(5 or 10 mg/kg) also failed to produce conditioned avoidance responses in dogs conditioned with *l*-epinephrine.

This type of conditioning can be due to peripheral physiological effects having an afferent influence centrally, or to direct drug effects on the central nervous system, or to aspects of both (*4*). The experiments involving pressure in jejunal Thiry-Vella loops suggest that peripheral stimulation alone can act as a conditioned stimulus. Physiological effects produced by *l*-epinephrine, *l*-norepinephrine, or acetylcholine can play a role in the development, as well as the maintenance, of a conditioned avoidance response in dogs.

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20 November 1959

Glucose-6-Phosphatase and the Exchange of Glucose with Glucose-6-Phosphate

Abstract. An equation is presented which makes it possible to estimate the exchange activity of glucose-6-phosphatase, as a percentage of the hydrolytic activity, for a given concentration of substrate and acceptor. The quantitative significance of the exchange-inhibition phenomenon is discussed.

Hepatic microsomal glucose-6-phosphatase catalyzes the exchange of glucose with glucose-6-phosphate (G-6-P), and this exchange is correlated with an inhibition of the hydrolytic activity (*1*). The purpose of this communication is to evaluate, in so far as possible, the significance of this exchange-inhibition phenomenon. Neither the exchange nor the inhibition appears to be quantitatively significant for normal hepatic carbohydrate metabolism, but the exchange activity could be important in liver slice experiments of the type described by Cahill *et al.* (*2*) where high concentrations of glucose have been used and where it has been assumed that the conversion of glucose to G-6-P is carried out solely by an enzymatic phosphorylation using a phosphate donor such as adenosine triphosphate.

The exchange activity equals the inhibition of the hydrolytic activity, and the inhibition can be estimated by using Eq. 1 (*3*, *4*) or, for a typical set of conditions, from Fig. 1.

$$\frac{v_i}{v} \cdot 100 = \frac{1+\frac{Km}{S}}{\left(1+\frac{I}{K_i}\right)+\left(\frac{k_2}{k_1}\cdot\frac{I}{K_i'}\cdot\frac{1}{S}+\frac{Km}{S}\right)} \cdot 100$$

where v is the velocity in the absence of inhibitor; v_i is the velocity in the presence of inhibitor; S is the substrate concentration; I is the inhibitor concentration; Km is the Michaelis constant; K_i' is the concentration of inhibitor that gives 50 percent inhibition when $S = \infty$; and k_2/k_1 is the dissociation constant of the enzyme-substrate complex.

It should be pointed out that Km ($6.1 \times 10^{-3}M$), K_i' ($8.8 \times 10^{-2}M$), and k_2/k_1 (8.3×10^{-4}) were determined at *p*H 6.0, which is the *p*H optimum of glucose-6-phosphatase. The assumption that these constants can be used to estimate the exchange-inhibition phenomenon *in situ* (*p*H of 7.4) is based on the observation that the percent inhibition by glucose is constant over a wide *p*H range (*3*). In summary, Eq. 1 can be used to calculate the percentage activity for a given concentration of substrate and inhibitor. The absolute quantity of inhibition (exchange) would also require an estimate of the velocity of the hydrolytic activity (v or v_i).

The inhibition by glucose of hepatic glucose-6-phosphatase in the rat, using the data of Steiner and Williams (*5*) for G-6-P (normal, 0.47 μmole/gm; diabetic, 0.13 μmole/gm) and glucose (normal, 5.9 μmole/gm; diabetic, 30 μmole/gm), would be 1.3 percent in the normal and 5.0 percent in the diabetic. Even if glucose-6-phosphatase were the limiting step in gluconeogenesis, this estimated inhibition does not appear to be significant. Under these conditions the exchange activity would also be unimportant, since the transfer of a phosphoryl group from one glucose to another is of no consequence. Molecules which are structurally related to glucose also act as acceptors and inhibitors, but glucose is the best naturally occurring inhibitor and acceptor. Furthermore, the "glucose analogues," known to be present in liver, are present in low concentrations, and their 6-phosphate esters can be formed more readily by other pathways.

