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A Comparative Approach to Fish Respiration*

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In many fields of biology two basic approaches may be adopted when considering the range of form and adaptations shown by different species. The first involves a detailed study of the morphology, physiology, ecology or behaviour of one particular species which, ideally, is representative of the whole group. In the second approach a whole range of species is studied with especial attention to the particular phenomena under consideration (e.g. respiration) and the way in which different species illustrate features relevant to the general study. This is the *comparative approach* which is known classically with regard to the anatomy of animals and has produced valuable information, not only concerning their structure but also their relationships.

Both approaches have advantages and disadvantages; clearly the first approach has the great advantage of concentrated effort resulting in very detailed knowledge. But there are dangers if a particular case is considered generally applicable. For example, the frog sartorius had been investigated in very great detail before much was known about the muscle and nerve/muscle physiology of other animals. It has now become clear that the frog sartorius is by no means a 'typical' muscle.

With the comparative approach, the danger of specialized knowledge is far less because such investigators are aware of the great variety of forms and systems in the animal kingdom. A disadvantage of this approach is that it must inevitably be lacking in depth. During research in any branch of biology, one approach may be best at one stage, but later another becomes most appropriate. Thus at a symposium in 1964, attention was drawn to the great need for detailed investigation of some aspects of the gas exchange in fish[1], using a single species. Since that time some valuable studies have been carried out and there has been a greater concentration of effort on individual species. Similar concentration has also been true with respect to the mechanics of ventilation in which it was thought that the trout was a typical example. At the present time there is a need to extend investigations into the whole range of different fish forms and their adaptations. In neurophysiology the comparative approach has been extremely successful at revealing preparations in which particular aspects of nervous function can be investigated better than in others. Notable examples are the giant fibre and stellate ganglion preparations of the squid, the giant neurones of *Aplysia* and the Mauthner cells in lower vertebrates. At a later stage, more detailed investigations of these systems were carried out from a more biophysical and biochemical viewpoint. Clearly a most important role for zoologists is to survey the different systems that have evolved.

In the present review, examples will be chosen to illustrate the value of a comparative approach in fish respiration.

To survey this whole field requires more space than is available but we can summarize the main branches of fish respiration which are being investigated at present: (1) Measurement of oxygen consumption and its relationship to environmental conditions. (2) The structure of the gills, especially the water/blood pathway. (3) Investigations of the pumping mechanisms ventilating the gills. (4) Measurement of parameters of the ventilation system, e. g. the minute volume, respiratory frequency and gill resistance. (5) Measurements on the perfusion side of the gill exchanger including determination of cardiac frequency, stroke volume and physico-chemical properties of the blood. (6) Measurement of P_{O_2}, P_{CO_2} and pH in the water and blood from different parts of the respiratory system provide information about the mechanisms of gaseous exchange. (7) Finally, mechanisms whereby these systems are controlled and respond to various stresses are being actively investigated in relation to the concept of respiratory homeostasis.

In this review, attention will be given particularly to the first four headings although reference to some of the others will be mentioned 'en passant'.

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[1] G. M. Hughes, Symp. Soc. exp. Biol. *18*, 81 (1964).

1. *Oxygen consumption*

The measurement of oxygen consumption in fish is fraught with many difficulties as emphasized by FRY[2] and his school. Great difficulty is experienced in maintaining the fish under constant conditions with respect to exercise and environmental factors, as well as their previous experience. The methods available usually involve confinement of the fish in a respirometer and until recently it was difficult to study swimming fish. Recent studies by BRETT[3] using a respirometer in which the fish maintains a constant swimming speed against a flow of water have proved most valuable. Even so, this type of respirometer may involve the accumulation of carbon dioxide and other metabolic products in the medium so that conditions do not remain constant throughout the experimental period. Another valuable approach is to use a species which lives in a restricted environment, and hence the confined space of a respirometer is not so abnormal. Species such as the sea-horse, *Hippocampus*, have been used in small respirometers and more recently a cardinal fish[4] which normally occupies niches within coral reefs has proved a valuable experimental animal. Other fish[5,6] which spend most of their time at rest on the sea-bottom also provide good material for this kind of work, as have eels[7,8] and dogfish[9] which rest in long tubes quite readily.

2. *Gill structure and function*

As most gas exchange takes place across the gill epithelium, it is clear that knowledge of its nature is essential to the whole study of fish respiration. Gas exchange takes place in the secondary lamellae, which are folds running at right-angles to the main axis of the gill filament (Figure 1). The basic arrangement of the gills has been known for a long time, although details of their function, especially changes during a respiratory cycle such as differences in resistance to water flow and of the diffusion barrier[10] to gaseous exchange, are only now being investigated. But it is most surprising that it is only in recent years that some gross morphological features have been clarified. Normally the edges of the secondary lamellae are free and water flows through a sieve as indicated in the diagram (Figure 1,B). But in some species the free edges of the secondary lamellae are fused to one another by development of a secondary septum as in *Amia* (BEVELANDER[11]). A similar development of interlamellar fusions is also found in fast oceanic fish such as the skipjack tuna. Other tunas, in addition to the lamellar fusions, also have both the leading and trailing edges of the gill filaments considerably modified by the development of an overlay structure (MUIR and KENDALL[12]) such that water can only enter the interfilamentar spaces by a number of holes spaced at intervals along the length of the filament (Figures 2 and 3). Consequently the path of the water across the gills is more complex and water can only enter and leave the space containing the secondary lamellae at these points. In some other fast-moving oceanic fish, such as the marlin and swordfish, this type of development is present, but the interlamellar fusion is absent.

