tested by two methods: (1) the rate of filtration in the toad's kidney and, (2) the rise of arterial pressure in the cat.

In both cases this destruction of hypertensin was markedly accelerated by oxidized cytochrome and sharply retarded by reducing agents such as ascorbic acid and cysteine.

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## *Pityrosporum ovale*—A Lipophilic Fungus. Thiamin and Oxaloacetic Acid as Growth Factors.*

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The cultivation of *Pityrosporum ovale* by the addition of fats to wort agar has been reported previously[1] and corroborated by Emmons.[2] Of a number of fats and fatty acids, oleic acid seemed the most favorable. In subsequent experiments[3] it was found that if oleic acid was supplied, this organism would sometimes grow feebly on an inorganic solution, similar to Currie's medium, containing $NH_4NO_3$, $KH_2PO_4$, $MgSO_4$ and dextrose. Substitution of asparagin as a nitrogen source caused increased growth. If thiamin was added, some growth was regularly obtained even with $NH_4Cl$ as a nitrogen source, and there was increased yield with asparagin. It seemed that further study might clarify the growth requirements of this fungus and throw some light on the function of thiamin in its metabolism.

As a basal media a modified Currie's solution was used. It contained $KH_2PO_4$, 1 g $MgSO_4$, 0.25 g per liter. Either asparagin 1.25 g or $NH_4Cl$ 2.5 g was added, and a parallel series of flasks containing in addition 50 g of dextrose was usually prepared. A solution of the soluble constituents was distributed in amounts of 50 cc in Erlenmeyer flasks and autoclaved. One-half cc of oleic acid was then added to each flask. Brom cresol green was used as an indicator. Solutions of the supplements to be tested were added by pipette in the following amounts: $B_1$ 5 gamma, ethyl oxalacetate 5 cc of a 5% solution, L-Keto-glutaric acid 2 cc of an M/10 solution. The pH of these media before the addition of oleic acid and the supplements varied from 4.4 to 4.8. The pH was not changed with $B_1$, but rose to 5 with the ethyl oxalacetate and the keto-glutaric acid. The growth did not seem to be influenced by the pH within the range tested.

Stock cultures were maintained on wort agar tubes to which oleic acid had been added, as previously described. The growth appears, first as tiny cream-colored colonies which later fuse, becoming dry and wrinkled with the color varying from cream to tan or orange. This growth was removed, ground with distilled water to make a heavy suspension. The suspension was then centrifuged, washed several times with distilled water and diluted for use. (0.1 cc of packed fungus cells to 4.9 cc of water). Two or 3 drops of this suspension were used as inoculum. Growth was permitted for 4-6 weeks at room temperature. At this time the growth was harvested and the dry weight obtained. This was done by centrifuging the mold specimen with an equal volume of 95% ethyl alcohol. The sediment was then washed with 50% alcohol and finally

* The writer wishes to acknowledge her indebtedness to Professor Edgar A. Miller for advice and guidance in conducting these experiments.

1 Benham, R. W., *J. Invest. Dermat.* 1939, **2**, 187.

2 Emmons, C. W., *Public Health Rep.*, 1940, **55**, 1306.

3 Benham, R. W., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 176.

TABLE I.
Showing Average Yields, Maximum and Minimum in Mg Dry Weight.

| Media* | + Dextrose | | | | 0 Dextrose | | | |
|---|---|---|---|---|---|---|---|---|
| | No. of measurements | Avg. S.E.† | Max. | Min. | No. of measurements | Avg. S.E. | Max. | Min. |
| Asparagin | 15 | 44±11.3 | 123 | 0 | 8 | 24± 8.4 | 54 | 0 |
| " + $B_1$ | 15 | 123±14.6 | 220 | 52 | 7 | 57± 8.8 | 107 | 22 |
| " + Oxaloacetic | 9 | 174±17.1 | 275 | 110 | 7 | 96± 9.6 | 127 | 50 |
| Asparagin + Oxaloacetic + $B_1$ | 4 | 114± 2.9 | 123 | 110 | 3 | 72± 6 | 85 | 60 |
| Ammonium Chloride | 12 | 6± 1.6 | 13 | 0 | 8 | 4± 1.5 | 10 | 0 |
| " " + $B_1$ | 8 | 20± 3 | 40 | 10 | 4 | 24± 6.2 | 42 | 10 |
| " " + Oxaloacetic | 7 | 145±20.6 | 240 | 80 | 4 | 108±30.4 | 200 | 45 |
| Ammonium Chloride + Oxaloacetic + $B_1$ | 3 | 171± 4.6 | 182 | 165 | 3 | 104±26.1 | 140 | 40 |

* The modified Currie's solution with the additions as listed.

† $S.E._{Avg.} = \sigma/\sqrt{N}$ when $\sigma$ is the standard deviation of the measurements composing the avearge and measures their variability, and N is the number of measurements.

with acetone. The solid material of the fungus was then dried in a desiccator over calcium chloride and weighed. The results are shown in the accompanying table.

