either alone, suggests that the INH may be split into toxic products which are separately conjugated with the respective chemicals either directly or by enzyme action.

The recent report of Ragno and others(12), suggests the explanation that the amino acid combines with ammonia liberated from the INH. These investigators have used glutamic acid as a detoxifying agent with INH in patients with tuberculous meningoencephalitis. With such combined therapy they have been able to use larger oral doses of INH without toxic disturbances, and have obtained higher concentrations of the drug in the spinal fluid.

Summary. Attempts have been made to find a means of increasing the amount of isoniazid (INH) which mice will tolerate. In the DBA strain of mice, INH alone was lethal for 45% of the animals when 5 mg/20 g mouse (250 mg/kg) were given orally. With an 8 mg dose of INH, lethal *per se,* simultaneous administration of as little as 25 mg glycine and 10 mg sodium glucuronate monohydrate was sufficient to keep alive all the animals tested. By the use of appropriate amounts of the 2 detoxifying chemicals, all mice survived either a single oral dose of 25 mg INH, or 3 doses of 8 mg daily, or 13 doses of 10 mg each given at 72-hour intervals. Larger amounts were not tolerated. A study of the blood plasma concentrations of INH showed that, whereas 24 hours after a 4-mg dose alone only a negligible amount remained, appreciably higher concentrations, approximately 6-fold, persisted for a longer period following 8 or 10 mg INH in combined treatment. In the proportions tested, sodium glucuronate and glycine had no effect on the bacteriostatic action of INH *in vitro* on one human strain of tubercle bacilli.

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Nitrogen Requirements of *Glaucoma scintillans* and *Colpidium campylum.** (21202)

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The ciliated protozoans, *Glaucoma scintillans* and *Colpidium campylum* were isolated and established in axenic (bacteria-free) culture in 1941(1). They were grown in a medium containing yeast cell fragments, and because growth failed when the particles were withheld it was concluded that they were phagotrophic. Later Peterson(2) was able to grow *Colpidium* in a non-particulate medium containing yeast protein fractions. We have found that *Glaucoma* also is dependent not on particles but on peptides and it is our analysis

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TABLE I. Stock Medium for *Glaucoma* (G) and *Colpidium* (C). Amounts in γ/ml of final medium.

	G	C
I. Amino acids		
DL-alanine	55	—
L-arginine HCl	43	—
L-aspartic acid	61	—
Glycine	5	—
L-glutamic acid	116	—
L-histidine HCl • H_2O	21	—
DL-isoleucine	63	—
L-leucine	97	—
L-lysine HCl	76	—
DL-methionine	34	—
L-phenylalanine	50	—
L-proline	87	—
DL-serine	77	—
DL-threonine	44	—
L-tryptophan	12	—
DL-valine	66	—
II. Protein		
Casein (Labco)	10000	10000
III. Carbon source		
Glucose	2500	2500
Tween 80	5000	10000
IV. Nucleic acid derivatives		
Guanylic acid	30	30
Adenylic acid	20	20
Cytidylic acid	25	25
Uracil	10	10
V. Vitamins		
Ca pantothenate	1.0	1.0
Nicotinamide	.1	.1
Pyridoxal HCl	1.0	1.0
Pyridoxamine HCl	1.0	1.0
Riboflavin	.1	.1
Thiamine HCl	1.0	1.0
Folic acid	.01	.01
Biotin (free acid)	.005	.005
Choline Cl	1.0	1.0
DL-6-thioctic acid	.004	.004
VI. Salts		
$MgSO_4 \cdot 7H_2O$	100.0	100.0
$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$	25.0	25.0
$MnCl_2 \cdot 4H_2O$	.5	.5
$ZnCl_2$	.05	.05
$CaCl_2 \cdot 2H_2O$	50.0	50.0
$CuCl_2 \cdot 2H_2O$	5.0	5.0
$FeCl_3 \cdot 6H_2O$	1.25	1.25
K_2HPO_4	500.0	400.0
KH_2PO_4	500.0	600.0

of the growth conditions for these 2 organisms which is to be reported here.

