interpreting the paper of Darlington in particular, it is important to recognize that (a) there is an extensive experimental basis for the acceptance of a nuclear hereditary system; (b) there is increasing evidence for the existence of an extra-nuclear, mitochondrial hereditary system; (c) there is *no* evidence for a diffuse cytoplasmic hereditary system (Sonneborn's data[19] quoted by Darlington as evidence can be differently interpreted on the basis of experiments supplied by Marshak and Walker[20]); also, mitochondrial exchange has not been excluded in Sonneborn's experiments. Darlington does not even mention mitochondria, but only plastids of green plants); and (d) the development of simple non-hereditary cytoplasmic proteins into hereditary systems is entirely without experimental proof. A casual student might find illuminating and thought-provoking the postulate that a non-hereditary protein gradually develops into an hereditary protein, and this, via a particulate hereditary system, into a nuclear hereditary system. Such a view implies that any change in any part of the cell might be the cause of cancer and is, therefore, of little help to the person who has to make a choice among the many experimental approaches to the cancer problem.

It is possible that hereditary particulates of the cytoplasm, other than the mitochondria or plastids, may exist and may develop into pathogenic agents, but the important issue is that we do not have adequate evidence for their existence. On the other hand, we do have proof of the occurrence in nature of known cytoplasmic particulates that can become pathogenic and that can be connected with viruses by gradual steps in a "spectrum of variegation."

In the plastid-induced variegations of plants we have access to concrete facts which may well be applied to the solution of the cancer problem. We have at least been able to set up a theory which reconciles both the virus and so-called non-virus theories of cancer. In addition, it offers an explanation for such phenomena as latency, fluctuations in activity of tumor cells, etc. Also, the precise cellular structures which are changed at the induction of a cancerous condition are indicated. The data obtained from studies on cytoplasmic particulates of plants can offer leads for studies in the much more difficult field of animal cytoplasmic diseases.[21] They will help the workers in the field of cancer to make a choice among the great number of scientific approaches possible in their complex field.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

AN ANTIBIOTIC FROM A BEE PATHOGEN

THE fact that honyebee larvae dead of American foulbrood (scales) almost invariably contain pure cultures of *Bacillus larvae* suggested that this organism might produce an antibiotic. When such scales were placed on the surface of freshly seeded nutrient agar plates of a soil suspension, and of market milk, definite inhibition zones were noted. Since antibiosis against a wide bacterial flora was thus shown, qualitative studies were made employing cultures of Gram-positive, Gram-negative and acid-fast bacteria.

Inhibition zones were produced around scales placed on plates seeded with the following cultures: *Staphylococcus aureus, Staphylococcus albus, Streptococcus agalactiae, Escherichia coli, Aerobacter aerogenes, Brucella abortus, Brucella melitensis, Bacillus subtilis, Bacillus alvei, Mycobacterium tuberculosis* var. *hominis, Mycobacterium tuberculosis* var. *bovis.* No inhibition occurred with *Mycobacterium avium* or an unidentified acid-fast bacterium from a skin lesion. Secondary or "clearing" zones appeared progressively around the original inhibition zones with *B. subtilis* and *E. coli,* but not with *Staph. aureus.* Such zones produced no growth on subculture, even though, with *B. subtilis,* numerous spores were present.

The size of the inhibition zone around scales on agar plates seems to be a function of the diffusion properties of the antibiotic rather than a measure of its concentration or activity against a particular organism. Thus, approximately equal zones are produced around *E. coli* and *B. subtilis;* yet an aqueous scale extract which inhibited *B. subtilis* in nutrient broth at a dilution of 1:20,000 inhibited *Staph. aureus* at only 1:2,000. The cup assay method has not proved feasible.

What appears to be the identical antibiotic is produced in culture by *B. larvae,* but only, as has been shown for certain enzymes,[1] when sporulation occurs. Sporulation has not been attained in broth culture.

A peculiarity was noted when *B. larvae* was grown on serum-glucose-potato extract agar in the presence

[19] T. Sonneborn, *Proc. Nat. Acad. Sci.,* 29: 329, 1943; *id.,* 29: 338, 1943.
[20] A. Marshak and A. C. Walker, *Amer. Jour. Physiol.,* 143: 235, 1945.
[21] H. G. duBuy and M. W. Woods, *A.A.A.S. Research Conference on Cancer,* 4: 162, 1945.
[1] E. C. Holst and A. P. Sturtevant, *Jour. Bact.,* 40: 723–731, 1940.

of the human or bovine strains of the tubercle organism. On this medium *B. larvae* cultures grow well, but fail to sporulate. When the tubercle organisms are present, however, sporulation occurs. Since the human and bovine strains studied show a different inhibition response to the presence of *B. larvae* and since the avian strain is not inhibited, a cultural method of differentiating such strains might be possible.

