A Ciba Foundation Symposium

IONIZING RADIATIONS AND CELL METABOLISM

CIBA FOUNDATION SYMPOSIUM
ON
IONIZING RADIATIONS
AND
CELL METABOLISM

Editors for the Ciba Foundation

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and

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PREFACE

THE Ciba Foundation, London, is an educational and scientific charity founded by a Trust Deed made in 1947. Its distinguished Trustees, who are wholly responsible for its administration, are The Rt. Hon. Lord Adrian, O.M., F.R.S.; The Rt. Hon. Lord Beveridge, K.C.B., F.B.A.; Sir Russell Brain, Bt.; The Hon. Sir George Lloyd-Jacob, and Mr. Raymond Needham, Q.C. The financial support is provided by the world-wide chemical and pharmaceutical firm which has its headquarters in Basle, Switzerland.

The Ciba Foundation forms an international centre where workers active in medical and chemical research are encouraged to meet informally to exchange ideas and information. It was opened by Sir Henry Dale, O.M., F.R.S., in June 1949.

In the first seven years, in addition to many part-day discussions, there have been 40 small international symposia, each lasting two to four days and attended by from twenty-five to thirty outstanding workers from many countries. Other symposia are planned at the rate of five or six a year.

The informality and intimacy of these meetings have permitted discussion of current and incomplete research and stimulated lively speculation and argument. They have also been the occasion for reference to much published and unpublished work throughout the world.

The reader will probably be well aware that there have been many conferences, national, international and inter-disciplinary, in recent years on the effects and hazards of radiation. This is partly due to rapid progress and expansion in this field of research, and partly to a quickening interest in the significance of the work shown by other scientists and by laymen. Most of these conferences have been on a fairly large scale, valuable for exchanges of information but usually affording little opportunity, except privately, for thorough discussion.

Dr. A. Hollaender and Professor A. Haddow made these points when approaching the Director late in 1954 with a request that a symposium on the Influence of Ionizing Radiations on Cell Metabolism should be included in the Ciba Foundation's programme. The Director readily agreed, subject to receiving their expert advice on its organization, in which they were later most helpfully joined by Professor J. A. V. Butler and Dr. L. H. Gray.

The symposium, which was realized in March 1956, and which was held under the skilful and kindly chairmanship of Professor Haddow, is amply recorded in this book. The Editors hope that their intervention has to some extent made for easier reading, but that the reader will be able to enjoy, as if he were a participant, the efforts made by the contributors on this friendly occasion to bring forward new information and to come to an understanding of each other's aims, methods, problems and interpretations.

List of those participating in or attending the Symposium on "The Influence of Ionizing Radiations on Cell Metabolism" held at the Ciba Foundation, 6th-9th March, 1956

P. ALEXANDER	Chester Beatty Research Inst., London
TIKVAH ALPER	Experimental Radiopathology Research Unit, Hammersmith Hospital, London
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J. N. DAVIDSON	Dept. of Biochemistry, University of Glasgow
A. FORSBERG	Inst. of Radiophysics, Stockholm
E. F. GALE	School of Biochemistry, University of Cambridge
L. H. GRAY	British Empire Cancer Campaign Research Unit in Radiobiology, Mount Vernon Hospital, Northwood
A. HADDOW	Chester Beatty Research Inst., London
G. DE HEVESY	Inst. of Organic Chemistry and Biochemistry, University of Stockholm
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ALMA HOWARD	British Empire Cancer Campaign Research Unit in Radiobiology, Mount Vernon Hospital, Northwood
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H. LASER	Molteno Inst., University of Cambridge
R. LATARJET	Laboratoire Pasteur de l'Institut du Radium, Paris

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ANTOINETTE PIRIE . .	Nuffield Laboratory of Ophthalmology, University of Oxford
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G. E. STAPLETON . .	Biology Division, Oak Ridge National Laboratory, Tennessee
L. A. STOCKEN . . .	Dept. of Biochemistry, University of Oxford
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CHAIRMAN'S OPENING REMARKS

