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.

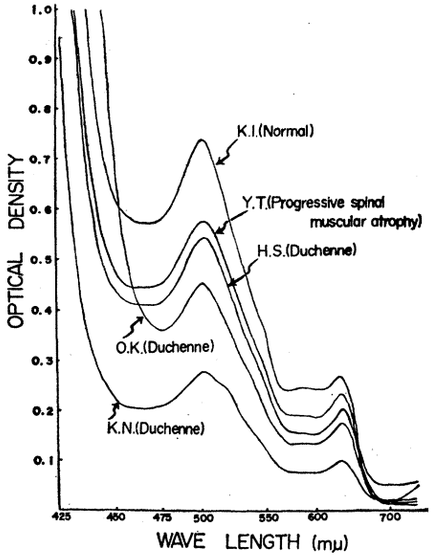

Fig. 1. Spectrophotometric absorption spectrum (visible region) of skeletal muscle met-myoglobin at *p*H 5.4 with protein concentration of 0.7 to 2.0 mg/ml. Results for three cases of Duchenne type of PMD and two controls are shown. The curves show the characteristic feature for met-myoglobin. Each curve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

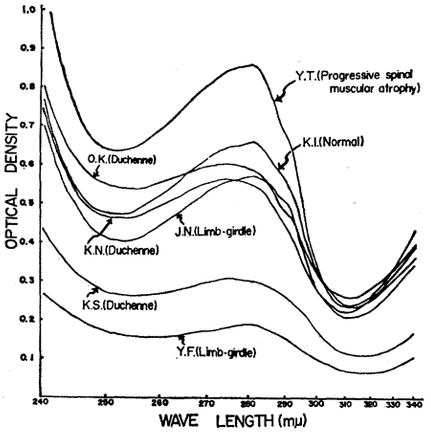

Fig. 2. Spectrophotometric absorption spectrum (ultraviolet region) of skeletal muscle met-myoglobin at *p*H 7.0 with protein concentration of 0.1 to 0.4 mg/ml. from cases of PMD and some representative controls. Each curve is indicated by the initials and condition of the respective individual. A definite 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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12 August 1963

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-

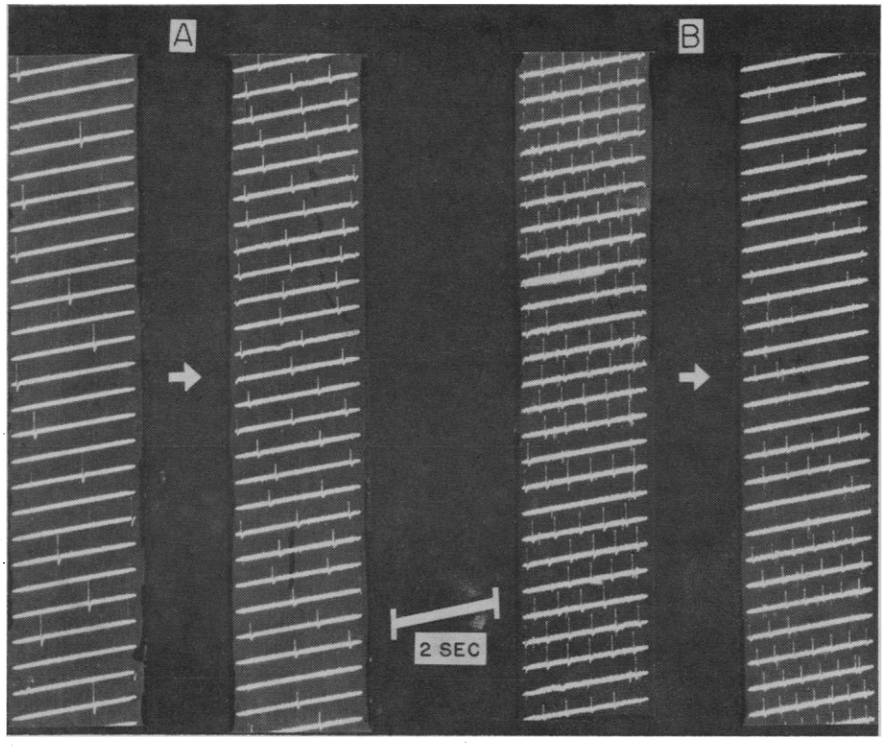

Fig. 1. Spike recordings, each of 1-minute duration, from two neurosecretory axons in the urophysis of *Tilapia mossambica*. *A*, Fish immersed in tap water for 1 hour before experiment: (left) in tap water, 2 minutes before changing the external solution, and (right) 9.5 minutes after replacing the tap water with 1.5 percent saline. *B*, Fish immersed in 1.5 percent saline for 1 hour before the experiment: (left) 2 minutes before changing the external solution, and (right) immediately after replacing the saline with tap water. Note that the spike frequency increases with time (from bottom to top) in A (right) but decreases in B (right).

ditions, the heart rate 4 hours after curare administration was virtually the same as the initial rate.

