essary to have as keen an edge as is required for paraffin sectioning. The photomicrograph (Fig. 2)

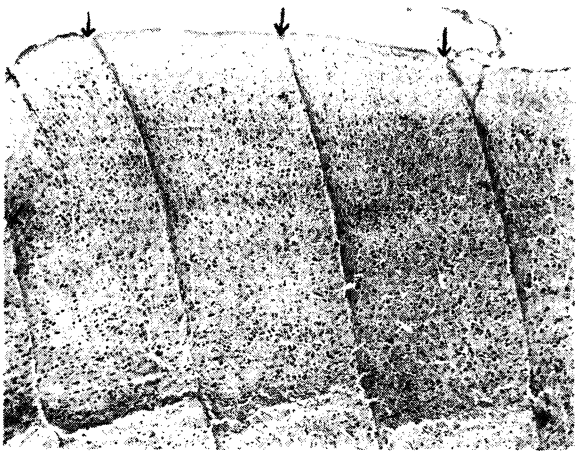

FIG. 2. Formalin-fixed block of sliced brain tissue, cut (7 μ) at right angles to slices (400 μ). Stained with hemotoxylin and eosin. Margins of slices are indicated by arrows.

shows a formalin-fixed preparation stained with hemotoxylin and eosin and cut at approximately right angles to the slices to show the edges of the sliced tissue. A study[3] of these sections showed that tearing and distortion of the tissue were minimal. The cut surfaces appear to be more a separation of the components of the brain on a plane than a sharp cut. The integrity of the cell structures along the line separating the slices appears to be preserved.

Preliminary preparations for embedding the brain consist in melting agar in a water bath, then transferring it to a constant temperature bath held at 39° C. For convenience in handling, a number of test tubes of agar may be prepared beforehand and kept stoppered until used. A large beaker of crushed ice for precooling a small, thin glass vial (2 × 8 cm) to receive the excised brain and the syringe and plunger is also prepared. A watch glass containing about 5 ml of the same solution to be used in the respirator flask is used to separate the slices transferred from the microtome in preparation for weighing and transferring the slices to the flasks. This is supported by a small beaker or shallow dish containing ice.

The actual procedure begins with the removal and transfer of the brain to the precooled vial in the ice bath, where it is left for about 1 min. The brain is then teased into the well formed by dropping the plunger about 2 cm from the top edge of the syringe barrel. This space is filled to the brim with the agar, and the brain oriented to the desired position, which will be maintained if the syringe is precooled and the agar held at 39°–40° C. The syringe, with a glass vial slipped over the tip to hold the agar in place, is then returned to the ice bath for about 30 sec, mounted on the microtome and the embedded brain cut by rotating the knife. It is important that the sections of cut tissue are not removed until the desired number of slices is obtained or the whole brain sectioned. This stacking of cut tissue helps keep the surrounding agar firmly around the tissues and prevents the slices from being peeled off and torn, as happens when the slices are removed one at a time as cut. Sagittal sections of a whole mouse brain may be cut in less than 10 sec. The entire stack is transferred to the precooled watch glass containing the tissue media. The slices are separated from each other and from the encircling rings of agar by gentle agitation of the solution and some teasing. The selected slices are then weighed and placed in the flasks in the usual manner.

The technique described here offers the advantage of rapidly preparing consecutive brain slices of constant thickness for tissue respiration studies. Further work to adapt this procedure to other tissues is now in progress and will be reported later.

[3] The authors are indebted to Fae Tichy, of the Neuropathology Department of this university, for her valuable assistance in evaluating this important phase of the technique.

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## Production of a Solid Rhenide

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The existence of uninegative rhenium in aqueous solution has been definitely established (*1–3*). Up to the present time the only method that has been successfully employed for the production of rhenide has involved the reduction of perrhenate in acid solution either by means of a Jones reductor or cathodically. A maximum concentration of approximately 0.001 *M* perrhenate has been quantitatively reduced to uninegative rhenium in acid solution (*4, 5*), and a rhenide concentration of 0.0026 *M* has been obtained in an acid solution containing pyridinium chloride (*5*).

We have successfully produced for the first time a solid rhenide material in admixture with potassium hydroxide by reduction of potassium perrhenate in ethylenediamine-water solutions by means of potassium metal. The reaction is carried out in a closed system under nitrogen atmosphere. The valence number of rhenium in the white solid was established in the following way: An aqueous solution of the solid was prepared, and separate aliquot parts were analyzed for rhenium content and for reducing power in terms of number of equivalents of standard oxidizing agent (potassium dichromate) required to oxidize the rhenium to the perrhenate state. Typical values of the valence number of rhenium in the solid established in this manner were: −0.97, −1.06, −1.00, −0.95, −1.16, −0.81.

[1] The authors wish to acknowledge the support of the Office of Naval Research in this investigation.

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.

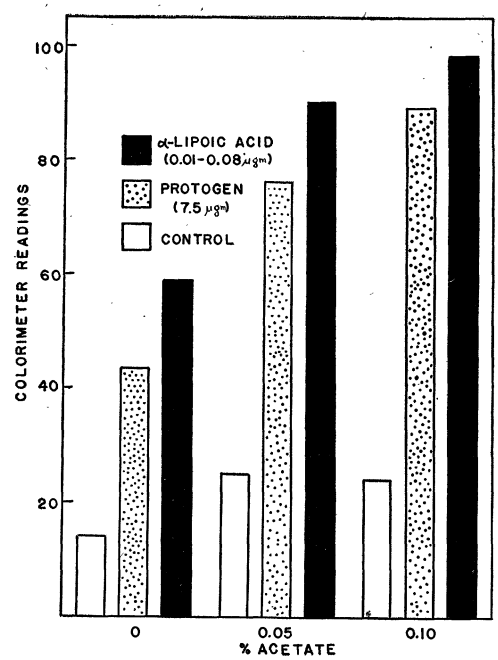


FIG. 1. Comparative effects of protogen and α-lipoic acid on maximum growth of *Tetrahymena*.

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

[1] Postdoctoral fellow in the biological sciences, United States Atomic Energy Commission.

[2] The aid of E. L. R. Stokstad, of the Lederle Laboratories Division, American Cyanamid Company, in supplying the protogen is gratefully acknowledged.

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