It has been shown by Smith (1946), working with trout eggs, that when an embryo develops the heat lost to the surroundings is balanced by the loss in the calorific value of its energy-rich storage substances. Other workers (e.g. Shearer, 1922) have shown that the heat lost by eggs and developing embryos is approximately equal to the available energy deduced from the observed respiration. It would seem, therefore, that only a very small fraction of the energy known to be available to the embryo from its aerobic metabolism can be stored as potential energy in the visible pattern of tissue structures which the embryo progressively develops. It has sometimes been concluded from this that little energy can be required for morphogenesis. This conclusion is doubtless true, but the argument is unsound, because no knowledge of the energy balance between the total energy being released by the embryo and the energy being lost to the surroundings can tell us anything about the work which may be required to overcome a potential energy barrier. For instance, consider this example which is used here as a simple illustration of the argument rather than as a model of what actually happens. It is conceivable that when a newt embryo gastrulates, considerable work must be done in so deforming the cells against their own elasticity that the blastula tissue may pass through the narrow blastopore opening. To perform such work energy is required from the chemical energy source; it will be stored as potential energy by deformed cells as they pass through the blastopore; yet when the constriction is passed, the cells relax to their equilibrium shapes once more and the energy is dissipated as heat. In such a case a knowledge of the energy-balance could tell us nothing about the work done in gastrulation. On the other hand, if we knew about the forces required to deform the cells we should be able to estimate the work required for gastrulation, using this particular model for the process.

Tuft (1953) working with Rhodnius made an indirect estimate of the proportion of the total energy available which could conceivably be used by an embryo in performing morphogenetic movements. As pointed out by Waddington (1956), one weakness of this estimate is the necessity of assuming a constant energy requirement for maintenance. Tyler (1942) found that two dwarf echino-
derm embryos respire at the same rate as one normal embryo of the same mass, but they take roughly 37 per cent. longer to gastrulate. Assuming maintenance requirements at a given stage are proportional to the living mass, this result may be interpreted as meaning either that two dwarfs require about 37 per cent. more energy for gastrulation than does one normal embryo (which agrees roughly with Tyler's theoretical estimate of 41 per cent.), in which case we can infer nothing about the relative magnitudes of the energies required for gastrulation and maintenance (see also Needham, 1942), or alternatively we may suppose that the energy needed for gastrulation is very small compared with that required for maintenance, in which case Tyler's results tell us nothing about the relative energies needed for gastrulation by dwarf and normal embryos. It seems likely that, to be in any sense reliable, an estimate must be based directly on measured values for the forces which the tissues of embryos can exert during their morphogenetic movements. The experiments of Moore (1941, 1945), who found the osmotically developed pressure within the blastocoel of an echinderm blastula which would just prevent gastrulation, led him to estimate that only a two-thousandth of the available energy from respiration would be required to perform the gastrulation process. The work required to change the shape of the embryo is evidently very small. Of course, considerable energy may be needed to produce the chemical syntheses associated with morphogenesis, but calculations based on physical measurements will give no information about this.

The present paper describes measurements of the forces which amphibian embryos can exert between their neural ridges during neural closure. The results lead to an estimate for the mechanical work done by an embryo during neurulation, and they provide data of value when one tries to consider how the neurulation process is carried out by the embryo. Such theories of neural closure as have been put forward in the literature have all attempted no more than a qualitative explanation of what is observed to take place. In future a satisfactory theory of neural closure should lead to a derivation of a neural closure force of the correct order of magnitude. The present paper is concerned, however, with the forces and work involved in neural closure rather than with theories of morphogenesis. A preliminary communication about this work was made by Selman (1955).

**EXPERIMENTAL PROCEDURE**

The method arose out of a suggestion made by Professor C. H. Waddington that it might be possible to apply the principle of the A.C./D.C. moving iron ammeter to the measurement of forces developed by embryos during morphogenesis. In the present experiment two small iron dumb-bells were mounted on the neural plate parallel to and against the neural folds. They were made to repel each other by arranging them in a uniform magnetic field produced by a pair of electromagnetic coils set up similarly to a Helmholtz arrangement (Text-fig. 1). The force of mutual repulsion between these induced magnets could be varied by adjusting the alternating current passing through the coils. By balanc-
ing this magnetically produced force against the force which the neurulating tissue could exert, an estimate of the latter could be obtained.

The dumb-bells themselves were found by careful searching in a single sample of iron shot which contained a majority of spherical iron particles. A typical dumb-bell might be described as two equal spheres or ellipsoids of diameter \( \sim 150 \mu \) connected by a cylindrical shaft about 30 \( \mu \) in diameter and 300 \( \mu \) long.