In the presence of C^{14}-G-6-P or C^{14}-glucose, the exchange reaction catalyzed by glucose-6-phosphatase (Eq. 2) would

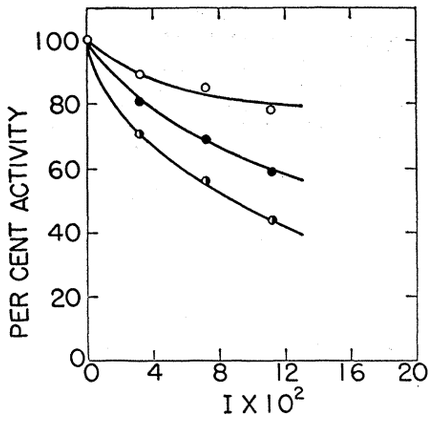

Fig. 1. Inhibition of glucose-6-phosphatase by glucose at different concentrations of G-6-P. The open circles, solid circles, and half-open circles represent data obtained in the presence of $1 \times 10^{-3}M$, $8.0 \times 10^{-3}M$, and infinite G-6-P, respectively. Values at an infinite concentration of G-6-P were obtained from the ordinate intercept of a Lineweaver-Burke plot (reciprocal velocity versus reciprocal substrate concentration). The experimental data used for this figure have been taken from the results for the microsomes prepared from an alloxan-diabetic rat given in Fig. 3 of a previous publication (*3*), but the inhibition data for microsomes from diabetic and normal animals are indistinguishable.

be indistinguishable from the hydrolysis of G-6-P by glucose-6-phosphatase (Eq. 3) or the phosphorylation of glucose by a kinase (Eq. 4).

$$\text{G*-6-P} + \text{Glucose} \rightleftharpoons \text{Glucose*} + \text{G-6-P} \quad (2)$$

$$\text{G-6-P} + \text{H}_2\text{O} \rightarrow \text{Glucose} + \text{orthophosphate} \quad (3)$$

$$\text{Glucose} + \text{ATP} \rightarrow \text{G-6-P} + \text{ADP} \quad (4)$$

The actual net effect of the exchange reaction on the rate of transfer of labeled G-6-P to glucose (Eq. 3) would be negligible, since the transfer by the exchange reaction is effectively canceled by the concomitant inhibition of the hydrolytic activity. The conversion of glucose to G-6-P by the exchange reaction, however, would be an apparent kinase (Eq. 4) and the contribution of the exchange reaction to the total apparent kinase could vary from a minor to a major rate, depending upon the conditions. It also should be emphasized that the net transfer of labeled material per unit time, with an exchange reaction, depends upon the difference in specific activity between the donor and acceptor.

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4. Eq. 1 is the quotient of the steady-state rate equations in the presence and absence of inhibitor for the following mechanism:

$$E + \text{G-6-P} \rightleftharpoons E - \text{G-6-P} \rightleftharpoons E - P + \text{Glucose}$$
$$H_2O \downarrow$$
$$E + \text{Orthophosphate}$$

The inhibition of glucose-6-phosphatase by glucose is not one of the classical types, competitive, noncompetitive, or uncompetitive [P. W. Wilson, *Respiratory Enzymes,* H. A. Lardy, Ed. (Burgess, Minneapolis, 1949), pp. 16–56], and is best described by the above mechanism and its rate equation (Eq. 3).
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10 November 1959

Mean Lifetime of Free Radical Chains Determined by a Flow Technique

Abstract. When radiolysis is induced in chloral hydrate solution flowing through a glass coil wound around a lead brick and irradiated from one side only, the rate of reaction depends on the flow rate. The effect resembles that for intermittent irradiation and makes it possible to estimate the mean lifetime of the free radical chain.

Intermittent irradiation techniques have been widely used in photochemistry (*1*) and to a lesser extent in radiation chemistry (*2*) for the determination of mean life-times of free radical chains. The usual method of securing intermittency is to employ a rotating sector between the source of radiation and the material being studied. Alternative methods include the reciprocating source method (*3*), in which the source is repeatedly removed from the reaction vessel and replaced in it after a short time interval, and the rotating source method, in which the cells containing the solution to be irradiated are mounted on a wheel which is rotated past an aperture in a shield containing a Co^{60} source (*4*). A somewhat different approach to the problem has been reported by Goldfinger and Heffelfinger (*5*). Radical formation was initiated in a mixture of styrene and benzoyperoxide by exposure to a mercury arc. After the stream of material had passed the illuminated region it fell freely, thus allowing chains to grow. Chains were terminated by means of a picric acid inhibitor, and by varying the distance between the source of radiation and the picric acid solution, the half-life of the styrene free radical chain could be determined.

