

BIOCHEMICAL SOCIETY SYMPOSIA NO. 6

THE BIOCHEMISTRY OF FISH

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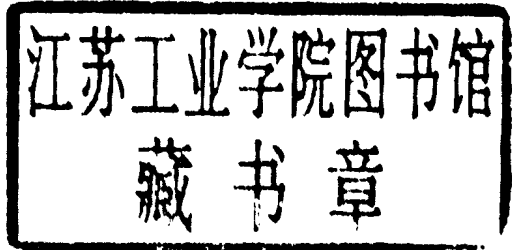
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BIOCHEMICAL SOCIETY SYMPOSIA NO. 6.

THE BIOCHEMISTRY OF FISH

*A Symposium held at
Derby Hall, University of Liverpool
on 22 September 1950*

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INTRODUCTION

BY R. A. MORTON

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Many of us like to regard Biochemistry as an independent science even if, in so doing, we put our own meaning into 'independent' and 'Biochemistry'. Accepting that as biochemists we have our own way of looking at things, it is beyond dispute that we draw freely upon other sciences for facts and ideas.

The biochemistry of fish is a good illustration of this. A study of the environment of fishes would have to begin with the inorganic solutes of fresh water and sea-water, a subject with very wide implications. The nutrition of fishes leads directly to photosynthesis in phytoplankton and its seasonal variation. The combination of photochemical, tracer and chromatographic techniques has in the hands of the Berkeley and Chicago Schools emphasized that photosynthesis is a central biochemical theme. The dependence of zooplankton populations on phytoplankton fluctuations is the first step in the biological sequence which determines the work of fishermen.

Investigation of the biochemistry of fish requires a brisk traffic to and from other fields of study. The results of this exchange of information and ideas are both stimulating and challenging whether the topic under consideration is reproduction, absorption, metabolism, pigmentation, vision, or a dozen other matters. May I mention a few of the questions within my own experience?

How are we to interpret the pigmentation of fish eggs and the evidence that some of the carotenoids are 'useful' but not indispensable? What are we to make of the extreme variability of squalene in fish-liver oils? What are its precursors and what is its fate?

Again, how much remains to be done in the field of the chemistry and biochemistry of the poly-ethenoid long-chain acids of fish oils? The regulation of growth in fishes is a fascinating problem with important implications for endocrinology.

It is often interesting to speculate how Biochemistry would have developed if some discoveries had been made in a different order. Vitamin A could easily have been first isolated from tunny liver oil or halibut liver oil or visceral oil, in all of which it is a major constituent. Vitamin D₃ might have been isolated from one of the tuna species. Such speculations illustrate a point of some importance. Hitherto a great deal of research has been directed towards isolating active principles responsible for previously defined physiological effects. Isolation techniques have now been so developed that some important

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substances are likely to be discovered in natural products before the *nature* of their significance. In such cases we shall need the biochemical insight which comes from a wide biological discipline.

From another point of view it is equally important to take a broad view of the *applied* biochemistry of fish. The facts about one sector of the fishing industry are eloquent enough. Fish Bulletin 64 of the California Department of Nature Resources (1946) tells the story of the soupfin shark. From 1930-36 the annual catch was on the average some 270 tons. The discovery in 1937 that soupfin shark livers were very rich in vitamin A resulted during the years 1938-41 in a 12-15-fold increase in landings. Prices rose 40-fold, and livers were sold at from 1.5 to 13 dollars per lb. The catch then dwindled rapidly and the 'return per unit-effort' fell greatly. The 'bonanza' conditions of war-time prices and plentiful supplies brought about the most obvious over-exploitation.

I am not sure what has happened since 1946 but possibly the advent of synthetic vitamin A may have had the indirect effect of allowing the species to recover.

The search for rich sources of vitamin A and D has stimulated interest in the nutritional value of fish in many parts of the world and it is all to the good that the general biochemistry of fish is attracting more attention now. Interesting results are being obtained in all directions, the range of effort covering academic studies in comparative biochemistry to intricate technological problems. We are indebted to the organizers of the Symposium for bringing together so many different aspects of the subject.

1. SOME COMPARATIVE ASPECTS OF THE BIOCHEMISTRY OF FISH

BY E. BALDWIN

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A fish, according to one dictionary I have consulted, is "an aquatic, cold-blooded, water-breathing, gilled vertebrate with limbs represented by fins". This is brief and to the point, and the definition goes on to add that "there are 12,000 species". This is probably a gross underestimate. It will at any rate serve to remind us that, even among 12,000 species, there is plenty of room for variations of shape, size, structure, habits and even chemistry. One possible scheme of classification of the fishes is given in Table 1.

