erals," *Natl. Acad. Sci.–Natl. Research Council Publ. No. 456* (1956), p. 322.
3. W. P. Kelley, *Am. Soil Survey Assoc. Bull.* **15**, 45 (1935); L. D. Whittig, *Soil Sci. Soc. Am. Proc.* **23**, 469 (1959).
4. Henneke and Josephene, unpublished; P. J. Zinke, thesis, University of California (1956).
5. I. Barshad, *Soil Sci.* **61**, 432 (1946).

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Epinephrine, Norepinephrine, and Acetylcholine as Conditioned Stimuli for Avoidance Behavior

Abstract. Conditioned leg-flexion responses in dogs were developed with electric shock as an unconditioned stimulus and intestinal stimulation or the effects of injections of various drugs as conditioned stimuli. It is concluded that physiological effects can play a role in the development and maintenance of conditioned avoidance behavior.

Many investigators have suggested the importance of physiological correlates of behavior (*1*). However, few workers have considered the possible role of physiological changes as stimuli (*2*). The studies reported here were conducted to determine whether physiological changes produced by *l*-epinephrine, *l*-norepinephrine, acetylcholine, or stimulation of a Thiry-Vella jejunal loop can become conditioned stimuli in avoidance conditioning.

Beagles, surgically prepared with Thiry-Vella jejunal loops, were restrained in a harness in a soundproof chamber. A balloon inserted into the Thiry-Vella loop could be inflated remotely with 10 cm-Hg pressure. The conditioned stimulus was a balloon inflation lasting 2 seconds, terminated by the unconditioned stimulus, a brief electric shock (of intensity sufficient to cause leg flexion) delivered to the left hind leg. After an appropriate number of trials, balloon pressure *alone* consistently produced leg flexion, the conditioned avoidance response.

Other experiments were conducted to determine whether physiological changes produced by pharmacological agents could also act as conditioned stimuli in avoidance conditioning. A polygraph simultaneously recorded respiration, electrocardiogram, and intestinal activity of Thiry-Vella jejunal loops. The electrocardiographic recordings were made with surface electrodes fixed over the heart apex and the right paravertebral line (modified CR_{CL} lead) in order to minimize artifacts from gross body movements (*3*). Two fine polyethylene catheters were inserted into the external saphenous vein of the right hind leg and attached to syringes outside the soundproof chamber. These catheters permitted the injection of *l*-epinephrine, *l*-norepinephrine, or acetylcholine under remote control.

In each conditioning trial, electric shock was delivered to the left hind limb 30 seconds after the start of the injection. Injections were spaced 5 to 10 minutes apart, to allow time for physiological responses to return to normal levels. No stimuli, other than the physiological effects produced by the injected agents, preceded the shock. Preliminary experiments demonstrated that the monitored physiological responses were generally maximal 30 seconds after injection. Leg flexions occurring within the 30-second interval automatically prevented the shock. Prior to conditioning, none of the agents studied produced leg flexion. The effects of *l*-epinephrine (10 μg/kg), *l*-norepinephrine (10 μg/kg), or acetylcholine (20 μg/kg) came to serve as conditioned stimuli for the avoidance response after an appropriate number of training trials.

Figure 1 shows representative curves for the development of avoidance behavior with the various types of conditioned stimuli. For comparative purposes, other dogs were conditioned to an auditory stimulus (tone) as the conditioned stimulus. Differences in rates of acquisition are apparent; however, direct comparison of these rates is limited because the patterns of stimuli vary, due to differences in dose and in the intensity of effects. Figure 2 is a polygraphic recording of a representative trial, showing physiological effects and conditioned leg-flexion avoidance response. Jejunal activity, respiration, and electrocardiograph were monitored for indications of the occurrence of physiological changes and the temporal relationships of these changes to conditioned leg flexion. It was observed that physiological changes consistently preceded the occurrence of the avoidance response.

