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STUDIES ON THE BRAIN CAPILLARIES OF PREGNANT RATS AFTER EXPERIMENTAL HYPOXIC EXERCISES

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Pathological changes of the blood-brain barrier can be induced experimentally by different procedures, and they can be detected in certain human diseases. The actual state of the barrier is important in the regulation of the neuronal activity through the blood supply. Nowadays, increasing interest comes to the hypoxic and hypokinetic exercises, because the flying and astronautics are more and more widespread. Beside this, the hypoxic exercise could give new data on the genetical malformations and other pathological alterations which are frequent with people living on high mountains (Alzamora et al. 1953, Ingalls 1960).

In our present studies we wanted to investigate the effect of the hypoxic and hypobaric environment on the fine structure of the blood-brain barrier of pregnant rats. On the other hand, using biochemical techniques, the ability of prostacyclin and prostaglandin synthesis after the exercises was also determined in the isolated brain capillaries in the kids of the rats that had been subjected previously to the exercises.

In the first group adult pregnant Wistar rats were placed in a low pressure chamber, which equals to 7000 m height above sea level for 6 hours. The animals in every group stayed altogether 42 hours with interruption of two days. In the second group oxygen tension was raised up to the normal atmospheric value maintaining the hypobaric state. In the third group hypobaric and hypoxic rats were treated with 50 mg progesterone capronate divided in four equal doses for the duration of exercise. The fourth group was the control one. For the electron microscopic investigations one day before the expected time of birth the animals were perfused with Krebs-Ringer solution and then with Karnovsky solution containing 5 per cent glucose. After decapitation small pieces of the parietal cortex were fixed in the latter solution for 4 hours at 4°C. After postfixation and dehydration, the samples were embedded in Durcupan (Fluka). Sections of gold and silver interference colours were cut on Porter-Blum ultramicrotome. After staining with uranyl acetate and lead citrate the sections were viewed under a JEOL 100B electron microscope.

Fig. 1 shows the ultrastructure of the blood-brain barrier of a rat after hypobaric and hypoxic exercises.

The transendothelial transport seems to be enhanced comparing to the controls. Actually, some signs of the oedematous swelling of the glial end feet system could be seen. The majority of capillary endothelial cells was found more or less contracted which is undoubtedly represented by the well pronounced contractions of the nuclei (Fig. 2). When the oxygen level was adjusted in the chamber to the normal value maintaining the hypobaric environment, only milder alterations were observed in the endothelium, but in the basal lamina, formation of collagen-like fibers was seen (Fig. 3). This kind of fiber-formation was observed after ATPase inhibition (Joó 1979). The prolonged progesterone treatment minimized the changes in the pregnant rats kept in hypoxic and hypobaric atmosphere. The fine structure of capillaries in this group was similar to those of control animals (Fig. 4).

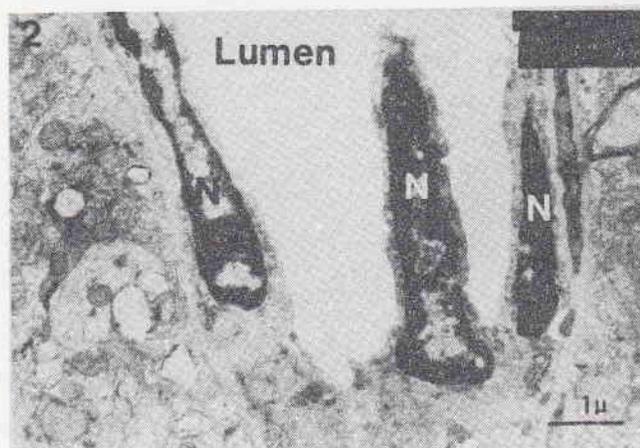
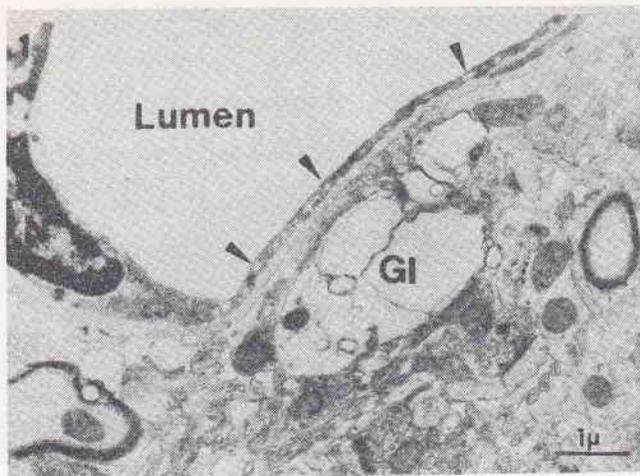
The other part of pregnant rats exercised in the same ways let give birth to their kids. The newborn animals were kept under normal laboratory conditions till the 8th week of life, which time had been reported as the end of sexual maturation (Piacsek 1978). Capillary-rich fractions were prepared from all groups of animals according to the method of Joó and Karnushina (1973). The prostacyclin and prostaglandin synthesis of the capillary fractions were assayed with $l\text{-}^{14}\text{C}$ -arachidonic acid as substrate in the presence of 2 mM reduced glutathion and 1 mM norepinephrine as cofactors. The incubations and determinations were made according to our earlier determination (Gecse et al. 1980).

