but appeared to remain continuous. Of the 7,026 vesicles observed, 14.9% appeared tubular by three-dimensional analysis (Figure). The tubular forms consisted of a single membrane-bound dense core while some were individual condensations of electron-dense material surrounded by a single limiting membrane. However, the length of the tubes or their existance in a network could not be determined as they usually extended from the top or bottom surfaces of the section. The tubular forms (both the single and multiple condensation types) appeared with greater frequency near the nucleus. The two types did not appear to be interconnected by a continuous membrane nor were they seen to be connected to the Golgi complex. Spherical vesicles were occasionally seen in a linear array.

The visualization of the tubular forms of vesicles may suggest that the granular matrix which begins to form in the Golgi complex enters a network of tubules, and at the terminal aspects of the system the granules condense and become enclosed by a separate membrane. It seems likely that the vesicles observed in a linear array represent the last stage in the maturation of the spherical granules. On the other hand, it is possible that the tubular vesicles remain as separate forms and may be functionally different from the more frequently encountered spherical vesicles. Perhaps the use of other heavy metal stains will provide better resolution of the membrane systems of the adrenal medulla for three-dimensional analysis and more precise information about the development of the catecholamine-containing vesicles can be obtained.

Comparing these data with that obtained from conventional electron microscopy, it seems likely that the irregular forms of vesicles (ovoid, elongated, comma-shaped, dumbell-shaped) reported by other investigators[6–8] result from tangential sectioning through these tubular forms. In ultrathin sections a recognition of tubular forms would be difficult. Even though interconnections were not found in a freeze-etching study[9] the possibility that these tubular vesicles interconnect should remain open for further investigation.

Zusammenfassung. Nachweis, dass die catecholaminhaltigen Vesikel des Nebennierenmarkes eine sehr spezifische dreidimensionale Form haben, die sich bei der gewöhnlichen Transmissionselektronenmikroskopie nur durch Unregelmässigkeiten bemerkbar macht, jedoch mit Hilfe der Stereo-Elektronenmikroskopie mit Hochspannung sichtbar gemacht werden kann.

S. W. CARMICHAEL and D. J. SMITH[10]

Department of Anatomy and Departments of Anesthesiology and Pharmacology, West Virginia University School of Medicine, Morgantown (West Virginia 26506, USA), 14 May 1974.

[6] R. E. COUPLAND, J. Anat. *99*, 231 (1965).
[7] J. G. WOOD, Histochemie *28*, 183 (1971).
[8] I. BENEDECZKY and A. D. SMITH, Z. Zellforsch. *124*, 367 (1972).
[9] H. PLATTNER, H. WINKLER, H. HÖRTNAGL and W. PFALLER, J. Ultrastruct. Res. *28*, 191 (1969).
[10] We thank G. WRAY, AL SZIRMAE, DRES. L. THOMAS and R. FISHER for their help and cooperation with the MVEM, MARYLON RIDER and MARILEE BENSON for technical assistance. This study is supported by N.I.H. Contract No. 70-4136 and N.I.H. Grant No. 5-S01-FR05433.

Development of Enterochromaffin and Dopamine Cells in the Gastrointestinal Tract of the Calf

The gastrointestinal (G-I) tract of the cow and some other species contains numerous amine-stroing cells[1]. Enterochromaffin cells (EC) are characterized by 5-hydroxytryptamine (5-HT)[2], whereas what are called 'dopamine cells' (DC) have been shown to contain dopamine[3]. The epithelial origin of the EC has been generally accepted[4,5], whereas it is presumed that DC belong to the mast cell category[6]. It is characteristic of G-I amine-strong cells that they appear at early stages of development, whereas accumulation of amines by these cells was shown to vary in vivo and in vitro experiments[7,8]. Because there are no data concerning the appearance of DC in the G-I tract, an attempt was made to correlate the development of DC with that of other amine-containing structures, using the specific histochemical method to demonstrate monoamines.

