either the mean intermitotic time or the doubling time of the tumor. Strictly speaking, it may not be possible to label every cell of a tumor regardless of how long the exposure is prolonged. The significance of nonproliferating tumor cells is an open question, depending on whether or not these cells still have the potential to proliferate and repopulate the tumor after therapy. In any case, the therapist is faced with the prospect of delaying the definitive step in tumor therapy while the tumor literally doubles before his eyes (*4*).

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8 September 1961

Food Production by Submerged Culture of Plant Tissue Cells

Abstract. In investigating the possibility of producing food by large-scale culture of plant tissue, several plant tissues were used. The yield for carrot tissue, which is especially capable of rapid growth, was an average of 4.6 grams per liter per day in 6 liters of medium. The results suggest that with improved techniques food could be produced by this method.

Within the past few years considerable progress has been made in the design of systems for accelerated food production. The goal of much of this work is to establish the requirements for a practical method of feeding men under unusual survival conditions or, ultimately, during prolonged space travel.

Our work (*1*) was undertaken as an exploratory study to determine the feasibility of producing food by tissue-culture techniques. That large amounts of plant material may be obtained by growing specific tissues of higher plants in liquid media has been established (*2*). The large-scale culture of plant tissue for food, however, has not been previously studied, which is rather surprising in view of the potentials of a successful system of this type.

Table 1. Yields of carrot tissue from submerged cultures in 6 liters of broth.

Culture No.	Days cultured (No.)	Age of inoculum (days)	Yield (wet weight)		
			Total (g)	g/lit.	g/lit. day
1	6	7	167.1	27.8	4.6
2	6	19	160.4	26.7	4.4
3	7	35	167.2	27.8	3.9
4	5	2	183.5	30.6	6.1
5*	5	2	122	20.3	4.0

* Malt extract used in place of coconut milk.

In seeking to discover the specific requirements for a large-scale food production system we used cultures of normal tissues of carrot and potato and of the stems of tomato, rose, grape, and tobacco (*3*). These tissues have been maintained on 0.6-percent agar "tobacco" medium at *p*H 5.7 to which coconut milk (15 percent by volume) and α-naphthaleneacetic acid (0.1 mg/lit.) were added. "Tobacco" medium without agar was used for the liquid cultures (*4*). All of the tissues grew well in submerged cultures, but, since the carrot tissue appeared to be especially capable of rapid growth, it was selected as the principal tissue for this study.

To scale-up liquid cultures to large volume we used Erlenmeyer flasks (300 ml), then Fernbach flasks (3 liters), and finally Florence flasks (12 liters). The inoculum for the largest flasks was prepared by transferring a piece of tissue from the agar to 100 ml of liquid medium in a 300-ml Erlenmeyer flask. This culture was agitated on a rotary shaking machine. When sufficient growth occurred (1 to 3 weeks), the contents were transferred to a Fernbach flask containing 1 liter of medium. This culture was also agitated on a shaker. After another period of growth, 200 to 300 ml of the culture were inoculated aseptically into 6 liters of medium in a 12-liter flask. The tip of a 100-ml volumetric pipette was cut off before sterilization to facilitate this transfer of tissue. To aerate and agitate the culture during growth, the flask was fitted with sterile tubing, by which compressed air, sterilized by passage through a 2-inch pipe packed with sterile cotton and two Seitz filter pads, could be injected into the flask. The pipe was connected to a compressed-air vent with a pressure regulator. Aseptic techniques were followed in all the procedures.

At first, cultures grown in the large flasks were frequently contaminated with mold and bacteria. To control this contamination without inhibiting the growth of tissue, antibacterial Tylosin (20 parts per million) (*5*) and antifungal Mycostatin (25 units) (*6*) were added to the liquid medium before inoculation. These two antibiotics—now used routinely in our tissue cultures of carrot, grape stem, and Queen Elizabeth rose stem—have produced no toxic effects.

The cultures on the shaking machine were incubated at 28°C while the culture in the 12-liter flask was of necessity subjected to the variations of room temperature. It is assumed that a constant optimum temperature would increase the growth rate and the yields of tissue.

The yields of tissue from five large-volume cultures are shown in Table 1. Our method of large-volume culture did not provide for removing any of the tissue or for supplementing the medium. Consequently, the tissues were harvested after 5 to 7 days in culture because heavy growth almost stopped agitation by the aeration system.

Inocula from a young, actively growing culture contributed to an improved yield and decreased the lag phase of growth in the large flask (culture No. 4). Culture No. 5, with malt extract in place of coconut milk, was inoculated at the same time as No. 4 with material from the same Fernbach flask. By the use of a Y-tube the two 12-liter flasks were aerated simultaneously.

Our yields from 6 liters of medium averaged 4.6 g/lit. per day. They are slightly higher than the yields obtained by Tulecke and Nickell from cultures of *Gingko*, Holly, and *Lolium* tissue grown in 10 liters of medium in 20-liter carboys (3.1 g/lit. per day). Their pilot-plant tank cultures of rose stem gave a higher yield (9.7 g/lit. per day). However, they say this figure is subject to interpretation, since the culture period was only 2 days and a heavy inoculum was used in 134 liters of medium.

