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ACTIVE TRANSPORT AND SECRETION

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PREFACE

This volume contains the papers read at a Symposium of the Society for Experimental Biology which was held at Bangor in July 1953. It is the eighth of an annual series of Symposium Reports. The Symposium for 1954 will be held at Leeds, on Fibrous Proteins.

In the present Symposium the first three papers are introductory in character. These are followed by four papers on water movements and four papers on active transport phenomena in red blood cells, yeast and bacteria. The next group of five papers are concerned with active transport of ions in plants, and are followed by four papers on active transport of ions in animal cells. The remaining three papers are concerned with active movements of proteins and fats, and with mechanisms of active transport.

The papers presented here should be considered in relation to a number of recent reviews, notably by Conway (1953) (*Biochemistry of Gastric Secretion.* Springfield: Thomas), Hodgkin (1951) (*Biol. Rev.* **26**, 339), Brown (1952) (*Int. Rev. Cytol.* **1**, 107), Goldacre (1952) (*Int. Rev. Cytol.* **1**, 135) and Sutcliffe (1953) (*Int. Rev. Cytol.* **2**, 179).

The Society is deeply indebted to the British Council, the Rockefeller Foundation and to Imperial Chemical Industries Ltd. for financial aid.

The Editors wish to thank the members of the Advisory Committee who assisted us in preparing the Symposium programme. We also wish to thank the Cambridge University Press for the kindness with which we were assisted in producing this report.

R. BROWN
J. F. DANIELLI
Symposium Editors
Society for Experimental Biology

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MOVEMENTS OF WATER AND ELECTROLYTES IN INVERTEBRATES

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I. INTRODUCTION

When I was asked to give this introductory paper it was suggested to me that I should first and foremost present the background against which modern developments can be seen in perspective. It would be neither practicable nor desirable to attempt a review of all the material available; instead, what I shall do is to trace the development of ideas in the subject, to consider why some lines of approach have prospered more than others, why some questions have been answered and others left unasked, to show how the existence of active transport mechanisms has been recognized and to put forward some suggestions as to how they may have been evolved.

A few analyses of the body fluids of invertebrates were published during the latter part of the nineteenth century, and work of this type continued sporadically throughout the first two decades of the twentieth. At this stage these investigations were not inspired by any precise theory as to the nature of the body fluids and the ways in which their compositions were maintained; nevertheless, it became apparent that the body fluids of animals were in general not unlike sea water. Owing to the relative ease with which freezing-point measurements can be made our knowledge of the osmotic pressure of the body fluids began to advance rapidly. Experiments were carried out to test the effects of changes in the external medium upon the osmotic pressure of the body fluid. On the other hand, determination of the constituents of the body fluids was beset by technical difficulties and advance was slower. The development of the subject can be followed in the reviews which have appeared from time to time, notably those of Duval (1925), Schlieper (1930, 1935), Pantin (1931), Krogh (1939) and Beadle (1943). By the time the subject became of sufficient importance to merit review it had also acquired a philosophy which conveniently rationalized its ecological and physiological aspects—Claude Bernard's now famous pronouncement 'la fixité du milieu intérieur est la condition de la vie libre'. For the subject now under discussion Claude Bernard's pronouncement has the following special implication: primitive marine animals have in general no means of regulating the composition of their body fluids, and penetration into fresh water is only possible for animals which have evolved such means. Although the reviewers I have mentioned may have been mainly concerned with the physiological mechanisms whereby constancy is achieved, they have very obviously accepted Claude Bernard's proposition and incorporated it into the background of their ideas.

Let us then begin by considering a primitive marine animal having no control over the composition of its body fluid. When such an animal is placed in dilute sea water it swells, which may be interpreted as due to the inward diffusion of water. After some time in dilute sea water the volume of the animal returns to normal, which may be interpreted as the result of the relatively slower outward diffusion of salts through the general body surface. This, of course, is to look upon the animal as little more than a bag containing sea water. But even a primitive animal is generally something more than this. We have to consider that it has an alimentary canal, that it takes in food together with some sea water, and that it voids faeces which also have some admixture of fluid. It has an excretory organ from which urine is eliminated. Even if the body fluid is isotonic with sea water and the net exchange across the external surface is zero, the animal continually gains water, partly along with its food and partly as metabolic water produced by the oxidation of the food within the body, and loses water with its urine and faeces. There is thus a current of water continuously maintained through the body, upon which other movements, such as occur when the animal is placed in dilute sea water, are superimposed. The recovery of normal volume in dilute sea water, which we considered a moment ago, is not primarily due to leakage of salts and water through the general surface; it is due to an increased flow of urine, and it is via the excretory organ that most of the water and most of the salt leaves the body. There are in fact three principal regions of the body through which exchanges with the external medium can and do take place: (1) between the body fluid and the external medium at the surface of the body, (2) between the body fluid and the fluid in the gut, (3) between the body fluid and the urine in the excretory organ. All of these can be the sites of active transport mechanisms.

