Discussion. 'Paralymphatic' pathways devoid of an endothelial lining and lymph vessels that play a vital role in the fluid circulation of intracranial structures, can readily be visualized by means of an intracisternal injection of lipiodol ultrafluid.

According to DAVSON[1] 'there is no free passage of fluid from the peri-optic subarachnoid space into the retinal tissue, the latter space being apparently sealed off at the lamina cribrosa'.

Our studies disprove this view and support the idea[2] that conjunctival and orbital lymph vessels, taking part in the fluid circulation of the eyeball proper, are in direct communication with the intracranial fluid circulation. The possibility of artifacts in the above studies can completely be excluded.

Zusammenfassung. «Paralymphatische» Bahnen sowie Lymphgefässe, welche im Flüssigkeitskreislauf der intrakranialen Strukturen eine wichtige Rolle spielen, können im Tierversuch durch intrazisternal injiziertes Lipiodol ultrafluid dargestellt werden. Nach DAVSON[1] scheint die Lamina cribrosa das freie Eindringen einer Flüssigkeit aus dem perioptischen Subarachnoidealraum in das Retinalgewebe zu verhindern. Auf Grund der beschriebenen Befunde, welche die Möglichkeit von Kunstprodukten mit Sicherheit ausschliessen, vertreten die Autoren den Standpunkt, dass die Lymphgefässe der Bindehaut und der Orbita mit dem intrakranialen Flüssigkeitskreislauf in direktem Zusammenhang stehen.

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The Content of Non-Esterified Fatty Acids and Glycerol in the Blood of the Hedgehog During the Hibernation Period[1]

The periodic spontaneous arousals of hedgehogs during the winter[2] give a good opportunity to investigate the influence of the body temperature upon the content of non-esterified fatty acids (NEFA) and glycerol in the blood of the hedgehog (*Erinaceus europaeus* L.).

Throughout the winter the hedgehogs must live on food reserves – fat deposits – which they have built up in their bodies. At least in hibernating (hypothermic) animals, the RQs are those of lipids.

For demonstration of the changes related to the hibernation cycle (to the periodicity of hibernation) in midwinter (late January) the 6 following groups were created on the basis of continuously monitored body temperature recordings via thermocouples implanted s.c. in the interscapular region[2].

Group 1. Animals at about the middle of their hypothermia period from 4–5 days in deep hypothermia. Body temperature (TB) less than 1 °C higher than prevailing ambient temperature (TA) in a cold animal room (TA constantly at 4.2 ± 0.5 °C).

Group 2. Spontaneously arousing animals with a body temperature of 6 °C (thermocouples implanted s.c. in the interscapular region).

Group 3. Spontaneously arousing animals. TB has reached 15 °C when analyzed.

Group 4. 'Fully aroused animals': as a result of the spontaneous arousal process the recorded TB had reached its 'normal' level.

Group 5. Animals entering hypothermia with TB of 20 °C.

[1] Dedicated to Prof. H. MISLIN on the occasion of his 60th birthday.

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The content of non-esterified fatty acids and glycerol in the blood of hedgehogs. Means ± SE (and number of animals) are presented.

	NEFA meq/l	Glycerol μM/ml
Animals awake in late summer (7.IX)	0.396 ± 0.039 (5)	–
Animals awake in late autumn (27.-29.X) just before the onset of hibernation	0.492 ± 0.034 (6)	0.306 ± 0.031 (8)
Hypothermic animals in the beginning of hibernation (1.XI-16.XI)	0.555 ± 0.040 (6)	0.208 ± 0.019 (5)
Group 1. In deep hypothermia. Late January	0.659 ± 0.053 (7)	0.187 ± 0.005 (6)
Group 2. 'Arousing'. TB + 6 °C. Late January	1.381 ± 0.057 (7)	0.333 ± 0.039 (6)
Group 3. 'Arousing'. TB + 15 °C. Late January	1.156 ± 0.102 (7)	1.440 ± 0.210 (4)
Group 4. 'Fully aroused animals'. Late January	0.993 ± 0.074 (7)	0.436 ± 0.043 (7)
Group 5. Entering hypothermia. TB + 20 °C. Late January	0.517 ± 0.042 (7)	–
Group 6. Entering hypothermia. TB + 10 °C. Late January	0.613 ± 0.057 (7)	0.228 ± 0.036 (4)
Animals awake in late spring-summer (18.V-11.VI)	0.412 ± 0.024 (13)	–
Animals awake in summer (16.VI-8.VII)	0.399 ± 0.034 (10)	–

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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