AN ANALYSIS OF DIFFERENTIATION AND REGULATION
IN THE MESENCEPHALON OF THE CHICK EMBRYO

by

WESLEY JOE BIRGE

A THESIS

submitted to

OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of

DOCTOR OF PHILOSOPHY

June 1955

APPROVED:

Associate Professor of Zoology

In Charge of Major

Chairman of Department of Zoology

Chairman of School Graduate Committee

Dean of Graduate School

Date thesis is presented *May 10, 1955*

Typed by Vivian I. Birge

ACKNOWLEDGMENTS

TABLE OF CONTENTS

AN ANALYSIS OF DIFFERENTIATION AND REGULATION
IN THE MESENCEPHALON OF THE CHICK EMBRYO

INTRODUCTION

Beginning with the turn of the century, much atten-
tion has been devoted to neural tube regulation in Am-
phibia. Many grafting and deficiency experiments have
been performed not only in an attempt to determine the
regenerative capacity of the amphibian neural tube, but
also in an effort to learn more of the developmental
nature and potentialities of the component subdivisions
thereof. Although the experimental papers relating to
these problems are too numerous to be critically re-
viewed here, some of the disclosures more pertinent to
the issue at hand will be cited.

Working with Ambystoma, Detwiler (17) showed that
following unilateral excision of the medulla during
stages 21 to 23, a gradual restitution of the excised
half may occur. In such cases the regulation was re-
ported to have resulted from an extensive proliferation
and migration of extra-ependymal cells of the intact
contralateral half of the medulla. In later studies,
Detwiler (21, 22) presented evidence to the effect that
a similar regulation occurs subsequent to unilateral

excision of the mesencephalon as well as of the brachial
region of the spinal cord in embryos of stages 20 to 21.
Here again this regulation was reported to result from
extensive proliferation and migration of cells from the
intact half. This was later observed to be augmented
by a proliferation of ependymal cells on the operated
side following the closure of the excision.

Also working with Ambystoma, Harrison (35) presented
further evidence in regard to the regenerative capacities
of the urodele neural tube. Subsequent to the removal
(during stage 14) of one lateral half of the presumptive
brain area of the neural plate, a restitution of the
deficient area was observed to result. This regulation
began as a regeneration from the opposite, intact side,
and was later augmented by a general hyperplasia. On
an average, the reconstituted half of the brain was found
to be 71 per cent of normal by the time of complete yolk
resorption.

More recently Holtzer (42) described in detail the
course of regulation following the unilateral extirpation
of spinal cord segments three, four, and five in Ambystoma.
On the basis of observations made, he showed the medullary
system to be mosaic in nature, at least in regard to the
type of tissue potentially derivable from the presumptive
plate areas. Thus the histological nature of the

reconstituted portion of the spinal cord was concluded to be dependent on the type of cells contributing to its restitution, and this in turn was found to depend upon the age of the embryo at the time of operation. It was observed that following operation prior to the very early neurula stage, the reconstituted side of the spinal cord compared favorably, even in fine structural detail, to the opposite intact side. In such instances presumptive motor, sensory, and internuncial cells were contributed from the intact half to the operated side. Through hyperplasia and further development these "stem" cells then differentiated into a comparatively normal lateral half of a spinal cord. However, progressive deficiencies in histological detail were found to occur concomitantly with increased age at operation. Such deficiencies were particularly pronounced with respect to the presumptive motor regions. For example, the motor outer mantle cells failed, at least for the most part, to contribute to the regulation when the operation was performed during the early neurula stage. Therefore, the normal derivatives of these cells were subsequently deficient or lacking.

Though the course of reparative events following hemilaterally produced deficiencies are quite well established for Ambystoma, such knowledge is somewhat

sketchy with regard to the chick. Following removal
of one half of the forebrain in 5 to 25 somite chicks,
Waddington and Cohen (64) concluded that the remaining
half could remodel itself into a complete forebrain.
Later, Spratt (63) performed similar operations on the
forebrain of 8 to 10 somite chick embryos and found
little or no reconstitution of the extirpated half.
Work now in progress by the author (unpublished) dem-
onstrates convincingly that following ablation of one
half of the forebrain in 26 to 38 hour chick embryos,
the contralateral, intact half may develop into a cer-
ebral hemisphere which is normal in size and gross
structure, and which resembles in no way a complete
forebrain, but rather one half thereof. Further details
concerning this matter are forthcoming.

In 1950, Wenger (70) published data on which she
concluded, among other things, that no regulation occurs
following hemilateral extirpation of the spinal cord in
15 to 25 somite chicks (at least under the particular
conditions imposed by her type of operation). Watterson
and Fowler (67) on the other hand showed conclusively
that distinct regulation can occur in the chick spinal
cord when the opposite sides are previously separated
during the 17 to 28 somite stage and a mesodermal block

inserted between them. They found that such restitution as did occur involved almost exclusively the alar plate derivatives. Birge and Hillemann (5) also demonstrated that regulation may occur following hemilateral alar plate reduction in the metencephalon of 28 to 38 hour chick embryos. Subsequent to such loss, an extensive compensatory hyperplasia resulted in the presumptive anterior and middle cerebellar lobe areas of the intact alar plate. Similar operations however, did not indicate the occurrence of regulation in the basal plate regions. Thus in the chick, it is clear that the alar plate areas are capable of regulating for certain hemilateral deficiencies, at least in certain areas of the early post-prosencephalic neural tube. Apparently the basal plate regions lack such a regulatory capacity at the developmental stages hitherto considered, and if such a potentiality ever exists it must be lost relatively early in development, probably by the early neural plate stage or shortly thereafter.

This paper relates to the development and regulation following the ablation of certain specific portions of the midbrain in the chick. The principal aims are these: (1) to learn whether any developmental relationships essential to normal, primary histogenesis exist between the several mesencephalic plate regions, and (2) to

determine the character or potentiality of these plates with respect to regulative and mosaic development.

MATERIALS AND METHODS

Of a total of 239 chick embryos used in this study,
144 were utilized for experimental purposes, while the
remaining 95 embryos were set aside as controls. In-
cubation temperature for both experimental and control
animals was maintained at 100 to 101 degrees Fahrenheit,
and at a relative humidity of 62 to 65 per cent. Alto-
gether, seven sets of operations were performed. Prior
to each set, three dozen White Leghorn eggs were in-
cubated for 28 to 42 hours. The embryos selected for
experimentation varied from 26 to 38 hours of development.
The fertility of the eggs used varied from 84 to 92 per
cent.

As stated previously, specific portions of the
mesencephalon of the early chick embryo were ablated by
thermal electrocoagulation. This was accomplished by
the use of microelectrodes used in combination with a
high-frequency current supplied by a Birtcher spark-gap
hyfrecator. The electrodes were fashioned after those
designed by Hillemann (38) for ablating the pituitary
rudiment in early chick embryos but with the principal
exception that straight-tipped, steel electrodes of
greater diameter (30 to 34 microns) were used. Approxi-
mately one third of the operations were performed with

the aid of the Hillemann micromanipulator (36). For the
remaining operations the microelectrode was adapted to
fasten directly in the chuck of the hyfrecator handle so
that the electrode could be manipulated manually. The
latter method made possible a free dissecting motion in
addition to the coagulating effect.