Next, let us consider what happens when this primitive marine animal evolves the ability to live in fresh water. This is a question which has been discussed at length by Beadle & Cragg (1940) and by Beadle (1943), and they have come to the conclusion that there are two stages in the process. I can most conveniently illustrate their thesis with examples drawn from the Crustacea (see Fig. 1). The spider crab *Maia* will serve to represent the primitive marine animal. It has virtually no powers of osmotic regulation, the osmotic pressure of its blood following that of the external medium over the whole of its viable range. The shore crab *Carcinus* shows some powers of osmotic regulation, being able to maintain the osmotic pressure of its blood above that of dilute sea water. It is commonly found in estuaries as well as in the sea but cannot maintain itself in fresh water. As an example of a crustacean fully adapted to fresh water we will take the crayfish *Astacus*. In this case the general level of the osmotic pressure of the blood is lower than in *Maia* and *Carcinus*, but it can be maintained at this level even in fresh water.

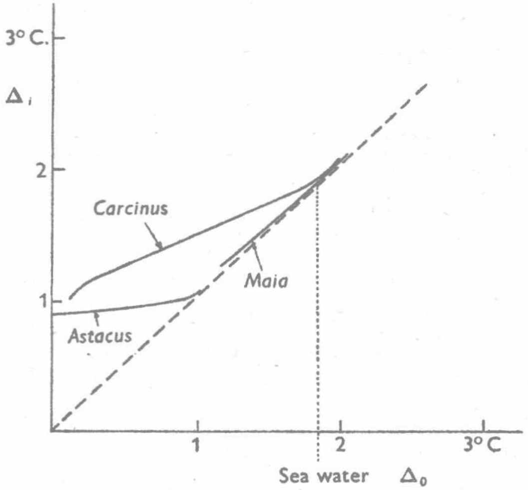

Fig. 1. Relation between the osmotic pressure of the blood, and the osmotic pressure of the external medium, for three crustaceans. *Maia* from Duval (1925), *Carcinus* from Duval (1925) and Schmidt-Nielsen (1941), *Astacus* from Herrmann (1931).

It might well have been imagined by the early investigators that these animals, when placed in dilute sea water, would follow what seems to us the obvious and logical course of pumping out the water which diffuses into them. But this does not seem to be the case. In a recent article, Robinson (1953)* has reviewed the evidence for the active transport of water in living systems, and as far as the aquatic invertebrate Metazoa are concerned the evidence is as yet circumstantial. It appears that these animals prefer to transport dissolved substances in such a way as to compensate for the passive movements of water under osmotic gradients.

According to Beadle & Cragg, in the first stage of the evolutionary process the animal develops the power of actively transporting salts from

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* I wish to thank Dr Robinson for allowing me to see his review in typescript before publication.

the external medium into the blood, to an extent which is sufficient to maintain the osmotic pressure of the blood significantly above that of the external medium. The excretory organ, however, does not back up the effort of the surface membranes. In *Carcinus* the urine is isotonic with the blood under all conditions, and beyond a certain point of dilution more salt is lost via the urine than can be gained by absorption through the surface. Ecologically this point lies for *Carcinus* some distance up the estuary, but definitely short of the river. But there is a crustacean which does in fact succeed in getting into rivers on this same inefficient physiological basis, and that is the Chinese mitten crab *Eriocheir*. As in *Carcinus*, so in *Eriocheir* the urine is isotonic with the blood under all conditions, but by sheer hard work, by absorbing salts from the river water at a great rate, this determined animal penetrates up rivers such as the Elbe for hundreds of miles, returning to the sea only for the purposes of breeding. For *Eriocheir* the ecological limit seems to lie not between brackish water and fresh water as for *Carcinus*, but between hard fresh water and soft fresh water; *Eriocheir* does not appear to be able to penetrate the softer waters of the Norwegian rivers (Schmidt-Nielsen, 1941).