Table 1. *Classification of the fishes*

(Craniata : Gnathostomata : Anamnia)

Class PISCES

Sub-class	Order	Sub-order	Examples
CHONDRICHTHYES	Elasmobranchii	Selachoidei Batoidei	Dogfishes, sharks Rays
	Holocephali		Rat-fish, rabbit fish
OSTEICHTHYES	Teleostei	Clupeiformes Anguilliformes Ostariophysi Haplomi Percososes Percomorphi Heterosomata Gadiformes	Herrings, salmones Eels Carps, catfish Pikes Mulletts Perch, tunny Flat fish Cod, haddock
	Aetheospondyli Protospondyli Chondrostei Polypterini Diphoi		Ganoids (One genus) Sturgeons (Two genera) Lungfishes

(After Shipley & MacBride, 1920. "Zoology". Cambridge.)

Not all of these groups will come up for discussion today, but perhaps it is well to realize from the outset that what goes for one fish does not necessarily hold for others, but rather that there are group-specific and probably even species-specific differences of a chemical as well as a morphological kind.

One striking and important phenomenon may be mentioned at once: namely that it is characteristic of the elasmobranchs that their tissues and body fluids generally contain remarkably large amounts of urea—about 2.5% in marine and about 1% in freshwater forms. This was first observed by Stadeler & Frerichs in 1858 and confirmed in the 1880's by Krukenberg. The Holocephali, which are believed on morphological grounds to be derived from an ancient and aberrant elasmobranch stock, share with the true elasmobranchs this state of physiological uraemia. So a chemical differentiation is possible between the two great sub-classes. Closely associated with this uraemia we find that, whereas arginase is confined to the liver in most fishes, amphibians and mammals, it is found in every tissue of the body in elasmobranchs and so far as the evidence goes, in the Holocephali as well. This association is probably more than fortuitous, for arginase, as every one knows, plays a central part in the ornithine cycle mechanism for urea production.

The essentially vertebrate character of the fishes is chemically demonstrated in at least two ways. First, all the fish muscles so far studied contain creatine phosphate and, if we neglect a single report of somewhat dubious origin, arginine phosphate is never present. Similarly, many fishes have been found to contain carnosine and anserine. These two aberrant dipeptides are known to occur very widely and perhaps universally in the muscles of vertebrates but, in spite of repeated attempts, have not so far been detected in invertebrates.

For some reason the distribution of carnosine and anserine has attracted a good deal of attention, and the reports in the literature serve well to emphasize my point that there are group-specific and perhaps even species-specific differences among the fishes. Clifford (1921), who studied the distribution of carnosine in the animal kingdom, stated her main conclusion in the following words: "The only relation brought out by this investigation is a morphological one. If the base is absent from one member of a zoological family, it appears to be absent from all." Thus, among the fishes, she examined four heterosomes and found carnosine absent from them all. Two gadiformes also contained no carnosine, while nineteen representatives of other sub-orders did contain it.

Quite apart from morphological considerations there is the ecological consideration of habitat. Some groups, e.g. the lungfishes, are wholly confined to fresh water; other groups again are exclusively marine, but there are a number of teleosts especially that can live in sea-water and fresh water alike. Some marine forms, such as the salmon, go into the fresh waters in order to spawn; others, such as the eel—normally a denizen of fresh water—spawn at sea. Realizing that the osmotic pressure of sea-water is at least 100 times that of fresh water, and remembering as we must that fishes *live* in water, it follows that there are large environmental differences between the two types.

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I do not wish here to do more than mention a few of the ascertained facts. Fresh water has an osmotic pressure which seldom exceeds $\Delta = -0.02^\circ$, sea-water being about -2.0° or even more. Yet the bloods of marine fishes, apart from the Chondrichthyes, have an osmotic pressure only a little higher than that of freshwater species, about -0.7° as against -0.6° .