Prior to operating, an opening approximately three
eighths of an inch in diameter was made in the egg shell
and the underlying shell membranes removed to expose the
embryo. Following this, a glass needle was inserted
through the roof of the posterior portion of the prosen-
cephalon. The needle was then extended back through the
mesocoel and lifted gently, splitting the roof of the
mesencephalon along the midline. The midbrain was then
spread open to expose most favorably the intended lesion
site, yet meanwhile separating this area as far as
possible from the remainder of the mesencephalic tissue.
The electrode was then inserted into the selected region
and the tissue ablated. Following this operation, the
coagulated tissue was removed with the aid of forceps
and glass needles to provide a clean wound. The most
suitable current was found to vary between settings 50
and 100 in the low intensity range (black outlet), and
the desired extent of tissue coagulation was controlled

by the time of exposure. Exposure periods of a few
seconds duration were repeated successively until the
spread of coagulation was seen to coincide with the in-
tended extent of the lesion. All operations were carried
out under semisterile conditions.

The presumptive mesencephalic areas ablated have
been grouped into the following lesion catagories: (1)uni-
lateral alar plate ablations, (2) unilateral alar and
basal plate ablations (in whole or in part), (3) bilateral
reduction of the prospective alar plates, (4) bilateral
ablation of the basal plate regions, and (5) complete
coagulation and removal of the entire midbrain. The
overall mortality was 42 per cent.

Subsequent to operation, the opening in each egg
was either covered with a glass window and sealed with
paraffin or closed with cellophane tape. Both methods
were found to work equally well. The eggs were then
returned to the incubator for further development. Ex-
perimental embryos were later sacrificed at various stages
of development from 2 to 20 days. Of the control embryos,
five were sacrificed daily from 2 to 20 days. In each
group the midbrain was sectioned in two animals while the
other three were preserved and the brains used for gross
comparisons. Both experimental and control embryos were

fixed in Bouin's solution, sectioning serially at 10 to 15 microns, and stained in Harris' hematoxylin and eosin-Y.

Following hemilateral ablations of the mesencephalic alar plates, a compensatory regulation in the form of a hyperplasia was found to occur. In establishing this to be a hyperplastic condition, cell counts were made in sample areas of the intact alar plate. These values were then contrasted with those gained from counts made in corresponding areas of control embryos in an attempt to compare cell density. In determining the extent of alar plate hyperplasia, tracings of the region in question were made (at a standard magnification of 18 X) of every 11th section in each slide series. The square area of each tracing was then determined with the aid of a polar planimeter. With this information together with the number and thickness of the sections in each series, it was possible to determine the approximate volume (in cubic microns) of the hyperplastic tissue. Such values were then compared with figures (derived in an identical manner) for similar areas in normal animals of corresponding age.

OBSERVATIONS

For ease in discussion, the observations are presented under five subheadings conforming to the lesion catagories mentioned under the section dealing with materials and methods.

Hemilateral alar plate ablations. As an initial step in analyzing any relationships which might exist among the several mesencephalic plates in their growth and differentiation, and in determining the capacity for regulation in the midbrain, the right alar plate was completely removed in a number of embryos. Except for the loss of the right alar plate and varying degrees of destruction to the roof plate, the remainder of the midbrain was left intact.

A total of 79 embryos survived this operation and were sacrificed at 2 to 20 days of development (Table 1). Histological examinations revealed that the development and differentiation of both right and left basal plates occurred in a completely normal manner in every instance (plate 3, figures 9, 10, 11). Moreover, these findings were verified also in the case of the floor plate. Thus the future motor and coordinative areas of the midbrain were found to be relatively unaffected by the loss of the

right alar plate. This holds true with respect to both their growth and differentiation, and to their rate of development.

The fate of the intact left alar plate subsequent to such operations was found to be in sharp contrast to that described for the underlying basal and floor plate areas. This alar plate region regularly underwent an extensive compensatory regulation, leading eventually to the formation of an optic lobe with a tectum comparable in size to the total tectal area of two normal optic lobes. The course of this compensatory regulation will be traced through developmental stages of 2 to 20 days (Table 1).

In each animal sectioned on day two, the lesion area was found to be entirely clear of coagulated tissue. The neuroepithelium of the intact dorso-lateral area (left presumptive alar plate together with approximately one half of the prospective roof plate) was flexed somewhat ventrally in the primary phase of closing the lesion area, though the lesion was seldom found to be closed over by more than one third. Little or no definite regulation could be substantiated on the basis of volumetric comparisons between normal and experimental material. Histological studies on the other hand, indicated that regeneration was in an initial phase at the medial margin of the intact dorso-lateral plate where mitotic figures were found to be

somewhat more numerous than in other areas. In these two-day embryos, as well as in all older embryos having open lesion areas, the head mesenchyme condensed up to the outer margin of the lesion, but extra-neural tissue was never seen to push into the open mesocoel with but few exceptions. In these rare instances, neighboring blood vessels had ruptured and numerous blood cells were found within the lumen of the neural tube.

By the third day definite regulative development was demonstrable by volumetric methods. Measurements on four animals revealed an expansion in the intact dorso-lateral region which on an average, attained a volume 55 per cent as large as that normally produced by both alar plates (together with the roof plate). This remaining alar plate region could under normal circumstances at least, be expected to constitute a mass equal to one half of the combined alar plate area of a normal embryo, but the additional volume increase amounted to a mass that was 10 per cent larger than the normal area of one dorso-lateral plate. Thus the average extent of mass regulation based on measurements in these four embryos was 10 per cent. For the overall range of regulation in this group refer to Table 2. Aside from the increased regulation, the condition of the midbrain at three days was essentially the same as that for the two-day animals, with the exception that the lesion had closed by one third to one half.

TABLE 1. CLASSIFICATION OF EXPERIMENTAL
ANIMALS IN ACCORDANCE WITH
LESION TYPES

MESENCEPHALIC TISSUE ABLATED	EMBRYONIC AGE AT SACRIFICE																
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	18	20
right alar plate	6	5	4	5	6	4	5	6	7	1	6	2	5	2	6	4	5
right alar and right basal plates complete	2	2	1		1		3		3		2		3		2	2	
partial		2	1	1		1	2		1		1		3			2	
right and left alar plates				1		1	2	1	1		2				2	2	
right and left basal plates				1	2		1	1			1	1					
total midbrain ablation				3	1		2	1	1		3						

The extent of regulation averaged 28 per cent by the fourth day of development, and the closing of the lesion ranged from one half to three fourths or slightly more at this time (plate 1, figure 1).

In about one half of the specimens the margin of the presumptive tectum bordering the lesion was neither even nor distinct as in the other embryos; instead, fine fibrils from the glia extended out into the lesion area. These fibrils appeared to have followed along the inner, membraneous-like surface of the condensed head mesenchyme bordering the external margin of the lesion opening. Furthermore, it seemed as though cells from the adjacent neuroepithelium had migrated along the course of these fibrils. Such occurrences, though not consistently found, might possibly afford an example of contact guidance (68). In cross-section, the regulating presumptive optic tectum (of left alar plate origin) extended well across the midline where it flexed toward the lateral margin of the right basal plate. Except for a reduction in size and a persisting lesion opening, the prospective tectum had arched itself over the subjacent basal plates in a relatively typical manner.

TABLE 2.   REGULATORY RATES IN THE INTACT ALAR PLATE
FOLLOWING HEMILATERAL ALAR PLATE ABLATION

AGE OF EMBRYOS IN DAYS	NO. OF EMBRYOS*	OVERALL RANGE OF REGULATION EXTENT	AVERAGE EXTENT OF REGULATION	PER CENT SIZE OF REGULATED LOBE COMPARED WITH COMBINED TECTAL AREA OF NORMAL
2	8 -	——	——	——
3	7 (4)	8 - 16%	10%	55%
4	5 (4)	18 - 38%	28%	64%
5	5 (4)	35 - 52%	44%	72%
6	7 (4)	47 - 68%	54%	77%
7	4 (4)	60 - 72%	70%	85%
8	8 (4)	102 - 119%	128%	114%
9	6 (4)	111 - 127%	118%	109%
10	10 (4)	75 - 93%	86%	93%
11	1 -	——	——	——
12	8 (4)	76 - 123%	98%	99%
13	2 -	——	——	——
14	8 (4)	96 - 109%	102%	101%
15	2 -	——	——	——
16	8 (4)	97 - 104%	100%	100%
18	6 (4)	97 - 112%	106%	103%
20	5 (2)	91 - 101%	96%	98%

* The number of embryos in each age group used
for the volumetric studies is given in parenthesis.