As these different types of fusion occur in phylogenetically unrelated groups, they must have evolved independently a number of times. Their convergence suggests a similarity of function and is probably true of the oceanic fish, but the presence of interlamellar fusions in *Amia* as well as skipjack tuna, is difficult to reconcile with such a generalization. In *Amia* it has been suggested that they may help maintain the patency of the sieve in air and so aid respiration

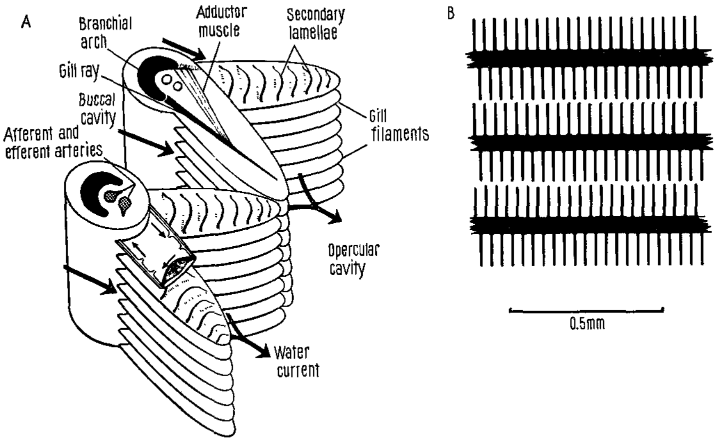

Fig. 1. Diagrams showing the general structure of teleost gills. (A) Arrangement of 2 gill arches with attached filaments and secondary lamellae. (B) Diagram of the profile presented by the sieve to the flow of water (after HUGHES[56]).

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[2] F. E. J. FRY, *The Physiology of Fishes* (Ed. BROWN; Academic Press, New York 1957), vol. 1, p. 1.

[3] J. R. BRETT, J. Fish. Res. Bd. Can. *21*, 1183 (1964).

[4] R. J. LIVINGSTON, J. mar. biol. Ass. U.K. *48*, 485 (1968).

[5] G. M. HUGHES and S.-I. UMEZAWA, J. exp. Biol. *49*, 565 (1968).

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[7] L. VAN DAM, Thesis, Groningen (1938).

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[11] G. BEVELANDER, *Copeia 3*, 123 (1934).

[12] B. S. MUIR and J. I. KENDALL, *Copeia 2*, 388 (1968).

(HUGHES[13]). For the oceanic species, the modification may be related to their fast swimming and ram-jet ventilation. However, other fish having similar ventilation (e.g. mackerel) do not possess these modifications, although there is some suggestion of a stiffened leading edge to the filaments. MUIR and KENDALL[12] have suggested that they may contribute to the rigidity of such a large sieve and this could certainly be the case, but its presence in the smallest tunas that have been obtained (MUIR and HUGHES[14]) seems to preclude size as being of great importance. Clearly the development of the overlay must have some protective function, and the need for this is suggested by the observation of marlin gills (Figure 4) in which whole filaments were severely damaged but showed partial regeneration. With ram-jet ventilation at high swimming speeds, it would be difficult for a fish to avoid certain objects in the water. Another possible functional significance is that the overlay acts as a baffle system whereby the speed of the water flowing over the secondary lamellae is reduced and the efficiency of the exchange is increased.

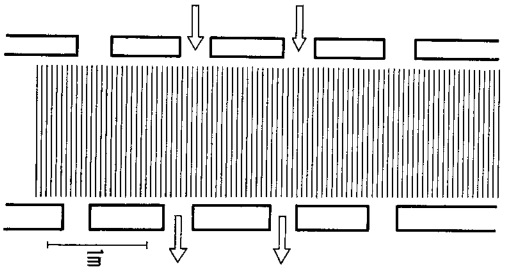

Fig. 2. Diagram illustrating the flow pathway of water across a filament of a tuna gill in which interfilamentar junctions are developed. On the inflow side, water enters through small pores, then passes through an interfilamentar space containing the secondary lamellae and leaves by a corresponding series of slightly smaller outlet holes into the opercular cavity.

Fig. 3. Photograph of a single gill arch from a marlin. Several gill filaments have been damaged and show regeneration. The formation of interfilamentar junctions leaving pores for the flow of water are clearly visible and correspond to those shown diagrammatically in Figure 2.

Further features of the gross morphology of fish gills have been investigated quantitatively[15].

$$\text{Total gill area (A)} = L \cdot \frac{2}{d'} \cdot bl$$

where L is total filament length (mm), $1/d'$ is the number of secondary lamellae/mm on one side of a filament, and bl is the average area on both sides of a secondary lamella. Thus estimation of the total number of secondary lamellae depends on measurements of the total length of the filaments and the frequency of the secondary lamellae along this length. Recent studies on tunas have made it possible to investigate variations within the sieve in more detail than has been possible previously (Figure 5) and to make allowance for differences by using a system of weighted averages

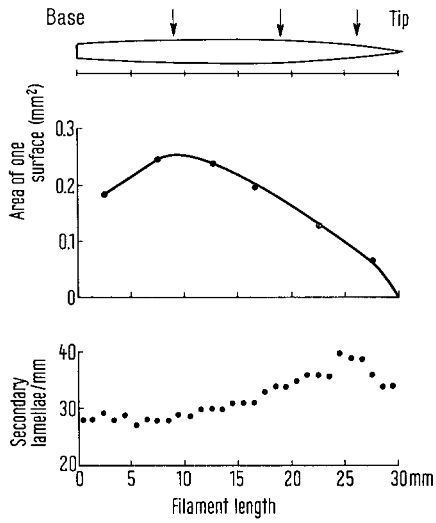

Fig. 4. Diagram showing variations in the areas and frequencies of secondary lamellae along a single filament from a tuna gill (after MUIR and HUGHES[14]).

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[13] G. M. HUGHES, Proc. R. Soc. Med. *59*, 494 (1966).

[14] B. S. MUIR and G. M. HUGHES, J. exp. Biol. *51*, 271 (1969).

[15] G. M. HUGHES, J. exp. Biol. *48*, 177 (1966).

(MUIR and HUGHES[14]). Comparative study has also shown that the way in which the gills grow among different fish is of significance. There is usually a relatively rapid increase in total number of filaments; but increase in gill area later in growth is mainly achieved by the greater length of the filaments and hence an increase in total number of secondary lamellae (Figure 6). Comparative studies have also emphasized the need to take into account the size of a fish when giving figures for the gill area. This was indicated by GRAY[16] and shows especially well when the data is plotted on log/log co-ordinates – the average slope being about 0.8. In tunas figures for gill area/g body weight are not exceptionally high, but when account is taken of their large size (Figure 7) they are clearly about an order of magnitude greater than most other fish.

