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Some Effects of Pressure Upon the Embryological Development of The Domestic Fowl (Gallus Domesticus)

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SOME EFFECTS OF PRESSURE

UPON

THE EMBRYOLOGICAL DEVELOPMENT

OF

THE DOMESTIC FOWL

(GALLUS DOMESTICUS)

BY

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MASTER OF SCIENCE THESIS

OF

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ABSTRACT

In an investigation of the effect of pressure on the developing chick embryo, all environmental factors excepting varying degrees of compression were kept constant.

Eggs were sealed in a compression chamber fitted with a thermometer for temperature control.

A lethal pressure was determined somewhat above sixty pounds. Pressures near this point caused gross malformations including an anamniotic condition.

the general effect of pressure is acceleration of structure formation along with diminution of body size.

I

INTRODUCTION

The writer's first interest in pressure as a factor in the environment of animal life was aroused several years ago when he came upon one of Pflüger's early accounts (1884) of the effect of compressing amphibian eggs between two plates of glass. Until that time he had given but little attention to any of the vital relationships between the growing embryo and its internal and external physical conditions. His conception of the cleavage of the fertilized egg as the differentiation of the potential adult had been essentially that held by most embryologists of the present time. Those chemical and mechanical complications of cell division which substantiated the chromosome theory he accepted at their value. The influence of the agent pressure, which is universally manifest in nature in one or more of its several forms (Appendix 1), was not until then considered a paramount factor in the existence and growth of the living substance.

Any consideration of the part pressure might play in the existence of life leads to a question of importance. How can such a part be studied to best advantage? The solution arises immediately: The animal embryo offers more possibilities than the adult, as the embryo is small; it develops gradually but quickly by differentiation; and it can be subjected to compression with ordinary apparatus. Obviously the independently de-

veloping embryo is more adaptable than the inter-uterine fetus.

The writer's decision to work with the eggs of the fowl was made in the fall of 1932 when it was his pleasure to hear that an English investigator (or group of workers) had recently subjected such eggs to compression with the result that embryonic development had been greatly accelerated. He attempted to secure the original account of the work; however, after fruitless search he decided to dispense with it and investigate the subject independently. It was obvious to him that such circumstances of acquaintance with antecedent work did not admit of corroboration or disagreement. Therefore, the present writer formulated his method of procedure in such a manner that it would, first of all, serve as an adequate basis for a study and determination of the general effect of compression. The possibility of subsequent specialized experimentation based upon subsequent findings was anticipated.

II.

THE REVIEW OF LITERATURE

A. Amhibian Eggs. Pflüger and his collaborators applied their apparatus to the segmenting eggs in such a way that hydrostatic pressure was created in the cytoplasm without change of any kind in the surrounding environment. It can be understood that such a pressure was not great because the maximum was that which did not tax the tensile strength of the cell membrane to the point of rupture. Even so, the internal pressure invariably

caused the first cleavage plane to appear at right angles with the parallel plates of glass. This effect is not yet satisfactorily explained. Whether protoplasm is to be regarded as a semi-solid and subject to compression lines, or whether a true liquid with equal pressure in all directions is a question which may be of significance in explaining the pressure effect as determined in this problem. In fact, certain observations subsequently recorded, it is hoped, will throw some light on Pflüger's phenomenon.

B. Pressure Teratogeny. Perhaps the earliest account of the application of extra-cellular pressures to the embryo is that of Weldon (1902). His work included introducing water through the shell and membrane of larger end of the developing bird's egg by means of a siphon from a reservoir. In that manner not only was water lost by evaporation replaced, but also a pressure was created. Although a definite morphologic change was not invariably produced, several cases of anamniotes resulted. Lillie (1903) attributes the production of such abnormalities to an hydrostatic pressure which so forced the embryo against the immovable shell that the normal up-folding of the amnion was more or less prevented.

In the same paper (1903), Lillie describes several experiments which throw some light upon the mechanical factors of amnion development. By partially and completely destroying the lateral amniotic folds, he was able to materially retard the closure of the somatopleure to form the body wall. Upon that evidence he assumed an interdependence between formation of

amnion and body wall.

As to the occurrence of anamniotic specimens without the aid of man, Dareste (1879) claimed that he had observed several, one of which was living and normal in all respects excepting the complete absence of amnion. He attributed many cases of teratogenesis to an antecedent condition of partial anamniotic development. (Appendix 2).

C. Modification of Cardiac Tissue by pressure. That hydrostatic pressure produces definite physiological changes in the beating heart of certain animals is demonstrated in the work of Edwards and Cattell (1928). Their report can be summarized as follows:

1. Moderately high pressures produce a marked increase in the amplitude of contraction of the frog and terrapin heart.

 a. The initial cycle (as recorded by electro-cardiograph) after compression is greatly augmented.

 b. The maximum effect is apparent after the third or fourth cycle.

 c. The effect of pressure as manipulated by these workers is immediate although not instantaneous.

 d. The increase in amplitude of contraction is not an enduring effect.

 e. The normal cycle is gradually resumed upon release from compression.

2. Pressure has the effect of increased heart rhythm.

 a. The normal cycle of the frog's heart occurs every 3.9 seconds while at the time of maximum amplitude

under pressure, it occurs every 3.6 seconds.

b. Upon release from compression, the cycle time of
4.2 seconds is assumed, whereupon the normal is
gradually attained.

D. Pressure Modifications of Cleavage and Heart Rate in
Marine Forms. Edwards and Draper (1932) have carried on fur-
ther experimentation with cardiac modification in the Fundulus
embryo. They conclude that hydrostatic pressure as high as 1900
pounds per square inch does not produce any evident changes in
cell constituents. In all test groups subjected to pressures
from 1300 to 1900 pounds, however, a definite retardation of
cell divisions was observed. A few abnormalities were produced;
these were types showing gross body distortion, cardiovascular
irregularities, and tendencies towards anopthalmos. Teratogenic
specimens, however, were not numerous and there were no dominant
anomalies.

