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Method for the Simultaneous Establishment of Many Axenic Cultures of *Paramecium**†

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SYNOPSIS. A method is described for the simultaneous treatment of 42 (or more) stocks of *Paramecium*, and their adaptation to growth in axenic culture. Samples of dense cultures of these ciliates growing with *Enterobacter aerogenes* are rendered bacteria-free by migration through 2 sets of tubes containing Adaptation Medium (Peters' salts solution, stigmasterol, vitamins, and autoclaved *E. aerogenes*). The 2nd set of tubes contains Adaptation Medium plus antibiotics. Bacteria-free samples containing ~ 100 animals are then transferred to test tubes containing Adaptation Medium without antibiotics. This medium also serves as a growth medium. It supports indefinite growth of all *Paramecium* stocks tested. After adaptation to this medium, the ciliates can be grown in the axenic medium developed by Soldo, Godoy & van Wagtendonk. On a single trial at least half of the stocks can be expected to produce axenic cultures within 5 to 10 days by these procedures. The method has been applied successfully to several of the species of the *Paramecium aurelia* complex, to all syngens of *Paramecium multimicronucleatum*, to several stocks of *Paramecium jenningsi*, and to 1 stock each of *Paramecium caudatum* and *Paramecium calkinsi*. A modification of the method also works for *Didinium nasutum*.

Index Key Words: *Paramecium aurelia* complex; *Paramecium multimicronucleatum*; *Paramecium jenningsi*; *Paramecium caudatum*; *Paramecium calkinsi*; *Didinium nasutum*; adaptation to growth in axenic culture.

A long-standing need has existed for an easy, practical method of transferring simultaneously many stocks of *Paramecium* feeding on bacteria to axenic culture. For certain types of biochemical analysis, the presence of living bacteria not only affects the expression of protozoan products but bacterial products can be detected (1, 5, 6). Obtaining a few stocks of *Paramecium* in axenic cultures has not been a problem. For genetic and evolutionary work, however, hundreds of stocks may be required to sample a cross, a taxonomic group, or a field population adequately. None of the current methods for removing the bacteria from *Paramecium* stocks is satisfactory for large numbers.

Three methods for freeing *Paramecium* stocks of bacteria have been described previously: (a) "handwashing" individual ciliates by micropipette (9); (b) electromigration (15, 16); and (c) continuous washing using an apparatus called "The Tree" (16). The 1st method is tedious, laborious, and the yield is extremely low. The 2nd and 3rd methods utilize bulky equipment and are not practical for large numbers of stocks. Both the 1st and 3rd methods were used in obtaining small numbers of axenic stocks of the 14 species of the *P. aurelia* complex for enzyme analysis (2, 3). To obtain large numbers of axenic stocks for such studies a new method was required. The method that we have devised is based upon the 3rd method (The Tree), which relies upon a behavioral trait of the ciliates (negative geotropism). They swim upward through several meters of glass tubing containing 0.15% (w/v) Cerophyl infusion and antibiotics (16). We have greatly simplified the apparatus so that large numbers of stocks can be handled simultaneously.

An important problem common to any method is the adaptation of *Paramecium* stocks to growth under axenic conditions. When transferred to the axenic medium of Soldo et al. (8), a long lag period follows before the organisms begin to divide, if they divide at all. The degree of success in establishing and/or maintaining growth in this medium is low, although highly variable for different stocks and species. Clearly needed were procedures which permitted better adaptation to growth in axenic culture so that the yield of established cultures could be improved. We introduce a new medium which facilitates adaptation.

The method described below permits not only the handling of large numbers of stocks but it also results in a high yield of successfully established axenic cultures. In addition, it appears to be applicable to several different species of *Paramecium*.

