organisms have contributed the largest amount of the calcareous part of the finer fraction. Some other organisms, however, may also have contributed, since additional spectrochemical analyses made for comparison did not differ greatly from that of the finer fraction (*cf.* [8]). The data did not completely preclude the possible existence of chemically precipitated carbonate in the sediment, however.

The conclusion that the sediments are largely organic in origin seems most significant in view of the fact that Red Sea oceanographic conditions should be, according to many proponents of the chemical deposition of $CaCO_3$ (*9–11*), ideal for such a mode of accumulation. First, the temperature of the entire water column is high throughout the year and seldom falls below 22° C even at depth during the winter. Second, the salinity ranges from $40^0/_{00}$ to $41^0/_{00}$, which is exceptionally high and is, in fact, among the highest recorded in any existing open sheet of water. Third, the diversified topography of the bottom varies from large shallow flats to deep and highly irregular basins. Fourth, the coast is reef-bound, and a tremendous supply of $CaCO_3$ nuclei should be present as the result of normal marine erosion and attrition. Fifth, although the phytoplankton is not too abundant, it is by no means inconsiderable. Sixth, some areas are continuously agitated; others essentially undisturbed. From the evidence here cited that these Red Sea sediments do not possess significant quantities of chemically precipitated $CaCO_3$, it seems valid to conclude that chemical deposition of $CaCO_3$ is negligible, at least in the present-day seas.

The optimum conditions for formation of calcareous deposits in the Recent seas are: (1) an environment conducive to proliferation of $CaCO_3$ shell-building organisms, (2) conditions under which large quantities of contaminating terrigenous materials are prevented from reaching basins of deposition, thereby masking the calcareous organic debris. For example, basins with reef-bound coasts and virtually no runoff because of aridity are probably the most favorable environments for accumulation of carbonate deposits. In the Red Sea itself, the carbonate percentage increases progressively offshore, irrespective of depth. It seems, therefore, that influx of terrigenous material, normally maximum near shore, reduces the total relative carbonate percentage.

It may also be worth while to note that the mineralogy of the finer calcareous fractions of the samples studied, as determined by x-ray diffraction measurements, indicate they are mainly calcite, with very little aragonite. This observation is of interest, as the coarser calcareous fractions from which the fine fractions are derived consist largely of aragonite (*2*). It seems, therefore, at least in the Red Sea, that inversion of aragonite to calcite requires not too much time during abrasion and transport. The stability of aragonite has long been a controversial subject, but these observations of the Red Sea sediments add evidence of the facility with which aragonite inverts to calcite under the described conditions.

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A Tissue Culture from Potato Tuber: The Synergistic Action of 2,4-D and of Coconut Milk[1]

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The conditions that enable actively proliferating tissue cultures to be established from otherwise mature nongrowing cells are of interest because they may contribute to an understanding of normal and abnormal growth—or furnish material by which the differences between growing and nongrowing cells may be investigated. Our work with these systems has been in progress for some time. This note describes a technique by which it has been possible to establish an actively growing tissue culture from the parenchyma of the potato tuber.

Proliferation of the parenchyma beneath lenticels is a familiar feature of cut potato tubers kept in a very moist atmosphere. Nobécourt (*1*) attempted to obtain a tissue culture from slices of potato tuber kept either on moist cotton or on an agar surface. These tissue slices were relatively large (250 mg), and although proliferation occurred and protuberances formed, the relative growth was small (the fresh weight doubled in 4 months). We, however, have established, apparently for the first time, actively growing tissue cultures from potato tuber which increased in fresh weight approximately 50 times in 5 weeks, and subcultures from these continue to grow actively. The technique by which this was accomplished has obvious and far-reaching implications. It seems appropriate, therefore, to describe it here.

The mature parenchyma cells of the potato tuber are certainly able to divide. When cut slices are exposed to moist air, the cell divisions near the surface lead to the formation, within the mature parenchyma, of a cork phellogen. Henceforward, the divisions of this cambium are orderly, but the behavior of the cells

[1] This work was carried out with the aid of a grant to one of us (F.C.S.) from the Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

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cut off by the cambium is circumscribed: the outer ones differentiate to form dead cork cells, the inner ones (fewer in number) remaining alive, though they mature and cease to divide. It is well known that Haberlandt (*2*) postulated quite early that this response of the cells of the potato tuber was stimulated by the then hypothetical wound hormone. He also believed that unless a small piece of tissue contained at least some sieve tubes (supposed to be the source of the hormone) they would not heal.

Later Bonner and English (*3*) claimed that the substances they called traumatin (1-decene-1,10-dicarboxylic acid) stimulated cell divisions in the young, inner epidermis of bean pods. They stated that this substance occurred in a variety of plants, including potato, but they did not themselves record any data which demonstrate that this substance stimulates cell division in potato cells.