That portion of the presumptive tectum to the left
of the midline presented a normal differentiation into
marginal and mantle layers by the fourth day. On the
right side, relatively normal differentiation was found
in the medial one half to two thirds of the tectum but
the lateral one third to one half narrowed progressively
to about one half or less of the normal thickness at
the lateral margin (adjacent to the lesion opening).
In this lateral portion of the prospective tectum,
distinct marginal and mantle layers were usually lacking,
in which case the tissue resembled a typical neuroepithe-
lium.

An analysis of animals sacrificed on day five
revealed an increased rate of regulation which averaged
44 per cent (Table 2). As in the four-day embryos, the
tectum presented normal differentiation on the left side
but a retarded development in the lateral portion of the
right side. Here again an opening between the right
margin of the tectum and the lateral margin of the right
basal plate usually persisted, though this gap was some-
what diminished in size in comparison with the four-day
stage.

Observations made on day six and seven embryos showed
a progressive increase in regulatory rate; this averaged
54 per cent and 70 per cent respectively. During this

period the lesion opening was generally closed by a
fusion of the right margin of the optic tectum with the
lateral margin of the underlying basal plate. Though
differentiation was again retarded by one to two days
in the extreme right portion of the tectum, marginal and
mantle layers were always presented (plate 1, figure 2;
plate 2, figures 5 and 6).

By the eighth and ninth days, a conspicious increase
in the rate of regulation had occurred. This averaged
128% on day 8 and 118% on day 9 (plate 2, figures 7 and
8; plate 3, figure 9). Thus the optic tectum in these
embryos constituted a mass larger than the combined
tectal area of normal embryos of comparable age (Table 2).

In the early stages of development (three to seven
days), the regulation consisted essentially of a
regeneration of the neuroepithelium in such a manner as
to close the lesion. However, beginning on the seventh
and eighth days, this regeneration was greatly augmented
by a general hyperplasia within the developing optic
tectum. This hyperplasia is cited as being chiefly
responsible for the extensive regulation occurring in
the older stages (7 to 20 days). The compensatory
volumetric increase affected all portions of the tectum
evenly, except for the non-cellular stratum opticum. In

embryos sacrificed on days 10 to 20 of development, the rate of regulation averaged close to 100% (plate 3, figures 10 and 11).

In summarizing these observations, it may be stated that following the removal of the right presumptive alar plate in the 26 to 38 hour chick embryo, the remaining (intact) prospective alar plate undergoes a course of compensatory regulation which eventually leads to the formation of an optic tectum comparable in size to the combined tectal area of normal embryos of corresponding age. Although this optic lobe expands over and onto the right side of the brain, bilaterality was never attained to such an extent that two optic lobes were distinguishable. Instead, the optic tectum was broadly rounded with never any appearance of a roof plate, even though the presumptive anlage of this plate was most always left partially or wholly intact at the time of the operation. With but two exceptions, the tissue of the hyperplastic optic lobes in eight day to twenty day embryos was normal in arrangement. First, the developmental retardation noted in the extreme right portion of the tectal area in the early stages (three to seven days) was present to a greater or lesser extent up to 12 to 15

days of development. In 18-day and 20-day animals this area was essentially normal. The second exception concerns the distribution of the optic fibers of the right optic tract. The distribution of these fibers can be grouped as follows: (1) stratum opticum on the right side of the optic tectum, (2) deep lying tissues on the left side of the tectum, and (3) irregular dispersal in the right basal plate. The distribution of these fibers usually involved at least two and often all three of the areas designated above. In most cases, at least some of the fibers were distributed to the stratum opticum. This latter area was usually thinner on the right side of the tectum than on the left, due to the partial distribution of the fibers of the right optic tract to other areas. Those fibers distributed to the right basal plate were often seen to have converged on developing nuclei in the vicinity.

Hemilateral alar and basal plate ablations. Thirty-five embryos having undergone this particular type of operation, were sacrificed on 2 to 18 days (Table 1). Of these, 21 had suffered complete removal of the right alar and right basal plates (plate 1, figures 1, 2, 3; plate 2, figures 5 and 6). In these embryos the opposite

(intact) alar plate followed a course of regulative development identical to that discussed above except that the right margin of the optic tectum fused with the dorso-medial margin of the left basal plate.

The fate of the intact basal plate area was found to be in distinct contrast to that described for the intact alar plate following hemilateral ablation. No distinguishable regulation was detectable in the intact basal plate following such experiments. In fact, the structure and development of this region, subsequent to the removal of the right alar and basal plates, was entirely normal as far as could be determined. Thus the absence of the right basal plate region had no noticeable effect on the primary growth and differentiation of the remaining portions of the midbrain within the period studied. In addition, no regeneration of the basal plate tissue removed, was observed to occur.

In some 14 animals fixed on 3 to 18 days, various portions of the right alar and basal plates had been previously ablated. Here again the ensuing results differ with respect to these different plates. In the basal plate region, the deficiencies proved to be permanent without regeneration or regulation of any sort occurring. The unablated portions of the right basal

plate, as well as the opposite, intact basal plate region, developed normally and at a normal rate (plate 2, figures 7 and 8). In several cases, portions of the anlage for the right oculomotor nerve were destroyed in part. The remainder differentiated in a typical fashion forming an oculomotor nerve reduced in proportion to the extent of destruction to the primordium.

Following partial alar plate reduction, the remnant portions always differentiated into tectal tissue. Also, varying degrees of regulation were observed. Though a volumetric analysis of the extent of restitution was not attempted in any such case, certain general observations may be worthy of note. The extent of regulation varied roughly in proportion to the size of the ablation, being greater in extent following large lesions than following smaller lesions. Also, no conspicious hyperplasia developed in the contralateral (intact) alar plate unless considerable portions of the right alar plate had previously suffered reduction. One further noteshould be added. The tendency for regulation to occur appeared significantly greater when portions were removed from the lateral border of the presumptive alar plate (leaving at least some tissue intact medially) than when the plate was entirely ablated for a certain distance along its longitudinal axis.

Bilateral destruction of the alar plates. The
results of these experiments were seen in 12 embryos
sacrificed on days 5 to 18 (Table 1). Following operation,
the basal plate regions fused together at their dorso-
medial margins. This fusion was usually accomplished by
the fifth or sixth day. Except for this, the basal
plates displayed typical gross histology, and evidently
were relatively unaffected in their development by the
absence of the overlying alar plates (plate 3, figure 12).

Bilateral removal of the basal plates. This
particular type of operation proved to be the most
difficult to achieve. It was never accomplished without
the partial reduction of the anterior portions of the
overlying alar plate areas. However, following such
operations in seven embryos, the alar plate remnants
were always observed to differentiate into tectal tissue.
This tissue usually rounded up to form a distinct lobe,
the ventral margins of which were fused to the anterior
border of the metencephalic basal plates. Thus the
absence of the mesencephalic basal plates appeared to
have little or no effect on the primary histogenesis
of the overlying tissue within the period studied
(Table 1).