To record the impulses from the urophysis, we used silver microelectrodes insulated with a glass capillary (with an outside tip diameter of 10 to 20 μ) and a conventional recording system with a Grass P-5 preamplifier, a Tektronix 502 oscilloscope, and a Grass C-4 oscilloscope recording camera. When the electrode was inserted into the urophysis two types of unit discharges were observed which differed in duration. Most spikes were of long duration (averaging about 10 msec), and consonant with intracellular records from caudal neurosecretory neurons (*4, 5*), they appeared to represent discharges of neurosecretory axons. Presumably, those of shorter duration (1 to 2 msec) were due to discharges of ordinary axons. Subsequent recordings were made only from axons showing the spikes of long duration.

Fish that had been treated with tap water for 1 hour before the experiments showed, initially, a low frequency of spontaneous discharges. When the tap water bathing the anterior part of the fish was replaced by a 1.5 percent sodium chloride solution, an increase in the frequency of these discharges was observed in single fibers from each of five fishes, sometimes with a long latent period preceding the response (see Fig. 1*A*). Three other fibers, presumed to be neurosecretory, showed no change when the tonicity of the external solution was increased, while two others were inhibited. In fish that had been treated for 1 hour with 1.5 percent NaCl, some of the neurosecretory fibers showed a much higher frequency of discharges initially. When the saline bathing the anterior part of the fish was replaced by tap water, the frequency of discharges decreased in seven out of ten neurosecretory fibers recorded from a total of eight fishes (Fig. 1*B*). In two instances (fish that had been treated initially for 1 hour with tap water), rapid responses to the change in external medium in both directions were observed in a single neurosecretory fiber.

The fact that some neurosecretory fibers in the urophysis were activated when the osmotic pressure of the environment was increased, while others were not, may be explained in at least three ways. (i) Those fibers which did not respond may have very long latent periods. If this is so, the latent period exceeds 30 minutes in some cases, which does not seem probable. (ii) Some fibers may have a higher threshold for response to hypertonic stimulation; they might have been activated if more intense stimulation had been applied. (iii) There may be more than one type of neurosecretory axon, with only one type responding to the particular experimental situation described above.

Active ion transport is presumably operative from the inside to the outside of the body in marine fishes and from the outside to the inside in freshwater fishes, to compensate for the movement of ions occurring as a result of concentration gradients. In the marine fluke studied by Bennett and Fox (*5*), injection of distilled water resulted in a fall in the osmotic pressure of the blood; hence the enhanced activity of urophysial neurons in this species might be correlated either with increased transport of ions inward, or with depressed active transport in the normal (outward) direction. Exposure of the freshwater teleost *Tilapia*, that we studied, to salt water presumably resulted in a higher osmotic pressure of the blood and, consequently, a need to increase the ion efflux and perhaps also to depress the normal (in this case, inward) active ion transport.

These data suggest that the urophysis may enhance ion movement in the direction opposite to that which is normal. However, other data suggest that removal of the neurohormonal factor(s) by urophysectomy might result in loss of the ability to regulate active transport of ions, leaving the organism subject to changes resulting from the passive movement of ions in accordance with the concentration gradient. Thus, urophysectomized *Tilapia* (*2*) show a lower concentration of sodium chloride in the serum when in fresh water, and a higher concentration when in salt water, than do control animals. That administration of urophysial extracts may stimulate normal regulatory processes is suggested by the experiment of Maetz *et al.* (*3*), in which urophysial extract increased the normal active up-

take of sodium by the gills of goldfish in fresh water.

