The optimum conditions for conversion of the perrhenate to solid rhenide are as follows:

1) Composition of solvent, 9.8 g. of water/100 ml ethylenediamine-water solution.
2) Initial concentration of potassium perrhenate, 3 mM/l.
3) Potassium requirement, 4 g/100 ml solution.
4) Initial temperature, room temperature. (The solution warms up to about 60° C in the course of the reaction.)

Under the conditions described above, a conversion of approximately 55% of the initial perrhenate to solid rhenide is obtained. The filtrate contains unreduced perrhenate but practically no rhenide.

An interesting reaction of the rhenide ion has been observed. Treatment of an aqueous solution of the solid mixture of potassium hydroxide and rhenide compound with thallous nitrate solution yields first a white precipitate, presumably thallous rhenide, which rapidly undergoes reaction to yield finely divided thallium metal and the perrhenate ion.

Work is in progress with view of separating the rhenide compound from the potassium hydroxide impurity in order to determine the composition and structure of the former. A full account of our studies will be reported at a later date.

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Comparative Biological Activity of α-Lipoic Acid and Protogen in the Growth of *Tetrahymena*

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Preliminary experiments in this laboratory indicate that α-lipoic acid can replace protogen completely in the growth of *Tetrahymena*. Snell *et al.* in 1937 (*1*) first demonstrated that acetate was stimulatory in the growth of lactic acid bacteria, and Guirard in 1946 (*2*) reported the occurrence of an acetate-replacing factor(s) in some biological materials. Snell and Broquist in 1949 (*3*) indicated that protogen, the pyruvate oxidation factor, and the acetate-replacing factor for *L. caseii* were either identical or closely related substances. Stokstad *et al.* (*4*) stated that more than one form of this material occurs naturally. Getzendaner (*5*) and Reed (*6*) have concentrated this factor, and Reed *et al.* (*7*) this year reported the preparation of a crystalline compound from liver which was able to replace acetate in the growth of certain lactic acid bacteria and which was required for the oxidative decarboxylation of pyruvate by these organisms. They have named this compound α-lipoic acid and state that its melting range is 47.5°–48.5° C. Kidder's group in 1950 (*8*) stated that one of the functions of protogen in *Tetrahymena* is concerned with the production of acetate.

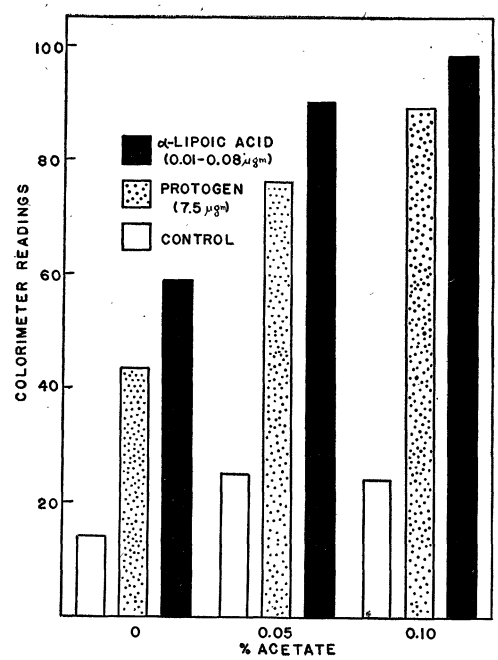

FIG. 1. Comparative effects of protogen and α-lipoic acid on maximum growth of *Tetrahymena*.

Protogen[2] and α-lipoic acid[3] have been compared for their growth effects and also for their ability to spare and replace acetate in *Tetrahymena geleii* (strain E). The protogen crude concentrate used had a value of 7.5 γ/unit, the concentrations of which ranged from 0.125 to 1.0 unit/ml. The α-lipoic acid was tested in concentrations of 0.01–0.08 γ/ml. Other concentrations of this material were not used because of limited availability.

The general methods employed were those of Slater (*9*). The temperature was 25° C, and the initial pH was set at 7.4. Initial inoculation was of the order of 1000 animals/tube. Tests were made in duplicate, and the entire series was repeated. The medium used was that recommended by Elliott (*10, 11*), with the exception that choline and biotin were omitted. Guanylic acid, adenylic acid, cytidylic acid, and uracil were used in 25 γ/ml quantities instead of yeast nucleic acid as recently advised by Elliott (*12*). Growth was measured turbidimetrically, and counts were made of final peak populations.

