A Study of the Effect of a MAO Inhibitor on the Early Development of the Chick Embryo

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Growth and differentiation of the avian embryo is largely dependent upon the activity of various enzyme systems. Embryonic development can therefore be studied by means of altering such enzyme mechanisms. The present study is an investigation of the effects produced by the inhibition of the metabolism of monoamines in the chick embryo during early development. The metabolic degradation of monoamines is dependent upon the action of an enzyme, monoamine oxidase (MAO) which is of major importance in the degradation of epinephrin, norepinephrin, dopamine, and 5-hydroxytryptamine (Goodman and Gilman, 1965). MAO inhibitors are drugs that produce an irreversible inactivation of the enzyme by forming a stable compound with it (Goodman and Gilman, 1965).

This study utilized the MAO inhibitor, phenelzine sulfate, manufactured by Warner-Chilcott Laboratories and sold for clinical use under the trade name Nardil. It is a hydrazine and possesses toxic properties when administered in excessive dosage (Remmen, et al., 1962).

Methods and Materials

Seventy-five eggs of the domestic fowl *Gallus gallus* were obtained and divided into experimental and control groups: 33 controls and 42 experimental eggs were selected at random. Following incubation at 40 C for 24 hr, all eggs were removed from the incubator and allowed to cool.

A solution of phenelzine sulfate in sterile water for injection was prepared in a concentration of 0.25 mg/cc. The standard tablet of Nardil was used. Preliminary studies revealed that a dosage of 0.125 mg did not prevent development when introduced into eggs prior to incubation.

Eggs were prepared for injection by drilling two small holes 90° apart on the median transverse circumference of each shell. One cubic centimeter of albumin was aspirated from each egg using a sterile hypoderim syringe and needle, exercising care to maintain sterility and avoid yolk damage. Withdrawn albumin was immediately replaced with 0.5 cc of phenelzine sulfate solution (0.125 mg) in experimental eggs and 0.5 cc sterile water in controls. Holes were paraffin-sealed and eggs returned to the incubator and rotated 180° daily to prevent embryos from adhering to the extraembryonic membranes. A second incubation period of 90 hr was allowed. Embryos were removed, examined in the live state, measured, and placed in Bouin's solution. Specimens were stained *in toto* with Mayer's carmalum and prepared as whole mounts. Serial transverse sections were prepared from selected paraffin-embedded specimens.

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RESULTS

The results of incubation together with crownrump measurements of living embryos obtained are presented in Table I.

TABLE I. EGG INCUBATION SUCCESS AT 134 HR, WITH CROWN-RUMP LENGTH (mm) RANGES, AVERAGES IN PARENTHESES.

GROUP	Incubated	Fertile	Infertile	Embryos Decomposed	Length
Control	33	10	23	1	10.0-12.0 (10.9)
Experimental	42	31	11	0	3.0-13.0 (7.43)
Total	75	41	34	1	— —

Low-power microscopic examination indicated no structural abnormalities among the control specimens. Observation of gross structure of living, treated embryos revealed many differences from the controls. The head regions appeared relatively large, and marked variety characterized the body curvatures. Some specimens displayed no flexures; many had exaggerated cervical flexures. A large percentage had an elongated tail process with either no caudal flexure or a reversal of the direction of the flexure to produce an S-shaped curvature of the tail region. There was great variation in the degree of brain development; in many the mesencephalon was more prominent than normal and the smallest specimens showed little or no brain differentiation. Some specimens lacked eye and lens vesicles; in others the otic vesicles were underdeveloped or absent. Heart development paralleled the size of the embryo. All except the smallest specimens were enclosed in an amnion: in one specimen blood was observed in the amniotic fluid. Rocking motions were observed in only the largest specimens.

