especially when nine or ten succeeding doses of the drug were given on alternate days. Even a single dose of IUdR, however, when given at the same site either immediately after the virus or 2 hours later, provided definite, though possibly somewhat less, protection. The effect of multiple injections of IUdR, when initiated 24 hours after the injection of virus, was difficult to evaluate because of the small number of hamsters that survived for 30 days in this group.

In both experiments, virtually every hamsters which developed tumors, despite treatment with IUdR, also developed complement-fixing antibodies to adenovirus type 12 viral antigen, which were similar in reactivity and titer to those described previously (*7, 8*). Those which did not develop tumors did not develop complement-fixing antibodies.

In previous reports (*7, 8*), we concluded that the specific complement-fixing "viral" antigens in adenovirus 12 induced hamster tumors, and the total absence of infectious virus could best be explained by the incorporation of genetic material from the viral genome into the genetic apparatus of tumor cells (*8*).

In the hamsters which developed tumors despite administration of IUdR, we therefore conclude that IUdR did not interfere with the transfer of viral genetic material, with its oncogenic activity or with its expression in the form of specific adenovirus antigens.

On the other hand, in those hamsters in which tumors were suppressed, it is possible to suppose that either IUdR prevented effective incorporation of viral DNA into the genomes of the cells which, in the absence of drug, would have been transformed, or that following incorporation into the cell genome, the oncogenic effect of the viral DNA was somehow rendered impotent.

Possible action of the drug on the tumor cells induced by virus cannot be ruled out; however, the preventive effect of a single dose of the drug given simultaneously or 2 hours after injection of virus makes this an unlikely mode of action, a conclusion supported by the studies already cited (*2–4*) concerning the mode of action by IUdR and related compounds on the replication of DNA viruses.

It is clear that IUdR can suppress the formation of tumors by adenovirus type 12 tumors in the newborn hamster, provided the drug is injected within 2 hours after the virus and in either the same or another subcutaneous site. One experiment in which the initial injection of IUdR was delayed until 24 hours after the injection of virus gave equivocal evidence of the inhibition of tumor development. It was also clear, however, that despite multiple injections of the drug, some of the tumors grew to sizes large enough within the 90-day observation period to kill the hamsters, and that hamsters with tumors developed typical complement-fixing antibodies to adenovirus 12 antigens.

These preliminary studies provide only qualitative data concerning the inhibitory action of IUdR on the development of these virus-induced neoplasms. Since replication of infectious adenovirus 12 has not been demonstrated in hamsters with or without tumors (*10, 11*), any effects of IUdR on this phenomenon could not be measured. Additional studies of the suppression by IUdR of the development of tumors initiated by adenovirus 12 may provide opportunities for gaining insight into the mechanism of adenoviral oncogenesis; at the same time a new means of evaluating the action of 5-halogenated pyrimidine deoxyribonucleosides on the differing host cell relationships exhibited by DNA viruses may be afforded.

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## Abnormal Myoglobin Ultraviolet Spectrum in Duchenne Type of Progressive Muscular Dystrophy

Abstract. *Met-myoglobin isolated from gluteal muscle of cases with Duchenne type of progressive muscular dystrophy showed an abnormal ultraviolet spectrum. The maximum of the spectrum at pH 7.0 was at 275 mμ in contrast to that at 281 mμ in normal met-myoglobin. Such an abnormality was not found in the limb-girdle type of dystrophy and in progressive spinal muscular atrophy. The results indicate the presence of an abnormal myoglobin in the Duchenne type of progressive muscular dystrophy.*

In 1962 Whorton *et al.* (*1*) reported that the myoglobin from cases of progressive muscular dystrophy (PMD), differed from normal controls in the visible absorption spectrum. Their results did not seem indisputable, however, because the optical density of their sample was too low (*2*). Perkoff *et al.* (*3*) analyzed the myoglobin from skeletal muscle by chromatography on a column containing diethylaminoethyl cellulose and said that the $F_3$, one of the three major components of normal myoglobin, preponderated in muscle samples obtained from two cases of childhood dystrophy.

We have been studying the etiology of PMD and have assumed that it might be a "myoglobinopathy" similar to a hemoglobinopathy. We have found that the ultraviolet spectrum of some types of PMD myoglobin definitely differs from that of normal myoglobin.

Ten cases of PMD, five cases each of the Duchenne and limb-girdle types, were examined. As controls we used muscle obtained at autopsy from cadavers showing no signs of muscular disease, and muscle obtained by biopsy from one patient with progressive spinal muscular atrophy. The met-myoglobin was isolated from the gluteal muscle of these subjects according to the procedure of Theorell and de Duve, modified by Singer *et al.* (*4*).

In a paper electrophoresis at *p*H 8.6, no obvious differences were found in the mobility of met-myoglobin from patients with PMD and that from controls.

Spectrophotometry in the visible region also revealed no recognizable differences in the absorption curves between the met-myoglobin from patients

with PMD and that from controls. In both cases, at *p*H 5.4, the maxima were at 500 and 630 mμ and the "plateau" was between 570 and 610 mμ (Fig. 1). At *p*H 8.6, the maxima of the absorption curve were at 495, 540, and 580 mμ. All of these findings correspond to those described (*4*) for muscle met-myoglobin from normal individuals.