A. HADDOW

A GREAT deal of work has been carried out on the elucidation of the changes in gross cellular structure produced by ionizing radiations, on the histopathology of radiation damage, and on the cytological and genetical effects. Yet what of the biochemical changes, the metabolic changes we have to consider? To quote Dubois and Petersen's review (1954, *Annu. Rev. Nuclear Sci.*, **4**, 351), although research on the biochemical effects of ionizing radiations has yielded a vast amount of information, no satisfactory explanation of the exact mechanism by which tissue damage is inflicted has yet been obtained. Research on the biochemical mechanisms has been under way for a relatively short period of time. A considerable amount of research on the subject during recent years was of necessity exploratory in nature. Many approaches to the problem of mechanism have been employed. A large number of the earlier studies dealt with *in vitro* systems. The information obtained from such studies has been valuable in indicating the chemical linkages and groups which are the most susceptible to alteration by ionizing radiations. However, attempts to apply *in vitro* findings with ionizing radiations to intact cells have been generally disappointing. Biologists have therefore turned their attention to the more difficult task of attempting to define radiation damage in terms of interference with biochemical systems, through research on irradiated animals and microorganisms. My colleague J. A. V. Butler has pointed out that the basic puzzle of radiobiology, one which has been stressed especially by L. H. Gray, is still unsolved—namely that comparatively small doses of radiation produce marked biological changes, although in general rather large doses are required to produce easily observable chemical changes. In Butler's

words, the passage of radiation through living tissues obviously initiates a long chain of events. We have the primary ionizations, the chemical consequences, and the biological events which follow. Although the physical nature of the primary actions has been well worked out, and the chemical consequences have been established, at least in numerous simple cases, the link between the chemical changes and the biological consequences is almost completely unknown. Discussing the radical initiated polymerizations of unsaturated substances, Butler points out that the radical merely acts as a catalytic agent in that it stimulates processes which can occur spontaneously. This recalls a recent impression that the chemical carcinogens may simply expedite processes which occur spontaneously at much lower rates. Again to quote Butler, we are at the moment in the position of a man who tries to elucidate the mechanism of a telephone exchange by throwing bricks into it and observing some of the results.

Our subject is at an elementary stage, yet it is always dangerous to say what will not happen in science. Even Lord Rutherford at one time thought little of the prospects of the release of atomic energy. From the study of the influence of ionizing radiations on cell metabolism may, however, flow the most profound consequences for the theory of ageing, for the theory of carcinogenesis, and for the theory of heredity. J. J. Thomson once said that if he were to start life again he would take up the study of biology, this being, as he thought, at the same stage as physics when he started his early career. Our own subject is the ideal region in which physics, chemistry and biology meet.

CYTOPLASMIC AND NUCLEAR STRUCTURE IN RELATION TO METABOLIC ACTIVITIES

J. BRACHET

University of Brussels

AMONG the numerous theories which have been proposed with a view to explaining the functions of the nucleus in the life of the cell, several have now been definitely rejected. This is true in particular for the hypothesis of Loeb (1899) who considered the nucleus as the prime centre of cellular oxidations, for we have now shown, both for amoebae and for the unicellular alga *Acetabularia mediterranea*, that removal of the nucleus does not appreciably reduce the rate of cellular oxidations, even after a considerable length of time (Brachet, 1955*a*). It is also now well known that isolated nuclei have an extremely low oxygen consumption and lack most of the oxidative enzymes, this being true for amphibian egg nuclei obtained by microdissection (Brachet, 1939) and for nuclei of liver homogenates prepared by differential centrifugation (cf. recent review articles by Dounce, 1955; Allfrey, Daly and Mirsky, 1955). Extensive research on liver homogenates has shown, in addition, that mitochondria are the primary, though not exclusive, site of the energy-generating reactions of the cell (oxidative phosphorylations). This work has been ably summarized in recent reviews by de Duve and his co-workers (de Duve and Berthet, 1954) and by Hogeboom and Schneider (1955). An interesting exception, as yet unconfirmed, has been reported by Rubinstein and Denstedt (1954): bird erythrocytes lack mitochondria and contain oxidative enzymes (cytochrome oxidase and succinic dehydrogenase) in their nuclei.

The fact remains, however, that the metabolism of enucleated cytoplasm is never entirely normal. In the case of

amoebae, removal of the nucleus leads to considerable disturbances of phosphorylation. ^{32}P-incorporation into non-nucleated halves slows down almost immediately (Mazia and Hirshfield, 1950), while their ATP content undergoes an increase in aerobiosis which probably reflects a block in the utilization of the phosphate-bond energy of ATP (Brachet, 1955*a*). Under anaerobic conditions, on the other hand, non-nucleated cytoplasm shows a markedly reduced ability for keeping ATP in phosphorylated form (Brachet, 1955*a*). Moreover, the general metabolic disturbance of non-nucleated cytoplasm is also revealed in other biochemical systems. As we have shown (1955*a*), the utilization of lipid and carbohydrate reserve products is considerably reduced in non-nucleated halves of amoebae.

