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.

ANNE F. BYRNE
ROBERT B. KOCH*
Quartermaster Food and Container Institute for the Armed Forces, Chicago, Illinois

References and Notes

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* Present address: Honeywell Research Center, Minneapolis-Honeywell Regulator Co., Hopkins, Minn.

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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

1). The increased glucose uptake was not associated with a detectable change in glycogen content.

In four dogs the activities of phosphorylase and uridine diphosphate glucose–glycogen synthetase were compared on the irradiated and nonirradiated skin of the thighs. The irradiation was administered 24 hours before the assays were made. Phosphorylase activity was weaker on the irradiated side in all animals by an average of 20 percent. The synthetase activity was assayed with a uridine diphosphate glucose (UDPG) substrate labeled with C^{14} in the glucose moiety (*6*). This activity was enhanced in the irradiated skin in all experiments by an average of 23 percent. Thus it appears that a dysbalance of phosphorylase and synthetase activities, brought about by radiation, disturbs the dynamics of glycogen metabolism.

The effect of x-rays on the uridine diphosphate glucose–glycogen synthetase of the skin was also tested in mice 1, 2, 3, 4, 5, and 7 days after irradiation with one erythemal dose. A progressive increase of synthetase activity was observed up to 3 days after irradiation; then there was a gradual return to preirradiation values 7 days after irradiation.

Recently we found that ultraviolet irradiation with a hot mercury vapor lamp may have an effect similar to that of x-rays if the skin is analyzed 24 hours after irradiation with a minimal erythemal dose, but these results have not been consistent.

Experiments with a UDPG substrate labeled with C^{14} in the glucose moiety indicate that the UDPG pathway is the main route of glycogenesis in the skin. The in vivo synthesis of glycogen from labeled glucose and the in vitro synthesis of glycogen from labeled UDPG by the skin are of the same order of magnitude.

In histochemical studies of human skin performed in cooperation with the members of the biology department of Brown University (*7*), phosphorylase and uridine diphosphate glucose–glycogen synthetase were found in the same sites—that is, in the eccrine glands, hair follicles, muscle, and epidermis.

KENJI ADACHI*
DONALD C. CHOW
STEPHEN ROTHMAN
Argonne Cancer Research Hospital, University of Chicago, Chicago, Illinois

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* Present address: Department of Biochemistry, Yokohama University School of Medicine, Urafune-cho, Minami-ku, Yokohama, Japan.

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Barriers on the Surfaces of Dispersed Particles

Abstract. A study of some of the factors affecting the extractability of methyl stearate from aqueous methanol has provided evidence for the existence of a disperse phase from which the ester is released at a very slow rate. It is suggested that this phenomenon may be relevant to some problems of binding and activation of substrates in biological systems.

When methyl stearate is present in water in solution it is readily extracted by *n*-heptane. When it is prepared as a dispersion by the addition of water to a methanolic solution it is almost completely unextractable. As the methanol content of the aqueous phase approaches 70 percent, the visible dispersion gives way to a clear solution and extractability increases greatly, rising from about 25 percent in 62-percent methanol to nearly 90 percent in 75-percent methanol. The extractability in 50-percent methanol (about 10 percent) can be improved by addition of sodium chloride (Fig. 1, curve *A*). If sodium stearate is present, as an additional component, extractability is greatly reduced (Fig. 1, curve *B*).

These observations suggest that the particles of the dispersed phase are surrounded by a barrier that exerts a controlling effect on transfer between the dispersed phase and the surrounding medium. To study the process further, I prepared the dispersed phase in media which allowed a slow, moderately reproducible transfer of methyl stearate from the dispersion into the heptane layer.

Dispersions were prepared by adding 5 ml of 0.2-percent sodium chloride to 5 ml of a methanolic solution containing 1.0 mg of methyl stearate and 0.13 mg of sodium stearate, contained in glass-stoppered 20- by 200-mm test tubes. The faintly cloudy mixtures became more turbid during the first few minutes after mixing. After 10 minutes, 10 ml of *n*-heptane was added and extraction was begun by rocking the tubes 30° to either side of the horizontal at a rate of 25 cy/min (*1*). The rocking was interrupted every 3 minutes for a 3-minute period. At the end of the extraction period colorimetric ester determinations were performed on aliquots of the heptane layer.

Curve *A* of Fig. 2 illustrates the moderate rate of extraction observed when sodium stearate is not a component of the mixture. The pronounced decrease in rate in the presence of sodium stearate is apparent from curve *B* of Fig. 2.

The slow transfer of methyl stearate from the aqueous suspension to the upper layer may be due to a slow breakdown of the structure of the dispersed phase, or to a slow rate of equilibration between heptane and the dispersed phase, or to a combination of both of these factors.

If there is a two-way transport between the dispersed phase and the heptane, then addition of methyl stearate to the heptane prior to extraction could decrease the rate of accumulation of

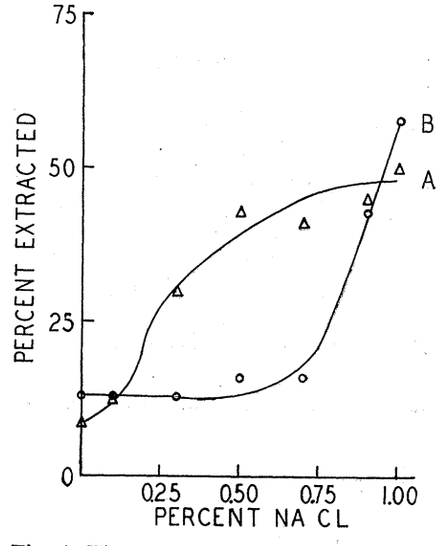

Fig. 1. Three-minute extraction of methyl stearate from a methanolic solution (see text).