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.

tion have been detected paralleling the graying and regenerative stages studied during recovery.

We have also been able to turn the fur of one black guinea pig completely gray by a diet deficient in this factor and have produced graying of hair about the mouths of two young Boston bull pups.

Apparently many of the physical changes of senescence have been produced in these animals in the course of a few weeks by depriving them of the unknown dietary factor which affects particularly the function of the adrenal cortex.

The curative concentrates have been made from yeast, rice bran and liver. Whether the filtrate factor in question is the same as the anti-chick pellagra filtrate factor of Lepkovsky, Jukes and Krause[2] is not at present known.

A full report of these experiments will appear elsewhere.[3]

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BACTERIAL LEAF-SPOT DISEASES[1]

AN investigation has been made of the leaf-spot of Pennsylvania cigar-leaf tobacco, commonly known as "wildfire." Evidence obtained in these laboratories indicates that the organism associated with the leaf-spot, *Phytomonas tabaca,* is a transitory physiological adaptation of the common saprophyte, *Pseudomonas fluorescens.* This organism, in various physiological adaptations, is found in large numbers on normal tobacco. It is apparent from the data obtained in these investigations that all Pennsylvania cigar-leaf tobacco is exposed to this organism throughout the growing and ripening period and that infection in the field is due not to the mere presence of the organism, which is ubiquitous, but to improper host nutrition.

Single cell isolations of various adaptations of the organism have been the subject of physiological and serological studies. The particular adaptation, or what might be termed by the plant pathologist "relative virulence" has been found to vary greatly according to the source of the isolation. Distinct physiological characteristics have been found to be typical of these various isolates. Rapid changes may be made in the laboratory at will, both in the direction of increased and decreased virulence. Serologically, it appears that the "virulence" of the organism is associated with the specific nature and amount of the capsular material of the cell. Although the particular adaptation of the organism is probably related to severity and rapid spread of infection in those cases in which the tobacco plant is suffering from improper nutrition, the evidence indicates on the other hand that the most "virulent" adaptations of the organism are unable to cause economic loss in those cases in which the nutrition of the plant is satisfactory, other things being equal.

The normal tobacco plant of the cigar-filler type is very resistant to infection of economic severity during the growing season. Leaves of such a plant ordinarily contain at maturity between 3 and 4 per cent. nitrogen and between 4 and 5 per cent. potassium. Changing agricultural practices of the past quarter century have resulted in the general production of an abnormal tobacco from the standpoint of nitrogen and potassium content.

Investigations have shown that, contrary to the opinions expressed in publications by other workers, it is not the exact nitrogen level within reasonable limits that is important but the ratio of nitrogen to certain minerals within the plant and, of extreme importance, the stage in which high nitrogen uptake occurs.

Any condition which leads to the accumulation in the plant of a greater quantity of nitrogen than potassium results in poor quality and susceptibility to leaf spot troubles. It is not to be inferred from this that potassium is the only other element which must be available to the plant in order to insure quality and disease resistance. Potassium is, however, the element needed in greatest amount and apparently most frequently deficient in the Pennsylvania area.

It is not during the period of active growth but during the period of ripening that faulty agricultural practices may lead to the greatest susceptibility to leaf-spot. Our investigations have shown that, regardless of the nutrition of the plant prior to this period, the uptake of significant quantities of nitrogen during the ripening period results in an appreciable lowering of the normal resistance of the plant to wildfire. In Pennsylvania certain practices are necessary which accentuate the abnormal condition brought about by significant nitrogen uptake during ripening and greatly increase the susceptibility of those plants taking up large quantities of nitrogen during the ripening period. These include "topping" and "suckering" which take from the plant the possibility of utilizing large amounts of nitrogen at this period in the proliferation of new tissue. It is significant that wildfire was not a problem in Pennsylvania a number of years ago when agricultural practice precluded the possibility of any appreciable ammonification and nitrification at the ripening period.

Practices in Pennsylvania which have been found

[2] Lepkovsky, Jukes and Krause, *Jour. Biol. Chem.,* 115: 557, 1936.

[3] We acknowledge with gratitude help in the interpretation of the tissue changes from Dr. Jesse Carr, of the Department of Pathology, University of California School of Medicine.

[1] Authorized for publication on March 27, 1939, as paper No. 898 in the journal series of the Pennsylvania Agricultural Experiment Station.