These metabolic injuries can be accounted for, as we have already suggested (Brachet, 1955*a*), by assuming that the cell nucleus is involved in the synthesis of nucleotide coenzymes, which are essential for glycolysis and cellular oxidations. This hypothesis is in agreement with most recent findings. Hogeboom and Schneider (1952) have shown that, in the liver, the complete enzyme system for the synthesis of diphosphopyridine nucleotide (DPN) from ATP and nicotinamide nucleotide is located in the nuclei. In the starfish oöcyte, as shown by our co-worker E. Baltus (1954), the same enzyme system is concentrated in the nucleoli, which are fifty times more active than entire oöcytes in this respect. If one of the biochemical functions of the cell nucleus consists in the production of DPN-like nucleotide coenzymes, enucleation should result in a rapid loss of these coenzymes from the cytoplasm and Baltus (1956) has found that this is indeed the case: the DPN content of fasted amoebae drops much faster in the non-nucleated than in the nucleated halves.

Certain conclusions can be drawn from these various results. At first it appears that the presence of the nucleus is by no means essential to keep up the normal rate of cellular oxidations and that those cytoplasmic granules which are specially active in cellular oxidations, in particular mitochondria, are

largely independent of the nucleus. The latter, however, does exert an indirect control by regulating these oxidation processes through the synthesis of the nucleotide coenzymes. It appears probable that these coenzymes are protected from hydrolytic enzymes when bound to the mitochondria, in which case removal of the nucleus can have little effect on bound DPN and cannot interfere strongly with cellular oxidations. On the contrary, free coenzymes, those not bound to mitochondria, would appear to be left unprotected against hydrolysis and this should result in a rapid drop of glycolysis with an incomplete utilization of the stored glycogen after removal of the nucleus. Thus non-nucleated cytoplasm, with its low content of free DPN and the resulting deficient glycolysis, should no longer keep up its normal ATP content in anaerobic conditions.

Such a direct action of the nucleus might be postulated not only for the synthesis of DPN, but also for that of the other nucleotide coenzymes (triphosphopyridine nucleotide, flavine-adenine dinucleotide, coenzyme A, etc.). The experiments to prove it have yet to be done but it remains an attractive hypothesis, in view of the extremely important part taken by the nucleus in the metabolism of a polynucleotide, ribonucleic acid (RNA). We already know from ^{32}P experiments by Marshak (1948), Marshak and Calvet (1949), Jeener and Szafarz (1950) and Barnum and Huseby (1950), that nuclear RNA shows a much higher specific activity than cytoplasmic RNA. Studies with other radioactive precursors such as orotic acid (Hurlbert and Potter, 1952), glycine (Bergstrand *et al.*, 1948), formate (Payne *et al.*, 1952; Smellie *et al.*, 1953) have confirmed these results. In all cases, incorporation by nuclear RNA was very high, higher in fact than that by any cytoplasmic fraction.

There has been much debate as to whether, as suggested by Jeener and Szafarz (1950), nuclear RNA is a precursor of cytoplasmic RNA. Recent mathematical work by Barnum, Huseby and Vermund (1958), as well as measurements showing that nuclear and cytoplasmic RNA's have different

molecular compositions (Crosbie, Smellie and Davidson, 1958; Elson, Trent and Chargaff, 1955) give little probability to the idea of nuclear RNA being the sole precursor of cytoplasmic RNA. On the other hand, Goldstein and Plaut (1955) recently succeeded in grafting, in normal and in non-nucleated amoebae, nuclei which had been labelled with ^{32}P. These experiments strongly suggest that nuclear RNA can give rise to cytoplasmic RNA, but they do not demonstrate that nuclear RNA is the sole precursor of cytoplasmic RNA, nor do they prove that nuclear RNA is not degraded prior to its conversion into cytoplasmic RNA. It appears rather as if both forms of RNA are synthesized independently, though at a faster rate in the nucleus than in the cytoplasm. We shall see later that major differences are also found in the fate of RNA in various enucleated organisms.

Let us next consider another aspect of the rôle of the nucleus in the life of the cell, the possible relations of the nucleus with protein synthesis. As early as 1881, Verworn had suggested a control by the nucleus of the cell's anabolism, making this hypothesis in order to explain the usual incapacity of non-nucleated cytoplasm to regenerate. Caspersson (1941, 1950) has taken up this old hypothesis of Verworn and extended it. On cytochemical grounds he has postulated that the nucleus plays a fundamental rôle in protein synthesis, a suggestion we shall now consider in the light of recent experimental results from a number of laboratories.

