OBSERVATIONS ON THE ORIGIN AND SEQUENCE OF THE PROTOZOAN FAUNA OF HAY INFUSIONS

LORANDE LOSS WOODRUFF

From the Sheffield Biological Laboratory, Yale University

FIFTEEN FIGURES

CONTENTS

I. GENERAL INTRODUCTION

Although hay infusions have been one of the chief means of providing organisms for microscopists from the early days of Leeuwenhoek, there are comparatively few published data which have been secured through a careful study of the origin, relative number, and sequence of the various organisms which abound in them. It is a well known fact that a hay infusion presents a kaleidoscopic series of phenomena from its inception until it finally reaches a stage of sterility, or, in the presence of sunlight, of practically stable equilibrium in which animals and green plants become so adjusted that a veritable microcosm exists; and it is also generally accepted, largely on the basis of casual observation of infusions made up for one purpose or another, that the organisms appear and disappear in quite a regular sequence.

It seemed desirable, accordingly, to attempt to study the fauna and flora of representative infusions by some comparatively exact methods. The first intention was to make a comprehensive tabulation of the entire animal and plant life of the infusions studied, including bacterial counts, as well as to follow the chemical and physical changes in the medium. This proved to be impossible without the aid of more assistance than was available.[1] Consequently the study was chiefly confined to a careful observation of the Protozoa which appeared, and especially to certain characteristic forms which were present in large numbers in practically all the infusions studied.

This general problem has been considered by Peters,[2] but more data were needed for points of attack on the biological effects of one type of organism on another, and this survey of the protozoan

[1] Certain chemical analyses, chiefly in regard to the acidity of the infusions, were made by Dr. M. S. Fine, and his results are published independently in the following paper, in this journal, entitled: Chemical Properties of Hay Infusions with Special Reference to the Titratable Acidity and its Relation to the Protozoan Sequence.

[2] Metabolism and division in Protozoa. Proc. Amer. Acad. Arts. and Sci., vol. 39, no. 20, 1904. Chemical studies on the cell and its medium. I. Methods for the study of liquid culture media. Amer. Journ. Physiol., vol. 17, no. 5, 1907; II. Some chemico-biological relations in liquid culture media. Amer. Jour. Physiol., vol. 18, no. 3, 1907.

fauna of hay infusions is preliminary to further studies on the interactions of particular species on each other.

II. ORIGIN OF THE PROTOZOAN FAUNA OF HAY INFUSIONS

The point first considered was the source or sources from which the protozoa which appear in infusions of hay are derived. This general problem has, of course, been treated at length in the long series of experiments on spontaneous generation which occupied the attention of biologists for several centuries. The present experiments were planned to determine the best method of making up infusions for the purpose of the study of their biological cycle, and incidentally to show the relative importance of air, water and hay, and whether some forms appear in infusions chiefly through one of these channels and others through another.

Hay is generally considered the chief source of the protozoan life of infusions. Kent[3] in 1879 studied the question

> from whence (are) derived all these myriad organisms frequently produced in such abundance as to literally jostle each other for room in every drop of water extracted for examination? . . . . hay from different localities was placed in maceration and examined continuously from its first contact with the fluid medium, from periods varying in duration from a few days only to several weeks. The water added to the hay was of the purest possible description, and was frequently boiled for some time to prevent the introduction of extraneous germs. In all instances, the results obtained were broadly and fundamentally the same, and differed only with respect to the specific types found living together in the separate infusions. Even here, however, the general dominance of two or more special forms was notably apparent.

Kent was satisfied, then, that the organisms were derived from the hay, and microscopical examination of the mode of distribution of the cysts upon the lowermost blades, colored brown or yellow from incipient decay, led him to conclude that "all the essential conditions of their life cycle had been passed in close connection with it." He put this conclusion to a practical test by gathering grass saturated with dew during a heavy fog and studying it without the addition of any water.

[3] A manual of the infusoria. London, 1880, pp. 135-141.

> In every drop of water examined, squeezed from the grass or obtained by its simple application to the glass slide, animalcules in their most active condition were found to be literally swarming . . . . Their purpose in life, as in the case of the animalcules inhabiting artificial infusions, is to break down and convert into new protoplasmic matter this otherwise waste product . . . . To maintain the balance here, however, and to check the too rapid increase of the various herbivorous monads, we find other types . . . . developed side by side with and feeding in turn upon the plant-eating species.

Following Kent's method, I have examined grass from the campus wet with dew and light rain, and have obtained substantially the same results. Active forms of various flagellates, chiefly monads, and ciliates such as Colpoda, Chilodon, etc., were observed swimming in the moisture on the blades of grass. I have not found them in such great abundance as described by Kent, but still in sufficient numbers to make an interesting demonstration. Goodey, also, in his recent study of the Protozoa of the soil found a number of active forms among the surface vegetation.[4]

A. EXPERIMENTS

Twenty-four hay infusions were made up, and kept in a well lighted room in the laboratory at room temperature. Twelve contained hay cut near the laboratory, and twelve practically pure timothy hay from a farm near New Haven. These two sorts of hay were designated, for convenience, Y and T respectively. Each infusion consisted of about 5 grams of hay in 1 liter of tap water, and was contained in a flask with a capacity of 1500 cc. These infusions were divided into four groups of from four to eight infusions, and in each of these four groups, half of the flasks contained Y, and half T hay. The four groups of infusions were designated by the letters, *A*, *W*, *H* and *WH* respectively.

A Infusions. In this group of eight infusions the hay was put into the various flasks and then subjected to seven pounds pressure of steam for one hour in an autoclave. The water was likewise subjected to the same conditions and when it had again

[4] A contribution to our knowledge of the Protozoa of the soil. Proc. Royal Society, Series B, vol. 84, 1911.

reached the room temperature it was added to the sterile hay in the flasks. Four of these infusions were left exposed to the air, and four were plugged with cotton, sterilized dry at a temperature of 180° C. for one hour.

W Infusions. The hay in these six infusions was sterilized exactly as in the case of the *A* series, but the water was not sterilized. All the flasks were plugged with sterile cotton.

H Infusions. The water used in this series of six infusions was sterilized as in the case of the *A* series, and to this water was added fresh hay. Sterile cotton plugs were inserted in each flask.

WH Infusions. These infusions, four in number, were made by simply adding fresh hay to ordinary tap water. The tops of the flasks were covered with inverted beakers.

The twenty-three infusions may be tabulated as follows:

A Series—to determine the organisms derived from the atmosphere.
- *At1, At2, Ay1, Ay2* = sterilized hay and water. Exposed to the air.
- *At3* = control; sterilized hay and water. Not exposed to the air, but plugged with cotton.
- *At4, Ay4* = control; sterilized hay and water. Inoculated with pure cultures of Paramaecium aurelia and caudatum, and also with Oikomonas. Plugged.

W Series—to determine the organisms derived from tap water.
- *Wt1, Wt2, Wt3, Wy1, Wy2, Wy3* = sterilized hay and fresh water. All the flasks plugged with sterile cotton.

H Series—to determine the organisms derived from the hay.
- *Ht1, Ht2, Ht3, Hy1, Hy2, Hy3* = sterilized water and fresh hay. Flasks plugged.

WH Series—control.
- *WHt1, WHt2, WHy1, WHy2* = fresh water and fresh hay. Mouth of flask covered with inverted beaker.

The experiments were started on July 29th. The infusions were examined at intervals of approximately one week during the following six weeks, and at irregular intervals thereafter until November 11th, when the remaining infusions were destroyed. It was planned to carry the observations for six weeks, but at the end of this time it seemed advisable to continue certain ones longer.

B. RESULTS

A Series. The *A* series gives evidence as to the general influence of the atmosphere as a source of protozoan life in laboratory infusions. The mouth of the containing flasks measured $1\frac{1}{2}$ inches in diameter and thus afforded ample exposure to the air without rendering rapid evaporation troublesome. The flasks stood during most of the time in a room in which hay was being used for various purposes, and consequently there was ample opportunity for the air to be contaminated with cysts, etc. Also, during the day time the windows at either end of the room allowed a considerable current of air to pass over the flasks. Again, certain flasks were placed on a shelf outside of the window where they were exposed to the air of the campus.

The results derived from this series are as follows: *Ay1* remained free from protozoa from the start to October 31st, at which time it was seeded with Paramaecium and Oikomonas. Two days later it showed a good growth of each of these forms, thus proving that it was a favorable fluid for protozoa. *Ay2* was sterile in regard to protozoa until September 24th when a very few tiny amoebae appeared, and remained until October 31st, when the culture was destroyed. *At1* contained on August 26th a few small hyaline bodies which seemed to be cysts. A week later there appeared a few tiny amoebae, and on September 24th a heavy growth of monads was observed which persisted to the discontinuance of the infusion on October 31st. *At2* remained sterile until September 24th when tiny amoebae appeared and continued to be present to the end. On October 31st the infusion was seeded with paramaecia and these had greatly increased in number by November 2nd when the infusion was destroyed. *At3*, which was kept plugged as a control, was first examined on September 2nd and was sterile. It was discontinued at this time. The *At4* and *Ay4* cultures were seeded at the beginning with Paramaecium and Oikomonas and showed heavy growths from the start—thus proving that the media, from the inception of the experiments, offered favorable conditions for protozoan life. These two infusions were discontinued on November 2nd.

It is believed that in this series of experiments exceptional opportunities were offered for infection of the culture medium by air borne cysts, etc., to occur, and the resulting protozoan fauna shows that the atmosphere is a negligible factor in the seeding of hay infusions used for laboratory study.

W series. The data from the *W* group of infusions show the protozoan life which was introduced with the laboratory tap water. *Wy1* showed from the start heavy growths of Chilomonas, Oikomonas, and Chilodon, and these persisted in varying numbers until November 9th. At this time the culture was seeded with paramaecia and two days later there was a considerable increase in their number. The culture was discontinued at this time. In the *Wy2* infusion there appeared several species of monads, including Oikomonas and Bodo. A rotifer (Rotifer vulgaris) was observed on August 12th and increased in numbers until there were about 2000 per cc. at the top of the infusion, when the culture was discontinued on November 11th. The culture was seeded toward the end with paramaecia which multiplied rapidly. *Wt1* and *Wt2* developed numerous species of monads and also considerable growths of a tiny amoeba. *Wt1* had as many as 5000 per cc. when it was lost by an accident on September 2nd. *Wt2* on the same day had 20,000 amoebae per cc., and on September 24th these were succeeded by myriads of Amoeba radiosa. The culture was seeded with paramaecia on November 9th, and was destroyed on November 11th when it contained a good culture of this animal. *Wy3* and *Wt3* remained plugged, as a control, until November 9th and when examined on this day they contained practically the same fauna as the other cultures of the *W* series as described above. A point worthy of special note, however, is that *Wt3* showed, in addition to many tiny amoebae, about twenty-five Amoeba proteus per cc. of the fluid at the top of the culture. It is interesting that in certain cultures heavy growths of tiny amoebae appeared; that in one culture these gave place to radiosa forms; and in a third, Amoeba proteus appeared. This suggests the possibility that Amoeba proteus was introduced in the form of extremely minute spores which became apparent as tiny amoebae, later became amoebae of the radiosa type, and in

one culture, developed as far as the typical proteus form (cf. p. 255).

Obviously tap water will vary from time to time throughout the year, and no emphasis is placed on the completeness of the experiment in respect to the species which can be introduced through this channel. However, the work is extensive enough to clearly show that an insufficient number of species of Protozoa is introduced with ordinary tap water to make this a practical method for seeding infusions for study.

H Series. The organisms which appeared in these cultures must have been encysted on the dry hay with which the infusions were made, and therefore they represent at least some of the forms which one may secure in the laboratory through this source. *Hy1*, *Hy2*, *Ht1*, and *Ht2* showed a closely similar series of forms, including all those which have been noted in the previously described cultures except Chilomonas and typical Amoeba proteus, and in addition several species of Colpidium, Colpoda, Oxytricha, and other hypotrichous forms, Glaucoma, Holophrya, Spathidium, Bursaria, etc. All of these infusions were seeded with Paramaecium on November 9th, and when discontinued there was a heavy growth of this organism in each, thus proving that a favorable medium was present for Paramaecium. *Hy3* and *Ht3*, served as a control, and were not examined until the end of the experiments when they contained essentially the same forms as the other members of the *H* series.

WH Series. This group of infusions, consisting of fresh hay and water partially exposed to the atmosphere, was carried as a control for the above experiments. The protozoan fauna which developed was somewhat more meager than that developed by the *H* series. The explanation of this fact is not at once apparent since the hay and the water employed came from the same source as that used in making the other infusions. It was evident that the cycle of the infusions of this series developed more rapidly than those of the other series, and a possible explanation is that the bacteria introduced with the water so augmented the initial processes of decay with their attendant phenomena that a medium less favorable for large growths of various protozoan forms was

produced. These data, though too meager to be conclusive, suggest that sterile water added to fresh hay may prove to be a better medium for the development of the protozoa encysted on the hay.

C. CONCLUSIONS

Viewed in their entirety, these twenty-three infusions indicate that: (1) Ordinary hay added to tap water usually will not produce an infusion which is productive of a sufficient number of representative forms to make it profitable for a study of protozoan sequence. (2) Air, water, and hay are all sources from which the Protozoa are derived, and increase in importance in the order given. Of these three, however, air is practically a negligible factor in seeding infusions.