Total reduction of the midbrain. Studies made on
11 embryos in which the complete mesencephalon had been
previously ablated at 26 to 38 hours of development,
revealed no apparent affect on the early histogenesis
of either the forebrain or the metencephalon, except
that both regions healed over to close the opening next
to the lesion area. The cerebellum, metencephalic basal
and floor plates, and the forebrain structures of all
the animals studied revealed essentially typical gross
form, size, and histology (plate 1, figure 4). No
attempt was made to analyze the effect of such a deficiency
on the fiber tracts and nuclear patterns in the older
embryos.

DISCUSSIONS AND CONCLUSIONS

On the basis of observations made subsequent to
hemilateral ablation of the right presumptive alar
plate in the midbrain of 26 to 38 hour chick embryos,
it may be concluded that the remaining (intact) alar
plate region possesses the potentiality to undergo a
course of regulation leading to the establishment of an
enlarged optic lobe with a tectum closely comparable
in size to the combined tectal area of normal stages.
In addition, the detailed histology of the tectum in
such experimental animals was found to be normal except
for a slight reduction in the thickness of the stratum
opticum on the operated side. In no case did the intact
alar plate region regulate for basal plate deficiencies.
It is thus evident that even though the presumptive
mesencephalic alar plate areas during these develop-
mental stages (26 to 38 hours) are potentially capable
of producing tissue in considerable excess of the volume
normally formed, yet they can give rise only to the
kinds of tissues which typically differentiate from the
alar plates, at least under the conditions of this
experiment. In other words, while such alar plate areas
are determinate with respect to the quality of dif-
ferentiation, they are less limited with respect to the

extent or quantity of development.

The initial phase of regulation occurring in the intact mesencephalic alar plate, begins on the second or third day of development as a regeneration stemming from the neuroepithelium along the medial border of the intact (left) dorsolateral area. Such regeneration results in the closure of the wound by day six or seven, and this regenerative process is greatly augmented beginning on days seven to eight by a general hyperplasia occurring throughout the tectal area. On the eighth and ninth days of development the tectal areas of the experimental embryos at least equal and usually exceed that of the combined tectal area of corresponding normals, and from days 10 to 20 the tectal area of such embryos compares closely with that of normal stages.

The course of this compensatory regulation differs in detail from that found by Birge and Hillemann (5) to occur subsequent to hemilateral alar plate ablation in the chick metencephalon. In the latter case, no regulation on the part of the intact alar plate was found to occur until after day nine with but one exception. There was a slow regeneration of a simple columnar epithelium which usually closed the lesion opening with a curtain-like regenerate by the 10th or 12th day. This structure greatly resembled the lamina

epithelialis of the roof of the fourth ventricle, and
was always seen to be continuous with the ependyma in
the cases studied. Subsequent to day nine, an extensive
hyperplasia was found to develop in the anterior and
middle cerebellar lobe areas.

It appears likely that the difference in the onset
of regulation in these two brain regions may stem from
basic differences in the normal growth rate of the alar
plates of the midbrain and metencephalon. Up to day
nine, the normal growth rate in the metencephalic alar
plates is much less than that for corresponding areas
in the midbrain. Subsequent to this stage however, a
rapid growth phase normally begins in the metencephalon.
This growth wave starts anteriorly in the alar plates
and spreads cephalo-caudally throughout the anterior
and middle cerebellar lobes and finally reaches the
posterior lobe area. The course of hyperplasia was
found to follow this growth wave very closely, though
little or no regulation occurred in the posterior
cerebellar lobe. This absence of posterior lobe
regulation may possibly be explained on the basis of
both the delayed acceleration of growth characteristic
of this region, and the somewhat slower rate of growth
lasting for several days thereafter. As indicated above,

the growth rate throughout early development and up to
day nine is much higher in the mesencephalic alar plates
than in the corresponding areas of the metencephalon. It
is possible that the earlier onset of regulation in the
midbrain is explainable on this basis. In conclusion,
it would appear that the onset and course of alar plate
regulation in the chick midbrain and metencephalon is
closely related to periods of general growth acceleration
which normally occur.

Though only gross observations were made on remnant
portions of the alar plate area subsequent to fractional
hemilateral ablations, certain tentative conclusions may
be drawn. In the first place, the extent of regulation
for such remnant areas varies roughly in proportion to
the extent of tissue lost, and it appears that no
conspicious hyperplasia occurs in the intact contra-
lateral alar plate unless considerable portions are
removed from the alar plate area of the operated side.
Pending further investigation, it may be suggested that
there is more of a tendency for extensive regulation to
occur in response to the removal of lateral portions
of an alar plate than when this plate is completely
ablated over a part of its longitudinal extent.

With respect to the fate of the remaining mesen-
cephalic basal plate following hemilateral basal plate
reduction, the data presented under the section dealing
with observations are consistent and of one accord. In
such cases the intact basal plate developed unaffected
by this loss, and no distinct or substantial basal plate
regulation was distinguishable. Furthermore, following
the partial reduction of a basal plate, corresponding
localized deficiencies were always found while the
uninjured tissue on the other hand, presented essentially
normal composition. It is then quite clear that the
mesencephalic basal plates of 26 to 38 hour chick embryos
represent a mosaic system in which the material potentially
derivable therefrom is distinctly limited with respect
to both the kind of tissue and its total volume.

Under observations, brief mention was made in
regard to the varying extent of damage suffered by the
presumptive roof plate during the electro-thermal
ablation of the right presumptive alar plate region.
In some cases the entire roof plate area was left intact
while in others it was almost completely destroyed, but
in most instances the extent of destruction varied between
these extremes. However, no roof plate tissue was ever
found to differentiate subsequent to such operations. On
this basis it is suggested that the presumptive mesen-
cephalic roof plate in the 26 to 38 hour chick is not a

mosaic system since it may form tectal tissue character-
istic of the alar plate areas. Thus it appears that the
roof plate region is the least determinate of any of the
mesencephalic plates. Even so, there was no evidence
that this area could differentiate into basal plate tissue
since deficiencies in the latter were never reconstituted
to any detectable extent.

The course of mesencephalic regulation described
in this paper is in general accord with the findings of
Watterson and Fowler (67), Fowler (24), and Birge and
Hillemann (5). Watterson and Fowler convincingly
demonstrated regulatory potentiality in the brachial
spinal cord of the chick. They found that subsequent
to the separation of the lateral halves of the brachial
spinal cord by mesodermal blocks during the 17 to 28
somite stages, high degrees of hyperplasia developed in
the alar plate regions in 11 of the 17 cases studied.
However, little or no distinct regulation was found in
the basal plate areas. Fowler (24) presented similar
results in connection with his study of spina bifida in
the chick, and while he noted extensive alar plate
regulation in the brachial spinal cord, he found little
or no distinct regulation affecting the basal plates.
Wenger (70), on the basis of hemilateral extirpation

experiments, concluded that the presumptive basal plate
regions of the brachial spinal cord in the 15 to 25
somite chick constitute a rigid mosaic system. However,
she failed to find any regulation relating to the intact
alar plate areas in any of the four embryos studied.
Even though the occurrence of alar plate regulation in
the chick brachial spinal cord has not been consistently
reported, the weight of the evidence at hand indicates
that this area at least possesses such potentialities,
and that under proper experimental conditions, it tends
to undergo regulation more often than not. Following
hemilateral ablations in the metencephalon of 28 to 38
hour chicks, Birge and Hillemann (5) demonstrated the
consistent development of high degrees of hyperplasia
in the intact alar plate, but failed to detect any
regulation on the part of the intact basal plate area.