Knowledge of microscopic structure of secondary lamellae has likewise gained by this approach. Ultrastructural investigations first showed that the water/blood pathway was made up of epithelial layers, basement membrane, and a flange of the pillar cell (HUGHES and GRIMSTONE[17]). Recent studies on a range of bottom-living teleosts and elasmobranchs indicate that the outer surface of the epithelial layer is frequently folded into many microvilli (HUGHES and WRIGHT[18]). They may be finger-like protuberances or more in the nature of ridges, as in the flatfishes *Pleuronectes* and *Solea*. Apart from interesting morphological differences there are also differences in the length of the water/blood pathway and of its detailed nature. In elasmobranchs, for example, there is nearly always a layer of vesicles below the microvilli. Studies on elasmobranch and teleost species from a range of habitats suggest the presence of lymphocytes between the epithelial layers, indicating the presence of lymphoid spaces. The total length of the water/blood pathway varies and appears to be least in the most active fish such as tunas, and greatest in the more sluggish forms such as ice-fish and flatfish; the greatest water/blood barrier being in the air-breathing fish such as *Anabas*, where the multilayered epithelium may be at least 15 μ thick[19]. In these air-breathing forms, the barrier between the air contained in the suprabranchial chambers and the blood of the accessory organs which line this cavity and also form the labyrinthine plates is extremely small (0.1–0.3 μ), being comparable to that of the mammalian alveolus. *Anabas* probably obtains most oxygen from the air and often lives in water containing very low oxygen tensions. Consequently there is a danger of oxygen being lost from the blood during its passage through the gills. Under these circumstances the thick diffusion barrier is clearly of functional significance.

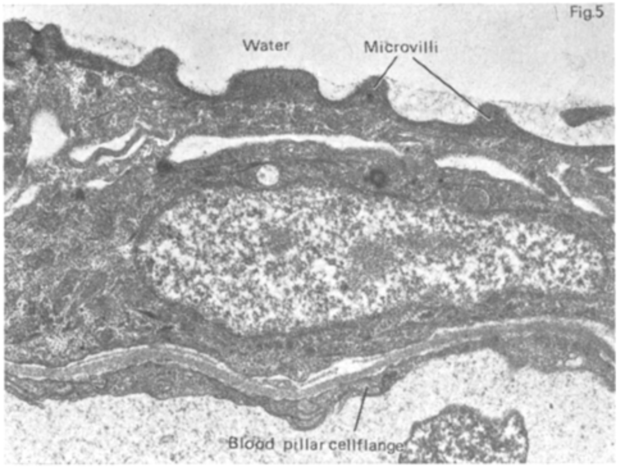

Fig. 5. Electronmicrograph showing the surface microvilli of a flatfish in which the muous layer is particularly clear (Afler HUGHES and WRIGHT[18] × 18,000).

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[16] I. E. GRAY, Biol. Bull. mar. biol. Lab. Woods Hole *107*, 219 (1954).

[17] G. M. HUGHES and A. V. GRIMSTONE, Q. Jl. micr. Sci. *106*, 343 (1965).

[18] G. M. HUGHES and D. E. WRIGHT, Z. Zellforsch., in press (1970).

[19] G. M. HUGHES and J. S. DATTA MUNSHI, Nature, Lond. *219*, 1382 (1968).

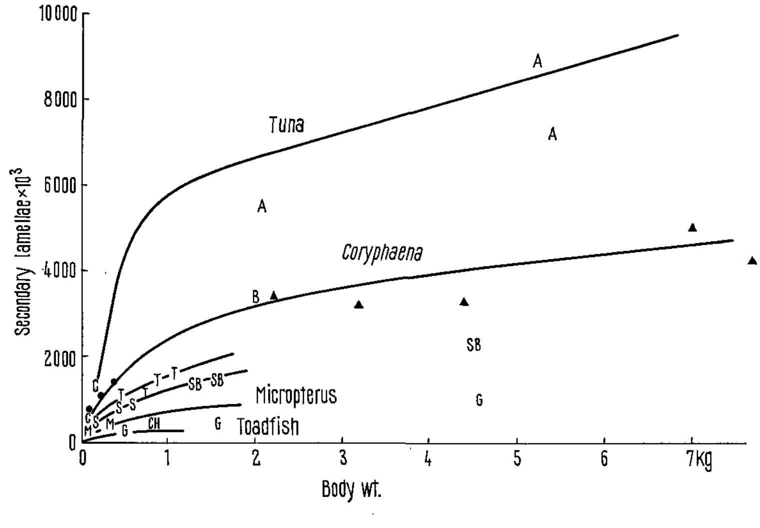

Fig. 6. Relationship between the number of secondary lamellae and body weight in a number of teleost fish. Most of the data is based on that of GRAY[16] and symbols are as follows: A, false albacore; B, *Bonito*; C, *Coryphaena*; Ch, *Chaenocephalus*; G, goosefish; M, mackerel; S, scup; Suc, striped bass; T, sea-trout.

There are indications that fish can reduce the barrier particularly around the edge of secondary lamellae, presumably when gas exchange with the water is more important.

Data plotted in Figure 8 indicates that active fish with larger gill areas also have shorter diffusion distances, and fish with small gill areas have longer diffusion distances. This suggests that selection operates on both parts of the system to increase the efficiency of gas exchange. The epithelial surfaces are frequently covered by a mucous film particularly in the clefts between microvilli. This film is probably normal and perhaps one of the functions of the microvilli is to anchor it. Possible functions of the mucus in addition to protection against irritants include reduction of drag and the presence of differential permeability properties with respect to ions and water as compared to the respiratory gases. Mucous coverings of the skin in a number of amphibious animals are permeable to gases but relatively impermeable to water and ions and so help reduce water loss. In fish, particularly in freshwater, there is a danger of ionic losses from such large surfaces of contact between the blood and external medium.

