vegetables and flowers for the family. . . . Accounts are given of a number of large-scale soilless-growth operations. . . . Finally, the authors have attempted to repudiate some of the widely circulated, but erroneous, claims made for soilless-growth practices. The reader is warned that plants grown without soil need attention just as do those grown in soil."

With a book intended for popular use, extreme care in the choice of terms must be exercised in order that what is written be scientifically accurate as well as simply stated. In courting simplicity, the authors have been led into some statements that will make scientific readers wince. For example, in speaking (p. 12) of "innumerable tiny pores on the under side of the foliage" they say: "On the proper functioning of these infinitesimal stomata and the microscopic cells within the leaves the entire life of the earth is dependent. For without proper breathing of the stomata the production of the entire food supply of all plants and animals would cease." (How about the evolutionary precursors of plants with foliage? And the authors quote the work of the N. J. Agricultural Experiment Station (New Brunswick, N. J.) where Dr. Selman Waksman has done so much work on the autotrophic bacteria). Page 15: "*Cells?* What are they? . . . They differ in size among plants, but are all formed in more or less the same way, and all serve the same purpose." (The differentiation of cells should at least have been mentioned.) Page 21: "In soil farming the purpose of plowing is to break the soil's upper crust and allow better circulation of air among the roots to take place." (Agriculturists recognize many other benefits, *e.g.*, plowing under of nutrient salts which migrate upward as noted on page 94.) Page 36: An important factor in the accumulation of solar heat in glass-covered containers is the penetration of the glass by the short-length incoming radiation and the impounding of the re-radiated longer heat waves which can not so readily pass out, although "cooling by evaporation of water," mentioned by the authors, is also to be reckoned with. Page 54: In discussing pH, a distinction ought to be made between the *total* acidity or alkalinity of a substance, and the *mobilized* acidity or alkalinity represented by the hydrogen ion concentration. The pH of human blood oscillates about 7.45, and even a few tenths of a unit may mean illness or death. Page 140: Algae are not fungi, nor do they arise from fungi. (Among the parasites, no mention is made of viruses or mosaics.)

All told, however, the authors have presented an interesting and useful résumé in an important new field. They are to be congratulated on the warning they give against high-pressure salesmen who try to sell equipment for this work under promise of miraculous results. They caution against excessive optimism or impatient pessimism. Experimenters are advised to construct their own containers and to use relatively inexpensive commercial grades of chemicals. Under ordinary farming conditions, fertilizing chemicals widely scattered are in part washed away by rain, whereas in hydroponic farming the plants are *protected against rain* so that the nutrient solution is not diluted. The effective utilization of chemicals is higher in tray agriculture, and the onslaught of pests and parasites inhibited or prevented.

It is still to be discovered how soilless growth will affect the quality and viability of seeds thus serially produced; also to what extent the fruits, seeds and plants so grown will carry certain substances (*e.g.*, vitamins, trace elements) needed by animals. From recent work it appears that traces of chromium in forage are essential for sheep and inferentially for other animals too. Possibly impurities in the industrial chemicals will serve to supply such substances, or they may be added. Thus most commercial iron salts contain traces of chromium.

The book is clearly and attractively printed.

JEROME ALEXANDER

SPECIAL ARTICLES

ISOLATION OF A FILTRABLE, TRANSMISSIBLE AGENT WITH "NEUROLYTIC" PROPERTIES FROM TOXOPLASMA-INFECTED TISSUES

A HITHERTO undescribed disease-producing agent, of especial interest because of its unique properties and origin, was encountered in the course of certain experiments with toxoplasma in mice. These toxoplasma (obligate, intracellular, protozoon parasites of large size[1]) have undergone numerous brain to brain passages in mice in the past three years, and repeated tests revealed that no other agent separable by centrifugation or filtration was involved in the disease they produced. After about the fiftieth passage, however, in an attempt to preserve the toxoplasma at −80° C. it was found that while they were invariably killed by this procedure, some other agent capable of producing central nervous system disease in mice either became manifest or was liberated in the process. The disease proved to be transmissible by intracerebral injection and the first strain thus isolated has now undergone more than 30 serial passages. At least six other strains obtained in other toxoplasma experiments by freezing or other procedures have been passaged 4 to 12 times. It has proved impossible, thus far, to isolate or demonstrate this transmissible agent in at least two lines

[1] A. B. Sabin and P. K. Olitsky, SCIENCE, 85: 336, 1937.

of toxoplasma which are being passaged separately in mice.

The transmissible agent, free from toxoplasma, has the following properties:

(1) It does not multiply in ordinary media with or without blood or ascitic fluid, nor in Noguchi's leptospira and bartonella medium, either aerobically or anaerobically.

