When ribosomes were isolated from the same muscles 6h after intraperitoneal injection of the steroids it was shown that the minimum effective doses capable of causing a significant ($P < 0.05$) depression in their amino acid-incorporating ability *in vitro* were: corticosterone, 20mg/kg body wt.; prednisolone, 20mg/kg body wt.; triamcinolone, 0.05mg/kg body wt.; betamethasone, 1mg/kg body wt.; dexamethasone, 0.05mg/kg body wt.; triamcinolone acetonide, 0.05mg/kg body wt. Although it is true that the low catabolic potencies of corticosterone and prednisolone were reflected in the high doses required to affect ribosomal activity *in vitro*, the other steroids, on this criterion, were in the wrong order, since betamethasone would be expected to be less catabolic than triamcinolone, which itself would be expected to have a catabolic potential similar to that of its acetonide.

A much more satisfactory correlation between catabolic potential and diminished ribosomal activity was observed on the basis of duration of steroid action. For example, 12h after a single dose (20mg/kg body wt.) of corticosterone muscle ribosomes had regained normal activity, whereas those from animals receiving a similar dose of prednisolone still retained diminished activity. Likewise it was possible to separate, as pairs, triamcinolone and betamethasone from dexamethasone and triamcinolone acetonide. Triamcinolone, the least catabolic of these four, ceased to be effective 18h after injection, whereas the acetonide was still effective 48h after administration.

Cardiac-muscle ribosomes were not affected as a result of steroid treatment, in agreement with the absence of any effect on heart weights.

These results serve to emphasize again the close correlation between changes in ribosomal activity and cellular determinants of catabolic decline in skeletal muscle. Nevertheless the mechanism of the decrease in incorporating ability of isolated ribosomes, which will be discussed, still remains obscure.

The Heterogeneity of Muscle Mitochondria Demonstrated by Discontinuous Density-Gradient Centrifugation

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Muscle mitochondria in the peripheral region of white fibres enlarge rapidly after the administration of triamcinolone acetonide to rats (Bullock, Christian, Peters & White, 1971). For this reason a method was sought that would separate normal organelles on the basis of size, at the same time preserving functional and morphological integrity.

Mitochondria were released from muscle without a homogenizer by using the method of tryptic lysis (Bullock, Carter & White, 1970) and centrifuged through a discontinuous sucrose density gradient at 105000g for 45min. Broken organelles (band 1) did not penetrate into the gradient, but the remaining mitochondria were collected into three bands at the interfaces of the following sucrose molarities: 1.07M/1.12M, band 2; 1.12M/1.17M, band 3; and 1.17M/1.22M, band 4. Mitochondria in band 2 were smaller than those in bands 3 and 4 and more homogeneous.

For testing with the oxygen monitor, mitochondria from different bands were diluted with medium in the absence of glutamate and pelleted at 11000g for 10min. Oxygen uptake was then measured in the presence of glutamate (10mM) with and without ADP to give the respiratory control ratios. These were 16.0, 10.5 and 3.3 for bands 2, 3 and 4 respectively.

For electron-microscopical examination the mitochondria were diluted and pelleted through medium containing glutamate (10mM) and incubated for 5min at 39°C in the presence of glutamate (10mM) and in the presence or absence of excess of ADP (1mM). They were then re-isolated by centrifugation and the pellet was fixed simultaneously with glutaraldehyde and osmium tetroxide by the method of Hirsch & Fedorko (1970).

Mitochondria in State 4 from all bands assumed a characteristic appearance with parallel cristae and fairly dense matrix, although those in band 4 appeared more swollen. In State 3 there was a shift of a large number of mitochondria to an open-mesh structure, presumably indicative of an energized conformation.

These results demonstrate a heterogeneity according to size among isolated muscle mitochondria and also a morphological change as a response to change in function. The latter could not be demonstrated by Kuner & Beyer (1970), possibly because of a harsher method of tissue homogenization.

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