this antibody constitutes a general immunological principle. Equally pertinent for investigation would be the role of anti-DNA in these diseases. However, in systemic lupus erythematosus sera, a variety of anti-nuclear antibodies have been detected, yet no proof has substantiated their role in contributing to the pathology associated with this disease (10).

From the foregoing, and the results described herein, it is suggested that the presence of antibodies of the general class of autoantibodies warrants study as to their significance in chronic diseases of obvious microbial etiology.

Summary. BDB indirect hemagglutination was determined to be an effective measure of antibody to thermally denatured DNA. It was found equally as effective as bentonite agglutination in detecting anti-DNA in SLE sera. Antibodies to thermally denatured DNA were detected in the sera of experimentally-infected tuberculous rabbits. The antibodies appeared 4-6 weeks post-infection, reached a maximum titer at approximately 8 weeks, and declined thereafter. Similar anti-DNA antibodies were also detected in the sera from patients with tuberculosis or histoplasmosis.

Dr. Roy L. Donnerberg, Benjamin Franklin Tuberculosis Hospital, Columbus, Ohio, generously provided the human tuberculous sera employed in this investigation.

Human histoplasmosis sera were obtained through the courtesy of Dr. J. A. Schmitt, Department of Botany, Ohio State University; Dr. Tom D. Y. Chin, Kansas City Communicable Disease Center; Miss Jo Ann Sparks, Infections Disease Center, University Hospital, Columbus, Ohio.

Normal human sera were obtained from the University Hospital Blood Bank, Columbus, Ohio. Only those serum samples from acceptable blood donors who demonstrated no overt signs of disease were employed.

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Mesodermal Alterations Induced by Dimethyl Sulfoxide.* (31235)

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Intraperitoneal administration of dimethyl sulfoxide (DMSO) to pregnant hamsters induces developmental malformations of their embryos(1,2). The most common malformation encountered is cranioschisis, with exencephaly in later stages of embryonic development. The cephalic malformation is easily reproduced, and occasionally all the embryos of a litter depict the same degree of abnormality(2). This suggests (1) a specific effect of DMSO upon development of the cephalic region of the hamster embryo worthy of further investigation. DMSO-induced cranioschisis represents an excellent opportunity for studying the mechanisms of the embryogenesis of this malformation, to be compared with other previously reported mechanisms of the embryogenesis of vitamin A-induced(3,4) cranioschisis and of the defect in man(5,6,7).

Methods. Fifteen pregnant hamsters were

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used for this study. Twelve were treated, each receiving 5500 mg/kg of DMSO† in a single intraperitoneal injection at 8 a.m. on the eighth day of gestation(2). The 12 treated animals were separated into 3 groups of 4 each, and sacrificed as follows: the first group 10 hours after, the second 12 hours after, and the third 24 hours after administration of DMSO. One uninjected animal, serving as a control, was sacrificed with each treated group. The uteri of treated and of control animals were submitted to the same procedure used in previous studies of experimentally-induced cranioschisis(4). This method consisted of dissection of the individual gestational areas of each pregnant uterus. From each gestational area the decidual cast with the embryonic cavity was dissected out. Each decidual cast was then divided into two halves. The one containing the embryo was fixed in Bouin's solution, the other discarded. Nine to twelve such embryonic cavities can, after 24 hours' fixation, be mounted in a single paraffin block. Each block was submitted for complete serial sectioning; all sections were stained with hematoxylin and eosin.

Results. Histological examinations of all of the embryos recovered from DMSO-treated hamsters show, in some, mesodermal alterations at the cephalic region and to a much lesser degree in the axial nonsegmented mesoderm of the embryonic body. Embryos recovered 10 and 12 hours after DMSO administration reveal severe and acute mesodermal alterations (Fig. 1 and 2) and those removed at 24 hours (Fig. 3) appeared to be recovering from the mesodermal injury. The embryos of all groups demonstrate, however, the results of the mesodermal injury, namely, mesodermal collapse in the first 2 treated groups, and failure of closure of the neural tube in the cephalic region in the treated embryos of the third group. The cephalic mesoderm of the embryos of the first 2 groups shows a reduction in size and in number of cells and an increase in size of the intercellular space. The vascular channels, especially branches of the aortae, and the peripheral capillaries are also greatly enlarged (Fig. 1 and 2). The increase in the intercellular space appears to be due to accumulation of edema fluid. This is also suggested by the marked shrinkage of the cytoplasm of the affected mesodermal cells (Fig. 1-3). The nonsegmented mesoderm of the embryonic body shows slight but similar changes to those of the cephalic region. Apparently, a direct consequence of these alterations is the mesodermal collapse of the cephalic region depicted by the treated embryos of the first two groups. This mesodermal collapse causes a flattening of the cephalic region of these embryos with exposure and exterioration of the neuro-ectoderm (Fig. 2), producing the false impression of an overgrowth of the neuro-ectoderm, since it comes to cover a previously collapsed mesoderm (Fig. 2).