(Plate, fig. A). The iron dumb-bells were approximately of similar form, but they varied in length \( L \) between about 450 and 800 \( \mu \), and were of mass \( w \) between 16 and 98 \( \mu g \) each. About 20 matching pairs of dumb-bells were selected on a basis of approximately equal weight, length, and general proportions.

Embryos of two species were used, the newt, *Triturus alpestris*, and the axolotl, *Siredon mexicanum* (sometimes called *Amblystoma mexicanum*), because these possess well-developed neural folds before neural closure. After removal of the jelly capsule and vitelline membrane with fine forceps, the undamaged embryo was placed in 1/10th strength Holtfreter’s saline, in a wax depression inside a small perspex box in the shape of a cube of side 5 mm. A pair of equal dumb-bells was then mounted symmetrically on the neural plate where the folds would normally first close. The box containing the neurula was then mounted between the two electromagnets so that the dumb-bells were approximately on the axis of the coils, midway between them, and parallel to the lines of force of an inducing magnetic field (Text-fig. 2). A small alternating current was then passed through the coils, so that the dumb-bells mutually repelled each other and pressed against the side of the neural folds (Plate, fig. C). After about 30 minutes the edges of the folds became partly moulded to the contours of the sides of the dumb-bells, and this helped greatly to reduce the tendency for the dumb-bells to slip sideways when greater magnetic fields were applied later. A fragment of coverslip
was placed across the top of the plastic box and in contact with the saline surrounding the embryo, so as to provide a plane window for observation. Throughout the experiment the neural closure and the behaviour of the dumb-bells were followed, using a microscope giving a magnification of $\times 100$ and having a scale in the ocular which enabled the separation $D$ between the dumb-bells to be continually recorded. Knowing the separation $D$ and the current $i$ passing through the coils, the force of repulsion between the particular pair of induced magnets could be calculated by reference to graphs prepared in separate calibration experiments to be described later. Alternating current was used throughout all experiments in order to avoid difficulties due to residual magnetism which might otherwise have arisen. The value of the current was smoothly raised or lowered from zero using an auto-transformer and rheostat (Text-fig. 1). The coils had soft iron cores with plane parallel circular faces 6-8 mm. in diameter.

In the majority of experiments an attempt was made to hold the separation between the neural folds near the dumb-bells at approximately the same value by adjusting the current through the coils. Meanwhile, the neural folds would continue to close at distances greater than about 500 $\mu$ on either side of the dumb-bells, and in some experiments the neural closure took place in the head region while the mid-fold region was held apart by the force between the induced magnets.
If a movement towards neural closure took place, so that the dumb-bells were forced nearer each other, the neurulating tissue was then known to be exerting a force greater than, and opposite to, the force applied magnetically, whose magnitude was known from the values of current $i$ and separation $D$. The neurulating tissue at this time was therefore capable of exerting a closure force at least as great as this. The movements towards neural closure which took place...
during experiment 14 with the newt, and experiment 13 with axolotl, are indicated against the letter \( N \) in Text-figs. 3 and 4 respectively. The corresponding numerical values, which have been calculated for the neural closure forces

![Graph](image)

**Text-fig. 4.** The graphs refer to experiment 13 performed on a neurula of axolotl. The upper and lower graphs are plotted like those in Text-fig. 3 for experiment 14, although the calibration employed was indirect. The middle graph shows the current \( i \) passed through the coils of the electromagnet during the experiment. In the lower graph the dotted line gives suggested values for the closure force which the neurula can exert. This force must be greater than, or equal to, the values depicted by the points \( o \) recorded during neural closure, and less than, or equal to, the values shown by the points \( x \) when there was no closure (see text).

exerted by the embryo against the dumb-bell-shaped magnets, are shown in Tables 1 and 2, also oppose the letter \( N \).

It will be seen that the design of the experiment is such that when the current \( i \) is held constant, any neural closure movement meets increasing resistance as the dumb-bells are carried nearer to each other by the neural folds. On the other
hand, when the current was maintained at a constant value for a period of time during which no movement towards neural closure took place, then the force of mutual repulsion between the magnets was known to equal or perhaps might