This means little to us, who are wholly unaffected by osmotic changes in our habitual environment, but to the fishes it means a very great deal. Freshwater species live under an ever-present threat of flooding and must balance endosmosis by urinary excretion. Marine forms, on the other hand, are in constant danger of dehydration, and the exosmosis in this case is balanced by drinking. We find, looking a little further, that the glomeruli, so numerous and well developed in the kidneys of freshwater fishes, which elaborate a voluminous but very hypotonic urine, are degenerate and non-functional in marine forms, so that in these latter there is little loss of water by urine formation. But the excretion of metabolic waste products is still a primary necessity even in fishes and, in marine species which lack the usual glomerular filtration apparatus, the excretory function of the kidneys is largely taken over by the gills: some 75% of the total nitrogen excreted by marine teleosts is extra-renally excreted through this channel.

Now a large proportion—up to 30%—of the total waste nitrogen of the marine teleosts is eliminated in the form of trimethylamine oxide. Freshwater fishes, on the other hand, produce little or no trimethylamine oxide. This is why they do not smell so 'fishy' when they are dead, but the fact probably has a deeper significance. As is well known, ammonia, the predominant nitrogenous excretory product of aquatic animals in general, is a highly toxic body, requiring abundant supplies of water for its elimination if a toxic level of ammonaemia is to be avoided. Freshwater fishes have all the water that is necessary for this purpose and more; but their marine counterparts are in a very different position. Now terrestrial or semi-terrestrial animals such as the amphibians, some chelonian reptiles and the mammals evade the dangers of ammonia poisoning by converting ammonia into urea, saurian reptiles and birds preferring to turn it into uric acid, and all these are adapted to more or less serious shortages of water. May it not be, then, that the production of trimethylamine oxide by marine, yet *never by freshwater fishes*, is in fact a chemical adaptation to the relative shortage of water to which these marine fishes are permanently subjected? Experiments have been made which appear to suggest that trimethylamine oxide is essentially exogenous in origin, but I know of no evidence tending to suggest that freshwater fishes take a diet which is radically different from that of marine species with similar dietetic habits, or that freshwater foodstuffs are free from trimethylamine oxide and its potential precursors. Possibly this is so, but even if it is, we

must still of necessity presume that there are differences in the enzymic equipment of freshwater as opposed to marine fishes. Certainly there is something peculiar here.

Another markedly peculiar fact to which I should like to make some reference is that, while vitamin A_1 forms the raw material for the prosthetic group of the retinal pigment of vertebrates generally, it is replaced in the eyes of freshwater fishes by A_2 . Is this, perhaps, another aspect of freshwater *versus* marine dietetics, or is the phenomenon more deeply seated? It is all the more striking, I think, that in euryhaline fishes, which are as much at home in the sea as they are in fresh water, A_1 and A_2 are both present, and that in those that hatch in fresh water A_2 predominates, A_1 predominating in those which are hatched at sea. Yet A_2 differs from A_1 only in containing an additional double bond in the ionone ring.

If, in conclusion, I may go back to the problem of nitrogen excretion among the fishes I would say this. The excretion of unchanged ammonia is a usual feature of aquatic animals, with exceptions only in the case of animals that have at one time lived on the land and made a subsequent, secondary return to the fresh waters; carrying their chemical adaptations with them. The replacement of ammonia by uric acid is a device that has been adopted by many terrestrial vertebrates, notably the snakes and the lizards, and by some terrestrial invertebrates also, in particular by the insects. The formation of urea from ammonia has, as I have mentioned, been adopted by those terrestrial vertebrates that did not adopt uricotelism, but seems never to have been discovered by invertebrates. In all these cases it would seem reasonable to suppose that the detoxication of ammonia, whether by conversion to uric acid or to urea, may be regarded as an adaptation to restriction of water supply. If, however, we accept this hypothesis, it remains to account for the fact that elasmobranchs excrete little or no ammonia. Probably the same is true of the Holocephali. Rather do they convert their waste nitrogen into urea, in spite of the fact that they are all aquatic. We have already seen that marine species suffer from a relative shortage of water, yet even freshwater elasmobranchs, with their superabundant supplies of water, still produce and retain urea on quite a large scale.

