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.

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19 JANUARY 1962

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14 November 1961

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

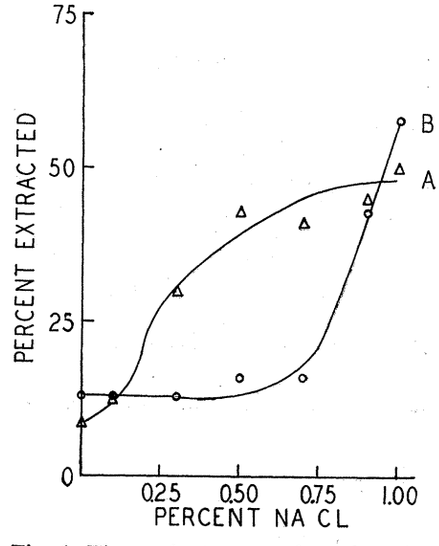

Fig. 1. Three-minute extraction of methyl stearate from a methanolic solution (see text).

the ester in the upper layer. Figure 2, curve *C* shows that this is indeed the case.

Microscopic examination of the cloudy lower layer obtained after 300 inversions (the 12-minute point in Fig. 2, curve *B*) reveals a range of particle size from 1 to 5 μ, with an average of about 3 μ. The total volume of the particles in 20 ml of lower phase, estimated from the average diameter and the total number, obtained by standard hematological technique, is about 0.015 cm^3.

It thus appears that the concentration of methyl stearate in the particles is about 50 mg/ml, and that the partition coefficient, concentration in particles/concentration in heptane, for equal volume of each, is about 1000 (*2*). Such an assertion implies that the structural organization of each dispersed particle is particularly suited to retention of the ester. A hypothetical model of such a particle is shown in Fig. 3. The hydrocarbon "tails" of the outer layers of the ester molecules are visualized as being oriented inward, so that the ester is essentially dissolved in a hydrocarbon environment. The polar carbonyl "heads" of the ester molecules would then constitute the inner surface layer of the particle. Water and methanol, the principal components of the surrounding medium, would then be adsorbed on this inner surface and constitute a barrier to disengagement of an ester molecule from the interior of the particle. Those regions of the surface containing adsorbed water would be expected to be a more effective barrier than regions containing adsorbed methanol. Adsorption of other solutes on the surface would be expected to alter the nature of the barrier. No conjecture concerning the thickness of the surface region is intended.

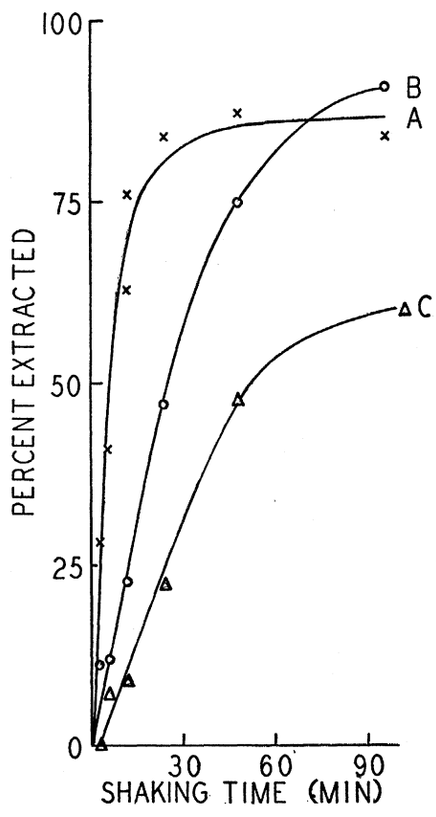

Fig. 2. Extraction of methyl stearate (see text). For curve *C*, 1.0 mg of methyl stearate was present in the heptane layer at zero time. The percent extraction was calculated from the increment of the zero time quantity.