III. RELATIVE NUMBER AND SEQUENCE OF REPRESENTATIVE PROTOZOAN FORMS IN HAY INFUSIONS

A somewhat regular sequence of organisms in infusions of one kind or another attracted the attention of the early devotees of the simple microscope, as is shown, for example, by the following paragraph from a letter written in September, 1702, by an anonymous person who was led by the writings of Leeuwenhoek to make such studies:

> In my observations of the *Animalcula in Waters* I have seen many of the same species in the several infusions, and even in Waters that have been exposed (especially at this time of the year) any time without any particular mixture, such as you find in the hollow of a Cabbage-leaf, or on the *Dipsacus*, etc., and I am confident that many of these are the same Creatures under different dresses. For I have noted such a regular process in them, and such a constant order of their appearance, that I am of opinion most of them are the product of the Spawn of some invisible *Volatile Parents*[5] . . . .

Nearly a century and a half later Dujardin, from his experience with infusions, wrote:

[5] Philosophical transactions, Royal Society, London, vol. 23, 284, 1703, p. 1366. This communication is accompanied by the first published figure of Paramaecium. From the description in the text, however, it is evident that the author at times confused Paramaecium and certain hypotrichous forms. These same figures are reproduced by Baker, in his treatises on the microscope.

Depuis l'instant de sa préparation, une infusion change incessamment, et plus ou moins vite, suivant la température; elle montre seulement d'abord le Bacterium termo, puis quelqu'autre Bacterium et le Vibrion linéole, puis des Monades, des Amibes et quelques autres Vibrions ou Spirillum; un peu plus tard, les Enchelys et les Trichodes commencent à s'y montrer avec des Kolpodes qui, grossissant rapidement, se montrent conformes au type nommé *Kolpoda cucullus;* enfin, viennent les Trachelius, les Loxodes, les Coccudina ou Ploesconia, les Paramécies, les Kérones, les Glaucomes et les Vorticelles, soit tous ensemble, soit séparément; mais toujours à peu près des mêmes animalcules, de ceux que Joblot nommait d'une manière très-significative les Cornemuses, les petites Huîtres, les Chaussons, que Gleichen appelait les gros et petits Ovales, les Pendeloques et les animalcules pantoufles. Le nombre en est assez restreint, et c'est à peine si les quinze genres que nous venons de citer fournissent en tout quarante ou cinquante espèces. Si les infusions sont conservées pendant longtemps, elles changent tout à fait de nature; pourvu que le liquide soit en quantité suffisante, la substance mise à infuser devient un sol sur lequel peuvent se développer des végétations, ainsi que sur la paroi du vasc; si la lumière est assez intense, on observe même des végétations vertes; alors, avec d'autres Infusoires on peut rencontrer dans les liquides des Systolides st des Diatomées.[6]

It is obvious, however, from the preliminary experiments outlined in this paper in regard to the origin of protozoan fauna of hay infusions, that the Protozoa which appear, when laboratory water is added to ordinary hay, are insufficient in variety to render their study profitable from the standpoint of the sequence of forms, because, to determine a sequence of any general interest, it is necessary that a large number of species be present initially so that the dominating forms may be selected for particular study. It would clearly be easier to work out the sequence of forms encysted on the hay, but by doing this a sequence would be obtained which would represent merely that of a special group of forms and this would obviously vary more or less with each lot of hay. Again, since paramaecia cannot be secured from dried grass, this form would not appear in the series.

It was necessary then to employ other means of making up and seeding the infusions, so that there would be no doubt but that all the more common protozoan forms were present at the beginning. It was also necessary to start as many infusions as could be carefully studied simultaneously, in order to have the record

[6] Histoire naturelle des Zoophytes. Infusoires. Paris, 1841, pp. 173–174.

sufficiently comprehensive to rule out as far as possible individual variations and give final results of some general applicability; for, as Dujardin quaintly expressed his own experience with infusions:[7]

> Rien de plus simple que de préparer des infusions et d'y voir se produire les Infusoires; mais rien de plus difficile que d'obtenir des résultats semblables de deux infusions préparées en apparence dans les mêmes conditions: c'est qu'en effet les circonstances ne peuvent jamais être exactement semblables. En supposant que la dose des ingrédients et la qualité de ces ingrédients soient les mêmes, la température, l'état hygrométrique et l'état électrique, ainsi que l'éclairage, et l'agitation ou le renouvellement de l'air, n'auront pas pu être les mêmes ou varier de la même manière dans les deux cas. Or, toutes les causes exercent sur le développement des Infusoires une influence qui, pour n'être pas scientifiquement déterminée, n'en est pas moins bien réelle et souvent bien considérable.

A. EXPERIMENTS

Twenty-six infusions were made up with nearly pure timothy hay and laboratory tap water. In every case 20 grams of hay and 5 liters of water were put into a glass battery jar with a capacity of about $5\frac{1}{2}$ liters. Each was loosely covered with a plate of glass to prevent undue evaporation and the entrance of dust. The jars were situated in a small room with windows on three sides so that all the infusions received practically the same illumination. The temperature was recorded with a maximum and minimum thermometer. With this as the general plan, three methods of procedure were followed, giving three types of infusions designated respectively, *A*, *B* and *C*.

A Infusions. In this series the hay was boiled for five minutes in approximately 250 cc. of water and then sufficient tap water was added to make 5 liters. This infusion was then 'seeded' with 5 cc. of material from laboratory infusions and aquaria rich in animal and plant life. The 'seed' used in this series and in the following *B* series was thoroughly mixed in a flask before being added, so that each was seeded as nearly the same as possible.

B Infusions. These were made up exactly the same as the *A* series, except that the hay was removed from the infusion by

[7] Loc. cit., pp. 170–171.

straining it through cheese cloth. This eliminated all but an insignificant number of the smallest fragments.

C Infusions. To make up this set, 20 grams of hay was put into five liters of tap water. It was neither boiled nor strained. A few drops of 'seed' was added, thus insuring the presence of all the chief forms seeded into the *A* and *B* infusions.

The twenty-six infusions were made up at intervals and were designated as follows:

April 1st: A-1, A-2, A-3, A-4, A-5, A-6, B-1, B-2, C-1, C-2, and C-3.
April 13th: A-21, A-22, B-21, and B-22.
April 24th: A-31, A-32, B-31, B-32, and C-31.
May 1st: A-41, A-42, B-41, B-42, C-41, and C-42.

Each of the infusions existing during April was studied daily from its inception to May 1st. After this date the observations were made for a while at forty-eight hour intervals, and then at somewhat longer intervals depending on the rapidity of change in the respective cultures. The last regular count was made on June 26th, 1909, but since that time up to the present (Oct., 1911) the infusions have been kept under general observation.

The methods of study consisted of an examination of samples of the liquid taken from the top, middle and bottom of the jars, and the enumeration of the different Protozoa, Rotifera, Algae, etc., which were present. The liquid was removed from the jar for study with a 5 cc. pipet. The 'surface' medium studied was taken from three points in the jar just under the surface film; one at the side nearest to the chief source of light, another at the side farthest from the chief source of light, and the third directly at the centre of the surface of the infusion. The 'middle' medium was taken from this portion of the infusion by inserting the point of the pipet quickly to the region, while the other end of the pipet was closed with the finger. The 'bottom' medium was taken in a similar manner. In 'middle' and 'bottom' counts care was exercised to move the tip of the pipet through the respective regions in order to get a representative sample. Only one pipetful was taken in each of these counts because of the possibility that a few organisms might get into the pipet when it was passing through the upper portion of the fluid on its downward

course, and such error as existed from this would only be augmented by passing the pipet more than once through this region. Various methods were tried to avoid this error entirely. For example, when the study of a sample suggested that possibly some of the organisms observed might have entered from the surface fluid, another sample was taken with a pipet in the tip of which a cork was inserted. When the pipet in this condition had reached the point from which the sample was desired, a wire was inserted through the pipet and the cork pushed out. The pipet, of course, immediately filled with water up to the level of the surrounding infusion and the cork itself rose to the surface. In the great majority of cases it was found that samples taken by this latter method simply corroborated those taken by the more expeditious means, and consequently it is believed that the data secured with the method generally used in the work possesses an error which is negligible.

After a sample of the infusion had been removed it was immediately put into a watch glass and stirred, and then 1 cc. was taken with a pipet and put into a Sedgwick-Rafter counting cell. As is well known, this consists of a glass slide upon which is cemented a metal rectangle. The dimensions of the space enclosed by the rectangle is 50 x 20 mm., and, as the metal is 1 mm. thick, when the rectangle supports a large cover glass it forms a cell which has a capacity of exactly 1 cc. The sample to be examined, then, was spread out on the slide to a depth of 1 mm., and presented to view a total of 1000 cubic mm. The contents of this cell was then at once examined under a microscope which was provided with an ocular micrometer so ruled that, with lenses and tube length properly adjusted, a square of the micrometer just covered 1 sq. mm. of the field, and by focussing through the depth of the liquid enclosed by the square, a volume of the sample equal to 1 cu. mm. was under observation. By counting the organisms which were included, during a unit of time, in the 1 cu. mm. under observation, and multiplying this by 1000, the number of organisms in the cell could be ascertained.[8] Usually ten such counts,

[8] For a detailed description of the apparatus, cf. Whipple: The microscopy of drinking water, 2d ed. 1910.

each of about one minute duration, were made for each sample and their average taken. This was the general method of observation employed, but in samples in which only a few comparatively large forms were present the number of each species was counted directly under a dissecting lens. Again, in cases in which myriads of the tiniest active monads were present it was impossible to count them satisfactorily and accordingly it was necessary to estimate the number present on the basis of the experience gained by the use of the exact counting system. In addition to the observations made with the compound microscope, in nearly every case the sample was also examined with a lens magnifying about ten diameters, in order that a comprehensive view of the slide could be secured which would serve to indicate the general distribution of the organisms on the slide, and act as a check on the more exact observations.

Accordingly, while the enumeration of the organisms varied as exigencies demanded, all the counts were made by one person and consequently the personal equation of the observer, which must influence to some extent the data collected from such a series of observations, remained the same. It is believed that the data secured are sufficiently comprehensive to give accurately the relative number and to show approximately the actual number of the various organisms present. It is obvious, of course, that the method employed does not give data which show the presence in the infusions of one or a dozen organisms. Therefore the terms employed, 'time of appearance' and 'time of disappearance,' indicate simply the presence or absence of a sufficient number of animals to be detected by the method. More than this, I believe, could not be secured without the expenditure of more labor than one individual could devote to it daily for a period of three months.

Obviously the rate of development of an infusion will depend upon the temperature to which it is subjected, and, within limits, the higher the temperature the more rapidly the sequence of forms will proceed.[9] The ideal way, therefore, to conduct such a series of experiments as these under consideration would be to

[9] Woodruff and Baitsell: The temperature coefficient of the rate of reproduction of Paramaecium aurelia, Am. Jour. Physiol., vol. 29, no. 2, 1911.

maintain a constant temperature throughout the work. This was impracticable when the observations were made and considerable fluctuations in temperature occurred. However, all the infusions of the same set were subjected to the same temperature and consequently the relative time of appearance of the different forms in these is directly comparable. As the work progressed, from April to June, the average temperature of the room increased (cf. table 1), and consequently the infusions made later than April 1st, were subjected from the start to higher temperatures than the former. Thus it is impossible to compare accurately the con-

TABLE I

DATE, 1909	TEMPERATURE (F.)		DATE, 1909	TEMPERATURE (F.)		DATE, 1909	TEMPERATURE (F.)	
	MAXIMUM	MINIMUM		MAXIMUM	MINIMUM		MAXIMUM	MINIMUM
April 4	52	49	April 29	76	70	May 27	71	65
5	59	48	30	71	52	29	78	65
6	69	53	May 1	63	60	30	71	67
7	75	72	2	69	65	31	73	67
8	80	71	3	75	69	June 1	76	70
9	76	62	4	76	67	3	77	66
10	62	52	7	85	67	4	75	70
11	56	50	8	74	64	5	70	67
12	59	50	9	72	61	6	68	66
13	60	54	10	64	60	8	75	70
14	66	58	11	66	64	10	74	65
15	78	62	12	73	60	11	79	75
16	72	56	13	74	63	12	74	68
17	65	55	14	75	66	13	77	70
18	75	59	15	71	70	14	75	70
19	83	75	16	79	69	15	78	74
20	82	61	17	72	65	16	79	70
21	65	57	18	66	60	17	80	70
22	67	61	20	68	62	18	74	70
23	78	65	21	67	58	19	73	66
24	63	53	22	61	57	20	72	68
25	78	51	23	69	59	23	85	72
26	60	58	24	79	71	25	92	83
27	73	54	26	78	64	26	89	81
28	70	59						

dition of, for example, the *A I* cultures at the end of the first fifteen days, with the *A III* cultures at the end of the same length of time without taking the temperature into account. However, it is fair to compare the relative time of appearance of the various organisms in *A I* and the relative time of appearance of the various organisms in *A III*; but even here an error is undoubtedly present, though, it is believed, it is not sufficiently marked to appreciably influence the general results though minor variations which occurred in particular infusions may well be due to it. This error arises from the fact that the different species of organisms in the infusions undoubtedly have their own optimum temperature for development and consequently it may be supposed that a particular form, which has a comparatively high optimum temperature, may reach its maximum later than another with a slightly lower optimum temperature, in the cultures existing during the early part of April when the general average temperature was lower, while it may attain its maximum earlier than the latter in the cultures which reached a corresponding stage of their development when the temperature was generally higher.

