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HISTOCHEMICAL STUDY OF NON-SPECIFIC ESTERASE ACTIVITY IN SMALL AND LARGE INTESTINE OF YOUNG RABBIT

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ABSTRACT : The distribution of non-specific esterase in normal duodenal, jejunal, ileal and large intestinal mucosa of the rabbit has been studied using histochemical methods. Thirty three New Zealand White rabbits were used ranging from 26-day old fetuses to 43-day old young (3 rabbits / age). Distribution and intensity (strong to very strong) varied little throughout the small and large intestine from the 30th day of foetal life and as after birth up to 43 days. At the 26 or 28th days of foetal life only mild to positive reactions were seen. I In the cæcum and in the upper colon, a low non-specific esterase activity was observed in the 7- and 15 day-old rabbits, although this activity was very strong in younger one-day-old rabbits, and in the 19-day-old and older rabbits.

RÉSUMÉ : Etude histochimique de l'activité de l'estérase non spécifique dans l'intestin grêle et le gros intestin du lapereau.
La répartition de l'estérase non spécifique dans la muqueuse du duodénum, du jéjunum, de l'ileum et du gros intestin du lapin a été étudiée en utilisant une méthode histochimique. Trente trois lapins néo-zélandais blancs allant de fœtus âgés de 26 jours jusqu'à de jeunes lapereaux âgés de 43 jours ont été utilisés (3 lapins à chaque âge). La répartition et l'intensité de l'activité (forte à très forte) varient peu dans le gros intestin et le grêle à partir du 30ème jour de vie fœtale et jusqu'au 43ème jour. Par contre, au 26-28ème jour de vie fœtale il y a une réaction seulement positive ou moyenne. D'autre part, il faut souligner que dans le caecum et le colon (partie haute) un faible activité de l'estérase non spécifique est observée chez les lapereaux âgés de 7 et 15 jours alors que l'activité est très forte chez les lapereaux d'1 jour et chez ceux de 19 jours et plus.

INTRODUCTION

Non-specific esterase (NSE-ase) is detected through the hydrolysis of organic esters. Since their physiological substrates are not yet precisely known, *in vitro* tests employ synthetic esters such as those of naphthol and nitrophenol with small carboxylic acids (TSITSILONI *et al.*, 1993). Considering their high tissue levels and their wide tissue distribution in vertebrate species, (COATES *et al.*, 1975 ; HARITOS and SALAMASTRAKIS, 1982 ; VEINI *et al.*, 1986 ; SALAMASTRAKIS and HARITOS, 1988 ; TZANNETATOU-POLYMERI and HARITOS, 1989), the function of non-specific esterases has to be important. It has been suggested for example that certain isoenzymes of NSE-ase participate in lipid transport in the mouse (BOCKING and DEIMLING, 1982).

There is no evidence that the alimentary tract of foetal rabbits has been investigated for NSE-ase although there are reports of such investigations in the adult (ZUTPHEN *et al.*, 1983). The surface epithelium of the duodenal villi of the adult rabbit was strongly positive and the underlying connective tissue and the cells of the glands of Brunner were faintly so. The surface epithelial cells of the colon were also positive for NSE-ase in the supranuclear positions of the cytoplasm (NACHLAS and SELIGMAN, 1949). A positive reaction in the epithelial cells of the rabbit duodenum as well as a faint or irregular reaction in the glands of Brunner was observed (CHESSICK, 1953, SHNITKA, 1960).

Likewise, in the adult rabbit it was observed that the esterolytic activity is homogeneously distributed throughout the intestinal tract in the enterocytes with some deeply stained granules in supranuclear positions. Furthermore, goblet cell cytoplasm was esterase positive and goblet cell secretion and brush borders were negative (WEIGEL and GEYER, 1971).

Based on the fact that in the quail and chicken, a stronger esterase reaction was observed upon hatching and thereafter (TSITSILONI *et al.*, 1993, VEINI *et al.*, 1986) the aim of this preliminary study was to determine the distribution of the NSE-ase activity in the developing intestine during the foetal and postnatal life of the rabbit.