This is, I think, a problem worthy of the few moments I would like to devote to it, if only because, in the end, it may teach us to regard fishes as something more exalted than mere 'lower vertebrates'. Marine elasmobranchs must at one time, we may suppose, have suffered from the osmotic disadvantages which still afflict their teleostean counterparts, and it would appear that they became adapted, or partly adapted, in essentially the same manner, for marine elasmobranchs, like marine teleosts, elaborate considerable quantities of trimethylamine oxide. Subsequently, it would seem, they antedated the amphibian

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discovery of the ornithine cycle and adopted urea as a predominant nitrogenous end product—a notable step in their chemical evolution. But subsequently they went even further: with the development of boundary membranes that are impermeable to urea, and the elaboration in the renal tubule of a special segment that reabsorbs urea from the glomerular filtrate, they became able to retain urea, to add its osmotic pressure to that exerted by the salts of their blood, and thus to raise their internal osmotic pressure to a level somewhat higher than that of the surrounding sea-water. Whether this explanation is or is not sound, the fact remains that, because so much urea is retained in the tissues, the marine elasmobranchs of the present day do indeed have an internal osmotic pressure that appreciably exceeds that of their environment, so that they are assured of a good water supply by mild endosmosis.

Finally, in those elasmobranchs which have returned secondarily to fresh water, where a high internal osmotic pressure is as great an embarrassment as a low osmotic pressure is for a marine animal, the urea content of the blood has dropped from some 2.5% to only about 1%. If this is an adaptation to freshwater existence, it is certainly a step in the right direction. But one wonders why this 'physiological uraemia' has not been altogether abandoned, for to abandon it would involve less intense endosmosis and a corresponding reduction of the work to be performed by the heart and the kidneys. But it would appear, if we may judge by the results of physiological experiments, that, after countless generations of exposure to a more or less severe degree of uraemia, the elasmobranch heart, and possibly other organs, can no longer function normally in the complete absence of urea.

These are some of the facts, and some too of the speculations, that came to mind when I was asked to address you. It may prove, as the day goes on, that they have little to do with the communications to which we are now to listen. But I would have you remember, nevertheless, that the fishes are a very heterogeneous and highly specialized group and, in a word, that, as fishes, they are as successful as we are in being men.

REFERENCE

Clifford, W. M. (1921). *Biochem. J.* **15**, 725.

2. THE PROTEINS OF FISH

BY G. HAMOIR

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A review of the biochemistry of fish proteins was made in 1948 by Geiger, and here more recent progress in the isolation and characterization of fish proteins will be described. No special consideration will be given to the nutritive value of these compounds or to their possible pharmacological action. It is intended in the present report to give a general picture of the composition of fish muscle and an account of the advances made in the study of the proteins of other fish tissues.

I. PROTEINS FROM SKELETAL MUSCLE

Previous work has shown that fish muscles contain myoalbumin, myogen, globulin X, myosins and stroma. The quantitative estimation of these different fractions was carried out some time ago following Smith's scheme (1937) by Reay (1935) and Reay & Kuchel (1936). At that time, however, the nature and properties of the muscle proteins were not accurately defined; a sufficiently comprehensive study of the conditions of extraction was lacking and the analysis of the protein mixtures was based only upon differences in solubility.

The influence of different factors on the extraction of fish muscle has been re-examined recently by Dyer, French & Snow (1950), and the analysis of extracts by electrophoresis has allowed a better identification of the different components (Hamoir, unpublished). As uncertainty has recently arisen concerning the nomenclature of muscle proteins, precise definitions of these based on our knowledge of rabbit muscle proteins will be given here.

Weber (1934) has given the name myogen to the albumins of the muscle juice. This definition can be generalized to the albumins of muscle extracted at low ionic strength. Recent research has shown that Weber's myogen contains the myoalbumin described by Smith (1937) (known as the component *h* of isoelectric point 4.65 by Jacob (1947, 1948)), together with a group of other proteins of much higher and fairly close isoelectric points (6.00, 6.20, 6.75) which we shall call myogens (Jacob, 1947). All these compounds exist in muscle juice or are extracted by water or dilute salt solution ($\mu \leq 0.25$), together with Weber's globulin X (1934). This last fraction is removed with denatured myogens by dialysis against distilled water

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and is isolated by redissolution in salt solutions. Globulin X represents therefore the muscle globulins extractable at an ionic strength insufficient to bring the myosins into solution. It must not be confused with other globulins such as protein-Y of similar solubility (Dubuisson, 1950) which can only be extracted at much higher ionic strength. The general term 'myosins' includes the 'crystalline' myosin of Szent-Györgyi (1943) and the actomyosin of Banga & Szent-Györgyi (1941-1942) which is a mixture of actomyosins (according to Portzehl, Schramm & Weber, 1950 resulting from the association of actin with the 'crystalline' myosin of Szent-Györgyi. The latter is called L-myosin in the sedimentation diagrams (Portzehl *et al.*, 1950) and β -myosin in the electrophoretic patterns (Dubuisson, 1946 *a, b*). Stroma protein can be defined according to Smith (1937) as the residue obtained after repeated extraction by dilute hydrochloric acid or sodium hydroxide.