MATERIALS AND METHODS

Stocks.—Bacterized cultures (with living *Enterobacter aerogenes*) of a large number of stocks of *Paramecium primaurelia*, *P. biaurelia*, *P. tetraurelia*, and *P. octaurelia*, stocks from all 5 syngens of *P. multimicronucleatum* and from the one syngen of *P. jenningsi*, and 20 stocks of *Paramecium* unidentified to species, were graciously supplied by T. M. Sonneborn and M. V. Schneller (Indiana University). In addition, we have small numbers of stocks representing each of the other species of the *P. aurelia* complex. Species names were introduced recently by Sonneborn for each of the species in the *P. aurelia* complex (13) thereby supplanting the numbered species designation introduced previously (12) and the older designation, syngen (10). Syngen designations are, however, still used for the 5 noninterbreeding groups of *P. multimicronucleatum* and the one known group of *P. jenningsi*. The bacterized stocks were maintained with *E. aerogenes* in 0.25% (w/v) Cerophyl infusion in the manner described by Sonneborn (9, 11). Axenic stocks of *P. caudatum* and *P. calkinsi* were kindly supplied by R. Napolitano (Brooklyn College).

Axenic Medium.—We use the medium developed by Soldo et al. (8) but we do not prepare it in the manner described. The medium, without the vitamins, is autoclaved under 15 lbs pressure for 20 min. The vitamins, sterilized by filtration, are then added. In some experiments autoclaved *E. aerogenes* was added at a final concentration of 100 μg dry weight/ml (or 5×10^8 bacteria/ml).

Adaptation Medium.—The components of this medium along with their final concentrations (μg/ml) and comments are shown in Table 1. The vitamins are added separately after being sterilized by filtration.

Antibiotic Wash Solution.—This is Adaptation Medium with the addition of antibiotics—either neamine (a gift from the Upjohn Company, Kalamazoo, Michigan; final concentration: 50 μg/ml) or Antibiotic-Antimycotic Mixture (obtained from

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† This paper is dedicated to Professor T. M. Sonneborn on the occasion of his 70th birthday.

TABLE 1. *Composition of adaptation medium.*

Components	Conc. (μg/ml)	Components	Conc. (μg/ml)
(A) Peters' salts solution*		(D) Vitamin mixture§	
NaCl	600	Ca-pantothenate	5
$CaCl_2 \cdot 2H_2O$	20	Nicotinamide	5
$MgSO_4 \cdot 7H_2O$	10	Pyridoxal-HCl	5
Na_2HPO_4	10	Pyridoxamine-HCl	2.5
KCl	10	Riboflavin	5
		Folic acid	2.5
(B) Stigmasterol†	5	Thiamine-HCl	15
(C) Autoclaved		Biotin	0.00125
Enterobacter aerogenes‡	100	DL-Thioactic acid	0.05

* See Ref. 11; solution adjusted to pH 7, with 1 N NaOH.

† Stock solution prepared in absolute ethanol (2 mg/ml); 2.5 ml of the stock solution added to 1 liter of solution A. Final concentration equals that in the medium (8) used for axenic cultures.

‡ Concentrated (100×) stock culture prepared in solution A and pressure-sterilized (121c, 151b, for 15 min). Sterile stock culture added to sterile mixture of solutions A and B to give a final concentration of 100 μg dry wt (5×10^8 bacteria)/ml.

§ Aqueous 100× stock solution sterilized by filtration and added to the medium (10 ml/liter) to give a final concentration equalling that in the medium used for axenic cultures.

Grand Island Biological Company, Grand Island, New York) which contains penicillin (10,000 units/ml), streptomycin (10,000 μg/ml) and fungizone (25 μg/ml), and is diluted to the final concentration of 1/100 (v/v).

Growth Medium Used Immediately before Elimination of Bacteria.—Enterobacter aerogenes is grown on Tryptic Soy Broth (Difco Laboratories, Detroit, Michigan) at 30 C to a concentration of 10^9/ml. The bacteria are then centrifuged at 1,085 *g* for 20 min and washed twice with modified Peters' salts solution containing stigmasterol and vitamins (Table 1). For optimum growth of *Paramecium* the final concentration of living *E. aerogenes* must be at least 10^8/ml as determined from a standard curve relating absorbancy to plate counts.

