Size as a species characteristic in coccidia: Variation under diverse conditions of infection [1].

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(With 5 figures in the text.)

The use of size differences as a criterion for the recognition of species of coccidia has been a customary procedure with many investigators up to the present time. The work of several recent authors who have employed this method almost exclusively will be discussed in some detail later in this paper. It has been brought out, however, by TYZZER (1929) that variations in dimensions alone may prove to be an unsatisfactory method of differentiating species since large and small races may exist within a single species. Moreover, the size of an organism may conceivably be influenced by a variety of environmental and physiological factors, such as the duration and severity of the infection, the location of the organism in the intestine, and the reaction of the host tissues to the invading parasite.

The present experiments were undertaken to determine first, the natural range of variation among organisms developing from a single oöcyst, and the influence on size of oöcyst of the age, breed of the host, and of the duration and severity of the infection; and

[1]) Additional data not included in this paper may be found in a thesis written in partial fulfillment of the requirements for the degree of Doctor of Philosophy deposited in the library of the Harvard Medical School, Boston, Massachusetts.

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second, whether, under the most favorable experimental conditions, two species, with oöcysts of distinctly different sizes, could be crossed, and an intermediate or modified size race produced.

Since part of the data bearing on the first set of problems was obtained in the course of the experiments on the second, the work on the attempted hybridization of two species will be described first.

Part I.

Attempted hybridization.

For this experiment, two species of coccidia, *Eimeria maxima* and *Eimeria acervulina*, were chosen. These are easily distinguished from each other by their size and general morphology. Furthermore their normal locations in the intestinal tract overlap to a certain extent, thus giving an opportunity for the sexual forms of each type to be present in the same area.

Description of *Eimeria acervulina* [1].

It has been shown by TYZZER (1929) that *Eimeria acervulina* is found principally in the upper half of the small intestine, but may also be scattered throughout the lower portion and even rarely may occur in the caeca near the outlet. The developing forms are found chiefly in the epithelium of the villi, but are sometimes found also in the glandular epithelium. Their location is characteristically superficial in the epithelium, organisms not being found below the nuclei of the epithelial cells.

Schizonts with fully developed merozoites are present three days after the infective feeding. Macro- and microgametocytes are present from the fourth day on. Oöcysts are found in the feces at the end of the fourth day of infection. They are egg-shaped, and range in size from 17.7 to 20.2 μ in length and 13.7 to 16.3 μ in breadth, the average being 19.5×14.3 μ. Sporulation is usually complete at twenty hours at room temperature. The oöcysts show polar inclusions.

[1]) The description of *Eimeria acervulina* and *Eimeria maxima* are taken from TYZZER's monograph (1929).

Description of *Eimeria maxima.*

Eimeria maxima occurs chiefly in the middle portion of the small intestine, but may also be found throughout the entire length. Schizonts and merozoites are found after seventy-two hours. They lie superficial to the nucleus in the epithelial cells of the villi, or occasionally beside the nucleus which is then flattened to one side. The sexual forms, however, found from the fifth day on, are at the lowest level of the epithelium. Cells containing gametocytes become rounded and the entire cell is displaced into the deep reticular portion of the epithelium, often into the subepithelial tissue.

The oöcysts are egg-shaped, but with one end more pointed than in *Eimeria acervulina* oöcysts, and range in size from 21.4 to 42.5 μ in length and 16.5 to 29.8 μ in width, the average being 29.3×22.6 μ. Sporulation occurs after forty-eight hours. The oöcysts contain a coarse polar inclusion.

It will be noted from the above descriptions, that the sexual forms of *Eimeria acervulina* and *Eimeria maxima* occur at different depths in the intestinal wall, *Eimeria acervulina* being found chiefly in the epithelium of the villi, superficial to the nucleus, while the *Eimeria maxima* forms are at the lowest level of the epithelium, and are even displaced into the subepithelial tissue. This fact may be a controlling element in the failure of these strains to cross with one another when a double infection occurs. However, since there must be a certain amount of migration of the sexual forms from cell to cell, the possibility of the gametocytes of one type meeting those of the other does exist, and it was thought that the chances were great enough to warrant the expectation of some cross fertilization between the two types unless this was inhibited by other biological factors. The difference in time of appearance of the gametocytes of the two species was allowed for by infecting with *Eimeria maxima* one, two and four days previous to infecting with *Eimeria acervulina.*

Hybridization experiment.

Material and Methods: The strains of *Eimeria maxima* and *Eimeria acervulina* employed in this experiment were developed by the isolation of single oöcysts from material used in previous experiments in the laboratory. Single oöcysts were procured by the dilution method, a portion of the material being diluted with distilled water until microscopic examination of small drops showed few to

no organisms in each drop. Strips of gelatin were then laid on the surface of a film of sterile water in a petri dish until soft, when they were put on a slide. Tiny drops of the diluted material were placed on the gelatin with a capillary pipette, and examined under the microscope at once. Those drops which contained only one coccidium were marked; the small square of gelatin surrounding them was cut from the strip and immediately placed well down the throat of a five-day old chick. Seven chicks were fed single oöcysts of *Eimeria acervulina* isolated in this fashion, and seven chicks, single oöcysts of *Eimeria maxima*. Infection resulted in four of the *Eimeria acervulina* fed chicks, and in five of those fed *Eimeria maxima*.

Each of these chickens was kept in an individual pen with a bottom of wire mesh coarse enough to allow the feces to fall through on to paper below, from which they were collected daily. This was done as an added precaution to insure that the infection resulted from a single oöcyst, giving the chick no opportunity to reinfect himself in the course of the experiment. Pens, wire bottoms, and covers, and all feeding dishes were sterilized before using. The mash and grit used for feeding were autoclaved. Hard boiled eggs were used to supplement the diet, and drinking water was taken from the hot water supply.

Since the infections resulting from the feeding of a single oöcyst were light, the material from the chick in each series showing the largest number of organisms was selected as the parent strain. Measurements were made on a series of fifty organisms of *Eimeria acervulina* from chick 113, which had been infected with a single oöcyst, and the remainder of the material was fed to a twenty-three-day old "clean" chick, 113 A, in order to obtain large numbers of organisms. The strain from 113 as the first parent strain will be designated *Eimeria acervulina* P_1; that from 113 A, *Eimeria acervulina* P_2.

Measurements were also made on a series of organisms of *Eimeria maxima* from chicken 115 infected with a single oöcyst (*Eimeria maxima* P_1), fifty measurements each being made on the material collected the first, second and third days on which oöcysts appeared in the feces, Oöcysts from 115 discharged on the seventh day after the infective feeding were fed to two twenty-five-day old "clean" chicks, 115 A and 115 B, and oöcysts collected on the eighth day were fed to two "clean" chicks, 115 C and 115 D (June 4). Measurements were made on material collected from 115 A—B and

C—D eight days later (June 11) and the material from 115 C—D was used as the *Eimeria maxima* strain for further experiments, and will be designated *Eimeria maxima* P_2.

Hybridization Experiment: Ten chickens, 118—127, thirty-two days old, were fed a massive dose of the *Eimeria maxima* P_2 strain, June 13, 1929; and the feeding was repeated on the following day On this day, June 14, a group of three chickens, 119, 121, 126, was also given a massive dose of the *Eimeria acervulina* P_2 strain. On the next day, June 15, the second group of four more chickens, 118, 123, 125 and 127 [1]), was fed *Eimeria acervulina* P_2 strain, and two days later, June 17, the third group of the remaining three chickens 120, 122 and 124 was given the *Eimeria acervulina* P_2 strain.

The first *Eimeria maxima* sexual forms should thus have been present in the intestine from June 18 for a period of several days, and sexual forms from the second infective dose from June 19. In the three chickens infected with *Eimeria acervulina* on June 14, the first gametocytes should appear on the 18th; in the four infected on the 15th, they should be present on the 19th; and in the three infected the 17th, they should be present on the 21st.

Oöcysts were collected June 20, the seventh day after the first infective dose with *Eimeria maxima*, from one chicken in each of the three *Eimeria acervulina* feeding groups, 118, 119, 120. On the 21st, material was collected from numbers 119, 120, 121, 122, 123, 124, 125 and 126. Numbers 122, 125 and 126 were killed on this date for microscopic examination. Material was collected June 22nd from numbers 118, 119, 120, 121, 123, 124 and on June 23 from numbers 121, 123, 124. Material was thus collected from the seventh to the tenth day after first feeding *Eimeria maxima*, and from the sixth to the ninth; fifth to the eighth; and third to the sixth days after feeding *Eimeria acervulina*.

Material was collected on three successive days from each chicken, and kept in 2.5 % potassium dichromate solution in petri dishes until sporulation occurred. One hundred oöcysts from each sample were measured. These oöcysts will be designated as F_1 oöcysts. Each of these samples was later fed, November 14, 1929, to a single eight-day old chicken as listed below, and material from each chicken was examined on three successive days, November 19, November 20, and November 22, 1929. One hundred measurements

[1]) Died, the second day of the experiment.

were made on each sample obtained. These oöcysts will be designated as the F_2. The F_1 material, the chickens to which each was fed, and the F_2 material obtained from them are listed below:

Second Generation:		F_2 material obtained:
a) Material from 118 on	6/20/29 fed 230	— 11/19, 11/20, 11/22.
	6/22/29 fed 231	— Died 11/20.
b) Material from 119 on	6/20/29 fed 232	— 11/20, 11/22.
	6/21/29 fed 233	— Very light infection, no material.
	6/22/29 fed 234	— Very light infection, no material.
c) Material from 120 on	6/20/29 fed 227	— Very light infection, no material.
	6/21/29 fed 228	— Died.
	6/22/29 fed 229	— 11/19, 11/20, 11/22.
d) Material from 121 on	6/21/29 fed 224	— 11/19, 11/20, 11/22.
	6/22/29 fed 225	— 11/19, 11/20, 11/22.
	6/23/29 fed 226	— Very light infection, no material.
e) Material from 123 on	6/21/29 fed 221	— 11/19, 11/20, 11/22.
	6/22/29 fed 222	— 11/22.
	6/23/29 fed 223	— 11/19, 11/20, 11/22.
f) Material from 124 on	6/21/29 fed 218	— 11/22.
	6/22/29 fed 219	— 11/19, 11/20, 11/22.
	6/23/29 fed 220	— 11/19, 11/20, 11/22.

