exhibiting the gametic types RP, Rp, rP, and rp, thus indicating induced parthenogenesis. If induced nucellus division had been indicated, the resulting plants must all necessarily have been red-fruited and pink-flowered.

Owing to the extreme lightness of the pollen of all the strawberry species, the earlier results were questioned, and the experiment was repeated under the most careful conditions. Of twenty-four plants which have now fruited, twelve are red-fruited and pink-flowered, three are white-fruited and pink-flowered, seven are red-fruited and white-flowered and two are white-fruited and white-flowered. These plants are all diploids ($2n = 14$).

Naturally the possibility is not excluded that a portion of these plants arose through the division of vegetative cells. On the other hand, at least a portion—and perhaps all—of the plants must have arisen through induced parthenogenesis. That is to say, the beginning of development must have been haploid. This being true, the ensuing diploidy is most likely to have come about through ordinary mitotic division. The plants are therefore complete homozygotes.

As yet I can not say whether the breeding results corroborate these conclusions. It will take three years more to grow selfed progeny of each maternal plant. But in looking back over data on Nicotiana species recorded before the war, I find that as high as 100 seeds per capsule yielded maternals in certain attempted crosses where the full complement of seed per capsule is about 400. Notes, photographs and measurements show that the populations resulting from these seeds were extremely uniform.

Since the production of maternals is common in many species when an attempt is made to cross distant species, since some of these cases are certainly instances of induced parthenogenesis with ensuing diploidy, I am publishing this note with the hope that geneticists working with each of the important agricultural and horticultural crops will undertake similar experiments. If the technique can be improved so that a reasonable percentage of parthenogenetic embryos can be forced to develop, and if the majority of these prove to be homozygous diploids, the importance of the procedure to agriculture can hardly be over-emphasized.

I can give very few suggestions regarding technique; but perhaps the following points may be helpful. The indications are that (a) pollen which will produce tubes that will enter the micropyle are likely to induce parthenogenesis whether or not hybrids are occasionally formed; (b) parthenogenesis is not induced when the two species are so similar that hybrids are usually formed; (c) diploidy is probably the result of mitotic division of the chromosomes without nuclear division, division then taking place normally. Possibly X-ray or radium treatment or other stimuli will produce similar results. The main point to be guarded in experiments is to use mother plants having a genetic constitution that will permit easy detection of parthenogenetic diploids.

If this means can be used for the production of complete homozygotes, the labor of producing homozygotes through long periods of self-fertilization will be eliminated. First generation hybrids of homozygous stocks of many perennials such as grapes, blackberries, strawberries, apples, plums, etc., can then be tested out. Moreover, it would be possible to use homozygotes of annuals produced in this way for plot-testing experiments, for physiological researches and for determining residual variability due to external conditions.

Professor R. A. Emerson writes to me stating that for several years he has been endeavoring to secure haploids parthenogenetically with the hope that some diploid seeds can be obtained by selfing them. Professor L. J. Stadler also has been working for and obtaining haploids by means of treatment with X-rays. In this connection it might be pointed out that possible non-disjunctions, translocations, inactivations of small portions of chromosomes, etc., in haploids might reduce the probability of obtaining complete homozygotes in this way; whereas mitotic division of each chromosome at the first nuclear division of the stimulated egg-cell (if that is what occurs) would practically insure homozygosis. Second, certain experiments can be set up which would insure the detection of parthenogenetic haploids which would not insure the detection of parthenogenetic diploids. Experiments should be planned to detect both types.

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THE PRODUCTION OF BACTERIA-FREE AMOEBIC ABSCESSES IN THE LIVER OF CATS AND OBSERVATIONS ON THE AMOEBAE IN VARIOUS MEDIA WITH AND WITHOUT BACTERIA[1]

SINCE the cultivation of *Entamoeba histolytica* by Boeck and Drbohlav five years ago, a number of investigators have attempted to obtain this amoeba free of bacteria, but without success. Various dyes have been placed in the cultures with the hope of inhibiting bacterial growth without inhibiting the growth of the amoebae or without killing them. Also the cysts have

[1] Aided by a grant from the DeLamar Mobile Research Fund.

been washed and treated with chemicals before being placed in culture media. We have tried these methods, too, and mostly without success. However, when the cysts were washed in sterile (Berkefeld) filtered tap-water four or five times daily for ten days and stored at 2° to 4° C. while not being washed, it was possible on three occasions to obtain a few bacteriologically sterile amoebae after treating the washed cysts for three minutes with a 1–1000 solution of bichloride of mercury, and, after four washings, allowing the amoebae to excyst in fresh horse serum-saline 1–6. But the amoebae obtained were few in number, even though several million cysts were treated, for most of the cysts were killed by the treatment.