As already stated, the first intention was to follow the entire fauna and flora which developed in the infusions, but this involved more labor than could be performed accurately by one observer. Consequently although a record was kept of all the animals and plants which actually were observed, these data will not be presented because I am not satisfied that they are sufficiently accurate or comprehensive. One who has not attempted to follow in detail a series of cultures, started in the manner described, has not, I think, an adequate realization of the wealth of forms which will develop. Some of the forms appear and disappear with such marvellous rapidity that if they are not immediately identified, in many cases it is impossible to do it later. Therefore, I repeat that the description which follows simply affords the data collected in regard to certain well-known genera and groups of Protozoa, which appeared in sufficient numbers, in a large majority of the infusions, to render their study of value in attempting to reach some general conclusions as to their sequence in such infusions under the conditions of the experiment. It is believed that the concentration of attention on these few forms is prefer-

able to a wider consideration of many transient species which appear apparently at random, for, if it is possible to reach any conclusions of value from the study of these few dominant forms, it may open the way for an explanation of the seemingly fortuitous distribution of the remaining species.

A tabulation of the fauna of the infusions showed that the first analysis of the results should consider the following groups and genera of Protozoa: Monads, Colpoda, Oxytricha and various closely related hypotrichous forms, Paramaecium, Vorticella, and Amoeba, because all these organisms were present in practically every infusion. The term 'monads' is used in a broad sense to include several different genera and a multitude of species of small flagellate Protozoa usually classified under the generic names Oikomonas, Monas, Bodo, etc. Colpoda cucullus is the most common member of the genus Colpoda which has appeared in the infusions. Occasionally the form of the organism has not agreed exactly with the specific description usually given, and it may well be that some of these organisms properly rank as other species of the genus, but as this could be determined only by following out the life history of the animals, it was necessary to assign the forms merely to the genus. In a number of cases species of Colpidium was found intermixed with the Colpoda. Colpoda and Colpidium are apparently adapted to practically identical conditions of the infusions and consequently it matters little which form is chosen for study. Since Colpoda has usually appeared in greater abundance than Colpidium, it has been selected, as the representative of this type of ciliate, for detailed study in this work. Among the hypotrichous ciliates which appeared, Oxytricha was probably the most common, but closely associated with this genus was Stylonychia, Urostyla, Gastrostyla, etc., and therefore the various species of these genera were considered as a unit and are designated in this work as 'Hypotrichida.' Also several members of the Vorticellidae appeared, nearly all of the genus Vorticella. The term 'Vorticella' accordingly is used to include all true members of this genus regardless of species. The same is true of the term 'Amoeba' as here employed, this name being used to include such forms as Amoeba guttula, radiosa, etc., as well as typical Amoeba proteus. 'Paramaecium' is applied to two species,

aurelia and caudatum, indiscriminately. It is apparent, then, that no attempt has been made to identify the various species, as this would necessitate a large amount of labor entirely incommensurate with the value of the information gained for the problem in hand. All of the forms included together are adapted to the same general environment (as the results which follow show), and therefore it is logical to consider them together as a unit without regard to the taxonomic variations of the individual moieties of which it is composed.

B. GENERAL OBSERVATIONS ON THE COURSE OF DEVELOPMENT OF HAY INFUSIONS

In infusions (*A*) made with boiled hay, which is allowed to remain in the jar, most of the hay sinks quickly to the bottom and remains there. In the cultures (*C*) made with unboiled hay most of the material floats near the surface for four or five days and then begins to sink gradually to the bottom. It is usually all at the bottom within two weeks. When the hay is allowed to remain in the infusion (*A*, *C*) this slowly disintegrates and is reduced to a more or less amorphous mass by the end of the second month. The rapidity of these changes, however, varies considerably with the temperature to which the cultures are subjected.

When hay and water are combined the liquid rapidly becomes straw colored, and within the first few days bubbles of gas appear entangled amongst the hay at the bottom, and these rise by degrees to the surface. At comparatively high initial temperatures the gas will frequently disturb the hay and sometimes raise it to the surface. Peters' observations show that this gas is chiefly CO_2. By the third or fourth day the color of the culture liquid appears darker and this becomes increasingly pronounced until finally the liquid is of a dark brownish color. One familiar with infusions can, of course, readily tell the approximate age of a culture by its color. Fine's studies on these infusions show that the light and yellowish shades of color are due to relatively high acidity; the darker and brownish shades to relatively low acidity.[10]

[10] Fine. Loc. cit.

When the infusions are first made up, the liquid, though colored, is transparent, but within forty-eight hours it becomes markedly turbid due to the development of countless bacteria. The bacteria at this time are equally distributed throughout the medium but on the third day a 'zoogloea' begins to be established and gradually increases in amount until it finally falls to the bottom and another is formed. In some cases, however, the 'zoogloea,' after reaching its maximum thickness, at approximately the end of thirty days, gradually thins out and practically disappears *in situ*. These variations in the transformation of the 'zoogloea' introduce a complicating factor in the study of the protozoan life of infusions, because in the cases in which it falls to the bottom, it changes the center of population of certain types quite suddenly, and thus causes a redistribution of some forms. The bacteria, then, at first are equally distributed throughout the fluid, then the largest number is at the bottom and top, while in the center of the volume of liquid there are comparatively few. The hay and smaller amount of oxygen at the bottom, and the more abundant supply of oxygen at the top, offer attractions for different forms with the result that apparently approximately the same number are to be found in each region. After the 'zoogloea' has fallen or disappeared the center of bacterial life is again at the bottom amongst the remnants of the disintegrating hay.

As soon as the bacteria have become numerous, and their action on the hay has put a certain amount of it in a form available for animal life, then occurs the great growth of Protozoa, comprising saprophytic, herbivorous, carnivorous and omnivorous forms, and this phase of the life of the infusions we shall consider in detail.

After the period of greatest protozoan fauna has passed, rotifers become numerous, and as the diatoms, desmids, and filamentous cyanophyceae and chlorophyceae flourish, under proper conditions of illumination, several species of Anguillula, copepods, etc., are more or less abundant. This condition of the fauna and flora merges imperceptibly into what may be called a condition of nearly stable equilibrium, in which green plants and animals, under

optimum conditions of light and temperature, are so adjusted that for a considerable period a practically self-supporting and self-sufficient microcosm exists—but with the balance of nature established neither the Protozoa nor bacteria can ever again attain their maximum abundance.

C. THE *A*, *B* AND *C* GROUPS OF INFUSIONS

All three types of infusions (A, B, C) which were made up gave the same general cycle of events, but the A and C series were slightly slower in development (as one would expect from the presence of the hay) than the B series. The cycle of the C series was essentially the same as that of the A series except that it progressed somewhat more slowly until the hay became thoroughly soaked. A practical disadvantage of the C series is presented by the fact that the unboiled hay, containing considerable air, has a tendency to float and so changes somewhat the distribution of the organisms until it sinks to the bottom at about the end of two weeks. This nuisance may be avoided by weighting the hay with glass. So far as length of cycle is concerned, however, both the A and the C series offered equal advantages for study, but the cycle of the B series (without hay) being considerably shorter, the sequence of the different types of organisms was more rapid, the number of organisms present was much smaller, and stable equilibrium of the infusions was attained sooner (cf. figs. 5, 6, 7). However, since the richness of the animal life was seriously decreased, this series did not prove to be the best for study, and accordingly such a method of making up cultures is not recommended for investigations of this character. Nevertheless, the results derived from all three types of cultures will be given here.

The data from each of the twenty-six cultures have been recorded (as already described), then these data from each culture of each set of experiments of the A, B, and C series, started at the same time, have been averaged together. Therefore, in discussing these data, I shall refer (unless it is specifically noted to the contrary) to the *average* number of organisms, time of appearance, etc., in infusions comprising each group as follows:

A-1, A-2, A-3, A-4, A-5, A-6, averaged and designated *A I*
A-21, A-22 averaged and designated *A II*
A-31, A-32 averaged and designated *A III*
A-41, A-42 averaged and designated *A IV*
B-1, B-2 averaged and designated *B I*
B-21, B-22 averaged and designated *B II*
B-31, B-32 averaged and designated *B III*
B-41, B-42 averaged and designated *B IV*
C-1, C-2, C-3 averaged and designated *C I*
C-31 designated *C III*
C-41, C-42 averaged and designated *C IV*

This method of treating the data was decided upon because it gives, it is believed, the fairest picture of the protozoan sequence in the infusions. As a matter of fact the individual infusions of the respective groups presented comparatively unimportant variations—except in certain cases which are mentioned. Three of the six infusions composing group *A I* were discontinued at the end of the first month because the variations between the individual infusions was not sufficient to warrant the study of so many. For a record of the surface sequence of a single infusion, reference should be made to *C III* (fig. 10). For the data of a single form (Paramaecium) at the bottom of two infusions comprising a single group, see fig. 12.

D. TIME OF APPEARANCE, MAXIMUM NUMBER AND DISAPPEARANCE OF REPRESENTATIVE PROTOZOAN FORMS AT THE SURFACE OF THE INFUSIONS

1. *Monad*

A I group. Monads were the first animals to appear in considerable numbers and their maximum was attained on the 7th day when there were about 5200 per cc. Their decline was equally rapid and by the 20th day of the life of the infusions none were observed in the samples studied.

A II group. These forms were the first to appear, reach their maximum of 2000 per cc. on the 4th day, and miminum on the 8th day.

A III group. Monads were practically absent from the two cultures of this group, and this is the only instance in which they did not appear in numbers sufficient to be considered. There were, perhaps, 100 per cc. at several counts. This dearth is accounted for, I think, by an exceptionally heavy growth of Colpoda, which occurred in this group very early (cf. table 2 and fig. 3).

A IV group. The monads appeared on the 2nd day, attained their maximum of 1000 per cc. on the 4th day and reached their minimum on the 6th day.

B I group. These forms appeared on the 2nd day, attained a maximum of 4200 per cc. on the 8th day, declined to 500 per cc. on the following day and then gradually became less and less until by the 23rd day their number was negligible. On the 36th day, however, they reappeared, attained the number of about 2000 per cc. on the 40th day, and reached extinction on the 60th day.

B II group. On the 4th day there were 1200 monads per cc., and on the 8th day they had entirely disappeared.

B III group. In this group the monads attained a maximum of 5000 per cc. by the 9th day, and by the 12th day there were none remaining.

B IV group. A maximum of 1400 per cc. was reached on the 3rd day of the life of the cultures, and then a rapid decline resulted in extinction by the end of the first week.

C I group. In these three cultures the average maximum number of monads, nearly 8000 per cc., occurred on the 15th day, and was followed by an abrupt decline ending with their disappearance on the 20th day. The maximum of Colpoda occurred on the same day as that of the Monads.

C III group. In this group, represented by a single infusion (*C-31*), the monads attained a maximum of over 8000 per cc. on the 8th day, declined rapidly to 2500 per cc. on the 13th day, and reached a minimum of practically zero on the 24th day.

C IV group. Here the monads rose to the number of 5000 per cc. on the 17th day, declined to about 2500 per cc. on the 21st day, then rose to their maximum of 7600 on the 27th day, and by the 32nd day had reached a minimum.

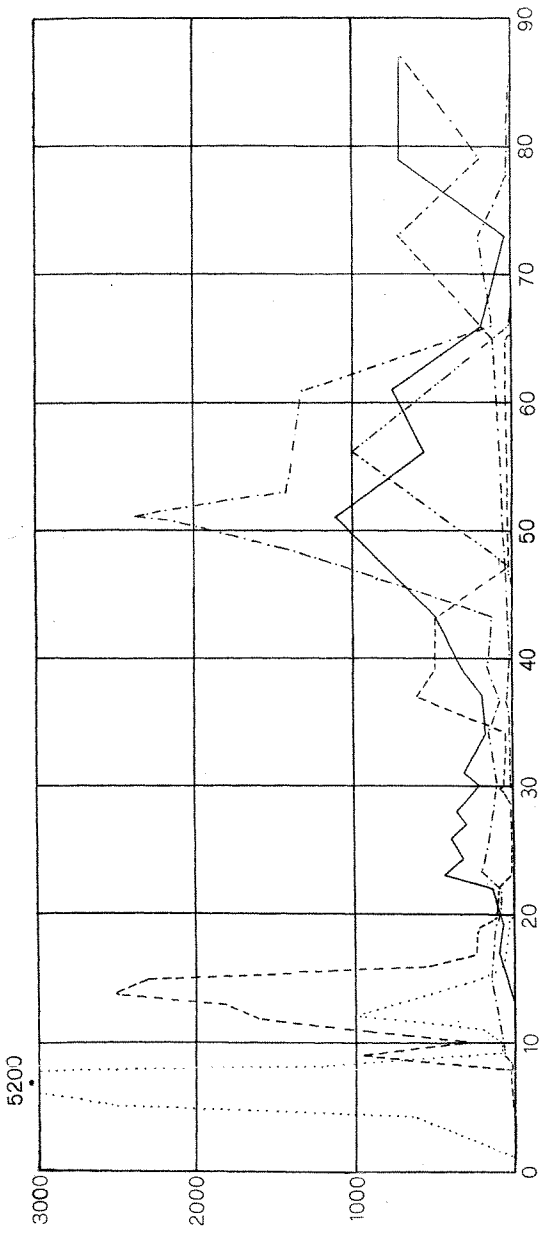

Fig. 1 *A I* group. Ordinates indicate the number of organisms per cc. at the surface. On the abscissa is plotted the number of days of the existence of the infusions. Monad = · · · · ; Colpoda = – – –; Hypotrichida = —·—; Paramaecium = ———; Vorticella = — — —. ; Amoeba = — · · —. (For details of methods cf. p. 225.)

