1. In describing new species of Algae special importance should be attached to the provision of illustrations and to maintenance of cultures of the species concerned.

2. The desirability of adopting further monographs as the starting-points of particular groups of Algae, as in the *Oedogoniaceae,* should be investigated.

3. A list of *Nomina dubia* of species, genera and families should be prepared, and also lists of *Nomina conservanda* and *rejicienda* of genera and families.

4. The desirability of retaining the Latin language for diagnoses of new Algae should be investigated.

(18) All proposals concerning mycology, submitted to the Amsterdam Congress, were referred to subcommittees to be appointed by the Special Committee for Fungi.

(19) Additions concerning paleobotany to be made to the rules and recommendations for the following objects:

1. To recognize as taxonomic groups, organ genera and artificial or form genera.

2. To ensure that the names originally given to detached organs or parts of plants shall only be used in their original signification, and shall not be employed in the designation of different organs, or of the plant as a whole.

3. To provide for the naming of an entire plant when it has been possible to reconstruct it by the association of its different organs.

4. To define how the names of the artificial genera are to be used.

5. To set up a permanent committee to consider the interpretation of the rules; to adjudicate in cases of dispute or difficulty; to draw up lists of *Nomina generica conservanda;* and to make such further recommendations as may prove necessary, including rules for the determination of types.

(20) Appointment of a Special Committee to report on the effects of the adoption of the proposed Art. A 19 and Appendix "IX," dealing with the rejection of certain works.—*Syn. Prop.* pp. 15, 77–80.

T. A. SPRAGUE

# SPECIAL ARTICLES

## THE EFFECTS OF PHYSIOLOGICAL AGENTS ON ADULT TISSUES IN VITRO[1]

THE dormant state of adult tissue cells has been studied by observing the effect of various agents upon the initial growth of the adult tissue *in vitro.* Fresh pieces of adult tissue (mainly chicken aorta) have been treated with these agents previous to planting in a dilute plasma medium. The resulting effects on the lag period preceding growth and on the rate of the initial growth have been recorded.

Furthermore, the physiological state of the cells has been studied by treating active cultures of adult tissue cells with various "factors" obtained from normal blood plasma.

A study of over 40,000 pieces of tissue has given the following results:

(1) The lag period preceding the first visible growth of fibroblasts from aortas of one-year-old chickens was normally three to five days (as contrasted with a few hours for embryo tissue).

(2) The lag period of aorta tissue from five- or six-year-old chickens was about the same as that of the one-year chickens, but the average rate of initial growth was 46 per cent. faster for the older tissue.

(3) Plasmas from the older chickens induced growth 9 per cent. sooner than young plasmas. The initial growth rate was 50 per cent. faster in two-year plasmas than in one-year plasmas; but it was 21 per cent. slower in the five-year plasmas than in the one-year plasmas.

(4) Trypsin stimulates the growth of adult tissue. Digestion of the tissue with trypsin previous to planting in a plasma medium reduces the lag period to less than one day and accelerates the rate of the initial growth. This has been repeated many times, not only on artery tissues, but also on liver, thyroid and some tumors. Papain stimulates in the same manner.

(5) The stimulating action of trypsin was found to result from the proteolytic digestion of the tissue. This apparently removed an inhibitor contained in the tissue. The digestion fluid after this treatment was found to contain an inhibitor which could be precipitated out. This "tissue inhibitor" is destroyed by heat. It seems to be widely distributed in normal adult tissues (and in tumors). It presumably plays a rôle in restraining growth in adult animals. It appears to be produced by cells in tissue culture.

(6) Embryo extract and spleen extract had little effect on the initial growth of adult tissue. Both contain inhibitors. Pituitary growth hormone was slightly stimulating, particularly in the presence of serum.

(7) Blood plasma contains a growth stimulant, the "A factor." It is present in a concentration more than adequate to induce growth *in vitro.* It is also present in tissues, in lymph, in urine, in serum and in the ultrafiltrate from serum. Serum ultrafiltrate is prepared routinely for use in washing cultures and as a basic medium in our sterile perfusion pump.

[1] These investigations have been supported by grants from the Josiah Macy, Jr., Foundation.

The A factor produces a definite reduction in the lag period and a stimulation of the initial growth. We have obtained no growth in its absence, and it appears furthermore to be needed by cells in a resting condition. It has a small molecular size. Electrodialysis shows it to be an acid. It can be precipitated with calcium or copper with subsequent recovery of activity. It is only slowly destroyed at 100° C., providing the pH is neutral.