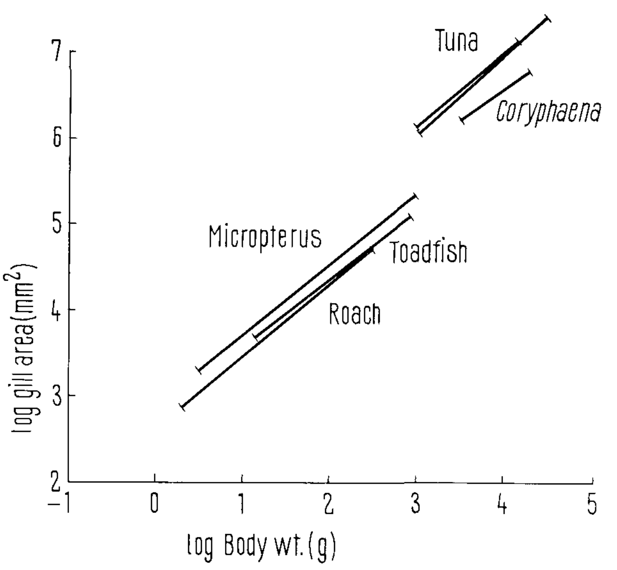

Fig. 7. Log/log plots showing relationship between gill area and body weight for a number of individual species. It is clear that the tunas have a very much larger gill area than other fish.

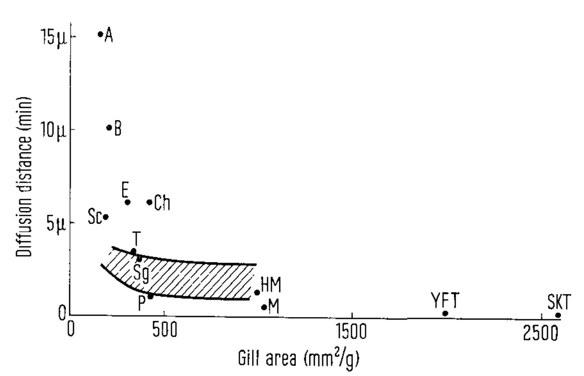

Fig. 8. Relationship between average gill area/g and the minimum diffusion distance for a number of species (after Hughes[57]). A, *Anabas*; B, bullhead; Ch, *icefish*; E, eel; HM, horsemackerel; M, mackerel; P, plaice; Sq, *Squalus*; Sc, *Scyliorhinus*; T, trout; SKT, skipjack tuna; YFT, yellowfin tuna.

3. *The respiratory pumps and their muscular basis*

Studies using modern pressure and movement recording techniques suggested that water flow depends on a double pump consisting of a pressure pump in front of the gills and a suction pump behind them. This mechanism was first elucidated on the trout where the two pumps appear more or less equally important. The differential pressure across the gills summarizes the interaction of the two pumps and strongly suggests that flow is almost continuous. The balance between the two pumps varies and may change during temperature and hypoxic stresses (Figure 9) (Hughes and Roberts[20]; Hughes and Saunders[21]). A comparative study (Hughes[22]) showed that the relative role of the pumps varies; in bottom-living forms the suction pump is most important (Figure 10). Gradual expansion of the opercular cavities draws water across the gills which is ejected during brief periods, often in a dorsally-directed stream through a narrow opercular opening (Dragonet) or a special portion of the opercular slit as in Gurnards. Among flatfishes and some other species, the reversal phase of the differential pressure is absent (Figure 10) and probably is associated with active mechanisms for closing the opercular valve. Such mechanisms may prevent sand entering through the opercular slit; entry of parasites by this route may also be hindered. Ventilation in cartilaginous fishes has similar features; the bottom-living rays have no reversal in the differential pressure curve.

From these studies it became clear that the basic mechanisms elucidated with the trout were of more general application, but further investigations have shown that the original model was an over-simplification. The two pumps are not independent but are coupled together by springs of varying compliance (Hughes[1]; Ballintijn and Hughes[23]). Electromyographic studies on the trout showed that similar movement patterns, with essentially the same pressure changes in the respiratory cavities, may be produced by different patterns of muscle activity. This is possible because of the extensive couplings between parts of

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[20] G. M. Hughes and J. L. Roberts, J. exp. Biol., in press (1970).

[21] G. M. Hughes and R. L. Saunders, in preparation (1970).

[22] G. M. Hughes, J. exp. Biol. *37*, 28 (1960).

[23] C. M. Ballintijn and G. M. Hughes, J. exp. Biol. *43*, 363 (1965).

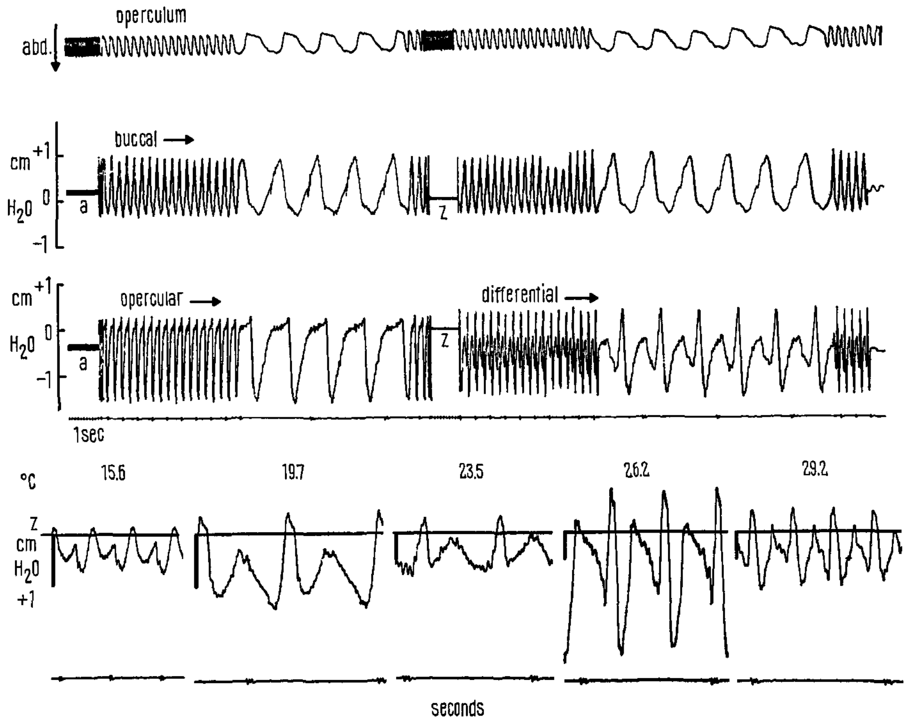

Fig. 9. Recordings of the buccal, opercular and differential pressures and opercular movements of a rainbow trout. Changes in the differential curve of another specimen at different temperatures are also shown. Differential pressure is positive when pressure in the buccal cavity exceeds that in the opercular cavity. Zero pressure (Z) is the pressure in the waterbath (after Hughes and Roberts[20]).

