Aus statistischen Gründen wurden Gewebeproben von 6 Tieren für jeweils eine Bestimmung verwandt. Es erfolgten mindestens 5 Bestimmungen für Haut bzw. Muskulatur. Die Elutionsdiagramme der durchgeführten Aminosäureanalysen lieferten insgesamt 39 peaks, von denen 29 eindeutig identifiziert werden konnten.

Der erste unbekannte peak erschien im Eluat nach Taurin und war offensichtlich von mehreren Verbindungen geringerer Konzentration überlagert (Peptide?). An dieser Stelle im Eluat ist auch Harnstoff zu erwarten, konnte jedoch wegen der Überlagerug nicht eindeutig identifiziert werden. Zwei weitere unbekannte Verbindungen erschienen im Eluat nach Alanin, eine nach β-Alanin. Während der basischen Analyse erschienen drei unbekannte peaks vor Trytophan; einer vor und zwei nach Ammoniak. Wegen der geringen Konzentration ($\ll 0.1$ nM/TG) dieser Verbindungen wurde auf ihre Identifizierung und quantitative Auswertung verzichtet. Cystein und Methionin konnten weder in der Haut noch in der Muskulatur registriert werden. Offensichtlich sind sie im Extremitätengewebe des Axolotl in freier Form nur in äusserst geringer Konzentration vorhanden. Asparagin und Glutamin hätten sich nur schlecht voneinander trennen lassen, sie wurden deshalb gemeinsam ausgewertet. Wie die Tabelle zeigt, sind die Aminosäurepools von Haut und Muskulatur der Axolotlextremität in ihrer qualitativen Zusammensetzung gleich. Eine Ausnahme bildet Cystathionin, das nur im Bereich der Muskulatur nachweisbar ist. Die quantitative Zusammensetzung der Gewebepools zeigt jedoch klare Unterschiede.

Nachweisbare ninhydrinpositive Verbindungen in Muskulatur und Haut der Vorderextremität von *A. mexicanum*

	Muskulatur	Haut
Alanin	4,89 ± 0,13	4,00 ± 0,19
β-Alanin	+	+
Arginin	2,04 ± 0,11	0,41 ± 0,01
Asparagin/Glutamin	4,52 ± 0,11	2,21 ± 0,10
Asparaginsäure	1,45 ± 0,04	2,93 ± 0,21
Carnosin	31,87 ± 2,06	1,04 ± 0,28
Citrullin	+	+
Cystathionin	0,33 ± 0,03	–
Cysteinsäure	2,19 ± 0,06	1,12 ± 0,05
Glutaminsäure	11,27 ± 0,34	13,12 ± 0,69
Glycerophosphoäthanolamin	0,55 ± 0,02	0,41 ± 0,01
Glycin	3,24 ± 0,10	1,29 ± 0,08
Histidin	1,34 ± 0,05	0,61 ± 0,03
Isoleucin	0,45 ± 0,02	0,57 ± 0,06
Leucin	0,97 ± 0,02	1,21 ± 0,17
Lysin	5,08 ± 0,19	1,13 ± 0,03
Methioninsulfoxid	+	+
1-Methylhistidin	0,39 ± 0,02	0,59 ± 0,06
Ornithin	+	+
Phenylalanin	0,32 ± 0,01	0,39 ± 0,04
Phosphoäthanolamin	0,36 ± 0,03	5,14 ± 0,23
Prolin	1,41 ± 0,12	1,23 ± 0,04
Sarkosin	+	+
Serin	3,08 ± 0,06	1,89 ± 0,03
Taurin	17,05 ± 1,10	0.93 ± 0,06
Threonin	3,89 ± 0,26	3,71 ± 0,18
Tryptophan	< 0,1	< 0,1
Tyrosin	0,37 ± 0,01	0,38 ± 0,03
Valin	0,74 ± 0,01	1,08 ± 0,06

Angegeben ist der Gehalt in Nanomol pro Milligramm (nM/mg) Trockengewicht (TG) und der absolute mittlere Fehler des Mittelwertes.

Unter den «*Nicht-Protein-Aminosäuren*» fällt in der Muskulatur der hohe Gehalt an Taurin und besonders an Carnosin auf. Beide Verbindungen zusammen machen fast 50% des Gesamtpools aus. Die Haut andererseits charakterisiert sich durch den vergleichsweise enormen Gehalt an Phosphoäthanolamin, der das 14fache der Muskulatur beträgt.