The results of the experiments as an effort at hybridization are negative as far as we are able to ascertain. A summary of the data is perhaps best visualized when presented in the form of a graph. Fig. 1 A shows the distribution of the lengths of the P_1 and P_2 *Eimeria acervulina* strains (material from chicks 113 and 113 A), ranging from 14.82 μ to 21.84 μ with the mean at 18.67 μ for 113 and at 16.83 μ for 113 A; Fig. 1 C shows the lengths of the P_1 and P_2 *Eimeria maxima* strains (material from chicks 115 and 115 C—D) ranging from 25.74 μ to 35.1 μ with the mean at 30.82 μ for 115 and at 29.91 μ for 115 C—D. Fig. 1 B—D, solid line, shows the distribution of the lengths of the oöcysts from all chicks on all days of the F_1 generation ranging from 14.04 μ to 35.88 μ with the mean at 17.76 μ for the *Eimeria acervulina* type oöcysts (Fig. 1 B); and at 30.17 μ for the *Eimeria maxima* type. The broken line shows the distribution similarly for the F_2 generation ranging from 12.48 μ to 35.88 μ with the mean at 17.25 μ for the *Eimeria acervulina* type oöcysts (Fig. 1 B), and at 30.10 μ for the *Eimeria maxima* type (Fig. 1 D). Fig. 2 A shows the distribution of the widths of the P_1 and P_2 *Eimeria acervulina* strains, ranging from 10.92 μ to 16.38 μ with the mean at 14.35 μ for 113, and at 12.37 μ for 113 A; and Fig. 2 C of the P_1 and P_2 *maxima* strains ranging

from 17.94 μ to 27.30 μ with the mean at 22.85 μ for 115, and at 20.55 μ for 115 C—D. Fig. 2 B—D, solid line, shows the distribution of the widths of the oöcysts from all chicks on all days of the F_1 generation, ranging from 10.92 μ to 28.08 μ with the mean for the *Eimeria acervulina* type at 13.29 μ (Fig. 2 B), and for the *Eimeria maxima* type at 21.38 μ (Fig. 2 D). The broken line shows the distribution for all the F_2 generation, ranging from 10.92 μ to

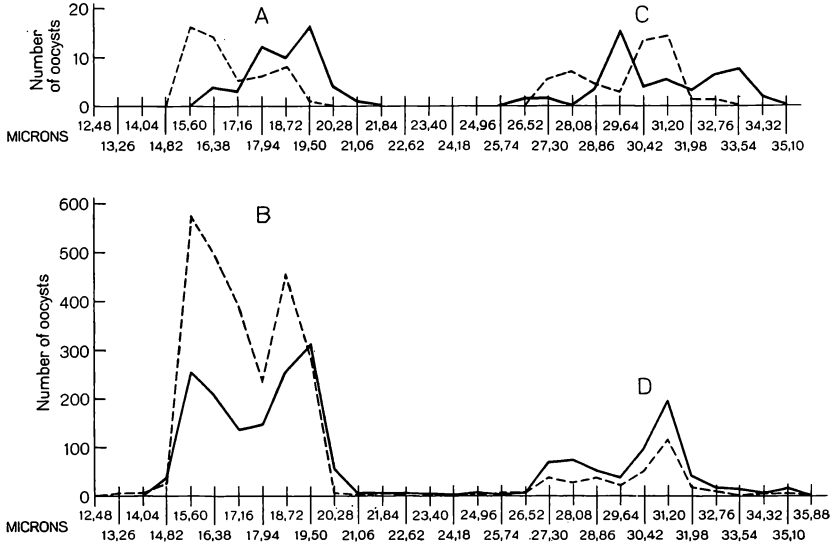


Fig. 1. Lengths. A *Eimeria acervulina*, parent strain. Solid line, single oöcyst infection; broken line, first transfer. One oöcyst per unit of ordinate. B *Eimeria acervulina*. Solid line, F_1 generation; broken line, F_2 generation. Ten oöcysts per unit of ordinate. C *Eimeria maxima*, parent strain. Solid line, single oöcyst infection (three oöcysts per unit of ordinate); broken line, first transfer (two oöcysts per unit of ordinate). D *Eimeria maxima*. Solid line, F_1 generation; broken line, F_2 generation. Ten oöcysts per unit of ordinate.

28.08 μ, with the mean for the *Eimeria acervulina* type at 12.75 μ (Fig. 2 B) and for the *Eimeria maxima* type at 21.49 μ (Fig. 2 D). It will be noted that of a total of 2000 measurements in the F_1 generation, there are nine possible intermediate forms, that is, nine F_1 oöcysts exceed in length the range of the P_1 and P_2 *Eimeria acervulina* series, but do not fall within the range of the P_1 and P_2 *Eimeria maxima* series. In the F_2 generation, there are three such forms. Two of these appeared on the fifth day after infection, while the third, and the nine F_1 intermediates were collected after seven days or more. These latter might then have been either

small *Eimeria maxima* forms or large *Eimeria acervulina* forms. The two F_2 intermediate forms collected on the fifth day, however, might be true hybrids, or *Eimeria acervulina* oöcysts which surpass in size any of those encountered elsewhere. It must be remembered, that the total number of measurements for the P_1 and P_2 *Eimeria acervulina* series was 150, and for the P_1 and P_2 *Eimeria maxima* series 250, while the F_1 series includes 2000 measurements. Had a similar number of measurements been made in the P_1 and P_2

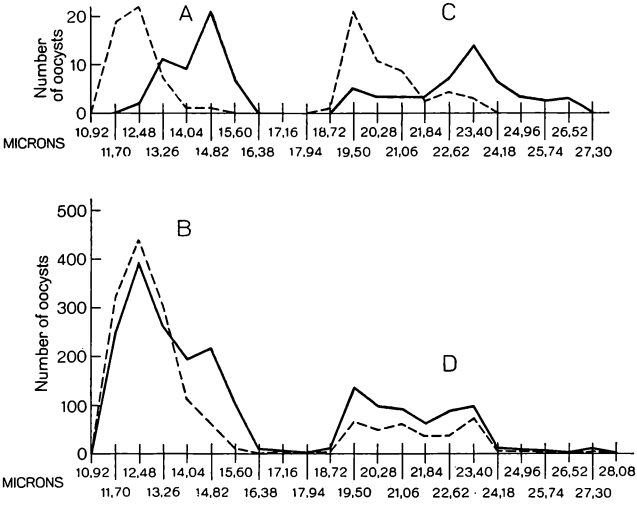


Fig. 2. Widths. A *Eimeria acervulina,* parent strain. Solid line, single oöcyst infection; broken line, first transfer. One oöcyst per unit of ordinate. B *Eimeria acervulina.* Solid line, F_1 generation (ten oöcysts per unit of ordinate); broken line, F_2 generation (twenty oöcysts per unit of ordinate). C *Eimeria maxima* parent strain. Solid line, single oöcyst infection (three oöcysts per unit of ordinate); broken line, first transfer (two oöcysts per unit of ordinate). D *Eimeria maxima.* Solid line, F_1 generation; broken line, F_2 generation. Ten oöcysts per unit of ordinate.

series, it is quite possible that the range of both parent types might have been extended and would then have included what now appear to be intermediate types. However, that these F_1 oöcysts are intermediates in size resulting from the crossing of the two parent strains is a possibility, but the small number encountered and their failure to appear on successive days in the chicken would suggest rather that they are simply extreme variations from one or the other parent type. Unfortunately, none of these intermediate

oöcysts could be isolated and a strain produced from them which might indicate their origin.

Had crossing taken place between *Eimeria maxima* and *Eimeria acervulina*, it is possible that some indication of hybridization would have been apparent in a modification of the external appearance of the oöcyst. An effect on size is perhaps the most obvious change to be expected, when the difference in size between the parent strains is remembered. Such an effect might have been expressed in one of several ways, such as the complete dominance of one or the other parent type in the F_1, or the production of an intermediate type in F_1, or a segregation into the two parent types depending upon which type of macrogametocyte was the mother cell. Since we are dealing with a population with probable opportunities for random mating, one would expect to find both parent types present in the F_1 even though there were complete dominance of one parent type over the other among the hybrids. In the F_2 from such hybrids, however, one would expect to find *Eimeria maxima* type oöcysts appearing from material containing only *Eimeria acervulina* type oöcysts, or *Eimeria acervulina* from material containing only *Eimeria maxima*, depending upon which size factors were dominant. The distribution in the F_2, however, gives no evidence of such an occurrence, the proportion of each parent type being apparently the same in each generation, and in those instances in which F_1 material containing only one type of oöcyst was fed, there was no case in which the other type was recovered in the second generation. As discussed above, the presence of so few intermediate types furnishes slight support to the second possibility. As to the third possibility, the influence of the size of the macrogametocyte cannot be ruled out completely without carrying the experiment a generation further, since in measuring the oöcysts, it is possible we are dealing with a character influenced or determined by the diploid stage of the life-cycle. Were this the case, oöcysts of the so-called F_1 generation would be influenced as to size by that parent strain from which the female gametocyte was produced. If, in turn, this size were determined by dominant mendelian factors, oöcysts of the F_2 generation, although heterozygous, would still resemble the parent strain, and only in F_3 would segregation occur.

Because of the size of the *Eimeria maxima* macrogametocyte, such might have been the case in the *Eimeria maxima* type of oöcysts appearing in F_1 and F_2. To test this possibility thoroughly, it would be necessary to select single oöcysts from many different

sources, and study the size of the organisms produced by them. As this was not feasible at the time, it was thought that the selection at random of a single oöcyst of the *Eimeria maxima* type from an F_2 culture in which many *Eimeria maxima* were present might give some indication of the purity or hybrid constitution of that particular strain. A single oöcyst of the *Eimeria maxima* type was therefore isolated from F_2 material from 230 of November 22, 1929 in which many *Eimeria maxima* were present. This was fed to chick 304, eleven days of age. The chick was kept in a separate cage on a wire bottom, and the feces were collected on the seventh day. From this material, one hundred oöcysts were measured. The distribution of the lengths and widths is shown in Fig. 3. The range in length is from 26.52 μ to 31.2 μ, and the mean is 29.30 $\pm$ .086. The widths range from 17.94 μ to 21.06 μ, with the mean at 19.70 $\pm$ .035. It is without doubt a pure strain of *Eimeria maxima*. This single instance is far from being proof that hybrid forms are not being masked by the size of the parent gametocyte, but in view of the lack of any other evidence that hybridization has occurred, it may perhaps be considered as an indication that it has not occurred, and because of that, be of interest here.

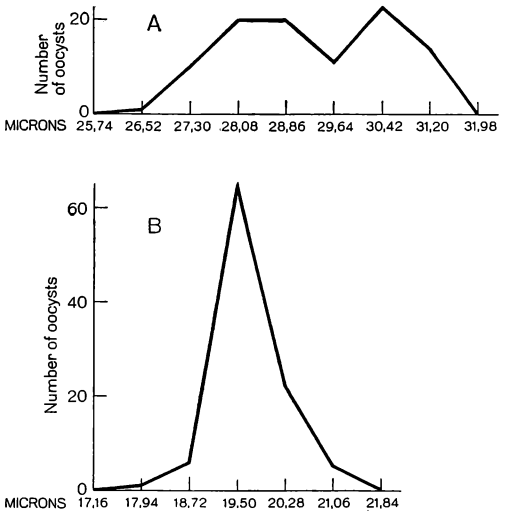


Fig. 3. Frequency curve of *Eimeria maxima* oöcysts, chick No. 304. Infection resulting from a single oöcyst isolated from an F_2 culture. A Lengths; B Widths.

In view of the negative results of these experiments, the only conclusion believed to be warranted by the data is that there is no convincing evidence that hybridization between *Eimera acervulina* and *Eimeria maxima* has occurred. Until such evidence is found, it seems justifiable to consider these two types of oöcysts as true species, and to maintain that size — within certain limits of variation — may be considered a species characteristic.

The material studied in the course of this experiment is of further interest, however, because of the amount of size variation encountered under different conditions.

Discussion of Measurements.

All the measurements presented in this paper were made with the use of a mechanical stage. The microscope was equipped with a micrometer eyepiece, each unit representing 3.9 μ. Readings were made to 0.2 of a division, or 0.78 μ, since this was felt to be to the limit of accuracy. Since all measurements were made by one individual, it is considered that the personal error involved is of the same magnitude in all series. However, the question of the degree of accuracy of the measurements is a troublesome one, since it may be argued that the precision of the mathematical formulae is too great to be applied to these data. No calculations were made on series of less than twenty measurements, and four times the probable error has been taken as the limit less than which differences are not considered to be significant.