A. Qualitative analysis of whole extracts

The amounts of total nitrogen, non-protein nitrogen, myosins and non-myosins nitrogen in extracts of cod muscles have been determined by Dyer *et al.* (1950) after extraction in the cold for 3-5 minutes in a specially adapted Waring Blendor, while an analysis, using a slightly modified Tiselius electrophoresis apparatus (Dubuisson, Distèche & Debot, 1950), of extracts from carp muscles has been made by me. In my investigations the muscles were cut with a freezing microtome into slices 40 μ thick (Dubuisson, 1947) and extracted in the cold with stirring.

(a) *Extracts at ionic strength 0.15 and pH 7-8.* Myosins could not be detected in extracts made under the above conditions by Dyer *et al.* (1950) or by me. Of the total protein content of cod muscle 21% went into solution under these conditions and, according to Smith's analytical scheme (1937), this was made up of 6% myogen, 7% myoalbumin and 8% globulin X. Electrophoretic patterns of one-hour duration of such extracts from carp muscles showed two main components and several others present in small concentrations migrating more rapidly (Fig. 1). Fig. 1 represents a mixture of myoalbumin, myogens and globulin X. As myoalbumin has a very low isoelectric point, it can only be represented by one of the small peaks in front. It is therefore not present in these extracts in a proportion corresponding to the above analytical value of Dyer *et al.* (1950). After dialysis of the extract of Fig. 1 against distilled water, an appreciable precipitate of globulin X and denatured myogens can be removed by centrifugation. The electrophoresis of the clear solution obtained shows a general decrease of all the components except the major one, which becomes very prominent and corresponds to 80-90% of the myogens. Its mobilities in a phosphate-NaCl buffer

of ionic strength 0.15 (0.1 phosphate and 0.05 NaCl) and pH 7.3 are -3.0×10^{-5} cm.²/volt/sec. on the ascending side and -2.5×10^{-5} cm.²/volt/sec. on the descending side.

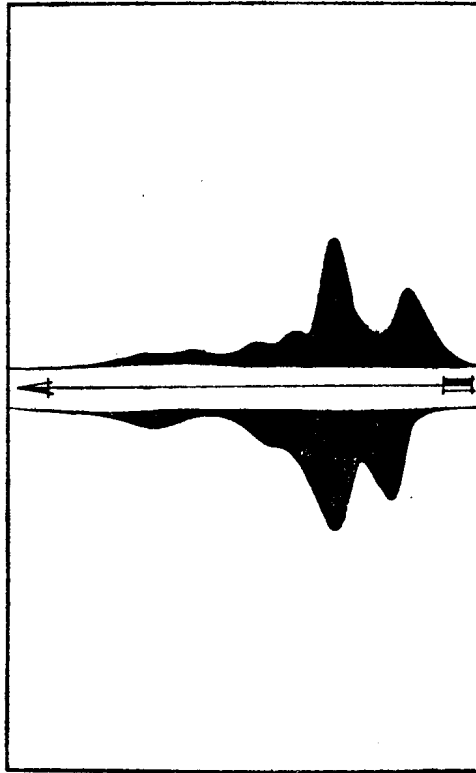


Fig. 1. Electrophoretic pattern of a one-hour extract from carp muscle at $\mu=0.15$ and pH 7-8. Ionic strength 0.15, pH 7.3. Migration to the left. Upper part: ascending limb. Lower part: descending limb. Duration of the electrophoresis: 14,100 sec. Electrical field: 3.80 v./cm.

(b) *Extracts at ionic strength 0.5 and pH 7-8. Conditions for maximum extraction.* When the ionic strength of the extractant is higher than 0.15, structural proteins also go into solution. Extraction of the myosins is already observable at ionic strength of 0.17 in cod muscles (Dyer *et al.*, 1950); in carp muscles, no myosins are detectable in extracts made at an ionic strength of 0.35 (Hamoir, 1949), but extraction at $\mu=0.5$ proceeds rapidly, giving turbid extracts of high viscosity and high protein content.

