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Dissociation of Uridine Diphosphate Glucose-Glycogen Transglucosylase from Phosphorylase Activity in Individual Muscle Fibers. (26690)

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The present concept of a cyclic glycogen metabolism in muscle(1) assumes the existence of different enzymatic pathways for glycogen synthesis and breakdown. The catabolic enzyme is represented by phosphorylase(2), and polysaccharide synthesis from glucose-1-phosphate is considered to depend on the uridine diphosphate glucose (UDPG)-linked pathway with the UDPG-glycogen transglucosylase reaction(3) as the final step.

Histochemical studies on the distribution pattern of these enzymes in resting skeletal muscle of the rat revealed a reciprocal relationship between phosphorylase and UDPG-glycogen transglucosylase activity in individual fibers. A different spatial arrangement of the two activities would be inconsistent with their operating as parts of a single enzymatic cycle.

Methods. Muscle tissue, excised from rats bled under ether anesthesia, was quenched in liquid oxygen. From tissue blocks oriented in a transverse or longitudinal plane fresh frozen sections were cut at 8 μ on a cold microtome (cryostat) at $-20°$ and mounted on coverslips. The activity of phosphorylase, branching enzyme (amylo-1, 4 $\rightarrow$ 1, 6-transglucosidase) and UDPG-glycogen synthesizing enzyme was demonstrated according to Takeuchi(4,5,6). The reaction for the last of these was carried out in the presence of .0005*M* glucose-6-phosphate. The glycogen content of untreated cryostat sections was assessed by the periodic acid-Schiff procedure after post-fixing the sections in Gendre's fluid. The methods employed for demonstration of various oxidative enzymes have been listed previously(7).

Results. A striking difference in enzymatic activity was noted in longitudinal and cross sections of muscles containing fibers of varying size. Both phosphorylase and branching enzyme were strongly active in fibers of large diameter(7). Conversely, high activity of UDGP-glycogen-transglucosylase was obtained in fibers belonging to the small ("red") type (Fig. 1, 2). Fibers of intermediate size showed varying degrees of activity with either type of reaction. Strong UDGP glycogen-transglucosylase activity was accompanied by high activity of oxidative enzymes, especially succinate and lactate dehydrogenases. In striated muscle of uniform fiber type (for instance, tongue, *M. levator ani*) little or no variation in enzyme activities was observed.

In contrast to findings in pigeon breast muscle(8) resting rat muscle showed intrinsic, diastase-labile, glycogen to be concentrated in the narrow fibers. In the same fiber type, polysaccharide formation was dependent mainly on the UDGP-transferase reaction.

The phosphorylase reaction was inhibited by substituting adenosine-5′-triphosphate

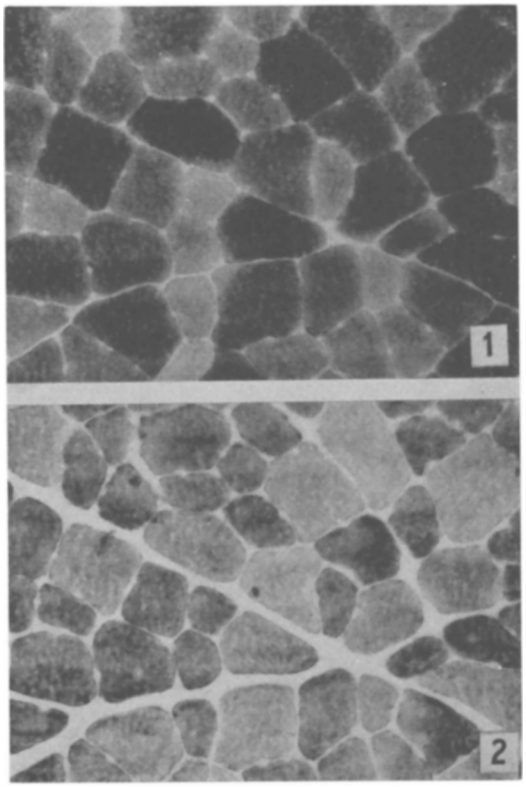

FIG. 1. M. rectus femoris of the rat. Phosphorylase. 170 ×.
FIG. 2. Serial section to Fig. 1. Reaction for UDPG-glycogen transglucosylase. 170 ×.

(ATP), adenosine-5′-diphosphate (ADP) or cyclic adenosine-3′,5′-phosphate for 5′-adenylic acid (AMP) in the medium. No influence of these nucleotides on glycogen formation from UDPG was observed. Addition of glucose or sodium-β-glycerophosphate (0.05M) caused marked reduction in polysaccharide formed from glucose-1-phosphate, but inhibited the UDPG reaction only slightly. Under the influence of inhibitors, no change in distribution pattern of the reaction products was observed.

