Biochemical Studies on the Nucleic Acid Metabolism of Embryonic Tissue and the Effect of Drugs. By D. NEUBERT, E. OBERDISSE, H.-J. MERKER, E. KÖHLER, and B.-R. BALDA

Almost nothing is known today on the biochemical mode of action of embryotoxic drugs. Embryotoxic effects at drug concentrations which do not significantly interfere with vital reactions of the maternal organism may be expected if qualitative or quantitative differences exist between metabolic pathways in the organism of the mother and the embryo.

Since there is an almost complete lack of knowledge of the biochemical events occuring during the different stages of development in the mammals, we have started systematic studies of metabolic reactions as they proceed in embryonic tissues. This paper is concerned with some results of experiments in which RNA and DNA metabolism of the rat embryo was studied between the 11th and 13th day of pregnancy. This time is interesting from the pharmacological point of view, because organogenesis takes place within this period. Furthermore, since so many enzymic steps are involved in the metabolic pathways leading to the formation of DNA and RNA, the possibility of an interference by embryotoxic substances is especially significant.

A metabolic pathway in the embryo can be investigated either *in vivo* by giving a radioactive precursor and following the incorporation into a metabolic product (e.g. DNA or RNA) or *in vitro* by homogenizing embryonic tissue, followed in many instances by an isolation of cell fractions, and measuring the activity of a special enzyme. Both of these methods have certain disadvantages, so that we have used both to get a close picture of the events taking place.

In order to be incorporated into embryonic nucleic acids in vivo, a precursor has to overcome the barrier between mother and embryo and to penetrate the cell membrane easily. Otherwise these reactions become rate limiting and not the enzymic steps. We would like to stress the point that a number of—otherwise very good—precursors (like orotic acid) do not fulfill this criterion so that they can not be used in such studies. Between the 11th and the 13th day of pregnancy thymidine penetrates into the cells of the rat embryo at a high rate which may allow metabolic studies. In order to overcome the difficulties mentioned and furthermore to measure the metabolic reaction over a longer period we have adapted a method in which DNA is pulse-labelled with H^3-thymidine and the specific activity is estimated subsequently for 24 hrs. The decline in specific activity measured in the DNA of isolated, highly purified nuclei is caused by the dilution of labelled DNA due to the formation of newly formed unlabelled DNA. It gives a measure of the rate of DNA synthesis during the experimental period. While this net DNA synthesis is almost

zero in the liver of an adult animal and is also comparatively small in a rapidly growing tumor (Table), a considerable amount of DNA—i.e. 4 times the amount present at 0-time—is synthesized in the embryo within the 24 hrs period. This rate of synthesis is decreased significantly by a single injection of as little as 50 μg/kg actinomycin D. No effect is seen on the activity of the DNA-polymerase 1 hr or 24 hrs after the injection of actinomycin. These results suggest—in agreement with other data obtained by our group—that the mode of action of actinomycin in these studies is located at some steps involved in the synthesis of nucleoside triphosphates [3].

Table. *Decline in specific activity of pulse-labelled DNA due to synthesis of unlabelled DNA within 24 hrs. 1 mC/kg H^3-thymidine (6 C/mmoles) was injected i.v. into pregnant (12th day) Wistar rats, followed 90 min later by a chase of 80 mg/kg thymidine (0-time). At 0-time and 24 hrs later (13th day of pregnancy) nuclei were isolated from pooled 50—100 embryos and the specific activity of the DNA was measured. A single dose of 50 μg/kg actinomycin D was given s.c. to one group of rats at 0-time. Experimental procedures otherwise cf [2]*

	dpm × μg DNA^{-1} in isolated, purified nuclei					
	number of experiments	0-time	number of experiments	24 hrs later	number of experiments	24 hrs actinomycin at 0-time
adult animals liver	15	40±12	15	38±11		
SHAY-chloroleucoma	2	280	2	225		
embryo	6	660±50	10	150±33	5	220±34

Data on the action of some embryotoxic drugs on RNA- and DNA-polymerase of nuclei isolated from embryonic tissue have been reported briefly elsewhere [1]. RNA- and DNA-polymerase of nuclei isolated from the whole embryo are found decreased in activity on the 12th day of pregnancy after i.p. injection of 7—10 mg/kg cyclophosphamide on the 11th day. This effect will be expected to became even more pronounced if instead of the whole embryo the enzymic activity of definite parts of the embryo is studied. This is suggested by morphological results which were obtained in extensive collaborative studies: The rate of mitosis in the inner layers of the vesicle which will form the telencephalon is tremendous at this stage of development. These mitotic figures are almost completely abolished after a treatment with 7—10 mg/kg cyclophosphamide. Furthermore, the epithelial cells of this vesicle contain as the result of the action

of cyclophosphamide numerous inclusions and vacuols. Electron microscopical examinations show these inclusions to be lysosomes which are packed with cell organells at different stages of degradation. Concomitant with these changes the adhesions between the cells decrease and many cells show a spherical appearance.

It has been the intention of this paper to show that it is possible to study the reactions leading to the synthesis of nucleic acids in the rat embryo—despite of the small amounts of tissue available—at this stage of development. The methods used may be helpful in clarifying—and perhaps even predicting—embryotoxic effects.

This investigation was supported by grant from the Deutsche Forschungsgemeinschaft.

References

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The Conversion of Angiotensin I to Angiotensin II in vivo. By K. K. F. NG and J. R. VANE

It has long been supposed that the decapeptide angiotensin I is rapidly converted to the much more active octapeptide angiotensin II by an enzyme in the blood. The present experiments show that, *in vivo*, the conversion in blood is relatively slow and probably unimportant; a much faster conversion occurs during passage through the pulmonary circulation.

Dogs were anaesthetized with chloralose. The blood-bathed organ technique (VANE, 1964) was used for the continuous estimation of angiotensin concentration in blood, as follows. Carotid blood was superfused at 15 ml/min in series over a rat stomach strip (VANE, 1957) to assay catechol amines (ARMITAGE and VANE, 1964) and over two rat colons to assay angiotensin (REGOLI and VANE, 1964a, b); the blood was then returned intravensously to the dog. Arterial pressure was recorded with a transducer.

To measure the conversion of angiotensin I to angiotensin II a length of silicone tubing was included in the extra-corporeal circulation and maintained at 37°C so that infusions of angiotensin I could be mixed with the circulating blood for different times before reaching the assay tissues. With this technique, there was negligible conversion of angiotensin