It will be observed that growth in the ammonium chloride media is always less than that with asparagin. The addition of thiamin regularly permitted growth in any of the basal media. When added with asparagin the growth was accelerated and the yield greater than that which occurred in any control tube. When $NH_4Cl$ replaced asparagin, growth with thiamin appeared later and the yield was far less. The addition of dextrose regularly increased growth in the asparagin series, and with thiamin gave yields practically equal to the best obtained. When dextrose was added to the $NH_4Cl$ series, with or without thiamin, there was no increase as compared with parallel flasks without dextrose.

The addition of ethyl oxalacetate caused a striking acceleration of growth. With asparagin the yields were slightly higher than those with asparagin plus thiamin. With $NH_4Cl$ the yields averaged about as good as those with asparagin. The addition of dextrose increased the yield both with asparagin and $NH_4Cl$. The addition of both thiamin and oxalacetate gave us greater average yield than did oxalacetate alone. The one exception to this would appear to be in the series with $NH_4Cl$ plus dextrose where the addition of both thiamin and oxalacetate gave an average dry weight slightly higher than that with oxalacetate alone. This difference however has been shown to have no significance statistically.†

In two series (results not shown on the table) L. keto-glutaric acid replaced the ethyl oxalacetate with similar results, growth approaching maximum. Lithium pyruvate, on the other hand did not duplicate the effect of the four carbon acids, in fact no growth occurred in any of the flasks to which it was added.

Growth was somewhat irregular in the control tubes in the asparagin series. Usually the yield was small but occasionally approached that obtained by the addition of thiamin. This was thought to be due to some accidental factor and occurred less often when the inoculum was small and carefully washed. This suggested that traces of thiamin may sometimes be transferred with the inoculum. To exclude the possibility that thiamin was present in the asparagin, the material was re-

† When comparing 2 averages, the difference in the averages is compared with the S.E. of the difference where $S.E._{diff.} = \sqrt{S.E._{av.1}^2 + S.E._{av.2}^2}$. The ratio of difference/$S.E._{diff.}$ must attain a value at least 2.5 to be called clearly significant. Values between 2 and 2.5 are of doubtful significance, and values less than 2 are called non-significant.

crystallized and tested against *Phycomyces blakesleanus*,[4] and no evidence of thiamin found.

*Summary and Conclusions.* Thiamin when added to a basal medium containing $KH_2PO_4$, $MgSO_4$ and with asparagin or $NH_4Cl$ causes an increase in the growth of *Pityrosporum ovale*. With asparagin the increase is much greater than with $NH_4Cl$. However, if ethyl oxalacetate replaces the thiamin, growth in the $NH_4Cl$ medium is just about equal to that in the asparagin medium. The addition of thiamin to the medium containing oxalacetate does not cause any further increase in growth. It would seem then that $NH_4Cl$ and ethyl oxalacetate can be substituted for asparagin and thiamin in the cultivation of *Pityrosporum ovale*. It appears that if a suitable keto acid is supplied neither thiamin nor an amino acid is necessary. On the other hand, the asparagin may furnish both the carbon and nitrogen source needed by the organism, yielding ammonia and a carbon skeleton, probably oxalacetate. Thiamin increases growth when added to the asparagin by hastening the production of the carbon skeleton in some way. When this carbon skeleton is supplied as oxalacetate, thiamin is no longer necessary.

[4] Robbins, W. J., and Kavanogh, Frederick, *Proc. Nat. Acad. Sci.*, 1937, **23**, 499.

## 14896

## Protective Tests in the Developing Chick Embryo With *Hemophilus influenzae* Type b.

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The assay of the protective power of immune sera versus *H. influenzae* type b is of some practical importance. Serum protection of the white mouse against infection with *H. influenzae* by the mucin method has been demonstrated.[1] However, this method seems not to have given very satisfactory results in routine use, and standardization of therapeutic anti-*H. influenzae* type b serum has been made by the determination of antibody nitrogen.[2] The identity of the precipitating antibody with the mouse protective power in the serum has been ascertained.[3]

The experience with the assay of the protective value of immune sera against *E. typhosa* and *Shigella*[4, 5] suggested an investigation whether a similar test could be developed for anti-*H. influenzae* type B serum.

The developing chick embryo has been previously found to be susceptible to infection with *H. influenzae*,[5,6] and the 6- to 7-day-old chick embryo has been employed as a test animal for the demonstration of the protective effect of sulfathiazole and sulfadiazine.[7] No reports have been found in the literature on the protective effect of immune sera against experimental infection of the developing chick embryo with *H. influenzae*.

Young cultures (5 to 6 h 37°C) either in a digest broth similar to the medium described by Silverthorne and Cameron[8] (but without agar) or in Levinthal broth were used through-

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[2] Alexander, H. E., and Heidelberger, M., *J. Exp. Med.*, 1940, **71**, 1.

[3] Alexander, H. E., Heidelberger, M., and Leidy, G., *Yale J. Biol. and Med.*, 1944, **16**, 425.

[4] Weil, A. J., and Gall, L. S., *J. Immunol.*, 1941. **41**, 445.

[5] Weil, A. J., and McFarlane, J., *J. Immunol.*, 1944, **48**, 291.

[6] Gallavan, M., *Am. J. Path.*, 1937, **13**, 911.

[7] Altura-Werber, E., *J. Bact.*, 1943, **45**, 195.

[8] Silverthorne, N., and Cameron, C., *J. Pediat.*, 1942, **20**, 9.