The fact that the IV of the traditionally produced palm oil is lower than that for the NIFOR palm oil (table 2) means that there is a lower amount of the unsaturated fatty acids in the traditionally produced palm oil. This is probably due to differences in the production methods. Essentially, the traditional "soft oil process" for producing palm oil in Nigeria involves the boiling of palm oil fruits in wooden or metal vats, manual kneading of the boiled fruits, separation of extracted oil from the fibrous residue and packaging. In all of these steps, the level of hygiene can, at best, be regarded as low. Air is not excluded as a matter of deliberate policy and the types of containers and vats used are not carefully selected to minimize contamination by pro-oxidant metals such as iron and copper. The oil is usually sold in glass bottles or open vat containers which are exposed to the warm humid environment and not shielded from light. All of these factors would tend to promote some oxidation of the palm oil. The NIFOR palm oil, on the other hand, is produced by modern scientific methods which take into account the need to retard lipid oxidation during processing and wholesale storage. The PV recorded in table 2 also indicate that the traditionally-produced palm oil had undergone greater oxidation than the NIFOR oil. The PV of the traditionally-produced palm oil is also indicative of rancidity[10]. The higher free fatty acid content of the traditionally-produced palm oil compared with that for the NIFOR palm oil indicates the lack of a refining process and probable contamination by lipase-secreting microbes. Since the SG of edible oils is related to the degree of unsaturation of the component fatty acids[4], there is consistency in the fact that the NIFOR palm oil which had the higher IV (higher unsaturation) also had the higher SG (table 2). The same consistency is observed with regard to the RI values which also tend to increase with increasing unsaturation, while the higher smoke point, shown in table 2, for the NIFOR palm oil, is consonant with its lower free fatty acid content.

The fatty acid profiles of both types of palm oil show high levels of the saturated fatty acids. The profiles are consistent with the low IV for the oils. While a low level of unsaturated fatty acids will enhance stability to oxidation and attendant spoilage, the relationship of low levels of lipid unsaturation to the development of certain disease conditions makes it nutritionally undesirable. The NIFOR palm oil had a higher content of the essential fatty acid linoleic acid and of linolenic acid, probably because it had been subjected to a lower degree of oxidative abuse during processing and subsequent storage.

Results in table 2 also show that increase in $A_w$ led to higher IV for the oil (higher unsaturation); this could be due to a decrease in lipid oxidation. This observation is in line with the reported retarding effects of high $A_w$ on lipid oxidation[7].

1 Association of Vitamin Chemists, (Method of Vitamin Assay), 3rd edn. N.Y. Intersc. Publ. 1966.
2 Bhattacharyya, G.K., and Johnson, R.A., in: Statistical Concepts and Methods, John Wiley and Sons, New York 1977.
3 Devine, J., and Williams, P.N., in: The Chemistry and Technology of Edible Oils and Fats, p. 161. Pergamon Press, N.Y./Oxford/London/Paris 1961.
4 Joslyn, M.A., in: Methods in Food Analysis, 2nd edn, p. 402. Academic Press, New York 1970.
5 Kamman, J.F., Labuza, T.P., and Warthesen J.J., J. Food Sci. *46* (1981) 1457.
6 Labuza, T.P., Proc. 3rd Intl. Cong., Food Sci. Technol., p. 618. Washington, D.C. 1971.
7 Loncin, M., Bimbenet, J.J., and Lenges, J., J. Food Technol. *3* (1969) 131.
8 Metcalfe, L.D., Schmitz, A.A., and Petka, J.R., Analyt. Chem. *38* (1966) 514.
9 Oyenuga, V.A., in: Nigeria's food and feeding stuffs, p. 79. Ibadan University Press, Ibadan, Nigeria 1968.
10 Pearson, D., in: The Chemical Analysis of Food, 7th edn, p. 495. Churchill Livingstone, London 1976.
11 Rockland, L.B., Analyt. Chem. *32* (1960) 1375.
12 Scheig, R., Am. J. clin. Nutr. *21* (1968) 300.
13 Ukhun, M.E., Food Chem. *14* (1984) 35.