A series of fifty measurements was made on the material collected from chick 113 (P_1) infected with a single oöcyst (shown by the solid line in Fig. 1 A and 2 A). The mean length is 18.67 μ $\pm$ .105 and the mean width 14.35 μ $\pm$ .082. The broken line (Fig. 1 A and 2 A) represents fifty measurements made on material from 113 A (P_2), which had been infected with oöcysts from 113. In this case, the mean length is 16.83 μ $\pm$ .113 and the mean width 12.37 μ $\pm$ .066. The difference between the lengths is 1.84 μ $\pm$ .154, which is 11.95 times the probable error, a mathematically significant difference. There are two points of difference between the infection in 113 and 113 A: 113 was five days old at the time of feeding; 113 A was twenty-three days old; and 113 was infected with a single oöcyst; 113 A was given a massive dose. It should also be pointed out at this time that neither distribution curve is symmetrical, 113 showing lesser peaks at 16.38 μ and at 17.94 μ; 113 A showing one lesser peak at 18.72 μ. The curves for the widths are more symmetrical, although 113 has a slight peak at 13.26 μ. The importance of this asymmetry will be discussed in a later paragraph.

In light infections, such as result from a single oöcyst, or from infection with a few oöcysts, there is no crowding of the developing forms in the epithelial cells. It would be expected, therefore,

that such oöcysts would attain their maximum size. In heavier infections, where every cell is parasitized, the developing forms may be too crowded to permit their reaching this maximum. It has been observed in some species after heavy infections, that some epithelial cells contain two or more parasites, and in such cases the developing forms are noticeably smaller than usually found in birds with light infections.

The *Eimeria acervulina* type of oöcysts appearing in the F_1 strains are represented in Figs. 1 B and 2 B by the solid line. The mean length of this generation is 17.76 $\mu \pm .027$, the mean width 13.29 $\mu \pm .020$. The *Eimeria acervulina* type in the F_2 strains are represented by the broken line in Figs. 1 B and 2 B. The mean length is 17.25 $\mu \pm .020$; the mean width 12.75 $\mu \pm .012$. The figures for the F_1 generation are based on 1410 measurements; for the F_2 generation on 2474 measurements.

The mean, standard deviation, and coefficient of variation for the lengths and widths of each group are presented in Table 1.

Table 1.

Biometrical data on Parent, F_1, and F_2 *Eimeria acervulina* Strains.

	Lengths in Microns	Widths in Microns
P_1 113	M — 18.67 ± .105 σ — 1.11 ± .074 CV — 23.13 ± 1.560	M — 14.35 ± .082 σ — 0.85 ± .059 CV — 23.21 ± 1.564
P_2 113 A	M — 16.83 ± .113 σ — 1.19 ± .082 CV — 27.56 ± 1.860	M — 12.37 ± .066 σ — .68 ± .043 CV — 21.39 ± 1.443
F_1	M — 17.76 ± .027 σ — 1.63 ± .020 CV — 35.81 ± .456	M — 13.29 ± .020 σ — 1.21 ± .016 CV — 35.48 ± .449
F_2	M — 17.25 ± .020 σ — 1.38 ± .012 CV — 31.20 ± .300	M — 12.75 ± .012 σ — .89 ± .008 CV — 27.33 ± .390

A summary of the means and their probable errors, and of the differences between the means is given in Table 2.

The variation within the group is so great that in only one case is the difference between the means less than four times its probable error, the majority being many times greater. However, it should also be noted that in no instance does the mean approach the limits of the range of *Eimeria maxima*, although there are sev-

Table 2.

Summary of the means and differences between the means of *Eimeria acervulina* Strains.

	Lengths in Microns		Widths in Microns	
(1) 113	18.67 ± .105		14.35 ± .082	
(2) 113 A	16.83 ± .113		12.37 ± .066	
(3) Total F_1	17.76 ± .027		13.29 ± .020	
(4) Total F_2	17.25 ± .020		12.75 ± .012	
Dif.		Dif. × P. E.		Dif. × P. E.
(1) and (2)	1.84 ± .154	11.95	1.98 ± .105	18.86
(1) and (3)	0.91 ± .108	8.43	1.06 ± .084	12.62
(1) and (4)	1.42 ± .107	13.27	1.60 ± .083	19.28
(2) and (3)	0.93 ± .116	8 02	0.92 ± .069	13.33
(2) and (4)	0.42 ± .115	3.65	0.38 ± .067	5.67
(3) and (4)	0.51 ± .034	15.00	0.54 ± .023	23.48

eral instances in which they are outside the range given by Tyzzer (1929) for the species.

Further analysis of the measurements made on the F_1 and F_2 generation material shows great variation within each group. This is particularly true of the *Eimeria acervulina* oöcysts, and their measurements will be discussed first. In the F_1 generation, material was collected on three successive days at varying intervals after the infective feeding of oöcysts. The question naturally arose, whether there was a difference among the oöcysts from the same chicken at successive stages of the disease. One hundred measurements were made on each of three successive days on material from three chickens. A summary of the *Eimeria acervulina* measurements giving means of the lengths and widths and their probable errors, and of the differences between the means is given in Table 3.

The figures for 119 show significant variation between the means of both lengths and widths for each day: 121 shows significant variation between the mean lengths on the first and second, and on the first and third days, but no significant variation between the mean widths on the first and second, and second and third days; while 123 shows no significant difference between the means of the lengths on any of the three days, but the difference in the widths is significant between the first and third days. Thus all combinations of variations seem to have existed, and it can only be said that material from the same chick on successive days may show significant variations from day to day.

Table 3.

Summary of means and differences between the means of *Eimeria acervulina* collected on three successive days from three birds.

	Lengths in Microns		Widths in Microns	
119 (1) 6/20	17.35 ± .117		13.09 ± .078	
(2) 6/21	16.03 ± .062		12.38 ± .043	
(3) 6/22	16.52 ± .086		12.68 ± .062	
Dif.		Dif. × P. E.		Dif. × P. E.
(1) and (2)	1.32 ± .132	10.00	0.71 ± .089	7.98
(1) and (3)	0.83 ± .145	5.72	0.41 ± .100	4.10
(2) and (3)	0.49 ± .106	4.62	0.30 ± .075	4.00
121 (1) 6/21	19.22 ± .090		14.40 ± .094	
(2) 6/22	18.31 ± .094		13.97 ± .078	
(3) 6/23	18.53 ± .098		13.86 ± .082	
Dif.		Dif. × P. E.		Dif. × P. E.
(1) and (2)	0.91 ± .130	7.00	0.43 ± .122	3.52
(1) and (3)	0.69 ± .133	5.19	0.54 ± .125	4.32
(2) and (3)	0.22 ± .136	1.59	0.11 ± .113	0.97
123 (1) 6/21	18.69 ± .074		13.72 ± .086	
(2) 6/22	18.65 ± .070		13.99 ± .066	
(3) 6/23	18.80 ± .062		14.33 ± .062	
Dif.		Dif. × P. E.		Dif. × P. E.
(1) and (2)	0.04 ± .102	—	0.27 ± .108	2.50
(1) and (3)	0.11 ± .097	1.13	0.61 ± .106	5.75
(2) and (3)	0.15 ± .094	1.60	0.34 ± .091	3.74

The question then arose, whether oöcysts from different chickens, but taken on the same day after the infective feeding would show similar variations. The data for the fifth, sixth, seventh, and eighth days after feeding *Eimeria acervulina* are presented in Tables 4, 5, 6 and 7. Material was collected from only one chicken on the fourth day after feeding (120 for 6/21/29). The mean length in this case is 15.85 $\mu \pm .055$; the mean width 12.13 $\mu \pm .062$. Similarly, material was collected from only one chick the ninth day after feeding (121 for 6/23/29). The mean length is 18.53 $\mu \pm .098$, and the width 13.86 $\mu \pm .082$.

Table 4 giving the means for material collected five days after the infective feeding shows significant differences in two of the three comparisons, the material from chick 124 being over 2 μ longer, and 1 μ wider than the material from the other two birds.

Table 5 giving the means for material collected six days after the infective feeding, shows significant variation in length in all but one out of six combinations, and in width in three out of six.

Table 4.

Means and differences between the means of *Eimeria acervulina* on the fifth day after infective feeding.

	Lengths in Microns		Widths in Microns	
(1) 118, 6/20	16.84 ± .101		12.61 ± .074	
(2) 120, 6/22	16.53 ± .078		12.48 ± .051	
(3) 124, 6/24	18.98 ± .086		13 81 ± .090	
Dif.		Dif. × P. E.		Dif. × P. E.
(1) and (2)	.31 ± .128	2.42	.13 ± .090	1.44
(1) and (3)	2.14 ± .133	16.09	1.20 ± .117	10.26
(2) and (3)	2.45 ± .116	20.82	1.33 ± .103	12.91

Table 5.

Means and differences between the means of *Eimeria acervulina* on the sixth day after infective feeding.

	Lengths in Microns		Widths in Microns	
(1) 119, 6/20	17.35 ± .117		13.09 ± .078	
(2) 123, 6/21	18.69 ± .074		13.72 ± .086	
(3) 125, 6/21	18.10 ± .113		12.93 ± .082	
(4) 124, 6/23	18.14 ± .082		13.14 ± .059	
Dif.		Dif. × P. E.		Dif. × P. E.
(1) and (2)	1.34 ± .138	9.71	.63 ± .116	5.43
(1) and (3)	.75 ± .163	4.60	.16 ± .113	1.42
(1) and (4)	.79 ± .143	5.52	.06 ± .098	—
(2) and (3)	.59 ± .135	4.37	.79 ± .119	6.64
(2) and (4)	.55 ± .110	5.00	.58 ± .104	5.58
(3) and (4)	.04 ± .139	—	.21 ± .101	2.08

Table 6 summarizes the means and the differences between them of material collected seven days after the infective feeding. Seven of the ten comparisons of the lengths are significantly different, and so also are seven of the ten comparisons of the widths.

Table 7 is a summary of material on the eighth day after the infective feeding, and here all three of the differences between the lengths are significant, and two of those between the widths.

Similar comparisons were made for material collected in the F_2 generation. Material from the same chick collected on successive days is considered first. Table 8 summarizes the measurements on *Eimeria acervulina* oöcysts made on material from eight chickens collected on November 19, 20, 22, 1929. (One hundred measurements were made on the material for each day.) Twenty-two out of the forty-eight comparisons (both lengths and widths) do not show

Table 6.

Means and differences between the means of *Eimeria acervulina* on the seventh day after infective feeding.

