may with their limited knowledge of the factors involved and with very considerable effort assess the result in a sufficiently great number of cases for statistically sound practice or non-practice in relation to presumed factors. (c) By research it may be possible to discover the most significant factors as a basis for scientific practice with the possibility of developing economic practice. Only the last should be recommended by the scientific worker, except when empirical success warrants continuation of practice.

To make salmon available for angling, they must be brought into the river from the sea. For this, knowledge is required of the factors determining salmon migration. In spite of an outstandingly successful trial on the River Grimersta in 1888[1], regular empirical use of artificial freshets to bring salmon in from the sea has not developed[2], and the rationale of the action of freshets has not been worked out. This is perhaps owing to man's thinking being preoccupied with the idea that the mature salmon in the sea is trying to find its way back to its home stream and that the scientific problem is to discover how it finds its way[3]. However, the facts indicate that, when at the river mouth, salmon may not try to enter for weeks on end until stimulated in some way. A freshet may provide the stimulus. In the very dry summer of 1942 on the Moser River, Nova Scotia, sharp artificial freshets resulted in the numbers of entering salmon and 'brook trout' (*Salvelinus fontinalis*) being doubled, as judged by the preceding three years[4]. This would not have been possible if these fish return only to the home stream. (Smolt marking indicated the next year that almost half the salmon were foreign.) High temperatures (higher than 70° F.) were considered responsible for the fact that very few of the salmon crowding the small river were taken with the fly. In 1943, the freshets were started as soon as salmon were moving in the estuary, and within a month a quarter of 474 salmon that had entered were taken by angling[5]. Then, a heavy flood and rising temperature stopped the angling and swept away counting fences and traps. Regular practice in water control is in prospect, and attempts are being made to elucidate factors that determine migration, such as current, turbulence, temperature, light and salinity.

To increase the salmon stock demands knowledge of the factors determining survival and growth of the fish. Since a female produces thousands of eggs, supply of new individuals is not apt to be much of a problem. Experiments are in progress to discover how cheaply salmon smolts can be produced in streams lacking native young salmon, by planting the young from hatcheries. Unless they can be produced economically as a preliminary to getting adult fish, there is no object in trying to compete with natural reproduction by planting hatchery fish in streams with native fish. Very great complexity of conditions in each stream makes scientific progress slow. Assessment of smolts, which was thought at first to be easily feasible by trapping them during descent, has given considerable trouble: (1) through failure of the trapping, as during heavy floods; (2) through the smolts not all descending, as with very low water or from above beaver dams; and (3) through anglers sometimes (low water) taking a very large proportion of them, and sometimes (high water) taking none. Assessment in the parr stage is seen to be needed and is being attempted. As the parr wander more or less, knowledge is required of the factors affecting not only their survival and growth, but also their movement. So far, predators are seen as the main explanation of disappearance of parr, so that control of bird predators[6] and provision of protective cover for the parr[7] are seen as promising practices.

This is an ecological problem, and the crux of it seems to have been given very little attention, doubtless because of the difficulties involved. In every case, the outcome depends upon *the response of the animal as a whole to what it faces where it lives*. To refer readily to this, a name is needed. 'Zoapocrisis' (ζῷον = animal, ἀπόκρισις = response) has been suggested. Its elucidation requires rather detailed knowledge of what the animal actually faces where it lives, which presents an initial task that is far from being an easy one. As an illustration, it is becoming apparent that salmon are, when in the sea, oriented riverward where there is a sufficiently steep gradient in salinity. Also, salmon evidently respond to current, which will take them upstream, only when it is sufficiently and finely turbulent. Zoapocrisis presents an almost virgin field for discovery.

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## Establishment of Cytochemical Techniques

IN his article on the "Establishment of Cytochemical Techniques"[1], Dr. J. F. Danielli directly or by inference questions the validity of very nearly the whole of cytochemistry. While there is no doubt that many workers have used cytochemical methods without fully considering the evidence for their validity, we feel that Dr. Danielli has gone to the opposite extreme.

Some of the tests that he mentions are usually made on fixed materials, where it must be supposed that part at least of the cell has been made insoluble. This process will cause just as much adsorption and shifting of substances as any cytochemical test applied afterwards. While it may be argued that artificial appearances are produced, yet something is shown that can be interpreted later in the light of our knowledge of the effects of reagents on cells. Every procedure that involves fixation is itself an experiment on a cell. The appearances produced by classical cytological methods do not represent exactly the structure of the living cell, but they enable us to make inferences about that structure. The same argument applies to cytochemical techniques.