Materials and methods. Glaucoma scintillans A is the strain originally isolated and established in axenic culture in this laboratory (1). It was found that it could be adapted to a non-particulate medium (proteose-peptone, liver fraction L, glucose) by loop transfers, providing sufficient time was allowed for the initial incubation (10 days). That this was adaptation and not mutation and selection was shown by isolating single cells into a large number of separate tubes of the non-particulate medium, in which case all produced flourishing cultures after a lag of many days. After the cultures had reached high concentrations and small numbers were transferred to fresh non-particulate medium growth was rapid, reaching maximum yields in 96 hours. Early failure to achieve growth in non-particulate medium was due to insufficient time allowed for adaptation. Organisms once adapted to non-particulate medium can be subcultured indefinitely and these were used for the experimental work.

The original strain of *Colpidium campylum* (1) has not been maintained. The strain reported here was obtained from the culture collection of Cambridge University, England. It was freed of associated bacteria and yeasts in this laboratory by a combined treatment with antibiotics and sulfonamides and a subsequent migration through small bore U-shaped tubing. This strain will be designated *Colpidium campylum* C.

The technics described previously in studies on *Tetrahymena* (3,4) were used here. All experimental setups were in triplicate, incubation tubes were slanted for maximum aeration and incubation times were 4 days for *Glaucoma* and 7 days for *Colpidium*. The initial pH of the media was 6.8 for *Glaucoma* and 6.0 for *Colpidium*. As was previously shown (1) the optimum pH for *Colpidium* is 5.4, but when using casein in the medium this much acidity results in turbid media, useless for turbidimetric measurement of growth.

Results. Early trials showed that neither ciliate would grow in the defined medium of *Tetrahymena* (5). The addition of proteose-peptone or casein to this medium did not improve it. It was found, however, that growth was limited by the level of pantothenate in the *Tetrahymena* medium and when this level was raised 10-fold, and casein added, good growth resulted. The pantothenate requirement will be discussed in a later communication.

Even with the high level of pantothenate neither organism grew in the absence of high molecular weight nitrogenous compounds. In the case of *Glaucoma,* the addition of peptone or casein hydrolysate (enzymatic) to a modified *Tetrahymena* medium (Table I) resulted in good growth, but not up to the level of whole casein. Casein was subjected to controlled digestion with a number of proteolytic enzymes but even short term action by the enzymes always reduced and never increased the activity.

To test the possibility of an unknown factor being a contaminant of the casein, thereby resulting in its activity, prolonged Soxhlet extraction with 95% ethanol was carried out. The casein was found to produce better growth after extraction than before (Table II), indicating that the growth-promoting activity resides in the casein and not in contaminating materials.

Bovine serum albumin (Armour) was substituted for the casein. The protein solution was dispensed aseptically after other components of the medium were sterilized by autoclaving. This protein was utilized readily

TABLE II. Response of *Glaucoma scintillans* A to Various Proteins and Protein Digests, Tested in "Stock Medium" (Table I) Minus II (Protein) and Plus Aspartic Acid (2 mg/ml). Figures represent optical density ×1000.

Additions	Amount added (mg/ml)* 1	5	10
Labco casein	214	479	585
" " ethanol extracted	235	556	623
Bovine serum albumin (Armour)	258	553	546
Casein enzymatic digest (NBC)	73	328	472
Proteose-peptone (Difco)	134	395	553
Tryptone (Difco)	155	340	434
Gelatin (Eastman)	63	121	132

* No growth with zero addition.

