Homogenization of Brain Tissue

Homogenization of cerebral tissue with Potter-Elvehjem tissue homogenizers may be incomplete because partially macerated shreds of brain adhere to the end of the rotating pestle. Complete homogenization may be obtained if a sphere 3 to 4 mm in diameter, carved out of a piece of black rubber (such as the wall of pressure tubing), is placed in the homogenizer tube containing the brain tissue, before insertion of the pestle. The rubber sphere rotates between the end of the whirling pestle and the inside of the homogenizer tube and dislodges tissue that may stick to the pestle. The rubber introduces no error, since it is insoluble and of high enough specific gravity to remain at the bottom of the aqueous suspension.

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Properties of Clearing Factor Obtained from Rat Heart Acetone Powder

The observation by Hahn (*1*) that injected heparin caused rapid clearing of alimentary lipemia led to the discovery of an enzyme, clearing factor, in the serums of such animals. This "*in vivo* clearing factor" will also "clear" lipemic serums or coconut oil emulsions *in vitro,* and it has been the general practice to assay for the enzyme by this decrease in the turbidity of fat emulsions (*2, 3*).

A preliminary experiment, in collaboration with Robert S. Gordon, Jr., demonstrated that the *in vitro* clearing of a coconut oil emulsion by *in vivo* clearing factor is associated with the hydrolysis of the triglyceride to glycerol and fatty acids. In the experiments discussed here, glycerol production was followed by the procedure of Lambert and Neish (*4*), modified so that 0.5 to 10 μg of glycerol in an aliquot of 0.05 ml could be determined accurately.

It has been found that an ammonia extract of an acetone powder of *normal* rat heart (1 ml of 0.025*N* NH_3 per 50 mg of powder) will catalyze the hydrolysis of chylomicrons. The rate of hydrolysis is stimulated by the addition of small amounts of heparin to the reaction vessel (Table 1, vessels 1 and 2). Further, if the acetone powder extract (APE) is preincubated with either 1*M* NaCl or 10^{-5}*M* protamine for 30 min at 0°C, all of its enzymatic activity is lost. Therefore, both the basal activity and that induced by heparin behave in a manner analogous to *in vivo* clearing factor (*3*).

The APE, with or without added heparin, catalyzes the hydrolysis of coconut oil only very slowly, if at all. The initial rate of glycerol production from chylomicrons is at least 40 times the rate of glycerol production from coconut oil (Table 1, vessels 3 and 4). In the presence of *normal* serum, however, coconut oil is hydrolyzed at half the rate of chylomicrons and if the serum and coconut oil are preincubated at 38°C (but not at 0°), the coconut oil is hydrolyzed as rapidly as the chylomicrons (Table 1, vessels 5 and 6). Serum does not stimulate the hydrolysis of chylomicrons, and taurocholate will not replace serum in the activation of coconut oil. Alcohol and ultracentrifugal fractionations of whole normal serum indicate that the activation of coconut oil is due to the alpha and beta lipoproteins only. Heparin stimulates the hydrolysis of "activated" coconut oil three- or fourfold.

In contrast to these results obtained with the rat heart APE, pancreatic lipase is neither stimulated by heparin nor inhibited by NaCl or protamine. Further, in the absence of serum, it hydrolyzes coconut oil at 5 times the rate it hydrolyzes chylomicrons.

It should be noted that, although in the experiments reported in Table 1 both albumin and Ca^{++} were used to accelerate the reaction, either may be used alone. The albumin, then, is not an obligatory component of

Table 1. Substrate specificity of clearing factor and "activation" of coconut oil.

Vessel	Substrate*	Additions at zero time†	Glycerol production (μM)	
			30 min	60 min
1	Chylomicrons (0.1 ml)	APE (0.1 ml)	0.11	0.26
2	Chylomicrons (0.1 ml)	APE (0.1 ml) + heparin (10 μg, 100 units/mg)	.24	.50
3	Chylomicrons (0.1 ml)	APE (0.2 ml) + heparin (10 μg)	.56	.92
4	Coconut oil (0.1 ml)	APE (0.2 ml) + heparin (10 μg)	.01	.04
5	Coconut oil (0.1 ml)	APE (0.2 ml) + heparin (10 μg) + normal serum (0.2 ml)	.20	.60
6	Coconut oil (0.1 ml) + normal serum (0.2 ml)‡	APE (0.2 ml) + heparin (10 μg)	.70	1.16