Material and methods. 19 embryonal calves of both sexes were studied. The age of the embryos was determined on the basis of body length[9]. The embryos were obtained within about 5 min after shooting the cow and small pieces were cut immediately from the corpus and antrum area of the stomach, the oral third of the duodenum and the jejunum, the ileum terminale and the oral third of the colon. The pieces were immediately frozen in isopentan precooled with liquid nitrogen. The histochemical method to demonstrate monoamines followed the principles outlined by ERÄNKÖ[10] and FALCK et al.[11]. The specimens were freeze-dried at −40 °C in vacuo for 24 to 48 h. Then temperature of the holder in vacuo was gradually increased to above room temperature. The specimens were treated with formaldehyde vapour at 80 °C for 1 h. Paraformaldehyde was equilibrated at 60% humidity. The paraffin sections were cut perpendicularly to the axis of the intestinum and contained all intestinal layers. The number of EC and DC was determined for the duodenum. The total cells counted for each specimen varied from 150 to 250. The volume of 5 µm adjacent sections was determined by planimeter and appropriate corrections were made[12].

Results. EC were first seen in the duodenum and in the other parts of the G-I tract before the 7th embryonic week. The cells were identified by their bright, strong

[1] A. PENTTILÄ, Acta physiol. scand. *69*, suppl. 281, 1 (1966).
[2] R. BARTER and A. G. E. PEARSE, Nature, Lond. *172*, 810 (1952).
[3] A. BERTLER, B. FALCK, N.-Å. HILLARP, E. ROSENGREN and A. TORP, Acta physiol. scand. *47*, 251 (1959).
[4] A. ANDREW, J. Embryol. exp. Morph. *11*, 307 (1963).
[5] A. PENTTILÄ, Annls. Med. exp. Biol. Fenn. *46*, 457 (1968).
[6] R. E. COUPLAND and I. D. HEATH, J. Endocr. *22*, 71 (1961).
[7] R. HAKANSON, CH. OWMAN and N.-O. SJÖBERG, Life Sci. *6*, 2535 (1967).
[8] R. HAKANSON, CH. OWMAN and N.-O. SJÖBERG, Acta physiol. scand. *75*, 213 (1969).
[9] O. ZIETZSCHMANN and O. KRÖLLING, in *Lehrbuch der Entwicklungsgeschichte der Haustiere*, 2nd edn. (Paul Parey, Berlin und Heidelberg 1955), p. 102.
[10] O. ERÅNKÖ, J. R. microsc. Soc. *87*, 259 (1967).
[11] B. FALCK and CH. OWMAN, Acta Univ. lund. II. *7*, 1 (1965).
[12] O. ERÅNKÖ, in *Quantitative Methods in Histology and Microscopical Histochemistry* (S. Karger, Basel and New York 1955).

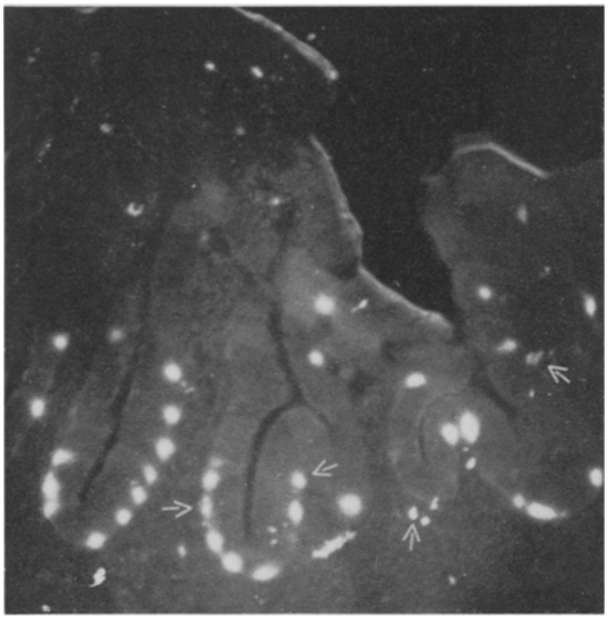


Fig. 1. Formaldehyde-induced fluorescence (FIF) photograph of the duodenum of a 17.5-week-old calf embryo. Greenish fluorescent granular DC (arrows) are smaller than EC. DC are seen in the epithelial layer, in the lamina propria and occasionally also in the submucous layer, whereas EC are located only in the epithelial layer. ×150.