### Table 1

<table>
<thead>
<tr>
<th>No. of expt.</th>
<th>Force of neurulation in dynes $\times 10^{-3}$</th>
<th>Time in minutes</th>
<th>Calibration</th>
<th>Separation of folds in $\mu$</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>$N = 3$, $6$, $10$, $15$, $31$, $38$</td>
<td></td>
<td></td>
<td>$0 - 7$, $10 - 13$, $15 - 19$, $19 - 34$, $38 - 41$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$N = 14$</td>
<td></td>
<td></td>
<td>$324 - 311$, $311 - 298$, $298 - 293$, $293 - 298$, $298 - 285$</td>
<td>Mid-fold</td>
</tr>
<tr>
<td>10</td>
<td>$N = 4$, $6$, $12$, $15$, $46.5$</td>
<td></td>
<td></td>
<td>$0 - 7$, $24 - 27$, $35 - 44$, $48 - 56$</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>$N = 18$, $18$, $46$, $38$</td>
<td>$0 - 36$, $\approx 90$</td>
<td></td>
<td>$179 - 153$</td>
<td>Mid-fold</td>
</tr>
<tr>
<td>2</td>
<td>$N = 39$, $28$</td>
<td>$\approx 30$, $40 - 90$</td>
<td></td>
<td>$\approx 205$, $\approx 210$</td>
<td>Mid-fold</td>
</tr>
<tr>
<td>3</td>
<td>$N = 7.4$, $9$, $8$, $15$, $50$</td>
<td>$3 - 8$, $8 - 28$, $41 - 43$, $48 - 74$, $74 - 78$</td>
<td></td>
<td>$313 - 294$, $294 - 268$, $294 - 263$, $250 - 227$, $229 - 205$</td>
<td>Mid-fold</td>
</tr>
<tr>
<td></td>
<td>$N = 6$, $7.3$, $9$, $12.6$, $15$, $30$</td>
<td>$55 - 60$, $60 - 70$, $427 - 375$</td>
<td></td>
<td>$427 - 375$</td>
<td>Torn side</td>
</tr>
<tr>
<td></td>
<td>$N = 34$, $43$, $40$, $150 - 165$</td>
<td>$244 - 230$</td>
<td></td>
<td>$230 - 161$</td>
<td>Mid-fold</td>
</tr>
<tr>
<td></td>
<td>$N = 21$, $40$, $10 - 50$</td>
<td>$161$</td>
<td></td>
<td>$161$</td>
<td>Head region</td>
</tr>
</tbody>
</table>
through or below some points, and through or above others, depending upon the experimental conditions as described above.

It was found that the magnetically applied force necessary to keep the folds at approximately the same separation during at least the first 40 minutes of the experiments must be gradually increased with time. This is clearly illustrated by experiment 14 (Text-fig. 3). This effect is partly a consequence of the elasticity of the neural tissues themselves and the manner in which most experiments were performed. Under conditions where neural closure is opposed by an externally applied force which is increased gradually, the neural closure effort is applied to compressing the tissues which are trying to push the magnets together. In experiments 2, 4, and 8, however, with neurulae of newt, much of the elastic compression of the tissues was taken up at the beginning of the experiment by applying a fairly large magnetic force, of the order $30 \times 10^{-3}$ dynes, which naturally opened the neural folds about 40 $\mu$ at the moment it was applied. Nevertheless, in these cases, at least 30 minutes had to elapse before a neural closure

### Table 2

<table>
<thead>
<tr>
<th>No. of expt.</th>
<th>Force of neurulation in dynes $\times 10^{-3}$</th>
<th>Time in minutes</th>
<th>Calibration</th>
<th>Separation of folds in $\mu$</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>$\leq 10.8$</td>
<td>$0-60$</td>
<td>DIRECT</td>
<td>$481 \rightarrow 586$</td>
<td>Torn side Head region</td>
</tr>
<tr>
<td></td>
<td>$N \geq 6 \rightarrow 8$</td>
<td>$135-145$</td>
<td></td>
<td>$350 \rightarrow 402$</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>$N \geq 27 \rightarrow 39$</td>
<td>$0-10$</td>
<td></td>
<td>$312 \rightarrow 286$</td>
<td>Mid-fold</td>
</tr>
<tr>
<td></td>
<td>$N \geq 27 \rightarrow 38$</td>
<td>$34-40$</td>
<td></td>
<td>$312 \rightarrow 299$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$N \geq 38 \rightarrow 52$</td>
<td>$40-50$</td>
<td></td>
<td>$299 \rightarrow 278$</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>$N \geq 52 \rightarrow 55$</td>
<td>$52-70$</td>
<td></td>
<td>$296 \rightarrow 301$</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>$N \geq 74 \rightarrow 65$</td>
<td>$70-75$</td>
<td></td>
<td>$299 \rightarrow 285$</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>$N \geq 74 \rightarrow 83$</td>
<td>$75-109$</td>
<td></td>
<td>$285 \rightarrow 275$</td>
<td>Mid-fold</td>
</tr>
<tr>
<td></td>
<td>$N \geq 92$</td>
<td>$109-117$</td>
<td></td>
<td>$285 \rightarrow 285$</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>$N \geq 6 \rightarrow 7.5$</td>
<td>$44-60$</td>
<td>INDIRECT</td>
<td>$324 \rightarrow 298$</td>
<td>Torn side Mid region</td>
</tr>
<tr>
<td></td>
<td>$N \leq 7 \rightarrow 8$</td>
<td>$60-70$</td>
<td></td>
<td>$123-126$</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>$N \geq 69 \rightarrow 92$</td>
<td>$132-137$</td>
<td></td>
<td>$285 \rightarrow 307$</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>$N \geq 74 \rightarrow 83$</td>
<td>$137-145$</td>
<td></td>
<td>$285 \rightarrow 300$</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>$N \geq 48 \rightarrow 65$</td>
<td>$16-30$</td>
<td></td>
<td>$265 \rightarrow 254$</td>
<td>Head region</td>
</tr>
<tr>
<td></td>
<td>$N \leq 70 \rightarrow 87$</td>
<td>$30-47$</td>
<td></td>
<td>$251 \rightarrow 265$</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>$N \geq 26 \rightarrow 38$</td>
<td>$0-32$</td>
<td></td>
<td>$445 \rightarrow 405$</td>
<td>Mid-fold</td>
</tr>
<tr>
<td>6</td>
<td>$N \geq 76 \rightarrow 106$</td>
<td>$32-45$</td>
<td></td>
<td>$405 \rightarrow 392$</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>SUBSIDES TO 55</td>
<td>$45-47$</td>
<td></td>
<td>$392 \rightarrow 419$</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>$N \geq 56 \rightarrow 118$</td>
<td>$47-74$</td>
<td></td>
<td>$419 \rightarrow 314$</td>
<td></td>
</tr>
</tbody>
</table>
movement took place. Irrespective of how the experiment was performed, at least 30 minutes had to elapse before a closure force of the order $40 \times 10^{-3}$ dynes could be exerted by the newt neurula.

