TABLE 1
COMPARATIVE ENRICHMENTS FOR *Azotobacter agilis* WITH WINOGRADSKY'S AND MODIFIED MEDIUMS

No.	Sources of the water	Original W. medium	Modified medium	Observations
1	Lake Mendota (Madison, Wisconsin) sample No. 1	–	+	.....
2	Lake Mendota (Madison, Wisconsin) sample No. 2	+	+	First growth in modif. medium
3	Lake Mendota (Madison, Wisconsin) sample No. 3	–	+	.....
4	Sewage effluent (Madison, Wisconsin)	+	+	First growth in modif. medium
5	Lower Crystal Spring Reservoir (San Francisco, Cal.)	–	+	.....
6	San Andres Reservoir (San Francisco, Cal.)	+	+	.....

Total number of samples examined for the presence of *Az. agilis:* 22.

faint gold or purple pigment in liquid mediums depending on the nature of the carbon source. In contrast to these, the strains isolated from sewage produced a definitely greenish pigment and grew better in agar mediums. Pigment production in liquid mediums was stronger than in the other group of cultures. The strains of both groups are very motile, and in general the morphological and physiological characters agree with those of the descriptions by the previous investigators. The size of the cells is 2.4–2.8 × 2.5–4.5 μ (taken from pictures originally magnified 200 ×). The cultures used for measurement were grown on Winogradsky's medium with 1 per cent. agar and 0.5 per cent. ethyl alcohol, and a small amount of calcium carbonate. No cultures grew with the use of mannite as a source of carbon, either in liquid or in solid mediums.

For comparison a culture of *Az. Vinelandii* from the Department of Agricultural Bacteriology of the University of Wisconsin was included in this study. Morphologically it differs from *Az. agilis* strains by having elongated cells (1.4–1.6 × 2.5–3.5 μ), usually in pairs, and it is less actively motile. In contrast to the *Az. agilis* the strain of *Az. Vinelandii* grew readily on both solid and liquid mediums with mannite as a source of carbon, producing a greenish fluorescent pigment.

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LIVER EXTRACT AS A SUBSTITUTE FOR SERUM IN THE CULTURE MEDIUM FOR ENDAMOEBA HISTOLYTICA[1]

THE first practical method for the cultivation of *Endamoeba histolytica* was published by Boeck and Drbohlav[2] in 1925. The medium consisted of a solid egg slant overlaid with a liquid composed of eight parts sterile Locke's solution and one part of sterile human blood serum. The following year Dobell and Laidlaw[3] used horse serum instead of human serum in the liquid portion of the medium and demonstrated that the addition of sterile rice starch produced more abundant growth of the amoebae and prolonged the life of the cultures, thus requiring less frequent transplants. This medium is used extensively at the present time in the cultivation of *E. histolytica.* Although many suggestions for the improvement of this medium have been made, they have consisted of changes only in the solid portion of the medium. The liquid portion of the medium has consisted in all cases of dilutions of human or animal serum or egg albumen, the most widely used being horse serum-Ringer (1–6). Numerous substitutes have been tested, notably by Cleveland and Collier,[4] but without success.

During the past six months we have been using a 0.5 per cent. solution of liver extract in an 0.85 per cent. solution of sodium chloride as a substitute for horse serum-Ringer. The results obtained have been fully as good as with the serum medium, and the advantages of liver extract in both experimental and diagnostic work are many.

The liver extract which we have used most extensively is Lilly's liver extract No. 343, which is employed in the treatment of pernicious anemia.[5] The powdered commercial product is dissolved in normal saline and sterilized in an autoclave at 15 pounds pressure for 30 minutes. The solution need not be filtered, as there is very little sediment. The solution of liver extract is then added to the sterile solid medium together with a small amount of sterile rice flour. The medium is then tested for sterility by incubating for 24 hours, and is stored in the refrigerator until used.

[1] Assisted by a grant from the Division of Medical Sciences of The Rockefeller Foundation.

[2] W. C. Boeck and J. Drbohlav, *Amer. Jour. Hyg.*, 5: 371–407, 1925.

[3] C. Dobell and P. P. Laidlaw, *Parasitology,* 18: 283–318, 1926.

[4] L. R. Cleveland and J. Collier, *Amer. Jour. Hyg.*, 12: 606–613, 1930.

[5] Kindly furnished for experimental purposes by Eli Lilly and Company.

