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.

BILL GAROUTTE
SARAH CHEIKER

Departments of Neurology and *Anatomy, University of California School of Medicine, San Francisco*

12 July 1954.

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.