For newt embryos, the maximum force tending to produce closure which the neural folds could exert against the dumb-bells was found to be between 40 and $45 \times 10^{-3}$ dynes. The maximum force which the neural folds of an axolotl neurula could exert was found to be about $90 \times 10^{-3}$ dynes in one case (experiment 13) and about $110 \times 10^{-3}$ dynes for another case (experiment 6). It should be remembered that the embryo of the axolotl is larger than that of the newt.

Under the conditions of these experiments the neural closure movements, or increases in the force which the neural folds exerted against the magnets, were made intermittently between intervals of inactivity. Closure took place, or tended to take place, in steps each of about $13 \mu$. The maximum force which the neural folds could develop was likewise exerted intermittently between intervals when only a much smaller force could be produced. Then, while the current through the coils was held constant at a high value, the neural folds periodically opened a little as they moved apart under the magnetically applied force, then approached their former positions as the forces of neural closure increased to their former value, and then opened again. This behaviour is illustrated in experiment 13 (Text-fig. 4 and Table 2).

The measured values for the force exerted by the tissues during neural closure were independent of the lengths of the dumb-bell pairs used in the separate experiments. This result is an expression of the rigidity of the neural-fold tissue and provides confirmation that it is appropriate to express the neural-closure forces exerted by the tissues in dynes rather than in dynes per unit length of neural fold.

All experiments except two were carried out with the dumb-bell pair on the mid-fold region of the neural plate, where the folds are normally first to close. It was easiest to carry out the experiment in this position, because the dumb-bell shape fitted the contours of the embryo here, and the dumb-bells were therefore less liable to slip sideways. Two experiments, however, 7 and 9, were made on the head region of the neurula, but the results were insufficient to establish whether the neural folds in the head region could exert as great a closure force as in the mid-fold region.

The tissues of the neural folds showed elastic behaviour. For newt, a repulsion force $40 \times 10^{-3}$ dynes opened the mid-folds about $50 \mu$. A plot of applied force against the resulting separation was roughly linear up to this point. The folds returned to their former positions in a few seconds after removing the force. During the last 5 minutes of experiment 14 the magnetic force which stressed the folds was removed and then restored by stages, while the corresponding positions of the folds were observed and plotted (Text-fig. 3). These results do not lead to a value for the elastic modulus of the tissues, because it seems impossible to define what is being stressed without ambiguity.
All the experiments (with the exception of experiments 5, 11, and 12) were performed upon embryos which were normal and quite undamaged in any way before the experiment, during the experiment, or after the experiment. The wax depression which supported the neurulae during the experiments was shaped to the contours of the ventral half of the embryo so that it was not flattened. The weight of the dumb-bells themselves was easily supported by the neural plate without obvious flattening. When the dumb-bells were removed at the end of the experiment, the imprint of the ball-shaped ends of the dumb-bells was left on the neural folds, which were slightly retarded by the experiment, but no damaged cells were seen. A few hours later the folds had closed normally along their entire length and no sign of the experiment was visible.