The automaticity of the Fundulus heart is slowed and can
even be completely inhibited by pressure. Upon release from
compression the original speed of contraction returns within a
few minutes.

That arrhythmia, local block, and isolated fibrillation
are not a result of the direct action of compression on the
inhibitory nerve mechanism is proved by Edwards and Draper by
sectioning all nerves controlling heart action.

III

THE INVESTIGATION

A. Object. The purpose of carrying on the series of experiments was two-fold. A determination of any change in embryonic development which might be brought about by increasing the absolute pressure of the environment was necessary; and such an aim involves a detailed study of the developmental structure of the embryos of definite chronological ages, as well as investigation of possible abnormal growth, whether such growth is general or restricted to certain local organs by dysmorphic differentiation, had to be presumed. These probable results were outlined for convenience of later definition as follows:

1. Changes of either accelerating or retarding nature with or without material increase or decrease in body size.

2. Increase or decrease in body size without acceleration or retardation.

3. Possible teratogenesis accompanying either of the above conditions.

4. No alteration of form or size.

The second aim evolved itself from the expected findings as a consideration of the causal factors concerned with change. In all probability changes effected in the embryo by the action of an increased pressure environment would involve a modified physical and chemical relationship of cells and organs.

B. Apparatus. The very fact that pressures were dealt with in these experiments necessitated the solution of a mechanical problem. After much experimentation with various contri-

vances, the author hit upon an apparatus suitable for the re-
quirements of the investigation.

A compression cylinder was designed of solid brass three inch
piping. One end was permanently sealed by screwing on and solder-
ing in a thick head of the same metal, while the other was fitted
with a trap cover which could be turned very tightly upon a firm
rubber washer. Through both the sealed cap and the removable
cover a three-eighths inch brass pipe was sealed with litharge.
A needle valve was attached to the opening at the cover; from this
a rubber tubing led to a beaker of water for the purpose of quan-
titative determination of air circulation through the chamber.
A third small brass pipe was screwed into the side wall of the
apparatus in such a way that a thermometer could be inserted
and sealed by fitting with a short piece of high pressure tubing
and two tubing clamps. To the inlet was clamped a four foot
length of pressure tubing which at its other end was attached
to the compressed air cylinder and its reducing gauge.

By means of a system such as described above, a continuous
flow of air under pressure as high as one hundred pounds could
be maintained for long periods. Since the volume of the compres-
sion chamber was known (400 cubic centimeters), the speed of
displacement could be computed at all times during incubation.
This precaution was necessary to keep the carbon dioxide per-
centage below .01, which is the optimum described by Romanoff
(1930).

An electric laboratory incubator of ordinary design was
used in all test work, while a hot water Buckeye model of accurate

adjustment served for controls. Each proved efficient in its special capacity; the one because it was easily regulated for long and continuous use, and the other because it was better fitted to maintain the adequate humidity level and could be operated independently of test work upon occasion.

A discrepancy of temperature was found inside the pressure chamber. This increase of five and one-half degrees Fahrenheit was at first not understood. Later however, it was attributed to high conductivity of the metal used in construction, and possibly a greater conduction of compressed air.

C. Materials. The eggs used in these experiments were from a single flock of Rhode Island Reds owned by Senator Jessie H. Metcalf. Their fertility and hatchability was unusually high, as evidenced by excellent results in the Sachem Farm incubators. Each was graded as closely as possible for size, shape, shell formation, and freedom from blood spots and other visible imperfections. Weighing, however, was not resorted to as a means of securing uniformity, although the usual grading process was performed by the hatchery operator. No eggs more than twenty-four hours in age were used, and the greater part of them were less than twelve.

The compressed air used was secured under a guarantee of purity and freedom from oil and toxic substances.

D. Method of procedure.

1. Uniformity of incubation. After the completion of several tests and experiments, the object of which was a

determination of the most accurate method for designating the chronological age of the test embryos, it was decided that the two eggs to be subjected to test conditions at one time should be sealed in the chamber before either the eggs or the chamber were heated.   Upon connection with the rest of the system, the pressure unit was placed immediately into the pre-heated incubator.   A low compression was then admitted, after which the maximum of pressure was gradually but quickly increased within a half hour.   Incubation time was recorded from the moment at which a temperature of 103° Fahrenheit was attained.   In every case this period was found to be just two hours after the unit had been placed into the incubator.

Such a procedure, the author believed, if followed diligently, was the most that could be done to maintain accuracy and uniformity of timing.   Never-the-less, the fact that the eggs of birds are fecundated in the oviduct and vary in the speed of their passage to the exterior leads to an unavoidable lack of uniformity in the development of freshly-laid eggs.

Equally exacting precautionary measures were carried out with the control specimens.   Although the aim in this case was not primarily to incubate controls simultaneously with the tests, but rather, to produce embryos of as nearly as possible the chronological age of those developed within the pressure chamber.   Emphasis was placed upon the representative development age; and to accomplish such an aim, a large number of eggs for each age group studied was incubated under normal conditions.

That specimen which upon careful examination proved to be analo-
gous to the mean of development of all the rest (any abnormalties
excepted) was designated the control, whether or not it corres-
ponded in all structural and developmental details with the
commonly designated chronological age.

2. Compression Methods. Without involving unnecessary
detail the author lists briefly the various procedures he carried
out in his preliminary handling of compression.

a. A two hour period of warming followed by gradual
rise of compression over a period of five hours.

b. The same preliminary warming followed by a com-
paratively quick, though gradual, increase of
pressure during a period of thirty minutes.

c. Gradual increase of pressure over five hours
without a period of warming.

d. Quick compression simultaneous with heating.

e. Normal incubation up to certain ages (24, 33,
48, 60, and 72 hours) outside of the compression
chamber, followed by gradual and comparatively
quick pressures.

f. Gradual compression before heating.

g. Comparatively quick compression before heating.

