnitude. Dobell (3) also observed that under

TABLE 5

The occurrence of small and large races of E. histolytica

	NUMBER OF CASES	PERCENTAGE
		per cent
Small race only	179	56.7
Large race only	117	37.0
Small & Large races	20	6.3
Total	316	100.0

certain conditions cyst sizes of a given strain became temporarily modified, noting in the case of larger strains an increase in size from 12–14 to 15 microns or more in diameter after treatment, the size later becoming smaller again. Recently Meleney and Frye (12) have recorded slight changes in size in a single strain when a small race was passed through successive cultures and through animals. Their measurements were on trophozoites and showed variations well within the limits of the small race. These observations strongly suggest that environment may play a role in accounting for the minor variations seen in mean diameters of strains belonging to either race. This variation, however, so far as is indicated by any evidence that has been presented, is sharply limited within the size range of either race.

Comparison of cyst and trophozoite sizes

Upon this matter we have been unable to find published reports, although the impression seems to be that cyst and trophozoite size are not necessarily related. In our data there are a number of cases for which both cysts and trophozoites were measured. There were 40 small race cases in which the means of both cysts and trophozoites were obtained, and an additional 22 cases with

trophozoite means only. Among the large race cases there were 19 with both, and 9 with only trophozoite measurements. In table 6 these data have been arranged to show the mean size of all cysts and all trophozoites measured. In these data the difference between the mean cyst and trophozoite measurements for the small race is not a significant one, while that for the large race is (critical ratio of 1.7 and 6.0 respectively). The differences between the standard deviations of the distributions are both significant; the critical ratio for the small race is 3.5 while for the large race it is 9.9. None of the large race trophozoites measured were from dysentery cases, i.e., the giant organisms so characteristically seen in acute cases. Had such measurements been included the disproportionately large sizes of the trophozoites, as compared with cysts, would have been even more marked. The suggestion is that trophozoites of the small race conform closely to the sizes of their cysts whereas trophozoites of the large race may attain sizes much larger than their cysts. This relationship suggests

TABLE 6

Comparison of cyst and trophozoite sizes of the large and small race

RACE	NUMBER	MEAN	STANDARD DEVIATION	STANDARD ERROR OF THE MEAN
		μ		
Small:				
Cysts	703	7.05	0.92	0.034
Trophozoites	383	7.17	1.08	0.060
Large:				
Cysts	744	11.52	1.09	0.040
Trophozoites	163	12.85	2.47	0.190

another important biological difference between the two races, and further, the possibility of being able to differentiate races on trophozoite measurements alone.

Differences in races of E. histolytica other than size

The concept of five or possibly more races of *E. histolytica* is not one which would encourage studies to determine whether these races vary in other characteristics than that of size alone. The bipartite division of the organism into small and large races, however, is a natural one which has already been observed by many. The result is that important differences have been reported by several workers despite the previous lack of evidence which would justify a biracial division of the species. These differences appear to be important and should be considered in addition to the size differences of the two races. It is our purpose to mention briefly some of these observations made by others and by ourselves.

Observations on morphology. A number of workers, Drbohlav (13), Spector (14), and Frye and Meleney (15), remark on the absence of ingested red cells in trophozoites of the small race. Spector (14) reports that the small trophozoites have less motility than the large. Our own observations upon these two points

are similar. Both of these characteristics noted in the small race contrast sharply with those exhibited by the large race in amebic dysentery where ingestion of red cells and vigorous motility are classical features.

Pathogenicity in animals

Several workers report that the smaller race is less infective and less pathogenic than the large race when inoculated in kittens. Drbohlav (13) reports that the small race does not produce dysentery or ulceration in kittens. Spector (14) succeeded in infecting only 8 of 37 kittens with small cysts *per os* and of these only two showed pathology, viz. minute ulcers of the large bowel. In contrast 10 of 22 were infected with large forms. Intra-rectal inoculation of kittens with large cysts all produced severe amebic ulceration, while the small race consistently failed to produce such results. Unfortunately this writer considered cysts measuring less than 12 microns as belonging to the small race, an assumption that we have shown to be unjustified. It is possible that such lesions as were ascribed to the small race may have actually been produced by the organisms properly belonging to the large race. Frye and Meleney (15) and Meleney, Frye and Leathers (12) report on the pathogenicity of several strains in kittens. Among their strains one was small with cysts less than 10 microns in diameter. This strain had a "pathogenic index" of 0.35, the smallest found in any of their human strains which had indices of 0.55, 0.90, 1.00 and 1.10. Of the five kittens they were able to infect, four showed only a few small shallow lesions, while one had a few shallow and one small deep ulcer. They say, "These observations strengthen the belief that a small race of *E. histolytica* exists which is relatively fixed in its size and pathogenic activity, and that it possesses a low power of invasion of the tissues of man and experimental kittens."