Experiments 5, 11, and 12 were made upon neurulae having wounds in the side of the embryo outside the neural folds but in line with the dumb-bells. Neurulation forces of only $10^{-2}$ dynes and less were recorded, although ample time was allowed for the experiment. Neural closure did, however, take place slowly when the inducing current was switched off altogether. These observations seem to suggest that the greater part of the force which the neural folds can exert is transmitted through the ventral ectoderm, and not as a result of a rolling-up movement of the neural plate itself. However, there are obvious dangers in interpreting results obtained on damaged embryos. It is not known how far-reaching were the effects of the wounds; nor were the effects of the healing reactions upon the measurements known. Moreover, it would have been better to have measured a normal neural closure force on an undamaged embryo, and then to have made the experiment on the same embryo after wounding, which would at least have proved the normality of the embryo at the beginning of the experiment. The work of previous authors (Glaser, 1914; Boerema, 1929; Brown, Hamburger, & Schmitt, 1941; and Holtfreter, 1944) strongly supports the conclusion that the capacity for folding and the closure of the neural tube is intrinsic in the tissues of the neural plate itself. The presence of other tissues is not necessary for neural closure. It is not known, however, in what degree the other tissues, in particular the lateral and ventral ectoderm, help or hinder the folding and closure of the neural tube.

Direct calibration

The forces of mutual repulsion between the dumb-bell pairs were calibrated against a quartz-fibre torsion-balance which was constructed like that illustrated on p. 215 of Strong's *Modern Physical Laboratory Practice*. The various ancillary techniques for handling quartz fibres which were used in its construction were those described by Nehr (1938) in the same book. The torsion-balance was calibrated, with its quartz cross-arm in the horizontal position, by weighing a series of microweights of between 50 and 100 µg., each prepared from known lengths of tungsten wire of 0·0005 in. diameter, 51·4 cm. of which had previously been found to weigh 1·393 mg. The torsion-balance was found to have a sensi-
tivity of 3.85 \mu g. per degree deflexion of the cross-arm, when the weighed object was fixed 4.6 cm. from the torsion fibre. When it was used with the cross-arm vertical and observed under the conditions of the measurements described below, it could measure forces to a quarter of a microgramme weight (i.e. to an accuracy of about 0.25 \times 10^{-3} \text{ dynes}).

Throughout the measurements by which the magnetically applied forces used in the experiments on neural closure were evaluated, the dumb-bells were mounted in precisely the same position with respect to the electromagnetic coils as in the illustration of Text-fig. 2, but while one of the dumb-bells was mounted on a strong rigid quartz support, the other was mounted at the tip of the quartz cross-arm which was in the vertical position of equilibrium. The cross-arm was 4.6 cm. long from dumb-bell to torsion fibre. The perspex box, which contained the embryo in the main experiments, was of course absent, and the dumb-bells were surrounded by air. The dumb-bells were fixed to the quartz fibres by minute amounts of sealing-wax with the aid of an electrically warmed wire loop. A small correction was applied for the weight of the sealing-wax. The dumb-bells were sealed so as to remain always parallel to each other and in the same horizontal plane, but while one dumb-bell was immovable the other was free to swing away from its partner when a current passed through the coils. The pair of electromagnetic coils were correctly positioned on an adjustable cradle which was clamped across the top of the draught-proof perspex case which contained the torsion-balance. A plane glass window was let into the box immediately above the dumb-bells, so that they could be viewed by the microscope with a scale in the ocular to record the position of each dumb-bell and the separation between them. The equilibrium position of the movable dumb-bell for zero current and therefore for zero torsion in the quartz system was first noted. Then when a known current was passed through the coils, this dumb-bell moved away from its partner and came to rest in a new equilibrium position, when the magnetic force of mutual repulsion was equal to the opposing couple applied by the torsion in the horizontal quartz fibre. From a knowledge of the previously measured torsion constant of the balance, the magnetic repulsion force between the dumb-bells was then easily calculated for the particular value of the current through the coils and the particular separation between the dumb-bells. By making a small measured alteration to the position of zero equilibrium, it was then possible to record a new value for the magnetic repulsion force corresponding to a new current value, but with the same separation between the dumb-bells. Then the magnetic repulsion force for the same current value, but a different separation, was recorded. In this way the forces of neural closure were, in effect at least, directly calibrated by a method of weighing.

Some small corrections were applied to the readings of the torsion-balance when it was used with its cross-arm in the vertical position, using a graphical calibration procedure. The corrections were for a small amount of sag in the torsion fibre and for the resolved part of the weight of the movable dumb-bell.
tending to deflect the balance whenever the cross-arm was not exactly vertical and adding slightly to the deflexion caused by the magnetic repulsion between the dumb-bells. The exact values of these corrections varied with the mass of each dumb-bell.