We have experimented with several other materials beside whole egg-Ringer for the solid portion of the medium, such as Cleveland and Collier's liver infusion agar, plain agar, plain agar made up in 0.5 per cent. liver extract and Loeffler's blood serum. The best results have been obtained with whole egg and with Loeffler's serum. The other materials have not produced good growth. It is interesting that Cleveland and Collier's liver infusion agar, which produces excellent growth when overlaid with horse serum-Ringer, gave very poor growth when overlaid with liver extract.

Various dilutions of the liver extract have been used, ranging from 0.2 to 2.0 per cent. The best results have been obtained with the 0.5 per cent. solution. We have also found that the addition of horse serum to the liver extract solution in varying dilutions does not produce any better growth than the liver extract solution alone.

In addition to Lilly's liver extract No. 343 we have tested the following preparations and have found them equally serviceable in 0.5 per cent. dilution: Lederle's "Solution Liver Extract Parenteral," Lederle's "Liver and Iron" in powder form, Wilson's "Liver Extract" solution with 0.5 per cent. phenol and Valentine's "Solution Liver Extract."

Approximately six months ago four strains of *E. histolytica,* which were being maintained in the egg-horse serum-Ringer medium, were transferred to the egg-liver extract-saline medium. These four strains had been under cultivation in our laboratory for a period of from two to six years. The cultural characteristics of these four strains have remained the same in the liver medium as in the horse serum medium. Cyst production is equally good in the two media, in both tube and flask cultures. At the present time we are using the liver extract medium in all our experimental work.

The use of 0.5 per cent. liver extract in the cultivation of *E. histolytica* directly from stool specimens also has certain advantages. We have never failed to initiate growth from a fresh stool in which cysts or motile forms of *E. histolytica* have been found by microscopic examination, and the growth has been uniformly more abundant after 24 hours than in the horse serum medium. *Blastocystis hominis,* which often interferes with cultures in the horse serum-Ringer medium, does not multiply in the liver extract medium.

We have not studied extensively the use of liver extract in the cultivation of the other intestinal amoebae of man. Attempts to cultivate *E. coli* and *Endolimax nana* have failed. Several cultures of both *Trichomonas hominis* and *Chilomastix mesnili* have been obtained from stools and have been maintained until discarded. A few attempts to cultivate *Giardia lamblia* from cysts have failed.

Other advantages of the liver extract over horse or human serum are that it can be resterilized several times without injury, it is inexpensive, it is available as a commercial preparation, it is easily prepared for use, and it requires much less aseptic manipulation than horse or human serum.

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ADRENAL ATROPHY AND SENESCENCE PRODUCED BY A VITAMIN DEFICIENCY

IN the course of experiments with young rats on the symptoms produced by deficiency of the factors in the vitamin B_2 complex[1] it was noted that the fur of black and brown rats gradually turned gray, coarse and lifeless when they were deprived of the filtrate factor or factors. The growth of these animals was usually subnormal and the graying developed only after eight to sixteen weeks of depletion, when the animals were twelve to twenty weeks old.

If the mothers were deprived of the factor from the day of the birth of the young the young rats developed the graying as early as eight weeks of age. If the mothers were deprived of the factor from the day of mating the litters were of normal size and weight, but none could be reared to weaning age. Filtrate factor deficiency is decidedly more damaging to milk production than is deficiency in either vitamin B_6 or riboflavin.

When the gray rats are kept in the deficient state for several months there occurs a peculiar sloughing of spots and patches of the skin, sometimes an inch or more in diameter with lazy ulcers resulting, which remain unchanged for months. Crystalline vitamin B_6 in large doses has no curative effect on these ulcers, but administration of concentrates of the filtrate factor brings about rapid healing. These ulcers are reminiscent of the "leg ulcers" of nutritional origin reported from the tropics.

The graying and all accompanying changes can be cured in a few weeks by administration of filtrate factor concentrates or, the graying at least, by injection of relatively large doses of adrenal cortex extract. The symptoms are not relieved by additional B_1, B_6, riboflavin, copper or iron, or by nicotinic acid or epinephrin. The effect of thyroxin is still in doubt.

Histological study of the skin, hair, adrenals and gonads of these animals have revealed striking and consistent atrophy of the adrenals, loss of elastic layer of the skin, failure of spermatogenesis, atrophy of hair follicles. Intermediate stages in this degenera-

[1] Morgan, Cook and Davison, *Jour. Nutr.*, 15: 27, 1938.