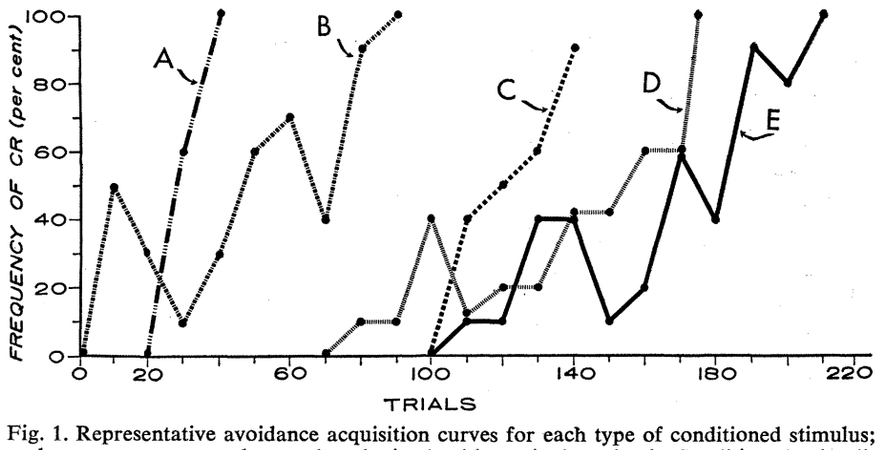

Fig. 1. Representative avoidance acquisition curves for each type of conditioned stimulus; each curve represents the results obtained with a single animal. Conditioned stimuli: *A*, tone; *B*, acetylcholine; *C*, *l*-norepinephrine; *D*, jejunal pressure; *E*, *l*-epinephrine. Different dogs were used in each experiment. Each point represents the percentage of conditioned leg flexions occurring in blocks of ten successive trials.

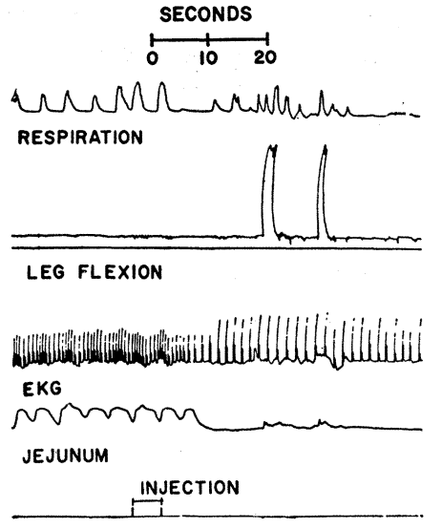

Fig. 2. Polygraphic record showing a trial in which the dog avoided shock. The leg flexion (avoidance response) followed the compensatory bradycardia and decreased intestinal motility produced by the injection of epinephrine.

After dogs had been conditioned to the effects of 10 μg of *l*-epinephrine per kilogram, doses of 1 or 2.5 μg of *l*-epinephrine per kilogram could produce avoidance responses. In a similar manner, dogs conditioned to 20 μg of acetylcholine per kilogram manifested avoidance responses after the injection of 10 μg of acetylcholine per kilogram. Saline, in comparable volumes, was injected on a random basis with all drug injections through the second catheter; saline injections never produced an avoidance response. These control injections eliminate the possibility that local sensation at the site of injection or volume changes acted as the conditioned stimulus. Injections of glucose

(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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References

1. W. B. Cannon, *Bodily Changes in Pain, Hunger, Fear and Rage* (Branford, Boston, Mass., ed. 2, 1953); F. Dunbar, *Emotions and Bodily Changes* (Columbia Univ. Press, New York, ed. 4, 1954).
2. K. M. Bykov, *The Cerebral Cortex and the Internal Organs*, W. H. Gantt, Trans. (Chemical Publishing Co., New York, 1957); G. Razran, *Science* **128**, 1187 (1958).
3. N. Lannek, *A Clinical and Experimental Study on the Electrocardiogram in Dogs* (Hoeggströms, Stockholm, 1949).
4. A. B. Rothballer, *Pharmacol. Revs.* **11**, 494 (1959); H. Weil-Malherbe, J. Axelrod, R. Tomchick, *Science* **129**, 1226 (1959).

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 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).

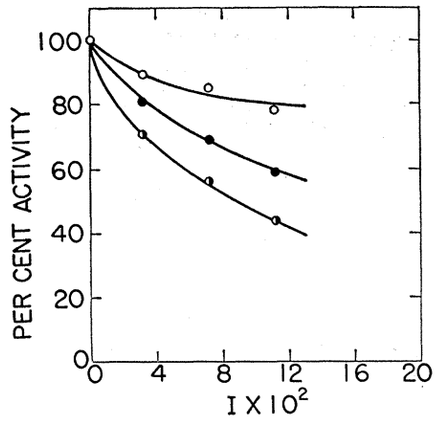

Fig. 1. Inhibition of glucose-6-phosphatase by glucose at different concentrations of G-6-P. The open circles, solid circles, and half-open circles represent data obtained in the presence of $1 \times 10^{-3}M$, $8.0 \times 10^{-3}M$, and infinite G-6-P, respectively. Values at an infinite concentration 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.

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

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

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

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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