Some time ago we attempted to establish a sterile, proliferating tissue culture, using the mature parenchyma of the potato tuber. We had found coconut milk to be successful in stimulating the mature secondary phloem of the carrot root into active growth (*4*). This fluid contains some growth factor, or combination of factors, which is designated, pending its isolation and identification, as the coconut milk factor (CMF). Although the CMF was effective in stimulating cells from other plants into active growth (e.g., Jerusalem artichoke tuber), it had no appreciable effect upon potato tuber tissue, which did not grow even when in contact with the medium (basal medium supplemented by coconut milk) that supported rapid growth of carrot cells. Attempts were made to stimulate the potato cells into an actively proliferating tissue culture by other means. Among these, treatment with traumatic acid and 2,4-D (2,4 dichlorophenoxyacetic acid) solutions was tried. So far as our experiments went, the former substance produced no appreciable effect as measured by continued increase of fresh weight, whereas the latter, when added to the nutrient medium, caused a real and provocative increment of fresh weight, which did not continue (maximum effect at 7.0 mg/liter). Even so, 2,4-D alone did not lead to a culture able continuously to grow and divide.

TABLE 1

GROWTH IN FRESH WEIGHT[1] OF 3-MG EXPLANTS FROM POTATO TUBER AT 26° C DURING 5 WEEKS IN ASEPTIC NUTRIENT SOLUTIONS SUPPLEMENTED BY 2,4-D AND COCONUT MILK

Coconut milk (% by vol)	Mg/liter 2,4-D					
	0	0.3	1	3	6	18
0	2.7	3.1	2.4	3.1	2.5	3.2*
0.3	3.4	6.3	5.4	6.3	4.8	3.3
1	2.6	7.4*	16.4**	21.0**	43.2**	11.1
3	3.6*	30.0*	32.5*	47.7**	44.8*	38.0**
6	5.7*	39.6	56.5*	90.9***	81.2**	44.3*
18	9.0**	93.7*	102.8**	156.3**	164.0**	68.4

[1] Fresh weight data represent mean of 5 replicates, except where indicated as follows: * mean of 4, ** mean of 3, *** mean of 2.

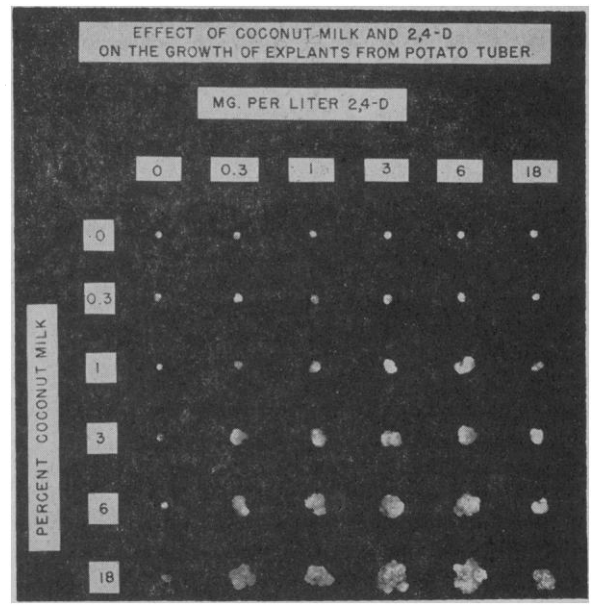

FIG. 1.

Fortunately we were led to try the effect in *combination* of coconut milk and 2,4-D. Whereas either substance *alone* produces only slight effects on the potato cells, and *neither* will stimulate the tissue into active proliferating growth, in combination they act as a powerful stimulus to growth. This is shown by the following data.

Explants (individually weighing about 3.0 mg) were removed aseptically from the medulla of the tuber, using a surgical cannula and a device to cut the cylinders so removed into standard lengths. For these experiments, the medulla was used because it was easier to obtain a large number of explants from one tuber. The cortex, however, responds in a similar but essentially more uniform manner to the medulla. The growth of the small cylindrical explants was then tested by exposing them to the basal medium (*4*) supplemented by coconut milk and/or 2,4-D.

Experiments have been made with explants grown on the surface of nutrient agar in tubes, but the more instructive data were obtained when the tissue cultures were grown under the special conditions that have been found suitable for carrot tissue (*5*). Under these conditions, the tissue is exposed alternately to air and liquid in special tubes slowly revolved (1 rpm) around a shaft slightly inclined to the horizontal.