All of the above tests were made in an endeavor to deter-
mine a set of conditions which would be workable as well as
optimum in the ensuing experiments. Since methods d, f, and g
proved to be of equal advantage from the standpoint of results,
that of comparatively quick compression simultaneous with

heating was adopted because of its convenience. However, the results of some of the other methods have a definite bearing on the problem; these will be dealt with specifically in the general results of this paper.

3. Histological Technique. For the purpose of uniformity, only two general methods were used in the preparation of embryos for study. The one was admirably adapted for preserving seventy-two hour and younger specimens. It comprised fixing in formol-nitric (Appendix 3) with subsequent treatment in Worcester's fluid. After a rather light staining with alum cochineal (Appendix 4) the specimens were mounted entire. A painstaking and uniformly timed schedule was followed throughout, and the resulting beautifully stained and cleared preparations served their purpose well. Older embryos were fixed in Bouin's (modification 3), sectioned in paraffin, and stained with Delafield's haematoxylin and eosin. Counterstaining made possible a study of cytological as well as morphological structures.

E. Results. Several methods of observation were employed with discrimination in a specific study of each stage and circumstances of variation of the embryos under consideration; viz, comparison of test and control specimens on a basis of observation of structure, and determination of gross- and micro-measurement; comparison of histological and cytological detail, where applicable; and comparison on a basis of relative change due to varying degrees of compression. Anatomical details will be given for the different age groups.

Effects of Various Conditions of Incubation.

Eggs normally incubated up to 24, 33, 48, 60, 72, and 122 hours were dead in most cases when removed after periods of compression at twenty-four or thirty pounds. The same result existed whether quick or relatively slow pressure was applied. In the forty-eight hour specimens and older specimens death was in most cases caused by hemorrhage. Extravasation of blood into the amniotic cavity through a rupture of the atrium was the predominant form, while in the 122 hour chick the bleeding occurred along the aorta. In almost everycase death took place immediately upon the onslaught of pressure, for no specimen given such treatment advanced beyond its development at the time of compression.

The application of various pressures after a period of warming longer than five hours always resulted sooner or later in death at an early age. No actual mounts could be made of such specimens because of disintegration before removal. However observations pinted to death before the fourteenth hour.

Two eggs which had been incubated normally for seventy-nine hours were subjected to thirty pounds of pressure applied very gradually (maximum not reached until the ninety-first hour). One chick had died, while the other, although its appearance was at first that of death, proved to be in a condition of cardiac fibrillation. An arrythmical beating set in soon after removal. As an explanation, it is reasonable to assume that the chick would have soon died under continued compression. This case of fibrillation was not a result of lowering the pressure; at least, no other occurrence of such cardiac modification came to

the writer's notice in all subsequent work. At any rate it is
very probably that fibrillation and heart block caused the death
of the first embryo.

Twenty-five-and-one-half hour embryos.

Four newly laid eggs were subjected (during two experiments)
to test conditions of thirty pounds of compression for twenty-
five and one-half hours. Six more were incubated under normal
conditions. Of these one was removed thirty minutes before the
twenty-five-and-one-half hour period had been completed; two at
the period, and one respectively at periods one, two and three
hours later.

Body form. Although one egg was sterile, the remaining
three test specimens presented the appearance of normal embryos.
All three had formed definite head folds. Two fully formed
somites were present in each, and in none was the third somite
completed. All three were more advanced than either of the two
twenty-five-and-one-half hour controls. In fact, the only con-
trol specimens which showed more development were those removed
at twenty-seven-and-one-half and twenty-eight-and-one-half hours.
The twenty-seven-and-one-half hour control was almost identical
with the test specimens, excepting a very slight increase in
development of the third somite. (See Plate 1, Figures 1 and 2.)

Body size. Careful measurements of all test and control
specimens showed that pressure brought about a slight diminution
in size.

Forty-seven-hour Embryos subjected to thirty pounds of

pressure (Plates 3 and 4).

Body form. Considerable variation was apparent in these embryos. All six of the test specimens showed flexure and torsion of a more advanced stage than any of the controls. In most cases the looping of the test hearts was more pronounced than in the control embryos. No form of gross abnormality appeared in these embryos.

Extra-embryonic structures. The extra embryonic splanchnopleures of the test specimens were somewhat smaller in diameter than those of the controls; and blood islands were not in the same advancement of coalescence.

Sixty-six-and-one-half hour embryos subjected to thirty-five pounds of pressure. (Plates 5 and 6).

Body form. The only appreciable difference between three test specimens and control embryos is a slight advancement of the tail fold of the amnion and an accompanying more advanced rotation of the caudal portion. (See Plates 4 and 5).

Seventy-two hour embryos subjected to sixty pounds of pressure.

Body form. Four embryos were removed at this stage. These show more abnormality than any other group. Plates 6 and 7, show the two types of teratogeny encountered. Plate 1 is a camera lucida drawing of that one of the group showing most variation, while Plate 2 is typical of the other three.

All specimens are somewhat advanced in anatomical structure. For example, an average of about thirty-nine somites compares with normal embryos older than eighty-three hours. Anterior

and posterior limb-buds show advancement up to eighty hours.

Notwithstanding such structural advancement, these chicks are only about one half the length of normal ones. This condition of diminution and emaciation is partly accounted for by an excessive thoracic flexure. A lack of cephalic flexure, however, leads one to attribute the extreme smallness of these embryos to the direct effect of pressure.

Heart structures in these embryos are extremely large and well developed. In fact, when camera lucida drawings are super-imposed upon those of normal seventy-two hours hearts, they are larger. Thickness of heart wall is in all cases nearly twice that of controls.