In contrast to these reports which agree that the small race is definitely less pathogenic than the large race is a report by Kessel (16). This author says, "Amoebae from races which produce both large and small cysts respectively, have been used and both produce the same pathological condition in kittens." Careful examination of his detailed tables of gross and histopathological findings, however, does not support this conclusion. Four of eight kittens were infected with a small race, 8 microns or below in diameter, and twelve of twenty-four kittens were successfully infected with strains of 10 microns or larger. His description of the gross findings with the large race indicates considerably more extensive ulceration than with the small race which showed only superficial necrosis or ulceration in 3 of the 4 kittens which he succeeded in infecting. The fourth kitten (187), however, according to his report did show minute pinhead ulcers throughout the colon. He failed, however, to find amoebae penetrating tissues in histological studies of the small race infections, whereas, penetration was demonstrated in several kittens infected with the large race.

It is apparent from the above studies that, even though results have been somewhat conflicting, to date experimentation suggests a distinct difference in the animal pathogenicity of the small and large races of *E. histolytica*.

Culturability

During a two year period we have made routine cultures from strains of *E. histolytica* encountered in examinations of stools from the hospital wards. Our records show uniform failure to culture the small race organisms in over one hundred attempts. In contrast, cultures of the large race succeeded in over 80 per cent of the inoculations although the same media (liver infusion) and technique were employed in all cases. Spector (14) reporting that the small race grows infrequently and with difficulty in Cleveland-Collier medium, succeeded in only 5–8 per cent of her attempts while with the large race she experienced better than 95 per cent success. In addition she found that the small race had to be subcultured every 24–48 hours, whereas, a longer period, 48–72 hours sufficed for the large race. The difficulty in culturing the small race suggests still another important difference between the large and small race.

Pathogenicity of small and large races in man

From a clinical standpoint we have the remarkable situation that neither amoebic dysentery nor liver abscess caused by the small race of *E. histolytica* has been reported in man. In our own work embracing clinical studies on many patients with chronic dysentery, the disease has always been associated with the large race, never with the small. In addition we have been unable to ascribe symptoms of any considerable severity to the presence of the small race although we have now personally interviewed and examined over 179 cases with small race infections. Not uncommonly, very mild complaints are presented by such patients, but in these cases it is exceedingly difficult to be certain that the small race is responsible. However, in a few cases of mild symptoms the administration of carbarsone gave relief, and from these experiences together with the fact that there is only negative evidence as regards the possible existence of severe symptoms, we must admit the possibility of the small race being harmful.

Remarkably enough, pathological lesions, either intestinal or elsewhere in man, definitely ascribed to the small race, do not appear to have been reported except in one instance and in that, the evidence of pathogenicity is not conclusive. Faust (17) in studying the intestine in 202 autopsies in cases of accidental death, found what he considered convincing evidence of tissue invasion in five cases, in four of which small races of *E. histolytica* were demonstrated. A few pin-point ulcers or shallow crater-like lesions were observed and the organisms were demonstrated from scrapings of the lesions. Although tissue sections were made, the writer does not state whether or not trophozoites were found actually penetrating the tissue in the small race cases, a crucial point in establishing that the lesions were caused by the organisms and not merely in coincidental association with them. While his work suggests that possibly minor lesions in the human gut may be caused by the small race, it is apparent that more evidence on this important point is needed.

We may summarize this presentation of evidence comparing small and large races by noting: (1) the small race trophozoites have not been observed to ingest red blood cells, and do not show the vigorous motility of which the large

race is capable; (2) cyst and trophozoite sizes remain essentially the same in the case of the small race; whereas the trophozoite of the large race is capable of growth to giant size; (3) the small race fails to grow or to be successfully cultured *in vitro* with the same success possible with the large race; (4) kitten inoculations definitely tend to be less pathogenic in the case of the small race; (5) conclusive demonstration of tissue invasion on the part of the small race in the intestine or elsewhere in man is lacking; (6) dysentery and other serious clinical conditions have been definitely proved to be caused by large races of *E. histolytica*, but it has not yet been definitely established that any particular clinical syndrome is associated with small race infections.

These observations clearly indicate the importance of clarifying the respective roles of the two races of *E. histolytica*. The suggestion exists that the small race might possibly be a distinct species. Such a suggestion, however, is premature as there is great need for further evidence on the various apparent differences exhibited by the races of the organisms. Until this is forthcoming, and certainly for practical purposes, conservatism demands equal respect for the potential harmfulness of either race.

SUMMARY AND CONCLUSIONS

1. A study of the mean diameters and size distributions of cysts of *E. histolytica* from 99 cases demonstrates the existence of large numbers of strains having mean diameters intermediate between and beyond the 5 races postulated by Dobell and Jepps. These strains overlap greatly in size distribution, do not show size constancy, and differ in no other respect than that of size alone. They, therefore, cannot properly be designated as races of *E. histolytica*.

2. Further analysis, on the other hand, both of individual strains and the pooled size distribution of 7495 cyst measurements from 320 cases, demonstrates the existence of two significantly distinct races, a large and a small. These are not only distinctly unlike in size, but differ as regards motility, culturability, and pathogenicity in man and lower animals.

3. In practice, the 10 micron line may be generally used to distinguish living cysts of the large or small race. For measurements of wet-fixed stained cysts, mounted in Canada balsam, the 9 micron line serves.

4. The evidence indicates racial constancy both as regards size and physiological characteristics exhibited by the large and small race of *E. histolytica*.

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