We feel that these yields are not indicative of the best results that could be obtained under better conditions of

aeration and medium replenishment. It is conceivable that food could be produced by submerged culture techniques of plant tissue with a continuous cell generator having a capacity of ten or more liters and operating under optimal conditions for a long period of time.

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References and Notes

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6. We express our thanks to E. R. Squibb and Sons for their generous gifts of Mycostatin.

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14 November 1961

Metabolism of Glycogen in Skin and the Effect of Radiation

Abstract. Continuous biosynthesis of glycogen was demonstrated by perfusion of the skin of dogs with uniformly labeled C^{14}-glucose. If the skin is irradiated in vivo with an erythemal dose of x-rays 24 hours prior to perfusion, the uptake of radioactive glucose into glycogen is significantly increased. The irradiation effect appears to be due to enzymatic dysbalance.

Most of the little that is known about the metabolism and biological role of glycogen in the skin is based on histochemical evidence. This evidence seems to indicate that certain cells of the skin accumulate glycogen when they are in a resting state and subsequently utilize it as a source of energy when their metabolism is heightened in order to perform some function (*1*). In addition, it has been assumed that epithelial cells accumulate glycogen if their metabolism is experimentally suppressed (*2*).

Pure biochemical work on cutaneous glycogen has yielded contradictory results. The methods used for isolation and estimation have been crude and unreliable, and modern biochemical research methods, particularly those using radioactive tracers, have not been applied (*1*).

In our work the following method was used for the quantitative extraction and isolation of chemically pure glycogen from dog skin: the skin was lyophilized and ground to a powder which was defatted, digested with hot 30 percent potassium hydroxide, dialyzed, and deproteinized with 10 percent trichloroacetic acid. The supernatant was dialyzed and transferred to a Dowex-1 ion-exchange column to remove mucopolysaccharides. Glycogen in the effluent solution was precipitated with alcohol. Chemical purity was tested by spectrography of the glycogen-iodine complex, optical rotation, phosphorylase degradation, β-amylase degradation, and elementary analysis. All these data were in the range of those obtained from a pure preparation of liver glycogen. The amount of glycogen thus found in the skin was around 30 mg/100 g (dry wt.), which is only 1/10 to 1/5 of the amounts previously reported.

In the last few years it has become possible to perfuse the skin of dogs in the same manner that liver, kidney, and adrenal glands are being perfused. The inner surface of the thigh of the dog has a saphenous artery, the cutaneous branch of which has very few minor muscular branches; these branches can easily be tied off. This cutaneous artery and the saphenous vein can be cannulated, and the skin supplied by the artery can be isolated and perfused under standardized experimental conditions (*3*). Metabolism in the isolated perfused skin has been maintained for 8 hours at 37°C and 100 percent humidity (*4*).

This preparation was utilized to study glycogen synthesis in the skin by perfusing it with uniformly labeled C^{14}-glucose. Six experiments were carried out with saline perfusion, eight experiments by perfusion with heparinized dog blood; the maximum time of perfusion was 2½ hours. Radioactive glycogen was isolated in all 14 experiments. In this way, continuous incorporation of glucose residues into the glycogen molecule by the skin was demonstrated. Specific activities of glycogen were greater by a factor of about 4 after perfusion with blood than after perfusion with saline to which 0.1 percent unlabeled glucose was added. After blood perfusions with C^{14}-glucose, 1 percent of the total cutaneous glycogen was found to be labeled.

Table 1. Effect of x-rays on incorporation of C^{14}-glucose into glycogen. A single dose of 800 r (250 kv, 1 mm Al, 30 ma) was given to one thigh 24 hours prior to perfusion. Uniformly labeled C^{14}-glucose (20 μc) was used for each perfusion experiment. The specific activities after saline perfusion are extremely high because in these experiments no carrier glucose was added to the saline to compete with the labeled glucose for incorporation into glycogen.

Activity in glycogen (count/min mg)		Increase (%)
Nonirridiated skin	Irridiated skin	
	Saline perfusion	
36,900	69,200	82
28,700	61,500	114
26,600	49,200	85
	Blood perfusion	
4,110	5,580	36
2,290	3,680	60
5,130	11,730	115
4,730	11,440	140

Degradation experiments on the radioactive cutaneous glycogen with β-amylase showed that, after blood perfusion with C^{14}-glucose, the specific activities of outer and inner tiers were approximately equal, indicating true biosynthesis of glycogen in the skin. After saline perfusion, in the majority of experiments, mainly the outer branches were found to be labeled. The average relation of specific activities in outer to inner tiers was 3:1. In a few experiments epidermis and dermis were separated after perfusion, and the radioactivity of glycogen was estimated separately in the two layers. The specific activity was 2 to 3 times greater in epidermal than in dermal glycogen after saline perfusion, but after blood perfusion the specific activities were about equal.

X-rays were used to study cutaneous glycogen after experimental depression of cellular metabolism. One thigh of the dog was irradiated in vivo with an 800-r dose from a Maxitron 250 machine (250 kv, 30 ma, and 1 mm of Al filtration), a dose corresponding to an "erythemal dose" in the dog (*5*). The skin flaps of both irradiated and nonirradiated thighs were perfused under identical conditions 24 hours later. Glycogen was isolated from both sides and specific activities were counted. Seven such experiments were performed. In all experiments the uptake of radioactive glucose into glycogen, per milligram, was found to be significantly greater on the irradiated side by an average of 90 percent (Table