MASTER

EMBRYO CULTURE IN TERATOLOGICAL SURVEILLANCE
AND SERUM PROTEINS IN DEVELOPMENTProgress Report
for Period 1978 to 1979

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INTRODUCTION

The present progress report covers the period from July 1978 to July 1979. In addition to this report the reader is referred to the accompanying manuscripts and abstracts. Two of the manuscripts are concerned with our work on teratological testing. One has been accepted for publication by the journal, Teratology, and the other has been submitted to Science. In addition, several abstracts of papers presented at meetings covering this same material are included. Last year a manuscript demonstrating that endoderm is the exclusive site of serum protein synthesis in the chick embryo yolk-sac was submitted to the Proceedings of the National Academy of Science for publication. This journal rejected the paper and it has now been submitted to Developmental Biology for publication. This journal requested some changes and it has now been resubmitted. Finally, an abstract is included of a paper to be presented at the Cell Biology meetings. This paper deals with studies of serum protein synthesis by cultures of pure endodermal cells.

TERATOLOGICAL SURVEILLANCE

Two initial studies on the use of cultured rat embryos for teratological surveillance have been completed. Each study will be summarized. In a third section preliminary studies on the teratology of several energy related fuel substances will be considered.

1. Cadmium and Cyclophosphamide. To test the feasibility of using cultured rat embryos to monitor serum for teratogenicity two recognized teratogens were selected, cadmium chloride and cyclophosphamide. Cadmium and cyclophosphamide were injected into rats and the rats were sacrificed for their blood at various intervals up to 24 hours for cadmium and to 48 hours for cyclophosphamide. With both substances the earliest serum samples taken after injection were embryo lethal while later samples caused abnormalities or embryo growth reductions.

This observation clearly demonstrated that rat embryo cultures could be used to monitor serum for change in teratogenicity with time. Next, cadmium and cyclophosphamide were added directly to control serum. With cadmium we found that the embryo response was closely related to the extent to which the embryos had passed through the head fold stage. The types of abnormalities were distinct from those obtained with serum from cadmium injected animals. Cultured rat embryos were resistant to cyclophosphamide when added directly to the culture medium. These observations show the value of passing a teratogen through an animal prior to testing in that, at least for cyclophosphamide, this approach more closely approximates the situation in the intact animal. Finally, cadmium was injected for two weeks and rats were sacrificed for their blood one, two and three weeks after the last injection. The serum from these animals was not harmful to the cultured embryos. Although a long term effect of cadmium was not demonstrated the feasibility of using this approach in long term studies was demonstrated.

2. Human Serum. During the past year we found that rat embryos could be cultured successfully on human serum provided that the human serum was supplemented with glucose. This finding made it possible to extend our testing to human subjects. First, we tested the serum from 19 control subjects and found that the serum from three gave abnormal embryos. For experimental groups we used sera from five subjects undergoing cancer chemotherapy and six subjects receiving anticonvulsants for epilepsy. All of these sera samples caused either embryo lethality or abnormal embryos. Thus, it appears that rat embryos cultured on human serum may provide a useful means of testing human serum for potential teratogens.

3. Energy Related Substances. Four energy related fuel substances were obtained through Dr. M. Guerin of the Oak Ridge Laboratory. Each sample was

diluted 50% by volume with safflower oil and injected i.p. at 2 ml/kg of body weight. The rats were sacrificed for their serum 12, 24, 36 and 48 hours after injection and the sera samples were used as culture media for head-fold stage rat embryos. In our first series of experiments the following were observed. The growth of embryos as measured by protein and DNA contents with petroleum crude #1 was more severely inhibited with the late serum samples (in reference to time after injection) than with the initial samples. Shale oil gave the opposite results in that the early samples of serum were more growth inhibitory than the late samples. Coal derived #6 and coal derived #7 were consistently growth inhibitory at each of the rat sacrifice times. Abnormal embryos were only detected with serum from animals sacrificed 12 hours after injection with shale oil. Although these results are preliminary, possible long-term effects appear implicated for petroleum crude #1 and the coal derived oils #6 and #7. Shale oil does not appear to have a long term effect but at comparable dosages it was the only substance that was teratogenic.

SERUM PROTEINS

1. Endoderm Cell Cultures. During the past year procedures were developed for the isolation of pure endodermal cells and for their subsequent culture. It was found that pure endoderm cells could be obtained by briefly "vortexing" 3-day chick embryo yolk-sacs. Following filtration and centrifugation these cells were plated out in plastic culture dishes and provided with Eagle's Minimal Medium supplemented with 10% fetal calf serum. After one, three and five days of culture the cells were exposed to radioactive valine and the medium was analyzed for total serum protein synthesis and for synthesis of individual serum proteins. For total serum protein synthesis determinations antisera to chick embryo serum proteins was used and showed that total synthesis remained constant at 50% throughout the culture period. However, changes in

synthesis of individual serum proteins ^{as} ~~or~~ measured by polyacrylamide gels were observed. The synthesis of transferrin, albumin and prealbumin in cultured cells followed the developmental changes of the intact yolk-sac. The synthesis of alpha-globulin-a and -b were not detected after the first day of culture and these two serum proteins were found to be synthesized by the intact yolk-sac.

2. Messenger RNA for Serum Proteins. During the past year a study was completed on developmental changes in translatable levels of serum protein mRNA's in the yolk-sac of the chick embryo. Poly A containing mRNA was isolated from chick embryo yolk-sacs on days 4, 10 and 16 of development. This mRNA was translated into protein by a system isolated from rabbit reticulocytes. Antiserum to serum proteins was used in conjunction with polyacrylamide gels to analyze the proteins that were synthesized. The results varied for the individual serum proteins. For prealbumin II, the amounts synthesized by the intact yolk-sac followed the levels of translatable mRNA for this protein at each stage examined. The translatable mRNA synthesis of prealbumin I exceeded the amount of prealbumin I synthesized by the intact yolk-sac on day 10 but as the amount synthesized by the intact yolk-sac increased to day 16 the mRNA remained constant. The translatable levels of mRNA's synthesized for transferrin and the alpha globulins remained low relative to the intact yolk-sac at all time points. The possibility that this reduced translation was the result of poor initiation rather than reduced mRNA levels could not be excluded.

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