When these embryos were first removed it was noticed that the extra embryonic structures were very poorly developed and very small. The peripheral third of the vascular network was found to be atrophied to the extent that circulation was very much hindered. Such a condition may explain a very pronounced arrythmia which was observed in the embryo pictured in Plate 7. Two specimens were without anterior amniotic structures of more than rudimentary formation.

Ninety-six-hour embryos subjected to fifty pounds of pressure.

Body form. Only two specimens of this group were removed. One was somewhat smaller than the typical ninety six hour control. In structural development it was advanced. The other specimen was dead at the eighty-fifth hour stage and showed

hemorrhage through a rupture of the left auricle. The extra-embryonic circulatory systems of both were somewhat poorly developed. Gross abnormalities of flexure and torsion were consummated in an contra-flexion in the cervical region of the living specimen. Histological observations did not reveal other malformations.

General results of subjection to pressure higher than sixty pounds.

All attempts to produce living embryos under these conditions failed. Sixty-five, eighty, and one hundred pounds invariably caused death at an early age. It is of interest however, that death occurred earlier when compression was high than when sixty-five pounds were used. With one hundred pounds death occurred at the thirty-three hour stage; with eighty, at the thirty-eighth (Plate 8); and with sixty-five, at the forty-second or forty-fourth.

DISCUSSION

In the agent pressure we have an instrument of two-fold affect on the growth of the chick embryo. Almost invariably in these experiments, a decrease in size has been accompanied by an acceleration of structural development. This fact might be considered paradoxical in view of some of the results of earlier observations. Draper and Edwards (1932) show that relatively high pressures retarded cell division in the early cleavage of Fundulus. It is reasonable to consider that mitosis might have been likewise inhibited by a change in viscosity or ionic disturbance in the chick protoplasm. However, such a change, although it might account for the general diminution of body size in embryo chicks, could not be responsible for the present acceleration which indicates that cleavage and cell division have proceeded at a faster rate than normally; however, it may be that differentiation has proceeded at an increased rate without an accompanying increase in rate of cell proliferation. It is also possible that relatively low pressures produce a lowering of viscosity, especially where heat is a factor, instead of an increase. There is no specific evidence at hand in support of this view. However the author hopes to carry on experiments in the future in order that he may reach some conclusion.

The almost complete absence of the formation of the anterior portion of the amnion in two of the seventy-two-hour embryos subjected to sixty pounds of pressure substantiates the observations of Weldon (1902). In all probability the rise of the anterior

amnion folds was mechanically inhibited by contact with the shell. The statement of $D_a$reste (Appendix 2) applies adequately in this case, for both specimens show greater degree of monstrosity than any of those in which amnion formation has not been prevented.

The fact that embryos subjected to low pressures show a relatively small developmental acceleration leads to a comparison with the large amount of acceleration with higher pressures. In fact, there is sufficient evidence to believe that acceleration is proportional to increase in pressure.

In contrast to the above, it is also true that the stunting effect is proportional to the amount of pressure. This conclusion is based upon the fact that all embryos subjected to thirty pounds, including the sixty-six-and-one-half-hour specimen, were far nearer the normal size than were those incubated under fifty and sixty pounds.

All of these considerations come to an end with the application of more than sixty pounds of pressure, for death resulted at an early age in each case of such application. Upon this evidence the author assumes a lethal pressure somewhere above sixty pounds, although an exact lethal point has not yet been satisfactorily determined. No evidence of early malformation as a fore-runner of death due to a lethal pressure has yet been observed, for none of the young embryos subjected to that pressure showed gross malformations. The writer would be more prone to assume that death was caused by a lack of oxygen somehow brought about by a change in gas tension. At any rate,

the lethal factor operates in proportion to pressure applied, for death occurred earlier when pressure was increased.

V

CONCLUSIONS

The fact that an increased pressure accelerates the morphological development of the embryo chick on the one hand and retards normal growth in size on the other cannot be explained on a basis of viscosity change of protoplasm.

There is a possibility that protoplasm may become less viscous when subjected to both pressure and heat.

A lethal pressure exists. Fowl embryos developed in a pressure just below it show a high degree of gross mal-formation.

VI

SUMMARY

Pressures up to sixty pounds cause an acceleration of structural development in the embryo chick.

This acceleration is proportional to the degree of compression.

Such acceleration is accompanied by a marked decrease in normal development of size.

Teratogenesis is brought about by pressures near the lethal point. The predominant forms of malformation are: abnormal flexure and torsion, large hearts with greatly thickened walls, failure of formation of the anterior fold of the amnion, and atrophy of the extra-embryonic circulatory system.

A pressure of sixty pounds is sufficient to prevent the rise of the anterior fold of the amnion.

Stunting is proportional to the degree of compressions.

A lethal pressure exists somewhat above sixty pounds.

VII

ACKNOWLEDGEMENTS

VIII

APPENDIX

I. Pressure as designated in these experiments is in reality a hydrostatic force because the avian egg is enclosed in a thickness of semi-liquid albumin. The air compression utilized completely surrounds the porous shell and is contacted with the albumin and other egg constituents in the form of hydrostatic pressure.

II. Dareste (1897) "J'ai signale, depuis longtemps, l'arret de development de l'amnios et les anomalies nombreuses que cet arret partiel determine chez l'embryon. C'est la cause la plus frequente des monstrouosites simples.

III. The fomol-nitric fluid used for fixing young embryos in this investigation was made according to the following formula:

    40% formaldehyde -----5 parts
    Water ----------------90 parts
    Nitric acid (807) ---- 5 parts

IV. It was found that alum cochineal diluted with one third of its volume of distilled water stained more selectively than a heavier stain followed by acid treatment.

IX

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All illustrations are camera-lucida drawings to scale (27X).

Below is 27 X 1 M. M. which can be used in comparing the various figures.

/- M.M.
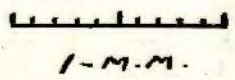
PLATE I.