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## Effect of epidermal growth factor on growth and maturation of fetal and neonatal rat small intestine in organ culture[1]

C.N. Conteas, J.M. DeMorrow and A.P.N. Majumdar

*Department of Medicine, Veterans Administration Medical Center, Martinez (California 94553, USA), and Departments of Medicine and Biological Chemistry, University of California, Davis (California 95616, USA), 30 September 1985*

*Summary.* Small intestinal explants from pre- and post-natal rats were incubated in an organ culture system in the absence and presence of epidermal growth factor (EGF). The rate of synthesis of small intestinal DNA and protein as well as the activity of lactase and alkaline phosphatase increased rapidly between 17 and 20-day gestational age, whereafter they declined. The maximal incorporation of $^3$H-thymidine and $^{14}$C-alanine into DNA and protein, respectively, was significantly stimulated by EGF (100 ng/ml). EGF had no effect on the activity of either lactase or alkaline phosphatase in the small intestinal explants.
*Key words.* Small intestinal growth; DNA synthesis; protein synthesis; disaccharidases; EGF; fetal development; organ culture.

Epidermal growth factor (EGF), which is structurally and functionally similar to urogastrone[2] has long been known to stimulate proliferation and differentiation (keratinization) of the epidermis[3,4] and to enhance growth and maturation of the fetal pulmonary epithelium[5]. More recently, it has come to light that EGF also promotes growth of the digestive tract during the early stages of development, as evidenced by increased DNA synthesis, ornithine decarboxylase activity and protein and nucleic acid content of the stomach and small intestine following administration of EGF to growing rats and mice[6-8]. This, together with the fact that milk possesses EGF-immunoreactivity[9] and that both EGF and milk stimulate DNA synthesis in cultured fibroblast[10] suggests further that EGF may play an important role in regulating growth during the early stages of life. Recently we have demonstrated that prolonged administration of EGF to non-weaned suckling rats (undernourished) not only stimulates growth of the stomach and small intestine but also increases body weight[8] indicating that EGF induces overall growth of the animals. On the other hand, Calvert et al.[11] have observed that in mice administration of EGF during the later stages of pregnancy produces no change in either fetal body weight or small intestinal DNA or protein content but significantly increases intestinal alkaline phosphatase, trehalase and glucose-6-phosphatase activities suggesting that prenatal functional maturation, but not growth of the small intestine, is stimulated by EGF. To further determine the role of EGF in the regulation of growth and

maturation of the gastrointestinal tract, at early stages of development, the present investigation examines the responsiveness of the small intestine from pre- and postnatal rats to EGF on DNA and protein synthesis and on the activity of lactase and alkaline phosphatase in an organ culture system.

*Materials and methods.* Timed pregnant Sprague-Dawley rats were obtained from Bantin and Kingman, Freemont, CA, and were supplied with ad libitum food and water throughout. At 17, 19 and 21 days of gestation they were anesthesized with pentobarbital (i.p.; 20 mg/kg) and fetuses were removed by laparotomy under sterile conditions. Some animals were allowed to give birth, and neonatal rats of 1 (8–12 h after birth), 4 and 7 days of age were used. The animals were decapitated, and the proximal 5 mm of the small intestine was resected and cultured as described below.

About 10 mg of small intestinal explants from each rat was placed on a 1 × 1 cm raft prepared from lens papers (Sight Saver, Dow Corning, Midland, MI). For preparation of raft, lens papers were first washed with ether, then with 95% ethanol and finally with water. The papers were air-dried, siliconized and after washing with water they were dried at 150 °C for 60 min. Each raft containing intestinal explants was placed in the petri dish containing 2 ml of Dulbecco's modified Eagles medium (DMEM) – 5% fetal calf serum (M. A. Bioproducts, Baltimore, MD), and equilibrated for 6 h at 37 °C under carboxygen atmosphere. After equilibration, the rafts were transferred into a petri dish containing 2 ml of fresh medium, and incubated for 24 h in the absence (basal) and presence of EGF (100 ng/ml). One μCi of [5-6-$^3$H]thymidine or [6-$^{14}$C]-alanine was added 2 h prior to termination of the incubation.