	Lengths in Microns		Widths in Microns	
(1) 119, 6/21	16.03 ± .062		13.09 ± .078	
(2) 121, 6/21	19.22 ± .090		14.40 ± .094	
(3) 126, 6/21	18.92 ± .105		14.07 ± .101	
(4) 118, 6/22	16.30 ± .074		12.38 ± .051	
(5) 123, 6/22	18.65 ± .070		13.99 ± .066	
Dif.		Dif. × P. E.		Dif. × P. E.
(1) and (2)	3.19 ± .109	29.27	1.31 ± .122	10.74
(1) and (3)	2.89 ± .122	23.69	0.98 ± .128	7.66
(1) and (4)	0.27 ± .097	2.78	0.71 ± .093	7.63
(1) and (5)	2.62 ± .093	28.17	0.90 ± .102	8.82
(2) and (3)	0.30 ± .138	2.17	0.33 ± .138	2.39
(2) and (4)	2.92 ± .117	24.96	2.02 ± .107	18.88
(2) and (5)	0.57 ± .114	5.00	0.41 ± .115	3.57
(3) and (4)	2.62 ± .128	20.47	1.69 ± .113	14.96
(3) and (5)	0.27 ± .126	2.14	0.08 ± .121	—
(4) and (5)	2.35 ± .102	23.04	1.61 ± .083	19.40

Table 7.

Means and differences between the means of *Eimeria acervulina* on the eighth day after infective feeding.

	Lengths in Microns		Widths in Microns	
(1) 119, 6/22	16.52 ± .086		12.68 ± .062	
(2) 121, 6/22	18.31 ± .094		13.97 ± .078	
(3) 123, 6/23	18.80 ± .062		14.33 ± .062	
Dif.		Dif. × P. E.		Dif. × P. E.
(1) and (2)	1.79 ± .127	14.09	1.29 ± .100	12.90
(1) and (3)	2.28 ± .106	21.51	1.65 ± .088	18.75
(2) and (3)	0.49 ± .113	4.34	0.36 ± .100	3.60

significant differences and eighteen of these twenty-two are between lengths and widths on the same sets of material, the variations in length and in width on the same days apparently showing some correlation in this case.

A comparison of the mean lengths and widths of material collected on the same day after the infective feeding from the different F_2 chickens was likewise made. Of the mean lengths and widths for material collected the fifth day after the infective feeding, fifteen of the twenty-eight differences in lengths compared are not significant, and thirteen of the differences between the widths. Eleven

Table 8.

Means and differences between the means of *Eimeria acervulina*, F_2 generation, from eight chickens on three days.

	Lengths (μ)		Widths (μ)	
219 (1) 11/19	18.03 ± .086		13.24 ± .066	
(2) 11/20	18.06 ± .086		12.93 ± .062	
(3) 11/22	16.74 ± .101		12.34 ± .051	
Dif.		Dif. × P. E.		Dif. × P. E.
(1) and (2)	.03 ± .122	—	.31 ± .091	3.41
(1) and (3)	1.29 ± .133	9.70	.90 ± .084	10.71
(2) and (3)	1.32 ± .133	9.92	.59 ± .080	7.38
220 (1) 11/19	17.25 ± .098		12.59 ± .055	
(2) 11/20	16.71 ± .070		12.65 ± .055	
(3) 11/22	17.36 ± .090		12.80 ± .070	
Dif.		Dif. × P. E.		Dif. × P. E.
(1) and (2)	.54 ± .120	4.50	.06 ± .078	—
(1) and (3)	.11 ± .133	—	.21 ± .089	2.36
(2) and (3)	.65 ± .114	5.70	.15 ± .089	1.69
221 (1) 11/19	16.25 ± .070		12.25 ± .043	
(2) 11/20	17.30 ± .086		12.82 ± .059	
(3) 11/22	16.60 ± .070		12.50 ± .051	
Dif.		Dif. × P. E.		Dif. × P. E.
(1) and (2)	1.05 ± .111	9.46	.57 ± .073	7.81
(1) and (3)	.35 ± .099	3.54	.25 ± .067	3.73
(2) and (3)	.70 ± .111	6.31	.32 ± .078	4.10
223 (1) 11/19	17.23 ± .094		12.57 ± .051	
(2) 11/20	17.98 ± .078		12.92 ± .051	
(3) 11/22	17.23 ± .094		12.70 ± .055	
Dif.		Dif. × P. E.		Dif. × P. E.
(1) and (2)	.75 ± .122	6.15	.35 ± .072	4.86
(1) and (3)	—	—	.13 ± .075	1.73
(2) and (3)	.75 ± .122	6.15	.22 ± .075	2.93
224 (1) 11/19	17.53 ± .094		12.96 ± .066	
(2) 11/20	17.55 ± .078		12.92 ± .051	
(3) 11/22	16.10 ± .062		12.12 ± .039	
Dif.		Dif. × P. E.		Dif. × P. E.
(1) and (2)	.02 ± .122	—	.04 ± .083	—
(1) and (3)	1.43 ± .113	12.65	.84 ± .076	10.05
(2) and (3)	1.45 ± .100	14.50	.80 ± .064	12.50
225 (1) 11/19	16.75 ± .094		12.51 ± .062	
(2) 11/20	16.90 ± .082		12.66 ± .055	
(3) 11/22	16.45 ± .086		12.24 ± .043	
Dif.		Dif. × P. E.		Dif. × P. E.
(1) and (2)	.15 ± .125	1.20	.15 ± .083	1.81
(1) and (3)	.30 ± .127	2.36	.27 ± .075	3.60
(2) and (3)	.45 ± .119	3.78	.42 ± .070	6.00

Continuation from Table 8.

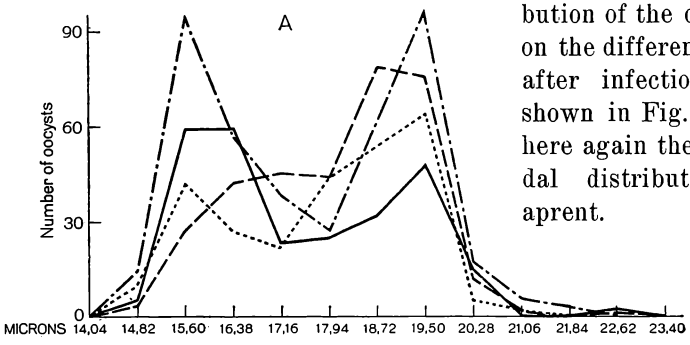
	Lengths (μ)		Widths (μ)	
229 (1) 11/19	17.06 ± .090		12.55 ± .062	
(2) 11/20	17.75 ± .086		12.89 ± .051	
(3) 11/22	17.60 ± .094		13.13 ± .070	
Dif.		Dif. × P. E.		Dif. × P. E.
(1) and (2)	.69 ± .124	5.56	.34 ± .080	4.25
(1) and (3)	.54 ± .130	4.15	.58 ± .094	6.17
(2) and (3)	.15 ± .127	1.18	.24 ± .087	2.76
230 (1) 11/19	17.07 ± .094		12.70 ± 0.55	
(2) 11/20	17.77 ± .086		13.17 ± .062	
(3) 11/22	17.96 ± .121		13.16 ± .094	
Dif.		Dif. × P. E.		Dif. × P. E.
(1) and (2)	.70 ± .127	5.51	.47 ± .083	5.66
(1) and (3)	.89 ± .153	5.82	.46 ± .109	4.22
(2) and (3)	.19 ± .148	1.28	.01 ± .113	—

of these differences are for lengths and widths of the same sets of material.

Of the mean lengths and widths for material collected the sixth day after the infective feeding, fourteen of the twenty-eight differences in lengths of which comparisons were made are not significantly different, while twenty-five of the twenty-eight differences in widths are not significant. Thirteen of these are of lengths and widths of the same material. In this case, however, there seems to have been much less variation among the widths than in the lengths.

Of the means of material collected on the eighth day after infection, ten of the twenty-eight differences between the lengths are not significant, and eleven of those between the widths, eight of these are on the same material. It would appear that the amount of variation on this day was somewhat greater than on the two previous days, and in this case the lengths and widths show approximately the same amount of variation.

The mean length and width were also calculated on all F_2 material collected on successive days. A comparison of the means is given in Table 9. Two of the six comparisons between the mean lengths, and one of the six between the mean widths are not significant. Because of the great variation between the material collected on the same day, such variation between material collected on different days was to be expected. Curves showing the size distri-



bution of the oöcysts on the different days after infection are shown in Fig. 4 and here again the bimodal distribution is aprent.

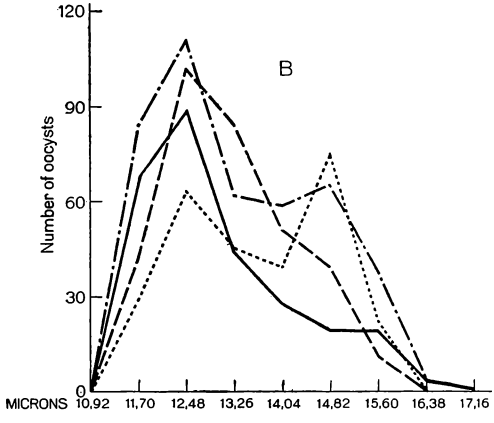


Fig. 4. Frequency distribution of *Eimeria acervulina* oöcysts on different days after the first infective feeding. Solid line, fifth day; broken line, sixth day; dot and dash, seventh day; dotted line, eighth day. A Lengths; B Widths.

Table 9.

Comparison of mean lengths and widths of *Eimeria acervulina*, F_2 generation, by days after infection feeding.

	Lengths in Microns		Widths in Microns	
(1) 5 days	17.50 ± .070		12.99 ± .051	
(2) 6 days	18.07 ± .051		13.20 ± .035	
(3) 7 days	17.65 ± .055		13.33 ± .039	
(4) 8 days	17.83 ± .062		13.62 ± 0.51	
Dif.		Dif. × P. E.		Dif. × P. E.
(1) and (2)	.57 ± .087	6.55	.21 ± .062	3.39
(1) and (3)	.15 ± .089	1.69	.34 ± .064	5.31
(1) and (4)	.33 ± .094	3.51	.63 ± .072	8.75
(2) and (3)	.42 ± .075	5.60	.13 ± .052	2.50
(2) and (4)	.24 ± .080	3.00	.42 ± .062	6.77
(3) and (4)	.18 ± .083	2.17	.29 ± .064	4.53

A summary of the data for *Eimeria maxima* in the four generations is presented in Figs. 1 and 2. The solid line (Figs. 1 C and 2 C) represents 150 measurements on 115 (P_1). These measurements were made on material collected on three successive days after oöcysts first appeared in the droppings. A comparison of the means of this material (Table 10) shows no instance in which the difference is more than four times the probable error.

Table 10.

Eimeria maxima from 115 on three successive days after infection from a single oöcyst.

	Lengths in Microns		Widths in Microns	
(1) 5/31	$30.72 \pm .158$		$22.78 \pm .162$	
(2) 6/1	$31.19 \pm .145$		$23.32 \pm .158$	
(3) 6/2	$30.55 \pm .168$		$22.45 \pm .158$	
Dif.		Dif. $\times$ P. E.		Dif. $\times$ P. E.
(1) and (2)	$.47 \pm .214$	2.20	$.54 \pm .226$	2.39
(1) and (3)	$.17 \pm .231$	—	$.33 \pm .226$	1.46
(2) and (3)	$.64 \pm .222$	2.88	$.87 \pm .223$	3.90

The dotted line (Figs. 1 C and 2 C) represents the P_2 generation combining 115 A—B, and C—D. Each of these cultures is composed of the combined material from two chickens. The mean length of 115 A—B is $29.45\,\mu \pm .098$ while that for 115 C—D is $29.91\,\mu \pm 101$. The difference is $0.46\,\mu \pm .141$, which is 3.26 times its probable error. The mean width for 115 A—B is $20.44\,\mu \pm .070$, and for 115 C—D, $20.55\,\mu \pm .082$. The difference is $.111 \pm .108$, or 1.08 times the probable error.