Purified α-lipoic acid was able to replace protogen

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[3] The author is indebted to Lester J. Reed, of the University of Texas, for the α-lipoic acid.

TABLE 1

COMPARATIVE ACTIVITY OF α-LIPOIC ACID AND PROTOGEN IN THE GROWTH OF *Tetrahymena geleii* E.

Acetate (%)	Protogen (unit/ml)	Count at 120 hr	Final pH
	1	83,200 ± 12,640*	6.9
	0.5		3.9
0	.25		3.8
	.125		3.5
	0.000		3.4
	1	83,200 ± 5,880	7.3
	0.5		7.2
0.05	.25		6.3, 4.5†
	.125		4.1‡
	0.000		5.3
	1	97,200 ± 14,520	7.5
	0.5		7.5
0.10	.25		7.2
	.125	47,440 ± 1,988	6.2
	0.000		6.0
	α-Lipoic (γ/ml)		
	0.08	95,000 ± 13,520	7.1
	.04		7.4
0	.02		7.4
	0.01	114,800 ± 16,840	7.2
	0.08	111,600 ± 11,880	7.5
	.04		7.5
0.05	.02		7.5
	0.01	131,200 ± 11,200	7.4
	0.08	114,000 ± 8,690	7.6
	.04		7.7
0.10	.02		7.5
	0.01	127,200 ± 10,320	7.6

* This count represents both live and dead animals, since many of the cultures had begun to die here after 72 hr.

† Approximately one half of the cultures had precipitated after 96 hr. Tubes containing the precipitate exhibited the lower pH.

‡ All these cultures had precipitated after 96 hr. Upon microscopic examination, the precipitate was found to consist only of dead cells.

completely in the growth of *Tetrahymena* (Fig. 1). In concentrations ranging from 0.01 to 0.08 γ/ml, α-lipoic acid is more active than protogen in acetate-replacing properties. As noted by Kidder *et al.* (*8*), protogen is active in sparing acetate. Low levels of protogen (0.125–0.5 unit/ml) resulted in considerable acid production in media containing 0–0.05% acetate. Lower population densities were also obtained (Table 1). Concentrations of protogen at 10 units/ml were found by the author (*9*) to be slightly inhibitory in media with 0.1% acetate. Maximal growth (approx 120,000 animals/ml) was obtained by the use of α-lipoic acid in media containing 0.05–0.1% acetate. Considerably lower population densities (approx 80,000 animals/ml) resulted from the use of protogen at the 0.05% acetate level.[4]

[4] Since this paper has gone to press, it has been found that half-maximal growth can be obtained by means of 0.0005 μ of α-lipoic acid.

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The Effects of Cortisone and Heparin on Eosinophils in Defibrinated Human Blood [1, 2]

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Four hours after injection of corticotropin (ACTH) into healthy individuals the blood eosinophil count is found to be significantly reduced from pre-injection levels. This adrenocortical eosinopenia also develops in man after treatment with 17-hydroxycorticosterone (Compound F), corticosterone (Compound B), and cortisone, but the latter steroid does not have as striking an eosinopenic effect as the other hormones. During the past 3 years we have tried to reproduce adrenocortical eosinopenia *in vitro* by incubation of human blood with cortisone. Baldridge *et al.* (*1*) and other investigators have also tried to do this, but without success. Both Baldridge and ourselves used heparin as an anticoagulant for blood samples.

Recently we incubated, under sterile conditions, defibrinated blood from 15 patients ill with various diseases associated with eosinophilia. They included patients ill with asthma, polyarteritis nodosa, and other diseases. Incubation of their blood for 4 hr at 37.5° C, alone, with saline, or with nonsteroidal microcrystals (e.g., sulfonamides), had only slight effects on the level of eosinophils in the sample. On the other hand, when a commercial microcrystalline suspension of cortisone acetate or pure crystalline cortisone acetate (1 mg/3 ml blood) was mixed with the defibrinated blood, the absolute eosinophil counts were significantly reduced after 4 hr incubation. This effect of cortisone on the eosinophil count could be blocked by adding heparin (40 u) to the defibrinated blood before introducing the cortisone. The data from our experiments are shown in Table 1. In some experiments with Compound F we have found that it can also produce significant eosinopenia when incubated with defibrinated blood.

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[2] We wish to thank Elmer Alpert, of Merck & Co., Inc., Rahway, N. J., for supplying the pure cortisone acetate, and Curtiss E. Meyer, of the Upjohn Company, Kalamazoo, Mich., for supplying the Compound F used in this study.