the musculo-skeletal system concerned with changing the volume of the respiratory cavities. Comparative electromyographic studies have also helped with the general problem of the phase relationship between the buccal and opercular movements. In the 18th century, Du Verney thought that movements of the mouth and operculum were synchronous. Some later workers (Duvernoy[24]) thought of them as alternating, but Bert[25] and others believed in synchrony. François-Frank[26] and Kuiper[27] considered them to be slightly out of phase as did Baglioni[28]. Willem[29] believed in synchrony because of the couplings between skeletal elements in the fish head. Modern recording techniques (Hughes and Shelton[30], Hughes[22]) show that movements of the lower jaw usually precede lateral movement of the operculum, although these movements would also be produced by lateral movements of the buccal cavity. The trout was taken as a typical example and the phase delay incorporated in a model (Hughes[31]). Studies of the muscular anatomy (Ballintijn and Hughes[23]) show that the trout has some specialized features such as a single continuous muscle on the inner aspect of the palatal arch and operculum. In the carp this adductor arcus palatini et operculi is replaced by three separate muscles, adductor arcus palatini, adductor operculi and levator operculi. Differences between the trout and carp in the timing of their lower jaw movement relative to the operculi are due to differences in the phase of the cycle when these muscles are active (Ballintijn[32]). Lowering of the jaw in the carp and most other teleosts that

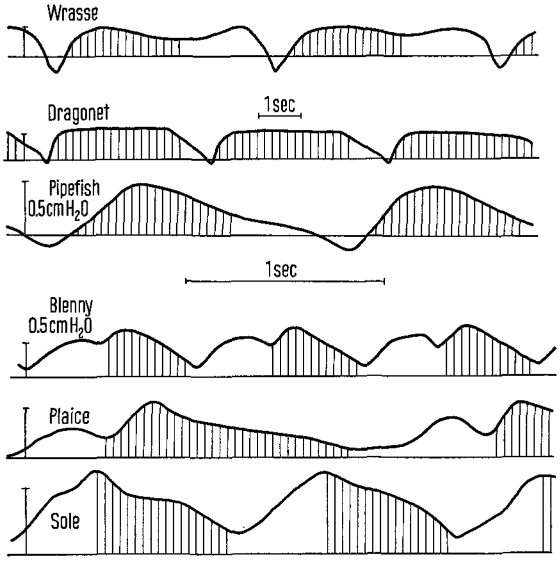

Fig. 10. Differential pressure curves from a variety of teleost fish. The cross-hatched portion represents the opercular suction pump, which is more developed in the benthic species (after Hughes[22]).

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[24] M. Duvernoy, Annls. Sci. nat. Zoologie *12*, 65 (1839).
[25] P. Bert, C. r. Soc. Biol. *1*, 330 (1869).
[26] Ch. A. Francois-Frank, C. r. Soc. Biol. *60*, 339 (1906).
[27] J. Kuiper, Archo. ital. Biol. *45*, 393 (1906).
[28] S. Baglioni, Z. allg. Physiol. *7*, 177 (1907).
[29] V. Willem, Bull. Acad. r. Belg. Cl. Sci. *26*, 211 (1940).
[30] G. M. Hughes and G. Shelton, J. exp. Biol. *35*, 807 (1958).
[31] G. M. Hughes, New Scient. *11*, 346 (1961).
[32] C. M. Ballintijn, J. exp. Biol. *50*, 569 (1969).
[33] G. M. Hughes and C. M. Ballintijn, J. exp. Biol. *49*, 583 (1968).
[34] J. Osse, Netherl. J. Zool. *19*, 290 (1969).
[35] G. M. Hughes, unpublished observations on *Balistes*.

have been investigated electromyographically (BALLINTIJN and HUGHES[33], OSSE[34], HUGHES[35]) is usually produced by contraction of the levator operculi operating through the operculum, hyoid and stylohyal. As can be seen from Figure 11, the timing of the levator operculi is in phase with the adductor arcus palatini of the carp which fires at about the same time as the adductor arcus palatini et operculi in the trout. Thus lowering of the jaw in the trout is associated with the adduction phase of the operculum due to the combined action of the different portions of the adductor arcus palatini et operculi. In the carp the levator operculi portion of this muscle is separate and is active during the opposite phase of the cycle. Hence lowering of the jaw can occur at the same time as abduction of the operculum. Thus differences in muscle anatomy make it possible to interpret differences in relative timing of the jaw and opercular movements.

Many variations occur in fishes according to the precise nature of their skeletal connections and muscle morphology. From the functional point of view changes in volume of the two cavities are most important; exactly which muscles are involved is of less importance. Sometimes the lower jaw plays an important part in closing the buccal cavity but in other species its movements are negligible, reflux of water during the compression phase being reduced by well-developed maxillary and mandibular valves. Even in such forms (e.g. *Balistes*) with very stiff skins, the mechanism appears to be basically the same[36].