Regarding the subject of regulation in the neural
tube of the chick, certain general conclusions can now
be presented on the basis of existing information.
These general conclusions are made with respect to the
brachial spinal cord, metencephalon and mesencephalon
of the early chick embryo (four to twenty-eight somites)
and probably also hold true for the remainder of the

post-prosencephalic neural tube. These conclusions
are as follows: (1) one presumptive alar plate is
potentially capable of extensive alar plate regulation
when separated from the opposite alar plate region;
(2) the tissue produced as a result of such regulation
may be as much as twice the volume normally derived from
one alar plate, but histologically always characteristic
of the tissue of the dorso-lateral sensory area; (3) the
basal plates under similar experimental conditions
exhibit no appreciable regulation, but behave on the
order of a relatively rigid mosaic system; (4) it is
tentatively suggested that the roof plate area is the
least specialized of all the plates from the standpoint
of differentiation potential since it can under certain
conditions develop into tissue characteristic of the alar
plate regions.

In comparing neural tube regulation in the chick
and in Ambystoma, the most essential difference concerns
the basal regions. In early Ambystoma embryos (prior
to early neurula stage) Holtzer (42) among others, has
shown that one presumptive basal plate is potentially
capable of reconstituting the opposite basal plate
following hemilateral excision. However, basal plate
regulation in Ambystoma decreases markedly in extent

concomitantly with increased age at operation. Though
the fact that distinct basal plate regulation has not
been demonstrated in the chick may possibly be ascribed
to basic differences between these presumptive ventro-
lateral motor areas in the chick and in Ambystoma, a
more acceptable explanation can probably be found in
considering the correlation existing between the age
of operation and the extent of regulation which is
known to exist in Ambystoma. It is quite possible that
the presumptive basal plate regions in the chick embryo
are potentially capable of regulative growth at some
time during early development (probably during the
neural plate stage). Thus it is quite possible that
the potentialities for neural tube regulation in the
chick and in Ambystoma are quite similar.

As mentioned earlier, a part of this study was
directed toward ascertaining whether or not any develop-
mental relationships exist among the several mesen-
cephalic plates of the young chick embryo (26 to 38
hours), essential to their normal differentiation. On
the basis of analyses presented under observations, and
made subsequent to the different hemilateral and bilateral
lesion types, certain conclusions become evident. By
26 to 38 hours of development, a mesencephalic basal

plate is potentially capable of undergoing primary
growth and differentiation in the absence of either the
opposite basal plate or the overlying alar plate regions.
Thus it may be concluded that by 26 hours of develop-
ment, no relationships essential to primary basal plate
development exist either between the two basal plates
themselves, or between the basal plate areas and the
overlying alar plates. Basal plate regions which
developed under these experimental conditions were
at least grossly normal in their histological structure.
Likewise, it appears that the alar plate areas possess
the potentiality to undergo typical histogenesis
independently of the basal plate regions. However, a
certain inter-alar plate association may exist under
normal circumstances, since following hemilateral alar
plate ablation, the intact dorso-lateral sensory area
undergoes a course of regulative development. Apparently
this association is one which pertains to the extent of
tissue production, but not to the type of tissue produced.
These conclusions are in close agreement with those of
Birge and Hillemann (5) regarding the chick metencephalon.

As previously stated, it was found that subsequent
to the complete ablation of the midbrain, the remaining
brain regions displayed relatively normal development

within the period studied. These findings indicate that
the mesencephalon exerts no appreciable morphogenetic
effect on the development of the other brain regions, at
least not after 26 hours of development. These findings
are likewise in accord with those concerning the chick
metencephalon (5). Similarly, Detwiler (18) working
with Ambystoma concluded that the forebrain exerts no
morphogenetic influence on the development of the
medulla.

SUMMARY

1. In 144 chick embryos ranging from 26 to 38 hours of development, either the presumptive mesencephalic plate regions were ablated individually or in various combinations, or the entire midbrain was destroyed. Subsequent to such operations the embryos were sacrificed at developmental stages varying from 2 to 20 days, and the brains were removed and histologically analyzed.

2. On the basis of observations made subsequent to hemilateral removal of the right mesencephalic alar plate area, it is concluded that the remaining (intact) alar plate region possesses the potentiality to undergo a course of extensive regulation.

3. The initial phase of this regulative development begins on days two to three as a regeneration stemming from the neuroepithelium along the medial border of the intact alar plate area. This regeneration accomplishes the closure of the wound by day six or seven, and on days seven to eight it is greatly augmented by a general hyperplasia which occurs throughout the regulating tectal area. By the eighth and ninth days of development, the regulating tectal area reaches on

an average a size larger than the combined tectal area
of a normal embryo, and from 10 to 20 days of develop-
ment the size of such regulating optic lobes compares
favorably with the combined tectal area of normal
animals.

4. Following hemilateral basal plate ablation,
the intact basal plate area followed a normal course
of differentiation and exhibited a normal growth rate.
Since no basal plate regulation was found to occur, it
may be concluded that the basal plate regions in the
26 to 38 hour chick embryo represent a rather rigid
mosaic system.

5. The roof plate area was found to be the least
determinant of the mesencephalic plates during this
period of development (26 to 38 hours). While the
basal plates lack the capacity for regulative develop-
ment during this period, and while the alar plates are
capable of regulating for alar plate deficiencies only,
the roof plate can take part in alar plate regulation
and thus give rise to a type of tissue other than that
normally produced.

6. In the discussion, certain general conclusions
are considered which relate to regulation in the post-
prosencephalic neural tube of the chick, and a comparison

is made regarding neural tube regulation in the chick
and in _Ambystoma_.

7. It is also evident that by 26 hours of develop-
ment, no relationships essential to primary basal plate
development exist either between the two basal plates
themselves, or between the basal plate areas and the
overlying alar plates. Similarly it appears that the
alar plate areas possess the potentiality to undergo
typical histogenesis independently of the basal plate
regions. However, a certain degree of inter-alar plate
association relating to the extent of tissue production
appears evident on the basis of the course of regulative
development occurring in the alar plate region subsequent
to hemilateral ablation.

8. Following the complete destruction of the mid-
brain, the remaining brain regions developed normally
(within the period studied). Therefore the mesen-
cephalon exerts no appreciable morphogenetic effect on
the primary development and differentiation of the
remaining brain regions, at least not after 26 hours
of development.

BIBLIOGRAPHY

1. Barron, Donald H. Observations on the early dif-
   ferentiation of the motor neuroblasts in the
   spinal cord of the chick. Journal of
   comparative neurology 85:149-168. 1946.

2. Bell, E. T. Experimentelle Untersuchung über die
   Entwicklung des Auges bei Froschembryonen.
   Archiv für mikroskopische Anatomie und
   Entwicklungsmechanik 68:279-296. 1906.

3. Bell, E. T. Experimental studies on the development
   of the eye and the nasal cavities in frog
   embryos. Anatomischer Anzeiger 29:185-194.
   1906.

4. Bell, E. T. Some experiments on the development of
   the eye and the nasal organ in frog embryos.
   Wilhelm Roux' Archiv für Entwicklungsmechanik
   der Organismen 23:457-478. 1907.

5. Birge, Wesley J. and Howard H. Hillemann. Meten-
   cephalic development and differentiation
   following experimental lesions in the early
   chick embryo. Journal of experimental
   zoology 124:545-569. 1953.

6. Bueker, E. D. Differentiation of the lateral
   motor column in the avian spinal cord.
   Science 100:169. 1944.

7. Crelin, Edmund S. Jr. Excision and rotation of the
   developing Amblystoma optic tectum and
   subsequent visual behavior. Journal of
   experimental zoology 120:547-577. 1952.

