R. G., Moorhead, P. S., Saksela, E., J. Cell. Comp. Physiol., 1962, v59, 281.

5. Jensen, F., Koprowski, H., Ponten, J. A., Proc. Nat. Acad. Sci., 1963, v50, 343.

6. Gerber, P., Science, 1963, v140, 889.

7. Pagano, J. S., Crichlow, R. W., Jensen, F., Cornog, J. L., Ravdin, R. G., Koprowski, H., Proc. Am. Assn. Cancer Res., 1964, v5, 50.

8. Shein, H. M., Enders, J. F., Palmer, L., Grogan, E., Proc. Soc. Exp. Biol. and Med., 1964, v115, 618.

9. Gilden, R., Taguchi, F., Defendi, V., to be published.

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Electrophoretic Analysis of Abnormal Development.* (29742)

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A maternal dietary deficiency of pteroylglutamic (folic) acid is markedly teratogenic in the rat, and furthermore, the types and incidence rate of malformations vary in relation to duration, severity and timing of the deficiency during pregnancy(1). A 48-hour maternal transitory deficiency of this vitamin early in the second week of pregnancy (days 8 and 9), which is a period of rapid differentiation and organogenesis in the rat embryo, will result initially in 100% embryonic malformation and ultimately in embryonic death (2). A reduction in histochemically detectable phosphatase in the neural tube as early as day 9½ was demonstrated(3) as a first sign of incipient malformation. This reduction in phosphatase was followed on day 10 by a partial metaphase block and by reduced numbers and uneven staining of ribosomes as seen with electron microscopy(4). Retardation in the embryonic growth rate was followed by multiple frank malformations on days 11 and 12 and finally by cessation of embryonic heartbeat by the end of the second week(2) of the 3-week gestation period. Intensive study of several organs and organ systems(5) has demonstrated that such rates of development were unequally retarded and that this asynchronism was fundamental to the genesis of structural malformations. The object of the research reported here was to ascertain whether there were similar changes in the multiple molecular forms of enzymes associated with the development of structural malformations in the mammalian embryo.

Methods. A purified diet(2) lacking pteroylglutamic acid (PGA) but containing 20 mg% of the antagonist 9 methyl-PGA was fed on days 8 and 9 of gestation to pregnant, Long-Evans rats which had been inseminated by males of the same strain. The period of deficiency was terminated by feeding a diet which lacked the antagonist but which contained a high level of the crystalline vitamin. On days 10, 11, 12 and 13 of pregnancy embryos were removed from the uteri of PGA-deficient mothers for comparison with normal controls of the same gestational age. Homogenates of these embryos were subjected to zone electrophoresis in starch gel by methods previously described (6). The multiple molecular forms of phosphatases were visualized by standard biochemical means(7), and a semiquantitative representation of staining intensity and band width as observed in the starch zymograms (8) is depicted diagrammatically in Fig. 1 and 2.

Results and discussion. At pH 8.2 a maximum of 4 molecular forms of phosphatase were detected (Fig. 1) with Na alpha naphthyl acid phosphate as substrate in conjunction with the diazo salt Blue RR. At day 10, two forms of the enzyme family (II and IV) were present in both control embryos and in those from mothers maintained by the PGA-deficient diet for the 2 previous days. By day 11 the controls had formed

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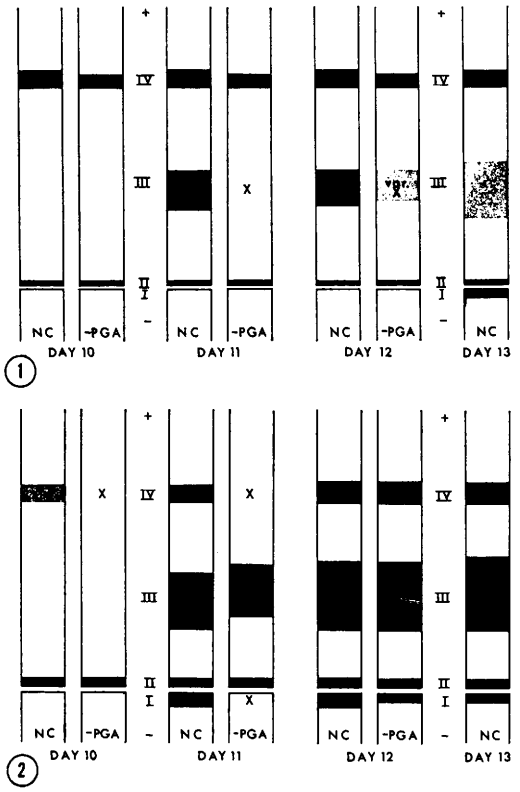

FIG. 1. Drawings of zymograms produced by alkaline phosphatases (pH 8.2) from embryos at 4 gestational ages. N. C. = normal control embryos; —PGA = embryos from PGA-deficient mothers; + = anode; — = cathode; var. = variable (a molecular form only rarely encountered); X = an active molecular form not detected in the abnormal embryo but present in controls of the same gestational age; I, II, III, IV = arbitrary designations for the stained bands in the gels (see text).