Then in the second stage of the evolutionary process two things happen. First, the excretory organ becomes awakened to a proper sense of its responsibilities and produces hypotonic urine, thus conserving the salt content of the body; secondly, the general level of the osmotic pressure of the blood is lowered to about half that found in marine animals. This reduces the strain on the active transport mechanisms at the body surface and in the excretory organ. It also involves some readjustment of the salt and water balance between the tissues and the blood.

It emerges from this survey that the important sites of active transport lie in the general body surface—or specialized parts of it—and in the excretory organ. There is as yet very little evidence of active transport in the gut, at least as far as the digestive epithelium is concerned. We will therefore now proceed to further consideration of the surface membranes and of the excretory organs in aquatic invertebrates.

II. THE TRANSPORT OF IONS BY SURFACE MEMBRANES

The first demonstration that an animal can maintain the osmotic pressure of its blood by uptake of salts against a concentration gradient was given by Nagel (1934) for *Carcinus*. Having confirmed that *Carcinus* had the power of hypertonic regulation in brackish water and having demonstrated that the urine was always isotonic with the blood, Nagel carried out the following well-planned experiment. He took a number of crabs and allowed them to become adapted to a medium of considerable dilution. Some of the crabs were killed and measurements were made of the osmotic pressure and chloride content of their blood. The rest of the crabs were then placed in another medium, more concentrated than the first medium but less concentrated than the blood of the crabs which had become adapted to the first medium. After 24 hr. in the second medium the crabs were killed and their blood taken for analysis. Nagel's figures (Table 1) show that in the second lot of crabs both osmotic pressure and chloride concentration of the blood had increased. Since the body volume remained more or less constant the increase of osmotic pressure and chloride concentration could only be explained by the uptake of salts from the external medium against the concentration gradient. This uptake was not affected by blocking the mouth and Nagel assumed that it occurred at the gills.

Table 1. *Demonstration of active transport of chloride by* Carcinus

(From Nagel, 1934)

	External medium		Blood	
	Δ° C.	Cl (mg./ml.)	Δ° C.	Cl (mg./ml.)
I.	0·89	8·57	1·42	12·0
			1·20	11·9
			1·23	12·3
			1·42	13·0
			1·28	11·5
			Av. 1·31	12·1
II.	1·18	11·45	1·50	14·0
			1·52	14·3
			1·48	14·2
			1·73	15·4
			1·56	15·3
			1·57	14·2
			1·51	14·0
			Av. 1·55	14·5

Three years later, Krogh (1937 *a*, *b*) showed that fresh-water fishes and Amphibia are able to take up chloride from extremely dilute external media, and he later extended this work to other ions and to invertebrates (Krogh, 1938). His method was to keep the animals in a current of distilled water until their salt reserves were depleted and then to place them in measured volumes of dilute solutions whose final composition was determined by analysis at the end of the experiment. He was able to demonstrate active uptake of chloride in *Astacus*, in a variety of fresh-water molluscs and in the horse leech. Active uptake of chloride from dilute solutions has also been demonstrated by Koch (1938) for mosquito larvae, by Maluf (1939) for the earthworm, by Boné & Koch (1942) for caddis larvae and by Holm-Jensen (1948) for *Daphnia*. It is therefore of very widespread occurrence; but there are some fresh-water animals in which it has been looked for but not found, for example, in the eel and in the larva of the alder fly *Sialis* (Beadle & Shaw, 1950).

One of the invertebrates which Krogh studied in particular detail was *Eriocheir*, and he was able to show that there was active uptake of sodium, potassium, chloride, bromide, cyanate and thiocyanate; that nitrate diffused inwards rapidly under a concentration gradient, iodide slowly and sulphate not at all. He was also able to show that the mechanisms for uptake of anions and cations were independent, e.g. chloride, but not ammonium, taken up from NH_4Cl and replaced by bicarbonate; sodium, but not sulphate, taken up from Na_2SO_4 and replaced by ammonium. *Eriocheir* does not appear to show any discrimination between sodium and potassium or between chloride, bromide and thiocyanate when these are present in the same solution. *Astacus* (Schmidt-Nielsen, 1941) will absorb sodium but not potassium from solutions in which both are present, but does not distinguish between chloride, bromide and thiocyanate.