From the study of the monads in all the cultures it is clear that in every instance they were the first type of protozoon to appear and the first to reach a maximum. This is undoubtedly to be explained by the fact that these forms, combining holozoic and saprozoic methods of nutrition, are able to feed on the bacteria which are developing so rapidly at this period, and also to absorb various substances entering into solution from the hay. The monads under consideration are also the first forms to decline and practically disappear, and this is probably due, in part, to the rapid decrease in numbers of the bacteria brought about by the monads themselves and by the rising generations of Colpoda.

2. Colpoda

A I group. Colpoda was the second protozoon to appear in considerable numbers and its maximum was attained on the 14th day when there were about 2500 per cc. Its decline was equally rapid and by the 25th day very few active individuals were seen. Beginning at about the 30th day, however, more were observed and on the 37th day there were about 600 per cc. This second rise in numbers was followed by a more gradual decline which ended in the extinction of this form by the 66th day of the life of the infusions.

A II group. This form was the second to appear and very slowly attained its maximum of 1000 per cc., which took place on the 27th day, then it fell in number to about 200 per cc., rose again to about 500 per cc. on the 44th day, and then became extinct on the 49th day.

A III group. Colpoda was the second protozoon to appear in considerable numbers in these infusions, the cycle of the monads being apparently aborted. Colpoda arose abruptly to the great number of 15,000 per cc. on the 10th day, fell to about 11,000 per cc. on the following day, and by the 15th day very few active forms were observed. However, almost immediately it had another period of reproductive activity which brought up the number to about 4000 per cc. on the 29th day. After this second high point it decreased in number, but persisted until the 63rd day

of the infusion's life. The growth of Colpoda in this group of infusions is remarkable for its abundance and persistence, for during the greater part of the life of the infusion, Colpoda was the form which dominated.

A IV group. Colpoda was the second form to attain its maximum, which occurred on the 13th day with 2500 per cc. present. This number persisted to the 17th day, and then a very quick decline ended in the extinction of the form four days later.

B I group. Colpoda was the third to attain its maximum, being preceded by the monads and the hypotrichida. Its maximum occurred on the 14th day and this was followed by a slow decline resulting in the disappearance of Colpoda on the 30th day.

B II group. Colpoda attained its maximum abundance on the 6th day, then rapidly proceeded to its extinction on the 15th day. The notably small development of Colpoda in this group of infusions is paralleled by that of all the other organisms in *B II.*

B III group. In this group of infusions Colpoda rose rapidly to a maximum of 8000 per cc. on the 18th day, and then fell even more rapidly to extinction on the 29th day. In this series of infusions Colpoda was again the dominant form, greatly outnumbering the hypotrichida and paramaecia whose small maxima occurred before its own.

B IV group. The appearance of Colpoda occurred relatively late, none being observed until the 6th day, and its maximum growth occurred on the 12th day, and its extinction on the 16th day. In this series it was the fourth form in point of time to reach its greatest abundance.

C I group. In these three cultures the average maximum number of Colpoda, 4500 per cc., occurred on the 15th day, after a rapid rise from the 7th day. Then there was an equally sudden decline to about 40 per cc. by the 22nd day, and this number gradually decreased until it became negligible at the 46th day.

C III group. Again in this culture the growth of Colpoda over-shadowed that of all the other forms. Appearing on the 4th day it gradually increased until a maximum of about 15000 per cc. was attained on the 33rd day. This was sustained for four days and then a remarkable decrease brought it down to about

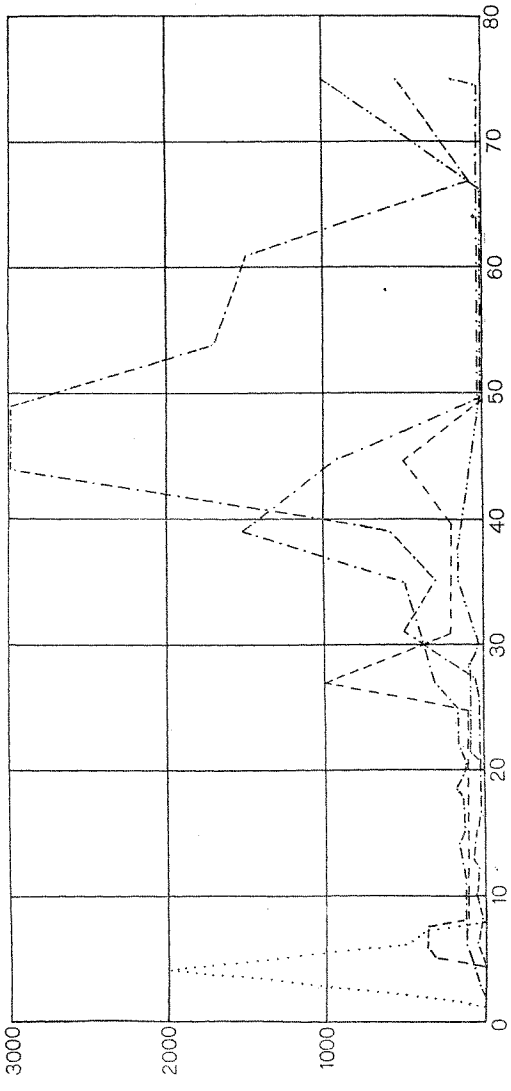

Fig. 2 *A II* group. Ordinates indicate the number of organisms per cc. at the surface. On the abscissa is plotted the number of days of the existence of the infusions. Monad = · · · ·; Colpoda = – – –; Hypotrichida = — · —; Vorticella = — — ·; Amoeba = — · · —.

600 per cc. on the 43rd day, and 60 per cc. on the 49th day. Approximately this number persisted until the end of the observations on the 76th day.

C IV group. Colpoda developed in greater abundance in this group than in any other, attaining, comparatively gradually, a maximum of 25,000 per cc. on the 32nd day, falling to 15,000 on the 37th day, and to 100 per cc. by the 44th day, and practically reaching extinction by the 56th day.

An analysis of the above data in regard to Colpoda shows that this form is adjusted to those surface conditions of infusions which exist when the monads have about run their course.

3. *Hypotrichida*

A I group. Representatives of the hypotrichida were the third to appear in considerable number and their maximum of about 2400 per cc. was attained on the 51st day after a long gradual rise. Their decrease in number was somewhat more abrupt, resulting in their extinction by the 85th day of the life of the infusions.

A II group. These forms were the second to appear and the third to reach a maximum growth. This was attained on the 39th day after a long gradual increase. A rapid decline reduced them to about 40 per cc. on the 49th day, and they persisted in this number to the 74th day. There was a slight increase in number on the 75th day, at which time the observations were discontinued.

A III group. A few hypotrichida appeared on the 6th day, gradually increased until there were about 400 per cc. on the 15th and 16th days, then declined to the 24th day. Their maximum of 700 per cc. was reached on the 29th day, after which they declined until, on the 43rd, they became extinct.

A IV group. The maximum consisting of 1600 per cc. followed those of the monads and Colpoda and occurred on the 26th day. A continuous decline brought the number down to 100 per cc. by the 43rd day; and this number persisted to the 50th day,

after which there was a slight increase up to the end of the observations on the 57th day.

B I group. These forms attained a maximum of 560 per cc. on the 11th day, and then very gradually declined until they became practically extinct on the 55th day.

B II group. In this group the maximum of the hypotrichida was reached on the 11th day, and extinction on the 29th day.

B III group. The hypotrichida were practically negligible as the maximum number which occurred on the 10th day was less than 40 per cc., and the animals disappeared completely by the 15th day.

B IV group. Here these forms reached their greatest abundance, 250 per cc., on the 7th day, declined to about 20 per cc. by the 11th day, gradually rose to 150 per cc. on the 26th day, and disappeared by the 35th day.

C I group. In these three cultures the average maximum of about 360 per cc. occurred on the 19th day, and was followed by a continuous decline until the 49th day, when about 40 per cc. were seen. From this time on slight fluctuations in numbers occurred, and at the last observation on the 86th day, about 40 per cc. were still to be seen.

C III group. In this group, represented by a single infusion, these forms first appeared on the 20th day, reached their greatest abundance, 300 per cc., on the 24th day, then declined to about 40 per cc. on the 28th day, and persisted in approximately this number to the end of the observations.

C IV group. Here the hypotrichous forms reached a maximum of 500 per cc. on the 32nd day, declined to about 40 per cc. on the 37th day, rose to 400 per cc. on the 44th day, declined again to about 20 per cc. on the 51st day, and remained at about this number to the last observation on the 56th day.

From the study of the hypotrichous ciliates which appeared in all the cultures, it is clear that these forms are adjusted to conditions at the surface of the infusions which are present, in most cases, after the monads have nearly disappeared and Colpoda has passed its period of greatest abundance.

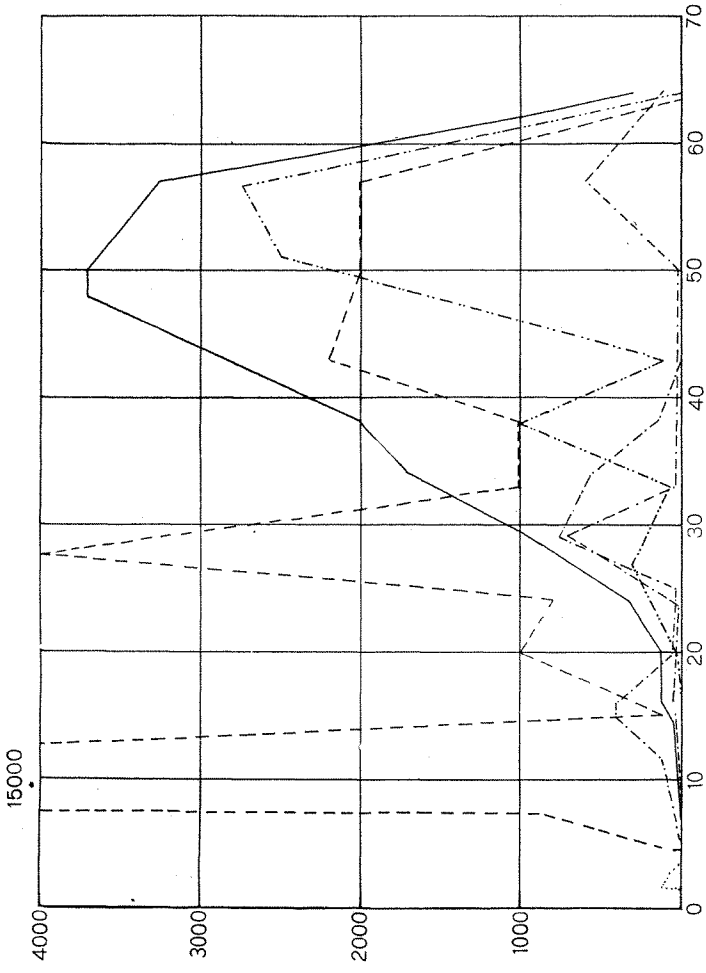

Fig. 3 *A III* group. The ordinates indicate the number of organisms per *cc.* at the surface. On the abscissa is plotted the number of days of the existence of the infusions. Monad = ... ; Colpoda = - - - ; Hypotrichida = —.—; Paramaecium = ———; Vorticella = — — · ; Amoeba = —

4. *Paramaecium*

A I group. Paramaecium appeared in considerable numbers about the 14th day and after various fluctuations in numbers reached a maximum of 1100 per cc. on the 51st day. This was succeeded by a general decline in numbers to the 73rd day when only about 40 per cc. were present; but after this their number increased to about 700 per cc. on the 79th day and continued so to the end of the observations on the 86th day.

A II group. The data for the paramaecia of these two cultures will be considered later, because the presence of Didinium so altered the paramaecium cycle that it cannot be fairly compared with that in the other cultures.

A III group. Paramaecium made its appearance here on the 7th day and gradually increased to its greatest abundance on the 48th to 50th days, at which time about 3700 individuals per cc. were present. After this the number rapidly fell to 300 per cc. on the 64th day when the last count was made.

A IV group. This organism appeared on the 4th day, attained the number of 2500 per cc. on the 31st day, and this maximum continued for fourteen days after which there was an abrupt decline to about 50 per cc. on the 57th day, when observations were suspended.

B I group. Paramaecium appeared on the 10th day, attained a low maximum of less than 100 per cc. on the 20th day and then very gradually reached extinction by the 65th day.

B II group. This form was present in small numbers practically from the start (3rd day) and reached a small maximum of about 160 per cc. on the 7th day, from which time they gradually decreased until they became extinct on the 24th day. This culture also was influenced by the activities of Didinium, and this will be discussed later.

B III group. Paramaecium appeared on the 11th day, reached its maximum on the 17th day when 160 per cc. were counted. From this time to the 65th day, when the last count was taken, it continued to persist in varying numbers under 100 per cc.

B IV group. The cycle of Paramaecium will not be considered at this time as it was so altered by Didinium that it is not at all comparable with that of the other cultures.

C I group. Paramaecium appeared on the 21st day, gradually multiplied until 600 per cc. were counted on the 40th day, then increased rapidly to 2500 per cc. on the 45th day, fell to 1200 per cc. on the 48th day, and to 200 per cc. on the 61st day. From this time there was a gradual decline to the last count on the 86th day, when about 80 per cc. were present.