Bei den «*Protein-Aminosäuren*» sind ebenfalls grosse quantitative Unterschiede zwischen Muskulatur und Haut zu verzeichnen. Die Lysin-Konzentration übersteigt beispielsweise in der Muskulatur fast das Vierfache, die Glycin- und Serinkonzentration das 2-, bzw. 1,5fache der entsprechenden Konzentrationen in der Haut. Diese weist andererseits gegenüber der Muskulatur den doppelten Gehalt an Asparaginsäure auf.

In beiden Geweben dominiert Glutaminsäure, möglicherweise Ausdruck der zentralen Bedeutung dieser Aminosäure im Intermediärstoffwechsel. Die als essentiell bekannten Aminosäuren Threonin, Isoleucin, Leucin, Phenylalanin, Histidin und Tryptophan erscheinen in sehr geringer Konzentration. Ist Threonin mit 3,7 bzw. 3,9 nM/mg TG noch verhältnismässig stark vertreten, so liegt Tryptophan mit weniger als 0,1 nM/mg TG bereits an der quantitativen Nachweisgrenze des gewählten Analysenverfahrens.

Summary. On investigating the free amino acid pool in skin and muscles of the stylopode of *Amblystoma* forelimbs, 39 ninhydrine positive substances could be detected, from which 29 have been identified. High quantitative differences between skin and muscles could be shown.

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Polysome Profile and Free Alkaline Ribonuclease Activity of Placenta and Maternal Liver of Intact, Starved and X-irradiated Mice

Protein synthesis is known to be affected by ionizing radiations[1–3]. Enhanced protein synthesis in liver following irradiation has been correlated with increase in polysomes[2,3]. Starvation also induces changes in polysome profiles[4,5]. This study is on hepatic and placental polysome profiles of fed, starved and X-irradiated mice. The activity of free alkaline ribonuclease has also been measured.

Materials and methods. 3-month-old Swiss mice were used on the 15th day of gestation in accordance with an earlier study[6]. The mice were X-irradiated at a dose of 1000 R, 100 R/min[1,7]. All except the starved mice were fed ad libitum a balanced diet. During the 24-h period of starvation the animals received only water. The X-irradiated mice were sacrificed at either 2 or 8 h after exposure. All the animals were sacrificed by cervical dislocation; placenta and maternal liver were collected for further processing.

For the study of polysome profiles, the tissues were homogenized in a medium containing 0.25 M sucrose and

Effect of starvation and X-irradiation on free alkaline ribonuclease activity in placenta and maternal liver of mice

Treatment	Tissue	
	Maternal liver	Placenta
None (control)	0.28 ± 0.014 (8) [a]	0.31 ± 0.016 (8)
Starvation (24 h)	0.67 ± 0.038 (5) $P < 0.001$	0.63 ± 0.040 (5) $P < 0.001$
X-irradiation (2 h after exposure)	0.50 ± 0.038 (5) $P < 0.001$	0.55 ± 0.040 (5) $P < 0.001$
X-irradiation (8 h after exposure)	0.35 ± 0.018 (5) $P > 0.05$	0.44 ± 0.024 (5) $P > 0.05$

Difference in O.D. at 260 nm/mg protein/30 min. [a] Values are mean ± S.E.M. Treated groups are compared with control. Number of animals in each group is given in parenthesis.

0.5 m*M* EDTA in 50 m*M* *Tris*, 200 m*M* NH_4Cl, 150 m*M* KCl, 10 m*M* $MgCl_2$ buffer at pH 7.6[8]. The subsequent steps involved in the preparation of ribosomes and polysomes were the same as those employed in a recent study[9]. The O.D. of the fractions was measured at 260 nm in a Beckman DB-G spectrophotometer.

Free alkaline ribonuclease activity was estimated by the method of KRAFT et al.[10]. Protein was assayed by the method of LOWRY et al.[11].