FIG. 2. Same as Fig. 1, but of acid phosphatases (pH 5.9).

a third enzyme (III) which was not detected in the abnormal embryos even though the mother had been receiving a complete ration for the 24 hours preceding autopsy. However, 24 hours later this enzyme was found, though not invariably, in the abnormal embryos. It might be safe to assume that those instances where this enzyme was present at day 12 represent either less severely affected embryos or accumulation of the enzymatic activity to a level just achieving the range of sensitivity of the methods.

As judged on the basis of the amount of dye deposited on the gel, which is a rough estimate of enzyme activity, it is obvious that in general the enzymatic activity increased with gestational age. Though sufficient living embryos could not be collected for analysis from the PGA-deficient mothers on day 13, it is of interest that in normal embryos at day 13 a fourth enzymatic form (I) had appeared, but enzyme III was now present to a lesser degree after having been a major band on the 2 preceding days. Such a phenomenon is not unique to the early mammalian embryo for it also has been observed in other systems undergoing rapid morphogenesis(7) wherein the adult repertoire of enzymatic forms is attained by both additions to and deletions from those present at preceding morphogenetic stages.

At pH 5.9 there were also 4 forms of enzymes capable of hydrolyzing Na alpha naphthyl acid phosphate (Fig. 2). The changing patterns of these enzymes in the various preparations examined were somewhat different from those at the alkaline pH. Two forms were normally present at day 10 but one of these (IV) was absent from the abnormally developing embryos both at day 10 and at day 11, though it was detected at day 12. Similarly, in the embryos from PGA-deficient mothers at day 11 enzyme I had failed to form, but another enzyme (III) did form in apparently normal fashion. Though abnormally developing embryos achieved a normal complement of molecular forms capable of hydrolyzing the substrate by day 12, they did not achieve this complement until 24 hours after the controls; and furthermore, as judged by staining intensity, the enzymes were present in markedly reduced concentrations when they did appear. The appearance on time of enzyme III in conjunction with the 24-hour delay in formation of number I and the 48-hour delay in the formation of number IV indicates a selective retardation. At the morphologic level, selective or asynchronous retardation has been intensively studied and is intimately related to the genesis of frank malformations. For example, retarded growth of the umbilical arteries appeared to interfere with normal ascent of the kidney from the pelvis and to result thereby in the anomalous situation of a pelvic kidney(5). Such a highly selective

phenomenon could possibly be related to altered differential gene expression. At day 11 the abnormally developing embryo had a molecular complement of acid phosphatases unparalleled in comparable normal development. This biochemical situation is similar to structural paraplasia wherein an abnormally developing embryo forms a structure "entirely foreign"(9) to normal embryology. Such structural paraplasia was illustrated by the presence of horseshoe kidney in the offspring of vitamin A-deficient rats.

Summary. As a result of a teratogenic maternal folic acid deficiency, discrete changes are produced in the molecular composition of abnormally developing embryos. Furthermore, these changes continue in the embryo after the mother has been returned to a complete ration. The adverse effects on the development of new enzymatic forms appeared to be both qualitative and quantitative. Although there was a general trend for delayed and asynchronous appearance of specific molecules in the abnormally developing embryos, this was not invariably the case and some of the enzymes did appear on schedule and at or near control amounts in the experimental animals. Thus the retardation in the appearance of certain enzyme forms resulting from folic acid deficiency would appear to be selective.

1. Nelson, M. M., Congenital Malformations, Wolstenholme, G. E. W., O'Conner, C. M., ed., Little, Brown & Co., Boston, CIBA Found. Symp., 1960, pp. 134-151.

2. Johnson, E. M., Nelson, M. M., Monie, I. W., Anat. Rec., 1963, v146, 215.

3. Johnson, E. M., ibid., 1962, v142, 244.

4. ———, ibid., 1964, v149, 49.

5. Monie, I. W., Nelson, M. M., Evans, H. M., ibid., 1957, v127, 711.

6. Johnson, E. M., Analyt. Biochem., 1964, v8, 210.

7. Solomon, E. P., Johnson, E. M., Gregg, J. H., Develop. Biol., 1964, v9, 314.

8. Hunter, R. L., Markert, C. L., Science, 1957, v125, 1294.

9. Wilson, J. G., Warkany, J., Am. J. Anat., 1948, v83, 357.

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Preliminary Observations on Spontaneous and Radiation-Induced Leukemia in Germfree Mice.* (29743)

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The induction of leukemia by ionizing radiation in conventionally reared mice has been a subject of intensive study for more than 20 years(1). With the implication of viruses in the pathogenesis of these neoplasms(2) it has become increasingly important to study the influence of host microbial flora on induction of leukemia and to determine the mode of transmission of any etiologic agents. Preliminary observations on X-ray-induced and spontaneous leukemia in germfree mice, associated with the appearance of "virus-like" particles in the leukemic tissues, are reported at this time because of their significance to further consideration of the pathogenesis of these neoplasms.

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Materials and methods. The mice used in this study included: 1) germfree and conventional noninbred ICR mice at least 4 generations removed from germfree ancestors supplied by the Lobund Laboratory of the University of Notre Dame, and 2) mice of the RF strain. The latter comprised mice from our noninbred production stocks and from our inbred line, which was originally brought to Oak Ridge by Furth in 1949(3,4). The germfree RF mice (RFM/Uf) were developed by caesarian section of a full-term