SESSION VIII

IV. INVESTIGATIONS OF ISOTOPE EFFECTS IN BIOLOGICAL SYSTEMS

Comparison of Effects of D_2O and X-ray Irradiation on Lipid Composition of Tissue Culture Cells—G. H. ROTHBLAT, *Wistar Institute of Anatomy and Biology, U.S.A.*

Question: Have you any idea where the delays might occur in the generation time and have you made any estimates of DNA or of the ploidy patterns of the exposed cultures? J. POST, *Goldwater Memorial Hospital, U.S.A.*

Answer: No definitive cytological studies have been carried out. However, the large number of polynucleated cells observed during growth in D_2O may provide a clue. Generation times given for L5178Y cells were derived from logarithmically grown cells. All the cells used were heteroploid in nature.

No DNA estimates have been made. ROTHBLAT

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Question: You indicated increased synthesis of various components as steroids or carglycerides, but the conditions observed could also be explained by higher synthesis rate or decreased rate of degradation. Have you done any turnover studies to determine the actual cause? S. ARONOFF, *Iowa State University, U.S.A.*

Answer: Yet another possible cause would be increased incorporation of exogenous lipids, since they are grown in serum. A combination of increased synthesis and decreased breakdown or increased incorporation of lipids would also be possible.

We have carried out only one preliminary isotope study on lipid synthesis, which disclosed increased synthesis of both sterol and triglycerides in 5178 saline, but have done no turnover or uptake studies. Generally speaking, however, synthesis does appear to increase. ROTHBLAT

Dose-determined Effects of H^3TDR as DNA Label upon the Liver Cell Replication Time and Pattern in the Growing Rat—J. POST, *Goldwater Memorial Hospital, U.S.A.*

Question: How did you remove the fixative and did you use cold thymidine during fixation? F. ANTONI

Answer: The fixatives were treated in the usual ways, by passage through organic solvents. No cold thymidine was used during fixation. POST

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Question: When you injected 1, 2 and 20 μc of ^{3}H-thymidine, was the actual amount of thymidine injected identical or not? F. ANTONI

Answer: The amounts of thymidine added varied with the dosage levels used; the same specific activity of ^{3}H-thymidine was used throughout. POST

Mitosis and Macromolecule Synthesis in Cells Exposed to D_2O—P. R. GROSS *et al., Brown University, U.S.A.*

Question: Has D_2O-induced protein synthesis in unfertilized eggs been carried out with more than one radioactive amino-acid? For instance, with the D_2O-"squeezing" of the ribosomes induce protein formation from any type of amino acid? If so, some of the protein obtained might differ from the normal, or natural, protein derived from the polyribosomes obtained after fertilization. D. KRITCHEVSKY, *Wistar Institute of Anatomy and Biology, U.S.A.*

Answer: We have done experiments of this sort with leucine, valine, and lysine, labelled with both ^{14}C and tritium. It would, of course, be of great interest to determine what sort of protein is being made during the time spent in D_2O, but we have only indirect information as yet, to the effect that some, at least, of the protein made in D_2O contributes to the assembly of cytasters and to cell division. GROSS

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Question: Is the cytoplasm of the sea urchin egg organized by means of membranous structures which could undergo configurational changes as the result of D_2O treatment? P. D. KLEIN, *Argonne National Laboratory, U.S.A.*

Answer: That appears rather unlikely, since the ribosomes in sea urchin eggs, as in many other types of egg and rapidly-growing or dividing cell, are largely free in the ground cytoplasm, and not attached to membranes; *i.e.*, "rough endoplasmic reticulum" is very greatly reduced by comparison with what is seen in some differentiated tissue cells. This follows also from the mechanical properties of the ground cytoplasm, as has been discussed in detail elsewhere†. GROSS

Question: Can the possibility of protein synthesis in enucleated cells be

† P. R. Gross, D. E. Philpott, and S. Nass *J. Biophys. Biochem. Cytol.* **7**, 135 (1960).

interpreted to mean that they contain pre-existing messenger RNA? L. E. ERICSON

Answer: It was precisely an uncertainty about the interpretation that you have just proposed, *i.e.*, that sea urchin and other eggs apparently have DNA in the cytoplasm as well as the nucleus, which led us to do the actinomycin experiments and those reported in our paper here. The type of DNA is unknown, but it is almost certainly highly degraded DNA, perhaps a product of the nerve-cells feeding the growing oocyte. We hope to demonstrate that protein synthesis can occur without RNA synthesis. GROSS

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Question: Does respiration stop in D_2O? S. ARONOFF, *Iowa State University, U.S.A.*

Answer: I have never measured it, but it is hard to believe that it would stop, because protein synthesis is critically dependent on the supply of ATP. GROSS

Question: Have you investigated what kind of proteins are formed when amino-acids are added to these organisms which have been in D_2O? It would be useful to know if they are normal proteins. S. ARONOFF, *Iowa State University, U.S.A.*

Answer: No, we have not done so yet, but this is, of course, an extremely interesting question, and the investigations are now in hand. The proteins are clearly associated with cell division and it would be most interesting to see whether the spectrum of proteins being made changes with time when the eggs are kept in heavy water for a long time, as it almost certainly does under normal conditions. GROSS

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Question: Is the amount of protein synthesized greater than that corresponding to the turnover time suggested for messenger RNA? In other words, are you merely using up, so to speak, the rest of the messenger RNA until it is degraded, after which protein synthesis stops, or do you find more than is to be expected from that source? S. ARONOFF, *Iowa State University, U.S.A.*

Answer: I can only offer the following information: If eggs are treated with Actinomycin D until there is no sign of RNA precursors and are then labelled in pulses with amino-acids for a long time after fertilization, it is usually found that the protein synthesis rate increases at the beginning and then levels off for a time; later it begins to rise again very sharply, this second rise occurring at the time when the primary mesenchyme is beginning to migrate and all sorts of complicated morphogenetic events

are taking place. In this case, there is demonstrable messenger synthesis *ab initio*.

If the same experiment is carried out with a population of eggs which have been treated with Actinomycin D, and in which no messenger synthesis can be measured, there is, if anything, a slight increase in the protein synthesis rate for many hours; only after 10 hours or so does the rate begin to decline. The suggestion is, therefore, that those early messages are, by ordinary criteria, extremely stable; they must have half-lives of the order of 20 hours or so. GROSS

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Question: Have you actually measured the molecular weight of gelatine in increasing D_2O concentrations? S. ARONOFF, *Iowa State University, U.S.A.*

Answer: No, I have not, nor do I know how to do it properly, because of the many technical difficulties in sedimentation experiments. GROSS