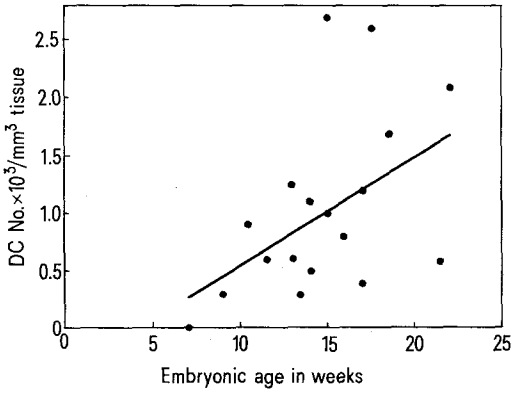


Fig. 2. The number on DC determined per unit of volume of the duodenal mucosa (see Methods). The formula of the regression line was $y = 5.4 + 0.07 * X$.

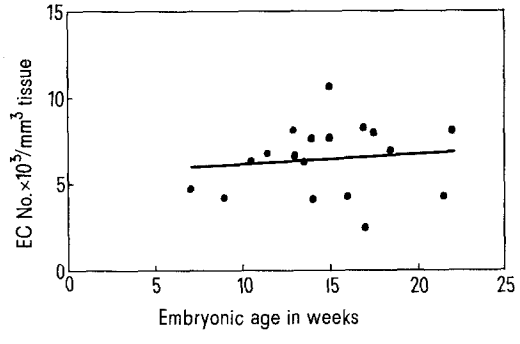


Fig. 3. The number of EC determined per unit volume of the mucosa similiarly to that in Figure 2. The number of EC remained constant during the embryonic period investigated. The formula of the regression line was $y = -0.4 + 0.1 * X$.

and yellow fluorescence and coarse cytoplasmic granules. The EC were always located among other epithelial cells of the mucous membrane and mainly at the crypts of Lieberkühn (Figure 1). Form and size of cells corresponded to that of adult animals[1]. Counts of EC per unit of volume remained quite constant following their first appearance (Figure 3). The EC were the only structures of the G-I tract showing bright yellow formaldehyde-induced fluorescence.

DC were first identified in the intestinal tract at the age of 8 weeks. In the stomach DC were first seen about 1 week later. Duodenal DC counts were much lower than the respective EC counts (Figures 2 and 3). The same observation was made also in other areas of the intestinum. During embryonic development a clear increase in DC count was registered (Figure 2). The DC were easily identified by their greenish, strong cytoplasmic fluorescence. The cells were also clearly smaller than EC and of more irregular shape. The cytoplasm was characterized by few large, coarse granules. Most of the DC were located between epithelial cells of the mucous membrane at early stages of development. Later individual cells and groups of them were seen in the lamina propria and in the submucous layer. During early embryonic stages, no DC were seen in the muscular and subserous layers, but at the age of 10 to 15 weeks and later, individual cells were seen in these areas also. Greenish fluorescent nerve fibers were seen in the muscular and submucous layers of the intestinum even at the age of 7 weeks. These structures did not have any apparent connection with the DC, which were seen at that stage and later most abundantly between epithelial cells and in the villous areas of the mucous membrane. Individual DC were mostly adjacent to vascular channels in the submucous and muscular layers.

Discussion. The present results are consistent with earlier observations on other mammalian species[1, 4, 5] that 5-HT accumulates in the EC during early embryonic stages. Because the EC were the only 5-HT-containing structures in the G-I tract of the calf, and because the cells were always located among the epithelial cells of the mucous membrane and inside the basement membrane, it seems obvious that the cells are specialized in the intestinal epithelial layer and are presumably of epithelial origin as suggested earlier[4, 5]. The present observations on specialization of the cells to produce 5-HT at the age of 7 weeks of intrauterine life are in agreement with earlier observations when specific stainings were used to demonstrate the cells[13].