The antibiotic is soluble in water, but not in the common organic solvents or alcohols. It is adsorbed on activated charcoal or infusorial earth, but no eluent has been found. The material does not pass through either a Cellophane or a parchment membrane. It possesses moderate heat stability and can be sterilized by pasteurization without appreciable loss of potency. Active preparations have been obtained from scales held for over four years in the laboratory. Antibiotic activity is greatly inhibited by the presence of glucose, but not by sucrose, glycerol, xylose or cysteine.

A certain amount of toxicity was demonstrated when a scale extract was injected intraperitoneally into mice, but no toxicity was evident upon oral adminstration. Experiments to determine possible therapeutic use are in progress.

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THE CONTINUOUS CULTIVATION OF MICRO-ORGANISMS

IT was in the belief that micro-organisms could be so handled that the substrate could be continuously collected, spent organisms removed and fresh substrate added that the following two experiments were performed.

Experiment 1. The cultivation of Penicillium notatum. All glassware was chemically clean and sterile before the start of the run and all manipulations were done in a manner to maintain sterility or rather to prevent the entrance of organisms foreign to the desired ones.

The *Penicillium notatum* used was an experimental surface-growing strain that under normal handling had been producing 50 units per cubic milliliter[1] of substrate. The substrate was the standard corn steep composition and four days was chosen as the incubation period.

Daily cultivations from the removed matt showed that during the course of the run no morphological change occurred to the organism and no change in its penicillin production could be detected. Daily estimations of the penicillin level of the outflow from the apparatus showed no change.

The diagram of the apparatus is self-explanatory. Sizes of glassware were dictated by availability and may not have been optimum. The prime requisite of the set-up is the regulation of the flow and removal of the matt at such a rate that self-inoculation occurs with the minimum lag period and the removal of adult organisms before they can mutate.

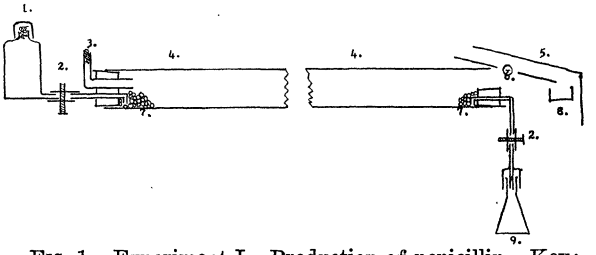

FIG. 1. Experiment I. Production of penicillin. Key: 1. Container for sterile substrate. 2. Screw clamps to regulate inflow and outflow, in this case 20 ml per hour. 3. Aeration tube (cotton plugged). 4. Fermentation tube in this case 4 cm × 200 cm and containing 200 cc of fermenting substrate. 5. Glass baffles to protect open end of tube. 6. Emery covered roller to remove matt. 7. Glass beads to fill lower portion of fermentation tube so that a thin film of fluid floats the matt. 8. Jar to hold removed matt. 9. Shielded container to hold penicillin containing outflow.

Experiment II. Cultivation of Yeast. To determine the possibility of utilizing the principles involved in the Penicillium experiment with other organisms the following experiment was set up.

A commercial strain of brewers' yeast was used. The details of the apparatus had to be varied due to the difference in characteristics of the organism. The wire screens indicated in the diagram serving as baffles worked with relative satisfaction, the outflow being only slightly turbid. The yeast cells in the collecting columns compacted to remove most of the fluid and yielded a pasty mass of cells.

The alcohol estimation of the outflow was based on the specific gravity and is quite inaccurate, the per cent. on this basis was indicated as about 4 and did not vary more than a very small amount from day to day. Daily culture of the yeast at the removal point did not show any change in characteristics.

The diagram (Fig. 2) is self-explanatory. As with the first experiment sizes were determined by availability and are probably not optimum.

Aeration of the reaction chamber in both experi-

[1] Recently strains of Penicillium have been isolated having much greater yields than the strain used. This, however, does not have any bearing on the experiment itself.