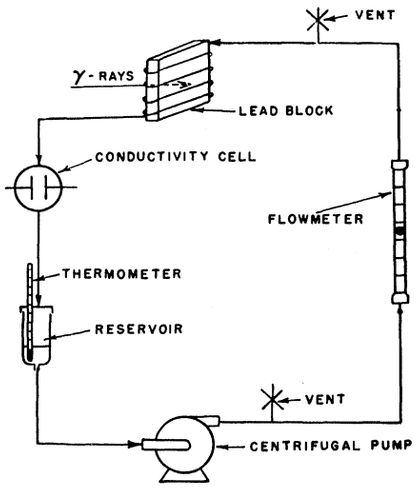

Fig. 1. Diagram of flow system; a 90-c Co^{60} source is housed in the lead cylinder.

Yet another method of achieving intermittent irradiation is to use a flow system in which the material flows past the Co^{60} source in such a way that it receives successive bursts of radiation. A brief description of the application of this method to chloral hydrate solutions follows. The mean lifetime of the free radical chains in 1*M* chloral hydrate solutions had previously been determined to be about 0.1 second by Freeman *et al.* (*6*), who used a rotating sector technique and gamma rays. The corresponding experiments for beta rays have also been reported (*7*).

Fisher U.S.P. chloral hydrate was used without further purification, and the amount of acid formed on irradiation was measured with a conductivity cell (*8*). For details see (*9*).

A 90-c Co^{60} source, housed in a concrete irradiation cave, was used as a source of gamma rays (*10*). The radiation dose was measured with the usual Fricke ferrous sulfate dosimeter and was found to be approximately 640 rad/min. The flow system is indicated diagrammatically in Fig. 1. The solution was pumped by means of a Cole-Parmer polyethylene 1/35 hp centrifugal pump through a flow meter, a reaction cell, a conductivity cell, and a reservoir. The reaction cell consisted of a glass coil of ten turns wrapped around a lead block in such a manner that a given volume element of solution would be subject to alternate "dark" and "light" periods. Flow rates were varied from 10 to 500 ml/min. The inside diameter of the tubing was 5.1 mm. The lengths of tubing exposed ("light" period) and unexposed ("dark" period) were 13.7 and 20.4 cm, respectively. Thus, for a flow rate of 500 ml/min, the "light" and "dark" periods were 0.34 and 0.52 seconds, respectively.

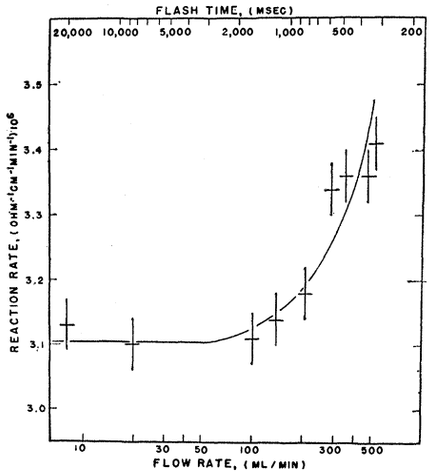

Fig. 2. Change in reaction rate versus flow rate.

Figure 2 shows the change in rate of reaction with rate of flow for 0.5*M* chloral hydrate solution at 25°C and at a *p*H of 4.60. The curve resembles that obtained by the rotating sector method (*6*) and indicates that the irradiation time in the region of reaction-rate increase is comparable to what would be expected if there were a strict parallel between intermittency in space and time. If we assume a complete parallel, the indicated mean lifetime of the chain is of the order of 1/5 second. Because of the dimensions of the reaction zone (4 by 4 in.) and its proximity to the Co^{60} source, the dose rate was not uniform, and thus a more detailed analysis of the experiment is not warranted. However, the results do serve to illustrate the possibilities of the method, which would appear to have wide applicability in both radiation chemistry and photochemistry.

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2 November 1959