Apparatus for Sterilization.—The 3 types of tubes used are diagrammed in Fig. 1. Two types of assemblies, which are employed in sequence (to be described below) are shown in Fig. 2. A wooden rack (not shown) was designed to accommodate 42 of these assemblies.

Growth of Paramecium before Elimination of Bacteria.—Approximately 100 ciliates are added to test tubes containing 5 ml of a living bacterial suspension (~ 10^8 bacteria/ml) in modified Peters' salts solution. The ciliates are grown for ~ 1 week at 23 C (or until a dense culture is achieved).

Procedure for Obtaining Axenic Cultures.—A Pasteur pipette with a cotton plug in the end (Fig. 1: tube A) is initially filled by capillary action with 500-1,000 ciliates from a dense bacterized culture (fluid volume 0.1-0.2 ml). The open end of the pipette is then sealed with heat. All air bubbles are removed from the tube by lightly tapping the pipette.

The cotton plug from tube A is then removed, and the lip of the pipette is flamed. The aluminum foil from tube B (Fig. 1) is removed, and the top of the Pasteur pipette is inserted into the rubber sleeve. The assembly is then mounted in a wooden rack. A 5-ml aliquot of Adaptation Medium is added to the assembly, all air bubbles are removed from the column, and the assembly is filled to the top with Adaptation Medium. The inverted culture tube is removed from tube B, and the aluminum foil is removed from tube C. The top of the glass tube (B) is inserted into the rubber sleeve of tube C. The complete assembly (assembly 1) is shown in Fig. 2a. The final tube is filled to the top with Adaptation Medium, the clamp is opened, and the ciliates are allowed to migrate overnight.

The next morning after the ciliates have migrated, the clamp in tube C is closed. Tube C is removed and then connected via the rubber sleeve to a new tube B. This assembly (assembly 2) is shown in Fig. 2b. A 5-ml aliquot of the Antibiotic Wash Solution is added, air bubbles are removed, and the tube is filled to the top with this solution. Migration of *Paramecium* begins. After 2-6 h a sufficient number have migrated to the top of the column so that a sample can be removed.

The ciliates are removed by capillary action from the top of Assembly 2 with sterile Pasteur pipettes which have previously been drawn to a fine point. This procedure is carried out by laying Assembly 2 horizontally on a dissecting microscope in a sterile chamber and observing the process at low magnification. The paramecia, now in the Pasteur pipette, are delivered to screwcap culture tubes containing 5 ml of growth medium. In the present study, 3 different growth media were tested: Axenic Medium, Axenic Medium plus autoclaved *E. aerogenes*, and Adaptation Medium. As is evident from the results, Adaptation Medium gives the best yield; therefore, it is the medium we now use routinely for adaptation to axenic culture.

Adaptation of Paramecium to Axenic Culture.—We have found that Adaptation Medium containing autoclaved *E. aerogenes* also serves as an excellent growth medium. After growth in Adaptation Medium, all the stocks we have tested grow successfully in Axenic Medium.

Adaptation Medium was developed as follows. The individual components of Axenic Medium (proteose-peptone, trypticase, yeast nucleic acid, TEM-4T, stigmasterol, and vitamins) were removed one at a time, adding autoclaved *E. aerogenes* at a final concentration of 100 μg/ml. Only when the vitamins were removed did the ciliates stop growing immediately. In the absence of stigmasterol, their growth ceased upon the 2nd transfer. Stigmasterol and vitamins in the concentrations used in Axenic Medium were added to Peters' salts solution containing 100 μ/ml of autoclaved *E. aerogenes*. This medium supported the growth of all *Paramecium* stocks and species indefinitely.

Tests for Sterility.—After transfer of the presumably bacteria-free samples of *Paramecium* to test tubes containing growth medium (Axenic, Axenic plus bacteria, or Adaptation), the test tubes are examined over the next week for signs of bacterial contamination. Tubes which appear free of bacterial contamination are subjected to a sterility test. From these tubes 0.1 ml samples are inoculated into test tubes containing 5 ml of Tryptic Soy Broth and also plated onto Tryptic Soy Agar. In addition, to test for bacteria which may have requirements for components present in Axenic Medium, additional test tubes and agar plates are prepared with Tryptic Soy Broth containing 20% Axenic Medium (v/v). All test tubes and plates are incubated at 30 C for 2 weeks.