The present observations indicated that during embryonic development DC were easily distinguished from EC. At early stage of development, surprisingly most of the DC were located between intestinal epithelial cells. They were, however, readily identifiable by their fluorescence, size, shape and cytoplasmic granularity. DC did not seem to have any apparent connection with adrenargic nerves in any phase of development. The present results on embryonic calves seem to support earlier findings on adult animals that DC are specialized dopamine-storing cells and are obviously one type of mast cells characteristic only of some ruminants[3]. Concomitant differentiation of EC and DC in all parts of the G-I tract of the calf embryo at the 6th to 8th week of intrauterine life may be an indication of the significance of these cell types for G-I function even during early stages of development.

[13] R. Faustini, Am. J. vet. Res. *16*, 397 (1955).

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.

A. AHONEN and A. PENTTILÄ[14]

[14] This study was supported by a grant from the National Research Council for Medical Sciences of Finland and the Sigrid Jusélius Foundation.

Department of Anatomy and Department of Forensic Medicine, University of Helsinki, Siltavuorenpenger 20b SF-00170 Helsinki 17 (Finland), 10 May 1974.

Teratogenic Effects of Tryptophane on the Development of Chick Embryo

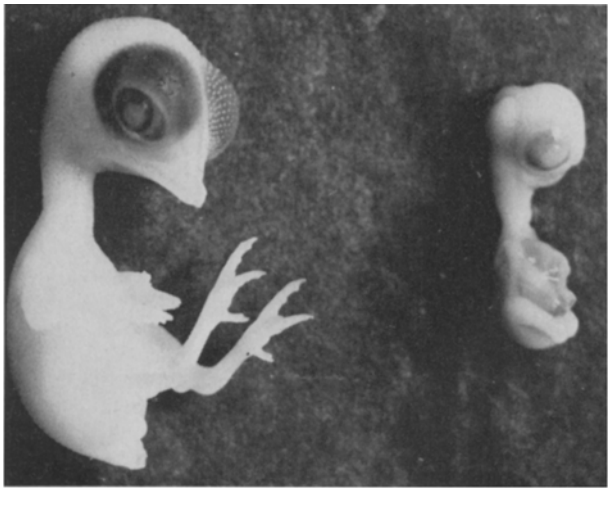
Though congenital anomalies were known to be produced by ionizing radiations[1], viruses and bacteria[2,3], antimetabolites[4], vitamins[5], alkaloids[6] and a variety of chemicals[7–9] work on the toxicity of amino acids is relatively meagre. HERRMANN[10] and ROTHFELS[11] reported the incidence of developmental 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).

[1] J. N. YAMAZAKI, Pediatrics. Springfield *37*, 877 (1966).
[2] N. M. GREGG, Trans. opthal. Soc. Austr. *3*, 35 (1942).
[3] C. G. BROWN, Adv. Teratol. *1*, 55 (1966).
[4] J. B. THIERSCH, Am. J. Obstet. Gynec. *63*, 1298 (1952).
[5] K. VERMA and WEIKING, Indian. J. exp. Biol. *5*, 49 (1967).
[6] DE MYER, Extr. Archs Anat. Histol. Embryol. *40*, 181 (1965).
[7] R. J. BLATTNER, J. Pediat. *66*, 1102 (1965).
[8] D. G. TRASLER, Lancet *1*, 606 (1965).
[9] W. HUGHES, Br. J. Pharmac. *25*, 317 (1965).
[10] H. HERRMANN, J. Embryol. exp. Morph. *1*, 291 (1953).
[11] U. ROTHFELS, J. exp. Zool. *125*, 17 (1954).
[12] T. V. N. PERSAUD, West Indian med. J. *18*, 34 (1969).
[13] T. V. N. PERSAUD and S. KAPLAN, Life Sci. *9*, 1305 (1970).
[14] R. M. BERGSTROM, T. ERILA and R. PIRSKANEN, Experientia *23*, 767 (1967).
[15] R. CHANDRAMOHAN NAIDU, Aust. J. exp. Biol. med. Sci. *51*, 553 (1973).
[16] S. KAPLAN and C. T. GRABOWSKI, J. exp. Zool. *165*, 325 (1967).
[17] P. SWAN and L. M. HENDERSON, in *The Encyclopedia of Biochemistry* (Eds. R. J. WILLIAMS and E. M. LANSFORD; Reinhold Publishing Corp., New York 1967), p. 582.