C III group. In this group, comprising but a single infusion, paramaecia appeared on the 23rd day, increased to about 440 per cc. on the following day and fell in number until only about 10 per cc. were observed on the 34th day. From this point they

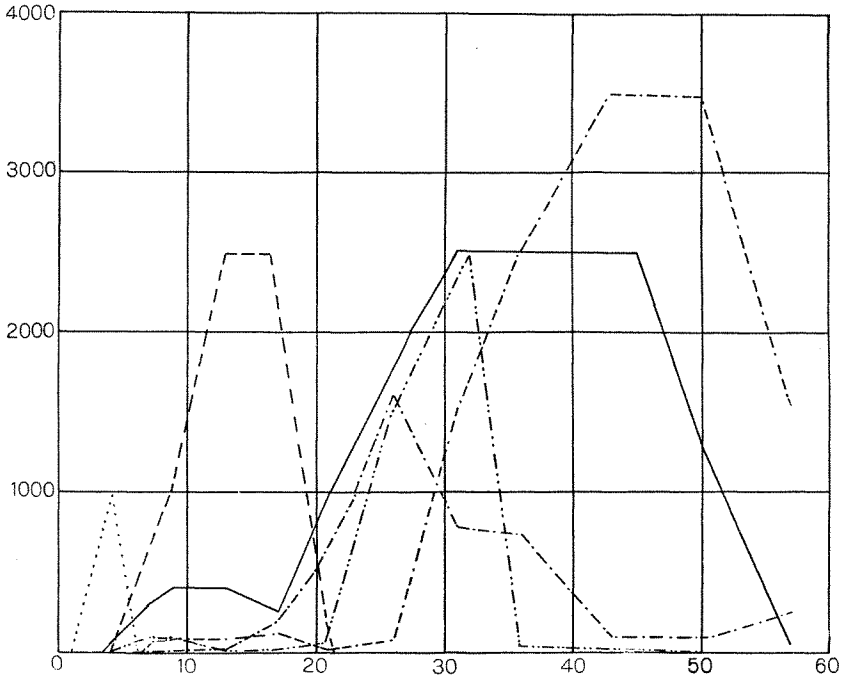

Fig. 4 *A IV* group. The ordinates indicate the number of organisms per cc. at the surface. On the abscissa is plotted the number of days of the existence of the infusions. Monad = · · · · ; Colpoda = – – –; Hypotrichida = — · —; Paramaecium = ———; Vorticella = — — · ; Amoeba = — · · —.

rose to a maximum of about 500 per cc. on the 37th to the 42nd day after which there was a general decline to 200 per cc. at the last observation on the 64th day.

C IV group. This organism appeared on the 31st day, attained its greatest abundance on the 44th day, with about 600 per cc. present, and then declined to 200 per cc. on the 65th day, when the final examination was made.

The results given above, when compared with those of the hypotrichida, show that Paramaecium usually attains its maximum numbers at the surface when the hypotrichous ciliates have passed their period of greatest abundance.

5. *Vorticella*

A I group. Vorticella made its appearance on the 30th day and increased gradually in numbers until the 65th day when about 100 per cc. were seen. Then it rose rapidly to a maximum of 700 per cc. on the 73rd day, declined equally rapidly to 200 per cc. by the 79th day, and then rose again to nearly 700 per cc. on the 86th day when the last observation was made.

A II group. This form appeared on the 5th day and persisted in small numbers to the 27th day. From this point it rose to 3000 per cc. on the 44th to the 49th day, and then quite rapidly declined to about 80 per cc. on the 67th day. Another rise to about 500 per cc. occurred on the 75th day when the culture was discontinued.

A III group. Vorticella appeared on the 8th day and continued in almost negligible numbers until the 25th day when it began to multiply rapidly until the maximum of 700 per cc. occurred on the 29th day. It then decreased to less than 20 per cc. by the 33rd day and remained in about this number to the 50th day, after which it again increased to 600 per cc. on the 57th day and declined to about 120 per cc. on the 64th day, when the last count was taken.

A IV group. This form made its appearance on the 7th day and persisted in comparatively small numbers until the 26th day,

when it began a period of great abundance, reaching a maximum of 3500 per cc. from the 43rd to the 50th day. It then declined to about 1600 per cc. on the 57th day when the last count was taken. Vorticella attained a higher maximum than any other protozoon in this group of infusions.

B I group. Vorticella appeared on the 18th day, rose to about 200 per cc. on the 21st day and fell to 20 per cc. on the 26th day. It continued in about this abundance up to the 34th day when it multiplied rapidly and produced a maximum of about 1000 per cc. by the 37th day. This was followed by a rapid decline for a few days and then a slow decline to the 67th day when the form became extinct.

B II group. Vorticella appeared on the 5th day and fluctuated in numbers under 200 per cc. until the 45th day when the maximum of 300 per cc. occurred. This was followed by a rapid decline resulting in extinction by the 50th day.

B III group. This genus appeared on the 16th day and persisted in very small numbers, reaching its maximum of 120 per cc. on the 59th day. It had decreased somewhat by the 64th day when the last observation was made.

B IV group. In this group Vorticella appeared on the 2nd day and persisted in numbers less than 200 per cc. until the 11th day, then arose abruptly to 3500 per cc. on the 16th day, and fell almost equally abruptly to about 20 per cc. by the 26th day. It persisted in approximately this number to the last count on the 36th day.

C I group. In this group of three infusions, the curve for Vorticella shows a peculiar series of fluctuations. The form appeared on the 44th day, rose to 500 per cc. by the 57th day, fell to 20 per cc. by the 67th day, rose again to practically 500 per cc. by the 74th day, fell again to about 20 per cc. by the 81st day and then had still another rise which brought the organism to its maximum on the 86th day, when the last observation was taken.

C III group. Vorticella appeared and attained its maximum of 100 per cc. on the 38th day in this infusion. From this time it very gradually decreased in numbers until the 65th day when the final count was made which showed about 50 per cc.

C IV group. Again in this series Vorticella passed through a series of fluctuations, beginning on the 19th day, reaching a maximum on the 32nd day of approximately 500 per cc., falling to nearly zero on the 37th day, and thereafter increasing in number until there were about 240 per cc. present at the final count on the 56th day.

A study of the data presented above shows that Vorticella usually attains its greatest abundance later than Paramaecium.

6. Amoeba

A I group. Amoeba was first seen on the 47th day, rose to a maximum of about 1000 per cc. by the 56th day and decreased until none were present on the 67th day.

A II group. This form appeared on the 21st day and fluctuated in numbers until the 50th day when it completely disappeared. It reappeared again on the 66th day and reached a maximum of about 1000 per cc. on the 75th day when the last count was made.

A III group. Amoebae appeared on the 17th day and after various fluctuations attained a maximum of 2700 per cc. on the 57th day. After this they declined rapidly and had disappeared by the 64th day when the last count was taken.

A IV group. These forms were first seen on the 5th day and continued to be present, though in practically negligible numbers, until the 21st day. Then they began to multiply rapidly and attained a maximum of 2500 per cc. on the 32nd day. A sudden decline brought the number down to about 40 per cc. on the 36th day and zero was reached by the 50th day.

B I group. Amoebae did not appear at all in one of the cultures of this group, and in the other a total of 22 amoebae were observed at different times from the 40th to the 87th day.

B II group. Starting on the 23rd day, this form reached a period of greatest abundance on the 45th day when about 100 per cc. were counted. Extinction occurred by the 55th day.

B III group. This form appeared on the 14th day and reached a maximum of 1000 per cc. on the 25th day. They completely disappeared by the 37th day.

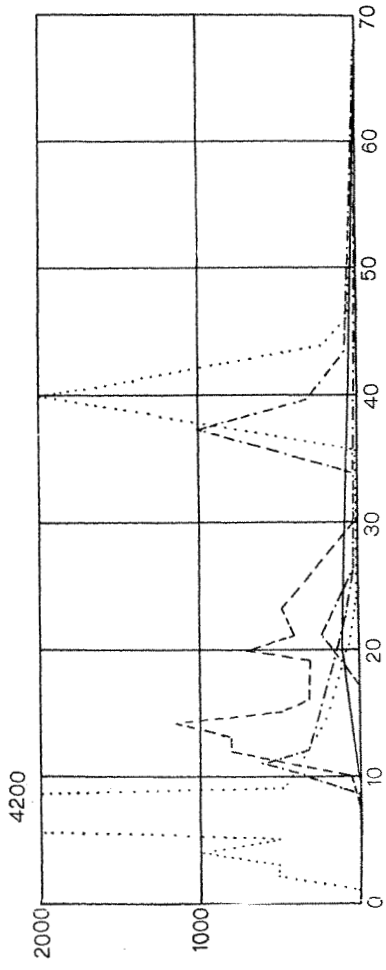

Fig. 5 *B I* group. The ordinates indicate the number of organisms per cc. at the surface. On the abscissa is plotted the number of days of the existence of the infusions. Monad = ... ; Colpoda = - - - - ; Hypotrichida = — . —; Paramaecium = ———; Vorticella = — — .

B IV group. About 80 amoebae per cc. were observed on the 7th day, and from this they gradually decreased until the 18th day when the last individuals were seen.

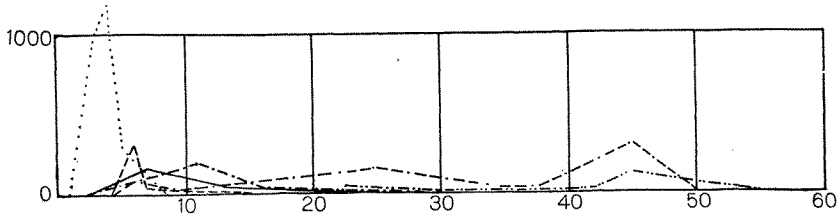

Fig. 6 *B II* group. The ordinates indicate the number of organisms per cc. at the surface. On the abscissa is plotted the number of days of the existence of the infusions. Monad = · · · · ; Colpoda = – – – ; Hypotrichida = — · — ; Paramaecium = ———; Vorticella = — — · ; Amoeba = — · · —.

C I group. Amoeba was first seen on the 46th day, reached its highest point one day later and maintained this maximum of nearly 300 per cc. for five days, after which a slow but steady decline brought it to extinction by the 86th day.

C III group. This form appeared on the 42nd day, reached its greatest abundance, 120 per cc., on the following day, and disappeared by the 53rd day.

C IV group. In this culture amoebae reached a greater development than in any of the others. They were first seen on the 6th day and reached 5000 per cc. from the 18th through the 22nd day. Then they declined quickly to about 500 per cc. on the 27th day, only to rise again almost as fast to their maximum of 5700 per cc. on the 38th day. From this point they fell to 200 per cc. by the 51st day, and at the last observation, on the 56th day, about 1000 per cc. were again present.

A careful analysis of the above data shows that amoebae attained their greatest development slightly later than Paramaecium and earlier than Vorticella at the surface of the infusions.

IV. SUMMARY OF SURFACE COUNTS

TABLE 2

		DAY OF APPEARANCE	DAY OF MAXIMUM	DAY OF DISAPPEARANCE
A I	Monads	2	7	20
	Colpoda	6	14	66
	Hypotrichida	9	51	85
	Paramaecium	14	51	—*
	Vorticella	30	73	—*
	Amoeba	47	56	67
A II	Monads	2	4	8
	Colpoda	5	27	49
	Hypotrichida	3	39	—
	Paramaecium	† 0	0	0
	Vorticella	5	44	—
	Amoeba	21	75	—
A III	Monads	2	2	3
	Colpoda	5	10	63
	Hypotrichida	6	29	43
	Paramaecium	7	48	—
	Vorticella	8	29	—
	Amoeba	17	57	64
A IV	Monads	2	4	6
	Colpoda	5	13	21
	Hypotrichida	6	26	—
	Paramaecium	4	31	—
	Vorticella	7	43	—
	Amoeba	5	32	50
B I	Monads	2	8	60
	Colpoda	8	14	30
	Hypotrichida	9	11	55
	Paramaecium	10	20	65
	Vorticella	18	37	67
	Amoeba	0	0	0

*A dash in this column indicates that the organism was still present when the last observation was made.

†Omitted because Didinium affected the sequence.

‡Disturbed by Didinium.