MATERIALS AND METHODS

Thirty three New Zealand White rabbits from litters of seven kits were used including 26- 28- and 30 day old foetuses and young 1, 7, 15, 19, 20, 24, 27 and 43 days old. Three rabbits were observed at each age. All animals were sacrificed by use of chloroform in a glass container. The abdominal cavity was immediately opened and the alimentary tract from the cardia of the stomach to the rectum was removed. The intestine was then unraveled and freed from mesentery. The contents

of the small and large intestine were washed out with formol-calcium solution.

Segments of duodenum, jejunum, ileum, caecum and proximal colon taken from each rabbit were placed and fixed in formol-calcium at 4°C. These specimens were trimmed, orientated and mounted in a support medium on metal chucks and were immediately immersed in liquid nitrogen until frozen. They were then sectioned at 6 µm on a Bright cryostat model OTF/AS/M microtome at -20° C. The sections were fixed on slides without adhesive and were dried at room temperature.

Cryostat sections of the small and large intestine were treated with a naphthyl acetate method (GOMORI, 1952, DAVIS and ORNSTEIN, 1959) for the demonstration of the esterase reaction product, which stains reddish brown. The sections were incubated at 37° C for 15 minutes in the following incubating medium:

Substrate solution	0.25 ml
(a Naphthyl acetate, 50 mg)	
(Acetone, 5 ml)	
Buffer solution	7.25 ml
(Sodium disodium orthophosphate, 2.75g)	
(Distilled water, 1,000 ml)	
Sodium nitrite solution	0.4 ml
(Sodium nitrite, 400 mg)	
(Distilled water, 10 ml)	
Pararosanilin-HCl stock solution	0.4 ml
(Pararosanillin hydrochloride)	
(2N hydrochloric acid, 500 ml)	
Distilled water	2.5 ml

Equal parts of sodium nitrite and pararosanilin-HCl stock solution were first mixed together before adding other ingredients (pH 7.4)

After incubation, the sections were washed in running water, dehydrated rapidly through fresh alcohol, cleared in xylol and mounted in DPX.

OBSERVATIONS

Average results of the observations are summarised in table 1, according to age and site of observation.

1. **Duodenum**:

By the 26th and 28th day of foetal life the epithelium possesses a homogeneously distributed esterolytic activity in the cytoplasm with some deeply stained granules (Fig.1). The reaction tails off in the epithelial cells of the deeper parts of the villi and it is either absent or weak in those of the crypts. The reaction is stronger on the 30th day and the crypt cells also are positive at this stage.

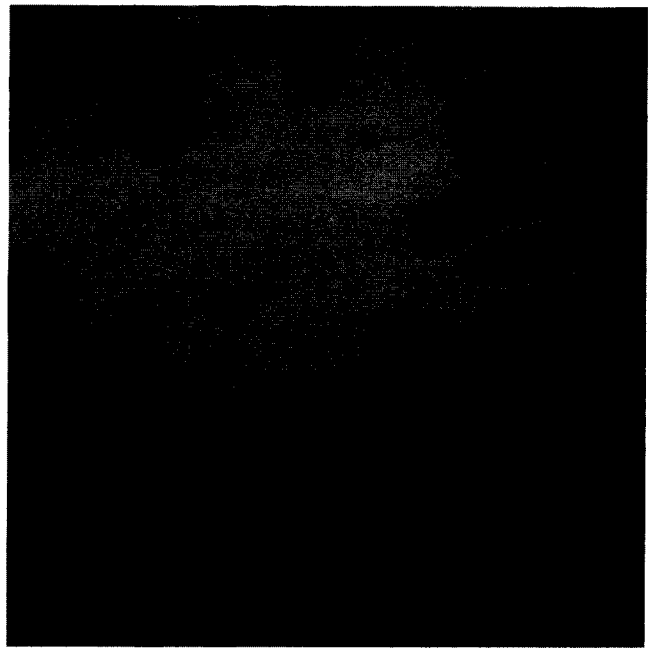

Figure 1 : Duodenum-28day fœtus
Homogeneous and granular positive reaction of cytoplasm of epithelial cells for non specific esterase, tailing off towards the bases of villi and crypts. A naphthyl acetate method for determining NSE-ase was used. X 140

