These compounds probably would be stored for an appreciable length of time in small algivorous rotifers, which are commonly eaten by *Asplanchna*. The absence of MF-inducing activity in the blue-green alga *Anacystis nidulans* (*2*) is now also explicable, for this organism is devoid of α-tocopherol and α-tocopherylquinone (*19*).

The function of vitamin E compounds in invertebrates is very poorly known. They have been suspected of enhancing vigor and fecundity in the cladoceran *Daphnia* (*20*); they stimulate egg sac formation in copepods (*21*) and are essential for spermatogenic activity in the cricket *Acheta* (*22*). Our report appears to be the first to show that these compounds can initiate either a transition from parthenogenetic to sexual reproduction or a marked change in body morphology.

The vitamin E control of MF production in *Asplanchna* could be very adaptive if this vitamin was required for spermatogenesis, as it is in crickets, for it would benefit a population to make mictic females only under conditions when functional males could be produced. Hence a possible explanation for the biological significance of the response to plant lipids considered earlier (*2*) can now be offered.

There are many occasions when the biological activities of natural and synthetic vitamin E compounds need to be determined. For these, bioassays using the MF- and hump-producing responses of *A. brightwelli* and *A. sieboldi* appear admirably suited. Although the responses are probably very complex, they are discrete, extremely sensitive, and very easily and quickly detected. Furthermore, the practical convenience of this system over those requiring tedious maintenance of vertebrates deficient in vitamin E is obvious. Also for these reasons, *Asplanchna* may prove useful in studies of the mechanism of the action of vitamin E.

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Myoglobin Subfractions: Abnormality in Duchenne Type of Progressive Muscular Dystrophy

Abstract. *Human metmyoglobin was separated electrophoretically into four subfractions: Mb_1, Mb_2, Mb_3 and Mb_4, which divide into at least two biochemically independent groups: Mb_1 and Mb_2, and Mb_3 and Mb_4. In normal subjects, Mb_1 constituted the predominant component; Mb_2, Mb_3, and Mb_4 were the minor components in this descending order. In the Duchenne type of progressive muscular dystrophy, on the contrary, a remarkable decrease in Mb_1 and a concomitant increase in Mb_3 were observed. This unique abnormality in the relative distribution of myoglobin subfractions was recognized only in the Duchenne type and not in other types of progressive muscular dystrophy or in other myopathies.*

We have already reported (*1*, *2*) that the absorption maximum in the ultraviolet spectrum of metmyoglobin, in the Duchenne type of progressive muscular dystrophy (PMD), was about 275 mμ, in contrast to the value of 281 mμ for metmyoglobin from the other types of PMD, spinal progressive muscular atrophy, polymyositis, myoglobinuria, and normal subjects. Further studies of the myoglobin of PMD have shown that this spectral abnormality is ascribable to an abnormal distribution pattern of myoglobin subfractions.

Rossi-Fanelli *et al.* (*3*) and Perkoff *et al.* (*4*) reported that human skeletal myoglobin was heterogeneous and was separated into three to four components by paper electrophoresis or DEAE cellulose–column chromatography. Kossman *et al.* (*5*), however, reported that minor components of myoglobin were artifactual. Existence of the myoglobin subcomponents, therefore, is still problematical, and rigorous physicochemical characterization of the subcomponents has not been reported.

Perkoff (*6*) claimed that myoglobin was separated into three fractions on DEAE cellulose–column chromatography, and named them F_1, F_2, and F_3. He observed that several diseases, including childhood dystrophy, dermatomyositis and myoglobinuria, caused a decrease in F_1 and F_2, with a concomitant increase in F_3; there was similar change in fetus myoglobin.

In our studies the metmyoglobin was isolated and purified by Singer's method (*1*) from the skeletal muscles of normal subjects and patients having various myopathies. The metmyoglobin

was then subjected to electrophoresis on cellulose acetate membrane and polyacrylamide gel.

Metmyoglobin from normal subjects (12 cases) and from patients having various types of PMD [Duchenne type (six cases), limb-girdle type (four cases), and facioscapulohumeral type (two cases)], polymyositis (four cases), myoglobinuria (one case), or spinal progressive muscular atrophy (two cases) was examined by this technique. On both cellulose acetate membrane and polyacrylamide gel, myoglobin from normal subjects and myopathies was separated into four subfractions that were designated, respectively, Mb_1, Mb_2, Mb_3, and Mb_4, starting from the cathodic side of the cellulose acetate membrane (Fig. 1).

All four of these subfractions were benzidine-positive and made positive precipitation against antimyoglobin rabbit serum. In a repetitive electrophoresis of each of four myoglobin subfractions, obtained from normal skeletal muscle by polyacrylamide-gel electrophoresis, Mb_1 and Mb_2 were found to be partially interconvertible, and the same was true of Mb_3 and Mb_4. However, no interconversion was observed between the two groups: Mb_1 and Mb_2 on the one hand, and Mb_3 and Mb_4 on the other.