The *Eimeria maxima* type of oöcysts appearing in the F_1 strains (Figs. 1 D and 2 D) have a mean length of $30.17\,\mu \pm .051$, and a mean width (Fig. 2 D) of $21.38\,\mu \pm .043$. Those in F_2 (Figs. 1 D and 2 D, broken line) have a mean length of $30.10\,\mu \pm .062$, and a mean width of $21.49\,\mu \pm .059$. The figures for the F_1 generation are based on 581 measurements, and for the F_2 on 326. The mean, standard deviation, and coefficient of variation for the lengths and widths of each group are presented in Table 11. A chart showing the size distribution of these oöcysts on the different days after the first infective feeding is given in Fig. 5.

A summary of the means and their probable errors, and of the differences between the means is given in Table 12.

Here again, there is a mathematically significant difference between the means of the two parent strains themselves, and

between the means of the parent strains, of the F_1, and of the F_2 generations. There is not, however, a significant difference between the F_1 and the F_2 strains, either in length or in width. The reason for this is not entirely clear. However, there is much less variation within the *Eimeria maxima* strain than was apparent in *Eimeria acervulina*. This is evident in a comparison of *Eimeria maxima* material from different chicks taken

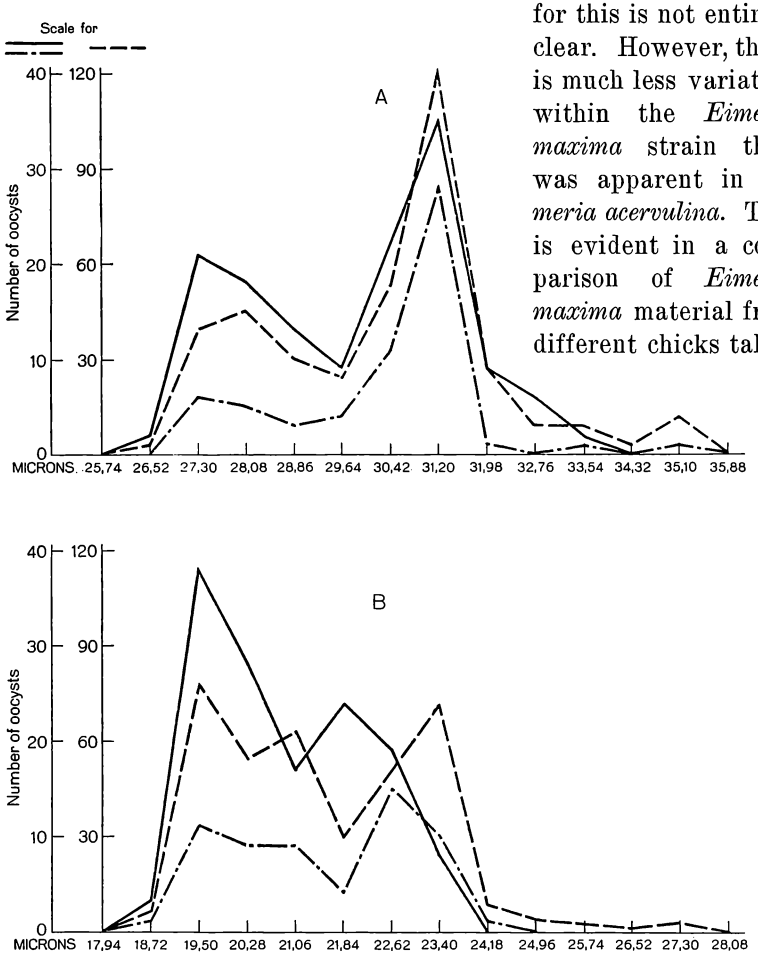


Fig. 5. Frequency distribution of *Eimeria maxima* oöcysts on different days after the first infective feeding. Solid line, seven days (one oöcyst per unit of ordinate); broken line, eight days (three oöcysts per unit of ordinate); dot and dash, nine days (one oöcyst per unit of ordinate). A Lengths; B Widths.

on the same day after the infective feeding, and is indicated in the one F_1 chick in which *Eimeria maxima* appeared on three successive days (Table 13).

In this case the only significant difference between the means appears in the comparison of the mean lengths on the first and

Table 11.

Biometrical data on parent, F_1, and F_2 *Eimeria maxima* strains.

	Lengths in Microns	Widths in Microns
Total 115	M — 30.82 ± .092 σ — 1.68 ± .066 CV — 17.95 ± .696	M — 22.85 ± .092 σ — 1.71 ± .066 CV — 24.73 ± .960
Total 115 AB—CD	M — 29.68 ± .070 σ — 1.48 ± .051 CV — 19.48 ± .659	M — 20.49 ± .051 σ — 1.12 ± .035 CV — 21.22 ± .714
F_1 Total	M — 30.17 ± .051 σ — 1.83 ± .035 CV — 24.87 ± .488	M — 21.38 ± .043 σ — 1.62 ± .031 CV — 29.59 ± .585
F_2 Total	M — 30.10 ± .062 σ — 1.71 ± .043 CV — 22.13 ± .585	M — 21.49 ± .059 σ — 1.54 ± .039 CV — 27.88 ± .737

Table 12.

Summary of the means and differences between the means of *Eimeria maxima* strains.

	Lengths in Microns		Widths in Microns	
(1) 115	30.82 ± .092		22.85 ± .092	
(2) 115 AB—CD	29.68 ± .070		20.50 ± .051	
(3) F_1	30.17 ± .051		21.38 ± .043	
(4) F_2	30.10 ± .062		21.49 ± .059	
Dif.		Dif. × P. E.		Dif. × P. E.
(1) and (2)	1.13 ± .116	9.74	2.35 ± .105	22.38
(1) and (3)	.65 ± .105	6.19	1.47 ± .102	14.41
(1) and (4)	.72 ± .111	6.49	1.36 ± .109	12.48
(2) and (3)	.49 ± .087	5.63	.88 ± .067	13.13
(2) and (4)	.42 ± .094	4.47	.99 ± .078	12.69
(3) and (4)	.07 ± .080	—	.11 ± .073	1.51

Table 13.

Summary of means and differences between the means of *Eimeria maxima* collected on three successive days.

	Lengths in Microns		Widths in Microns	
(1) 121, 6/21	31.29 ± .090		22.56 ± 2.15	
(2) 121, 6/22	30.19 ± .062		22.07 ± .148	
(3) 121, 6/23	30.92 ± .296		22.02 ± .304	
Dif.		Dif. × P. E.		Dif. × P. E
(1) and (2)	1.10 ± .109	10.09	0.49 ± .261	1.88
(1) and (3)	0.37 ± .309	1.20	0.54 ± .372	1.45
(2) and (3)	0.73 ± .302	2.42	0.05 ± .338	—

second days. The difference between the mean widths on these days is not significant.

A comparison of material in the F_1 generation from different birds, collected on the eighth day after the first infective feeding is shown in Table 14.

Table 14.

Means and differences between the means of *Eimeria maxima*, F_1 generation, collected on the eighth day after the first infective feeding.

	Lengths in Microns		Widths in Microns	
(1) 120	30.22 ± .137		21.11 ± .133	
(2) 122	29.71 ± .117		20.85 ± .101	
(3) 125	30.00 ± .265		21.35 ± .172	
(4) 126	30.48 ± .187		21.09 ± .199	
(5) 123	30.26 ± .254		22.39 ± .246	
(6) 124	30.47 ± .129		21.91 ± .121	
Dif.		Dif. × P. E.		Dif. × P. E.
(1) and (2)	.51 ± .180	2.83	.26 ± .167	1.56
(1) and (3)	.22 ± .298	—	.24 ± .217	1.11
(1) and (4)	.26 ± .232	1.12	.02 ± .239	—
(1) and (5)	.04 ± .289	—	1.28 ± .280	4.57
(1) and (6)	.25 + .188	1.33	.80 ± .180	4.44
(2) and (3)	.29 ± .290	1.00	.50 ± .199	2 51
(2) and (4)	.77 ± .221	3.48	.24 ± .223	1.08
(2) and (5)	.55 ± .280	1.96	1.54 ± .266	5.79
(2) and (6)	.76 ± .174	4.37	1.06 ± .158	6.71
(3) and (4)	.48 ± .324	1.48	.26 ± .263	—
(3) and (5)	.26 ± .367	—	1.04 ± .300	3.47
(3) and (6)	.47 ± .295	1.59	.56 ± .210	2.67
(4) and (5)	.22 ± .315	—	1.30 ± .316	4.11
(4) and (6)	.01 ± .227	—	.82 ± .233	3.52
(5) and (6)	.21 ± .285	—	.48 ± .274	1.75

In only two cases out of the fifteen comparisons between the mean lengths is there a significant difference; but there are seven significant differences between the mean widths.

Material was collected from only one chick on the seventh day after the first infective feeding, 120 on June 20, 1929. The mean length for this material was 29.95 μ ± .113 and the mean width 17.01 μ ± .082.

A comparison of the F_2 generation material from four different chicks collected on the same day is shown in Table 15. There are no significant differences between the mean lengths. Between the mean widths, however, there are two differences which are significant, indicating again a greater variation in the widths than in the lengths.

Table 15.

Means and differences between the means of *Eimeria maxima*, F_2 generation, from four birds on the same day after infective feeding.

	Lengths in Microns		Widths in Microns	
(1) 218	30.06 ± .125		21.21 ± .113	
(2) 222	30.24 ± .101		21.78 ± .109	
(3) 230	30.23 ± .117		21.52 ± .101	
(4) 232	30.71 ± .257		22.04 ± .156	
Dif.		Dif. × P. E.		Dif. × P. E.
(1) and (2)	.18 ± .161	1.12	.57 ± .157	3.63
(1) and (3)	.17 ± .171	—	.31 ± .151	2.05
(1) and (4)	.65 ± .286	2.27	.83 ± .193	4.30
(2) and (3)	.01 ± .154	—	.26 ± .148	1.76
(2) and (4)	.47 ± .276	1.70	.26 ± .190	1.37
(3) and (4)	.48 ± .282	1.70	.52 ± .186	2.80

From the data presented here it is evident that there is a more fundamental factor involved in the production of variation than the factors of age of the host or day of infection (within the period considered). The asymmetry of the distribution of the original parent strains (as shown in Figs. 1 and 2) indicates the presence of different types among the offspring of a single oöcyst. In the *Eimeria acervulina* measurements, the curve for 113 (lengths) shows three peaks, or shoulders, one at 16.38 μ, one at 17.94 μ and one at 19.5 μ. The width curve shows two peaks, one at 13.26 μ and one at 14.82 μ. Likewise 113 A shows two peaks in the length curve, one at 15.6 μ and one at 18.72 μ. The width curve shows only one peak at 12.48 μ. When the curves of the F_1 and F_2 *Eimeria acervulina* measurements are compared with those of the two pure strains, it is interesting to note the distribution of the peaks. A summary of the peaks of the F_1 and F_2 curves is given in Table 16. The principal peak is given in one column and minor peaks or "shoulders" are given in another. The length curves for F_1 chicks 118, 119 and 120 have their principal peaks at 15.6 μ (with one exception which is at 16.38 μ), with minor peaks at 19.5 μ, 18.72 μ and 17.94 μ. These correspond more or less closely to the peaks for 113 A. The width curves, for the most part, have only one peak, at 12.48 μ. The two exceptions are at 11.70 μ, and 14.82 μ. In contrast to these, the material from the other three F_1 chickens approaches more nearly the distribution of 113. The majority of the peaks in these curves come at 18.72 μ and 19.5 μ for the lengths, and at 14.82 μ, 13.26 μ and 12.48 μ for the widths.