RESULTS AND DISCUSSION

Three crucial steps can be identified as necessary for successful production of axenic cultures of *Paramecium*. The 1st step involves the pretreatment of the ciliates, the 2nd step removal of bacteria, and the 3rd step adaptation to axenic conditions. Pretreatment requires a dense bacterized culture of *Paramecium* so that a very small fluid volume is introduced into the sterilization apparatus. This could be achieved by concentration (see Ref. 16). An additional requirement, however, is adaptation to the environment during the washing procedure. By growing

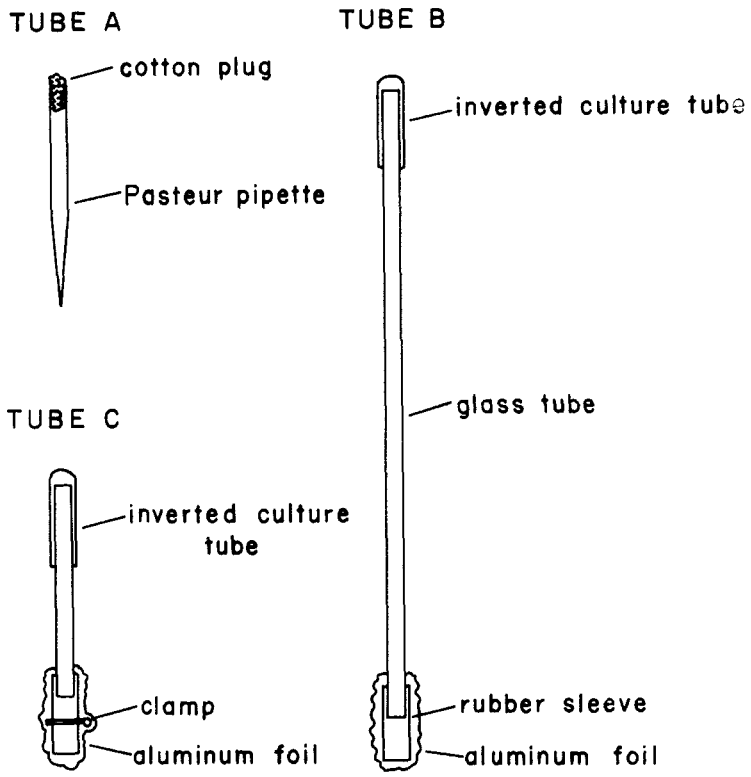

Fig. 1. Diagrams of component tubes used for the sterilization assembly. A, prepared from a 23-cm Pasteur pipette; B, glass tubing 7 OD × 5 ID × 600 L mm, with a culture tube 10 OD × 8 ID × 75 L mm; C, glass tubing 7 OD × 5 ID × 230 L mm with a culture tube as for B.