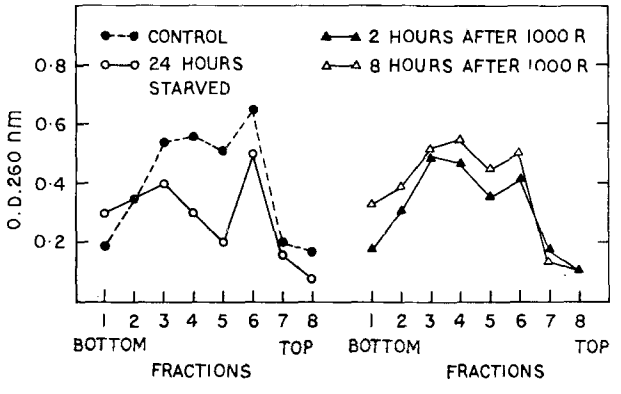

Fig. 1. Liver polysome profiles of control and treated 15-day pregnant mice.

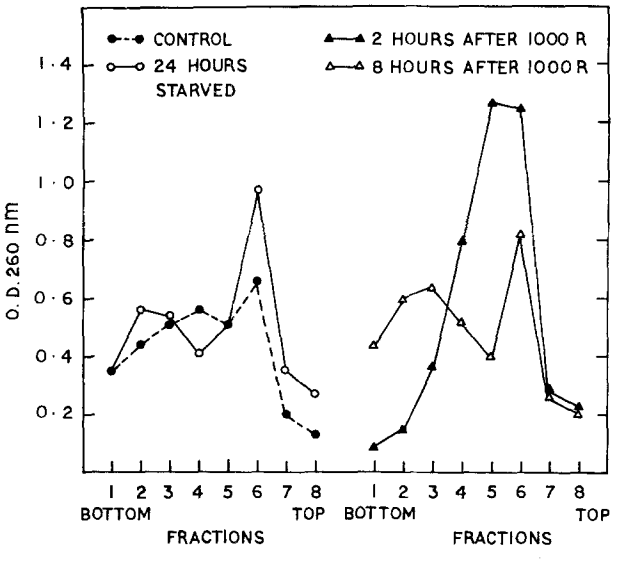

Fig. 2. Placental polysome profiles of control and treated 15-day pregnant mice.

Results. Polysome profiles of maternal liver and placenta of fed, starved and X-irradiated mice are presented in Figures 1 and 2. Starvation for 24 h results in a marked disaggregation of polysomes in placenta and liver. X-irradiation, however, seems to have a time-dependent effect on the polysomes. The radiation-induced disaggregation of polysomes 2 h after exposure is more pronounced in placenta than in liver. The proportion of disomes and monosomes to polysomes is higher in placenta than in maternal liver where only a slight change in the heavy-light polysome equilibrium occurs. An enrichment of polysomes is observed 8 h after irradiation in liver but not in placenta.

Free alkaline ribonuclease activity in control, starved and X-irradiated mice is shown in the Table. The enzyme activity in the control (fed) animals seems to be almost the same in placenta and maternal liver. The level of the enzyme is significantly enhanced ($P < 0.001$) 2 h after X-irradiation and 24 h after starvation. The enzyme activity tends to return to control value 8 h following irradiation.

Discussion. The present results on radiation-induced disaggregation of polysomes and a corresponding change in free alkaline ribonuclease activity supplement our earlier observations[1] on in vivo protein synthesis following X-irradiation. Enrichment of polysomes has been observed in liver of guinea-pigs[2] and rats[3] after irradiation. Enhanced incorporation of amino acids into hepatic proteins[1] and increase in polysomes 8 h after irradiation, thus, agree with their findings. A pronounced shift in the heavy-light polysome equilibrium occurring in placenta 2 h after irradiation appears to be related to the enhancement in free alkaline ribonuclease activity. The possibility that endogenous nucleases might disaggregate polysomes during their preparation has been eliminated by taking precautions as suggested by LAGA et al.[8].

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A reduction in heavy polysomes after starvation has been observed in liver[4,5]. Our results show that starvation for 24 h causes disaggregation of polysomes and increase in free alkaline ribonuclease activity. Such alterations in polysomes following starvation have been attributed to increase in ribonuclease activity[5].

Résumé. Le profil des polysomes et l'activité de la ribonucléase alcaline libre se modifient dans le placenta et le foie maternel des souris à la suite d'inanition et de l'exposition aux rayons X.