Figure 5 shows the distribution of synthesized products from arachidonic acid in the different groups of rats. The synthesis of prostacyclin, which is represented by its metabolite, 6-oxo-PGF₁₀, as well the PGD₂ remained unchanged in all four groups. The most striking effect of hypoxia is expressed in the enhanced synthesis of PGF₂₀ and PGE₂. The increase is undoubtedly due to the hypoxic conditions, because the synthesis in animals kept in hypobaric normoxic atmosphere was similar to the control values. Progesterone treatment of the mothers normalized the changes in synthesizing ability of the kids. The ultrastructure of the brain capillaries of the kids were without any oedematous changes.

In an other experiment, we investigated the effect of similar exercises on adult male rats. After 92 hours exercises, oedematous ultrastructural alterations were not seen. Preparing capillary-rich fraction from the brain, milder alterations of the prostaglandin synthesizing ability could be seen than in the first group. In the adult male animals, the synthesis of PGF₁₀ and PGE₂ was also enhanced but to a lesser degree. The stimulation of synthesis of PGF₁₀ was 40 per cent and this of the PGE₂ was 80 per cent of the stimulations in the capillaries of the kids.

The hypoxic and hypobaric exercises seem to have damaged the blood-brain barrier of pregnant rats but the alterations were reversible and could be prevented by prolonged administration of progesterone. This protective effect is probably due to the well-known anti-oedematous effect of steroids (Reulen and Schürmann 1972).

It has recently been published that, among other tissues, the blood vessels are able to synthesize prostacyclin and prostaglandins (Raz et al. 1977). The synthesized PGs might contribute to the regulation of vascular tone (White et al. 1971, Denton



Figs. 1-2. The fine structural appearance of brain capillaries in rats subjected to hypoxic and hypobaric exercises. Signs of increased transendothelial transport are labelled by arrowheads. Gl : swollen glial process, N : contracted endothelial nucleus.

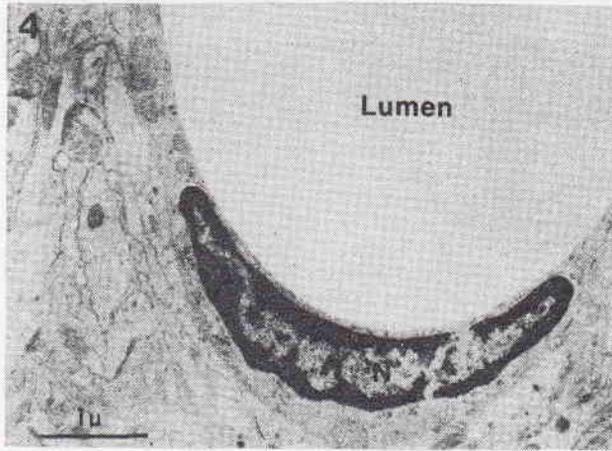
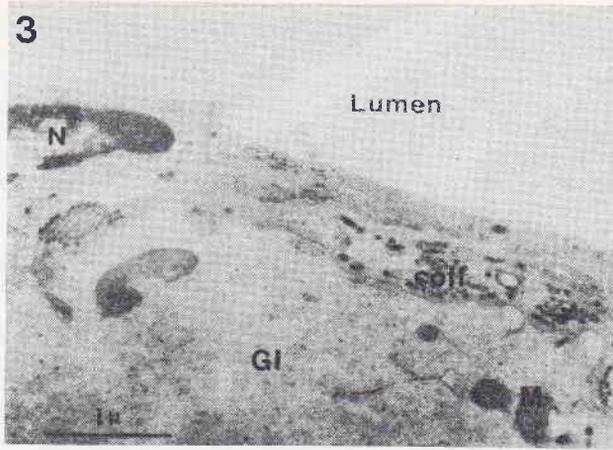


Fig. 3. Collagen-like fibers (coll) have appeared in the basal lamina in rats after hypobaric normoxic exercises.

Fig. 4. Progesterone treatment of rats subjected to hypoxic and hypobaric exercises prevented the pathological changes of brain capillaries.