The last group of embryos shows the beginning of recovery from the mesodermal injury and from the mesodermal collapse which is less marked (Fig. 3). However, the mesodermal cells remain slightly reduced in size and number, and the intercellular space is still enlarged, mainly the vascular channels (Fig. 3). The functional incompetence of the cephalic mesoderm of these embryos is manifested by the failure of closure of the neural tube, which at this time should be already closed, encountered in some of them (Fig. 3). The head of these embryos appears smaller than in controls, and the cephalic neuro-ectoderm shows early signs of folding, probably the result of the functional incompetence of the surrounding mesoderm (Fig. 3). Mesodermal concentrations in the cephalic region, such as the maxillary and the mandibular processes, show focal cellular necrosis which appears to be more severe than in control embryos. No distinct damage is encountered in the somites; however, they are surrounded by affected nonsegmented mesoderm and some of them may assume an abnormal shape. No alterations are found in any of the other embryonic tissues. The neuro-ectoderm of these embryos appears undamaged by DMSO.

Discussion. The mesodermal alterations described lead to a mesodermal collapse which then fails to accompany, to support and finally to enclose the developing neural tissue of the cephalic region. As a result, the neural

† Dimethyl Sulfoxide (DMSO) was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.

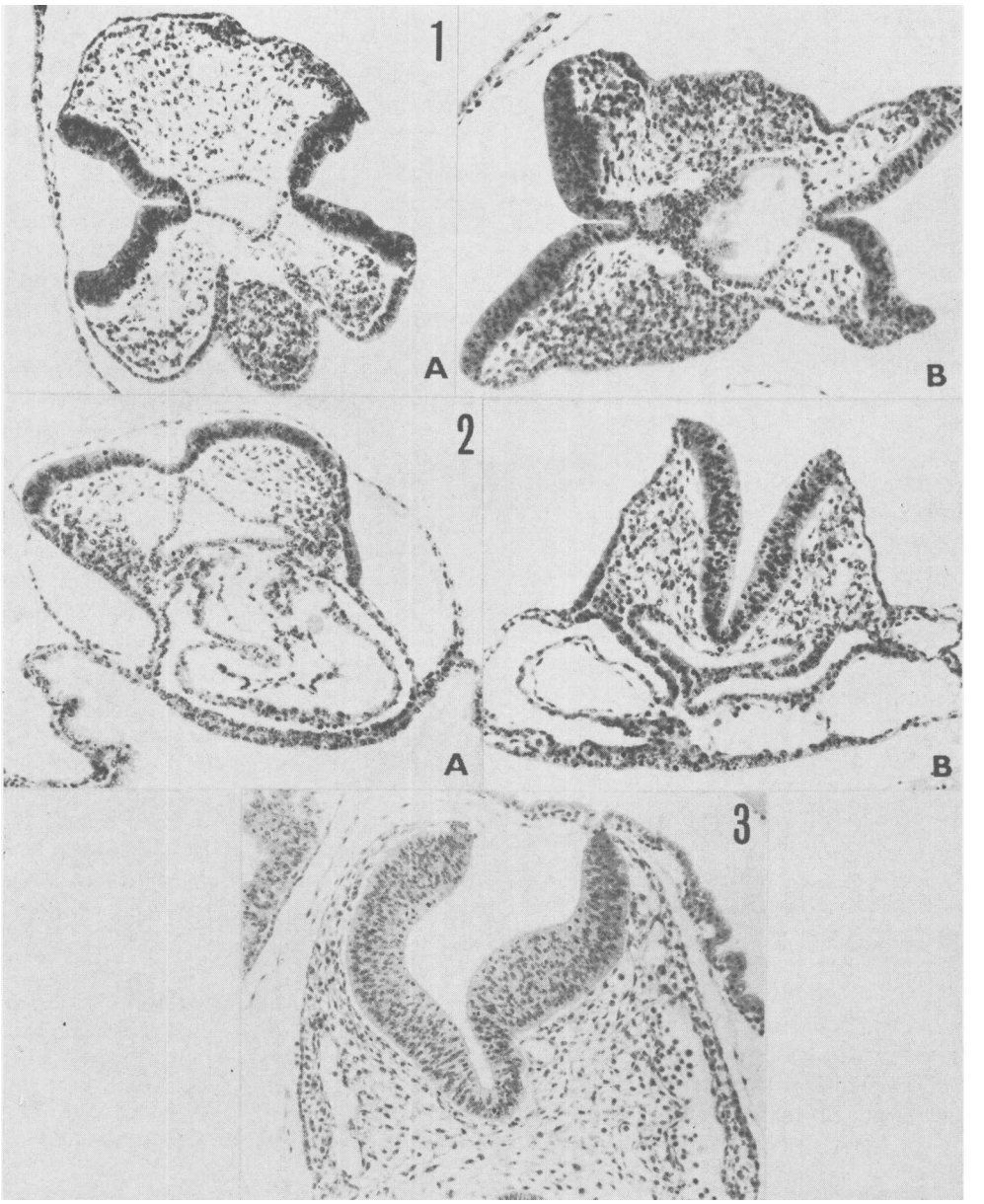

FIG. 1. *A*. Cross section of the head of an embryo recovered 12 hr after DMSO administration showing scanty cellularity of the mesoderm, shrinkage of the cell cytoplasm and increase in intercellular space. *B*. From a control embryo showing the richness of cells in the mesoderm and lack of intercellular edema. (H & E × 150).

FIG. 2. *A*. Section of an embryo recovered 12 hr after DMSO administration showing the mesodermal collapse. Notice the false impression of overgrowth of exposed neuro-ectoderm which covers the collapsed mesoderm. *B*. From a control embryo showing abundance of the cells in the mesoderm and lack of mesodermal collapse. (H & E × 150).

FIG. 3. Section of the head of an embryo recovered 24 hr after DMSO administration showing failure of closure of the neural tube which at this time, 9th day of gestation, should be closed. Notice shrinkage of mesodermal cells, numerous dilated intercellular spaces and greatly enlarged peripheral vascular channels. The neuro-ectoderm shows early signs of folding. (H & E × 175).

tube remains unclosed. Such failure explains the cephalic malformation (cranioschisis) encountered in later stages of embryonic development of treated hamsters(2). The mesodermal alterations described above are morphologically similar but less severe than those induced by hypervitaminosis A also in the golden hamster(3,4). The embryogenesis of cranioschisis induced by excessive vitamin A and by excessive DMSO in the golden hamster appeared to be the result of a similar phenomenon, namely, failure of closure