* Both the chylomicrons and coconut oil contained approximately 15 μM of neutral fat per milliliter.
† 0.2 ml of albumin (10 percent), 0.02 ml of $CaCl_2$ (1*M*) and 0.28 ml of NH_3—NH_4Cl buffer (0.25*M*, *p*H 8.5) were added to all vessels at zero time. All vessels contained a total volume of 1 ml.
‡ The coconut oil and normal serum were preincubated together for 30 min at 38°C before the other additions were made.

the system, but it can serve as a fatty acid acceptor (*5*) and is replaceable by other acceptors.

It would appear from the foregoing data that (i) clearing factor is primarily a tissue enzyme and is present in normal rats without the injection of heparin; (ii) clearing factor is a heparin-activated "lipoprotein lipase" that catalyzes the hydrolysis of chylomicrons but not simple triglycerides; and (iii) the incubation of coconut oil with serum makes it available as a substrate for clearing factor perhaps through the formation of a protein-triglyceride complex.

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Exposure Duration in the Perception of Shape

Visual perception is characterized by a compromise between the physical properties of the retinal image and the tendency to recognize the color, size, and shape of objects independently of the specific retinal image pattern. This tendency is described by the terms *color, size,* or *shape constancy,* which emphasize the stability of perception under varying conditions of observation (*1*). For example, when a circular object is presented at various angles to the line of vision, subjects will match the object with ellipses that are more circular than would be predicted from the geometry of the retinal image (*2*). The matches tend to approach the "law of shape constancy," a theoretical condition in which perceived circularity is independent of the angle of inclination. The data represented by the circles in Fig. 1, obtained for a white disk at a 1.0-sec exposure duration, demonstrate the effect. Matches for all subjects lie above the line representing the "law of the retinal image," a theoretical condition based solely on geometric relationships, and tend by varying degrees, depending upon the subject, to approach the line representing shape constancy.

If the time the subject is allowed to view the test object is reduced to 0.01 sec, crosses of Fig. 1, the matches no longer exhibit the "constancy" effect. The data for all subjects are in good agreement with predictions made on the basis of geometric theory. Similar results were obtained using a half-dollar coin as test object and, for either the coin or disk, comparing the 1.0- with a 0.1-sec exposure duration.

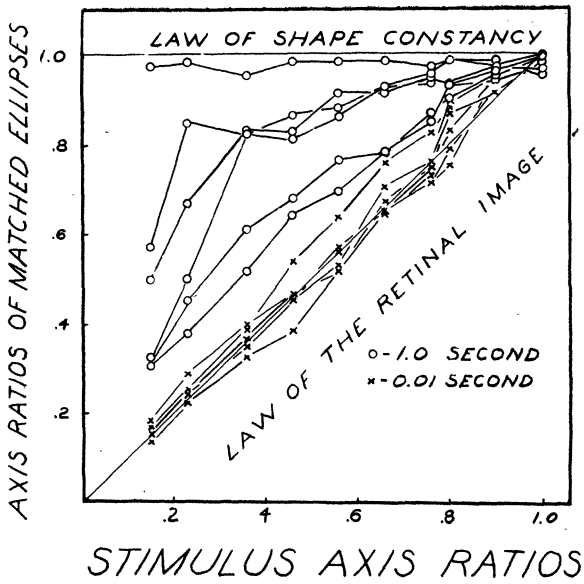

Fig. 1. Axis ratios of matched ellipses as a function of the stimulus axis ratios of a disk test object presented at various angles of inclination for two durations of exposure.

The absence of constancy effects and the resulting perceptions that are predictable from retinal image theory have been previously demonstrated for size and brightness judgments (*3–5*). In all cases, constancy was destroyed by the removal from the visual field, by means of a reduction screen or similar device, of "additional" stimuli other than the discriminative stimulus. The results of the present study (*6*) demonstrate that, in the case of shape discrimination, reduction of exposure time is perceptually equivalent to the removal of such "additional" stimuli which are necessary for constancy judgments.

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