Myoglobin subfractions thus obtained were then examined by spectrophotometry (*1*). In a visible region, the characteristic spectrum of myoglobin, displaying a plateau between 570 and 610 mμ, was observed in all subfractions. In an ultraviolet region, the absorption maximum of Mb_1 and Mb_2 was found at 281 mμ, while that of Mb_3 and Mb_4 was at about 275 mμ (Fig. 2).

We may conclude that human myoglobin can be separated into four subcomponents by electrophoresis on cellulose acetate membrane or polyacrylamide gel, and that among these four there are at least two independent groups (Mb_1 and Mb_2, Mb_3 and Mb_4) representing different protein constructions.

The relative amounts of these myoglobin subfractions separated on cellulose acetate membrane were relatively constant in normal subjects: Mb_1, 69.2 to 88.9 percent (mean of 12 cases, 78.3 percent); Mb_2, 8.2 to 14.3 percent (mean, 11.8 percent); Mb_3, 2.7 to 13.1 percent (mean, 7.0 percent); and Mb_4, 0.8 to 6.9 percent (mean, 2.9 percent) (Fig. 1a).

On the contrary, myoglobin from

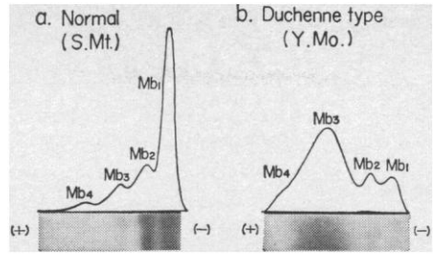

Fig. 1. Electrophoretic patterns of metmyoglobin from human skeletal muscle on cellulose acetate membrane. Electrophoresis was performed in a discontinuous buffer system (anode, 0.2*M* tris buffer, *p*H 9.1; cathode, barbital buffer, *p*H 8.6); electrophoretic strip was stained with amido black 10B. The metmyoglobin of normal control (a) was separated into four subfractions, Mb_1 predominating. In the Duchenne type of progressive muscular dystrophy (b), a marked decrease in Mb_1 and a corresponding increase in Mb_3 are apparent.

the Duchenne type of PMD displayed a unique change in the pattern of myoglobin subfractions in all six cases examined: Mb_1, 5.8 to 48.4 percent (mean, 25.5 percent); Mb_2, 1.3 to 29.1 percent (mean, 13.2 percent); Mb_3, 32.4 to 90.3 percent (mean, 58.4 percent); and Mb_4, 1.3 to 6.1 percent (mean, 3.1 percent). A marked decrease of Mb_1 and an increase of Mb_3 were characteristic of myoglobin in the Duchenne type of PMD (Fig. 1b). The wide discrepancy in the relative contents of the subcomponents may be due to a partial loss of Mb_3 and Mb_4 in our analytical procedures.

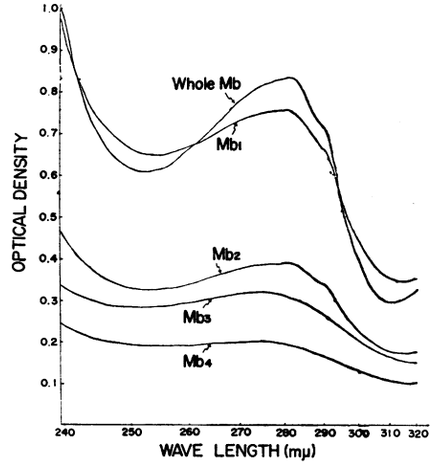

Fig. 2. The ultraviolet-absorption spectra at *p*H 7.0 of four subfractions of metmyoglobin from normal skeletal muscle. Subfractions were separated by polyacrylamide-gel electrophoresis, with tris–EDTA–borate buffer, *p*H 8.6. The absorption maxima observed were 281 mμ for subfractions Mb_1 and Mb_2, and about 275 mμ for Mb_3 and Mb_4.

This abnormal feature of myoglobin was not observed in neurogenic muscular atrophy and other myopathies, including other types of PMD, polymyositis, and myoglobinuria, although some variations in the relative amounts of Mb_1 and Mb_2 were often observed in these cases. Our results indicate that the blue shift of the ultraviolet-absorption maximum of metmyoglobin in the case of the Duchenne type of PMD (*1*) is due to an abnormality in the relative contents of the myoglobin subfractions.

The apparent contradiction between our results and Perkoff's (*6*) may be due to the difference in the methods of isolation and fractionation of myoglobin. Perkoff has claimed that the content of F_3 changes rather nonspecifically in various types of myopathies; this claim indicates that F_3 in his definition is grossly contaminated with nonmyoglobin proteins. Kossman's failure (*5*) to detect myoglobin subfractions also may be due to the difference in the isolation of myoglobin, in that Mb_3 and Mb_4 in our definition are lost during the isolation.

We consider the described abnormality in the pattern of myoglobin subfractions of the Duchenne type of PMD to be of great importance in understanding of the pathogenesis of the PMD.

To establish a biochemical and genetic entity of the subfractions of myoglobin, amino acid analysis of each myoglobin subfraction has been carried out with an automatic amino acid analyzer. A preliminary result suggests no significant difference between the amino acid compositions of Mb_1 and Mb_2, but that the composition of Mb_3 differs apparently from that of Mb_1 and Mb_2 (*7*).

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