After birth, the cytoplasm of the cells of the villi exhibits a strong positive NSE-ase reaction especially in the 20, 27 and 43 day-old rabbits. The reaction is more intense in the supranuclear region of the cells and marks out granules within a homogeneous background. The cells lining the crypts exhibit a similar reaction. The reaction is milder in the newborn. Brunner's glands are faintly positive containing positive granules in the apical part of the cells. The *lamina propria* exhibits a moderate reaction in the 20 and 27 day-old and a more intense one in the 43 day-old rabbit. Only in the 43 day-old is the brush border positive for NSE-ase . The secretion of the goblet cells is NSE-ase negative.

2. **Jejunum:**

A strong NSE-ase reaction is seen in the cytoplasm of the epithelium. The reaction may be homogeneous and/or granular and it is more intense in the apical cytoplasm. From the 26th day the cells at the bases of the villi and those of the crypts exhibit a milder reaction which has the appearance of positive NSE-ase granules in a homogeneous positive background.

The cytoplasm (Fig. 2) of the epithelial cells of the villi shows a strong NSE-ase reaction, that is more intense in the luminal region of the cell cytoplasm. The cells of the crypts also show a reaction. The *lamina propria* shows a slight

Table 1 : Average intensity of the non specific esterase reaction

			Days of gestation			Days after Birth							
			26	28	30	1	7	15	19	20	24	27	43
DUODENUM	Villi	Apex of villi	++	++	++++	++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
		Sides of villi	++	++	++++	++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
		Bases of villi	+	+	++++	++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
	Crypts		-	-	++	++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
JEJUNUM	Villi	Apex of villi	+++	+++	++++	++++	++++	++++	++++	+++++	++++	+++++	+++++
		Sides of villi	+++	+++	++++	++++	++++	++++	++++	+++++	++++	+++++	+++++
		Bases of villi	++	++	++++	++++	++++	++++	++++	+++++	++++	+++++	+++++
	Crypts		+	+	++	+++	++	++	++++	+++++	++++	+++++	+++++
ILEUM	Villi	Apex of villi	+	+	+++	+++++	+++	+++	++++	++++	++++	+++++	+++++
		Sides of villi	+	+	+++	+++++	+++	+++	++++	++++	++++	+++++	+++++
		Bases of villi	+	+	+++	+++++	+++	+++	++++	++++	++++	+++++	+++++
	Crypts		+	+	+	+++	++	++	+++	+++++	+++	+++++	+++++
CAECUM	Surface	Epithelial cells	+	±	+++	+++++	++	++	++++	+++++	++++	+++++	+++++
	Glands		+	+	++	+++++	+	++	++++	+++++	++++	+++++	+++++
COLON	Surface	Epithelial cells	+	+	+	+++++	+	+	++++	+++++	+++++	+++++	+++++
	Glands		+	+	++	+++++	+	+	++++	+++++	+++++	+++++	+++++

Reaction: +mild, ++positive, +++strong, ++++very strong, +++++ extremely strong -no reaction

reaction in the 19 day-old and a moderate one in the 20, 27 and 43 day-old. The brush border is negative except in the 43 day-old where it is positive. The secretion of the goblet cells is NSE-ase negative.