It so happens that for technical reasons the best evidence for active absorption of ions from the external medium comes from studies of fresh-water animals, and there is no doubt that among fresh-water animals these powers are well developed. Yet it would be wrong to suppose that they are wholly confined to fresh-water animals.

The most recent and most accurate analyses of the body fluids of marine invertebrates are those of Robertson (1939, 1949). Although the body fluids of some primitive marine animals resemble sea water very closely they are never identical with it owing to the Donnan effect which is set up by the proteins. Robertson used the method of comparing the ionic composition of the blood drawn from the animal with that of blood which had been dialysed against sea water. In this way the Donnan effect is eliminated from consideration, and it is possible to ascertain how far the differences between internal and external media are actively maintained. Some of Robertson's figures are reproduced in Table 2. From this table it can be seen that no active transport of ions is needed to maintain the composition of the sea-urchin's coelomic fluid. In the case of the lugworm *Arenicola* the only ion showing a significant difference in concentration is sulphate. But these are the exceptions. *Pecten*, the scallop, *Loligo*, the squid, and *Cancer*, the edible crab, are all typical marine invertebrates showing, like *Maia*, virtually no osmotic regulation; yet it appears likely that mechanisms of active transport are at work to maintain the generally higher concentration of potassium.

From this we may infer that the active transport of ions by the surface membranes, which is largely responsible for the hypertonic regulation of brackish- and fresh-water animals, is not of itself a novelty of adaptive evolution but is more probably the specialization of a mechanism which was already in existence in their marine ancestors.

Table 2. *Concentrations of various ions in body fluid as percentages of their concentrations in dialysed body fluid*

		Na	K	Ca	Mg	Cl	SO_4
Echinodermata	*Echinus*	100	102	101	100	100	101
Annelida	*Arenicola*	100	103	100	100	100	92
Mollusca	*Pecten*	100	130	102	97	100	96
	Loligo	95	219	102	102	103	29
Crustacea	*Cancer*	108	120	119	51	97	87
	Carcinus	109	117	108	34	103	60

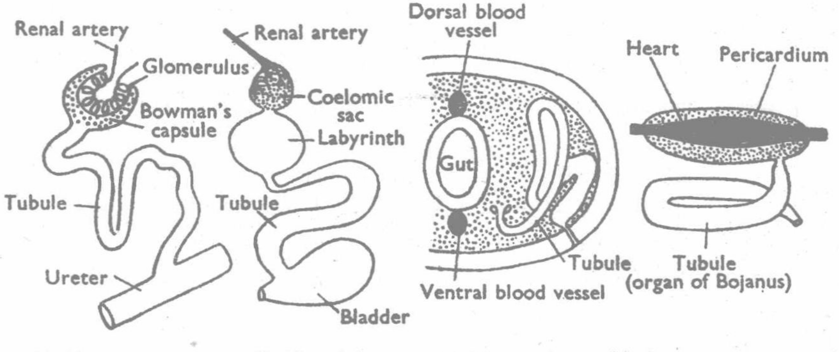
Carcinus figures from Webb (1940); the rest from Robertson (1939, 1949).

III. THE TRANSPORT OF IONS IN EXCRETORY SYSTEMS

The functional unit of the vertebrate kidney is the nephron which consists of a knot of blood vessels (glomerulus) projecting into a small coelomic vesicle (Bowman's capsule) from which a tubule leads to the exterior. The hydrostatic pressure of the blood in the glomerular vessels is sufficiently in excess of the colloid osmotic pressure of the blood to cause ultrafiltration, and the fluid in Bowman's capsule is identical with plasma except that it contains no protein. As this fluid passes down the tubule it is modified by the reabsorption of substances from it and the secretion of other substances into it. As is seen from Fig. 2*a*, the course of the fluid is: blood → coelom → tubule → exterior.