of the cephalic neuro-ectoderm caused by a collapse of the underlying cephalic mesoderm. Furthermore, the mesodermal defects caused by vit A and DMSO appear to be the result of similar mesodermal alterations characterized by shrinkage of the cell cytoplasm and by an increase in the intercellular space. The primary biochemical actions of DMSO (or vit A) upon the embryonic mesodermal cells leading to the alterations described above are unknown. However, the shrinkage of the cell cytoplasm and the possible accumulation of fluid in the extracellular space suggest alterations of cellular permeability. From other sources it is known that DMSO (as well as vit A(8)) affects the permeability of biological membranes(9,10). Also, it has been suggested that DMSO increases cellular permeability(11) and that it reduces the water-binding capacity of the cells of the horny layer of the skin(9). It is possible to speculate that DMSO alters the cellular membrane and reduces the water content of the mesodermal cells of the hamster embryos. The cephalic mesoderm is not more severely affected by DMSO or vit A than is mesoderm elsewhere; the lesion merely reflects the vulnerability of the cephalic mesoderm during this period of embryonic development. This vulnerability is due to the significant role the cephalic mesoderm plays during the morphogenetic mechanisms of closure of the cephalic neural tube. Probably any damage to the cephalic mesoderm at this time, regardless of the nature of the teratogen, would be followed by a delay or a failure in the closure of the cephalic neural tube. The consequence of such delay or failure is the development of cephalic malformations such as exencephaly (cranioschisis). In this regard, it should be mentioned that such different substances as trypan blue(12), triethanomelamine (TEM or 2,4,6-tri(ethyleneimino)-1,3,5-triazine)(13) and nitrogen mustard derivatives(14) cause in the mouse embryo mesodermal alterations similar to the one here described. These substances also cause exencephaly (cranioschisis) in the mouse embryo. Further investigation of the biochemical effects of DMSO and of vit A as teratogenic agents in the golden hamster is needed.

Summary. The observations presented above support the previously advanced concept of the embryogenesis of human and experimental cranioschisis(3-7). They also support the idea that any mesodermal alteration causing its derangement and collapse *before the complete closure of the neural tube* will necessarily be followed by failure of closure of the neuro-ectoderm, regardless of the nature of the teratogenic agent used. This concept supports the following facts: the closure of the cephalic neuro-ectoderm is a critical and significant period of embryonic development; the cephalic mesoderm plays an important role in its final closure; and cranioschisis (anencephaly), the most commonly induced experimental malformation, is the direct consequence of the failure of the cephalic mesoderm to fulfill its function of enclosing and lodging the cephalic neuro-ectoderm.

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