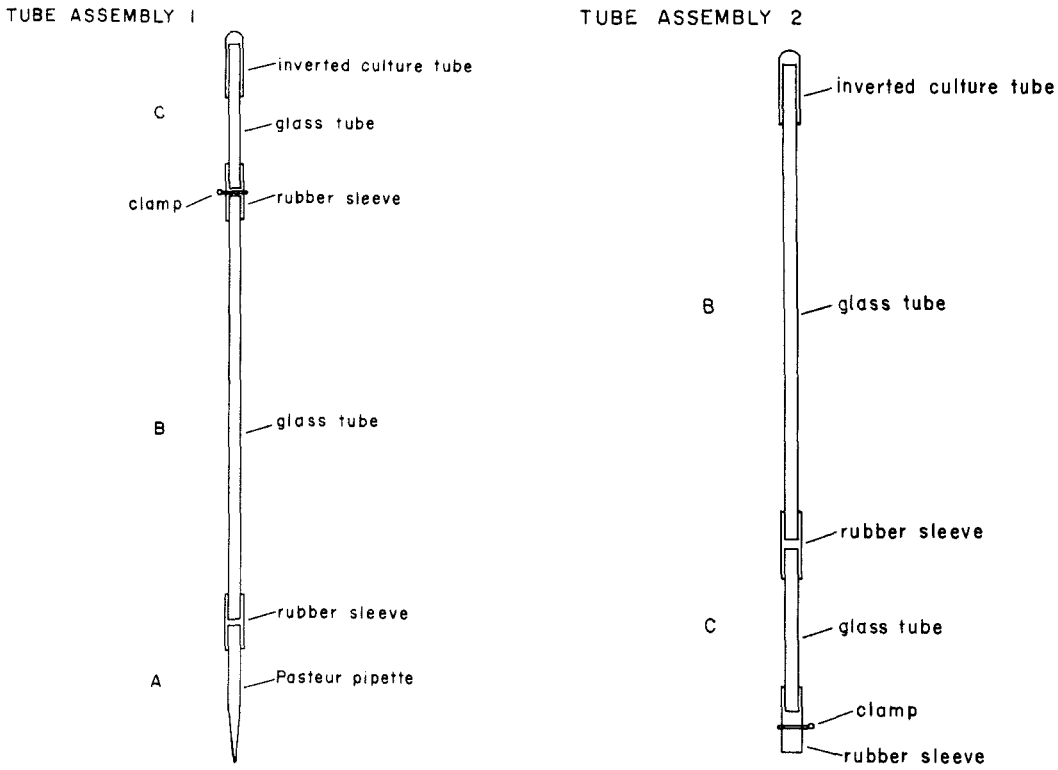

Fig. 2. Assemblies used for sterilization. Assembly 1 consists of tubes A, B, and C; Assembly 2 consists of Tubes B and C.

TABLE 2. *Comparison of the effectiveness of neamine and the antibiotic-antimycotic mixture in removing bacteria.*

Bactericidal agent	Species	Stocks	Tubes
		No. sterile/total	No. sterile/total
Neamine	*P. primaurelia*	27/36	73/136
	P. biaurelia	22/33	57/109
	P. triaurelia	5/12	13/35
	P. tetraurelia	15/25	54/94
	P. multimicronucleatum		
	Syngen 1	1/1	4/4
	Syngen 2	7/17	25/57
	Syngen 3	3/4	8/13
	Syngen 4	3/7	10/25
	Syngen 5	1/2	3/7
	TOTAL	84/137	247/480
Antibiotic-antimycotic mixture	*P. biaurelia*	29/31	76/104
	P. tetraurelia	8/10	29/41
	P. septaurelia	2/2	7/7
	P. octaurelia	34/38	98/150
	P. multimicronucleatum/2	12/14	37/51
	P. jenningsi/1	6/6	20/31
	TOTAL	91/101	267/384

Paramecium in a medium similar to Adaptation Medium except for the presence of living instead of autoclaved bacteria, this objective is met. The ciliates that we use are in good condition and readily accept the substitution of the autoclaved for living bacteria. Older methods used *Paramecium* feeding on bacteria in a Cerophyl infusion which were washed in sterile Cerophyl infusion or in sterile salts solution (3, 16). These solutions differ from each other, and they also differ drastically from Axenic Medium.