Figures 1 and 2, respectively, are typical of several test and control specimens subjected to 30 pounds of pressure. Figure one shows a $25\frac{1}{2}$ hour embryo which is very nearly comparable to the $27\frac{1}{2}$ hour specimen, (Fig. 2) in structural advancement. It is somewhat smaller, however, than the control.
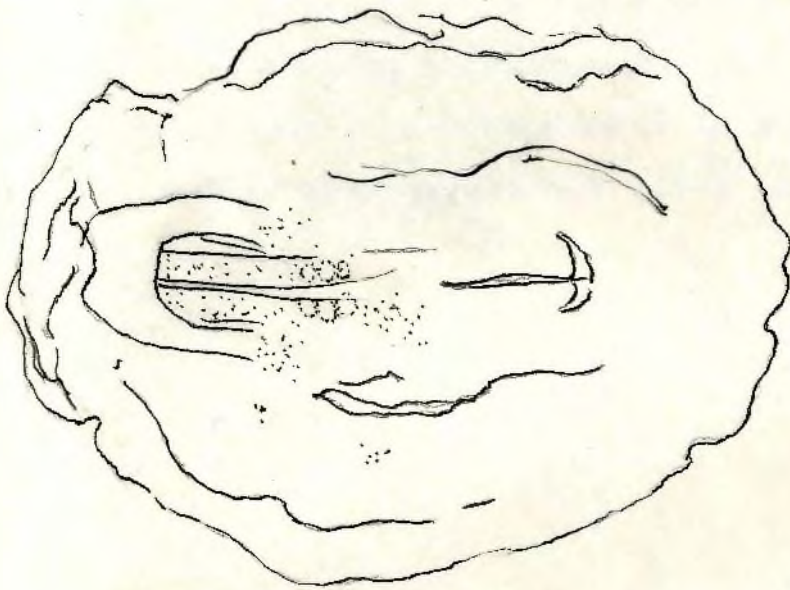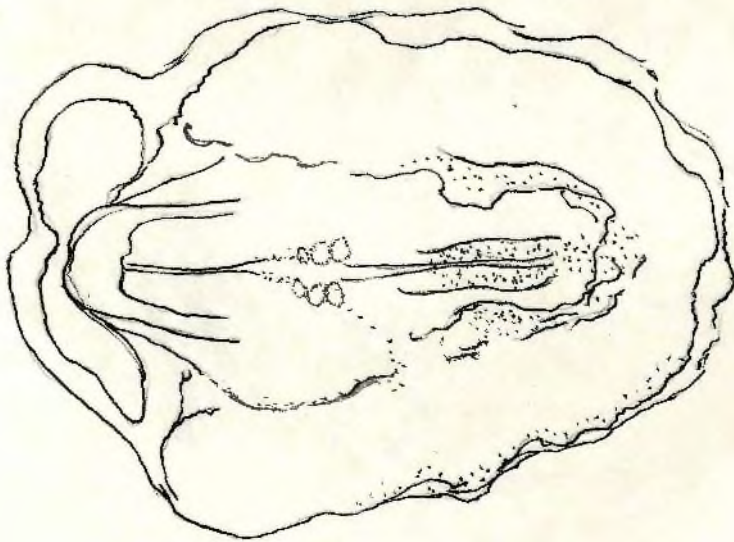
PLATE I

Fig. 1

Fig. 2

PLATES 2 AND 3

Figures 1 and 2 of Plate 3 are camera lucida drawings of typical test 47 hour specimens. They differ from the control (Plate 4) in that they show more advanced flexure and torsion, and more pronounced heart loopings. As can be determined by measuring according to the accompanying scale, the tests are smaller than the control.

