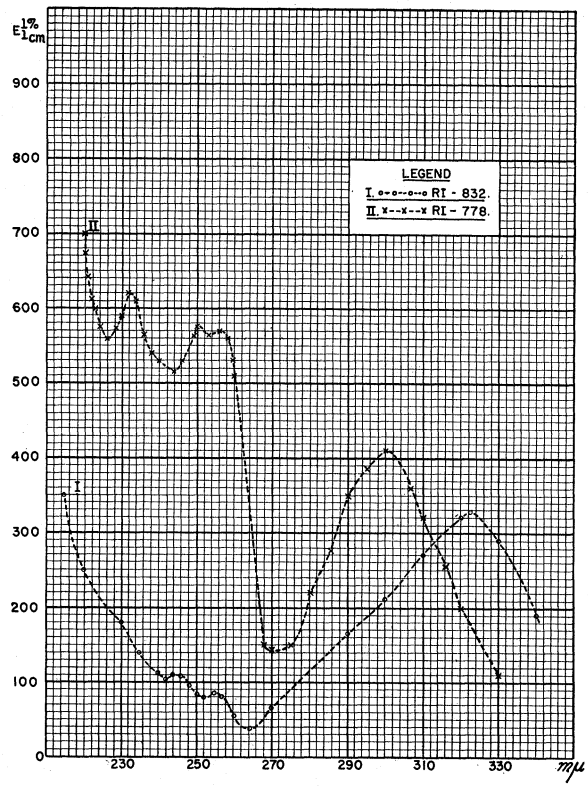

FIG. 1.

given in Fig. 1 is similar to the curve obtained by Cavallito and Rockwell. Anal:[1] C, 66.84%; H, 7.28% (calcd for $C_{21}H_{28}O_6$: C, 66.98%; H, 7.50%). Its molecular weight, determined cryoscopically, is 373 (calcd, 376.45).

Insofar as we are aware, this is the first description of the isolation of a crystalline compound from the amorphous visnagan fraction of khella extracts that has the ultraviolet absorption properties of the main amorphous product. Three preparations of the compound (RI-832) have been made, two of which have been tested by the Hazleton Laboratories, of Falls Church, Va., in isolated rabbit hearts and found to possess a vasodilating effect approximately eight times that of khellin (Table 1, RI-832 and RI-832-3).

The eluate having the specific rotation $[\alpha]_D + 50°$ also yielded a crystalline compound (RI-778), melting point, 157°–159°, and $[\alpha]_D + 96°$ after repeated recrystallization from ethyl acetate. Anal: C, 65.15%; H, 5.80% (calcd for $C_{15}H_{16}O_5$: C, 65.20%; H, 5.84%). The molecular weight by cryoscopic method was found to be 276 (calcd, 276.28). The ultraviolet absorption spectrum of this substance is given in Fig. 1.

This compound may correspond to the "crystalline impurity" of Cavallito and Rockwell, since it also yields a hydrochloride with ethereal hydrochloric acid. The hydrochloride is markedly different from the oxonium salt of khellin or visnagin in being readily soluble in excess ether. The ultraviolet absorption closely resembles the absorption spectrum reported by Davies and Norris (*4*) for dihydrokhellin. The basic structure of the compound seems, therefore, to be a dihydrofuranochromone. Its vasodilatory activity is given in Table 1.

[1] Microanalyses by Schwarzkopf Microanalytical Laboratory, Middle Village, L. I., N. Y.

References

1. HUTTRER, C. P., and DALE, E. *Chem. Revs.*, **48**, 543 (1951).
2. SAMAAN, K. *Quart. J. Pharm. Pharmacol.*, **4**, 14 (1931).
3. CAVALLITO, C. J., and ROCKWELL, H. E. *J. Org. Chem.*, **15**, 820 (1950).
4. DAVIES, J. S. H., and NORRIS, W. L. *J. Chem. Soc.*, 3195 (1950).

Manuscript received October 12, 1951.

Association of Enzymatic Activity with Submicroscopic Particles

Alton Meister

National Cancer Institute,
National Institutes of Health, Bethesda, Maryland

Considerable study of the mitochondrial fraction of tissues has shown that these particulates possess the major portion of the activity of a number of tissue enzyme systems (*1*). Although preparations of submicroscopic particles (microsomes) have been found to possess a few enzyme activities in relatively high concentration, previous studies (*1*) have not indicated exclusive localization of an enzymatic activity in these particles. In the course of investigations on the hydrolysis of triacetic acid lactone by rat kidney homogenates, it was noted that almost all the lactonase activity could be sedimented at $18{,}000 \times g$ (*2*). Fractionation according to the methods of Schneider (*3*) and Schneider and Hogeboom (*4*) indicated that about 70% of this activity was associated with the submicroscopic particles, whereas the mitochondrial frac-

TABLE 1

INTRACELLULAR DISTRIBUTION OF TRIACETIC ACID LACTONASE IN RAT KIDNEY

Fraction	Expt. 1*		Expt. 2†		
	Activity‡	Percentage	Activity‡	Percentage	Activity: Nitrogen‖
Whole homogenate	8.95	(100)	7.94	(100)	0.164
Nuclei + whole cells	1.66	18.5	1.55	19.5	.139
Mitochondria	.667	7.5	.474	6.0	.054
Submicroscopic particles	6.34	70.9	5.55	70.0	.607
Supernatant	1.00	11.2	.700	8.8	0.040
Sum of fractions	9.67	108	8.27	104	—

* Fractionated by the procedure of Schneider (*3*).
† Fractionated by the procedure of Schneider and Hogeboom (*4*) using 0.25 *M* sucrose.
‡ Activity expressed in terms of the rate constant, $k \times 10^3$ (*5*).
‖ $k \times 10^3$/mg nitrogen.

tion possessed approximately 7% of the total activity (Table 1). Since the slight activity of the mitochondrial and supernatant fractions can probably be attributed to contamination by submicroscopic particles, the data suggest that the lactonase activity is an exclusive function of microsomes. It is possible that other systems may also be entirely localized in these tissue particles (see, for example, *6, 7*).