The dispersed phase can be so altered by physical means that quantitative extraction is easily accomplished. Centrifugation after 30 inversions results in an upper layer containing all of the methyl stearate.

It thus appears that a water-insoluble compound can be dispersed in an aqueous medium in such a way that it equilibrates only very slowly with the surrounding medium. The significance of this observation for quantitative extraction is obvious. It may also have some relevance to the availability of substrates in the polydispersed systems commonly found in biological structures.

For example, if we were to encounter such incomplete extraction of a substance from a biological fluid, our usual interpretation would be in terms of binding of the substance with other non-extractable components of the fluid. The concept of unavailability because of retention within a stabilized disperse phase may, in some cases, provide a reasonable alternative "explanation."

Another example could be formulated as follows: Suppose that we added an esterase to a dispersed phase of methyl stearate and sodium stearate, and followed the rate of appearance of one of the products. Since the substrate is only very slowly in equilibrium with the aqueous medium, we might find that the rate of enzyme action is negligible until we add an "activator." Calcium ions have been reported (*3*) to activate pancreatic lipase. It is of interest, therefore, to determine whether the rate of release of an ester from the dispersed phase can be augmented by the addition of calcium chloride. To determine this, the 0.2-percent sodium chloride used to dilute the methanolic solution of methyl stearate and sodium stearate in one of the experiments described above was made $1.3 \times 10^{-4} M$ in calcium chloride. Extraction was performed as above by addition of *n*-heptane and inversion for 3 minutes. The methyl stearate found in the upper layer was 56 percent of the total and five times greater than the amount found when no calcium chloride was present (*4*). It seems likely, therefore, that in this case at least part of the function of the "activator" is to increase the rate of release of the substrate from the dispersed phase.

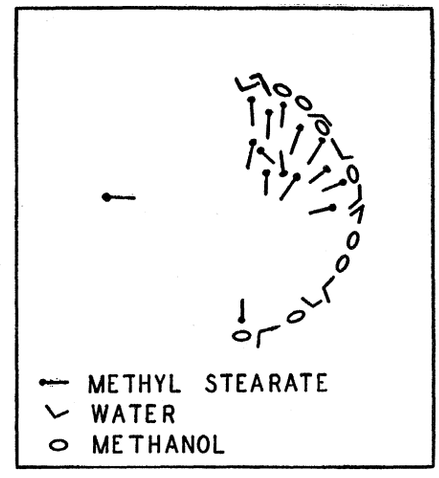

Fig. 3. Hypothetical model of certain particles described in the text.

It is generally recognized that the rate of enzyme action on heterogeneous systems is related to the nature of the emulsion. However, emulsification has generally been regarded as a device to present more surface to the enzyme (*5*).

The hypothesis presented here assumes a different effect. The substrate is contained within the dispersed phase in high concentration and protected from ready contact with the aqueous phase by some kind of a barrier. Its rate of release into the aqueous phase, and hence availability to the enzyme, can be altered by various physical means.

The observations presented in this paper are not intended as a proof of the hypothesis. However, it does seem as though some of the biological phenomena presently classed as storage,

binding, and activation could be "explained" just as readily by assuming slow equilibria between polydispersed phases and the surrounding aqueous medium. Further experimentation is needed to distinguish between these viewpoints (*6*).

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31 July 1961

Arlington Springs Man

Abstract. Bones of a man were found at a depth of 37 feet in waterlaid sediments on Santa Rosa Island, California, and dated by radiocarbon at 10,000 years before the present (B.P.). Two later occupational levels are dated at 7350 and 2090 years B.P. No artifacts are associated with the oldest bones, which are believed to be an accidental burial on the edge of a cienaga.

Human bones (*Homo sapiens*) protruding from a cut bank at a depth of 37 feet below the surface in Arlington Canyon, Santa Rosa Island, California, were discovered in 1959 by the 13th Santa Rosa Island expedition of the Santa Barbara Museum of Natural History. The bones were left *in situ*, and a thorough geological study was made of the Santa Rosa Island formation (*1*), in which they occur.

