strain of poliomyelitis virus(22,23), or the virus of western equine encephalomyelitis(23). Ainslie *et al.*(24) reported that large doses of ACTH in monkeys seemed to make them more susceptible to infection with the Minnesota strain of poliomyelitis. Cortisone has been shown to markedly enhance poliomyelitis infection in mice and hamsters(22). It increased the susceptibility of old mice to Coxsackie virus(25), of mice to influenza virus (26), and to West Nile, Ilheus and Bunyamwera viruses(27), and of guinea pigs to vaccinia virus(28). Moreover, cortisone treatment of embryonated eggs has resulted in significantly greater concentrations of both influenza and mumps viruses in the allantoic fluid(29).

Our findings that ACTH, in the doses employed, produced little effect on PVM in mice whereas cortisone treatment resulted in rather striking increase in the severity of the infection are in general agreement with the findings reported in other virus infections. It is quite possible that the ACTH employed may have lost potency through storage and that larger dosage or more frequent treatment might have resulted in effects similar to those of cortisone. The mechanism through which this effect takes place is obscure but the evidence presented in this paper and elsewhere(1) suggests that the enhancing effect of urethane on PVM infection may work through a similar mechanism.

Summary. The treatment of mice with ACTH did not influence their susceptibility to PVM infection but slightly enhanced virus multiplication. Cortisone treatment enhanced both the virus infection and the multiplication of virus to a greater degree. Adrenalectomy rendered mice slightly less susceptible to infection and retarded virus growth.

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Growth Studies on *Polytomella agilis*. (19122)

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The nutrition and metabolism of phytomonads and related species have been studied by several investigators. Doyle(1) has reviewed the problem of physiological classification of protozoa on the basis of their known nutrition. That ethanol and acetate are utilized by various species has been known for many years(2,3). A number of green and colorless flagellates such as Euglena, Astasia, Polytoma, Polytomella, Chlorogonium, and Hyalogonium have been found to be unable to utilize sugars and hence are dependent on simple acids and alcohols. Lwoff, Ionesco, and Gutmann(4) showed that *Polytomella caeca* lacks the hexokinase required for utilization of exogenous sugars. Albaum *et al.*(5) investigated this problem in Euglena.

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Experimental. This report deals with the growth responses of an organism which we have isolated and identified as *Polytomella agilis*. This organism was freed from bacteria by cultivation in media containing 50 μg per ml of aureomycin, penicillin, and sulfadiazine. It was first grown in a complex, defined medium supplemented with any one of a number of concentrated fruit extracts, the active principle of which proved to be ethanol. Modified from a medium developed for *T. cruzi*(6) this medium was far more complex than necessary. The organic constituents were as previously reported(6). It was found that substances such as glucose, levulose, and hexose diphosphate are not utilized by *P. agilis*. Substances such as glutamine, adenine, creatine, guanosine, nucleic acid, uracil, and uric acid were not essential but were found to be readily utilized as nitrogen sources. Urea was inactive. While the various constituents of the medium were not rigorously purified to eliminate trace elements, it was possible to demonstrate a requirement for magnesium and calcium. Cultures maintained by weekly transfer in this basal medium supplemented with 0.2% ethanol were used to inoculate sets of 5 tubes containing 10 ml volumes of the medium supplemented with various substances tested for ability to replace ethanol. The inoculum was 0.1 ml and the densities, measured turbidimetrically, reached their peak in 4 to 5 days. Cultures having density of "30" were found able to initiate growth when diluted 1-100,000.

Substances utilized for carbon-nutrition. Seven substances stimulated growth of this organism: Ethanol, butanol, sodium acetate, ethyl acetate, *n*-butyl acetate, sodium propionate, and sodium butyrate. The optimum range of activity of these substances was 0.1-0.2%. Ethanol, sodium acetate, and butanol were tested at several levels. The minimum requirement for ethanol was found to be around 0.03%, and the minimum requirement for sodium acetate and butanol appeared to be nearer 0.04%. It seems interesting to note that cultures maintained on 0.03-0.04% of ethanol remained at peak density for only 2 or 3 days whereas cultures maintained on 0.06 through 0.2% were active for successively longer periods up to 2 weeks. Ethanol was the only substance well tolerated at the 0.4% level. The organism tolerated concentrations of ethanol up to 2%. The utilization of propionate by our organism is of interest in view of the problem presented by odd-numbered carbon acids. Huennekens *et al.*(7) have investigated the role of the liver cyclophorase system in the complete oxidation of propionate.

Substances found inactive. Thirty-two substances were found unable to replace ethanol in our tests. They are: Formic acid, methanol, acetaldehyde, sodium oxalate, acetone, allyl alcohol, glycerol, isopropyl alcohol, lactic acid, malonic acid, methyl cellosolve, propylene glycol, sodium citrate, cellosolve, malic acid, succinic acid, amyl alcohol, diethyl ketone, mannitol, methyl amyl alcohol, pentanol-3, *n*-propyl acetate, glucose, butyl cellosolve, dulcitol, diacetone alcohol, inositol, methyl isobutyl ketone, sorbitol, triethanolamine, ethyl butyl ketone, heptanol-3. The inactivity of acetaldehyde is interesting in view of the studies of Stadtman and Barker (reviewed by Krampitz)(8) on the conversion of ethanol and acetate through acetaldehyde to fatty acids by *Clostridium kluyveri*.

TABLE I. Composition of Simplified Basal Medium.

