Group 6. Animals entering hypothermia with TB of 10 °C.

All the hibernating animals were caged individually and had neither food nor water available in their cages. 'Semiarousals' did not appear in recordings.

Blood samples were taken by heart puncture. Analyses were made from blood serum. Hemolysed samples were discarded. Analyses of the NEFAs were made according to DOLE[3], analyses of the glycerol according to WIELAND[4].

From the results obtained it can be stated that, in normothermic active hedgehogs in late spring and in summer, the mean NEFA level is very constant. In late autumn, just before the onset of hibernation, the level has risen. It is further on the rise in hypothermic animals. The highest NEFA level we have found in the arousing and fully aroused animals during the hibernation period. It is interesting to note that the lipolytic activity is very intensive even at a body temperature of + 6 °C measured from the neck of the animal. It is obvious that the brown fat of the hedgehog is very active when the animals awake from the hypothermic state. According to our investigations, the brown fat is able to preserve the enzymatic activity at a relative low body temperature[5]. Plenty of energy is released from the brown fat by the aid of mitochondriae and the temperature in it increases. The heat is distributed to the anterior part of the body so that in the arousing animal this becomes warm sooner than the hind part of the body. The level of the total lipids in the blood serum of the hedgehog is very constant during the year[6].

Zusammenfassung. Es wird gezeigt, dass der Gehalt der freien Fettsäuren im Blutserum des Igels während des Erwachens aus dem Winterschlaf sehr kräftig steigt. Die Lipolyse ist schon bei einer Körpertemperatur von + 6 °C sehr beträchtlich. Das braune Fettgewebe ist während des Erwachens sehr aktiv.

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Experimental Production of Chick Embryo Neural Tube Opening 'in vitro'

Mechanisms underlying the production of congenital malformations known under the name of dysraphia have been a matter for discussion since the 18th century (GARDNER[1]). Several authors have studied human foetuses with the malformations mentioned, although the state of preservation of this material was not sufficiently good to permit detailed analysis. PATTEN[2] studied 3 well-preserved human foetuses and observed that an overgrowth of neural tissue was present in the unclosed portion.

An experimental analysis of the problem was undertaken by ANCEL[3] who produced spina bifida in chick embryos through the use of diverse drugs; FOWLER[4] obtained similar results using mechanical means; BRACHET[5] succeeded in interfering with neural tube closure in amphibian embryos through the use of several sulphur-containing substances, and POHL and BRACHET[6] had obtained similar results using the same substances on whole chick embryos maintained in vitro.

In a previous work (ADLER and NARBAITZ[7]) we observed that spinal cord portions of 44–48 h chick embryos, when cultured on media containing rat submaxillary gland extracts (SGE), reopened in a short lapse of time and afterwards showed an increased growth of neural tissue; the histological picture was very similar to that observed by PATTEN in the malformed foetuses. This fact showed that the technique used by us could be used to analyze the action of substances on neural tube. In the present work we study the action on neural tube of SGE and 2 proteolytic enzymes.

Material and methods. White Leghorn chick embryos were used in all cases. 9 series of experiments were made, as described in the Table. The explants were removed at stages 11–13 (HAMBURGER and HAMILTON[8]). Neural tubes were sectioned at the spinal cord level; explants were 4 somites long, and the ectoderm and endoderm were cut laterally to the external limit of somites (see ADLER and NARBAITZ[7]).

Cultures were made following the technique of WOLFF and HAFFEN[9] with a slightly modified medium (medium 'A', ADLER and NARBAITZ[7]). In series 1–4 unmodified submaxillary gland extracts prepared as described in the same work were added to the medium. SGE dyalized against various changes of Hank's solution at pH 7.4 and 4 °C was used in series 5, while SGE heated at 90 °C for 10 min was added to media in series 6. These SGE modifications and the unmodified extracts were added to media in a final concentration of 0.5 mg of protein/ml being the protein concentration of the extracts, determined following the technique of LOWRY et al.[10].

Two proteolitic enzymes were assayed. Trypsin (1:250, Difco) was added to the medium in a concentration of 0.07–0.5 mg/ml; papain (N.F., Difco) was used in a concentration of 0.06 to 0.6 mg/ml.

Cultures were maintained at 37 °C and observed under a dissecting microscope at various intervals. The explants were fixed in Bouin's fluid and subjected to routine histological procedures; some of the explants were stained with the PAS technique. An approximate measure of the volume of neural tissue cultivated was obtained by projection and drawing of the 10μ serial sections on thick paper and weighing the resulting paper images (paper weight).

Results. The first series of experiments (see Table) was undertaken in order to discover if the neural tube aperture

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produced by SGE and described previously[7] was a reversible phenomenon. As in the experiments mentioned, after 6 h of culture some of the explants showed opened neural tubes, while after 24 h all of them were widely open. If after 6, 12 or 24 h explants were changed to media without SGE, neural tubes began to close again; when 72 h of culture were completed the aspect of these explants was similar to controls.