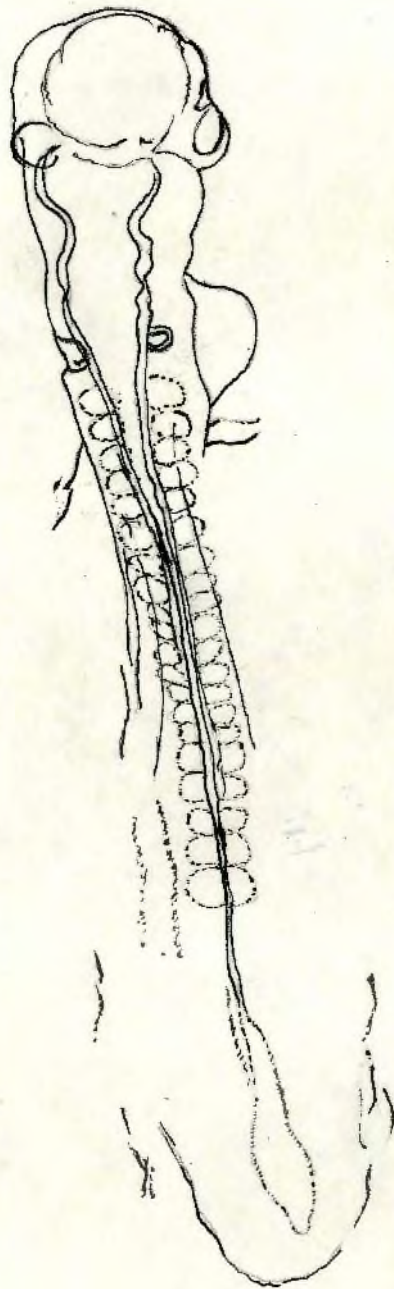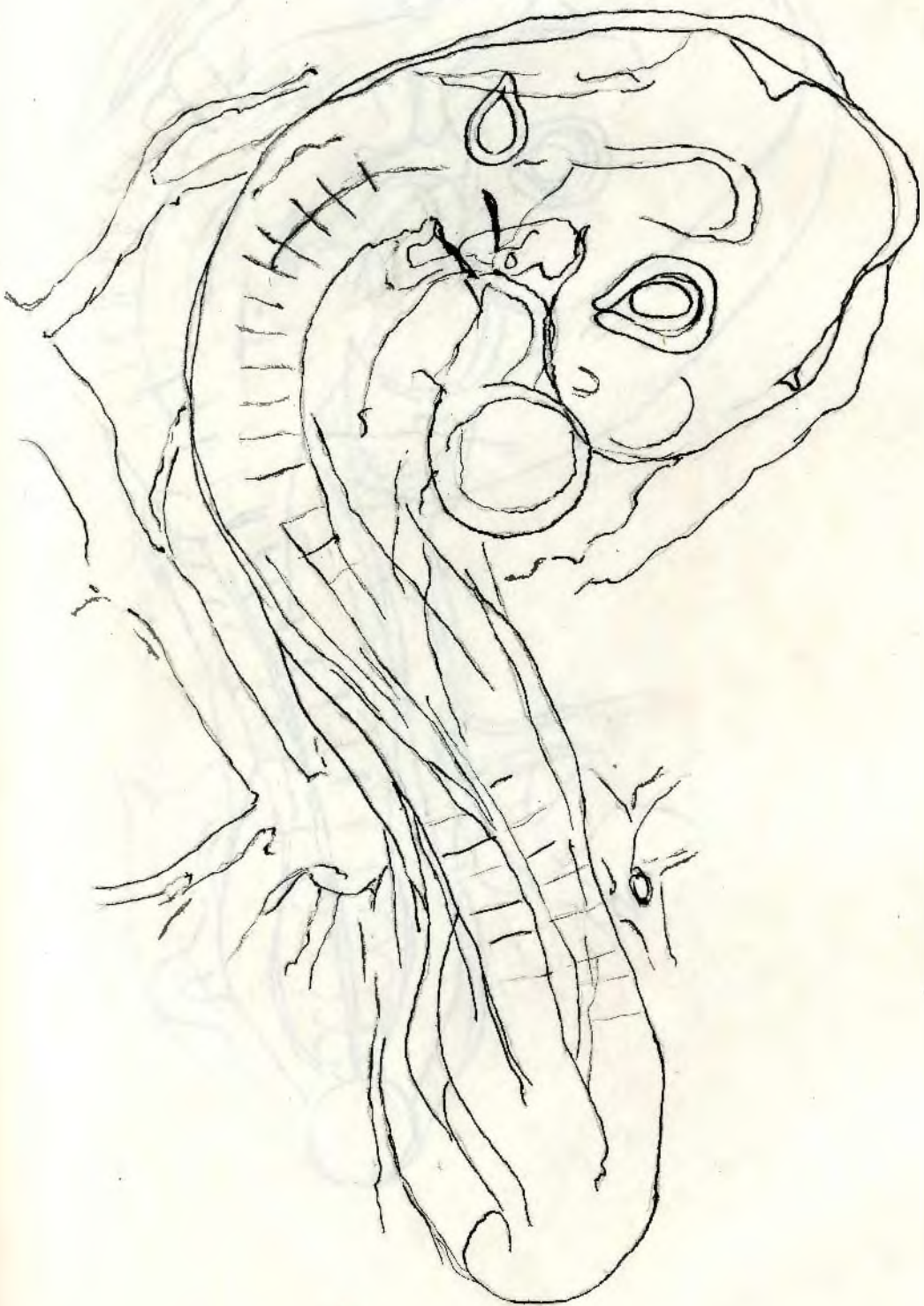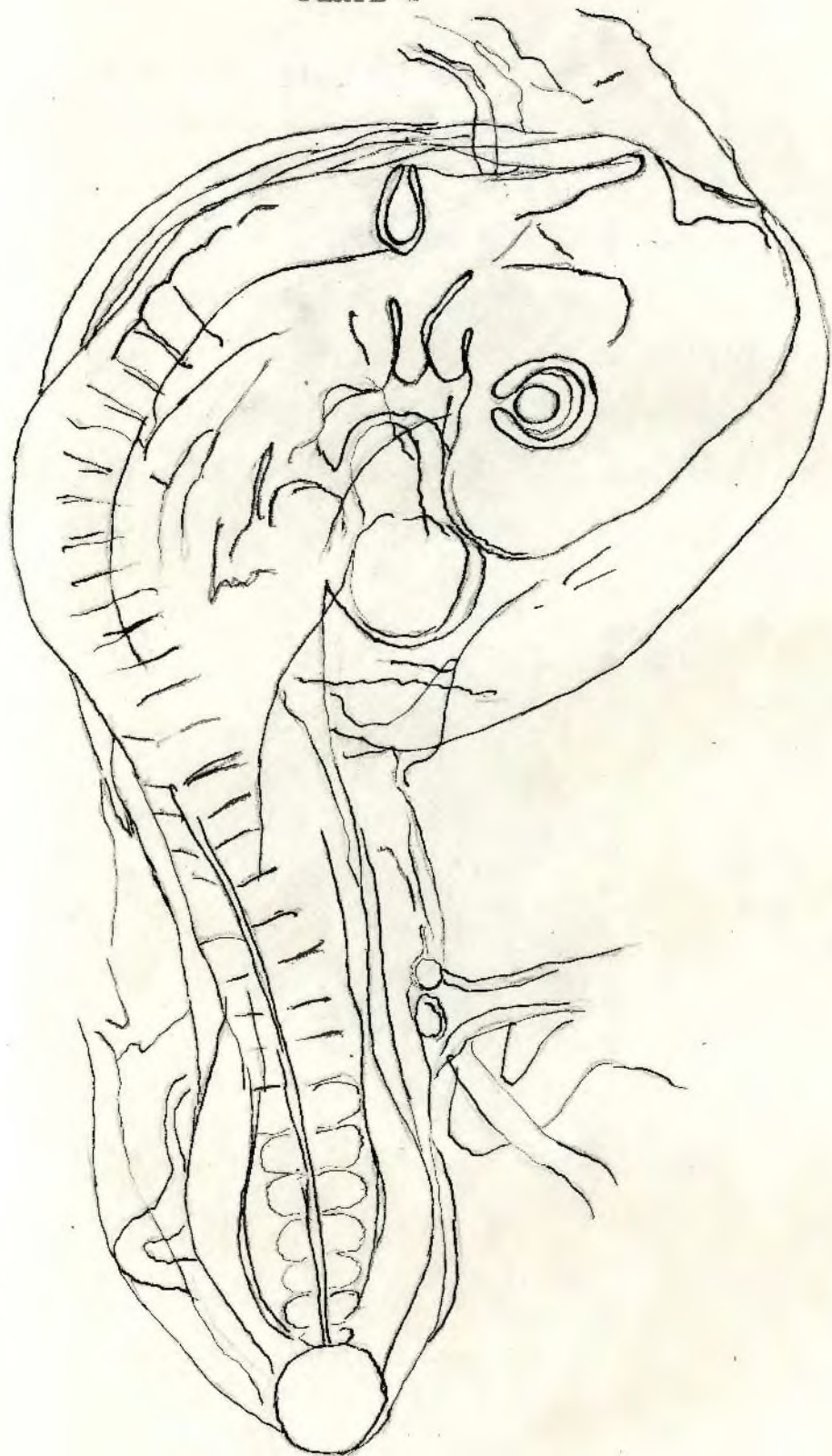PLATE 2