In 1960 a field conference was held, attended by prominent scholars representing archeology, paleontology, geology, geography, and oceanography (*2*), who viewed the bones *in situ*. Limited excavation and the collection of soil and radiocarbon samples were performed by various members of the party.

Following the conclusion of the field conference, the human bones, which consisted of two femora, were removed in a block, and excavation was carried out designed to develop an understanding of the rather complicated stratigraphy.

This is a stratified site exposed in the side of Arlington Canyon. On the surface is an Indian shell midden and a cemetery [C^{14} date, 2090 ± 200 years B.P. (M-1147)], which is underlain by several feet of tan, sandy silts, followed by a heavy black humus zone at about 10 feet, containing red abalone shells (*Haliotis rufescens*). This is separated from another black humus zone by a 1-foot layer of white sand. From the top of this black humus zone, and the bottom of the white sand, a red abalone shell was collected by J. B. Griffin (*3*) and dated at 7350 ± 350 years B.P. (M-1133) by H. R. Crane, University of Michigan–Memorial Phoenix Project Radiocarbon Laboratory.

About 5 feet below this dark humus layer are horizontal banded silts, and a dark humus line which dips sharply to the south, marking the old land surface, and levels out at 37 feet below the present surface of the valley fill, where it evidently was the bottom of a small cienaga, or hillside marsh, since filled with fine buff-colored silts, and interlined with narrow humus bands.

The human bones were found in this humus zone, along with thousands of bones of the island field mouse (*Peromyscus sp.*) and iron-stained casts of reeds. The human bones were lying at an angle to each other, and parallel to the sloping stratigraphy, and both showed pre-burial weathering. It is apparent that the bones do not represent a formal burial, but rather an accidental deposition along an edge of a small cienaga filled with rushes or cattails. The great number of mouse bones may be accounted for by the animals' struggling through these reeds until they became exhausted and drowned, a condition which may be observed today in many cienagas. About 6 feet below the bone layer is an active spring which forms a modern cienaga, and a number of other cienagas occur in the immediate region.

When the human bones were first discovered, a small sample of organic earth containing flecks of charcoal was removed adjacent to the bones, and radiocarbon dated by W. S. Broecker of Lamont Geological Observatory (L-568-A) at 10,400 ± 2,000 years B.P. (*4*). The high plus-or-minus factor was due to insufficiency of the sample. In May 1961 a second sample (L-650) was collected by Broecker, William Farrand, and me, and the date of 10,000 ± 200 years B.P. (*5*) was secured as an average of several runs.

Well-formed small gypsum crystals in rose form are found immediately above the dated level. Dwarf mammoth bones are found within 150 feet on either side, and Indian artifacts are found in the upper 10± feet of the deposits. However, no artifacts or mammoths have been found *directly* associated with these human bones.

Elsewhere on the island, a repeating pattern of burned mammoth bones, fire areas, abalone shells at considerable depth in terrestrial deposits, and chipped stone tools are found, and have been dated variously from 12,500 back to 29,700 years B.P. (L-290-R). The evidence suggests the presence of man during the Wisconsin glacial stage (*6*, *7*).

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7. This report is Santa Barbara Museum of Natural History contribution No. 2 and Western Speleological Institute contribution No. 18. This work is the result of long-term activities, sponsored by the trustees of the Santa Barbara Museum of Natural History and the Western Speleological Institute, with the cooperation of Vail and Vickers Co., owners of Santa Rosa Island; the Adventurers' Club of Chicago, the Wenner-Gren Foundation for Anthropological Research; the Max C. Fleischmann Foundation of Nevada; and the Leighton Wilkie Foundation. Radiocarbon dating was done by Lamont Geological Observatory, Columbia University, and the University of Michigan–Memorial Phoenix Project Radiocarbon Laboratory.

23 October 1961