Fish muscle differs from mammalian muscle in its very low content of stroma proteins, these being 3% instead of 16% (Reay & Kuchel, 1936; Smith, 1937). These determinations were carried out after extraction of the pulp with hydrochloric acid (0.05 to 0.005-N) or sodium hydroxide (0.1 to 0.005-N) (Reay & Kuchel, 1936); it is

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desirable, however, to use a more gentle method of extraction in order to remove the myosins quantitatively without denaturation.

Some progress has been made in this direction by Dyer *et al.* (1950) by examining the influence of grinding, ionic strength, pH and nature of the extractant, temperature of extraction and cold storage.

It is most important for maximum extraction that the subdivision of the muscle fibrils must be carried as far as possible, and that any denaturation is avoided. Good recovery was obtained by these authors by homogenization of the muscles for 3-5 minutes in a Waring Blendor provided with a plate to prevent the formation of foam. The amount of residue obtained from extractions carried out in these conditions, using a solution 0.85M-NaCl and 0.02M-NaHCO₃ to give a pH of 7 to 7.5, varied between 3 and about 15% "depending on the conditions of the fish" (Dyer *et al.*, 1950). Varying the ratio of fish muscle to extractant solution between 1/90 and 1/18 was without influence. The nature of the salt used was of secondary importance (Table 1) and the greater differences previously

Table 1. *Efficacy expressed in per cent. of protein N extracted of normal solutions adjusted to pH 7-7.5 of various salts as protein extractants of cod muscle (after Dyer et al., 1950)*

BaCl ₂	CaCl ₂	KBr	KCl	KI	K ₂ HPO ₄	K ₂ SO ₄	, LiCl
84	87	80	82	91	86	87	86
MgCl ₂	MgSO ₄	NaAc	Na ₃ Citrate	NaCl	NaHCO ₃	Na ₂ SO ₄	
91	83	77	83	91	77	83	

observed (Smith, 1937) were probably due to an insufficient subdivision of the muscle fibrils. By the systematic study of these factors, Dyer *et al.* (1950), could separate quantitatively the myosins from the stroma at pH 7-8 by extraction with potassium iodide, magnesium chloride or sodium chloride. It is probable that similar results could be obtained by quickly freezing the fresh muscle solid with a freezing microtome, cutting it immediately in thin slices (Dubuisson, 1947) and grinding the slices with sand in the cold.

Such extracts are unsuitable for electrophoretic analysis because of their high viscosity. They present all the properties of actomyosin solutions, but are from that point of view contaminated by their high content of other proteins. By altering the pH of extraction, extracts of lower viscosity can be obtained giving a general electrophoretic pattern of the different proteins present in fish muscle.

(c) *Extracts at ionic strength 0.5 and pH 5-6.* Extractions at high ionic strength and varying pH have been carried out by Dyer *et al.* (1950), on cod muscles. As shown in Fig. 2, the myosins begin to go into solution at pH 4.3, the extractability increasing slowly to pH 5.5 and the process is practically complete at pH 6.0. Maximum extraction is obtained between pH 7 and 9. Similar results (unpublished) were found by me with carp muscles, a shift of the curve to the right being observed with muscles kept for several weeks in frozen state. It is therefore possible, by working at pH 5-6, to get extracts containing only a small proportion of myosins. Such extracts have been analysed by electrophoresis (Hamoir, unpublished).

