white blood count, multiplying the result by 1000 to obtain the number per cubic centimeter, and then multiplying the result by the number of cubic centimeters of semen.

It has previously been observed that in the rat, atrophy of the testes is caused by prolonged injections of large doses of α-estradiol benzoate.[2] Also, in the brown leghorn cock, extreme atrophy of the testes and comb could be produced by prolonged administration of large doses of this material, but when the injections were discontinued, the comb growth took place and the testes increased in size.[3]

Summary. The subcutaneous injection of 6,000 to 10,000 r.u. of α-estradiol benzoate, 4 to 6 times weekly, for 26 weeks, produced an azoospermia in a 72-year-old man. The duration of treatment, the daily dosage, and the variation in the spermatozoa counts are presented in the graph.

11927 P

Cultural Characteristics of *Pityrosporum ovale*—A Lipophylic Fungus. Nutrient and Growth Requirements.

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In an earlier paper[1] the successful cultivation of *Pityrosporum ovale* was reported. The addition of fats to the basal medium, wort agar, was found necessary to obtain subcultures. Of the pure fatty acids tested oleic gave the best results. Oleic acid was used by Fleming[2] for cultivation of the acne bacillus and has since been used in a number of bacteriological media. Cohen and Mueller[3] have recently shown its importance for *C. diphtheriae*. However, the *Pityrosporum* is probably the first microörganism reported to need fats or fatty acids as an indispensable factor for growth. Consequently further study of its nutritional requirements seemed worthwhile.

A simple solution devised by Currie for the study of the citric

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2 Del Castillo, E. B., et Sammartino, R., *C. R. Soc. de Biol.*, 1938, **129**, 870.

3 Emmens, C. W., *J. Physiol.*, 1939, **95**, 379.

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

2 Fleming, A., *Lancet*, 1909, 1035.

3 Cohen, Sydney, and Mueller, J. Howard, PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 244.

acid fermentation of *Aspergillus niger* served as a basic medium with the exception that dextrose was substituted for sucrose. It had the following composition: NH_4NO_3 2-2.5 g, KH_2PO_4 0.75-1 g, $MgSO_4$ 0.2-0.25 g, dextrose 50 g, water 1000 cc. Fifty cubic centimeters were distributed in 150 cc Erlenmeyer flasks (or 100 in 250 cc flasks) and the substances to be tested added. A suspension of the fungus was washed in Currie's fluid or distilled water in the centrifuge and resuspended in fresh fluid. Two or 3 drops of a thick suspension was used as the inoculum.

In such media with various supplements the *Pityrosporum* grows both in granules which settle to the bottom of the flask and as a film or scum covering the surface and spreading up the sides of the flask above the level of the medium. When oleic acid is present the earliest growth appears as fine particles in or attached to the globules of acid which float on the surface. Inability to obtain even suspensions which could be measured in a colorimeter has prevented the making of quantitative records of growth. Dustlike particles scattered through the medium are recorded as "faint growth," a heavy suspension of particles with a thin film on the surface as "good growth," and larger granules in the depth with a thick membrane on the surface and adhering to the sides of the flask as "heavy growth".

In the Currie solution no definite development occurs if the inoculum is well washed. A faint growth of dustlike particles usually appeared after 2 or 3 weeks in earlier experiments when unwashed cells were used for seeding.

The addition of oleic acid .01% permits a slow faint growth. Increasing the oleic acid up to 1% favors growth, but beyond this point no increase was observed. In a 1% solution faint growth is obtained in 4 weeks.

The addition of 0.5% Asparagin does not permit growth in plain Currie broth but increases the growth in the presence of oleic acid. Asparagin plus 1% oleic acid gives good growth in 1-2 weeks and heavy growth in 2-4 weeks.

On account of the demonstrated relationship of another unsaturated fatty acid, linoleic, and pyridoxin in rat nutrition[4, 5] it seemed of special interest to test the action of this constituent of Vitamin B. Pyridoxin accelerates growth in the presence of oleic acid with or without asparagin. The effect seems more evident when the oleic acid is reduced to .01%, which retards growth in the controls.

[4] Buch, T. W., *J. Biol. Chem.*, 1938, **124**, 775.

[5] Gross, Paul, *J. Invest. Dermat.*, 1940, **3**, 505.

Thiamin has a more marked accelerating effect in similar combinations, but the effect is just as evident in the cultures containing 1% oleic acid as in those containing .01%.

With .01% oleic acid and 0.5% asparagin and thiamin, good growth is obtained in 5-10 days. At this time flasks without thiamin show only a trace, but after 4 weeks both show heavy and apparently equal growth. These effects were observed when as little as 1 gamma of the vitamin was added to 50 cc of medium. On the other hand, amounts of pyridoxin and thiamin up to 2.5 mg per 50 cc were without effect in the absence of oleic acid.

Conclusions. The *Pityrosporum ovale* grows in the presence of inorganic salts, glucose and oleic acid. The addition of asparagin accelerates growth and increases the final yield.

Thiamin and pyridoxin accelerate development but are not essential to the growth of this fungus under the conditions studied.

11928

Non-Availability of Gum Acacia as a Glycogenic Foodstuff in the Rat.

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No biochemical analyses have been made to test whether the gum acacia molecule passes entirely undigested through the gastrointestinal tract. Observations on poorly controlled feeding experiments, cited in the older literature,[1] are reported to have shown that gum acacia, without other dietary supplement, is a deficient foodstuff. Since gum acacia is a rather important physiological tool,[1] any information concerning its chemistry or physiological properties is of some interest.

The question arose whether all of the sugar components of the molecule[2] are tightly bound into a "main" chain molecule, or whether some of them are possibly attached as "side" chains to the main molecule. In the latter case, they might be vulnerable to the digestive enzymes, and thus become materials available for glycogenesis.

1 Amberson, W. R., *Biol. Rev.*, 1937, **12**, 48.

2 Butler, C. L., and Cretcher, L. H., *J. Am. Chem. Soc.*, 1929, **51**, 1519.