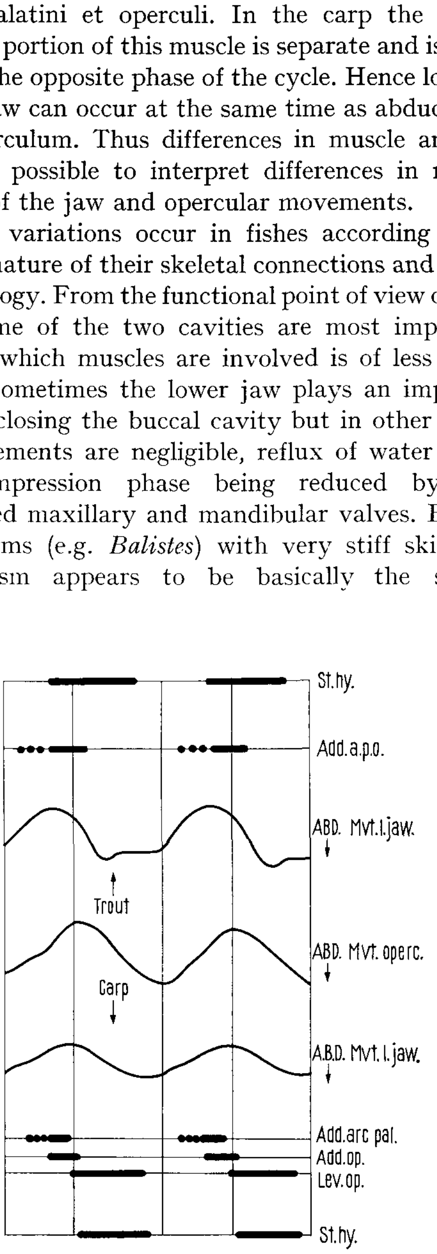

Fig. 11. Comparison between lower jaw movements (Mvt. l. jaw) and timing of muscle activity in the carp and trout. Opercular movements (Mvt. operc.) are superimposed. Striking differences in timing of activity in the adductor arcus palatini et operculi (add.a.p.o.) of the trout and the levator operculi (lev.op.) in the carp relative to the jaw movements are very clear. Abbreviations for other muscles are: st.hy., sternohyoideus; add.arc.pal., adductor arcus palatini; add.op., adductor operculi. (After BALLINTIJN[32]).

Extension of these studies to cartilaginous fishes (HUGHES[37], HUGHES and BALLINTIJN[38]) showed that the basic mechanism consisted of an active compression of the branchial region by the constrictor muscles leading to an increase in pressure before the gills which was greater than that in the parabranchial cavities, water being forced across the gills and out of the external slits. Expansion normally results from the passive elastic properties of the branchial skeleton and ligaments. The hypobranchial muscles are inactive during normal ventilation. Following experimental hypoxia, electrical activity could be recorded in the coraco-hyoideus and coraco-branchialis muscles but it is doubtful whether this occurs in normal life. However, these muscles, and especially the coraco-mandibularis, are active when the fish opens its mouth and expands the branchial region before biting. Similar patterns of activity have been recorded in the ray (HUGHES[39]); in this case muscles concerned with active opening and closing of the spiracle are also important.

It appears that the relaxed condition of the branchial system in elasmobranchs is an expanded one and can be observed in fully anaesthetized dogfish. During swimming, water enters the mouth and ventilates the gills without any active pumping. When sharks begin to swim there is a gradual fall in the frequency of respiration as the swimming speed rises (HUGHES[37]). The tonic activity of the adductor mandibulae during the mouth-open phase would regulate the flow of water into the mouth during swimming.

This ram-jet ventilation has been known for a long time and given particular attention following HALL's studies[40] on the mackerel which indicated that the P_{O_2} level in the dorsal aorta fell below normal if the swimming speed was reduced. In recent years a number of observers (BAINBRIDGE[41], BRETT[3], SMITH et al.[42]) have noted the effect of swimming speed on the respiratory movements of teleosts and it is often observed that the frequency falls as speed increases. The comparative approach provides the ideal species for such studies. The *Remora*, normally attached to the back of sharks, obtains a free ride and the possibility of getting food. But it is now clear that it also obtains free ventilation of the gills! When attached by their sucker to the wall of a water tunnel, the effect of changing water velocity on the frequency of the respiratory movements could be studied in a more

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[36] G. M. HUGHES, Experientia *23*, 1077 (1967).
[37] G. M. HUGHES, J. exp. Biol. *37*, 28 (1960).
[38] G. M. HUGHES and C. M. BALLINTIJN, J. exp. Biol. *43*, 363 (1965).
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[40] F. G. HALL, Biol. Bull. mar. biol. Lab. Woods Hole, *61*, 457 (1931).
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[42] L. S. SMITH, J. R. BRETT and J. C. DAVIS, J. Fish. Res. Bd. Canada, *24*, 1775 (1967).

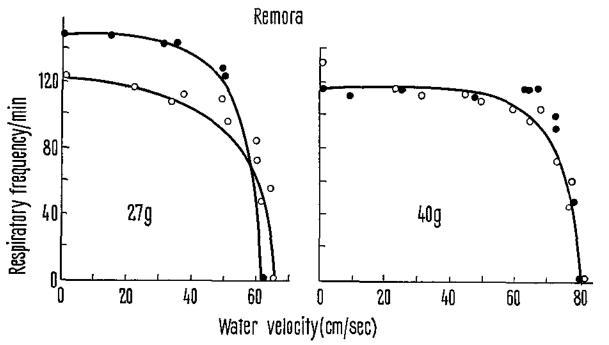

Fig. 12. Relationship between frequency of respiratory movements and water velocity for 2 specimens of Remora in a water-tunnel (after Muir and Buckley[43]).

controlled way (Muir and Buckley[43]). Figure 12 shows that above velocities of about 50 cm/sec the frequency of ventilation movements rapidly falls to zero.

Several studies have shown recently that during normal swimming only the red muscles of the trunk are active (Bone[44], Rayner and Keenan[45]). Clearly these are the muscles doing the work required to overcome the gill resistance during ram-jet ventilation. If they are more efficient than the branchial muscles at converting chemical to mechanical energy, then there could be a net gain to the fish. Nevertheless, the proportion of the resistance to forward propulsion provided by the gills appears to be relatively small; thus in a 6.7 kg tuna estimates suggest that it is less than 10% of the total frictional losses during swimming.

4. *Measurement of ventilation volume in fish*

The performance of the respiratory pumps is best assessed by measuring the flow of water across the gills. Two main methods have been used.