The 2nd step is removal of the bacteria. The 2 antibiotics appear to differ in their effectiveness (Table 2). For each antibiotic the 1st column lists the species included, the 2nd and 3rd columns show the number of different stocks in a species rendered sterile and the 4th and 5th columns show the number of sterile tubes, irrespective of stock (i.e. all data from a species pooled). On the average, there were 3.5 tubes per stock for each run with neamine and 3.8 tubes per stock for each run with the Antibiotic-Antimycotic Mixture. In a single run replicate tubes for a given stock were not highly correlated. This led to the higher values seen for the stock data compared to the tube data, since there were several opportunities for each stock to become sterile. It is evident from the pooled data for all species that 61.3% of the stocks (84/137) and 51.5% of the tubes (247/480) were bacteria-free after exposure to neamine, while 90.1% of the stocks (91/101) and 69.5% of the tubes (267/384) were bacteria-free after exposure to the Antibiotic-Antimycotic Mixture. Clearly the Antibiotic-Antimycotic Mixture appears to be more effective, although a much higher percentage ("approaching" 100%) was reported by van Wagtendonk & Soldo (16) who used the Tree method and neamine in the same concentration as the one used; however, they were dealing with a very limited number of stocks.

The 3rd step is the adaptation of *Paramecium* to axenic conditions. In our experience this has been one of the chief hurdles in obtaining axenic cultures. Certainly there is some variation among stocks within a species as well as among species in the ease with which they adapt. This is documented in the review by van Wagtendonk (14) with respect to the variation between species of the *aurelia* complex. We will return to this aspect later. More important than the species or stock variation are the conditions under which adaptation is carried out. We have found that the introduction of autoclaved bacteria improves our yield of sterile stocks which actively divide under axenic conditions. Our experiences with different growth media after treatment with neamine or the Antibiotic-Antimycotic Mixture are summarized in Table 3. Some of the ciliates that were freed of bacteria by neamine treatment were transferred only to tubes containing the Axenic Medium of Soldo et al. (8) and are indicated by an asterisk. Another group of similarly treated paramecia were transferred to tubes containing Axenic Medium or this medium plus autoclaved *E. aerogenes*. Ciliates freed of bacteria by the Antibiotic-Antimycotic Mixture were transferred to tubes containing 3 different media, (a) Axenic Medium, (b) this medium plus autoclaved *E. aerogenes*, and (c) Adaptation Medium. The data are given both by stock and by tube in the manner described for Table 2. With 1 exception, wherever there were multiple tubes per stock, the stock values were higher than the tube values. This reflects a low correlation between the performance of cultures in tubes of the same stock in the same run. The exception concerns the data in the last part of the table dealing with the Antibiotic-Antimycotic Mixture-treated paramecia transferred to Adapation Medium. The distribution of tubes is distinctly nonrandom. Where there were 2 tubes per stock, 30/32 sets were positive (one set of 3 tubes was also positive). Of the stocks with only 1 tube per stock, only 5/43 were positive.

For either antibiotic, the yield of actively growing ciliates was greatly improved by the addition of autoclaved bacteria to the growth medium, although there was also an effect of the type of antibiotic on the yield. If we focus on the tube data, we see that, after neamine treatment, 40% of the cultures grew in Axenic Medium while 61.7% of the cultures grew in Axenic plus autoclaved bacteria. Of the Antibiotic-Antimycotic Mixture-treated paramecia, 20.2% of the cultures grew in Axenic Medium, 36.8% of the cultures grew in Axenic plus autoclaved bacteria, and 62.7% of the cultures grew in Adaptation Medium. Clearly neamine appears to be less injurious to *Paramecium* than the Antibiotic-Antimycotic Mixture. The addition of auto-