TABLE 2—Continued

		DAY OF APPEARANCE	DAY OF MAXIMUM	DAY OF DISAPPEARANCE
B II	Monads	2	4	8
	Colpoda	5	6	15
	Hypotrichida	3	11	29
	Paramaecium	‡3	7	24
	Vorticella	5	45	50
	Amoeba	23	45	55
B III	Monads	5	9	12
	Colpoda	5	18	29
	Hypotrichida	8	10	15
	Paramaecium	11	17	—
	Vorticella	16	59	—
	Amoeba	14	25	37
B IV	Monads	2	3	7
	Colpoda	6	12	16
	Hypotrichida	3	7	35
	Paramaecium	†0	0	0
	Vorticella	2	16	36
	Amoeba	7	7	18
C I	Monads	6	15	20
	Colpoda	8	15	46
	Hypotrichida	16	19	—
	Paramaecium	21	45	—
	Vorticella	44	86	—
	Amoeba	46	47	86
C III	Monads	3	8	24
	Colpoda	4	33	—
	Hypotrichida	20	24	—
	Paramaecium	23	37	—
	Vorticella	38	38	—
	Amoeba	42	43	53
C IV	Monads	2	27	32
	Colpoda	5	32	56
	Hypotrichida	18	32	—
	Paramaecium	31	44	—
	Vorticella	19	32	—
	Amoeba	6	38	—

TABLE 3[11]

	TIMES APPEARED FIRST	TIMES ATTAINED MAXIMUM FIRST	TIMES DISAPPEARED FIRST
Monad.....	10	10	10
versus			
Colpoda.....	0	0	1 (*B I*)
Colpoda.....	8	6	8
versus			
Hypotrichida..	3 (*A II, B II, B IV*)	4 (*BI, BIII, B IV, CIII*)	2 (*A III, B III*)
Hypotrichida..	7	7	4
versus			
Paramaecium..	1 (*A IV*)	0	0
Paramaecium..	8	7	2
versus			
Vorticella.....	1 (*C IV*)	2 (*A III, C IV*)	0
Paramaecium..	7	7	0
versus			
Amoeba.....	1 (*C IV*)	1 (*C IV*)	6
Vorticella.....	7	4 (*A II, A III, C III, C IV*)	1 (*B II*)
versus			
Amoeba.....	3 (*A IV, B III, C IV*)	5	7

From the above tables the most frequent sequence for the *entire series* of infusions is found to be as follows:

APPEARANCE	MAXIMUM	DISAPPEARANCE
(1) Monad	(1) Monad	(1) Monad
(2) Colpoda	(2) Colpoda	(2) Colpoda
(3) Hypotrichida	(3) Hypotrichida	(3) Hypotrichida
(4) Paramaecium	(4) Paramaecium	(4) Amoeba
(5) Vorticella	(5) Amoeba[12]	(5) Paramaecium
(6) Amoeba	(6) Vorticella	(6) Vorticella

[11] Cases in which both of the organisms being compared attained the condition on the same day are not included. The fact that both the organisms frequently survived the period of observation (except in Series B) is responsible for the relatively few cases included in the third column.

[12] The figures for Amoeba versus Vorticella are so nearly the same that the variation is well within the error of the experiments.

A similar analysis of the data of the *A*, *B*, and *C* series of infusions separately shows the following sequence:

A Series

APPEARANCE	MAXIMUM
(1) Monad	(1) Monad
(2) Colpoda	(2) Colpoda
(3) Hypotrichida	(3) Hypotrichida
(4) Paramaecium	(4) Paramaecium
(5) Vorticella	(5) Vorticella, Amoeba
(6) Amoeba	

B Series

APPEARANCE	MAXIMUM
(1) Monad	(1) Monad
(2) Colpoda, Hypotrichida	(2) Colpoda, Hypotrichida
(3) Paramaecium	(3) Paramaecium
(4) Vorticella	(4) Amoeba
(5) Amoeba	(5) Vorticella

C Series

APPEARANCE	MAXIMUM
(1) Monad	(1) Monad
(2) Colpoda	(2) Colpoda
(3) Hypotrichida	(3) Hypotrichida
(4) Paramaecium	(4) Paramaecium
(5) Vorticella	(5) Vorticella
(6) Amoeba	(6) Amoeba

V. PROTOZOAN FAUNA AT THE MIDDLE OF THE INFUSIONS

It is evident, from the observations on these infusions, that the protozoan fauna of the middle of the infusions is meager, compared with that of the top and bottom. Practically all the organisms which have been observed at either the top or bottom have been found in the middle counts; but either in such small numbers, or so irregularly, as to make a detailed tabulation of the records of little value. Therefore they are not presented here. Biologically, the middle of the infusion clearly offers a less favorable environment than either the top or the bottom, and is therefore tenanted chiefly by a free-swimming population brought there by an overcrowding at the top or bottom, and by forms emigrating from the top to the bottom as the cycle proceeds. Naturally

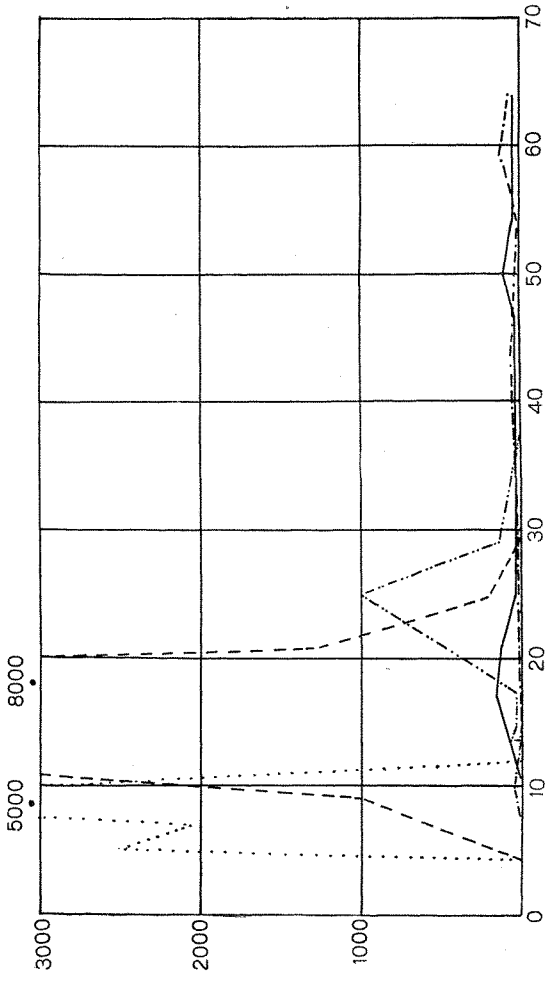

Fig. 7 *B III* group. The ordinates represent the number of organisms at the surface. On the abscissa is plotted the number of days of the existence of the infusions. Monad = ... ; Colpoda = - - - ; Hypotrichida = — · —; Paramaecium = ———; Vorticella = — — · ; Amoeba = — · · —.

those protozoa, like Paramaecium, which are strong swimmers are most frequently found in this region.

VI. PROTOZOAN FAUNA AT THE BOTTOM OF THE INFUSIONS

On account of the marked difference in the bottom fauna of the *A*, *B*, and *C* infusions, it is more convenient to consider each of these types of infusions separately.

1. A Infusions

Monad. The types of monads recorded in the surface fauna were observed in inappreciable numbers at the bottom, so that it is evident that when these forms disappear from the surface their cycle is over. Certain other species of monads appeared irregularly in comparatively small numbers at the bottom, but it is unnecessary to recount them here.

Colpoda. In groups I and II Colpoda did not appear at all at the bottom. In group III comparatively few Colpoda (approximately one-twenty-fifth as many as at the top) appeared just during the top maximum. Group IV showed a maximum of 500 per cc., which coincided with the top maximum of 2500 per cc.

Hypotrichida. These forms occurred in negligible number in groups II, III and IV. In group I there was a small maximum of 60 per cc. on the 38th day.

Paramaecium. Practically no paramaecia appeared at the bottom in any of the *A* cultures except *A2*, where, toward the end of the observations, one count of 300 per cc. was taken.

Vorticella. Vorticella were not observed in group I until near the end (76th day) when a maximum of 40 per cc. was attained. Group II, however, showed the largest number for the *A* series, with a maximum of 400 per cc. on the 72nd day, i.e., near the end of the observations. In groups III and IV Vorticella was not observed until nearly the end of the study when maxima of about 40 per cc. were reached.

Amoeba. In all the groups of infusions, amoebae were in greater abundance at the bottom than at the top. A maximum of 3000 per cc. occurred from the 55th to the 60th day in *A I;* a maximum of 6000 per cc. on the 76th day in *A II;* a maximum of 2000 per

cc. from the 36th to the 50th day in *A III;* and a maximum of 6500 per cc. on the 23rd day in *A IV*.

2. *B Infusions*

Monad. In groups *B I* and *B II* monads were practically absent. *B III* had none at the bottom during their presence at the top, but later a few were observed at the bottom from the 35th to the 55th day. In *B IV* monads appeared in numbers be-

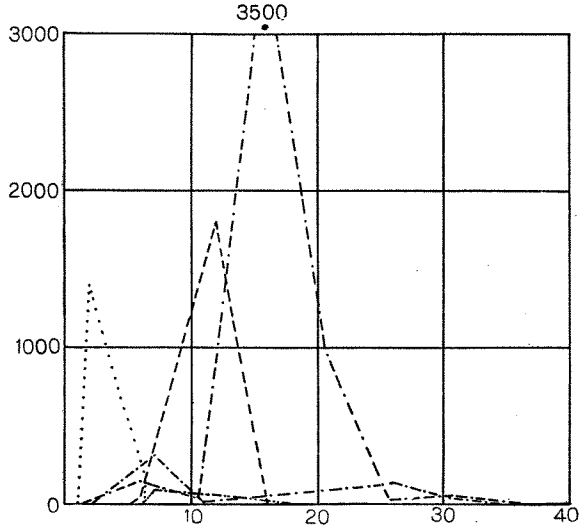

Fig. 8 *B IV* group. The ordinates represent the number of organisms at the surface. On the abscissa is plotted the number of days of the existence of the infusions. Monad = · · · · ; Colpoda = - - - ; Hypotrichida = — · —; Vorticella = — — · ; Amoeba = — · · —.

tween 500 and 100 per cc. from the 20th to the 35th day. Their appearance was coincident with the descent of paramaecia. It will be recalled that few monads were observed at the surface of the infusions of this group.

Colpoda. Considerable diversity existed between the Colpoda in the *B* groups. In *B I* and *B III* they attained a temporary maximum of approximately 15000 per cc., just after their disappearance from the top (cf. fig. 13). In *B II* and *B IV* practically none were seen at the bottom.

Hypotrichida. These forms appeared in negligible numbers at the bottom in groups II and III, while in group I the largest bottom count was taken, i.e., 110 per cc. on the 33rd day (cf. fig. 14). Group IV also showed a relatively large bottom count as compared with the top count, having a maximum of 80 per cc. on the 24th day.

Paramaecium. *B I* showed a larger number of Paramaecia at the bottom than at the top. Conjugating specimens were seen at the bottom only (cf. figs 5 and 12). *B II* showed practically no paramaecia at the bottom, but this is explained by the fact that Didinium early exterminated them in this group. A heavy growth appeared in *B III* after conjugation was prevalent at the top (cf. fig. 13). *B IV* had very few paramaecia at the bottom until the 34th day and then there appeared about 500 per cc. All disappeared by the 45th day. Their appearance at the bottom was coincident with a decline at the top which was brought about by Didinium.

Vorticella. In group I this form attained a maximum of 175 per cc. on the 38th day, while in group II they reached a maximum of 240 on the 20th day. Vorticella appeared in group III in small numbers at both top and bottom, the bottom maximum being 60 per cc. on the 31st day. In group IV, however, the largest bottom count was recorded, i.e., 600 per cc. on the 28th day (cf. fig. 15).

Amoeba. There was a great difference in the amoeba fauna of *B1* and *B2* of group I, so that it is better to present these separately. *B1* had a maximum of 10000 per cc. on the 53rd day, while *B2* contained practically no amoebae at any time. The *B II* group showed a large growth which attained a maximum of 2500 per cc. on the 50th day and terminated on the 58th day. There was a relatively small maximum of 250 per cc. on the 37th day in *B III* and the amoebae had disappeared by the 45th day. A maximum of 2000 per cc. was in existence in *B IV* from the 35th to the 45th day, when the last regular observation was made. This culture, however, supplied countless Amoeba proteus for class use for two years thereafter.

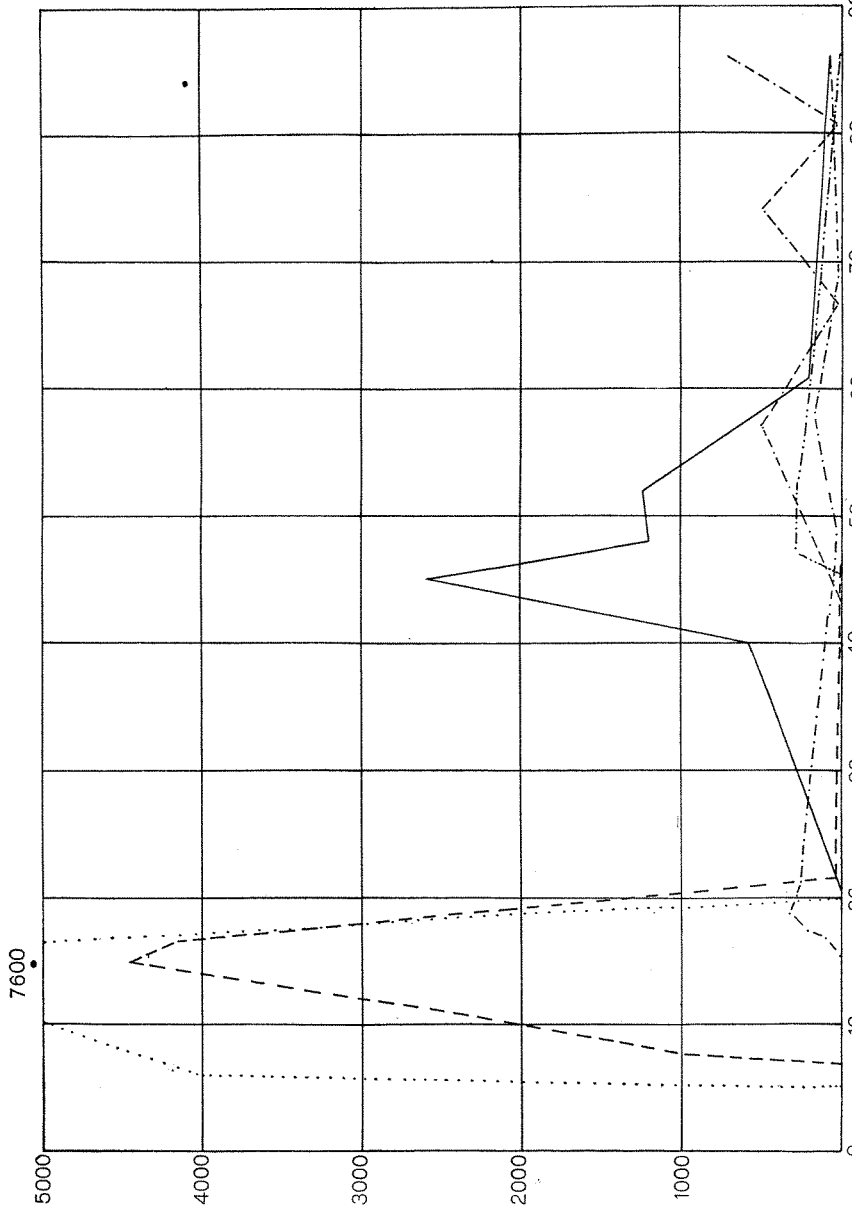

Fig. 9 *C I* group. The ordinates represent the number of organisms per cc. at the surface. On the abscissa is plotted the number of days of the existence of the infusions. Monad = ... ; Colpoda = - - - ; Hypotrichida = — . — ; Paramaecium = ———; Vorticella = — — . ; Amoeba = — . . —.