At the end of the incubation period, the explants were washed with fresh incubation medium containing excess of cold thymidine or alanine. Each explant was homogenized in 0.2 M $HClO_4$ and after centrifugation at 2000 × g for 10 min, an aliquot of the supernatant was counted for radioactivity (acid-soluble radioactivity) as described previously[12]. Protein and DNA were extracted from the $HClO_4$-precipitates, and counted for radioactivity as described elsewhere[12,13]. Protein content was measured by the micro method of Bradford[14]. Because of the limited size of the samples, no effort was made to determine DNA content. The results were expressed as dpm/ng protein.

For determination of lactase and alkaline phosphatase activities, the explants were homogenized in cold double distilled water. Lactase and alkaline phosphatase activities were determined by the method of Dahlqvist[15] and Bessey et al.[16], respectively.

*Results.* DNA and protein synthesis in small intestinal explants were determined by measuring the rate of incorporation of $^3$H-thymidine and $^{14}$C-alanine, respectively, following a 2 h pulse during a 24-h incubation period. The results are depicted in figure 1. The rate of DNA synthesis in the explants in the absence (basal) and presence of EGF revealed a steep rise between 17 days gestational age and 1 day after birth; whereafter it decreased sharply (fig. 1A). On the other hand, fetal small intestinal protein synthesis attained its peak on the 19th gestational age and then fell sharply with no further change occuring between 1 and 7 days postnatally (fig. 1B).

Addition of EGF to the organ culture system significantly stimulated the rate of incorporation of $^{14}$C-alanine and $^3$H-thymidine, into protein and DNA, respectively, of small intestinal explants, without affecting their pattern of synthesis during this developmental period (figs 1A and 1B). In the presence of EGF the maximal incorporation of $^3$H-thymidine and $^{14}$C-alanine was stimulated by 110% ($p < 0.001$) and 65% $p < 0.025$), respectively, when compared with the corresponding basal levels. Acid-soluble radioactivity between the basal and EGF-treated explants was not found to be significantly different (data not shown).

In the small intestinal explants, both lactase and alkaline phosphatase activities attained their respective peaks one day before birth and then decreased slightly and remained at that level up to 7 days postnatally (figs 2A and 2B). Exposure of the explants to EGF produced no apparent change in either lactase or alkaline phosphatase activity (figs 2A and 2B).

*Discussion.* In rats and mice, morphogenesis and cytodifferentiation of the small intestine occur at an extremely rapid rate during the last 3–4 days of gestation[17,18]. Our present findings are in line with such findings. We have observed that in both control and EGF-treated small intestinal explants the rate of synthesis of

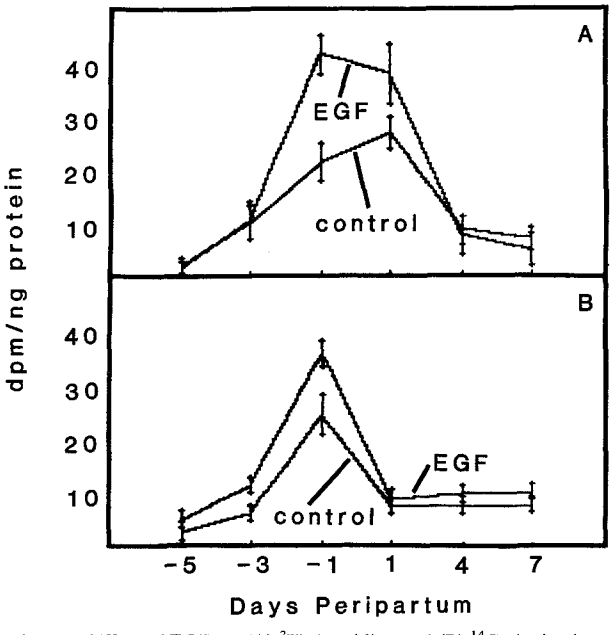


Figure 1. Effect of EGF on (A) $^3$H-thymidine and (B) $^{14}$C-alanine incorporation into small intestinal DNA and protein, respectively, in organ culture system. Small intestinal explants, obtained from fetal and neonatal rats were incubated in the absence (control) and presence of EGF (100 ng/ml) for 24 h. Each value represents the mean ± SE of 5–7 experiments.