Now consider the excretory organs of invertebrates, some of which are shown diagrammatically in Fig. 2*b*, *c* and *d*. It is conceivably possible that the antennary glands of Crustacea are homologous with the nephridia of annelids, but it is not easy to refute the assertion that all these types of excretory organ have been independently evolved. Yet it appears that in all of them the course of the urine is the same, i.e. blood → coelom → tubule → exterior. If they have this much in common, is it possible that they have other features in common? Is it possible that, like the vertebrate nephron, they operate on the ultrafiltration-reabsorption basis?

The suggestion that the primary process of urine formation in invertebrates was a process of ultrafiltration was first seriously put forward by Picken (1936, 1937), whose main contribution to this thesis was to measure the colloid osmotic pressure of the blood in various crustaceans and molluscs and to show that it was always low in relation to the hydrostatic pressure. Ultrafiltration was thus a possibility. Paying particular attention to *Anodon*, Picken showed that the pericardial fluid was isotonic with the blood, whereas the urine collected at the excretory pore was hypotonic and therefore modified in composition during its passage through the tubule. He drew off the pericardial fluid and showed that it was continuously and fairly rapidly renewed. Further confirmation came from the work of Florkin & Duchâteau (1948), who found that the concentrations of calcium, chloride and phosphate in the blood and in the pericardial fluid were identical (Table 3).



(*a*) Vertebrate (*b*) Crayfish (*c*) Earthworm (*d*) Fresh-water mussel

Fig. 2. Diagrams of the excretory organs of a vertebrate and of *Astacus*, *Lumbricus* and *Anodon* to show the relation between blood system (black), coelom (stippled) and tubule.

For the others the evidence is less complete. In *Astacus*, Peters (1935) succeeded in withdrawing small samples from various parts of the antennary gland and determined the concentration of chloride (Table 4). His figures show that within the limits of accuracy the fluids in the coelomic sac and labyrinth are isotonic with the blood and that the urine becomes hypotonic during its passage through the tubule. I carried out similar investigations upon *Lumbricus*, measuring the freezing-point depression of blood, of coelomic fluid and of samples collected from different parts of the tubule. I was able to show that the coelomic fluid is isotonic with the blood and that the fluid passing down the tubule becomes hypotonic in the region known as the 'wide tube' (Ramsay, 1949).

Although these results are not in themselves sufficient to prove the truth of the ultrafiltration-reabsorption theory for invertebrate excretory organs they are at least compatible with it and can be said to raise it from the level of mere speculation to that of a reasonable working hypothesis. But we are still in the speculation stage in regard to the factors which have been at work in the independent evolution of this same physiological process in so many different animals.

Table 3. *Analysis of blood, pericardial fluid, Bojanus fluid and urine of* Anodon

	Blood		Pericardial fluid (%)	Bojanus fluid (%)	Urine (%)
	Absolute	%			
Chloride (mM./l.)	18·0	100	99	55	—
Calcium (mM./l.)	7·0	100	100	75	—
Inorganic phosphorus (mM./l.)	0·144	100	101	90	—
Δ° C.	0·06*	100*	100*	—	60*

'Bojanus fluid' is fluid withdrawn from the excretory organ through its internal opening into the pericardium, while 'urine' is fluid withdrawn from the excretory organ through its opening to the exterior. Figures marked * from Picken (1937), the rest from Florkin & Duchâteau (1948).

Table 4. *Chloride concentration (in mM./l.) in the blood and in fluids collected from different parts of the excretory organ of* Astacus

(From Peters (1935) as recalculated by Krogh (1939).)

Blood	Coelomic sac	Main labyrinth	End of labyrinth	Tubule	Bladder
196±3	198±2	209±7	212±7	90±6	10·6±0·6

In pursuing this line of thought we may next ask ourselves what happens in animals such as the echinoderms in which there are no recognizable excretory organs. These animals must take in water with their food and must produce metabolic water like other animals. How do they get rid of it? I am not aware that anyone has ever put this point to experimental test, but it would not surprise me to be told that this water simply escapes by seepage through the surface membranes of the body. I would be prepared to risk a guess that if fluid were injected into an echinoderm so as to distend its body and increase its internal hydrostatic pressure the result would be an outward seepage of salts and water, only proteins being retained—in fact, ultrafiltration through the thinner parts of the general body surface.