The present data, which were obtained in the course of investigations on polyketo acids, are presented in the belief that they may be of interest to those concerned with the study of cellular particulates. The finding of localization of enzymatic activity in the submicroscopic particle fraction should be considered in the light of the suggestion (*8*) that these submicroscopic particles are formed as a result of mitochondrial disintegration.

References

1. SCHNEIDER, W. C., and HOGEBOOM, G. H. *Cancer Research*, **11**, 1 (1951).
2. MEISTER, A. *J. Biol. Chem.*, **178**, 577 (1949).
3. SCHNEIDER, W. C. *Ibid.*, **165**, 585 (1946).
4. SCHNEIDER, W. C., and HOGEBOOM, G. H. *Ibid.*, **183**, 123 (1950).
5. MEISTER, A. *J. Natl. Cancer Inst.*, **10**, 75 (1949).
6. PRICE, V. E., *et al. J. Biol. Chem.*, **181**, 535 (1949).
7. HERS, H. G., *et al. Bull. soc. chim. biol.*, **33**, 21 (1951).
8. HARMON, J. W. *Exptl. Cell. Research*, **1**, 382, 394 (1950).

Manuscript received September 24, 1951.

Membrane Resistance Changes in the Course of Axonal Spikes Modified by Low Na^+ Concentration[1]

Harry Grundfest, Rita Guttman, Charles D. Hendley, and Irwin B. Wilson

Department of Neurology, College of Physicians & Surgeons, Columbia University, New York, Department of Biology, Brooklyn College, Brooklyn, New York, and *Department of Neuropsychiatry, Tulane University, New Orleans*

The size, duration and propagation velocity of the axonal spike are known to be altered by changing the environmental Na^+ concentration (*1*). This means of reversibly altering the properties of the spike affords an opportunity to study the course of the correlated resistance changes of the excitable membrane during activity of the nerve fiber.

The resistance change was measured on cleaned giant axons of the squid (*Loligo pealii*) essentially as was done by Cole and Curtis (*2*). Transversely oriented external Ag-AgCl electrodes, although not as satisfactory as the platinum-platinum black electrodes of Cole and Curtis, were used. The measuring electrodes were slightly misaligned. Therefore, in addition to the a-c bridge signal, they also recorded a derivative of the spike. It was deemed desirable to carry out all the measurements at room temperature (25°–26° C), at which the axons nevertheless remained functional for as long as 5 hr. At these high temperatures the spike is brief, and the a-c bridge was therefore supplied with a 25-kc sine wave input, to provide good resolution of the resistance changes. The detector was a differential amplifier flat to 50 kc (6 db down at 150 kc), driving one beam of a dual cathode-ray oscillograph. The second beam carried a simultaneous record of the first differential of the spike.

Fig. 1 illustrates the membrane resistance changes observed during activity of one axon, successively bathed in sea water or in artificial sea water in which 40% or 60% of the NaCl had been replaced by choline chloride. Despite the slower, smaller, and broader spikes in the Na^+ poor media the onset of the resistance change occurs in the same phase of the spike—namely, approximately at the maximum of the first differential of the latter. The shift occurs rapidly and reversibly on changing the bathing medium.

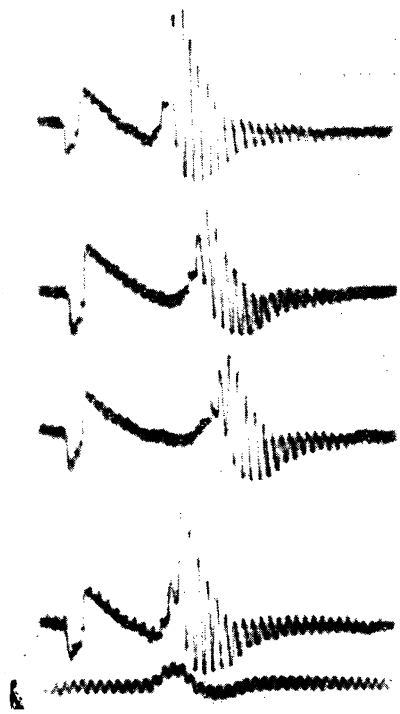

FIG. 1. The resistance changes of the active axonal membrane in relation to modifications of the spike form produced by altering the external Na^+ concentration. The first 4 traces represent resistance measurements in sea water, in artificial sea water containing only 60% and 40% Na^+, and again in sea water. The lowest record shows the diphasic spike recorded in sea water and at low amplification from electrodes on each side of the impedance measuring pair. A 25-kc signal is superimposed. The initial base lines of the upper 4 records show that the a-c Wheatstone bridge was balanced for the resting nerve fiber. After the stimulus artifact (lasting approx 70 μsec) and the electrotonic potential, there is seen the onset of the propagated spike. Before this has reached its crest, the membrane resistance falls, and the resulting bridge imbalance is signalized by the appearance of the 25-kc carrier. The maximum resistance changes from above downward are 3.2%, 2.1%, 2%, and 2.7%.

[1] This work was carried out at the Marine Biological Laboratory, Woods Hole, Mass., partly under a grant to one of us (H. G.) from the U. S. Atomic Energy Commission under contract AT (30-1)-1076.