Fig. 1
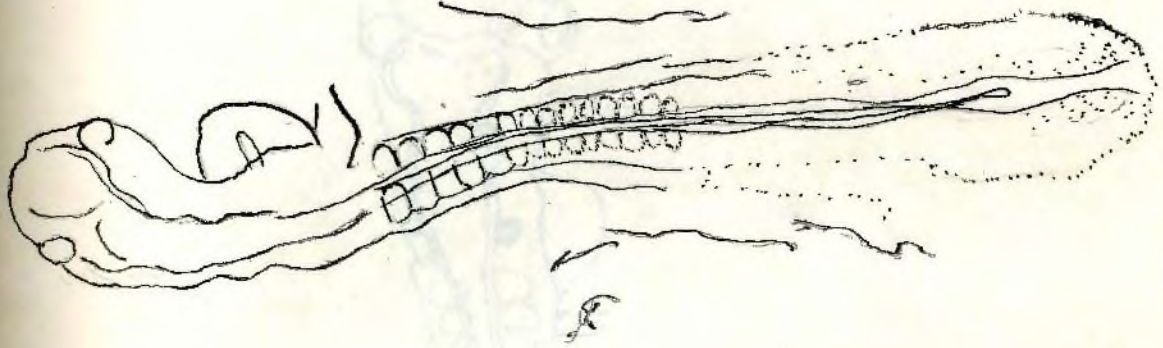
Fig. 2
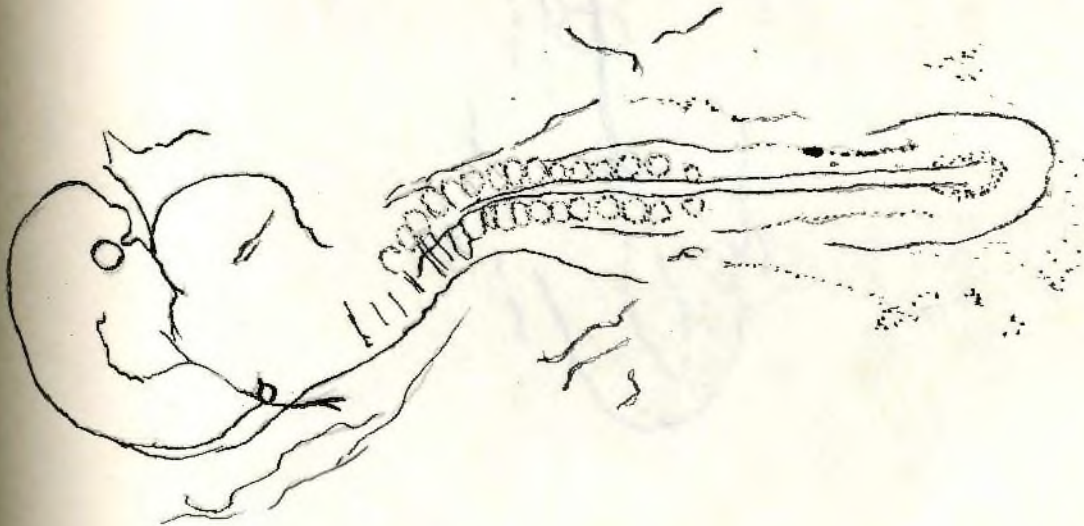
PLATE 3

PLATE 4

Plate 4 is typical of 66½ hour embryos subjected to 35 pounds of pressure. The only appreciable difference and the control (Plate 5) is a slight advancement of tail fold of amnion and caudal rotation.