8. Dalcq, A. Recent experimental contributions to
   brain morphogenesis in amphibians. Symposium
   on development and growth 6:85-119. 1947.

9. Detwiler, S. R. The transplantation of the medulla
   oblongata into the brachial region of the
   cord in Amblystoma embryos. Journal of
   comparative neurology 43:143-158. 1927.

10. Detwiler, S. R. Medulla injury in relation to
        cellular proliferation in Amblystoma
        embryos. Anatomical record 35:91-97.
        1927.

11. Detwiler, S. R. Further experimental studies upon
        morphogenesis in the anterior portion of
        the spinal cord. Journal of comparative
        neurology 50:521-544. 1930.

12. Detwiler, S. R. Growth acceleration and regulation
        in heteroplastic spinal-cord grafts. Journal
        of experimental zoology 61:245-276. 1932.

13. Detwiler, S. R. Further experiments upon
        accelerated growth in heteroplastic spinal-
        cord grafts. Journal of comparative
        neurology 56:465-502. 1932.

14. Detwiler, S. R. Experimental studies upon the
        development of the amphibian nervous system.
        Biological reviews and biological proceedings,
        Cambridge philosophical society 8:269-310.
        1933.

15. Detwiler, S. R. Unilateral reversal of the antero-
        posterior axis of the medulla in Amblystoma.
        Journal of experimental zoology 84:13-22.
        1940.

16. Detwiler, S. R. Unilateral substitution of the
        brachial region of the spinal cord by the
        corresponding half of the medulla in
        Amblystoma. Journal of experimental
        zoology 92:247-260. 1943.

17. Detwiler, S. R. Restitution of the medulla
        following unilateral excision in the embryo.
        Journal of experimental zoology 96:129-142.
        1944.

18. Detwiler, S. R. The results of unilateral and
        bilateral extirpation of the forebrain of
        Amblystoma. Journal of experimental zoology
        100:103-117. 1945.

19. Detwiler, S. R. A quantitative study of locomotion in larval Amblystoma following either mid-brain or forebrain excision. Journal of experimental zoology 102:321-332. 1946.

20. Detwiler, S. R. Midbrain regeneration in Amblystoma. Anatomical record 94:229-237. 1946.

21. Detwiler, S. R. Experiments upon the midbrain of Amblystoma embryos. American journal of anatomy 78:115-138. 1946.

22. Detwiler, S. R. Restitution of the brachial region of the cord following unilateral excision in the embryo. Journal of experimental zoology 104:53-68. 1947.

23. Detwiler, S. R. and B. L. Maclean. Cellular proliferation in the spinal cord of Amblystoma, transection and replantation of various embryonic cord segments. Journal of experimental zoology 62:433-451. 1932.

24. Fowler, I. Responses of the chick neural tube in mechanically produced spina bifida. Journal of experimental zoology 123:115-151. 1953.

25. Hamburger, Viktor. Die Entwicklung experimentell erzeugter nervenloser und schwach innervierter Extremitaten von Anuren. Wilhelm Roux' Archiv für Entwicklungsmechanik der Organismen 114:272-363. 1929.

26. Hamburger, Viktor. Motor and sensory hyperplasia following limb-bud transplantation. Physiological zoology 13:268-284. 1939.

27. Hamburger, Viktor. Differential mitotic activity in the spinal cord of the chick. Anatomical record 99:99. 1947.

28. Hamburger, Viktor. The mitotic patterns in the spinal cord of the chick embryo and their relation to histogenetic processes. Journal of comparative neurology 88:221-283. 1948.

29. Hamburger, Viktor and H. L. Hamilton. A series of normal stages in the development of the chick embryo. Journal of morphology 88:49-92. 1951.

30. Hamburger, Viktor and R. Levi-Montalcini. Proliferation, differentiation, and degeneration in the spinal ganglia of the chick embryo under normal and experimental conditions. Journal of experimental zoology 111:457-502. 1949.

31. Hamburger, Viktor and R. Levi-Montalcini. Some aspects of neuroembryology. In Genetic neurology. Chicago, University of Chicago Press, 1950. pp. 128-160.

32. Hammond, Warner S. Formation of the sympathetic nervous system in the trunk of the chick embryo following removal of the neural tube. Journal of comparative neurology 91:67-85. 1949.

33. Hammond, Warner S. and Chester L. Yntema. Depletions in the thoraco-lumbar sympathetic system following removal of neural crest in the chick. Journal of comparative neurology 86:237-253. 1947.

34. Harrison, R. G. The origin and development of the nervous system studied by the method of experimental embryology. Proceedings of the royal society of London 118:155-196. 1935.

35. Harrison, R. G. Wound healing and reconstitution of the central nervous system of the amphibian embryo after removal of parts of the neural plate. Journal of experimental zoology 106:27-83. 1947.

36. Hillemann, H. H. An embryological micromanipulator with an adjustable egg-holder attachment. Anatomical record 84:337-342. 1942.

37. Hillemann, H. H. A method and apparatus for opening and closing eggs which will permit turning at regular intervals during subsequent incubation. Anatomical record 84:331-336. 1942.

38. Hillemann, H. H. The design and use of micro-
        electrodes for the production of lesions in
        the pituitary rudiment of chick embryos.
        Anatomical record 84:343-357. 1942.

39. Hillemann, H. H. An experimental study of the
        development of the pituitary gland in chick
        embryos. Journal of experimental zoology
        93:347-373. 1943.

40. Hollinshead, May B. Wound healing and regeneration
        in the embryonic spinal cord of Amblystoma
        punctatum. Journal of experimental zoology
        119:303-331. 1952.

41. Holtzer, H. Differentiation of the regional action
        systems in the urodele spinal cord.
        Anatomical record 108:219. 1950.

42. Holtzer, H. Reconstitution of the urodele spinal
        cord following unilateral ablation. I. Chronol-
        ogy of neuron regulation. Journal of
        experimental zoology 117:523-557. 1951.

43. Holtzer, H. Reconstitution of the urodele spinal
        cord following unilateral ablation. II. Re-
        generation of the longitudinal tracts and
        ectopic synaptic unions of the Mauthner's
        fibers. Journal of experimental zoology
        119:263-301. 1952.

44. Hooker, D. Studies on regeneration in the spinal
        cord. I. An analysis of the processes
        leading to its reunion after it has been
        completely severed in frog embryos at the
        stage of closed neural folds. Journal of
        comparative neurology 27:421-449. 1915.

45. Hooker, D. Studies on regeneration in the spinal
        cord. III. Reëstablishment of anatomical
        and physiological continuity after
        transection in frog tadpoles. Journal of
        comparative neurology 38:315-347. 1925.

46. Hooker, D. Studies on regeneration of the spinal cord. IV. Rotation about its longitudinal axis of a portion of the cord in Amblystoma punctatum embryos. Journal of experimental zoology 55:23-41. 1930.

47. Huber, C. C. and C. C. Crosby. A phylogenetic consideration of the optic tectum. Proceedings of the national academy of science 19:15-22. 1932.

48. Jones, David Smith. Further studies on the origin of sympathetic ganglia in the chick embryo. Anatomical record 79:7-13. 1941.

49. Lehman, H. E. and L. M. Youngs. An analysis of regulation in the amphibian neural crest. Journal of experimental zoology 121:419-447. 1952.

50. Levi-Montalcini, R. Corrélations dans le dévelopment des différentes parties du système nerveux. II. Corrélations entre le développement de l'encéphale et celui de la moelle épinière dans l'embryon de poulet. Archives de biologie 56:71-81. 1945.

51. Lewis, W. H. Experiments on regeneration and differentiation of the central nervous system in amphibian embryos. American journal of anatomy 5:11. 1906.