3. *C Infusions*

Monad. Monads appeared only in inappreciable numbers in all the groups of infusions. In group IV, however, a number of monad forms, other than those included in the top counts, appeared in considerable numbers for a time.

Colpoda. Practically no Colpoda were recorded for groups I and III. Group IV showed a brief maximum of 2500 per cc. which coincided with that of the top.

Hypotrichida. The hypotrichous fauna was practically zero.

Paramaecium. Paramaecia were not observed, except in group I where 100 per cc. were recorded for five days after a rapid decline at the top.

Vorticella. Practically no Vorticella appeared in the bottom counts.

Amoeba. In group I a few amoebae appeared on the 45th day and reached a maximum of 250 per cc. within the next five days, and then disappeared with equal rapidity. A heavy growth of 10000 tiny amoebae was attained in group III by the twenty-fifth day, and all were practically gone within ten days. In group IV a maximum of 10000 tiny amoeba was recorded on the 20th day and from this time the number gradually decreased until the 47th day when very few were observed. This decline was followed by a rapid rise to about 2000 per cc. on the 56th day when the last count was taken.

VII. DISCUSSION AND CONCLUSIONS FROM THE OBSERVATIONS ON THE SEQUENCE OF THE SURFACE, MIDDLE AND BOTTOM FAUNA

1. *Surface fauna.*

These extended observations on the protozoa of typical laboratory infusions, made up by several different methods, clearly indicate a definite succession of certain representative forms at the *surface* of the water.[13]

[13] I am indebted to Mr. T. S. Painter, one of my students, who made for me a careful study of a number of similar infusions in the Yale Laboratory and also at his home in Salem, Virginia. His observations show an essentially comparable

The close agreement both of the sequence of appearance and of maximum numbers in all three series (*A*, *B*, *C*) is striking (cf. p. 244) and indicates that the sequence is not merely the result of factors incidental to the methods employed.

The data in regard to the time of disappearance is relatively meagre for the *A* and *C* series because many of the typical forms studied survived the period of the last observation. Consequently the sequence of time of disappearance is based chiefly on data from the *B* series, which, on account of the removal of the hay, passed through its cycle much more rapidly.

It is remarkably suggestive that the sequence (derived from the entire series of infusions) of all the forms at the time of appearance and at the time of maximum numbers and at the time of disappearance is *identical*, with the exception of Amoeba. The data indicate that the Amoeba cycle in the infusions is comparatively short since the position of Amoeba in the series advances progressively forward: it being last at the time of appearance, next to last (before Vorticella) at the time of maximum and third from last (before Paramaecium and Vorticella) at the time of disappearance. However, as has been already pointed out, the data is not sufficient to positively establish the relative position of Amoeba and Vorticella at the period of maximum numbers.

A study of the curves plotted from the surface counts of single infusions or groups of infusions reveals the fact that when once a great development is attained by a particular form, this maximum is seldom approached again. There are, however, some striking exceptions to this as, for example, Colpoda in group *A III* (cf. fig. 3) and the Hypotrichida in group *C IV* (cf. fig. 11). The curves further show that the major rise and fall in numbers are usually of about equal rapidity, though the final complete disappearance of an organism from the infusion may be long deferred. Careful searching in many of the *A* and *B* infusions

sequence of forms with the one here described. Among the monads, however, he found a large development of Chilomonas, while this form was relatively scarce in my infusions. Also, his Amoeba fauna was partially replaced by a considerable growth of Arcella. This latter result is interesting since it shows that somewhat closely related rhizopods fill substantially the same place in the economy of the infusions.

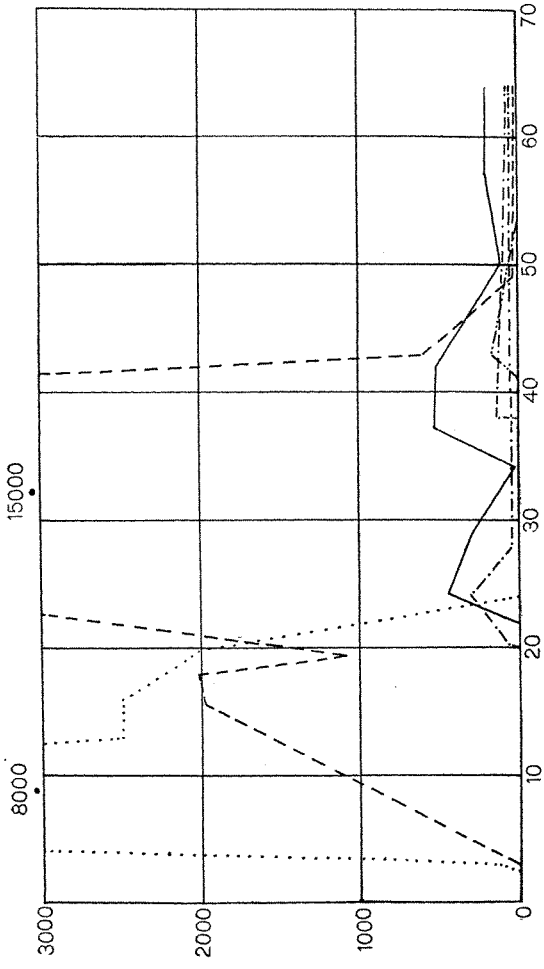

Fig. 10 *C III* group. The ordinates represent the number of organisms per cc. at the surface. On the abscissa is plotted the number of days of the existence of the infusion. Monad = ... ; Colpoda = - - - - ; Hypotrichida = — . —; Paramaecium = ———; Vorticella = — — . ; Amoeba = — . . —.

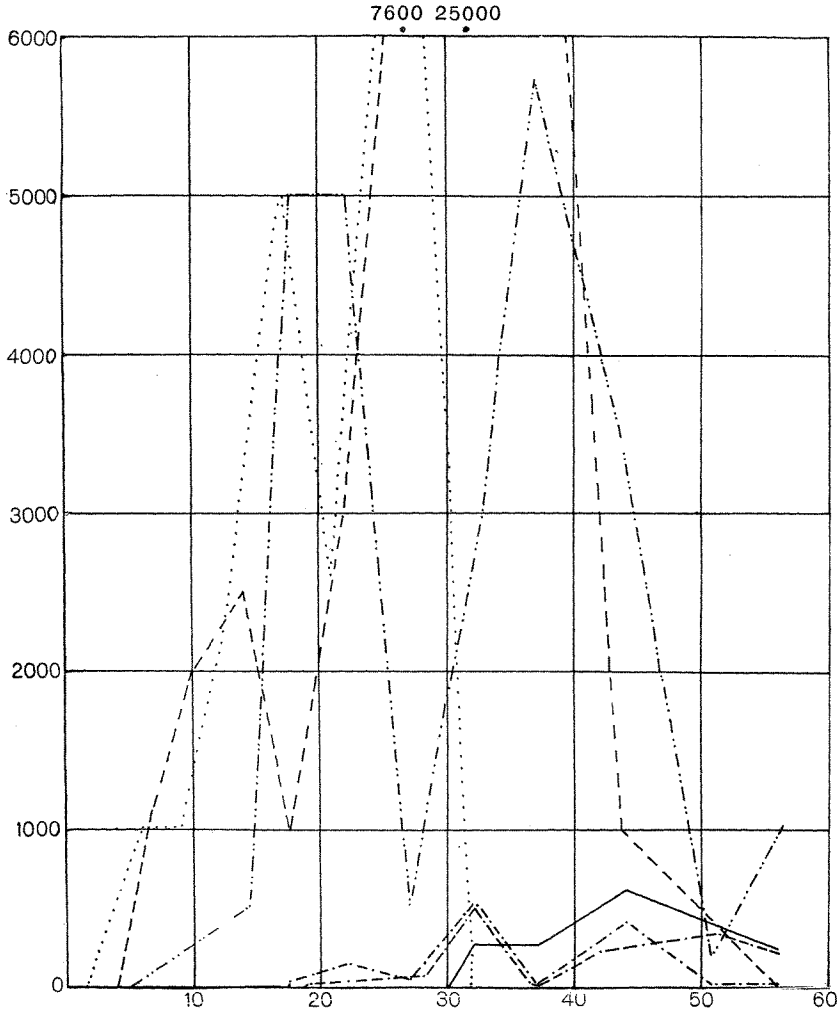

Fig. 11 *C IV* group. The ordinates represent the number of organisms per cc. at the surface. On the abscissa is plotted the number of days of the existence of the infusions. Monad = · · · · ; Colpoda = – – – ; Hypotrichida = — · —; Paramaecium = ———; Vorticella = — — · ; Amoeba = — · · —.

after a lapse of nearly three years showed a few survivors of nearly all the chief forms, mostly at the bottom among the algae and débris.

2. *Middle fauna*

It is impossible to determine any definite sequence of forms for the *middle* of the infusions—this region being, as already pointed out, a more or less neutral territory which is encroached upon from time to time by organisms from the top and bottom as conditions in these regions vary.

3. *Bottom fauna*

The *bottom* fauna also has not exhibited a definite succession similar to that of the top. A study of the data already presented shows that the protozoan forms under consideration, with the exception of many amoebae, are essentially surface dwellers and seldom resort to the bottom except during or after a period of great development at the top. However, there is no invariable correlation between a fall in numbers at the top and a rise in numbers of the same species at the bottom, and it seems clear that, in the majority of cases, when a species declines in one region, most of the animals encyst or die. The latter is certainly true for Paramaecium because many hundreds of passive and dying individuals, affording a feast for Coleps, may sometimes be seen among the débris at the bottom. Again, myriads of cysts of hypotrichous forms are frequently found at the bottom as the surface decline proceeds. Amoebae, among the protozoa under consideration, appear to give some evidence of migrating from the surface to the bottom which is their chief abode. The data on amoebae give the impression that some forms first appear in the infusions as amoebo-flagellates which gradually increase in size and before long are unable to assume the flagellated phase. The pesudopodia of these are first of the guttula type but become more and more long and slender until many typical radiosa forms are present, and these in turn give place to typical large A. proteus. Only in certain infusions has it been possible to trace such a series, but

in these it has been quite striking, and in one of the later infusions I was able to predict correctly that declining amoebo-flagellates would be replaced by typical amoebae. Such a cycle, of course, would not be remarkable in view of the results of some investigations on amoebae.[14] Although the data from these infusions by no means prove that the forms represented in this cycle are stages in the life history of a single species, nevertheless I lean toward the view that such will prove to be the case (cf. p. 211).

Taken as a whole, the study of the bottom fauna has proved to be less interesting than was anticipated, as I had expected to find

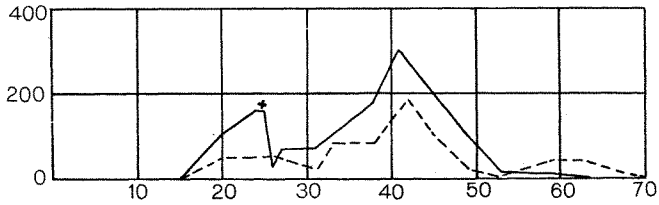

Fig. 12 Comparison of the Paramaecia fauna at the bottom of infusion *B1* (———) and infusion *B2* (– – –). x = point at which an epidemic of conjugation occurred in *B1*.

a much closer correlation between declines at the top and rises at the bottom, and *vice versa*. Apparently the bottom forms are largely independent of those at the surface, and the protozoan types under consideration, with the exception of the amoebae, are represented at the bottom by considerable numbers of active individuals chiefly when some sudden change, such as the falling of the zoogloea, brings them down, or by stragglers which manage to exist by avoiding the competition at the top. It is nearly always possible, by careful searching, to find at the bottom a few struggling individuals which have survived from an earlier prosperous surface population.

[14] For example, cf. Metcalf: Studies upon Amoeba. Jour. Exp. Zool., vol. 9, 1910.

4. Factors determining the sequence

The problem becomes enormously complex when an attempt is made to decide upon the chief determining factors of the observed sequence of organisms at the surface of the infusions, and is entirely beyond our power of analysis from the data extant. Therefore, I believe, it is preferable at this time not to enter into an extended discussion of this question. I shall, however, briefly mention some points which seem to indicate suggestive lines for future study.

