

zyme activity against duration of preillumination yielded a straight line (Fig. 1). This indicates that the inactivation follows the time course expected for a first-order

TABLE 2
EFFECT OF DURATION OF ILLUMINATION ON ACTIVITY OF
 α -AMYLASE SENSITIZED BY RIBOFLAVIN

Duration of exposure to light	Time (<i>t</i>) in min to complete disappearance of starch	$\frac{1}{t}$	Relative activity
0	34	.0294	100
10 min	39	.0256	87
30 min	48	.0208	71
1 hr	66	.0152	52
2 hr	114	.0088	30
3 hr	220	.0045	15
4 hr	360	.0028	10
5 hr	582	.0017	4
17 hr	∞	0	0

reaction and probably involves a "one-hit" mechanism. Subsequent experiments with antibodies and with bacteriophage have demonstrated that these types of pro-

teins are also rapidly photoinactivated in the presence of riboflavin.

As early as 1879 (1) it was reported that sunlight, especially the blue-violet wavelengths, could cause the aerobic photoinactivation of a crude enzyme preparation. Von Tappeiner (6) (see also other references in the review by Schomer [5]) later reported that the addition of fluorescent dyes such as eosin greatly accelerated such photoinactivation. It is perhaps not surprising, therefore, to find that riboflavin, which is strongly fluorescent in blue light, produces a similar effect.

Our investigations (unpublished) on the relation of light intensity and riboflavin concentration to the rate of photoinactivation strongly indicate that this type of reaction could proceed *in vivo*, at least in green plants. This reaction may therefore be of some importance in one or more of the light-growth reactions of plants such as phototropism, photoperiodism, and the light inhibition of internode growth.

It is also interesting to note that numerous investigators (4) have attributed the effects of ultraviolet irradiation of enzymes to a specific absorption by tryptophane. Since riboflavin is known (3) to cause the photoinactivation of tryptophane, this reaction may provide a mechanism whereby visible light produces the same sort of effect on enzymes as ultraviolet light.

References

1. DOWNES, A., and BLUNT, T. P. *Proc. roy. Soc. Lond.*, 1879, **23**, 199.
2. GALSTON, A. W., and HAND, M. E. *Amer. J. Bot.*, 1949, **36**, 85.
3. GALSTON, A. W. *Proc. Nat. Acad. Sci.*, 1949, **35**, 10.
4. GORBACH, G., and LERCH, K. *Biochem. Z.*, 1930, **219**, 122.
5. SCHOMER, H. A. *Biological effects of radiation*, edited by B. M. Duggar. New York: McGraw-Hill, 1936, Chap. 37.
6. VON TAPPEINER, H. *Ber. Deut. Chem. Ges.*, 1903, **36**, 3035.

A New Method for Isolation and Purification of Mammalian Striated Myofibrils¹

Armin F. Schick and George M. Hass

*The Rush Department of Pathology,
Presbyterian Hospital, Chicago*

The following method has been devised for isolating large numbers of mammalian skeletal and cardiac myofibrils in highly purified form without modifying their microscopic structure or reactivity to adenosine triphosphate.

The method with slight variations is suitable for isolation of myofibrils of man, rabbit, dog, and guinea pig. The present discussion is concerned principally with the isolation of skeletal myofibrils of rabbits and properties of the segregated fibrils.

A block (25 × 25 × 10 mm) of living white muscle from the anterior thigh was rapidly frozen and cut with a cold

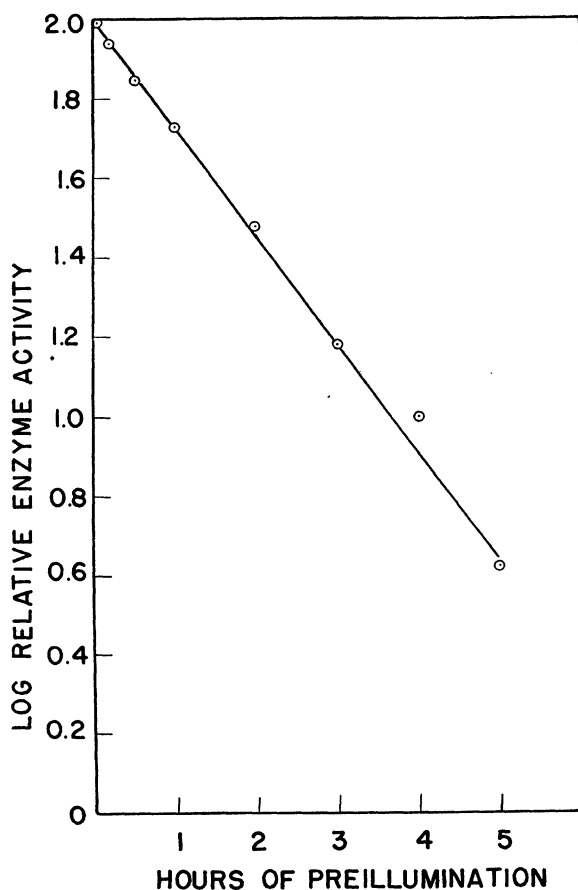


FIG. 1. Effect of duration of preillumination upon the inactivation of α -amylase.

¹ This research was aided by a grant from the Otho S. A. Sprague Memorial Fund.