TABLE 3. *Adaptation to axenic culture of bacteria-free ciliates.*

Bacteria eliminated by	Recipient medium	Species	Stocks	Tubes
			No. positive/total	No. positive/total
Neamine	Axenic Medium	**P. primaurelia*	14/20	22/56
		P. primaurelia	2/4	4/6
		**P. biaurelia*	18/22	24/57
		**P. triaurelia*	3/5	4/13
		P. tetraurelia	7/16	10/24
		P. multimicronucleatum		
		*Syngen 1	1/1	1/4
		*Syngen 2	6/7	11/25
		*Syngen 3	3/3	3/8
		Syngen 4	0/2	0/4
		*Syngen 5	1/1	1/3
		TOTAL	55/81	80/200
Neamine	Axenic Medium + autoclaved *E. aerogenes*	*P. primaurelia*	5/7	6/11
		P. tetraurelia	15/16	21/30
		P. multimicronucleatum		
		Syngen 4	2/3	2/6
		TOTAL	22/26	29/47
Antiobiotic-antimycotic mixture	Axenic Medium	*P. biaurelia*	3/22	4/23
		P. tetraurelia	3/8	3/9
		P. septaurelia	0/1	0/2
		P. octaurelia	5/28	9/35
		P. multimicronucleatum/2	2/10	2/11
		P. jenningsi/1	0/5	0/9
		TOTAL	13/74	18/89
Antiobiotic-antimycotic mixture	Axenic Medium + autoclaved *E. aerogenes*	*P. biaurelia*	7/18	7/18
		P. tetraurelia	2/6	2/6
		P. septaurelia	1/2	1/2
		P. octaurelia	10/26	11/28
		P. multimicronucleatum/2	2/9	2/9
		P. jenningsi/1	2/5	2/5
		TOTAL	24/66	25/68
Antibiotic-antimycotic mixture	Adaptation Medium	*P. biaurelia*	13/25	23/35
		P. tetraurelia	5/8	11/14
		P. septaurelia	1/2	2/3
		P. octaurelia	13/24	23/35
		P. multimicronucleatum/2	4/12	8/17
		P. jenningsi/1	1/5	2/6
		TOTAL	37/76	69/110

claved bacteria to the medium favored adaptation and improved the yield of axenically grown cultures. The final yield of axenic stocks was slightly better than half for each group run using neamine plus Axenic Medium with autoclaved bacteria or the Antibiotic-Antimycotic Mixture plus Adaptation Medium.

A 2nd measure of differences in adaptation to the 3 different growth media (Axenic Medium, Axenic Medium plus autoclaved bacteria, Adaptation Medium) is the time it takes for a culture to become established in a test tube after the introduction of the sterile sample. We did not keep accurate records of the "lag time" for each culture, so we do not have quantitative data. Our impression, however, is that it takes ~ 4 weeks for the establishment of most cultures in Axenic Medium and ~ 2-3 weeks for the establishment of most cultures in Axenic Medium plus autoclaved *E. aerogenes*. In contrast, it takes only 5 to 10 days for the establishment of a thriving culture in Adaptation Medium. Once adapted to Adaptation Medium, all of the stocks grew well without a time delay.

We have applied our method successfully to the species of *Paramecium* listed in Tables 2 and 3 as well as to the other species of the *P. aurelia* complex, to one stock each of *P. caudatum* and *P. calkinsi*, and to 20 stocks of *Paramecium* unidentified as to species. There is some stock variation within a species in their ability to adapt to growth in axenic culture. Thus, one stock of *P. primaurelia*, 6 in *P. biaurelia*, 5 in *P. octaurelia*, and 2 from *P. multimicronucleatum*, syngen 4, have required 3 or 4 attempts with 4 stocks still unable to grow in axenic culture. We now have 22 known species of *Paramecium* growing axenically. All stocks and species grow excellently in Adaptation Medium or in Axenic Medium with autoclaved bacteria. Some species grow well when transferred to Axenic Medium (*P. tetraurelia*, *P. pentaurelia*, *P. sexaurelia*, *P. septaurelia*, *P. octaurelia*, *P. quadecaurelia*, *P. jenningsi*, *P. caudatum*, *P. calkinsi*). Other species have fair to good growth, with stock variation (*P. primaurelia*, *P. biaurelia*, *P. dodecaurelia*, *P. tredecaurelia*). Still other species contain stocks which fluctuate in growth (*P. triaurelia*, *P. novaurelia*, *P. decaurelia*, *P. undecaurelia* and all 5 syngens of *P. multimicronucleatum*). The fact that all stocks and species grow indefinitely in Adaptation Medium or in Axenic Medium plus autoclaved bacteria and that almost all grow in Axenic Medium, although not indefinitely, suggest that the autoclaved bacteria contribute additional necessary growth factors. This subject is being pursued further (unpublished data).