In an attempt to reconcile the data currently available on the ion-regulatory activity of the caudal neurosecretory system in teleosts, we suggest that the urophysis may release two neurohormones: one which favors active ion transport in the normal direction and another that either inhibits the normal active transport or stimulates active transport in the opposite direction in response to abnormal osmotic circumstances. We are currently obtaining electrophysiologic data, of the kind described herein, on the effects of altering the internal osmotic environment by intravenous injections of saline solutions of various kinds. Results to date indicate the presence of two types of caudal neurosecretory fibers in individual *Tilapia*. One type, corresponding to the fibers stimulated by a hypertonic external environment, is depressed by intravenous injection of sodium-free hypotonic solution and stimulated by a subsequent injection of saline containing ten times the normal sodium chloride concentration; the other type of fiber shows exactly the opposite responses. It is possible that the first type of fiber is associated with an increase in total ion flux in a direction opposite to normal, whereas the second enhances normal osmoregulatory processes.

However, the situation regarding ion regulation by the urophysis must remain only conjectural for the present. It is assumed that impulse conduction by neurosecretory fibers (see *6*, *7*) bears an essential relation to neurohormone release, but this logical assumption has yet to be firmly established (*8*).

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Translational Control of Protein Synthesis in a Cell-Free System Directed by a Polycistronic Viral RNA

Abstract. *Observations with RNA isolated from the RNA virus indicate that regulation of protein synthesis occurs during translation. At least three electrophoretically separable proteins, one of which lacks histidine, appear as a result of adding purified viral RNA to a cell-free extract of* Escherichia coli. *This establishes that the RNA contains at least three cistrons. Comparison of the kinetics of appearance of the proteins that do and do not contain histidine reveals a control mechanism which determines the temporal order and the frequency of translation of each cistron. Such translational controls at the stage of message use provide additional devices for regulating protein synthesis which can supplement those functioning at the transcription step where genetic messages are produced.*

Currently accepted views of how information flows from the genome to the protein-synthesizing machines suggest two possible sites at which controls can be inserted. One is at the step (transcription) which produces the complementary RNA copies which serve as genetic "messages." The other would be where the genetic "messages" are translated into the protein molecules. Regulation of both production and use of message are not mutually exclusive and both mechanisms can, in principle, coexist. Recent experiments with the "gal" (*1*) and "lac" (*2*) loci of *Escherichia coli* have shown that inducers of specific enzyme synthesis stimulate the accumulation of the particular messages complementary to the homologous genetic regions. These results suggest a control of gene transcription. We now describe experiments from RNA from an RNA virus in a cell-free system showing that there is regulation at the point of protein synthesis.

An RNA molecule which can be translated into two or more proteins may be referred to as a "polycistronic" message. This concept immediately raises the possibility of regulated use in terms of the order and relative frequency of translation of the component cistrons. The RNA message fraction has been shown to contain molecules big enough to act as a code for several proteins (*3*, *4*), an observation confirmed with other systems (*5*, *6*). It has been proposed (*7*) that such polycistronic messages could arise from the continuous transcription of contiguous cistrons belonging to the same "operon." A simple physical basis is thus provided for the "operon" concept (*8*).

The RNA viruses guarantee the existence of polycistronic molecules and also provide a convenient material for their experimental analysis. Data on mutations of tobacco mosaic virus (*9*) show that viral RNA determines the amino acid composition of the coat protein. Further, it seems very probable that replication of viral RNA is mediated by an RNA-dependent RNA polymerase (*10*, *11*). Suggestive supporting evidence (*12*, *13*) has recently appeared without, however, proof of RNA dependence. Available evidence (*14*, *15*) makes it highly unlikely that a polymerase of this sort already exists in uninfected cells. The RNA polymerase discovered by August *et al.* (*16*), is not a likely candidate for fabricating specific heteropolymers. While it is stimulated by RNA, it apparently cannot catalyze the synthesis of anything but polyadenylate and the end product is uninfluenced by the "primer." We therefore predicted (*10*) that the structural program of the new polymerase is coded in the RNA of the virus particle. From these arguments, a viral genome must contain cistrons for at least two proteins, and hence all viral RNA molecules must be polycistronic. We present direct supporting evidence for this conclusion.

If a new polymerase must be synthesized before RNA replicas appear, the incoming viral RNA must obviously serve as a message and must furthermore be conserved while being translated into protein (*10*). The prediction of conservation was confirmed (*17*) through the use of double labeling (N^{15} and P^{32}) and by showing that the two isotopes can be recovered in the same RNA strands at the end of a complete lytic cycle.

Our principal concern here is with the conclusion that viral RNA must contain a device which dictates the number of times a particular cistron is