(2) After intracerebral injection in mice it produces a characteristic turning on the long axis of the body with or without other nervous signs after an incubation period of 1 to 10 days, but usually on the 2nd to 3rd day. Some of the mice die, some continue with choreiform signs for months, but the majority recover in a few days; some of the latter occasionally relapse. The characteristic lesion consists of an acute necrosis and almost complete dissolution of the caudal pole of the cerebellum (this is the reason for temporarily using the term "neurolytic" to characterize this agent); almost equally frequent is a disintegration of the structures around the lateral ventricles. Neither the meylin nor the axis cylinders are spared in the attack and free fat is formed in the process. A variable number of 21- to 30-day-old mice and the majority of those younger than 15 days or older than 2 months show no signs of disease not because they are immune (for the agent multiplies in them and periventricular lesions are present) but because the cerebellum is not involved.

(3) Nasal instillation with or without ether anesthesia, administration by stomach tube, intracutaneous, subcutaneous, intramuscular, intratesticular and intravenous injections of large amounts of active brain suspension have failed not only to produce any apparent disease or recognizable lesions but also to yield any evidence of local or systemic multiplication.

(4) After intraperitoneal or intrathoracic injection of large amounts about 20 to 40 per cent. of the mice develop convulsions and other nervous signs and almost invariably die, most of them 17 to 48 hours after injection, with an occasional one on the third or fourth day. Definite pathological changes were found only in the nervous system (chiefly the neopallial cortex, basal ganglia, cerebellum and nervous part of the retina) where extensive vacuolization and neuronal degeneration were seen. But while the agent has been found in the liver, spleen, kidneys, adrenals and lungs after intraperitoneal and in the lungs after intrathoracic injection, it could not be demonstrated in the brains of these mice (more than 15 experiments), nor in the peripheral blood either before or after the onset of nervous signs. The failure to find an inhibitor and certain other observations suggest that the brain lesions in these mice were not caused by the agent itself but rather by some non-transmissible substance which is formed when it acts on certain types of cells, or by some as yet inconceivable mechanism. After injection into the vitreous of the eye the agent undergoes marked multiplication, but nervous signs were observed in only 1 of 50 mice and that within 16 to 17 hours after inoculation before any appreciable multiplication is apparent.

(5) One intracerebral injection, whether or not it gives rise to clinical signs, renders mice immune to reinoculation by the same route. Their serum, however, contains no neutralizing antibodies; nor were any antibodies found in the blood of normal mice of various ages. Mice which have had one eye almost completely destroyed by the first inoculation are not immune to reinoculation in the other eye.

(6) The neurolytic agent does not so far appear to possess any definite pathogenic properties in rabbits, guinea-pigs, rats and *rhesus* monkeys.

(7) It keeps for months frozen at $-80°$ C. and also after being dried *in vacuo* in the Flosdorff-Mudd apparatus; it remained infective after one month in 50 per cent. buffered glycerol.

(8) An infected mouse brain may contain 1 to 2 million minimal doses, and this amount is not appreciably diminished when a suspension in broth is spun at about 2,000 r.p.m. for 1 hour on a horizontal centrifuge; most of it, however, can be sedimented by spinning for $\frac{1}{2}$ hour at 15,000 r.p.m. on an air-driven angle centrifuge. It could be filtered through Berkefeld V candles and it passed a 720 but not a 628 mμ gradocol membrane suggesting that the size of the smallest particle might be in the range of 314 to 360 mμ. Nevertheless, early attempts to visualize the agent microscopically were unsuccessful. After intraocular injection, however, films of the inner contents of the eye, stained with Giemsa or Wright's stain, constantly revealed peculiar minute structures, staining like the nuclear chromatin, which for the most part appeared in the form of rings or ovals with more intense staining at the poles and occasionally also in the form of larger rings or triangles, quadrangles or still other orientation in which a number of "elementary bodies" appeared to be linked by thinner intervening bonds. These structures were not seen in films stained by Gram's method, safranin, carbol-fuchsin, Ziehl-Neelsen or Abbott's spore stain, or after Cesares-Gil mordanting and counterstaining with carbol-fuchsin or methylene blue. Victoria blue stained them satisfactorily. With Rivers' modification of Castaneda's method staining was poor chiefly because the structures did not appear blue as do Rickettsiae or the elementary bodies of psittacosis, but faintly pink (or purplish depending on the differentiation) like the nuclear chromatin. They could not be visualized in the fresh state in ordinary light, while with the dark-field microscope only the

more unique forms could be discerned with any degree of certainty. The same structures were found free and in association with cells in films of the serous surfaces of the liver, spleen, omentum, lungs and heart of mice succumbing after intraperitoneal inoculation while films prepared in the customary way of the transverse sections of these organs usually revealed nothing. They are difficult to find or identify in brain films.