(A) *Indirect methods.* (1) Depends on measurement of the change in oxygen tension (ΔP_{O_2}) across the respirometer and the gills, together with the flow rate through the respirometer. Hence:

$$\text{Minute volume} = \frac{\Delta P_{O_2}\,\text{resp.}}{\Delta P_{O_2}\,\text{gills}} \times \begin{matrix}\text{Respirometer flow}\\ (\text{ml/min})\end{matrix}$$

This method requires very accurate P_{O_2} measurements. ΔP_{O_2} gills is especially difficult to determine, variations being obtained depending on where the opercular water is sampled. Nevertheless, this method has the advantage that the fish is interfered with relatively little and can be maintained at rest for long periods, but it can give little information on sudden changes in minute volume. (2) A dye-dilution method using dyes injected into the buccal cavity, where the dye must be well mixed, and it is sampled after its passage across the gills. This method has been used by Murdaugh et al.[46] for the shark *Squalus acanthias* and has given higher figures than those obtained by Piiper and Schumann[47] using a direct method with *Scyliorhinus stellaris*.

(B) *Direct methods.* For these measurements great use has been made of special adaptations. For example, *Uranoscopus* normally rests buried in sand, water being drawn into a depression above the mouth (Figure 13,a). By placing a thistle funnel over this opening and measuring the rate of movement of air bubbles along a tube connected to it, quantitative studies were made (Meyer[48]) on the ventilation volume under different conditions. In the puffer fish, Hall[49] inserted small tubes into the opercular openings and collected the water being forced out from them. In both these cases there was a danger of water loss and interference with the normal valve mechanisms.

Van Dam[7] employed a method for the eel and trout in which the chamber containing the mouth was separated by a rubber membrane from that containing the opercular openings (Figure 13,b). He made important measurements and showed the high utilization of oxygen. A similar method was used in the tench and the concept of gill resistance was developed by measuring the flow simultaneously with pressure changes across the gills (Hughes and Shelton[30, 50]).

In recent years the availability of special cements has made it possible to fasten membranes around the respiratory openings and so collect water leaving or entering with relatively little interference. In the dogfish, part of a rubber glove fixed around the external gill slits enabled the mixed expired water to be collected. Such experiments proved very satisfactory in *Scyliorhinus stellaris* (Piiper and Schumann[47]), but the smaller species, *S. canicula*, was not as suitable. The rubber chamber interfered with the ventilation movements, particularly the passive expansion phase (Hughes and Umezawa[9]). Among teleosts the dragonet (*Callionymus lyra*) has proved very useful[5, 6] because of the circular opening from each opercular cavity (Figure 13,d). In addition to directly determining the minute volume and following rapid changes during hypoxia, these methods give better samples for measuring oxygen consumption and percentage utilization. Differences in the ventilation/perfusion relationships within the whole gill system are evened out and the true mixed expired water is sampled. In all cases there is a danger of loading the system by the attachment of tubes and it is important that their bore is

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large (Hughes and Knights[6]). If animals of sufficient size are used, the increased O_2 consumption due to the loading is no greater than that resulting from the attachment of cannulae.

In *Callionymus* a correlation was obtained between stroke volume and size of the integrated myogram for several of the respiratory muscles (Hughes and Ballintijn[33]). The time course of changes in the integrated EMG during reduced P_{O_2} or increased P_{CO_2} of the inspired water gave evidence for a dual mechanism controlling ventilation. Variations in water flow could be produced by changing the hydrostatic pressure (Δp) across the system (Hughes and Umezawa[5]). With the hydrostatic pressure opposing the normal flow (Δp negative), the fish pumps more strongly (increased EMG) and *Callionymus* can maintain the flow against adverse gradients of 2 cm of water. Thus experimentally, ventilation could be increased in two ways: (a) by lowering the inspired P_{O_2} and (b) by increasing the Δp. Low P_{O_2}'s evoke bradycardia as well as hyperventilation. Corresponding increases in water flow without a change in P_{O_2} did not result in a bradycardia and it was concluded that bradycardia is a direct response to the P_{O_2} change and not to the secondarily produced hyperventilation (Hughes and Umezawa[5]). Experimentation with sluggish bottom-living fish clearly has many advantages although it must be remembered that their mechanisms may be specialized.