For each pair of dumb-bells a plot of the magnetic repulsion force $F$ against coil current $i$ gave a family of parabolas with one parabola for each particular value of the separation $D$ between the dumb-bells, each parabola having a common apex at the origin and a common force axis. This showed that the mutual repulsion force was proportional to the square of the coil current. A plot of $\log F$ against $\log i$ therefore gave a series of straight lines of slope 2, each for a particular value of $D$ (Text-fig. 5). Using this graph, the forces of mutual repulsion between the dumb-bells, which had been noted down during the neurulation experiments only in terms of the current $i$ and separation $D$, could then be
evaluated in dynes using the measured balance constants. In this way repulsion forces between eight separate pairs of matching dumb-bells having lengths and masses distributed evenly over the entire range used in the neurulation experiments were calibrated directly. Five of these pairs were used in actual neurulation experiments, and the values recorded for the forces of neural closure in these experiments are estimated to be accurate to about 5 per cent.

Indirect calibration

The forces of magnetic repulsion between dumb-bells in another nine experiments had to be indirectly calibrated because of the loss or damage to dumb-bells caused by accidents when handling these small objects, which occurred between a neural closure experiment and the completion of a direct calibration. A method for doing this was devised and the results obtained in this way are dependent upon the results of the direct calibrations with other dumb-bell pairs. All the eight dumb-bell pairs which had been used for direct calibration, and such of the others as remained whole, were weighed to a microgramme using the torsion-balance. All the dumb-bells used in the neurulation experiments had been previously sketched and carefully measured under a microscope. These measurements included the three principal diameters of each end of each dumb-bell, the length and average diameter of the shaft, and the total length. From these measurements their masses were calculated, and agreement to 7 per cent. was achieved with the results of the weighings.

The forces of mutual repulsion between dumb-bell pairs for particular values of separation \( d \) and coil current \( i \) were roughly proportional to the square of the mass \( w \) of the dumb-bell pair or the sixth power of the length \( L \), but pairs of identical length gave forces which were greater according as they were the more massive. Using the data from the eight dumb-bell pairs which had been directly calibrated, a plot of \( \log w \) against \( \log L \) was made and the best straight line of slope 3 was constructed. Then using the same data, \( \log F \) was plotted against \( \log L \) for particular constant values of separation \( d \) and coil current \( i \) and the best straight line of slope 6 was constructed. The deviation of each point, representing a particular matching pair of dumb-bells, was then measured as the perpendicular distance from the point on to the best straight line. This was done for both the above graphs, taking the deviations as positive or negative according as they were on the side of the line representing dumb-bell pairs which were respectively more or less massive than the average. When the deviations from the above two graphs were plotted against each other, a third straight line was obtained giving a good fit to the points. Then for any other dumb-bell pair of known mass and length, these three graphs gave a value for the force of mutual repulsion between the dumb-bells for the same particular values of separation \( d \) and current \( i \). The value of the repulsion force for different values of separation was found from a further family of curves, which was plotted, of the increments of \( \log F \) at constant coil current for other separations, again using data from the
dumb-bells which were directly calibrated. The value of the force for the other values of coil current was easily found, because an accurate dependence upon the square of the current could be assumed. The forces of neurulation, in the nine experiments where direct calibration was not possible, were evaluated indirectly in this manner to give values of an estimated accuracy of 15 per cent., which were in agreement with the results of those experiments which were calibrated directly.

DISCUSSION

According to the present author's knowledge of the literature, two previous measurements of forces exerted by embryonic tissues have been made. Waddington (1939) measured the magnetic force required to prevent movement of a steel sphere of 83 \( \mu \)m diameter embedded among cells of a gastrula of *Triturus alpestris*. He found that the maximum force exerted by the gastrulating tissues was equivalent to 0·34 mg./mm.\(^2\) of the hemispherical surface of the steel sphere. This means that the gastrulating tissue exerted a force of 3·6 \( \times \) \( 10^{-3} \) dynes on the embedded sphere. When the present author tried to perform a similar experiment, he observed that while a magnetic force was applied to a ball sufficient to hold it stationary, the gastrulating cells seemed still able to move round on either side of the ball, and in this way gastrulation continued. If these should have been the conditions under which Waddington's measurement was made, the maximum force which the gastrulating tissues were able to exert might be greater by anything up to a factor of say 10 times. In any case, Waddington's figure is of a similar magnitude to the present result for the maximum force which the neural tissue of the same species can exert, i.e. 40 to 45 \( \times \) \( 10^{-3} \) dynes. Moore (1941, 1945) found that the gastrulation of the sand-dollar *Dendraster excentricus* was prevented by the presence of sucrose of osmotic pressure 0·37 to 0·75 atmospheres in the blastocoel cavity. Taking the surface area of the invaginating cone of the gastrula as 707 \( \mu \)m\(^2\), Moore calculated that the necessary force to stop gastrulation in *Dendraster* was 2·6 to 5·5 dynes. (Moore actually calls this last figure a pressure, although he records it in milligrammes and clearly understands it as a force.) As Moore points out, his result is relatively enormous when compared with the measurements on newt, although the difference is not quite as great as Moore says it is.