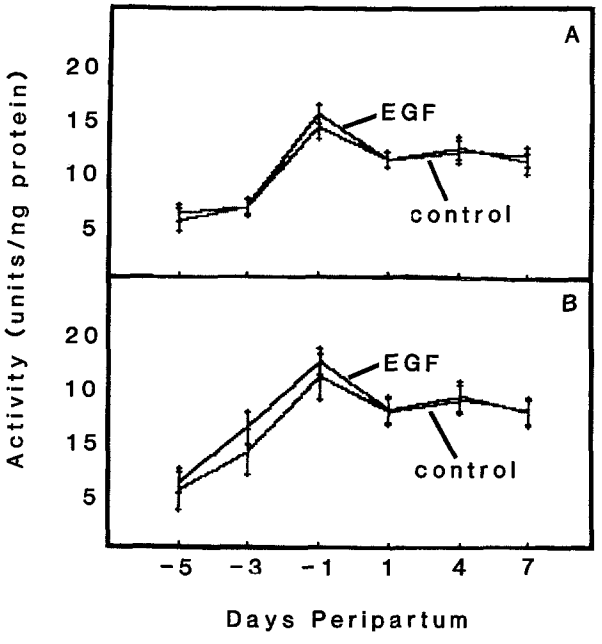


Figure 2. Effect of EGF on small intestinal (A) lactase and (B) alkaline phosphatase activities in organ culture systems. Small intestinal explants, obtained from fetal and neonatal rats were incubated in the absence (control) and presence of EGF (100 ng/ml) for 24 h. Each value represents the mean ± SE of 5 experiments.

both DNA and protein increase rapidly between 17 and 20 days of gestation. During the same period the activity of both lactase and alkaline phosphatase in the small intestine is also found to increase steadily. Taken together, the results indicate that growth and maturation of the fetal small intestine are extremely rapid during the last part of gestation.

In recent years, considerable evidence has accumulated to suggest that EGF, a mitogenic polypeptide, promotes growth of the digestive tract in both young and adult rats[6-8, 19, 20]. Our present observation that addition of EGF to the organ culture system containing fetal small intestinal explants stimulates the rate of synthesis of both DNA and protein suggests that (a) EGF promotes small intestinal cell proliferation, and (b) is independent of other hormone(s) or growth-promoting factor(s). The observed increment in incorporation of $^3$H-valine and $^3$H-thymidine into protein and DNA of small intestinal explants by EGF could not be attributed to an increased entry of the precursors. This interpretation is based on the observation that there was no significant difference in acid-soluble radioactivity (for both $^3$H-valine and $^3$H-thymidine) between the basal and EGF-treated samples.

Although under the present experimental condition EGF induced small intestinal growth as evidenced by increased DNA and protein synthesis, it had no affect on the functional maturation of the tissue. This interpretation is based on the observation that addition of EGF to the organ culture system produces no apparent change in either lactase or alkaline phosphatase activity when compared with the corresponding basal control. Such an observation is analogous to what we[8] as well as Dembinski and Johnson[9] have earlier observed in suckling rats. In contrast, Calvert et al.[11] have demonstrated that in mice administration of EGF during the later stages of pregnancy significantly stimulates alkaline phosphatase and trehalase activities in the fetal small intestine without affecting DNA and protein content in the tissue. However, since the observation was made after in vivo administration of the peptide to the mothers, it is difficult to assess whether the aforementioned findings are due to EGF by itself or secondary to EGF administration.

In conclusion, the present data demonstrate that whereas EGF promotes growth related processes of the fetal small intestine, it does not affect the functional maturation of the organ.