The images given by different processes of fixation and staining are often so similar that one can scarcely doubt that the picture of the fixed material bears a close relation to the living structure, whatever the submicroscopic changes may be. For example, in the case of salivary gland chromosomes, different fixatives may be used, or unfixed cells may be photographed in ultra-violet light, and the picture is essentially the same.

The fact that the bands in salivary gland chromosomes that react positively to the Feulgen test correspond exactly to those that strongly absorb ultra-violet light of 2675 A. must mean one of two things: either (1) the bands that absorb ultra-violet light contain desoxypentose nucleic acid, or (2) the 'diffusible' Feulgen reaction product always diffuses to those very places that have an absorption spectrum that indicates that they could contain nucleic acid. The principle of 'Occam's razor' surely decides in favour of the first alternative. If the reaction product is in fact diffusible in the circumstances under which the test is performed, are we to suppose that it diffuses into the fluid in the jar in which the reagents do their work and then attaches itself to certain other parts of the cell selectively? Or does it migrate through the section? The first alternative seems unthinkable, for the reaction product would be far too dilute to produce a perceptible result; and the second is also very unlikely, because if the reaction product can diffuse through the tissues away from the place where it was first formed, it can also diffuse into the fluid in the jar and thus be lost.

It must be allowed that metallic impregnations are far less trustworthy than staining methods. The minute structure of a metallic precipitate is irrelevant. The position of the precipitate as a whole within the cell, however, is not necessarily so.

Dr. Danielli seems to doubt whether nuclei can in fact be isolated by maceration. In certain cases they cannot; but there seems to be no doubt that when the cell membrane is weak and the nuclear membrane strong, such isolation is possible. It is true that nuclei may be changed by such isolation, but there is no special reason for supposing that the process would cause substances formerly present in the cytoplasm to migrate into the nucleus and collect there in appreciable quantities. This would be particularly unlikely in the case of substances with large molecules. The identification of isolated mitochondria is less certain than that of nuclei, but when the shape, size and staining reactions resemble those of mitochondria *in situ*, there is a high degree of probability as to their identity; in fact, there are not many more bases on which identity could be assumed. It is a debatable point, however, whether the submicroscopical particles exist as such in the living cell.

Dr. Danielli's criticisms of maceration techniques apply as much to bio- as to cyto-chemistry, for many of the classical biochemical methods of making tissue extracts are themselves maceration procedures.

It is true that many enzymes are not specific as to substrate; but ribonuclease, a depolymerase, is highly specific for ribonucleic acid. There may be slight proteolytic activity even in crystalline preparations, but this is destroyed by heating to 80°–100° C. at slightly alkaline *p*H, while the action on ribonucleic acid remains intact. Purified enzymes are beginning to open out a new and promising line of attack on cytochemical problems. Long ago, Unna thought that one could stain a particular object in a cell, apply various solvents, and then draw conclusions as to the chemical composition of the object from a knowledge of its solubility or insolubility. We know now that Unna's 'chromolysis' was invalid: the solubilities of cell-constituents are profoundly changed by association with other substances. Reactions to certain purified enzymes, however, are invariable, and thus a new and valid chromolytic cytochemistry is made possible, the lysis being achieved by the action of enzymes instead of solvents.

Dr. Danielli suggests that if an object were composed of nucleic acid, but were covered by a monolayer of protein, nuclease would be unable to exert any effect. We question the existence of such monolayers in the fixed tissues to which the nuclease is applied.

Although we cannot accept all Dr. Danielli's criticisms, for the reasons we have given, yet we fully appreciate the need for greater care in accepting the results of cytochemical tests at their face value. We think, however, that the tests themselves should be the main targets for criticism, rather than the localization of the reaction products. We suggest that any change of position, particularly in the case of large molecules, may be an affair of A. rather than of $\mu$; and it is with $\mu$ that cytologists are at present concerned. Many so-called histochemical and cytochemical tests are used without full consideration of the bases of their supposed validity. If Dr. Danielli's article encourages a more critical attitude in this respect, it will serve a very useful purpose.

We thank Prof. A. C. Hardy, Mr. P. B. Medawar, Dr. G. Bourne and Mr. A. J. Cain, with whom we have discussed Dr. Danielli's article.

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[1] Danielli, J. F., *Nature*, **157**, 755 (1946).

WHILE I welcome a measure of support which Drs. Baker and Sanders give to my criticisms of cytochemical techniques, their letter leaves no doubt that the principle underlying my article has evaded them. My remarks do not, and were not intended to, invalidate "nearly the whole of cytochemistry". What my article does is point out that, in cytochemistry, too much reliance has been placed on arguments which have not been rigorously demonstrated as true. There are suggestive inductive arguments in favour of many techniques in current use, and these arguments have in the past been of great value as a guide to further research. But the time has now come when their premises must be critically examined by experiment,