The data in Table 1 and Figs. 1 and 2 show the results of a symmetrical experiment in which the growth of the cultures was measured in 36 different solutions. The effect of coconut milk alone is shown by the growth in the basal medium supplemented by 0, 0.1, 1, 3, 6, and 18% by volume of sterilized, filtered coconut milk. The effect of 2,4-D alone is shown by

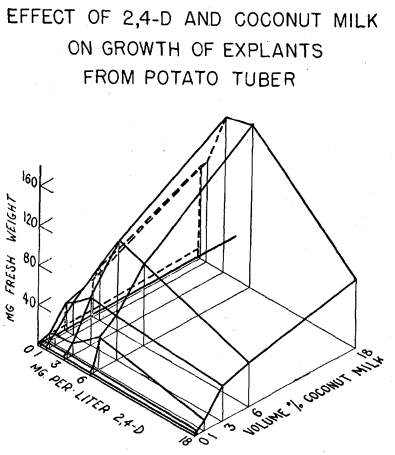

FIG. 2.

the growth in the basal medium supplemented by 0, 0.1, 1, 3, 6, and 18 mg/liter of 2,4-D. The interaction of the CMF with 2,4-D is shown by the growth in media containing both supplements in all the possible combinations of the dosages shown above.

The conclusions are self-evident from Fig. 1, which shows a representative culture harvested after 5 weeks from each treatment. Table 1 describes the growth in terms of the mean fresh weight of the cultures. In Fig. 2 these data are shown plotted on isometric paper. Occasional cultures that were not viable (for unknown reasons) were omitted when calculating the means. (Even including these, however, does not appreciably affect the results.)

Briefly, the conclusions are these. At zero, or very low, concentrations of coconut milk, the effect of 2,4-D on growth is small, and the specific effect of its concentration is also small. At zero, or very low, concentrations of 2,4-D, the effect of coconut milk is negligible at all concentrations. Cultures, however, in contact with *both* 2,4-D and the CMF grew, and the solid surface depicted in Fig. 1 shows that the optimum concentration of 2,4-D is of the order of 6 mg/liter. At this concentration of 2,4-D the tissue shows the maximum response to concentration of the CMF, and even at 18% by volume of coconut milk the limit of response has not been reached.

One further point should be stated. The experiment described in Table 1 and in Figs. 1 and 2 lasted some 5 weeks. For a relatively large part of this period (10–14 days) the tissue made but little growth. The significance for the future growth of this lag, or induction, period has yet to be investigated.

The implications of this work are clear. Whereas CMF alone may be a powerful tool in stimulating some cells into active growth, it is of no avail in some cases. At least in some such cases, the reason is that the cells cannot respond to CMF because their growth is also limited in some manner that requires the intervention of 2,4-D, or a similarly acting substance. Since the response to CMF is so clearly a function of the concentration of 2,4-D and vice versa, these two distinct stimulants appear to act synergistically on the cells of the potato tuber. Understanding of these relations may be expected to shed light both on the mechanism of the CMF on cells and also on the mechanism of the action of 2,4-D.

In view of the reported (*6*) but disputed (*7*) effect of onion juice to increase the growth-promoting activity of auxin or 2,4-D, experiments were conducted to test this further. Potato cultures were grown with combinations of onion extract and 2,4-D added to the medium. The results obtained clearly indicated that onion juice could not substitute for coconut milk. Also at relatively small concentrations (juice of 0.1 g of onion tissue/100 ml of culture medium) onion juice inhibits the growth of carrot even in the presence of the CMF.

The combined action of CMF and 2,4-D on cells of the potato furnishes a system of growing, randomly proliferating, cells that may be contrasted with the cells of the resting tuber, or with the metabolically active cells at the surface of a tissue slice which have, however, only a more limited ability to grow. When these contrasts are described in terms of protein synthesis, salt accumulation, and respiration, we should know more about the physiological characteristics of growing and nongrowing cells.

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Factors Involved in Blood Clearance of Bacteria[1]

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It was recently reported (*1*) that the injection of an antigen into a rabbit previously immunized to that antigen was followed in 15–30 min by a pronounced reduction of the hemolytic complement of the serum. Also observed were the previously noted phenomena of the so-called negative phase, including decreases in specific antibody (*2*), granulocytes, and platelets (*3*), and in the coagulability of the blood (*4*). Evidence was presented (*1*) to support the concept that the decrease of complement was due to the fixation of complement by an *in vivo* complex of extracellular antigen and extracellular antibody. It was suggested that this specific reaction might be applied to the experimental reduction of complement *in vivo* in order

[1] This paper is based on work sponsored by the Biological Department, Chemical Corps, Camp Detrick, Frederick, Md., under Contract No. W-18-064-CM-242 with Western Reserve University.