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2 Gregory, H., Nature *257* (1975) 325.
3 Cohen, S., J. biol. Chem. *237* (1962) 1555.
4 Cohen, S., Proc. natn. Acad. Sci USA *72* (1963) 1.
5 Sundell, H., Pediat. Res. *9* (1975) 371.
6 Dembinski, A.B., and Johnson, L.R., Endocrinology *116* (1985) 90.
7 Feldman, E.J., Aures, D., and Grossman, M., Proc. Soc. exp. Biol. Med. *159* (1978) 400.
8 Majumdar, A.P.N., J. Pediat. Gastroent. Nutr. *3* (1984) 618.
9 Hirata, Y., and Orth, D.N., Endocrinology *105* (1979) 1381.
10 Carpenter, G., Science *210* (1980) 199.
11 Calvert, R., Beaulieu, J.-F., and Menard, D., Experientia *38* (1982) 1096.
12 Kaysen, G.A., Majumdar, A.P.N., Dubick, M.A., Vesenka, G.D., Mar, G., and Geokas, M.C., Am. J. Physiol. *249* (1985) F523.
13 Wannemacher, R.J. Jr, Banks, W.L. Jr, and Wunner, W.H., Analyt. Biochem. *11* (1965) 320.
14 Bradford, M., Analyt. Biochem. *72* (1976) 248.
15 Dahlqvist, A., Analyt. Biochem. *22* (1968) 99.
16 Bessey, O.A., Lowry, O.H., and Brock, M.A., J. biol. Chem. *164* (1946) 321.
17 Moog, F., in: Textbook of Gastroenterology and Nutrition in Infancy, p. 139. Ed. E. Lebenthal. Raven Press, New York 1981.
18 Klein, R.M., and McKenzie, J.C., J. Pediat. Gastroent. Nutr. *2* (1983) 10.
19 Johnson, L.R., and Guthrie, P.D., Am. J. Physiol. *238* (1980) G45.
20 Konturek, S.J., Radecki, T., Brzozowski, T., Piastucki, I., Dembinski, A., Dembinska-Kiec, A., Zmuda, A., Gryglewski, R., and Gregory, H., Gastroenterology *81* (1981) 438.

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## Competition between retinol and 3,4-didehydroretinol for esterification in crude pigment epithelial cell fractions

A.T.C. Tsin

*Division of Life Sciences, The University of Texas at San Antonio, San Antonio (Texas 78285, USA), 6 September 1985*

*Summary.* The membrane fraction of the retinal pigment epithelium (RPE) of the frog *(Rana pipiens)* catalyzed the esterification of tritiated retinol to retinyl esters. This esterification reaction was inhibited in the presence of 3,4-didehydroretinol.
*Key words.* Retinol; 3,4-didehydroretinol; esterification; pigment epithelium; frog.

Many animals possess both rhodopsin (a retinal based visual pigment) and porphyropsin (a 3,4-didehydroretinal based visual pigment) in their retinas[1-6]. In these animals, both retinyl and 3,4-didehydroretinyl esters are also found in their retinal pigment epithelium (RPE)[7-11] located adjacent to the retina. The composition of rhodopsin/porphyropsin in the retina is similar to the composition of retinyl/3,4-didehydroretinyl esters in the pigment epithelium in some species[8, 12] but not in others[10, 13, 14]. In order to fully understand how the composition of visual pigments changes in response to light[10, 13, 15-18], temperature[10, 13, 18-20] and other factors[10, 21-23], it is necessary to study the pathway whereby retinol and 3,4-didehydroretinol are stored and utilized by the eye. Retinoids in the eye are found mainly in the RPE as retinyl esters of long chain fatty acids such as palmitic and stearic acids[24-26]. The present report shows how retinol and 3,4-didehydroretinol compete with each other for the esterification to palmitic acid resulting in the formation of retinyl and 3,4-didehydroretinyl palmitate.

The membrane component of the RPE/choroid from four frogs (*Rana pipiens*, average weight: 12 g) was obtained by homogenizing freshly removed RPE/choroid in amphibian Ringer solution (111.2 mM NaCl, 2.0 mM KCl, 1.1 mM $CaCl_2$ and 2.4 mM $NaHCO_3$; pH = 7.3) and centrifugation (100,000 × g; for 1 h). The membrane fraction (pellet) was then resuspended in 6 ml of Ringer solution and divided into six equal portions (tubes 1–6). Only tritiated all-*trans* retinol (0.2 nmol, $14 \times 10^6$ dpm, in 30 µl of ethanol) was added to the first tube. Tritiated retinol (quantity and radioactivity same as in tube 1) was mixed with cold (non-radioactive) all-*trans* retinol (50 nmol) and the mixture was dried by nitrogen and the residue re-dissolved in 30 µl of ethanol. The mixture was added to the second tube and this procedure was repeated for the third test tube. Similarly, tritiated retinol (quantity and radioactivity same as in tube 1) was mixed with cold all-*trans* 3,4-didehydroretinol (50 nmol) and the mixture redissolved in 30 µl of ethanol and added to the fourth tube (and this procedure was repeated for the fifth tube). Tritiated retinol