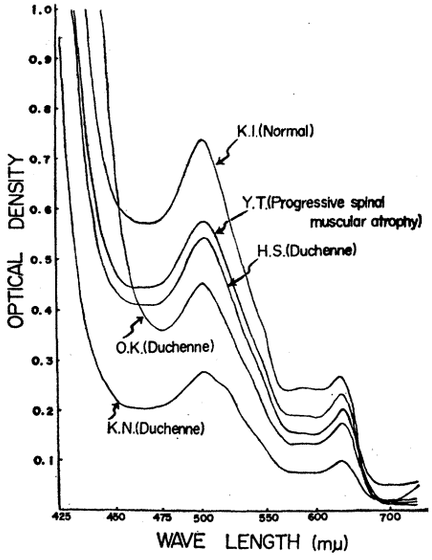


Fig. 1. Spectrophotometric absorption spectrum (visible region) of skeletal muscle met-myoglobin at *p*H 5.4 with protein concentration of 0.7 to 2.0 mg/ml. Results for three cases of Duchenne type of PMD and two controls are shown. The curves show the characteristic feature for met-myoglobin. Each curve is indicated by the initials and condition of the respective individual.

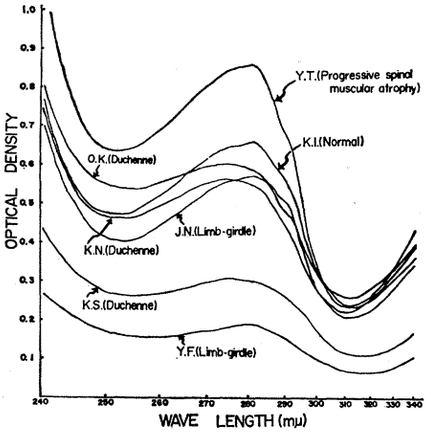


Fig. 2. Spectrophotometric absorption spectrum (ultraviolet region) of skeletal muscle met-myoglobin at *p*H 7.0 with protein concentration of 0.1 to 0.4 mg/ml. from cases of PMD and some representative controls. Each curve is indicated by the initials and condition of the respective individual. A definite blue shift by Duchenne type of PMD is apparent.

Spectrophotometry in the ultraviolet region revealed, in four normal individuals, an absorption curve of met-myoglobin with one maximum at 281 mμ, at *p*H 7.0 (Fig. 2). The finding was the same in another control with progressive spinal muscular atrophy. However, in the Duchenne type of PMD, of which we had three typical cases, spectrophotometry revealed a different feature from that of the controls; there was a maximum at 275 mμ in all of the three cases (Fig. 2). In the limb-girdle type, however, for two cases, the curve was similar to that of the controls (Fig. 2).

Judging by nitrogen content, the protein concentration of the samples was between 0.1 and 0.4 mg per milliliter. The molar extinction coefficient of the met-myoglobin at 280 mμ, for a molecular weight of 17,500, was 2.9 to 3.1 × $10^4$ at *p*H 7.4 in all of the cases; there were no apparent differences among normal control, spinal muscular atrophy, and muscular dystrophy.

Thus, in the Duchenne type of PMD there was a definite blue shift of the ultraviolet spectrum of met-myoglobin, indicating an abnormality in the myoglobin at least in some types of PMD.

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## Electrophysiologic Indications of the Osmoregulatory Role of the Teleost Urophysis

Abstract. Tilapia mossambica *when placed in salt water shows an increase in the frequency of spontaneous discharges in some neurosecretory fibers in the urophysis of the caudal neurosecretory system. Exposure to tap water usually depresses this activity. The nature of the ion-regulatory action of the caudal neurosecretory system of teleosts remains conjectural, but the data currently available indicate the possible production of two kinds of neurohormones, one which favors ion movement in the normal direction and another which has the opposite effect in response to abnormal osmotic circumstances.*

An osmoregulatory role for the caudal neurosecretory system of teleostean fishes was first suggested by Enami (*1*). More recently, additional evidence supporting this possibility has become available (*2*, *3*). It is now well established (*4*, *5*) that neurosecretory neurons of this system have the ability to generate and conduct action potentials of a much longer duration than those of ordinary neurons. In the course of their studies, Bennett and Fox (*5*) observed synaptic activation of the caudal neurosecretory cells as a result of the injection of distilled water into *Paralichthys dentatus*, a marine teleost. We now report the effect of a hypertonic environment on spontaneous discharges of caudal neurosecretory fibers in a euryhaline, fresh water teleost, the cichlid, *Tilapia mossambica*.

For 1 hour before each experiment, fishes weighing 185 to 350 g were placed in individual aquaria containing either 14 liters of tap water or 14 liters of 1.5 percent NaCl solution; the conditions were otherwise identical. Each fish was then immobilized by an intraperitoneal injection of curare in saline solution (approximately 10 mg *d*-tubocurarine chloride per kg of body weight). The urophysis (the neurohemal organ of the caudal neurosecretory system) was carefully exposed with minimum bleeding under a dissecting microscope. The fish was then placed into a three-compartment, divided holder. In the posterior compartment the dissected area was continuously perfused with a physiological saline solution for freshwater teleosts (128 m*M* NaCl, 2.65 m*M* KCl, 1.8 m*M* $CaCl_2$, 0.24 m*M* $NaHCO_3$). In the anterior compartment, the front end of the fish, including the gills, was immersed in either tap water or saline solution, the same as during the earlier 1-hour treatment; this then was replaced with the opposite solution during the course of the experiment. Under our experimental con-