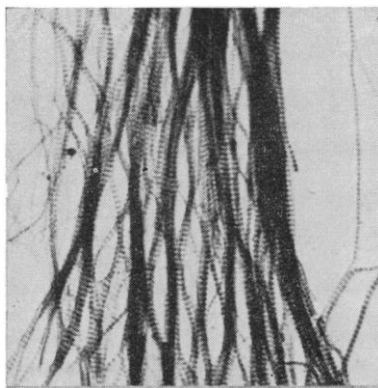


FIG. 1.

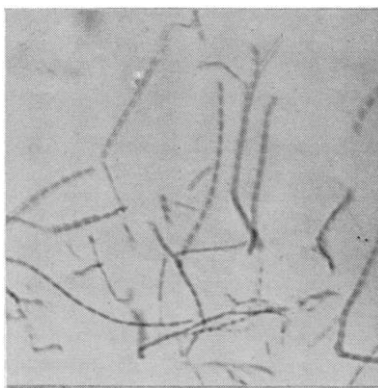


FIG. 2.

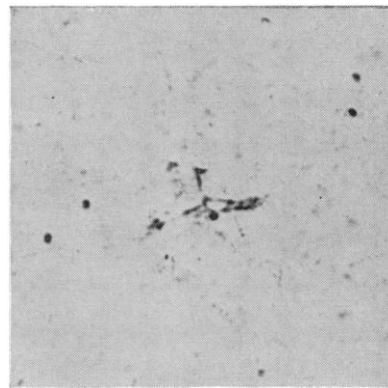


FIG. 3.

microtome into sections, 15 μ in thickness. The sections were transferred to 250 cc of potassium phosphate and citric acid buffer solution (pH 7, ionic strength 0.25, temperature 0° C) containing 5.0 cc of a 0.4% filtered solution of commercial trypsin. Sample sections were removed periodically, mounted under a cover slip and studied microscopically. When gentle pressure on the cover slip was sufficient to rupture the sarcolemma (Fig. 1) and permit the escape of segregated myofibrils (usually after 30–45 min of tryptic digestion), the sections were filtered from the enzyme buffer solution, washed, and resuspended in a potassium phosphate and citric acid buffer solution (pH 6.4, ionic strength 0.25). This suspension was agitated in a Waring blender for 5–10 sec. During this treatment the sarcolemma ruptured and myofibrils were released. This crude preparation was purified by repeated washing and centrifugation at 0° C. The final suspension of myofibrils formed a white flocculent mass containing only microscopic traces of collagenous and cellular debris. The myofibrils, usually single, but occasionally adherent in groups of two or more, were elongated, birefringent rods measuring 0.25–1.2 μ in diameter in fixed preparations. “A” and “I” disks

were distinct but “Z” lines were rarely seen (Fig. 2).

The solubility of the myofibrils was studied microscopically. They promptly dissolved in 5–10 volumes of a cold aqueous alkaline solvent (0.5 M potassium chloride, 0.03 M sodium bicarbonate) yielding a viscous solution showing birefringence of flow. In cold buffer solutions (pH 1–12, ionic strength 0.15), the myofibrils dissolved in the ranges: pH 1–4, and pH 10–12.

The influence of ionic strength and potassium chloride on the solubility of myofibrils was determined by adding potassium chloride to cold phosphate and borate buffer solutions (pH 6–11, ionic strength 0.05). The myofibrils dissolved in solutions (ionic strength 0.2) alkaline to pH 10; in solutions (ionic strength 0.3) alkaline to pH 8; and in solutions (ionic strength 0.5) alkaline to pH 6. Comparable results were obtained with potassium phosphate buffer solutions of varying ionic strength and pH.

When isolated myofibrils under direct microscopic observation were immersed in a solution (0.075%) of adenosine diphosphate and triphosphate at room temperature, they spontaneously and rapidly contracted into small round masses. This contraction was not reversible (Fig. 3).

Paleocene and Eocene Strata in the Bearpaw Mountains, Montana¹

Roland W. Brown and William T. Pecora

U. S. Geological Survey, Washington, D. C.

The dark, rounded features of the Bearpaw Mountains, rising some 4,500 ft above the surrounding plains in north central Montana, can be seen readily from railroad or highway between Chinook and Havre or between Havre and Big Sandy. Centennial Mountain, the most conspicuous land form in the western part of the mountains, has the appearance of a sleeping bear. This resemblance and the Indian legend associating the mountain with a prominent adjacent butte near Box Elder provided the original name “Mountain of the Bear’s Paw.” The

¹ Published by permission of the Director, U. S. Geological Survey.

softened contours of the Bearpaws are in large part the result of normal differential erosion of diverse rocks plus the effect of a forest mantle consisting of Douglas fir, ponderosa and lodgepole pines, cottonwood, aspen, white birch, service berry, cherry, hawthorn, rose, raspberry, alder, and dogwood—in brief, an outpost of the Rocky Mountain flora, 150 miles distant.

In this Bearpaw region irruptive magmas (now stocks, plugs, dikes, sills, laccoliths, flows, and agglomerate piles) in the Tertiary penetrated and at times overflowed a terrane originally of nearly horizontal sedimentary strata of late Mesozoic and early Tertiary age. These sedimentary formations exposed in and around the mountains are now tilted and faulted, with fault blocks of widely differing ages abutting against one another (?). The resulting complex geologic relations are unfortunately somewhat obscured in large areas by glacial deposits and bench gravel. The junior author began his geologic studies in 1937 with particular emphasis on the petrology