There is no serious disadvantage in this method of getting rid of water provided that the flow is normally very small—as it is likely to be in a marine animal whose blood is isotonic with sea water—and provided that the animal is not concerned to maintain the composition of its blood significantly different from that of sea water. But if the animal is maintaining some substance X in its blood in higher concentration than in the external medium and is actively transporting X against a concentration gradient, then it is wasteful simply to allow an ultrafiltrate to be swept away from the filtering surface by currents—for this reason, that less work is required to get X back again from the ultrafiltrate, in which it is *initially* at the same concentration as in the blood, than is required to get X from the external medium in which it is *always* at lower concentration than in the blood. This seems obvious, but it is only very recently that the point has been clearly put, by Potts.*

In the present context the interest of this conception lies in its evolutionary implications. We have seen the physiological parallels which can be drawn between the vertebrate nephron and the excretory organs of various invertebrates. Yet as far as we can tell they have all been evolved independently. I have suggested that ultrafiltration may be a widespread and primitive method of volume regulation; if this is true, then animals seem to have been at pains to restrict ultrafiltration to certain areas of the body and to arrange that the filtrate has to traverse some sort of tube before leaving the body. The point which Potts has made seems to me to provide the argument for selective advantage in this arrangement and enables us to understand why it has been evolved independently in different phyla of the animal kingdom.

If this is true it also implies that in marine animals having well-developed excretory organs the urine, although it may be isotonic with the blood, is probably not identical with the blood in composition. This is borne out by comparison of blood and urine in *Carcinus* (Webb, 1940) and *Cancer* (Robertson, 1939). In all these animals the urine is isotonic with the blood under all conditions, but as Table 5 shows there must be active transport of some ions in the excretory organ. As we saw in the case of the surface membranes, so now do we see in the excretory organs, that the active transport of ions, upon which depends the ability to penetrate fresh waters, is probably widespread among animals which are exclusively marine in habit.

Table 5. *Concentrations of various ions in urine as percentages of their concentrations in blood*

	Na	K	Ca	Mg	Cl	SO_4
Cancer (Robertson, 1939)	96	81	90	125	96	165
Carcinus (Webb, 1940)	95	78	94	390	98	224

It would appear therefore that the difference between *Maia* and *Carcinus* is not that *Carcinus* can actively transport salts while *Maia* cannot; it is likely that *Maia*, as well as *Carcinus*, has powers of active transport. The

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* I wish to thank Dr Potts for permission to quote his work which is as yet unpublished.

essential difference lies in the rate at which salts are transported *relative to the rate at which water diffuses passively in the same direction.* The degree of hypertonic regulation can be increased either by speeding up the active transport of salts or by reducing the permeability of the surface membranes to water, and we have perhaps paid too little attention to this second possibility.

There is some evidence which suggests that the surfaces of fresh-water animals are less permeable to water than the surfaces of their marine relatives. It is not easy to present this evidence in quantitative terms of permeability measurements because of the difficulties of measuring the surface area of an animal. But these difficulties are not insuperable, and it would be of great interest to know, for example, how far the success of *Eriocheir* as compared with *Carcinus* in penetrating fresh water is due to its powers of active transport and how far due to a decrease in the permeability of its surface to water. It would also be interesting to know if a decreased permeability to water is an active process in the sense that it demands a continuous supply of energy, as suggested by Beadle (1934) for the flatworm *Gunda*.

IV. OUTLOOK FOR THE FUTURE

Hitherto I have been concerned in tracing the growth of knowledge and ideas in what may be called the general field of osmotic regulation. In logical order, though not in chronological order, the problems were: first, to determine the general nature of the body fluids; secondly, to show that their composition was maintained by active transport; thirdly, to discover the sites of active transport in the body. There are, of course, a great many invertebrates, of which only a few have been studied, but as far as the major phyla are concerned it is fair to claim that sufficient ground has been covered to meet the first two points and it may be conceded that there is some progress to report in the identification of the sites of active transport. What is the next step to be? Are we to see the future merely as a process of filling in the details of a design whose main outlines are already clear?

I do not think so. On the contrary, it seems to me that the next few years will witness substantial changes in outlook.

Our present outlook is in fact still largely dominated by Claude Bernard and his 'fixité du milieu intérieur'. Primitive animals with no powers of regulation are condemned to live in the sea, those with some powers of regulation can work their way up estuaries and with the perfection of their mechanisms can graduate to fresh water. It is a good story and by and large it is true—but only by and large. On closer inspection the correlation between powers of regulation and ecological distribution is not so good.