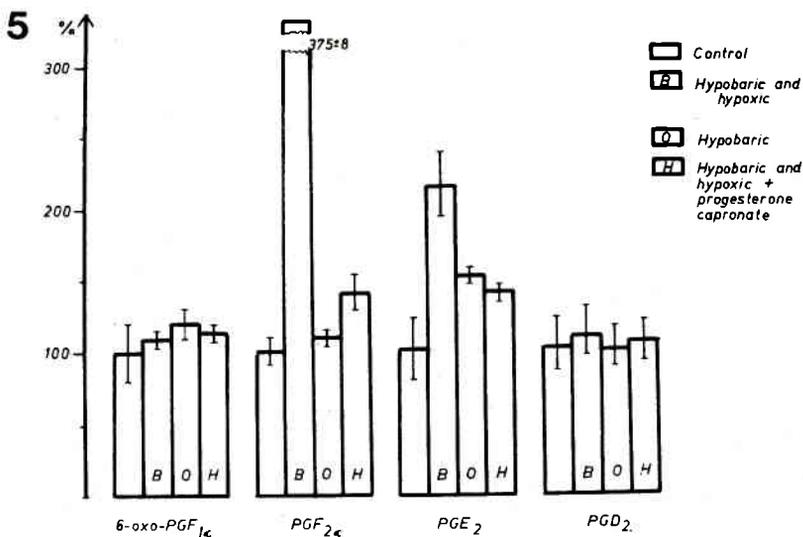


Fig. 5. The prostacyclin and prostaglandin synthesis in isolated brain capillaries of rat.

et al. 1972). In our experiment, the prostaglandin synthesizing ability of components PGF_{2α} and PGE₂ were stimulated even two months after the exercises in the kids. It is very probable that the oxygen dependent fatty acid cyclo-oxygenase enzyme is responsible for the increased synthesizing ability (Samuelsson et al. 1975). Intrauterine, the enzyme probably was adapted to the lower oxygen supply in those animals which were kept in hypoxic atmosphere. After birth, the normal partial pressure of oxygen was too high for the adopted enzyme and probably that is why the produced quantity of the two fractions were higher. The milder alterations in adult animals can be explained with the lower inductivity of enzymes in adult rats. The fact that both in kids and adult animals there were the PGF_{2α} and the PGE₂ involved suggests that these types of prostaglandins are responsible in the changes of vascular tone after such hypoxic exercises. Since the PGF_{2α} is known to have vasoconstrictor effect and the PGE₂ vasodilator on the brain capillaries (White et al. 1971, Denton et al. 1972) the enhanced synthesis of both fractions represents a balance at a higher level.

Summarizing the results of our present investigations, the hypoxic and hypobaric environment seems to be important in the actual state of the blood-brain barrier. The observed structural changes suggest that examination of the contractile proteins would be informative for the forthcoming studies in this field.

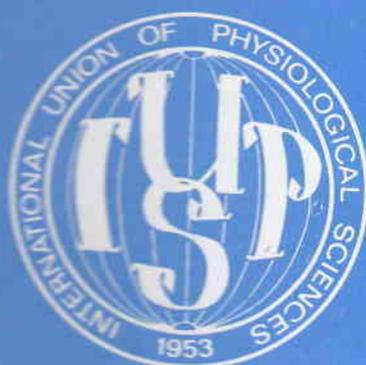
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ABSTRACTS
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AND VOLUNTEER
PAPERS

STUDIES ON THE BRAIN CAPILLARIES OF PREGNANT
RATS AFTER EXPERIMENTAL HYPOXIC EXERCISES.

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Biol. Res. Ctr. Hungarian Acad. Sci. Szeged,
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Adult pregnant Wistar rats were placed in a chamber, where the atmospheric pressure and oxygen tension could be experimentally adjusted. Rats were kept at 300 Hgmm with 65 Hgmm oxygen tension which equals to 7000 m height above sea level for 6 hours. The animals in every group stayed altogether 42 hours with interruption of two days. In the second series oxygen tension was raised up to the normal atmospheric value maintaining the hypobaric state. Hypobaric rats were treated with 50 mg progesterone capronate divided in four equal dose for the duration of exercise. Damage of brain capillaries consisting of increased number of pinocytotic vesicles, thickening of the basement lamina and swelling of glial end feet system in hypoxic brains were seen under the electron microscope. Progesterone treatment seemed to minimize the edematous alterations.

1290

PLASTICITY OF INTEROCULAR ORIENTATION DIFFERENCES IN CAT VISUAL CORTEX. M. R. Dureteler and R. von der Heydt. Dept. of Neurology, Univ. Hospital, Zurich, Switzerland

In the normal cat, the binocular cortical units have preferred orientations which are similar in the two eyes. We wondered whether this correspondence of orientations could be modified by an incongruent binocular environment. Four kittens were reared wearing goggles equipped with two meridional size lenses (axes of magnification oriented 45° left and right of vertical). Without affecting focus quality, these goggles produced simultaneously orientation disparities in opposite directions for horizontal and vertical contours (the respective image orientations in left eye/right eye were -3°/+3° and 93°/87°, for example). These disparities could not be compensated by eye torsions. At age 3-4mo, the preferred orientations of cortical units were determined and used to calculate interocular differences. We then compared these results for units with near-horizontal and near-vertical orientations. Their mean values differed in the predicted directions for all four cats. The effect was roughly of the expected size in two animals but smaller in the others. We conclude that a binocularly incongruent environment leads to cortical compensation during development.