52. Lewis, W. H. Localization and regeneration in the neural plate of amphibian embryos. Anatomical record 4:191-198. 1910.

53. Lillie, F. R. Experimental studies on the development of organs in the embryo of the fowl (Gallus domesticus). Biological bulletin 7:33-54. 1904.

54. Nicholas, J. S. The effects of the separation of the medulla and spinal cord from the cerebral mechanism by the extirpation of the embryonic mesencephalon. Journal of experimental zoology 55:1-22. 1930.

55. Nieuwkoop, P. D. Activation and organization of
    the central nervous system in amphibians.
    III. Synthesis of a new working hypothesis.
    Journal of experimental zoology 120:83-108.
    1952.

56. Piatt, Jean. Form and causality in neurogenesis.
    Biological reviews 23:1-45. 1948.

57. Piatt, Jean. A study of the development of fiber
    tracts in the brain of Amblystoma after
    excision or inversion of the embryonic
    demesencephalic region. Journal of
    comparative neurology 90:47-93. 1949.

58. Ramon y Cajal. Lóbulos ópticos de las aves.
    Trabajos del instituto Cajal de
    investigaciones biológicas 35:3-20. 1943.

59. Reddick, Mary Logan. The histogenesis of cellular
    elements in the postotic medulla of the
    chick embryo. Anatomical record 109:81-97.
    1951.

60. Rhines, R. and W. F. Windle. An experimental study
    of factors influencing the course of nerve
    fibers in the embryonic central nervous
    system. Anatomical record 90:267-283. 1944.

61. Sauer, F. C. Mitosis in the neural tube. Journal
    of comparative neurology 62:377-405. 1935.

62. Sauer, F. C. The cellular structure of the neural
    tube. Journal of comparative neurology
    63:13-23. 1935.

63. Spratt, N. T. An in vitro analysis of the
    organization of the eye-forming area in
    early chick blastoderm. Journal of
    experimental zoology 85:171-209. 1940.

64. Waddington, C. H. and A. Cohen. Experiments on
    the development of the head of the chick
    embryo. Journal of experimental biology
    13:219-236. 1936.

65. Watterson, R. L. Development of the glycogen body
        of the chick spinal cord. III. The paired
        primordia as revealed by glycogen-specific
        stains. Anatomical record 113:29-51. 1952.

66. Watterson, R. L. Development of the glycogen body
        of the chick spinal cord. IV. Effects of
        mechanical manipulation of the roof plate
        at the lumbosacral level. Journal of
        experimental zoology 125:285-330. 1954.

67. Watterson, R. L. and Ira Fowler. Regulative
        development in lateral halves of chick
        neural tubes. Anatomical record 117:773-804.
        1953.

68. Weiss, P. A. Growth and differentiation on the
        cellular and molecular levels. Proceedings
        of the international congress of experimental
        cytology (Stockholm) 6:475-482. 1949.

69. Wenger, Byron S. Determination of structural
        patterns in the spinal cord of the chick
        embryo studied by transplantations between
        brachial and adjacent levels. Journal of
        experimental zoology 116:123-163. 1951.

70. Wenger, Eleanor. An experimental analysis of
        relations between parts of the brachial
        spinal cord of the embryonic chick. Journal
        of experimental zoology 114:51-85. 1950.

71. Wolff, Etienne. La destruction expérimentale,
        totale ou partielle, de l'encéphale et ses
        conséquences chez l'embryon de poulet.
        Comptes rendus des seances de la société
        de biologie et de ses Filiales (Paris)
        127:440-442. 1938.

72. Woodard, T. M. and Sarah B. Estes. The mitotic
        index in the neural tube of the 48-hour
        chick as determined by the use of colchicine.
        Anatomical record 84:501. 1942.

73. Woodard, T. M. and Sarah B. Estes. Effect of
       colchicine on mitosis in the neural tube
       of the 48-hour chick embryo. Anatomical
       record 90:51-54. 1944.

74. Yntema, C. L. Experiments on the origin of the
       sensory ganglia of the facial nerve in
       the chick. Journal of comparative
       neurology 81:147-167. 1944.

75. Yntema, C. L. and W. S. Hammond. Depletions
       and abnormalities in the cervical sympathetic
       system of the chick following extirpation of
       the neural crest. Journal of experimental
       zoology 100:237-263. 1945.

APPENDIX

EXPLANATION OF PLATES

PLATE 1

Figure 1.   Cross-section through the head of a four-day
chick embryo at the level of the midbrain.
The right alar and basal plates were previously
ablated at 30 hours of development. The
optic tectum has been partially reconstituted
on the lesion (right) side, but the right
mesencephalic basal plate is entirely absent.
The left mesencephalic basal plate is normal
in structure however. 28 X.

Figure 2.   Cross-section through the midbrain of a
six-day chick embryo in which the right half
of the mesencephalon was ablated early in
development. The structural pattern is
essentially the same as that described for
figure 1, except the optic tectum shows a
greater extent of regulation (47 per cent).
28 X.

Figure 3.   Cross-section through the midbrain of a
nine-day chick embryo in which the right alar
plate, right basal plate, and the roof plate
were ablated at 32 hours of development. The
lesion has been closed by the fusion of the
optic tectum with the left basal plate. The
latter is normal in structure, but the right
basal plate is entirely lacking. 20 X.

Figure 4.   Cross-section through the anterior portion of
the metencephalon of an eight-day chick embryo.
The metencephalon is normal in structure
regardless of the absence of the midbrain
which was removed at 28 hours of development.
28 X.