The widths are intermediate between 113 and 113 A, 12.48 μ being the principal peak for 113 A, and 14.82 μ the major peak for 113.

The F_2 curves show greater variation in the distribution of their peaks. In cases where F_1 material showing two peaks was fed, the peaks of the F_2 material seem to fall at points between the F_1 peaks, the principal peak of the F_2 material never being at a point greater than that of the greater F_1 peak.

Variation within a strain of organisms produced from the isolation of a single oöcyst, such as has been encountered in the course of these experiments, leads one to believe that we may be dealing with a case similar to Jennings' biotypes in *Paramecium*. It will be remembered that Jennings (1909) found eight biotypes of which the four larger belonged to the species *Paramecium caudatum*, and the four smaller to the species *Paramecium aurelia*. Within a given biotype, if the largest and the smallest individuals were selected for reproduction, the offspring of each exhibited the same mean size.

A similar case is met with in *Difflugia corona* (Jennings, 1916) in which there are biotypes differing between themselves, and with these differences inherited, yet within each biotype there are similar differences and these are not inherited.

To determine the variation similarly in the coccidia it would be necessary to select single organisms from the extreme types encountered, within a strain, and from the strains produced by them discover their ability to reproduce the entire range of variation of the original strain.

In the species of *Eimeria* used in these experiments the means of the strains of *Eimeria acervulina* have never approached the means of the strains of *Eimeria maxima*. These have been distinct, and the distribution curves of the oöcysts have been distinct with the exception of the nine intermediate forms in the F_1 generation.

Within the strains themselves, the amount of variation has been great. It must be remembered than in this case, moreover, we are dealing with biparental inheritance, so that, although the strains were started from single oöcysts, the parent oöcyst may have been heterozygous for some of the factors determining size.

Part II.

Another series of experiments was later undertaken to provide further data on the question relating to the effect of age, breed of the host, and stage of infection on the size of the oöcyst. A third

Table 16.

Lengths								Widths							
No. F_1	Date	Major peak	Minor peak	No. F_2	Date	Major peak	Minor peak	No. F_1	Date	Major peak	Minor peak	No. F_2	Date	Major peak	Minor peak
					11/19	16.38	18.72						11/19	12.48	
118	6/20	16.38	19.5	**230**	11/20	17.16 18.72		**118**	6/20	12.48		**230**	11/20	13.26	
					11/22	17.16	18.72						11/22	12.48	
	6/22	15.6	18.72						6/22	11.7					
119	6/20	15.6	19.5	**232**	11/20	17.94 18.72	16.38	**119**	6/20	12.48		**232**	11/20	13.26	
					11/22	18.72	15.6						11/22	12.48 13.26	14.82
	6/21	15.6							6/21	12.48					
	6/22	15.6							6/22	12.48	14.82				
120	6/21	15.6						**120**	6/21	11.7					
					11/19	15.6 16.38	19.5						11/19	11.7	
	6/22	15.6	17.94	**229**	11/20	18.72	16.38		6/22	12.48		**229**	11/20	12.48	
					11/22	18.72	16.38						11/22	13.26	
					11/19	18.72	15.6						11/19	12.48	
121	6/21	19.5	16.38	**224**	11/20	16.38	18.72	**121**	6/21	14.82		**224**	11/20	12.48	
					11/22	15.6	18.72						11/22	11.7	
					11/19	15.6	18.72						11/19	11.7	
	6/22	18.72		**225**	11/20	15.6			6/22	14.82		**225**	11/20	12.48	15.6
					11/22	15.6	18.72						11/22	11.7	
	6/23	19.5	15.6						6/23	14.82	13.26				
					11/19	15.6							11/19	11.7	
123	6/21	18.72		**221**	11/20	18.72	16.38	**123**	6/21	14.82	13.26	**221**	11/20	12.48	
					11/22	16.38	18.72						11/22	12.48	
	6/22	19.5	17.16						6/22	14.04 14.82					
					11/19	15.6	18.72						11/19	12.48	14.82
	6/23	19.5		**223**	11/20	18.72	17.16		6/23	14.82	12.48	**223**	11/20	12.48	
					11/22	15.6	19.5						11/22	12.48	
					11/19	18.72	17.16						11/19	13.26	
124	6/22	19.5		**219**	11/20	18.72	17.16	**124**	6/22	13.26	15.6	**219**	11/20	12.48	
					11/22	15.6	18.72						11/22	12.48	
					11/19	15.6	18.72						11/19	11.7	
	6/23	18.72		**220**	11/20	16.38	18.72		6/23	12.48		**220**	11/20	12.48	
					11/22	15.6	18.72						11/22	12.48	

species of coccidia, a strain of *Eimeria tenella*, the oöcysts of which averaged somewhat larger than those of strains previously encountered, was being studied in the laboratory at the time, and was used for these experiments.

This strain was obtained from a Barred Plymouth Rock chicken, sixty-eight days of age. The bird was suffering from a typical *Eimeria tenella* infection, with severe hemorrhage from the caeca. Study of sections of the caeca from this bird and others infected with the same material showed the pathology to be identical with that of previous *Eimeria tenella* infections. The oöcysts, however, were somewhat larger than *Eimeria tenella* oöcysts measured at other times, the average, based on one hundred measurements, being $23.48 \times 19.04\ \mu$. This material was used to infect a Rhode Island Red chicken, seventeen days old. Oöcysts produced by this bird measured $21.88 \times 17.24\ \mu$ (average of one hundred measurements). The figures are shown in Table 17, together with the measurements of *Eimeria tenella* made on material from previous infections.

Table 17.

Comparison of size in microns of oöcysts in strains of *Eimeria tenella*.

	Average	Maximum	Minimum	Largest organism	Smallest organism
30 : 22 *E. tenella* (new strain)	23.48×19.04	26.52×21.84	20.28×16.38	26.52×19.5 or 24.96×21.84	20.28×17.94 or 22.62×16.38
Ist transfer of 30 : 22	21.88×17.24	26.52×19.5	16.38×12.48	26.52×18.72 or 25.74×19.5	16.38×12.48
E. tenella	22.6×19.0	26.1×22.8	19.6×16.3		

Heavy infections with this species produce severe hemorrhage from the fourth to the seventh days, after which the caeca may be filled with a core of hardened exudate, and become non-functional for a period of time. However, by feeding very small numbers of oöcysts, an infection may be produced in which hemorrhage does not develop, nor is the caecal core formed. Light infections, it was thought, repeated at short intervals, would induce immunity in the course of time, but more slowly than in a severe infection, and thus would give an opportunity of studying the effect of a developing immunity on the oöcyst. To test the effect of breed and age of the host at the same time, two groups of chickens were infected, one of Rhode Island Reds, one of Barred Plymouth Rocks.

Three chickens of each group, all three days of age, were fed a counted number of oöcysts on March 7. Four days later, they were again fed a small number (see Table 18 for numbers), and in addition, a fourth Rhode Island Red and a fourth Barred Plymouth Rock chick of the same age, but previously uninfected, were given a small dose of oöcysts. These two chicks served as a control for the age factor for the first four-day interval. The feeding of a small number of oöcysts to the six birds of the original group was repeated at four day intervals until March 24, making a total of five feedings, and at each feeding, two additional chicks, one of each breed, were fed as controls for the age factor. Not all of the control birds became infected at the later feedings, thus accounting for the discrepancy in the number of Barred Plymouth Rocks and Rhode Island Reds shown in the table.

The outline of the experiment is shown in Table 18. Two difficulties were encountered which made it impossible to carry out the original plan as exactly as it had been hoped. The first of these was the failure to obtain caecal droppings from all chickens at each observation; and the second was the rapid development of a partial immunity which reduced the number of oöcysts produced so greatly that it was impossible to measure a sufficiently large series from the later samples to compare with the earlier ones. The longest period over which oöcysts were produced in large enough numbers for counting was fourteen days in Barred Plymouth Rock chick 315. Three sets of measurements were made on material from this bird, on March 18, March 20, and March 31. The means, standard deviations, and coefficients of variation of these measurements are shown in Table 19.

A comparison of the means is given in Table 20.

The significant difference in size observed in this case is between material obtained after an interval of three days at the beginning of the infection, before any appreciable difference in age had occurred, or any active immunity had been developed. The means for the first series of measurements, on material collected eleven days after the first infective feeding, are not significantly different from the means for the third series, twenty-four days after the infective feeding.

In the Barred Plymouth Rock chick 316, four series of measurements were made on material collected the eighth, tenth, eleventh and twelfth days after the infective feeding. A comparison of the mean lengths and widths of these series is given in Table 21.

Table 18.

Date	Chick No.	No. Oocysts fed	Remarks	Control	No. Oocysts fed	Remarks
3/7	R. I. R. 311	6				
	R. I. R. 312	5				
	R. I. R. 313	6				
	B. P. R. 314	4				
	B. P. R. 315	6				
	B. P. R. 316	5				
3/11	R. I. R. 311	4		R. I. R. 317	4	
	R. I. R. 312	2		B. P. R. 318	3	
	R. I. R. 313		Died			
	B. P. R. 314	6				
	B. P. R. 315	3				
	B. P. R. 316	2				
3/14	R. I. R. 311		No caecal material	R. I. R. 317		Negative
	R. I. R. 312		Positive, moderate numbers			
	B. P. R. 314		Negative			
	B. P. R. 315		No caecal material			
	B. P. R. 316		No caecal material			
3/15	R. I. R. 311	3	No caecal material	R. I. R. 317		Negative
	R. I. R. 312	3	Positive, moderate numbers	B. P. R. 318		Negative
	B. P. R. 314	5	No caecal material	R. I. R. 319	3	
	B. P. R. 315	8	No caecal material	B. P. R. 320	3	
	B. P. R. 316	7	Positive, moderate numbers			
3/17	R. I. R. 311		No caecal material			
	R. I. R. 312		Positive, moderate numbers			
	B. P. R. 314					
	B. P. R. 315		No caecal material			
	B. P. R. 316		Positive, moderate numbers			

3/18	R. I. R. 311		No caecal material	B. P. R. 318		Negative
	R. I. R. 312		No caecal material	R. I. R. 319		Negative
	B. P. R. 314		No caecal material	B. P. R. 320		Negative
	B. P. R. 315		Positive, moderate numbers			
	B. P. R. 316		Positive, moderate numbers			
3/19	R. I. R. 311	4	Positive, very rare oocysts	R. I. R. 317	4	Negative
	R. I. R. 312	4	No caecal material	B. P. R. 318	6	Negative
	R. P. R. 314	2	Positive, very few oocysts	R. I. R. 319		Negative
	B. P. R. 315	2	No caecal material	B. P. R. 320		Negative
	B. P. R. 316	2	No caecal material	R. I. R. 321	6	
				B. P. R. 322	5	
3/20	R. I. R. 311		Negative	R. I. R. 320		Negative
	R. I. R. 312		Positive, very few oöcysts			
	B. P. R. 314		Negative			
	B. P. R. 315		Positive			
	B. P. R. 316		Positive, few oöcysts			
3/21	R. I. R. 311		Negative	B. P. R. 318		Rare oöcysts
	R. I. R. 312		Positive, few oöcysts	R. I. R. 319		Negative
	B. P. R. 314		No caecal material	B. P. R. 320		Negative
	B. P. R. 315		Negative			
	B. P. R. 316		Positive, very rare oöcysts			
3/24	R. I. R. 311	11	Negative	R. I. R. 317		Negative
	R. I. R. 312	10	No caecal material	B. P. R. 318		Negative
	B. P. R. 314	12	Positive, few oöcysts	R. I. R. 319		Very few oöcysts
	B. P. R. 315	8	No caecal material	B. P. R. 320		Numerous oöcysts
	B. P. R. 316	6	Negative	R. I. R. 323	5	
				B. P. R. 324	5	
3/28				R. I. R. 317		Few oöcysts
				R. I. R. 319		Few oöcysts
				B. P. R. 320		Negative
				B. P. R. 322		Negative
3/31	R. I. R. 311		Positive, very few oöcysts	R. I. R. 319		Negative
	R. I. R. 312		Positive. very rare oöcysts	R. I. R. 321		Negative
	B. P. R. 314		No caecal material	R. I. R. 324		Negative
	B. P. R. 315		Positive, very few oöcysts			
	B. P. R. 316		Negative			

Table 19.