(9) The neurolytic agent has a lower thermal death point (42–45° C. for 15 minutes) than is known for either pathogenic bacteria or mammalian viruses. Toxoplasma, under similar conditions, were not all killed at 45° C. for one half hour.

Tests with several thousand mice (inoculated with brain, blood or viscera or with normal broth) failed to reveal the presence of the neurolytic agent in the normal stock at the time that it was being isolated from the toxoplasma-infected tissues, nor has it been encountered among thousands of other mice of the same stock used in other experiments in the past three years. Furthermore, the properties of the agent are such that one can not at present conceive how it could possibly be transmitted naturally from mouse to mouse either by itself or through any insect vector. Experiments are still in progress on the possibility of a relationship between the neurolytic agent and toxoplasma, but any ultimate decision on its genesis, if that should ever be possible, must be postponed until further opportunity is given other investigators as well as ourselves to determine whether or not it may occur spontaneously in mice under conditions other than infection with toxoplasma.

ALBERT B. SABIN

THE ROCKEFELLER INSTITUTE
FOR MEDICAL RESEARCH
NEW YORK, N. Y.

INTERCELLULAR WOUND HORMONES PRODUCED BY HETEROAUXIN

WE have published investigations indicating that yeast[1] and animal tissue cells[2] injured by ultra-violet light and other means produce wound hormones which stimulate cellular proliferation. In the case of the wound hormone from yeast, we have been able to show that it probably contains adenine, guanine, pentose, phosphoric acid and possibly nicotinic acid, but not proteins or sulfur, and that it is thus similar to, but not identical with, coenzyme.[3]

[1] Fardon, Norris, Loofbourow and Ruddy, *Nature*, 139: 589, 1937; Sperti, Loofbourow and Dwyer, *Studies Inst. Divi Thomae*, 1: 163, 1937; Sperti, Loofbourow and Dwyer, *Nature*, 140: 643, 1937.

[2] Sperti, Loofbourow and Lane, SCIENCE, 83: 611, 1937; Loofbourow, Cueto and Lane, in publication.

[3] Cook, Loofbourow and Stimson, Tenth International Congress of Chemistry, Rome, Italy, May, 1938.

Leonian and Lilly[4] concluded from their investigations of the action of heteroauxin on various fungi, algae, etc., that it is a growth inhibitor rather than a growth promoter and that in instances in which it stimulates growth, its action is that of an irritant, leading to the increased production of growth substances by the plant cells.

We have studied the effect of heteroauxin on yeast, using the techniques employed in our wound-hormone experiments.[5] It was found to be toxic throughout a wide range of concentrations, and when yeast was subjected to it in toxic concentrations, wound hormones were produced.

In the toxicity determinations, methylene blue staining was used as a criterion of cell injury. *S. cerevisiae* standing in isotonic salt solutions containing heteroauxin in concentrations from 1:1000 to 1:100,000 showed an increasingly greater percentage of stained cells throughout the period of standing as compared with controls.

In the wound hormone experiments, suspensions of yeast at a concentration of 50 g per L. (wet weight) in isotonic salt solution were divided into two portions, to one of which heteroauxin was added in a concentration of 1:1000. After the suspensions had stood for 24 hours, heteroauxin was added in like concentration to the control suspension, both suspensions were centrifuged and the cell-free supernatant fluids were decanted and taken to dryness. These materials were made up in distilled water to approximately ten times their original concentration in the supernatant fluids, and tested for growth-promoting effect on yeast grown in rocker tubes for 24 hours.[5] The population densities at the end of this period were determined by a photoelectric method.[6] Heteroauxin alone, in the same range of concentrations in which it occurred in the yeast fluids, was added to a portion of the rocker tubes in each experiment.

The tubes to which heteroauxin alone was added showed less growth than controls. Those to which fluid from yeast which had stood with heteroauxin were added showed marked stimulation of growth, while those to which fluid from yeast standing in salt solution only was added showed little or no stimulation, depending upon the concentration of heteroauxin introduced with the fluid.

Separate experiments in which heteroauxin was added to rocker tubes in concentrations from 1:10 to 1:1,000,000 showed depression of growth except for some slight evidence of stimulation in the range near

[4] Leonian and Lilly, *American Jour. Botany*, 24: 135, 1937.

[5] Loofbourow, Dwyer and Morgan, *Studies Inst. Divi Thomae*, in publication.

[6] Loofbourow and Dwyer, *Studies Inst. Divi Thomae*, in publication.