The development of the Adaptation Medium which provides all the essential elements for the growth of *Paramecium* under axenic conditions opened the way for an attempt to establish axenic cultures of *Didinium nasutum*, a predator of *Paramecium*. *Didinium* was washed free of bacteria using the above method and inoculated into axenic cultures of *P. primaurelia*, stock 540, or *P. tetraurelia*, stock 29, growing with autoclaved *E. aerogenes* in Adaptation Medium. Under these conditions, the predator was able to consume its prey and grow. Attempts to culture *Didinium* with *Paramecium* growing in Axenic Medium were not successful indicating that this medium is unsatisfactory for the survival of the predator. Adaptation Medium *is* satisfactory. This means then that once *Paramecium* is consumed, a sterile culture of *Didinium* free of other contaminating organisms can be obtained for biochemical studies.

Butzel & Horwitz (4) indicated that *Didinium* was capable of growth with *P. tetraurelia* or *P. octaurelia* in the medium described by Soldo & van Wagtendonk (7), which is defined except for the inclusion of a yeast extract preparation. Both the medium and 1 of the species of *Paramecium* were different from those we used in our experiments. An explanation of these conflicting results is currently being sought.

The simplicity of the equipment used permits the simultaneous handling of a number of stocks of different species of *Paramecium* and potentially of other ciliate protozoa. Under optimal conditions the yield of bacteria-free cultures (69.5%) of paramecia which adapt to axenic growth (62.7%) is 44%. With multiple tubes per stock the yield is much higher. Thus on a single attempt one can expect at least half of the stocks handled to produce axenic cultures within 5 to 10 days. All the species of *Paramecium* we have tested using our method can be adapted to axenic cultivation. This new method opens the way for various kinds of biochemical, genetic, evolutionary, and population studies which require large numbers of stocks.

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BOOK REVIEW . . .

Bulla, Lee A., Jr. & Cheng, Thomas C., gen. eds.; Vávra, Jiři & Sprague, Victor, contrib. eds. 1976. *Comparative Pathobiology*. Vol. 1. *Biology of the Microsporida*. Plenum Press, 227 W. 17th St., New York, NY 10011. xvi + 371 pp. $37.50.

This is the definitive book on the Microsporida. In 1899 Labbé reviewed the literature on the group, and listed 33 named species. In 1924 Kudo did the same, and recognized about 170 species. At the time this book was written there were about 525. The electron microscope, tissue culture technics, cytochemistry and immunology have added a great deal of information on the group, and the possibility of using them for biological control of disease vectors has increased their popularity manyfold. The contributing editors think that the Microsporida are the largest group of parasitic animals, even though at present there are about half as many named species in the whole (sub)phylum as in the single coccidian genus *Eimeria*.

The book includes chapters by Vávra on *Structure of the Microsporidia* and on *Development of the Microsporidia*; by Earl Weidner on *Some Aspects of Microsporidian Physiology*; by John Paul Kramer on *The Extra-Corporeal Ecology of Microsporidia*; by Elizabeth U. Canning on *Microsporidia in Vertebrates: Host-Parasite Relations at the Organismal Level;* by Jaroslav Weiser on *Microsporidia in Invertebrates: Host-Parasite Relations at the Organismal Level*; by Richard Weissenberg on *Microsporidian Interactions with Host Cells*; by Michael Petri on *Microsporidia and Mammalian Tumors*; by Y. Tanada on *Epizootiology and Microbial Control*; by Vávra & J. V. Maddox on *Methods in Microsporidiology*; by Burdette W. Erickson, Jr. on *The International Protozoan Type Slide Collection: Its Origin and Goals*; and a glossary by Vávra & Sprague.

Volume 2 will deal with systematics of the Microsporida. With such an all-star cast, these 2 books are indispensable for anyone who wishes to work on the Microsporida. Further, they make it relatively easy for someone who wants to do research on this challenging group to get started.—NORMAN D. LEVINE, *College of Veterinary Medicine, Univ. of Illinois, Urbana, IL 61801, USA.*