There is experimental evidences that, broadly speaking, the potential of division decreases from monads to paramaecia; that is, for example, paramaecia, under optimum conditions, divide less frequently than the majority of the hypotrichida, and similarly, the latter divide less rapidly than Colpoda. In regard to Vorticella and Amoeba, however, sufficient data are not at hand to make a definite statement.

With this in mind a series of experiments were made on the time of appearance of maximum numbers of Monads, Colpoda, Hypotrichida and Paramaecium in separate flasks of infusion which were seeded with a single individual of one species. The multiplication of the respective forms in the various flask cultures was observed, and the results showed remarkable agreement with the sequence of maximum numbers as determined for these same forms in the regular infusions. Consequently it appears that the number of specimens of any particular organism initially introduced into the large infusions, or the time of emergence of encysted forms has not had an important influence on the sequence of maximum numbers in these infusions as determined for the complete series. It may well, however, account for at least some of the individual variations in the sequence of appearance in numbers sufficient to be included in the samples studied, and of maximum numbers, which are apparent in particular groups of infusions. Again the interaction of the different forms would appear, at first glance, not to be a crucial factor in the sequence of maximum numbers since, in the experiments cited, the 'sequence' was duplicated, when only one species of organisms was

in each flask of infusion. This conclusion nevertheless, does not necessarily follow from the data, because all of the forms under consideration can flourish on a bacterial diet, which, of course, was supplied in each case. The interaction of the various forms clearly plays a part in the duration of the maximum and the rapidity of the decline. Experiments by the slide method of culture, which I have employed in my pedigree culture work, show that in culture medium which is the same from day to day practically the same 'sequence' of maximum numbers occurs and in this case it is apparent that chemical changes in the environment are not responsible for the results. Further, it is possible to carry all the forms under consideration for at least one hundred generations by this slide method, and this is sufficiently long to show that enough organisms can be produced in a medium which is chemically constant to supply, many times over, the number of organisms recorded at the maxima in the regular infusions. Consequently I think that these observations indicate that the relative potential of division of the four forms under discussion is adequate, under certain conditions at least, to establish the observed sequence of maximum numbers, and clearly suggest that it may be an important factor in large infusions.

The data from these infusions lead me to believe that the strictly biological factors are of greatest importance, and that it is necessary to look to somewhat subtle chemical changes in the medium for the important chemical factors in the environment. Fine's studies[15] on these infusions are in accord with this view and indicate that such general chemical changes in the environment as, for example, titratable acidity are not determining factors, at least for these particular species. My work on the excretion products of Paramaecium shows,[16] however, that such substances have an inhibiting influence on the reproduction of this form, and it is quite probable that these products affect the sequence, maximum numbers, and decline of the various species. In fact Shelford, in his studies on the ecological succession of fish in ponds, believes that

[15] Cf. Fine: loc. cit.

[16] The effect of excretion products of Paramaecium on its rate of reproduction. Jour. Exp. Zool., vol. 10, no. 4, 1911.

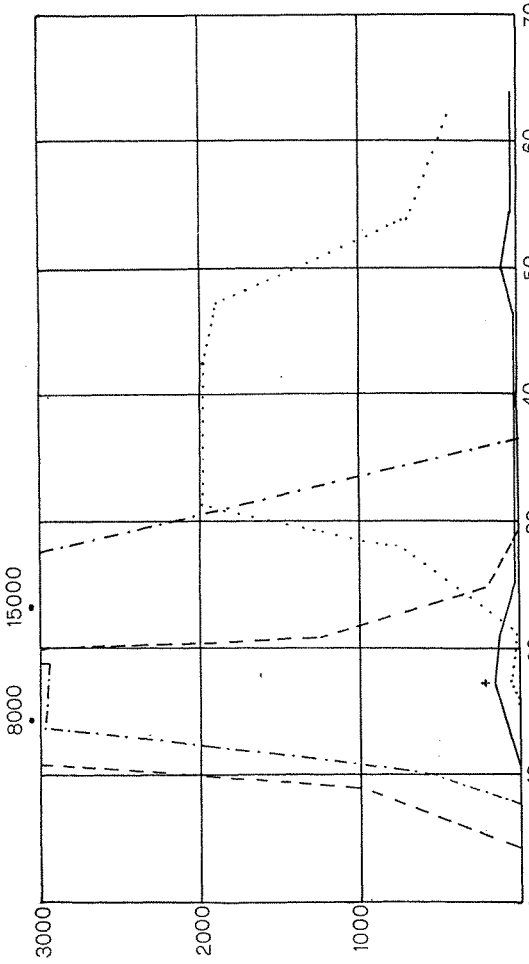

Fig. 13 *B III* group. Colpoda at the surface = - - - and at the bottom = — · —; Paramaecium at the surface = ——— and at the bottom = · · · · · · x = point at which an epidemic of conjugation occurred at the surface.

his data show that the succession of those forms is not determined by the kind of available food but to an unused increment of decomposition and excretory materials which, in the last analysis, affects breeding.[17]

5. *Decline in numbers*

Closely involved with the problem of the sequence of appearance and maximum of the various forms is that of their more or less rapid decline in numbers. Here again the accumulated data do little more than establish the fact. The decline of the monads is quite clearly due, in part at least, to the evident variation in the amount of food in solution and to the rising hosts of Colpoda. The decline of Colpoda may be similarly ascribed to the dominance of the hypotrichida. Most of the hypotrichous forms were literally filled with ingested Colpoda which formed their staple diet. The relations of Paramaecium and Vorticella to their predecessors, successors and to each other is not so apparent, but their abundance may well be influenced by a succession of the bacterial flora, for example, which unfortunately could not be followed in these studies, as well as to the host of other protozoan species. The competition between the various forms is so keen and the cycle is so rapid that even daily observations are, at times, insufficient to reveal the kaleidoscopic changes. Now and then, however, some prominent case of competition, such as that between Paramaecium and Didinium, is forced upon the attention and the reason for the extinction of one form is clear. Didinium, in fact, so quickly exterminated the paramaecia in groups *A II* and *B IV* that it was necessary to omit the records of paramaecia in the table of sequence of these infusions (cf. table 2). In *B II* also the paramaecia cycle was considerably aborted by Didinium. Among other instances of a similar nature, the destruction of hosts of Colpoda by the suctorian Podophrya may be mentioned. In other words, one who closely follows a series of infusions dayby day cannot but be impressed with the intense struggle for food and the eternal warfare in this microcosm, and become con-

[17] Biological Bulletin, vol. 22, no. 1, 1911.

vinced, though he cannot prove, that in the final anaylsis the paramount factor is food, though many other factors, such as excretion products, etc., may play a not unimportant part. Biometrical study of variation in certain Protozoa shows that the average size of the population is smaller after their period of greatest abundance in an infusion and that "there can be little doubt that one of the chief factors which induce saprophytes like Chilomonas to disappear from a culture is that the medium no longer furnishes proper food (either in amount or kind, or both)."[18]

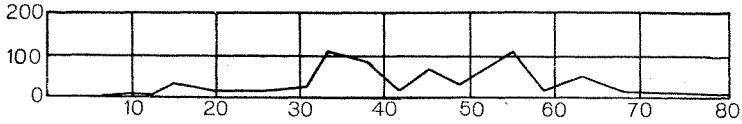

Fig. 14 *B I* group. Hypotrichous fauna at the bottom.

VIII. CONJUGATION

Comparatively few epidemics of conjugation were observed in this entire study, and these were chiefly among paramaecia, so that the data in this connection are quite meager. It therefore has not been possible to make any definite correlations between the presence of the phenomenon and the fate of the conjugating forms in the infusions. However, a study of the records in regard to Paramaecium seems to show that conjugation usually occurs when a comparatively large number of individuals are present and that immediately following an epidemic there is a temporary decline in the number of specimens observed. After this decline there may or may not be a large increase in the number of animals.

It seems clear that in many cases conjugation is coincident with sudden changes in the environment. In fact the phenomenon may occur in certain cases almost solely among individuals which have been carried to the bottom with falling zoogloea. But that this does not of necessity bring about conjugation is shown by infusions *B1* and *B2*. Conjugating paramaecia were not seen at

[18] Pearl: Variation in Chilomonas under favorable and unfavorable conditions, Biometrika, vol. 5, 1906–1907.

the surface of either of these infusions, and at the bottom it was only observed in *B1*. At the point marked x (fig. 12) fully 95 per cent of the animals were conjugating. Nevertheless the paramaecia fauna at the bottom ran practically the same course in each infusion, in fact it survived somewhat longer in the infusion in which conjugation was not observed. This culture also illustrates a case in which a temporary decline in numbers occurred immediately after an epidemic of conjugation (cf. fig. 12).

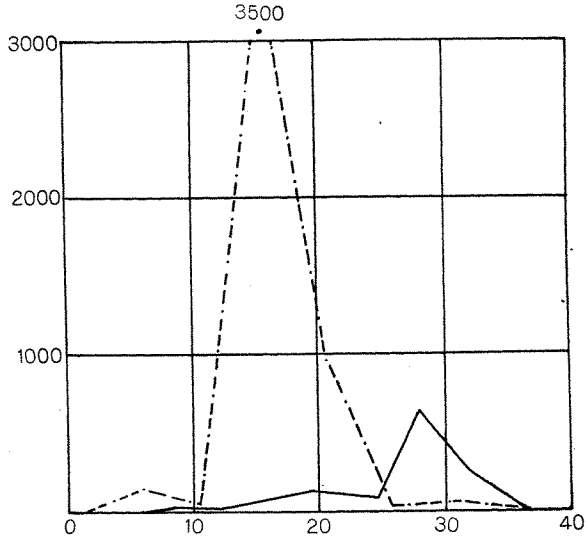

Fig. 15 *B IV* group. Vorticella fauna at the surface (- - - - -) and bottom (———).

In group *B III*, in which there was an exceptionally large bottom fauna, conjugation was observed among the paramaecia at the surface and bottom simultaneously, but was somewhat more prevalent at the top. Fig. 13 shows that the epidemic occurred at the period of the surface maximum and that the ensuing decline at the top was coincident with a remarkably large increase in the bottom growth.

Apparently many species of infusoria do not resort to conjugation to sustain rapid cell division when the environment is slowly changing and the data give no reason for believing that

conjugation effects 'rejuvenation.' In many cases encystment occurs and the organisms remain at the bottom when conditions become somewhat unfavorable; but undoubtedly the majority die after their period of maximum abundance. My experience with these cultures leaves me with the impression that conjugation will be found to be a means resorted to by many species to survive acute changes in the environment, which, for example, preclude encystment. It is suggestive in this connection that in forms like the hypotrichida, which, as is well known, have a decided tendency to encyst, and the cysts of which were observed in great abundance at the bottom of these infusions after the active forms passed their maximum, not a single syzygy was observed; while in Paramaecium, in which the power of encystment has never been established, conjugation is recorded comparatively frequently. However, it is also clear from this work that a condition which will induce conjugation in one race of Paramaecium will not always induce it in another, as epidemics have occurred between small races, while among giant races intermingled with them syzygies were not seen. This, of course, is in accord with Jennings' studies on Paramaecium.[19] The problem of the conditions inducing conjugation, and also of the effect of conjugation has recently become so complex from our increasing knowledge of the life history of various paramaecium genotypes, that the observations here recorded are interesting chiefly as throwing a side light on certain factors of the phenomenon as they appear in large cultures.

IX. SUMMARY

The following points may be emphasized:

1. Ordinary hay added to tap water will not produce an infusion which is productive of a sufficient number of representative protozoan forms to make it profitable for the study of protozoan sequence.

2. Air, water, and hay are all sources from which the protozoa of infusions are derived, and increase in importance in the order

[19] What conditions induce conjugation in Paramaecium? Jour. Exp. Zool., vol. 9, no. 2, 1910.

given. Of these three, however, air is practically a negligible factor in seeding infusions.

3. In hay infusions, seeded with representative forms of the chief groups of Protozoa, there is a definite sequence of appearance of the dominant types at the surface of the infusion, i.e., Monad, Colpoda, Hypotrichida, Paramaecium, Vorticella and Amoeba.

4. The sequence of maximum numbers and of disappearance is identical with that of appearance, except that apparently the position of Amoeba advances successively from the last (sixth) place to the fifth place and then to the fourth place.

5. A definite sequence of forms is not apparent at the middle or bottom of the infusions.

6. The middle of the infusions is tenanted chiefly by a free-swimming population brought there by an overcrowding at the top or bottom.

7. All of the protozoan forms considered (except Amoeba) are chiefly surface dwellers and it is evident that when they pass their greatest development at the surface this maximum is seldom approached again, and their cycle is practically over.

8. The major rise and fall in numbers are usually about equally rapid, though the final disappearance of an organism may be long deferred.

9. The appearance of any of the protozoan forms under consideration (excepting Amoeba) in appreciable numbers at the bottom is most often coincident with or immediately subsequent to its surface maximum, and portends its more or less rapid elimination as an important factor in the life of the infusion.

10. Numerous abnormal individuals and cysts are frequently to be found at the bottom in great abundance immediately after the surface maximum.

11. There is some evidence that amoebae migrate from the surface to the bottom which is their chief abode.

12. The observations give the impression that some amoebae appear as amoebo-flagellates which gradually increase in size and finally assume the form of typical A. proteus.

13. There is some evidence that the relative potential of division of the various forms may have an appreciable influence on the sequence of the maxima.

14. Emphasis is put upon the strictly biological interrelations (e.g., those involving food and specific excretion products) of the various forms as the most important determining factors in the observed sequence.

15. The observations suggest that conjugation will be found to be a means resorted to by many species to survive acute changes in the environment, which, for example, preclude encystment.