Biometrical data on strain of *Eimeria tenella* infection on three days during the infection.

Date	Lengths in Microns	Widths in Microns
March 18	M — 20.72 ± .183 σ — 1.36 ± .129 CV — 25.62 ± .245	M — 15.44 ± .101 σ — .76 ± .074 CV — 19.30 ± 1.841
March 20	M — 23.59 ± .078 σ — .80 ± .055 CV — 13.28 ± .897	M — 19.16 ± .062 σ — .66 ± .043 CV — 13.50 ± .909
March 31	M — 20.03 ± .242 σ — 1.79 ± .172 CV — 34.85 ± 4.914	M — 16.04 ± .222 σ — 1.65 ± .160 CV — 40.21 ± 3.841

Table 20.

Means and differences between the means of *Eimeria tenella* infection on three days during the infection.

	Lengths in Microns		Widths in Microns	
(1) March 18	20.72 ± .183		15.44 ± .101	
(2) March 20	23.59 ± .078		19.16 ± .062	
(3) March 31	20.03 ± .242		16.04 ± .222	
Dif.		Dif. × P. E.		Dif. × P. E.
(1) and (2)	2.87 ± .199	14.42	3.72 ± .119	31.26
(1) and (3)	0.69 ± .303	—	0.60 ± .244	—
(2) and (3)	3.56 ± .254	14.01	3.12 ± .230	13.56

Table 21.

Comparison of mean lengths and widths of *Eimeria tenella* from one bird on four different days during the infection.

Date	No. of days	Lengths in Microns		Widths in Microns	
(1) March 15	8	22.54 ± .086		18.16 ± .051	
(2) March 17	10	22.96 ± .082		18.07 ± .078	
(3) March 18	11	23.45 ± .094		18.85 ± .078	
(4) March 19	12	23.01 ± .109		18.53 ± .086	
Dif.			Dif. × P. E.		Dif. × P. E.
(1) and (2)		.42 ± .119	3.52	.09 ± .093	—
(1) and (3)		.91 ± .127	7.16	.69 ± .093	7.41
(1) and (4)		.47 ± .139	3.38	.37 ± .100	3.70
(2) and (3)		.49 ± .125	3.92	.78 ± .110	7.09
(2) and (4)		.05 ± .137	—	.46 ± .116	3.96
(3) and (4)		.44 ± .144	3.05	.32 ± .116	2.75

Assuming a difference of four times the probable error as being significant for these experiments, there is one significant difference between the lengths, between the eighth and eleventh days; and three significant differences between the widths, between the eighth and eleventh days, the tenth and eleventh days, and the tenth and twelfth days.

A comparison of the means of all the material from all the birds infected during the course of the experiment shows a range in size from a mean length of 20.72 μ and a mean width of 15.44 μ to a mean length of 23.59 μ and a mean width of 19.31 μ. When the means of these different series are listed approximately in the order of their magnitude, they form a perfectly graded series, as shown in Table 22. It will be seen from this table that apparently the size of the oöcyst is independent of the length of the infection, of the breed and age of the host, and of the severity of the infection. Large and small oöcysts appear equally in both breeds of chicks, apparently independently of the number of oöcysts being produced, and of the day of the infection on which they were collected.

Table 22.

Mean size of *Eimeria tenella* oöcysts in chickens of two breeds on different days after first infective feeding.

Day after first infective feeding	Breed	No.	Age of chick at first infection	Mean size of oöcyst in Microns	Infection
11	Rock	315	3	20.72 × 15.44	Light
8	Red	312	3	21.53 × 16.57	Heavy
24	Red	311	3	22.06 × 18.03	Light
8	Rock	316	3	22.54 × 18.16	Heavy
10	Rock	316	3	22.96 × 18.07	Heavy
12	Rock	316	3	23.01 × 18.53	Moderate
9	Red	317	14	23.01 × 18.80	Light
13	Red	319	10	23.21 × 18.75	Light
10	Rock	312	3	23.43 × 18.86	Heavy
11	Rock	316	3	23.45 × 18.85	Moderate
17	Rock	314	3	23.34 × 19.31	Light
9	Rock	320	10	23.56 × 19.00	Heavy
13	Rock	315	3	23.59 × 19.16	Moderate

Although, as we have seen, there is significant variation within the material from a single chicken on different days of the infection, when all the material from the Rhode Island Reds is combined and compared with the combined material from the Barred Plymouth Rocks, there is no significant difference between the two

series, the variations in material from individual birds in each series extending over approximately the same range.

Table 23.

Comparison of combined material from Rhode Island Reds and Barred Plymouth Rocks.

	Rhode Island Reds	Barred Plymouth Rocks
Lengths in Microns	M — 22.62 ± .066 σ — 1.68 ± .047 CV — 28.91 ± .796	M — 22.80 ± .043 σ — 1.46 ± .031 CV — 24.94 ± .546
Widths in Microns	M — 18.00 ± .062 σ — 1.59 ± .043 CV — 34.38 ± .944	M — 18.32 ± .043 σ — 1.34 ± .027 CV — 28.47 ± .620

The difference between the mean lengths is 0.18 $\mu \pm .079$ and between the widths 0.32 $\mu \pm .075$. The difference between the mean lengths is 2.2 times its probable error, and not significant; the difference between the mean widths is 4.2 times its probable error, and therefore just above the limit. In view of the greater variation in widths observed within all the series, this result is not surprising, and too much emphasis should not be put upon it.

At the same time that this experiment on young Rhode Island Reds and Barred Plymouth Rocks was started, the same infective material was also fed to three older birds, two Rhode Island Reds, 298 and 302, forty-three days of age, and one White Leghorn seventy-six days of age. These chickens received a massive dose of the material, sufficient to produce very severe hemorrhage on the sixth day after feeding. Oöcysts were present later in the bloody discharges, and were collected for measurement but after the cessation of the hemorrhage, the caeca became non-functional, and no other caecal droppings were obtained during the period of observation. The mean length of the material from the Leghorn was 20.06 $\mu \pm .098$; that from the Rhode Island Reds was 20.20 $\mu \pm .098$. The difference is 0.14 $\mu \pm .139$, which is not significant. The mean width of the Leghorn material was 15.94 $\mu \pm .062$; that of the Rhode Island Red material 16.06 $\mu \pm .082$. The difference is 0.12 $\mu \pm .103$, and not significant. Again no effect of breed of the host is apparent, although it is realized that with such a small number of experimental animals, the results obtained can only be suggestive.

In this experiment, there is a marked difference in size between these oöcysts collected from appreciably older birds, and those from

the younger group, — 20.06 μ and 20.20 μ in length in the older individuals as contrasted with 22.62 μ and 22.80 μ in the younger; and 15.94 μ and 16.06 μ in width against 18.00 μ and 18.32 μ (cf. Table 24).

A further experiment to determine whether there was any relation between host and size of oöcyst was then carried out using five three-day-old chicks. five thirty-day-old chicks and two forty-eight-day-old chicks. They were all Rhode Island Reds previously uninfected, and had been kept in the laboratory under comparable conditions. Material was collected on the seventh, eighth, ninth, tenth and eleventh days, but not on all days from all birds. Fifty oöcysts were measured from each lot of material. Those from the same bird on different days were considered together, and the mean length and width of oöcysts from each individual were calculated. In one instance, however, only one caecal dropping was obtained throughout the period of observation. Calculations on the total number of oöcysts from this bird were therefore based on only fifty measurements. From the other birds, material was obtained on two days in eight cases and on three days in the other three cases.

In Table 24, the mean length and width of oöcysts from the individual birds have been tabulated by age groups. The mean length and width of all oöcysts measured in each group are likewise shown, and a comparison of the means is made.

The differences between the measurements of oöcysts in the three-day-old group and the thirty-day-old group are the least, 0.24 μ for the mean lengths, and 0.28 μ for the mean widths. The differences between the three-day group and the forty-eight-day group are considerably greater, 1.11 μ for mean length and 0.84 μ for mean width. The differences between the thirty-day and the forty-eight-day groups are intermediate, 0.87 μ for lengths, and 0.57 μ for widths. In this particular series, therefore, there was a small but constant decrease in size of oöcyst with the increasing age of the host bird.

Considering these two experiments, it would appear that the size of the oöcysts decreases somewhat with the age of the host. But a comparison of the oöcysts from the older birds in both of these groups with those from the original strain also show marked variation. The Barred Plymouth Rock in which this strain of *Eimeria tenella* appeared was sixty-eight days old, and the first transfer of this strain was into a Rhode Island Red seventeen days old. The mean size of the original material was 23.48 $\mu \times$ 19.04 μ;

Table 24.

Mean lengths und widths of oöcysts from birds in different age groups. (In Microns.)

3-day old chicks			30-day old chicks			48-day old chicks		
No.	Mean Length	Mean Width	No.	Mean Length	Mean Width	No.	Mean Length	Mean Width
332	22.48	18.35	325	21.92	17.46	330	21.13	16.67
333	23.09	17.91	326	22.84	18.46	331	21.76	17.53
334	22.97	18.50	327	22.59	17.95			
335	22.69	18.03	328	22.12	17.24			
336	21.99	17.40	329	22.34	17.91			

Mean calculated from measurements on all oöcysts in each age group.

Age Group	Lengths in Microns		Widths in Microns	
(1) 3-day	$22.60 \pm .023$		$18.05 \pm .027$	
(2) 30-day	$22.36 \pm .027$		$17.77 \pm .027$	
(3) 48-day	$21.49 \pm .055$		$17.20 \pm .051$	
Dif.		Dif. $\times$ P. E.		Dif. $\times$ P. E.
(1) and (2)	$0.24 \pm .04$	6.0	$0.28 \pm .04$	7.0
(1) and (3)	$1.11 \pm .06$	18.5	$0.85 \pm .06$	14.1
(2) and (3)	$0.87 \pm .06$	14.5	$0.57 \pm .06$	9.5

that of the first transfer was $21.88\ \mu \times 17.24\ \mu$. Furthermore, the means of the series from these older birds in the later experiments ($20.06\ \mu$, $20.20\ \mu$, and $21.49\ \mu$) even overlap the smallest mean size observed in the younger series, $20.72\ \mu \times 15.44\ \mu$ (three-day-old chick 315, Table 19).