Practically every one who has attempted to free this amoeba of bacteria has felt that it would grow readily if separated from bacteria. But we have not found this to be true. None of the bacteria-free trophozoites which were obtained by the treatment given above lived longer than six days, and after the second day they were evidently abnormal organisms both in size and movement. But this may have been the result of the treatment employed.

So far as we know, no one has attempted to take advantage of the fact that the liver (of the cat) is capable of destroying many kinds of bacteria. We have found that it is sometimes possible for the liver of the cat to destroy the bacteria accompanying *E. histolytica* in culture and thus leave the amoebae in a bacteria-free amoebic abscess. This, of course, does not happen until the amoebae have been in culture for a month or more, probably on account of the fact that the bacteria are too virulent for the liver to destroy until they have been in culture for some time. Moreover, some pathogenic bacteria are probably overgrown and disappear from the cultures. All attempts to inoculate the amoebae directly into the liver from dysenteric stools have failed to produce bacteria-free abscesses; a majority of the animals inoculated in this way die within two to three days. It is possible, of course, that the inocula which we have used have been too large; had fewer bacteria and amoebae been inoculated, the bacteria, and not the amoebae, might have been killed. The inocula from dysenteric stools were from 0.2 to 0.3 c.c., and those from cultures were 0.5 c.c. each and contained about five million amoebae. Inoculation was made directly into the liver with a hypodermic needle following laparotomy. It was practically impossible to inoculate the amoebae into the liver without doing a laparotomy. The amoebae were cultivated on liver infusion agar slants covered with horse serum-saline (1–6) with a 5-mm loop of sterile rice flour added to each culture tube.

The best success we have had in producing bacteriologically sterile abscesses in the liver of cats was in a series of experiments in which thirty animals were inoculated. Of this number, abscesses developed in twelve, and the amoebae from eight of these abscesses were obtained in culture free of all bacteria. In many instances, however, the percentage of bacteria-free abscesses has been much smaller, sometimes not more than 5 per cent. being free of bacteria.

The following procedure was carried out in removing the abscesses from the liver and in testing them for bacteria. The cat was given ether and as soon as relaxation occurred was fastened to an operating board and the hair moistened with alcohol. When respiration stopped, the skin was removed from the ventral surface and the abdomen thoroughly seared with a large spatula. Then the liver was exposed by cutting through the abdominal wall with a red-hot platinum knife. That portion of the liver containing the abscess was severed with sterile instruments and the abscess placed in a Petri dish. Now, after searing the outer surface of the abscess and that portion of the liver which was removed with it, with a small platinum spatula, the abscess was cut into pieces from twice the size of a pea to one fourth this size, and these pieces were placed in tubes of sterile culture media, one piece in each tube. Many kinds of media were used and will be described presently. It was possible to inoculate from fifty to one hundred tubes from a single abscess, depending on the size of the abscess. The cultures were incubated aerobically and anaerobically for three to four days in determining whether bacteria were present or not. There is no doubt that some of the anaerobes which appeared in the cultures came from the liver of the cat instead of the culture (of amoebae and bacteria) which was inoculated into the liver, for it was possible sometimes to isolate similar organisms from uninoculated livers.

A fairly large series of experiments has been carried out in an effort to determine how soon after inoculation the bacteria are killed by the defense mechanism of the cat's liver. The earliest we have obtained the amoebae free of bacteria was seven days after inoculation. In our experience, from seven to nine days after inoculation is the best time to obtain the amoebae with no bacteria. We can not state definitely yet just what happens to the abscesses which contain no bacteria if the animal is not killed for examination, because we have not carried out a sufficient number of experiments. But the experiments which we have carried out indicate that soon after the abscess becomes free of bacteria it begins to heal and that within a few days, perhaps five to ten, no amoebae are present. This statement is based on

five observations: two where the abscesses were free of both amoebae and bacteria, and three where definite evidence of spontaneous healing was present.