Discussion. The reciprocal localization of the 2 possible metabolic routes from glucose-1-phosphate to glycogen (the phosphorylase and branching enzyme reaction on one hand and the UDPG-pathway on the other) in individual muscle fibers lends further support to the view that the contractile function of the red and white fibers of mammalian muscle depends on fundamentally different metabolic systems(9). The main reason for the observed difference may be a marked variation in levels of phosphorylase activity between individual fibers. Polysaccharide may, in fact, be formed from UDPG in broad fibers but escape detection because it may be broken down rapidly by the phosphorylase present in excess. If this were so, low demonstrable glycogen would not necessarily indicate actual low levels of UDPG enzyme. In resting muscle, low phosphorylase in the small fibers was accompanied by both high levels of intrinsic glycogen and glycogen synthesis from UDPG.

A catabolic action of demonstrable phosphorylase on polysaccharide formed from UDPG would fail to explain why, on the other hand, high activity of both enzymes can be shown histochemically in a single structure as, for instance, in heart muscle and in skeletal muscle of a uniform fiber type. Apparently, the levels of activity of enzymes acting on glycogen are of importance(10).

ATP, which has no influence on partially purified UDPG-glycogen transglucosylase (3), did not inhibit polysaccharide formation from UDPG in the histochemical reaction. If activation of catabolic phosphorylase *a* by ATP(11) takes place in tissue sections, the failure of the nucleotide to induce breakdown of glycogen synthesized from UDPG could indicate either very low levels of phosphorylase in small fibers or that phosphorylase is not capable of degrading the newly formed polysaccharide. The latter seems unlikely since both enzymes are presumed to act on 1,4-linkages of the glucose polymer(1). Histochemical polysaccharide formation from glucose-1-phosphate is dependent on the presence of AMP which is a cofactor required by phosphorylase *b*(12); ATP cannot substitute for AMP, and in the presence of ATP the histochemical reaction is inhibited.

An alternative explanation for the observed differences in activity of individual fibers would be the assumption of 2 separate polysaccharide forming mechanisms in muscle, one *via* phosphorylase and the other *via* the uridine linked pathway. The discussion of this possibility would require more evidence on the nature of the histochemical re-

action than is available. The most promising approach would appear to be the study of tissues known to be deficient in enzymes for glycogen utilization(13).

Summary. A reciprocal relationship between phosphorylase and UDPG-glycogen transglucosylase activity has been demonstrated by a histochemical technic in individual fibers of resting rat skeletal muscle. In general, polysaccharide formed from glucose-1-phosphate was deposited in the large (white) type of fiber and that from UDPG in the small (red) fibers.

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Effect of Adrenalectomy and Successive Hydrocortisone Replacement on Thyroid Secretion Rate in Male and Female Rats.* (26691)

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It has been suggested that adrenalectomy decreases thyroid function as a consequence of decreased metabolism(1), while other studies concerning exogenous administration of adrenocorticoids to animals have shown the following effects: a suppressive action of TSH release in normal rats(2,3); a stimulating action on TSH release in normal rats(4); a direct suppression on thyroid gland in hypophysectomized rats(5), normal rats(6) and normal chickens(7); reduction in peripheral utilization of thyroxine in normal rats(8); no effect in normal dogs(9); and a stimulating effect on thyroid gland in normal rats(10).

Determination of thyroxine secretion rate (TSR) has not been used to evaluate adrenocortical effects on thyroid function. Previous indices were I^{131} uptake and release and thyroid cell height. With accurate technics for determining TSR in rats available(11), present work was undertaken to determine TSR in individual animals before and after adrenalectomy and after subsequent adrenocortical replacement.

Materials and methods. Mature male (34) and female (25) albino rats of Sprague-Dawley-Rolfsmeyer strain were used. They were fed Purina lab chow *ad libitum* and were maintained at a constant temperature of 78 ± 1°F. Initial body weights ranged from 200 to 460 g.

TSR determinations were made with use of I^{131} by method of Pipes and Turner(12). Increments of 0.25 μg L-thyroxine (T_4) were used. Adrenalectomy (ADRX) was performed after normal secretion rates were obtained. Animals were maintained on 1% NaCl drinking water after ADRX and throughout glucocorticoid replacement therapy. Determination of TSR was commenced 2 weeks after ADRX. Replacement therapy

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