PLATE 2

Figure 5.   Cross section through the mesencephalon
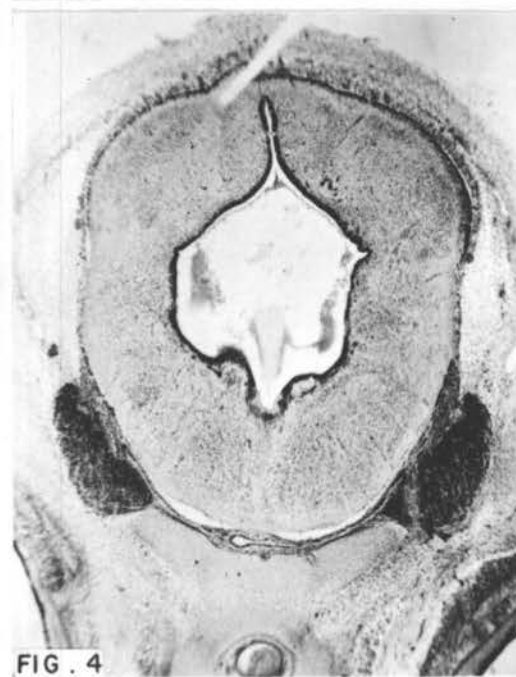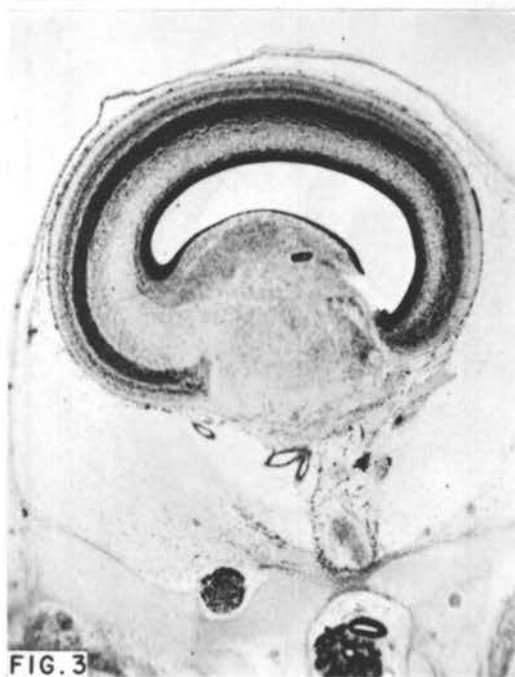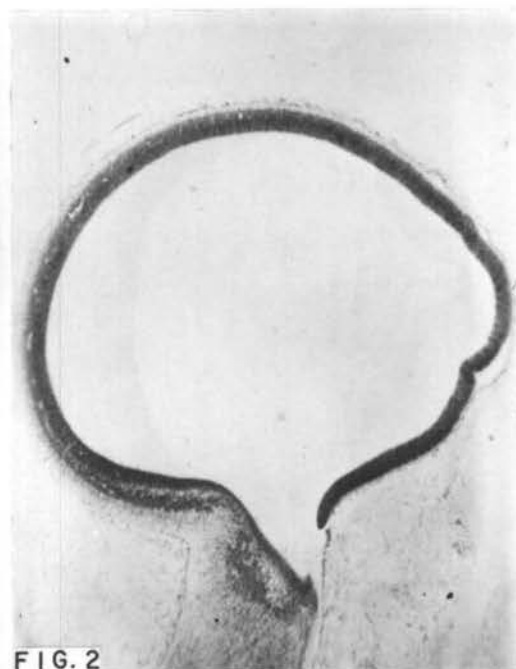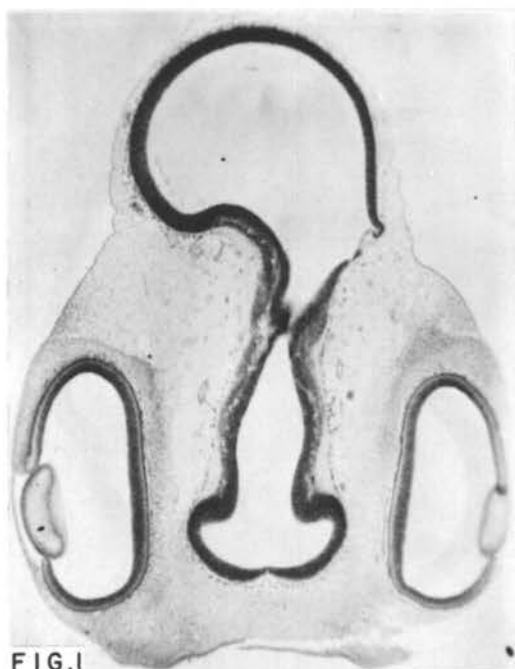of a six-day chick embryo in which the right

half of the midbrain was ablated at 30 hours of development. The structure of this region is essentially the same as that seen in figure 2, except a greater extent of regulation is indicated (68 per cent). 28 X.

Figure 6.   Cross-section through the midbrain of a seven-day embryo. In this animal the right mesen-cephalic alar plate and most of the under-lying basal plate were removed during the operation. The basal plate tissue, including the fragment on the right side, is normal in structure, and the optic tectum shows an average extent of regulation (70 per cent). 20 X.

Figure 7.   Cross-section through the anterior level of the mesencephalon of an eight-day embryo in which the right alar plate was previously removed. 20 X.

Figure 8.   Cross-section through the middle of the mesencephalon of the same embryo. At this level the right basal plate was slightly ablated during the operation. 20 X.

PLATE 3

Figure 9.   Cross-section through the midbrain of a nine-day chick embryo in which the right alar plate was ablated at 33 hours of develop-ment. 20 X.

Figure 10.   Cross-section through the mesencephalon of a 14-day chick embryo. The right alar plate was removed during early development. 15 X.

Figure 11.   Cross-section through the midbrain of a 16-day embryo in which the right alar plate was previously removed. 15 X.

Figure 12.   Cross-section made at the anterior level of the mesencephalon in a 12-day embryo. Both alar plate areas were ablated at 30 hours of development. The two mesencephalic basal plates are typical in structure. 20 X.
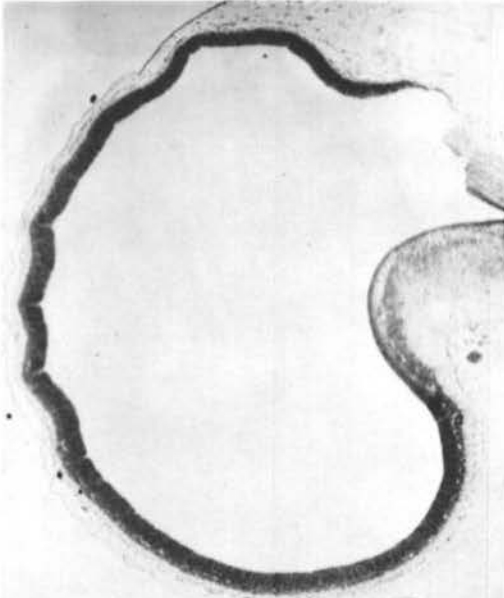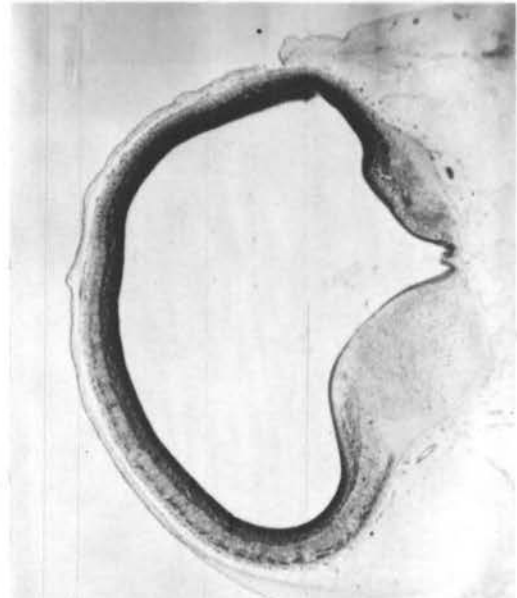
PLATE 1

FIG. I

FIG. 2

FIG. 3

FIG . 4
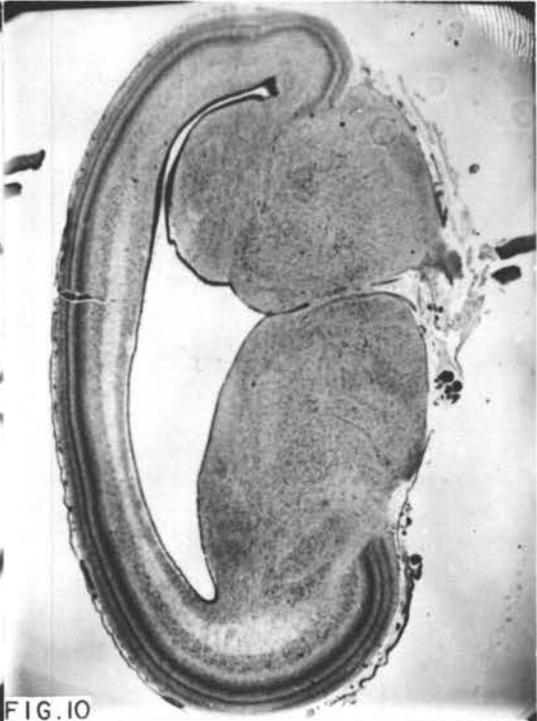
PLATE 2

FIG.5

FIG.6

FIG.7

FIG.8

PLATE 3

FIG.9

FIG.10

FIG.11

FIG.12