The observation that cells in which an active protein synthesis goes on have a particularly large nucleolus with a correspondingly high content of RNA, has led Caspersson (1941) to propose that the nucleus, and especially the nucleolus, is a key factor in protein synthesis. Simultaneously with Caspersson (1941) but working independently, we proposed the hypothesis that RNA plays a direct rôle in protein synthesis (Brachet, 1941). This was suggested by the exceptionally high RNA content of all cells actively synthesizing proteins. The hypothesis found further support in the results of Hultin (1950) and of Borsook and co-workers (1952), who

found that microsomes (the smallest cytoplasmic particulates, which have also the highest RNA content) are most active in the incorporation of radioactive amino acid into proteins. More recently, Gale and Folkes (1954, 1955) have found, in bacteria lysed by ultrasonics, that protein synthesis will only take place if RNA is left intact. Indeed this process is brought to a stop if the nucleic acid fraction is extracted by various means. In our laboratory also (Brachet, 1954, 1955*a* and *b*), it has been shown that ribonuclease, by specifically attacking or binding the RNA of normal, living cells (onion roots, amoebae, star-fish or amphibian eggs, etc.), has a powerful inhibitory action on the incorporation of amino acids into proteins, on the growth of the cell and on its overall protein synthesis.

It is now a generally accepted fact, as pointed out by Borsook (1955), Gale (1955) and Mirsky (1955), that nucleic acids are directly and fundamentally involved in protein synthesis. This is clear at least in the case of RNA (Gale and Folkes, 1954, 1955; Brachet, 1954, 1955 *a* and *b*), but appears less evident for DNA; some experiments of Allfrey (1954) and Allfrey and Mirsky (1955) do indicate that desoxyribonuclease inhibits amino acid incorporation into the proteins of isolated thymus nuclei; the inhibition, however, is not so strong as that by ribonuclease for the whole cell although ribonuclease does not inhibit amino acid incorporation into the proteins of isolated nuclei.

In view of the high nucleic acid content of cell nuclei and because of the now well-established importance of these compounds in protein synthesis, Caspersson's idea (1941, 1950) of a particularly important function of the nucleus in protein synthesis has been brought into focus again and several laboratories have initiated experiments on this problem. A simple method, used chiefly by Mirsky and co-workers (Daly, Allfrey and Mirsky, 1952) and by Davidson and co-workers (Crosbie, Smellie and Davidson, 1953; Smellie, McIndoe and Davidson, 1953), consists in injecting a radioactive amino acid into a living animal and then determining the specific

radioactivity of the various constituents of its liver cells (nuclei, mitochondria, microsomes and supernatant as obtained by differential centrifugation). This technique is open to some criticism. Results of the Mirsky group show that the methods of preparation of isolated nuclei used entail serious losses of some nuclear proteins. In this manner histones have been shown to incorporate amino acids only slowly, while the rest of the nuclear proteins do not differ much in activity from the whole of cytoplasmic proteins. But it must always be kept in mind that the current preparation processes may well extract from the nuclei some proteins of considerable metabolic importance.

In the light of these objections, we have taken up a different aspect of the same problem, one which appears more worthwhile from a biologist's standpoint. Together with a group of co-workers, we have investigated protein metabolism in nucleated and non-nucleated halves of unicellular organisms. We have deliberately selected two widely separated species: the amoeba (*Amoeba proteus*), the non-nucleated halves of which cannot regenerate, and the giant unicellular alga, *Acetabularia mediterranea*, in which the non-nucleated stems remain capable of extensive regeneration, as shown by the classical work of Hämmerling (1934, 1953). We shall next consider the results obtained in both cases.

If one cuts an amoeba into half, the non-nucleated fragment soon rounds up and stops feeding. The nucleated half keeps behaving normally and, if fed living micro-organisms, it can resume growth and divide. Since the biochemical changes in both halves should be studied under comparable conditions, both fragments must be kept fasting in the course of the experiment. Under these conditions the non-nucleated halves remain alive for 10–15 days and the nucleated fragments for about 8 weeks.

Our experiments have led us to the following conclusions. As already pointed out, the oxygen consumption of non-nucleated halves remains unaffected, but their ATP content rises aerobically. Under anaerobic conditions, however,