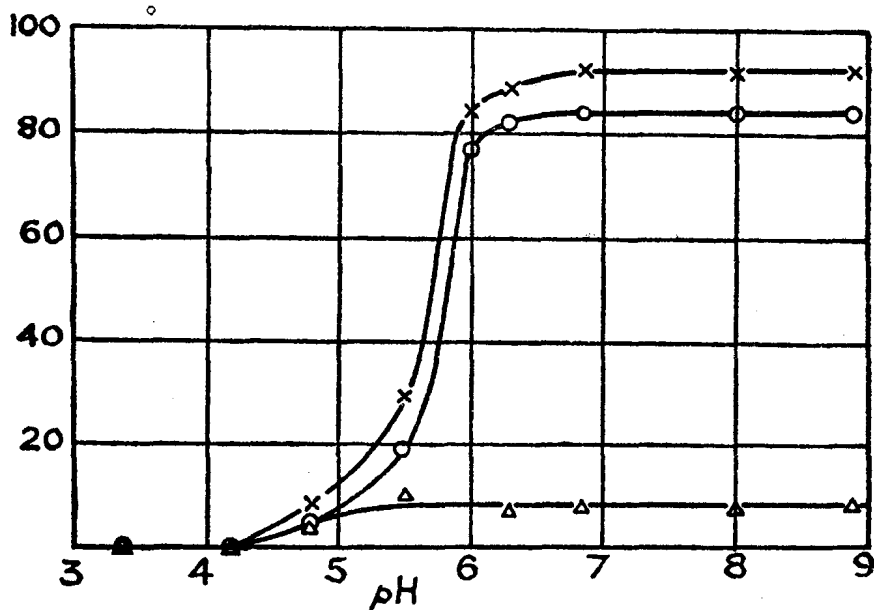


Fig. 2. Influence of the pH on the extractability of the muscle proteins of cod at ionic strength 0.85 (after Dyer *et al.*, 1950). Ordinate: per cent. of the total protein N extracted; \times — \times , as soluble protein N; \circ — \circ , as myosin N; Δ — Δ as non-myosin N. Abscissa: pH.

Carp muscles were extracted for ten minutes with phosphate-KCl buffers of ionic strength 0.5 ($\mu=0.3$ KCl and 0.2 phosphate) and of pH 5.8 or 5.1. After removal of the muscles debris by centrifugation, the extracts were dialysed against phosphate-NaCl buffers of ionic strength 0.35 and pH 7.1 ($\mu=0.1$ phosphate and 0.25 NaCl) or of ionic strength 0.15 and pH 7.3 ($\mu=0.1$ phosphate and 0.05 NaCl).

The electrophoretic patterns obtained are given in Figs. 3 and 4. At $\mu=0.35$ and pH 7.1, carp myosins are perfectly soluble, although they cannot be extracted at this ionic strength and pH (Fig. 3). On the ascending side, the myosins form two peaks migrating very closely together in front of the myogens, the rapid one being very sharp and

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the slower one showing a more symmetrical shape ; in front of these two peaks a new much more rapid component, representing only few per cent. of the protein content of the extract, can be observed. An important asymmetry exists between the ascending and the descending limbs due to the presence of the myosins : the components do not separate well on the descending side and the two peaks of the myosins are not visible. The mobilities of those different peaks have been determined ; mean values of several electrophoresis are given in Table 2.

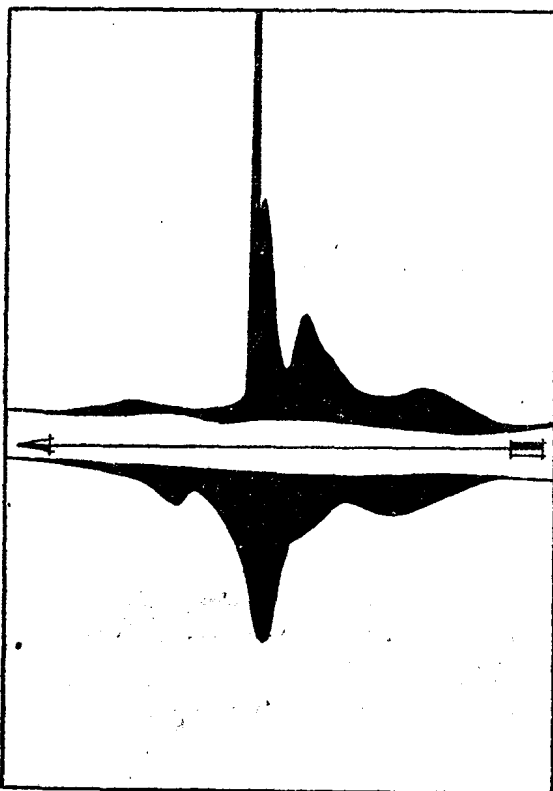


Fig. 3. Electrophoretic pattern of a ten-minute extract from carp muscle at $\mu=0.5$ and pH 5.8. Ionic strength 0.35, pH 7.1. Migration to the left. Upper part : ascending limb. Lower part : descending limb. Duration of the electrophoresis : 79,100 sec. Electrical field : 2.0 v./cm.

By dialysis of such an extract against a solution of $\mu=0.15$ and pH 7.3, a precipitate of myosins forms which can easily be removed by centrifugation. The composition of the supernatant is given in Fig. 4.

This electrophoretic pattern differs from the previous one obtained at low ionic strength (Fig. 1) by the presence of the peak of high mobility already observed in Fig. 3. This component behaves as a