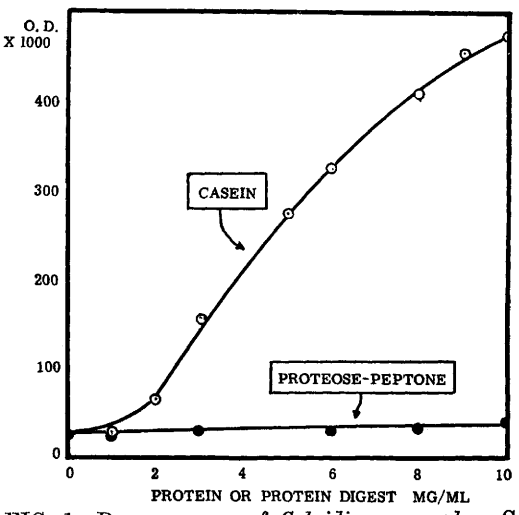

FIG. 1. Dose response of *Colpidium campylum* C to Labco casein and Difco proteose-peptone. The stock medium for *Colpidium* (Table I) minus protein was used at pH 6.0. Incubation time 7 days.

by *Glaucoma* (Table II) but not by *Colpidium.* Growth of *Colpidium* was obtained only when casein was present in the medium (Fig. 1).

Glaucoma can utilize free amino acids as evidenced by the fact that growth is increased by their addition (Fig. 2). The most active amino acid is aspartic acid. Asparagine is inert. With optimum casein (10 mg/ml) the addition of high levels of aspartic acid (2 mg/ml) increases the growth rate and raises the maximum yield. High levels of glutamic acid produce a similar effect, while the other amino acids (Table I) do not show marked stimulation individually but increase growth when added together (Fig. 2) even in the presence of high aspartic acid.

Free amino acids are inhibitory to *Colpidium.* The addition of single amino acids or combinations of amino acids to casein-containing media always reduces growth.

Discussion. The most striking difference between *Glaucoma* and *Colpidium,* on the one hand, and *Tetrahymena,* on the other, is the absolute requirement for polypeptides by the former two. *Tetrahymena* grows well on media based on crystalline amino acids (5). *Glaucoma,* while able to utilize free amino acids, is dependent on polypeptides. These do not seem to be specific, or if so they are not

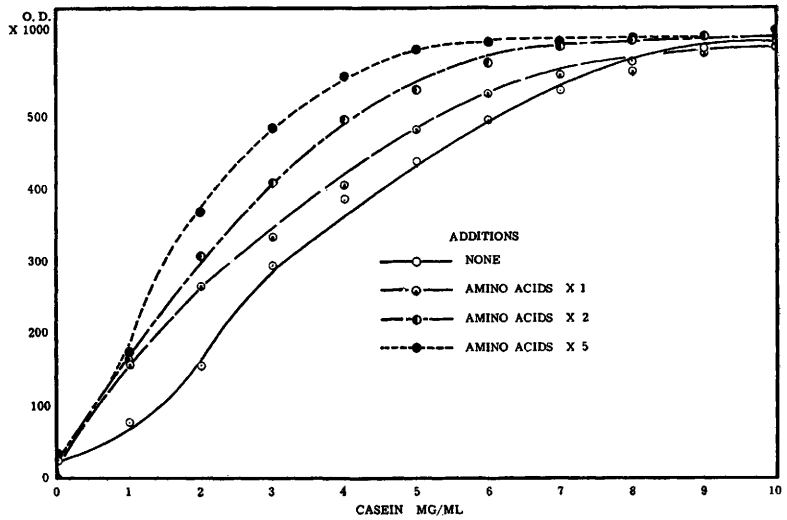

FIG. 2. Dose response of *Glaucoma scintillans* A to increments of Labco casein. The medium is the stock medium (Table I) amino acids (I) minus protein (II) and plus aspartic acid (2 mg/ml). Amino acids × = multiples of amino acids (I) in stock medium. Incubation time 96 hr.

released and made more available by various enzymatic reactions *in vitro*. Casein, treated to concentrate strepogenin(6,7) was used. In all trials the fractions containing strepogenin exhibited very low activity compared to the non-strepogenin containing fractions. The molecular size of the protein molecule appears to be the limiting factor in the growth of *Colpidium*. While the large casein molecule is adequate for this organism the much smaller albumin molecule is inert.