It would appear, therefore, that there are other factors influencing variation in size within this strain of *Eimeria tenella* such as there were in the strains of *Eimeria acervulina* and *Eimeria maxima* in the previous group of experiments.

Discussion.

From a consideration of the experiments presented in this paper, it would appear that the size of *Eimeria* oöcysts of a given species is subject to great variation within a more or less definite range. The limits of this range can only be determined by large series of measurements made on uncontaminated material, obtainable from pure infections in previously uninfected birds.

The use of measurements as a criterion of species would appear to be valid only when a sufficiently large series of measurements

gives a symmetrical and continuous curve. In those cases in which the curve shows several peaks without a complete break between them, it becomes necessary, in view of the variation within species encountered in these experiments, to demonstrate by other methods than the statistical treatment of measurements that these peaks represent different species, and not merely strains, or races within one species.

Although it has not been possible in these studies to show that size of the oöcyst is influenced by any of the environmental factors considered, there may, nevertheless, be conditions of which we are unaware, and consequently have not controlled, capable of producing size or other morphological variations.

These experiments indicate, however, that a mixed infection of two distinct types of *Eimeria* can be present in a single host without cross-fertilization and hybridization taking place.

In view of the variation encountered within pure strains of the three species of *Eimeria* considered in this paper, the use of measurements alone as a basis for the recognition of species would not appear to be adequate in the majority of cases. ANDREWS (1928) has used series of measurements in describing new species of coccidia from the skunk and the prairie dog. In one case, fifty measurements are given, with a calculation of the mean, standard deviation and coefficient of variation, and their probable errors. In the other case, twenty-five measurements were made, and similarly treated biometrically. In each of these series, the range is not great, and the coefficient of variation is small. It seems probable that each of these groups represents a pure strain although the numbers measured are very few. Difficulties are encountered, on the other hand, in interpreting the significance of series of measurements on organisms from the same host whose range is great, and whose peaks are not clearly separated. Such a case is found in the distribution of two hundred and fifty-two oöcysts presented by YOUNG (1929) on "*Eimeria avium* oöcyst measurements". Here the range given is from ten to thirty-two microns in length, and from ten to twenty-four microns in width. When these measurements are plotted on graph paper, two major peaks are found, one at eighteen and one at twenty microns among the oöcysts ranging from ten to twenty microns in length. The remaining measurements (on twenty-seven organisms) range from twenty to thirty-two microns with a peak (of ten organisms) at twenty-eight microns. Although it is extremely probable that this infection represents a

mixture of *Eimeria acervulina* and *Eimeria maxima*, the separation into two species could not justifiably be made on the basis of these measurements alone because of the many peaks in the lower range of the curve, the continuity of the curve, and the small number of organisms grouped about the extreme peak in the higher range.

The work of Boughton (1930) is another case in which a study of size leads to no conclusions. Boughton made a biometrical study of the coccidia of the English sparrow, *Isospora lacazei* Labbé, with the idea that "The condition of the parasite in relation to its host at the time the measurements are made, may have some bearing upon the results obtained". This author uses the volume of the oöcyst as the unit for study rather than the linear measures, since the use of one term makes the calculations of the biometrical constants easier. It should be considered, however, that no added accuracy is gained by this calculation, but rather it has the effect of masking any independent variations between lengths and widths which might appear in different strains.

Two sparrows are used as the host animals and oöcysts were collected from them over a period of thirty-four days in one case, and seventy days in the other. Both of these birds at the first observation were found to be negative, but several days later, oöcysts began to appear in the feces. The source of the infective material is not indicated. Whether these birds had a chronic light infection when brought under observation, and were then placed under conditions permitting them to reinfect themselves more heavily, or whether they were free from infection in the beginning and acquired it by contamination from other birds while under observation, is not known. If the birds were already infected when brought under observation, then the stage of the infection at which measurements were first made may have been far from being the first stage. Moreover, as the infection seems to have become heavier as time went on, it would indicate that the birds were being reinfected from some source, since coccidial infection, at least in poultry, dies out with greater or less rapidity according to the species, but in all within a fairly brief period if the birds are prevented from reinfection. It is quite possible, therefore, that the birds may have been infected with several types of *Isospora* at the time of the first observations, or that they acquired new infections as the experiment progressed. If two or more species of *Isospora* were involved in this case, and an immunity to one species, the larger one, were developed more rapidly than immunity to the

smaller one, it would account for the difference in size observed in the earlier and later periods.

This author is inclined to believe that "some group of factors brings about the production of smaller and smaller oöcysts during the sexual phase of the life cycle," to account for this marked decrease in size in the later periods, although he concludes that his data are insufficient to establish either this hypothesis, or to show that several species or strains of oöcysts were involved. Since BOUGHTON'S study is based on material from non-experimental sources of infection, there is no way of judging the period of infection, or the number of strains or species involved.

KARTCHNER and BECKER (1930) in a study of *Eimeria citelli*, n. sp., a coccidium of the striped ground squirrel, have made a series of measurements over nineteen days during an artificial infection. No gradual diminution in size is apparent, and these authors point out that their results do not agree with BOUGHTON'S. They also report no significant change in size or shape in a reinfection after a lapse of a week after the primary infection.

Throughout much of the work which has been done with measurements of coccidia, the size of the oöcyst has been treated as a species characteristic. As such it is regarded as a heritable morphological character which may be determined by a factor or factors within the chromosomes, and subject to the laws of mendelian inheritance.

In his essay on "The chromosome cycle of the sporozoa considered in relation to the chromosome theory of heredity" DOBELL (1925) argues that the chromosomes of the sporozoa (or of any organism) can not carry factors for heritable differences, since within the life cycle there are many different types of individuals all of which have the haploid number of chromosomes. In the life cycle of the *Sporozoa* in which meiosis has been adequately investigated, the only stage having the diploid or 2 N number of chromosomes is the zygote, or sporont. In this stage the chromosomes are paired and when division takes place they undergo reduction.

JENNINGS (1929) in discussing DOBELL'S theory, points out that the same type of relation holds for successive stages in the embryological development of a Metazoan. The chromosome number although diploid is the same throughout, yet the stages differ greatly.

There would seem to be no valid reason why the chromosomes of the *Sporozoa* should not behave in similar fashion to those of the

Metazoa. Although in the former there are many more cell generations in which the chromosomes are present in the haploid condition, there is no evidence but that in the one cell generation in which they are in the diploid number the process of meiosis is the same as in the *Metazoa.* Thus there is the same opportunity for an interchange of chromatin material between the chromosomes from the macro- and microgametes that there is in fertilization of the *Metazoa.*

The one case of hybridization in the *Protozoa* referred to by all writers is that of PASCHER (1916) on "species" crosses in *Chlamydomonas.* The two species differed in form, shape of the papilla-like point, position of chromatophore, shape of the eye spot, thickness of the membrane, and type of cyst wall. These forms (the "swarmers") at certain periods divide into smaller forms, or "gametes." Two gametes unite to form a zygote in the form of a cyst. The contents of the cysts later divide into four parts, which escape and again form "swarmers." It is in these divisions into "swarmers" that chromosome reduction is said to take place. PASCHER observed the union between gametes of the two species in a large number of cases, and among these, eighty hybrid cysts were formed intermediate in type between the two parent cysts. Of the eighty hybrid cysts, thirteen were seen to divide, and cultures of swarmers were obtained from eight of these. Two types of results were obtained: In one group, two swarmers resembling one parent, and two resembling the other were produced by the division of the cysts; in the other group, the four swarmers were of four different types, one resembling one parent, but differing slightly, one resembling the other parent (also with slight differences), and two which were intermediate between the parents.

In discussing these results of PASCHER's experiments, WILSON (1925) states: "A Mendelian segregation has therefore taken place during the germination of the zygote, i. e., its division into four zoöspores; these divisions, therefore, are almost certainly meiotic, and the ordinary vegetative individuals are haploid, as in *Spirogyra* or the desmids. If this is substantiated we shall have a complete proof of Mendelian disjunction in the meiotic divisions, exactly analogous to that demonstrated in *Sphaerocarpos* in respect to sex characters."

It would seem, therefore, that if one accepts the chromosome theory of inheritance for any group of organisms, there is nothing to invalidate its application in the life cycle of the *Sporozoa.*

Summary and conclusions.

The experiments reported here were undertaken to determine to what extent the use of size differences may be taken as a criterion for the recognition of species of coccidia. The range of variation within three different species has been studied under diverse environmental conditions to ascertain the effect of such factors as; age and breed of host; and stage and severity of the infection. An attempt has also been made to produce a race modified in size by providing opportunities for cross breeding between two species *Eimeria acervulina* and *Eimeria maxima* differing markedly in size. Nine oöcysts intermediate in size between the two parent species were found in the F_1 generation, and three in the F_2, two of which appeared on the fifth day. The possibility of these being true hybrids, or very large *Eimeria acervulina* oöcysts is discussed. However, there is no convincing evidence that hybridization has occurred so that it seems justifiable to consider the two parent strains here employed as true species.

Starting with infections from single oöcysts in two of the species used, the strains resulting from them have been studied to discover the range in size within a pure strain. It is realized that since an oöcyst is a sexually produced organism, we are dealing with a case of biparental inheritance and may therefore have selected to start with an individual heterozygous for size factors. The variation encountered within each species indicates the presence of different size races or strains, comparable in some respects to the biotypes in *Paramaecia* described by JENNINGS.

Eimeria acervulina oöcysts resulting from an infection with a single oöcyst were markedly larger than those from a massive infection. It is suggested that this may be due to the crowding of organisms in the epithelial cells in heavy infections which would not allow the growing forms to attain the maximum size.

Material from the same chick on successive days after infection may show significant variations from day to day.

In the hybridization experiment, samples of *Eimeria acervulina* material collected on the same day after infection from different chicks in a majority of cases differ significantly from one another, in the F_1 oöcysts. In the F_2, the amount of variation is somewhat less.

There is less evidence of variation in the *Eimeria maxima* strain in material collected from different chickens on the same day after the infective feeding in both F_1 and F_2 than in *Eimeria acervulina*.

The distribution curves of both *Eimeria acervulina* and *Eimeria maxima* show two distinct peaks. These are more marked in the *Eimeria acervulina* oöcysts appearing in parent, F_1 and F_2 generations.

Further experiments with *Eimeria tenella* have shown that the breed of the host chicken does not influence the size of the oöcyst.

Experiments to test the effect of age of the host on size of the oöcyst have given conflicting results. In some instances, oöcysts from infections of older birds were somewhat smaller than in younger birds. In other cases, oöcysts from infections in older birds were of approximately the same size as in the younger birds.

The stage of the infection in which oöcysts are produced was likewise shown to have no influence on size.

In view of the variations in size encountered in these experiments in strains of oöcysts resulting from infections with a single organism, it is felt that the use of size as a criterion in the establishment of new species of coccidia is valid only under certain conditions. When a distribution curve based on a sufficiently large series of measurements is symmetrical and continuous, it may be considered as satisfying these conditions. But when the curve is discontinuous or major and minor peaks are encountered, it must be demonstrated by other than statistical methods whether one species or more than one is present in the material measured.

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