PLATE 4

PLATE 5

PLATES 6 AND 7

Sixty pounds of pressure seems to be near the lethal
pressure as evidenced by the terato-logical effects shown
in Plates 6 and 7. Plate 6 shows a single specimen of
extreme abnormality, whereas, Plate 7 is typical of the other
embryos subjected to the near lethal pressure. Note the
extreme diminution of these embryos as compared the
advanced anatomical development. Extra-embryonic structures
were poorly developed. The peripheral third of the vascular
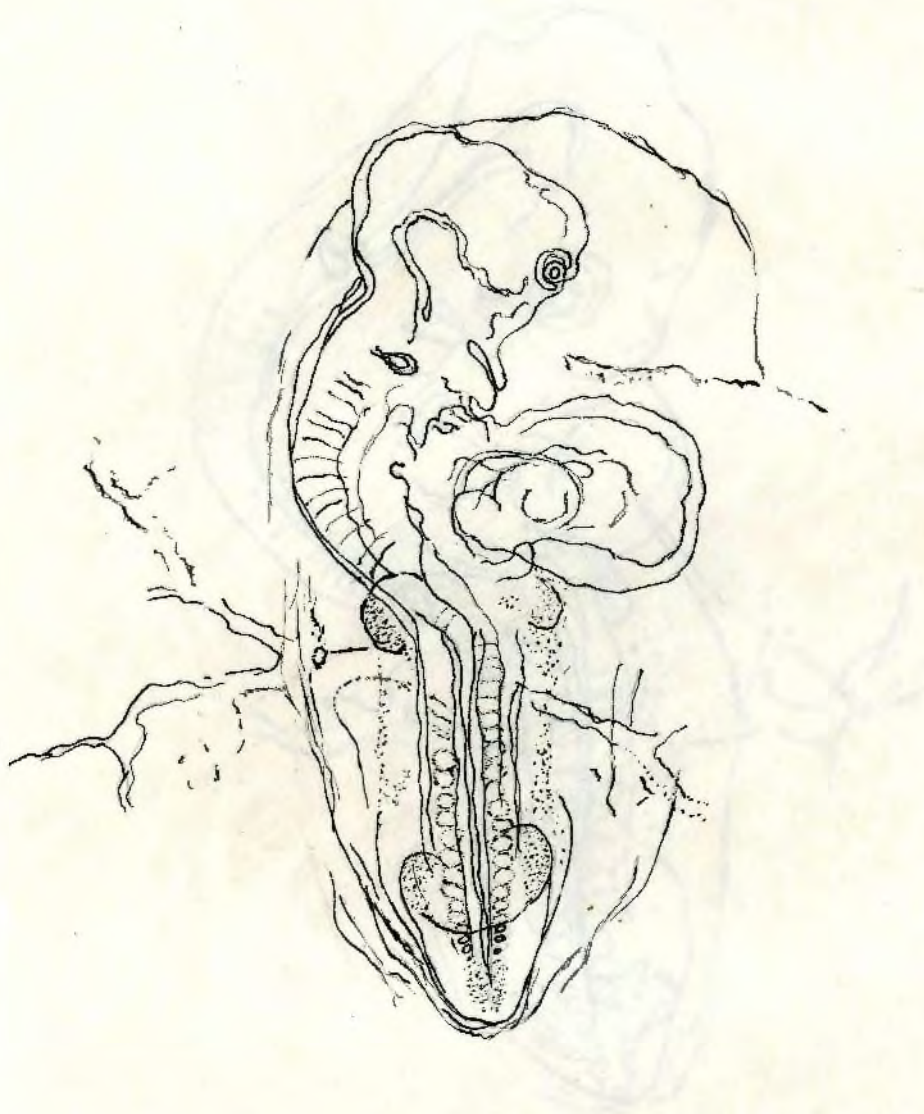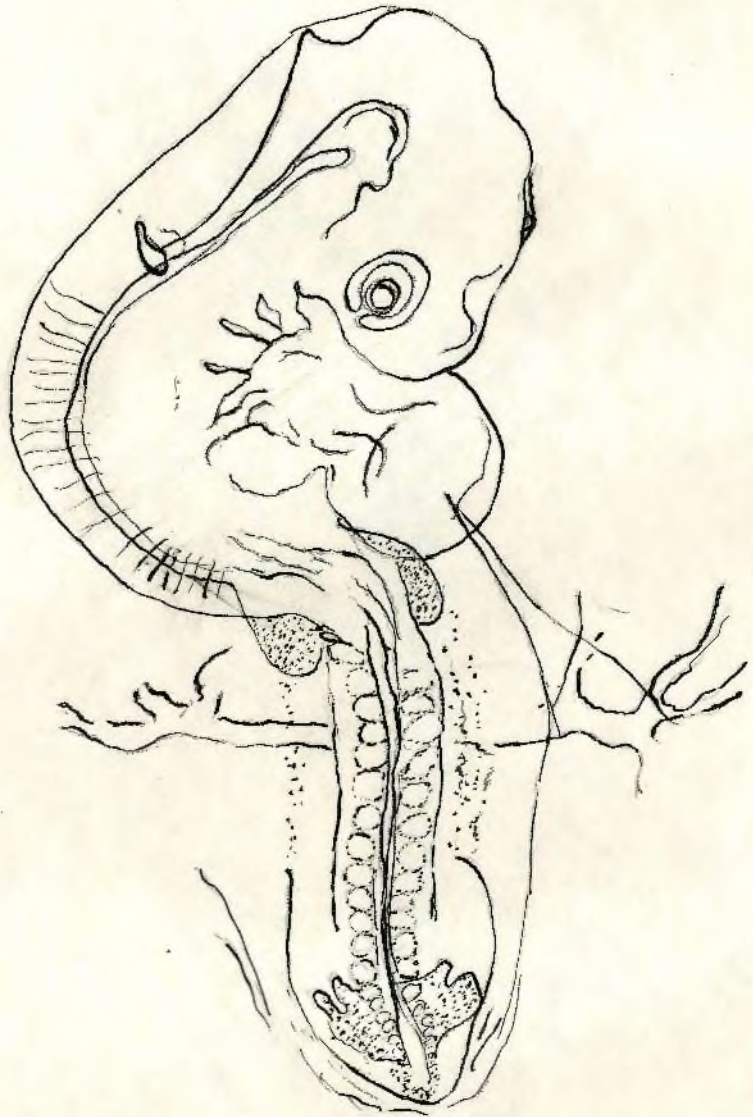network was so much atrophied that circulation was hindered.

PLATE 6

PLATE 7

PLATE 8

This embryo was dead when removed. With 80 pounds of pressure advancement did not proceed beyond the 22 somite stage. (See p. 18.)

PLATE 8