In order to gain knowledge on the possible chemical nature of the active substance in SGE, 2 additional series of experiments were made: SGE maintained its action after 24 h dyalisis (series 5), but lost its activity when heated at 90 °C for 10 min (series 6).

Trypsin added to the culture medium (0.07 mg/ml) produced the opening of neural tubes on the second day of culture. Neural tissue volume was shown to be higher in these explants (paper weight: 2.97 g) than in controls (paper weight: 1.64 g) this difference being statistically significant. Higher doses of trypsin proved to be toxic and produced diverse degrees of lysis and disorganization.

The addition of papain did not show any detectable action on cultured explants.

PAS technique permits the intense staining of the basal membrane which surrounds the neural tube. Although the continuity of this membrane is broken in the zone where the neural tube opens, its aspect appears normal in both SGE and trypsin-treated explants (Figure).

Discussion. PATTEN[2] showed that a notable overgrowth of neural tissue accompanies the opened portions of neural tube in dysraphic human foetuses. He concludes that 'myeloschisis may be the result of local overgrowth which interferes with its closure'. Nevertheless, FOWLER[4] produced spina bifida in chick embryos by opening with mechanical means the roof plate of the neural tube, and finding that an overgrowth of neural tissue followed the aperture. In agreement with this, in our results the proliferation of neural tissue was also secondary to tube aperture. Although these results obtained experimentally in chick embryos cannot be compared exactly with what happens in human foetuses, they tend to suggest that overgrowth may not be the cause, as PATTEN[2] supposes, but the consequence of neural tube opening.

On the basis of the histological observation of one dysraphic human foetus, LEMIRE[11] proposed that an alteration of the neural tube basal membrane may be the initial event in the genesis of the malformation. Although we have again to warn against comparing experimental findings with human pathological facts, we must remember that in our experiments tube opening and tissue proliferation occur without any visible alteration of the basal membrane.

An alteration of neural tube closure has been obtained experimentally in vivo[3,5] and in vitro[6] through the action of diverse substances. Our experiments differ from all of them in the fact that opening of previously closed neural tube, instead of interference with tube closure, is analyzed. The results of these types of experiments may be specially valuable, taking into account that it has been suggested[1] that human dysraphia may also be the result of secondary opening of a closed tube.

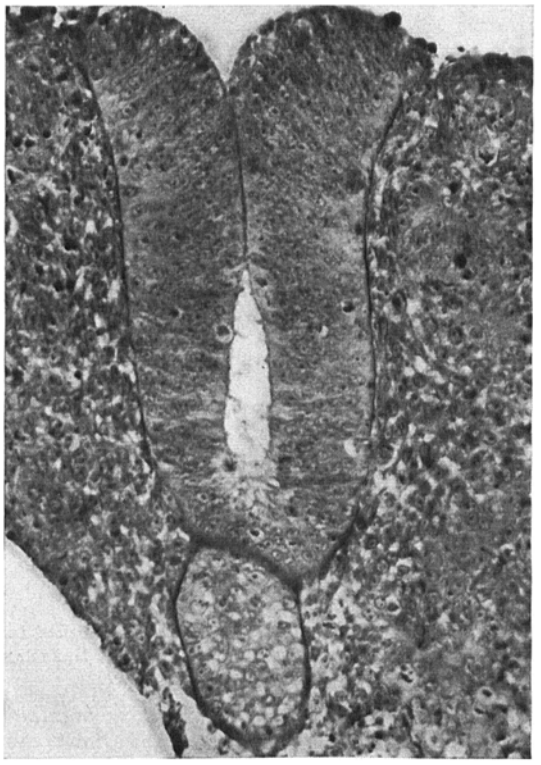
The fact that neural tubes which have been opened by the action of SGE close again, and develop normally if transfered to normal culture media, indicates that the extract produces its action without affecting definitively cell vitality.

The maintainance of SGE action after dyalisis and its inactivation by heat suggest that the active substance may be of proteic nature[12].

The different series of experiments

Series	No. of explants	1st culture period Medium[a]	Duration	2nd culture period Medium[a]	Duration
1	45	A plus SGE	6h	A	66 h
2	45	A plus SGE	12 h	A	60 h
3	45	A plus SGE	24 h	A	48 h
4	430	A plus SGE	6–12–24–72 h		
5	90	A plus dialysed SGE	24–72 h		
6	60	A plus heated SGE	24–72 h		
7	594	A plus Trypsin	24–72 h		
8	86	A plus Papain	24–72 h		
9	910	A	6–12–24–72 h		

[a] Medium 'A': see composition in ADLER and NARBAITZ[7].



Explant cultured for 24 h. Medium with submaxillary gland extract. PAS-hematoxylin × 380.

Resumen. La reapertura del tubo neural de embrión de pollo, obtenida «in vitro» mediante la acción de extractos de glandula submaxilar de rata, o de tripsina, es analizada.

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[12] Acknowledgments. This work was supported by a grant of the Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina. R. ADLER holds a Fellowship and R. NARBAITZ a permanent position in the same institution.