If we take the rate at which neural closure of *Triturus alpestris* can take place at 20° C. as 200 \( \mu \)/hour, against an externally applied force 40 \( \times \) \( 10^{-3} \) dynes, then the maximum possible rate at which a neurula can perform work against an externally applied system is \( 8 \times 10^{-4} \) ergs/hour or 1·9 \( \times \) \( 10^{-11} \) calories/hour. During the whole neural closure process the work which may be so done is about \( 8 \times 10^{-3} \) ergs. Tuft (personal communication) has measured the respiration of *T. alpestris* embryos. At 25° C. he found neurulae respired between 0·3 and 0·4 cu. mm. of oxygen each hour. If we take the respiration at 20° C. during neural closure to be 0·25 mm.\(^3\) of oxygen per hour and assume a calorific value
of $4.6 \times 10^{-3}$ calories per mm.$^3$ of oxygen consumed, then we know the total energy available to a neurula is about $1.15 \times 10^{-3}$ calories per hour. Assuming that metabolic energy is utilized by the neural closure process at an efficiency of 10 per cent., we find that the work which can be done by the folds of the neurula is about six million times less than the total metabolic energy available to the whole embryo. From the curve which Løvtrup (1953) published for the oxygen consumption of embryos of axolotl developing at 20° C., the oxygen consumed by an axolotl embryo at neural closure can be taken as about $0.45$ mm.$^3$ of oxygen per hour. Assuming a rate of closure of $200 \mu$/hour to be just possible against a force of $80 \times 10^{-3}$ dynes, a similar calculation for axolotl again shows that the ratio of energy available from respiration to the maximum work which can be performed by the embryo is just under $6 \times 10^6$.

If we take the result of Waddington (1939) that the gastrulating cells of Triturus were shown to exert a force of $3.6 \times 10^{-3}$ dynes, then assuming the tissues invaginated at a rate of $125 \mu$/hour, the gastrula was known to work at a rate of $4.5 \times 10^{-5}$ ergs/hour or about $10^{-12}$ calories/hour. If we assume that the gastrula respired $0.1$ mm.$^3$ of oxygen per hour at the room temperature of the experiment, then the total energy available to the embryo was about $4.6 \times 10^{-4}$ calories/hour, and assuming an efficiency of 10 per cent. for the gastrulation process we find the ratio of total energy available to the energy which can be used for doing work in gastrulation is about $5 \times 10^7$. If the argument is accepted that a gastrula is capable of exerting a force up to 10 times greater than Waddington's figure then the ratio agrees with the corresponding one calculated from the neurulation experiment.

Moore (1941, 1945) calculated for Dendraster that the energy available to this embryo from its respiration was 2,000 times greater than that which was used in gastrulation under the conditions of his experiment. This is, of course, a very different figure from that which has been calculated above for morphogenetic processes in amphibian embryos, but the mechanical work which the tissues can perform is still a minute fraction of the total energy available.

It is important to remember that all the experimental measurements of morphogenetic forces, whether made by this or other authors, are measurements of forces developed by embryos against an outside system introduced by the experimenter. They are not and cannot be measurements of the forces which an embryo normally exerts against its own tissues in order to bring about gastrulation or neurulation under normal conditions. When a morphogenetic movement is just brought to rest by applying an opposing force, the magnitude of the opposing force gives a measure of the force which the embryo can exert in excess of the force which it already is exerting against its own elastic and other physical properties. Therefore the estimates of work done derived from such experiments refer to the work which may be performed by the embryo in excess of that which it normally is called upon to perform. It would seem that the work performed in normal gastrulation and neurulation could only be calculated theoretically from
G. G. SELMAN—FORCES PRODUCING NEURAL CLOSURE 463

a complete knowledge of the process and all the physical constants involved in it. In the following way, however, it may be argued that the physical work which the embryo normally performs to change the shape and mutual dispositions of the cells is likely to be of the same order of magnitude as that which it can be shown to be capable of performing against an external system. The gastrulation and neurulation processes have evolved in such a manner that they can be successfully completed over a limited range of temperature and other environmental conditions, all of which must alter the physical properties of the cells themselves and therefore the work required to change their shapes. No quantitative estimates of the effect of temperature on the physical properties of amphibian cells are available, and one is forced to seek analogies elsewhere. For instance, Heilbrunn (1930) has shown that the 'plasm viscosity' of an amoeba varies in a complicated manner with temperature, and can take values which differ by a factor seven within a range of less than 10° C. Mitchison & Swann (1954) measured an increase in the stiffness of the cortex of unfertilized sea-urchin eggs of times 2·1 for a temperature drop of 18·5° C. Alfrey (1948) quotes many examples of polymer systems whose stiffness varies sixfold over 10° C.

