Zusammenfassung. Nachweis der ersten enterochromaffinen und dopaminen Zellen im Magendarmtraktus des Rindes während der 6. und 7. Woche der Embryonalentwicklung. Die Differenzierung der beiden Zelltypen gelang leicht mittels Fluoreszenz (Formaldehyd), topographischer Verteilung und Zellstruktur. Während die enterochromaffinen Zellen aus den Darmepithelzellen stammen, scheint es sich bei den dopaminen Zellen um Mastzellen zu handeln.

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Teratogenic Effects of Tryptophane on the Development of Chick Embryo

Though congenital anomalies were known to be produced by ionizing radiations[1], viruses and bacteria[2,3], antimetabolites[4], vitamins[5], alkaloids[6] and a variety of chemicals[7–9] work on the toxicity of amino acids is relatively meagre. HERRMANN[10] and ROTHFELS[11] reported the incidence of developmental abnormalities in explanted chick embryos with amino acid analogues. Leucine and leucine analogue, hypoglycine-A, were shown to be teratogenic in rat and chick embryos[12–14]. NAIDU[15] reported the effect of L-arginine hydrochloride on the development of rat embryos. The dysmorphogenetic effects were mainly localized in the hind limb development. The present study investigates the effect of tryptophane on the development of chick embryos and it is distributed in proteins at a low level.

Materials and methods. Freshly laid fertile eggs of white leghorn chickens were collected and incubated at 37 °C with 80% relative humidity. The eggs were divided into 2 groups. The control groups comprised a total of 20 eggs, and a set of 30 eggs formed the experimental group. Inoculation of the eggs was done by the method of KAPLAN and GRABOWSKI [16]. The eggs were removed from the incubator and swabbed with alcohol. The needle was inserted into the yolk sac lateral to the marginal vein and the solution was released just beneath the area vasculosa and subsequently sealed with paraffin wax and returned to the incubator.

After 72 h of incubation, half of the control group received 0.5 ml of saline and the experimental groups received 2.0 mg of tryptophane through 0.5 ml of saline (L-tryptophane was obtained from BDH pool, England). The remaining half of the control group were swabbed with alcohol daily and allowed to develop normally. The eggs were candled and the dead embryos were removed and examined for malformations, if any. After 8 days of incubation, the eggs were removed from the incubator and the embryos were isolated, washed in saline and fixed in 10% formaldehyde and observed for malformations.

Results and discussion. The results (Table) indicate that the amino acid tryptophane has a definite teratogenic potential. The main dysmorphogenetic effects were those of limb deformities, rumplessness (Figure) and visceral abnormalities with exposed intestines and other visceral organs indicating the sites of action. All the experimental embryos were smaller in size than the control group. Tryptophane is important as a raw material for the production of niacinamide. Kynurenine, quinolinic acid and nicotinic acid ribotide are the intermediate products in the metabolic pathway of tryptophane to nicotinamide[17].

Developmental abnormalities produced by amino acid and amino acid analogues have been attributed by HERRMANN[10], ROTHFELS[11] and PERSAUD[12] to be an inbalance in the amino acid pool which contributes to the

8-day-old embryos. Right: Experimental embryo showing the absence of the forelimb on one side together with rumplessness, reduced size and delayed growth in contrast to the control embryo (left).

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The dysmorphogenetic effect of tryptophane on the developing chick embryo

Type	Total No.	No. dead	Normal embryos	Abnormal embryos			
				Limb deformities	Rumplessness	Visceral abnormalities	Total
Control I	10	1	9	—	—	—	—
Control II	10	—	10	—	—	—	—
Experimental	30	4	5	10 (33.5%)	4 (13.33%)	7 (23.33%)	21 (70%)

embryonic development. Imbalance in the amino acid pool may be caused by the excess addition of one amino acid which results in relative deficiency of the remaining amino acids. Since tryptophane is an essential amino acid, there is every reason to infer that excess addition of tryptophane will cause a relative deficiency of the other essential amino acids. It may tilt the nitrogen equilibrium of the essential amino acids and may cause a total imbalance in the amino acid pool, ultimately bringing about dysmorphogenetic effects.

Hence the incidence of abnormalities may be due to the imbalance of the essential amino acids in the amino acid pool that is available for the synthesis of proteins of the developing embryo, or may be due to the toxicity of high tryptophane content or its accumulated metabolic byproducts.

Zusammenfassung. Nachweis einer teratogenen Wirkung von Tryptophan beim Hühnerembryo als mögliche Folge einer Störung der Proteinbiosynthese.

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Localization of the Labelled 5-Azacytidine in Cultured Mouse Embryonic Cells

5-Azacytidine is a pyrimidine analogue which affects primarily the synthesis of RNA thereby interfering also with the formation of DNA and proteins[1,2]. It has been observed that the drug causes chromosomal aberrations[3], affecting the cells predominantly in the S phase[4–7]. In the present work, we have studied the uptake of 5-azacytidine-4-^{14}C into cultured mouse embryonic fibroblasts and we observed its localization over heterochromatin.

Materials and methods. 5-Azacytidine-4-^{14}C (33.7 mCi/mmole) was prepared in this Institute. The cells were maintained in Eagle's minimal essential medium containing antibiotics supplement with vitamins and 10% calf serum. The primary cultures were prepared from 13-day-old mouse foetuses. The cells were grown in Roux bottles (1×10^6 cells/ml) and 10^{-5} *M* 5-azacytidine-4-^{14}C (0.2 µCi/ml) was added after 18 h of cultivation at 37 °C. 1 h later the radioactive medium was removed and a fresh medium was added. The cells were harvested at 2-h intervals following 1-h exposure to colchicine (0.05 µg/ml). Cultures were fixed in acetic methanol after hypotonic treatment (0.075 *M* KCl) for 12 min at 37 °C. The monolayer was than dispersed in 60% glacial acetic acid and drops of cell suspension were placed on clean glass slides prewarmed to 56 °C. After drying the slides were washed (5 min) 3 times in 5% trichloracetic acid, rinsed with distilled water and coated with a stripping film Kodak AR. 10. The exposition at −20 °C lasted for2 months. Following the development and fixation the cells were stained by Giemsa stain in a phosphate buffer (pH 6.8). The preparations for the evaluations were photographed using Orwo-film NP 15. In each instance 25–30 individual karyotypes were counted. The localization of grains over individual chromosomes was registered separately over proximal (centromeric), medial and distal parts. The labelling over the proximal part was considered to correspond to constitutive heterochromatin whereas that over the distal part of chromosomes to euchromatin. The number of grains over the medial segment was constantly low in all instances where the labelling of this region was disregarded.

Results and discussion. The number of nucleoli in mouse fibroblasts and their size considerably varied during the experiment. The largest nucleoli were found 8 h after the removal of medium containing 5-azacytidine-4-^{14}C. The nucleolar enlargement following this analog has been also observed by Voigt et al.[8]. Furthermore it has been shown recently[9] that 5-azacytidine inhibits the maturation of 45 S precursor ribosomal RNA to 28 S and 18 S rRNA.

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