Thus the dormancy of adult tissues appears to involve a balance between the non-diffusible "tissue inhibitor," on the one hand, and the stimulating A factor and proteolytic enzymes on the other hand (and probably other agents).

The physiological condition of adult tissue cells seems similarly to involve a balance between certain hormone-like "controlling agents." There are at least four of these in plasma, the A, B, C and D factors.

(8) The first of these, the A factor, is not only a stimulant, as mentioned above, but it appears furthermore to be needed by dormant cells. If a culture of adult fibroblasts is washed repeatedly with serum ultrafiltrate (containing the A factor) the B, C and D factors are thereby removed without depriving the cells of the needed A factor. The cells then become clear, stellate and free from fat granules. They can be kept in a healthy state indefinitely, merely by semi-weekly washing with serum ultrafiltrate.

(9) When one of these cultures of clear cells is treated with a solution containing the B factor (obtained from chicken plasma or dog serum) the cells become filled with fat granules. These can be seen in 24 hours and are very conspicuous in two or three days. This process can be reversed. Repeated washing with serum ultrafiltrate results in a complete absorption of the fat granules. Thus the B factor is an agent which causes the cells to produce fat granules—but it does not produce degeneration.

(10) The C factor, however, produces degeneration but does not produce fat granules. It is closely associated with the B factor. This degeneration is not reversible.

(11) There is also evidence for a "D factor" which produces cohesiveness between fibroblasts. The cultures which have been washed with serum ultrafiltrate contain independent isolated cells. On the addition of certain fractions these cells coalesce to form the usual reticulum.

(12) Segments of chicken innominate arteries were incubated in solutions containing the B factor. Frozen sections stained with Scharlach R showed that fat had been deposited in these arteries *in vitro*. The fat was seen as a thin layer along the intima with scattered droplets in the adjacent media. This same distribution of fat occurs spontaneously in chicken arteries *in vivo*.

Details will be published elsewhere. Further work is in progress.

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## BACTERIOLOGIC EXPERIMENTATION ON THE GUINEA PIG FETUS

ALTHOUGH bacteriologists are continually in search of new and more adaptable experimental animals, the possibility of using the fetus for bacteriologic studies appears to have been almost entirely overlooked. Most of the recorded investigations dealing with experimental fetal infections have been based on the passage of the infectious agent from maternal to fetal circulations through the placenta. Obviously, in such experiments there can be no control of the time of effective fetal inoculation or of the amount of inoculum which actually reaches the fetus. That direct manipulation of the mammalian fetus is possible, however, has been shown by Bors,[1] Wohlwill and Bock,[2] and others. Their studies have been concerned chiefly with developmental processes and pathologic changes. The latter authors studied particularly the cellular type of response of the guinea pig fetus to directly inoculated chemical and bacterial irritants.

The potential value of the fetus as an experimental animal for bacteriologic purposes is based on its inherent sterility and on the possibility that the fetus may present useful variations in susceptibility to certain disease agents in comparison with the postnatal representative of the same species. This possibility may be inferred from the fact that the fetus differs markedly from the postnatal animal not only in size and structure, but in physiological and biochemical processes as well. In order to investigate this possibility and to learn some of the technical applications and limitations in the use of the fetus as an immediate experimental animal for bacteriologic research, we[3] have inoculated fetal guinea pigs *in utero* with six infectious or toxic agents. They were selected to represent a wide range of host-parasite relationships, *viz.*, the poliomyelitis virus, for which the monkey is at present the only susceptible experimental animal; the vaccinia virus, which finds a relatively resistant host in the guinea pig; diphtheria toxin, for the study of which the guinea pig may be said to be the classic experimental animal; two strains of the tubercle bacillus (H37 and BCG), representing, respectively, bacteria virulent and relatively non-virulent for guinea pigs and other animals; and the submaxillary gland

[1] E. Bors, *Archiv. f. Entwick. d. Org.*, 105: 655, 1925. *Ibid., Deutsche Ztschr. f. Chir.*, 203–204: 669, 1927.

[2] F. Wohlwill and H. E. Bock, *Virchow Archiv. f. path. Anat. u. Physiol.*, 291: 864, 1933.

[3] *Am. Jour. Pathol.*, in press.