It seems possible that the basis for the above results lies in differences in reactions of the organisms regarding food-taking. While all 3 ciliates (*Tetrahymena, Glaucoma, Colpidium*) are bacteria feeders in nature, where the food organism evokes a swallowing response (food vacuole formation), only *Tetrahymena* has retained the ability to respond with vacuole formation to relatively small molecules in solution (drinking response). The swallowing response of *Glaucoma* is evoked by polypeptides and small proteins, while only large proteins evoke the response in *Colpidium* (Fig. 1). There are many ciliates in nature which appear to be much more exacting in their requirements for stimulation of the feeding mechanism than is *Colpidium* (*e.g. Bresslaua*(8), *Perispira*(9), etc.)), where only certain types of organisms will be accepted as food.

When polypeptide and whole protein are required for growth it is extremely difficult to determine the synthetic capacities of organisms regarding specific amino acids. While we know nothing of these specific capacities in the case of *Colpidium* it has been possible to show with *Glaucoma*(10) that, under certain conditions and with very low protein additions (producing low but transplantable growth), the omission of any one of the following amino acids results in immediate growth failure: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, serine, and proline. These, with the exception of serine and proline, are the "essential" amino acids for *Tetrahymena* (11,12). The failure in an animal to synthesize proline seems very unusual, but under certain conditions even *Tetrahymena* fails to synthesize proline and also serine(13).

The growth factor requirements, aside from that for high levels of pantothenic acid, mentioned above, are similar to those of *Tetrahymena*(3). Some interesting differences, however, will be discussed later.

This investigation is important for practical purposes in isolating and establishing new or-

ganisms in axenic culture. Attention must be directed to both the kind and amount of biologically active molecules present, and especially to the complexity of the nitrogenous molecules. In these 2 cases, for instance, the amount of pantothenic acid in the *Tetrahymena* medium(5) plus that found in peptones is far below the amount required for growth. Yeast extract supplies the required pantothenic acid level but not the required polypeptide or protein level. For initial establishment in axenic culture, therefore, it is not always correct to assume that crude peptones or extracts of natural origin will supply adequate amounts of the compounds usually regarded as being essential.

The requirement for a polypeptide or protein is not unknown among organisms. Sprince and Kupferberg(14) have found that the flagellate, *Trichomonas vaginalis* can be grown on a pancreatic digest of casein to which are added the B-vitamins, purines, a pyrimidine, acetate, ribose, asparagine, linoleic acid, and serum albumin. While the essential nature of all the ingredients could not be determined, growth was dependent on the protein supplement.

Similarly, Dougherty *et al.*(15) have found that rhabditoid nematodes (*Rhabditis*), when grown in axenic culture, require a heat labile protein found in liver and chick embryo juice.

Recently Williams and Grady(16) have reported that a specific protein (lactein) is an absolute requirement for the growth of a strain of the bacterium, *Lactobacillus bulgaricus*. This requirement appears to be for a specific configuration, as in the case of strepogenin(6,7) rather than for a certain molecular size.

Summary. The nitrogenous requirements of *Glaucoma scintillans* and *Colpidium campylum* in axenic culture have been investigated. Both ciliates require approximately 20 times as much pantothenic acid as does *Tetrahymena pyriformis* W. *Colpidium* appears to require whole protein molecules of the molecular weight range of casein. Digests of casein do not support growth, nor does bovine serum albumin. Free amino acids do not appear to be utilized and are somewhat inhibitory. *Glaucoma* will utilize free amino acids but will not grow when they are the sole nitrogen source. It appears to require peptides (as in enzymatic digests of casein or in peptones) but best growth occurs when whole protein molecules are present (casein or bovine albumin). The requirements of neither ciliate appear to be for a specific configuration (certainly not strepogenin) but rather for molecules of a certain minimum size. This may reflect the necessity for stimulation of the cell for the swallowing reaction.

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