	Amt per liter
Na_2HPO_4	.5 g
KH_2PO_4	.5
$MgSO_4 \cdot 7H_2O$	3.75 mg
$MgCl_2 \cdot 6H_2O$	20
$CaCl_2 \cdot 2H_2O$	18.75
$FeCl_3 \cdot 6H_2O$	.3
$MnCl_2 \cdot 4H_2O$	.01
$ZnCl_2$	.01
Glutamine	100
Thiamine	1
Vit. B_{12}	6 μg
Final pH: 6.8	

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TABLE II. Growth Responses of *Polytomella agilis.*

Medium	Carbon source, .2%	Avg photodensity readings (5 tubes) 1 day	3 day	6 day	8 day	10 day	12 day	Final pH
Simplified basal (Table I)	Ethanol	6	43	63	48	45	37	5.96
	Acetate	5	18	43	56	49	42	7.91
	Butyrate	5	29	63	63	60	52	7.81
Same minus B_{12}	Ethanol	7	24	37	37	37	28	6.37
	Acetate	5	14	24	29	32	32	8.48
	Butyrate	6	16	31	40	40	40	7.73
" " thiamine	Ethanol	7	33	43	41	43	40	3.31
	Acetate	4	34	48	49	43	37	8.23
	Butyrate	5	30	38	46	50	50	7.24
Same minus both B_{12} and thiamine	Ethanol	5	18	28	29	31	31	5.00
	Acetate	6	18	30	33	32	34	8.40
	Butyrate	7	16	31	40	41	40	7.82
Complex basal (51 organic constituents)	Ethanol	3	25	36	37	38	35	6.21
	Acetate	3	12	24	31	31	33	8.16
	Butyrate	2	20	42	46	45	43	7.91
	None	6	8	8	8	8	8	6.90

Albaum *et al.*(5) observed that malic and succinic acid are utilized by Euglena.

Experiments with a simplified basal medium. The medium described in Table I was developed as the result of a study of the effect of eliminating various constituents from the more complex medium. It will be noted that glutamine provides the only source of nitrogen; 100 mg per liter supported optimum growth. Under the conditions of our tests, neither glutamic acid, aspartic acid, nor glycine gave as good growth as glutamine or asparagine.

In contrast to our medium, Lwoff *et al.*(4) employed a medium for *Polytomella caeca* consisting of 1 g per liter of ammonium sulfate, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.5 g KH_2PO_4, 1 g sodium acetate, 3 ml ethanol, 0.01 mg thiamine, and 10 mg ferric citrate. They observed a requirement for thiamine but not for vit. B_{12} which is important in the nutrition of Euglena(9). Our organism grew poorly in this medium even when supplemented with vit B_{12}.

As shown in Table II, omission of thiamine and vit. B_{12} from our basal medium caused retardation of growth of our organism in the presence of ethanol, acetate or butyrate. The effect was more marked with vit. B_{12} than with thiamine. However, serial subcultures were not made to determine the absolute requirement of these vitamins.

Of particular interest are the final pH values obtained in each instance. Lwoff *et al.* (4) observed that growth of *Polytomella caeca* in the presence of ethanol leads to a marked acidification of the medium due presumably to the uncompensated liberation of $SO_4^=$ from ammonium sulfate, and that growth in the presence of sodium acetate leads to a marked alkalinization of the medium which they attributed to the liberation of sodium ions. In Lwoff's experiments this problem was met by using a mixture of ethanol and acetate.

The effect of thiamine and vit. B_{12} on acid production on the ethanol medium is shown by the pH readings in Table II. There is a slight drop in pH when both thiamine and vit. B_{12} are omitted. The omission of only thiamine results in a marked pH drop. This indicates that vit. B_{12} stimulates the production of acidic metabolites, which are not metabolized in the absence of thiamine. In the presence of acetate or butyrate, an alkaline pH is obtained which masks the effect of the two vitamins.

Polytomella agilis has been maintained 4 months by weekly subcultures on the simplified basal with 0.2% ethanol. *Euglena gracilis, E. gracilis var. bacillaris,* and *Parapolytoma satura* have also been successfully prop-

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agated in this medium for varying lengths of time.

Summary. The nutritional requirements of a strain of *Polytomella agilis* were investigated. Ethanol, butanol, acetate, propionate or butyrate were utilized as carbon sources. Glutamine was adequate as a sole source of nitrogen. Thiamine and vit. B_{12} were stimulatory. A simplified medium, adequate for continuous subculture of this organism, is presented.

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Effects of Adrenalectomy on Blood Cells of Mice with Special Reference to Eosinophiles.* (19123)

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Numerous references to the effects of adrenalectomy on the numbers of blood cells occur in the literature. The various investigators tend to agree that total removal of both adrenals results in an anemia which is manifested by decreases in total red cell count and in Hb values, provided hemoconcentration of the blood is prevented by supplying the salt factor(1-5). The situation is less clear with regard to the leucocytes. The results reported range from a decrease through little or no change to a significant increase, in total white count, in lymphocytes, and/or in neutrophils(1-13). Either there has been no consideration of eosinophilic leucocytes apart from neutrophilic or the statement has been made that the former seem unchanged(2,3,10).

In the course of a series of experiments with adrenalectomized animals some data were accumulated with regard to the white blood cell picture before and at various intervals after the operation(14) and later additional counts were made. Special attention has been given to variations in the numbers of eosinophils and bone marrow has been examined to determine the effects of adrenal insufficiency upon the myeloid-erythroid ratio and upon mitotic division.

Material and methods. All counts included in this report were made on 56 young male adult mice, hybrids of mixed ancestry, raised and maintained in wire cages in a warm animal room (72-78°F) and fed Purina dog chow supplemented with occasional milk and lettuce. Following adrenalectomy they were kept in wooden boxes and given 0.9% NaCl in tap water for drinking. Bilateral adrenalectomy was performed under light ether anesthesia following an intraperitoneal injection of 0.1 cc of 1 part Nembutal to 20 parts 0.9% NaCl solution. A subcutaneous injection of 5 cc of salt solution was given postoperatively.

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