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Formation of Lipoperoxide in Brain Edema Induced by Cold Injury

The mechanism of the occurrence of brain edema is one of the most urgent problems to be elucidated in the field of neurosurgery. As a mechanism of the occurrence of brain edema, DEMOPOULOS et al.[1] suggested that free radical reactions may be involved in the damage to the membrane structure in the brain. However, no such experimental data have been reported. Since the provocation of brain edema by cold injury, according to the method of KLATZO et al.[2], has been well established, we tried to adopt this experimental edema for demonstration of the participation of free radicals in this disorder.

Male Wistar rats, 150–200 g, were used as experimental animals. Cold injury was made as described by KLATZO et al.[2]. After the rats were anesthesized with urethane (1 g/kg body weight, injected i.p.), the parietotemporal cranial bone was exposed and fenestrated in a round form (1.5 mm in diameter). A column of dry ice was attached lightly to the dura mater for 20 sec. At definite time after the cold injury, the brain was removed and the right hemisphere was taken for the analysis of lipoperoxide. Lipoperoxide was determined by thiobarbituric acid method[3] modified by NISHIGAKI et al.[4] as follows. The hemisphere was homogenized in 5 ml of cold 0.9% NaCl solution and 4 ml of the homogenate was mixed with 1 ml of thiobarbituric acid reagent (0.67% thiobarbituric acid aqueous solution + glacial acetic acid, 1:1, v/v). The mixture was heated at 100 °C for 1 h. After cooling, it was shaken with 5 ml of chloroform, and centrifuged at 3000 *g* for 10 min. The supernatant was centrifuged further at 10,000 *g* for 10 min, and clear supernatant obtained was subjected to absorbance measurement at 532 nm. The animals which were treated in the same manner except for the cold injury were adopted as controls.

The results are summarized in the Table. As can be seen from the table, the lipoperoxide formation reached its maximum 12 h after the cold injury, but the development of the brain edema and the destruction of the blood-brain barrier were most prominent 24 h after the injury[5]. It is clear from these observations that the brain edema was established at a definite period after the injury. This means that the mechanism of the occurrence of the edema involves some successive processes of changes in the brain. The lipoperoxide formation, demonstrated in the present study, would occur in association with the above processes. Probably the damage that is brought about by the cold injury liberates the agent(s), such as hemoglobin, to provoke free radicals that promote the peroxidation, which brings about further enormous damage in the membrane. Consequently, water and other extracellular substances can permeate easily into the brain perenchyma.

Since the effect of steroids on the cold-injured edema was reported by PAPPIUS and MCCANN[6], the effect of dexamethasone on the lipoperoxide formation in the present edema was studied. The animal was injected intraperitoneally with 0.5 mg/kg of dexamethasone 1 h before the cold injury and further with 0.5 mg/kg of it 6 h after the cold injury to examine the effect of this drug on the lipoperoxide formation. The animal was killed 24 h after the injury, and the lipoperoxide in the brain was measured as mentioned above. As listed in the table, a remarkable preventive effect of dexamethasone on the lipoperoxide formation was found. This might be ascribed to its stabilizing effect on membrane, mainly during the stage of the cold injury.

The present data support the supposition of the usefulness of steroids in prevention of human brain edema. Also, the use of antioxidants is considered to be effective to prevent and treat the brain edema as described by ORTEGA et al.[7], but such antioxidants have to be non-toxic and preferably permeable to the blood-brain barrier.

Zusammenfassung. Biochemische Untersuchung zur Frage der Membranschädigung bei experimentellem Hirnödem: Lipoperoxid tritt 12 h nach der Traumatisierung in der höchsten Konzentration auf, was pathogenetisch bedeutungsvoll sein könnte.

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Increase in the amount of lipoperoxide in the cold-injured brain

Time after injury	Controls	Cold-injured brain
30 min	5.38 ± 0.31 (3)	6.01 ± 0.56 (4)
12 h	5.45 ± 0.56 (4) [a]	7.14 ± 1.13 (4) [a]
24 h	5.13 ± 0.25 (4) [b]	6.01 ± 0.50 (4) [b]
48 h	5.32 ± 0.38 (5)	5.63 ± 0.63 (5)
DM (24 h)	5.01 ± 0.19 (6)	5.26 ± 0.56 (6)

The data represent nmole malonaldehyde formed per g wet weight of the brain, obtained by thiobarbituric acid method[4]. Numbers of experiments are shown in parentheses. Statistically significant increase was observed in both [a] ($p = 0.05$) and [b] ($p < 0.05$). DM: treated with dexamethasone (see text).

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