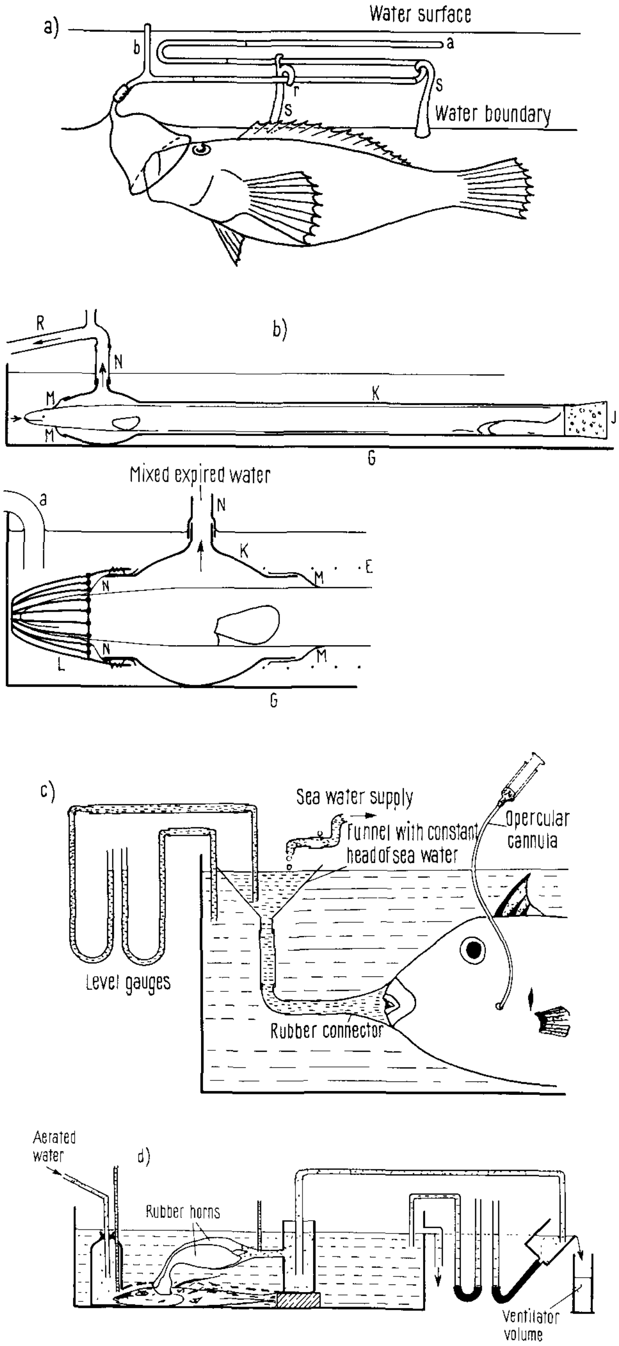

Fig. 13. Apparatus used for the direct measurement of ventilation volume in a variety of fish species. (a) *Uranoscopus* (after Meyer[48]); (b) eel (after Van Dam[7]); (c) *Balistes* (after Hughes[36]); (d) *Callionymus* (after Hughes and Knights[6]).

This type of experimentation together with refined cannulation of blood vessels, has greatly advanced our knowledge of gas exchange (Piiper and Schumann[51]). The results also give information about the flow across the gills. The high utilization and the relationship between inspired P_{O_2} and the P_{O_2} of blood leaving the exchanger support the presence of counterflow between water and blood. However, a multicapillary type of exchanger has been suggested for the dogfish gill by analogy with placental circulations, and fits in with what is known of the blood and water circulation.

There is no doubt that other fish will provide excellent material for the study of gas exchange. Already investigators interested in ionic exchange have found the anatomy of the eel ideal for this purpose and isolated gill preparations are readily obtained (Rankin[52]). With such preparations independent control of the water and blood flow will be possible and the permeability of the gill membrane to gases could be investigated. Studies on the swim-bladder of the eel have been greatly facilitated by the anatomy of gas gland rete in this species (Fänge[53] and Steen[54]).

A particularly interesting example of the comparative approach has recently been shown by the Antarctic fish which have no haemoglobin (Chaenichthyidae). In recent studies of gas exchange, observations have been made on the survival of trout after their haemoglobin had been poisoned by carbon monoxide (Holeton[55]). As might be expected from theoretical analyses[1], the reduction in O_2 solubility in the blood is compensated by increased cardiac output.

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The existence of fish without an oxygen-carrying pigment provided the obvious extension for such studies. The icefish (*Chaenocephalus aceratus*) has a large blood volume and circulates relatively large volumes through the gills in unit time. Both the ventilation and cardiac pumps operate at low pressures and thus help maintain a low total O_2 consumption[55].

From this survey it is apparent that fish respiratory studies have already gained a great deal from the comparative approach which is one of its most rapidly advancing areas. Nevertheless, the importance of detailed investigations using single species must not be neglected. The advantages of the two approaches are now more generally appreciated and in some laboratories both are going on simultaneously. It is important that communication between workers of the two sorts should be encouraged so that the advantages of the comparative approach are gained as rapidly as possible. In this way we can expect a continuing expansion of studies on fish respiration, especially as they have become significant in relation to work on pollution and underwater exploration.

Zusammenfassung. Es wird ein Überblick über neuere Forschungen gegeben, die das weite Arbeitsgebiet des Mechanismus der Fischatmung betreffen. Nachdruck wird auf den vergleichenden Standpunkt einer solchen Forschungsarbeit gelegt, wobei gezeigt wird, dass die Wahl besonderer Spezies ein förderndes Licht auf allgemein wichtige Probleme werfen kann, die bei Kiemenuntersuchungen sowohl auf der Makrostrukturstufe wie auf der Ultrastrukturstufe auftreten. Vom physiologischen Standpunkt aus gesehen, erweisen sich einige Arten auf Grund ihrer Lebensweise für experimentelle Untersuchungen ihres Sauerstoffverbrauchs besonders geeignet. Die strukturelle Anordnung des Pumpenmechanismus anderer Arten erleichtert wiederum äusserst günstig die Direktmessung des Ventilationsvolumens und anderer mit dem Gasaustausch zusammenhängender Parameter.

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SPECIALIA

The Monamycins, a New Family of Cyclodepsipeptide Antibiotics

An earlier communication[1] described the isolation, from cultures of *Streptomyces jamaicensis*, of monamycin, a crystalline preparation which inhibited the growth of various Gram-positive bacteria at high dilution. We have reinvestigated the production and isolation of monamycin and have examined further the chemical and biological properties of what has now been identified as a new family of antibiotics.

It has been established that monamycin production in deep culture is favoured by a medium based on neopeptone and glucose. Using a New Brunswick Fermacell 100 l fermenter, low aeration (0.25 v/v/min) and agitation rates, short fermentation times (42 h), low phosphate concentration and high carbon-nitrogen ratios gave optimum yields (150 mg/l). The crude mixture of crystalline antibiotics, which was obtained from a pentane extract of the culture fluid, was separated by recrystallization into 2 fractions. One consisted of compounds containing non-ionic chlorine, the other of non-chlorinated antibiotics. Extensive countercurrent distribution studies of each of these fractions (5500 and 7000 transfers), using the system ethyl acetate, cyclohexane, methanol, water (41:35:35:10) led to the isolation of pure peptides, or concentrates in which particular components predominated. The molecular formulae of the individual antibiotics were established by high-resolution mass spectrometry. The amino-acid and hydroxy-acid composition of acid hydrolysates was determined by isolation of individual components, by g.l.c. (for hydroxy-acids) and amino-acid analysis.

In addition to the acids L-proline, D-Valine, N-methyl-D-leucine, L-isoleucic acid and L-α-hydroxyisovaleric acid, all of which have been identified as metabolites of micro-organisms, the unusual amino-acids D-isoleucine (an abnormal isomer of the isoleucine group),[2] *trans*-4-methyl-L-proline, and 4 new C_5-amino-acids were present in the hydrolysates of the monamycins. Of the 4 new C_5-amino-acids[3], two, piperidazine-3-(*R*)-carboxylic acid (I) and 5-(*S*)-chloropiperidazine-3-(*R*)-carboxylic acid (II) have been identified as members of the D_s-series and a

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