As a result of the inoculation of the amoebae and bacteria into the liver, we have failed to produce abscesses in some instances while in other instances we have produced abscesses which ranged all the way from purely bacterial ones with no amoebae in them to those which contained only amoebae. In those in which only bacteria were present, the amoebae had probably been killed off or crowded out by the growth and activity of the bacteria, while in those where the activities of the bacteria were partly checked by the cat, it was possible for both amoebae and bacteria to live together as they do in the lumen of the intestine or in culture media. Whenever amoebae were present in an abscess, regardless of whether bacteria were present or not, there was no membrane or wall of granulation tissue at the edge or periphery of the abscess. This fact made it possible to tell at a glance whether amoebae were present or not. Whenever pus was present in the abscess, the amoebae were always found to be accompanied by bacteria. When a large amount of pus was present in the center of the abscess, as was sometimes the case when many bacteria were present, the amoebae were confined mostly to the outer portion near the uninjured tissue. But when no bacteria were present, the entire abscess was hard and dry and the distribution of amoebae was uniform throughout the abscess. In the abscesses which we have studied, there has been no indication that the amoebae ever bring about pus formation. However, if the abscesses were to run a long time—perhaps months or years as they are supposed to do in man—it is possible that pus might be formed.

We have estimated that each of the pieces of the bacteria-free amoebic abscesses which were placed in culture tubes contained from five to ten thousand large active trophozoites. These bacteriologically sterile amoebae have been placed in many kinds of culture media employed in the cultivation of bacteria, all the media that have been used in the cultivation of amoebae, and many others, but in no instance have they lived longer than fourteen days. There was some multiplication in several kinds of media, but the amoebae never multiplied so rapidly as they do when certain bacteria are present. The two kinds of media which gave the greatest promise of successful cultivation were: (1) egg slants covered with horse serum-saline (1–6) with one to three drops of laked blood added to each tube; and (2) liver infusion agar slants covered with horse serum-saline (1–6) with 1 c.c. of hydrolyzed haemoglobin and three drops of the sediment from autoclaved red cells of the horse added to each tube. Many substances were added to each medium; for instance, red cells of the cat, sterile cells from the liver, brain, spleen and kidney of the cat, rice flour, pure rice starch, various kinds of heat-killed bacteria, egg-white, egg yolk, glycogen, glucose, powdered milk, coagulated albumen, etc., but the amoebae were never successfully cultivated. In a few instances the growth of the amoebae appeared to be stimulated somewhat when the oxygen tension was reduced.

We have not spent a great deal of time in cultivating the amoebae with pure cultures of bacteria. In most of the experiments, a medium composed of liver infusion agar slants covered with serum-saline (1–6) and sterile rice flour has been used. After the amoebae had been definitely proved to be free of all bacteria, they were transferred to this medium and then the various bacteria were added. With some bacteria there was little or no multiplication of the amoebae. To this group belong certain of the spore-formers—*Bacillus megatherium, B. cereus* and *B. subtilis*—and *Proteus vulgaris, Escherichia acidi-lactici, Pseudomonas aeruginosa* and *Alcaligines fecalis*. With the spore-formers *B. niger, B. mesentericus* and *B. brevis*, the amoebae grew rather poorly for the first two or three subcultures, but after this they grew better and finally became abundant. They have been grown with *B. brevis* for almost a year and are no doubt capable of growing indefinitely. With *Escherichia communior, Vibrio comma* and *Neisseria catarrhalis* they grow well from the start.

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THE SPECIFIC ACTION OF A BACTERIAL ENZYME ON PNEUMOCOCCI OF TYPE III

A SYSTEMATIC search for enzymes capable of hydrolyzing the polysaccharides found in the capsular material of pneumococci of the various types has been carried on in this laboratory for several years. A number of enzymes from animal and plant sources, known to be active in the hydrolysis of simpler carbohydrates, were tested, but none of them were found capable of attacking the polysaccharides of pneumococcus origin. In addition, cultures of various moulds, yeasts, soil actinomycetes and bacteria, many of which were known to decompose cellulose, were tested without success. Recently, however, a bacillus has been isolated from the organic matter of soil taken from the cranberry bogs of New Jersey, which is able to split the specific capsular polysaccharide of pneumococci of Type III. The micro-organism is