Specimen A, smallest of the treated embryos (3 mm long), was studied as a whole mount. It consisted of a mass of densely packed cells centrally located in a germinal disc showing areas pellucida and vasculosa. The "body", exhibiting two caudal flexures, was thicker along the longitudinal axis and became thinner laterally. The very irregular lateral edges in many places were composed of a thin sheet of cells with cellular cords extending outward along the path of vessels within the area pellucida. No organization of structures was detected in the embryo and extraembryonic blood vessels and islands were few in number.

Specimen B, studied both as a whole mount and in serial sections, showed a greater degree of complexity and presented a bizarre appearance. A pale-staining, bubble-like structure surrounded the cephalic end and extended caudally, narrowing only slightly. A furrow appeared in this structure on the left side opposite the heart and paired, flattened processes extended laterally in the midportion. An elongated structure of dense tissue, with its cephalic end bilobed and expanded, was located centrally: this had the appearance of a neural tube except for the extreme caudal end which was bulbshaped. The midportion was flanked by five pairs of somites followed by mesodermal plates extending to the caudal end. An elongated, tortuous sac on the right side represented the heart. Four small, darkly stained, appendage-like structures were closely associated with one another at the caudal end of the embryo.

Study of serial sections gave a much clearer understanding of the structure of specimen B. Transverse sections taken cephalad showed

body ectoderm enclosing a mass of compactly arranged, deeply stained cells surrounding a double-layered, open-sided eyecup. The center of the cup was filled with mesodermal cells continuous with those of the body. A V-shaped invagination of ectoderm opposite the eyecup represented an attempt at lens formation. A solid mass of compact tissue presumably represented the brain.

A section through the heart showed this structure to be situated in the extraembryonic coelom on the right side. The heart was quite thick-walled, and its chamber was packed with large cells that showed little tendency toward differentiation into typical blood cells. No evidence of the presence of a gut was found.

Sections at the somite level showed a notochord for the first time. The neural tube was present and had the appearance of that of a 24-hr embryo, but the neurocoel contained numerous cells. Cylindrical somites flanked the neural tube on either side. Two large dorsal aortae were present as were the post-cardinal vessels. Mid-gut entoderm was prominent.

Sections farther caudad showed mesodermal plates on each side of a neural tube that was thin-walled dorsally and whose cavity was filled with cells. Lateral extensions of the amnion were more closely associated with the chorion than in previous sections.

An amnion surrounded the entire boly mass and cephalad the amniotic cavity was filled with loosely arranged cells, presumably of mesodermal origin. A deep fold in the membranes on the left side, almost separating the cavity into two compartments, produced the furrow observed in the whole mount. The amnion extending laterally with folding produced the appearance of flattened processes observed in the whole mount.

Study of the larger embryos by means of transverse and sagittal sections revealed that they deviated from normal in several respects. Body form appeared more cylindrical and neural structures showed less differentiation than normal. Retarded development of cranial and spinal ganglia was noted. Cellular elements often filled the neurocoele and incomplete closure of the neural tube dorsally was common. Study of the heart, liver, and lungs indicated an abnormally slow development of these structures, and the blood vessels appeared larger in diameter and fewer in number than normal. Delayed and/or abnormal limb bud development was observed. The mesonephros showed full development and often the number of tubules was greatly increased. Development of the hind-gut appeared to be retarded and generally the allantois was very small.

Discussion

It appears that specimen A represents a degeneration or replacement of originally developed structures into a disorganized mass of cells growing for the most part in an unorganized manner. Only the arrangement along a longitudinal axis and its two flexures suggest a relationship to a chick embryo. Unregulated invasive growth would explain the presence of masses of cellular elements within the amniotic cavities and neurocoeles of this and many other specimens. It is presumed that these cells were of mesodermal origin since their staining characteristics and size and shape were similar to cells within the heart and blood vessels. All of the treated embryos were found to be abnormal in one or more respects while no abnormalities were detected in the untreated control specimens.

After careful gross and microscopic studies of embryos treated with the MAO inhibitor phenelzine, it is concluded that the drug exerts a powerful influence upon embryonic development of the chick. The effects were nonselective and, in general, retarded growth and/or organogenesis.

REFERENCES CITED

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