It is not unreasonable, therefore, to suggest that embryos at less favourable temperatures must perform in morphogenesis several times the work that is needed at the most favourable temperature. On the other hand, it is unlikely that in newt or axolotl a gastrulation or neurulation process can have been evolved that is capable of exerting closure forces vastly in excess of that which is ever needed. It seems more likely that the tissues can normally exert a few times the force which is necessary, and this view is supported by the following tentative derivation of the work normally done by a newt neurula in closure. Without having to accept any particular theory of neural closure, a number of which are briefly discussed by Brown, Hamburger, & Schmitt (1941), it may be supposed that however the cells of the neural ectoderm become progressively more wedge-shaped, so that the plate becomes a tube, some work must be done against elastic forces. If the movements towards closure are pictured as taking place in little steps, during each one of which work is done in straining the cells slightly, then during the interval between each step of deformation we may suppose that the stresses have time to become completely relaxed, partly because the cells have plastic as well as elastic properties and partly because the cells can rearrange themselves slightly and take up positions of least strain within the tissue. Under the conditions of the present experiment, stepwise closure was observed and the steps were roughly of the order 13 μ, which happened to be just the distance moved in each of three closure movements in experiment 14 for example (see Table 1). From the present experiments a 13 μ deformation of the neural folds is known to be produced by a force of about 10^{-2} dynes, and the work required to produce it is the product of one-half this force times this final deformation, which gives 6·5 × 10^{-6} ergs, so that complete closure, which would demand about 154 of these steps, requires 10^{-3} ergs, which is a rate of working of about
10^{-4} \text{ ergs/hour. So this very rough estimate for the work which the neurula does against its own elastic properties at } 20^\circ \text{C. gives a figure which is an eighth part of the previously calculated maximum working rate against an external system.} 

**SUMMARY**

1. It is argued that an estimate of the fraction of the total energy available to an embryo which is necessary to achieve gastrulation or neurulation can only be valid when based upon measured forces exerted by embryos.

2. A method is described for measuring the forces which the neural folds can exert towards closure. A pair of dumb-bell-shaped electromagnetically induced magnets was placed on the neural plate against the neural folds. The force of mutual repulsion between the magnets was balanced against the closure force exerted by the embryo so that values for the latter were obtained.

3. The elasticity of the neural fold tissues was demonstrated.

4. Under the conditions of the experiment, neural closure movements took place between intervals of no movement. The force, which an embryo exerted, increased with time to a maximum value.

5. Neurulae of *Triturus alpestris* exerted forces up to between $40 \times 10^{-3}$ and $45 \times 10^{-3}$ dynes. Neurulae of axolotl, *Siredon mexicanum*, exerted up to between $90 \times 10^{-3}$ and $110 \times 10^{-3}$ dynes.

6. During the experiments with neurulae the repulsion forces between the dumb-bells were noted in terms of the separation between the dumb-bells and the current in the magnetizing field coils. The corresponding forces were evaluated in separate calibration experiments with the same dumb-bells by a method of weighing using a sensitive quartz-fibre torsion-balance. In other cases, calibration was indirect but based upon the measurements obtained in direct calibration.

7. The present results are shown to be of similar magnitude to those of Waddington (1939) for gastrulae of the same species. Calculations show that newt and axolotl neurulae can only do work in closure against an external system at a rate which is six million times less than the total energy used by the embryo. *Triturus alpestris* neurulae can work at $8 \times 10^{-4}$ ergs/hour at $20^\circ \text{C. Another more tentative calculation suggests that the work which a newt neurula normally does against the elastic properties of its own tissues is about an eighth of this.} 

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REFERENCES


EXPLANATION OF PLATE

Fig. A. A typical pair of dumb-bell-shaped induced-magnets used in these experiments.

Fig. B. An axolotl neurula at the stage chosen to begin an experiment on neural closure in the mid-fold region.

Fig. C. An axolotl neurula with a dumb-bell pair in position on the neural plate against the neural folds, in the mid-fold region, at the beginning of an experiment on neural closure.

Fig. D. An axolotl with a pair of dumb-bells in position against the neural fold towards the head region. The embryo is tilted so that the dumb-bells are horizontal.

(Manuscript received 10: ii: 58)