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THE PRL, LH, E₂, T, PGS, CORTICOIDS, T₃ ESTIMATIONS IN SOWS PLASMA DURING LACTATION AND SOME DAYS AFTER WEANING. L. Dusza, H. Krzymowska, S. Okrasa, J. Czernyszewicz, G. Kotwica, M. Koziorowski, S. Kuźnia, J. Czarnocki, J. Dębek, R. Nowicka. Institute of Animal Physiology and Biochemistry Academy of Agriculture and Technology, Olsztyn, Poland

The level of mentioned hormones in suckling sows kept in conditions of a big industrial farm was estimated using RIA method. The size of litter influenced not only the PRL level but also some other hormones. The individual differences in the quantity of determined hormones and the speed of decreasing of their level in course of lactation were stated.

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STUDIES ON THE BRAIN CAPILLARIES OF PREGNANT RATS AFTER EXPERIMENTAL HYPOXIC EXERCISES. E. Dux, *L. Dux, F. Joó, †L. Bognár, †P. Remes, †J. Hídeg. Biol. Res. Ctr. Hungarian Acad. Sci. Szeged, *Inst. Biochem. Med. Univ. Szeged, †Health Service of Hungarian People's Army, Kecskemét, Hungary.

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CHANGES OF PROTEOLYTIC ENZYMES IN SKELETAL MUSCLES AFTER CASTRATION
Dux, L. Sohár, I. Nagy, I. Guba, F. Inst. of Biochemistry, Univ. Med. Sch. Szeged, Hungary

The role of the proteolytic processes in the anabolic effects of the androgenic hormones was under study. Male Wistar rats were castrated before as well as after the puberty. 1, 2, 3 and 4 weeks later the activities of several proteolytic enzymes were determined in the levator ani, soleus, semimembranosus and extensor digitorum longus muscles. The measurements were made in muscles of castrated rats with testosterone-propionate, progesterone-capronate and "LEVADOSIN" substitution too. The changes of the proteolytic enzyme activities after castration, and the inhibition of this by androgenic hormone, suggests the participation of the suppression of the protein degradation in the anabolic effects on muscles. This participation seems to be different, depending on the age of the animals, and on the fibre composition of the muscles. The involvement of the different muscular proteolytic enzymes seems to be different too.

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SPATIAL GROWTH OF TUMORS: A SIMULATION STUDY. W. Düchting, G. Dehl. Dept. of Electrical Engineering, Univ. of Siegen, D-5900 Siegen 21, West-Germany

The present contribution tries to determine the spatial structure and the time behaviour of cell renewal systems. For this purpose a computer model for the two-dimensional cell space was developed by which selected case studies are simulated on a large digital computer (CYBER 76). Special emphasis was laid on

- the possibility of growing several cell systems with different mean life spans in competition,
- the variability of the mean life span of a cell and of the initial configuration,
- the description of mutual influences among the individual cells, their elimination comparable with a surgical removal or an irradiation and
- the consideration of tumor-cell loss.

In the future it seems to be possible to partly substitute long and expansive biological experimental test series by simulation with the help of these models.

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FEEDBACK ACTION OF ESTRADIOL IN 15 DAY OLD FEMALE RATS. E. Düker, K. Honma, W. Hilgendorf, Max-Planck-Inst. für biophysikalische Chemie, Göttingen, Germany

At the age of 15 days, female rats have high Estradiol (E2) and FSH levels, relatively high α -feto-protein (E2 binding protein) values and LH peaks predominantly during afternoon hours. The LH peaks might be spontaneous or due to a positive or an absence of negative feedback of E2. To examine E2 action, 8 days old rats were ovariectomized (ovx) and transplanted with E2 containing silastic tubes of different lengths. On day 15, sequential blood samples were taken at 1 hr intervals. In intact and in ovx rats transplanted with very short (1 mm) or very long (3 x 20mm) E2-implants basal LH levels were higher and more LH peaks occurred during PM than during AM hours. No LH peaks were observed in rats bearing intermediate length E2 implants (5, 10, 2x20 mm). The anti-estrogen tamoxifen completely blocks the occurrence of LH peaks in intact animals at doses which are ineffective in altering serum LH levels in ovx rats. The results demonstrate that E2 maintains a diurnal rhythm in 15 day old rats and that spontaneous LH peaks are E2 dependent.