3. Ileum:

A positive NSE-ase reaction is seen in the cytoplasm of the epithelial cells of the villi on days 26 and 28. The reaction is mainly homogeneous but also contains some granules. Bases of villi and crypts exhibit a milder reaction with distinct granules that, on the 28th

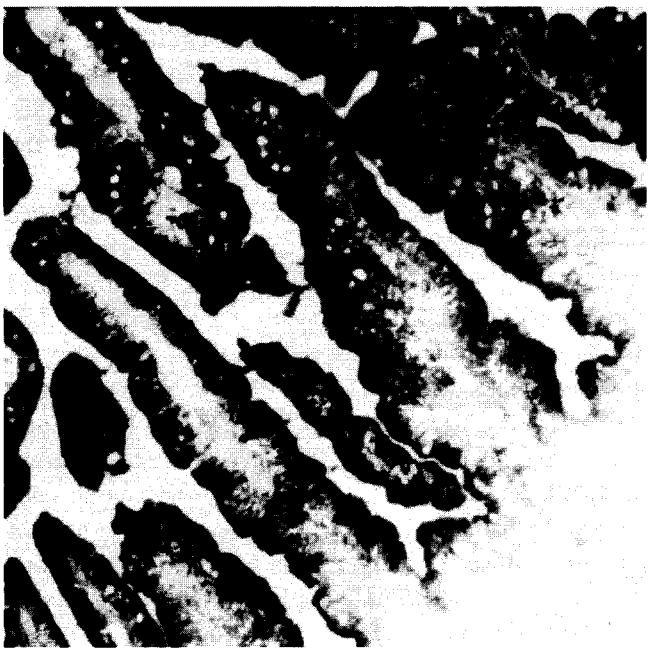

Figure 2 : Jejunum-7 day old
Strong non specific esterase positive reaction especially in the supranuclear region using a naphthyl acetate method for determining NSE-ase. Cells lining the crypts exhibit a weaker reaction. X 140

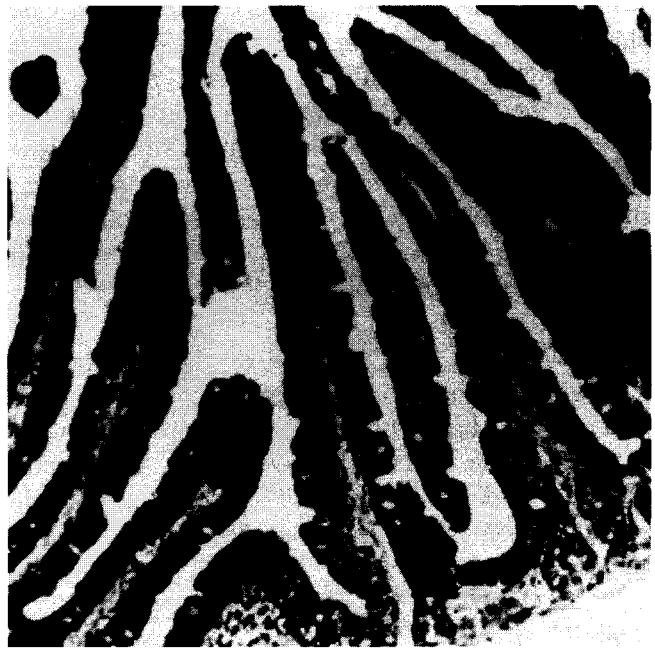

Figure 3 : Ileum-43 day old
Strong non specific esterase positive reaction in the cytoplasm of the epithelial cells using a naphthyl acetate method for determining NSE-ase.. X 140

Figure 4 : Caecum-20 day old
Very strong non specific esterase positive reaction using a naphthyl acetate method for determining NSE-ase. X 140

day, are in the apical part of the cell. The reaction is stronger on the 30th day and is centered particularly on the supranuclear region of the cell. Crypt cells contain mainly positive granules.

The epithelial cells of the villi show a homogeneous NSE-ase positive reaction (Fig. 3), which in the 7 day old also have superimposed granules. Their brush border is NSE-ase negative at all ages. The cells of the crypts show a granular reaction in 1day-old animals, otherwise they reveal granules in an NSE-ase positive homogeneous cytoplasm. The *lamina propria* shows a slightly positive reaction in the 19-day-old and a moderate one in the 20, 27 and 43-day-old animal. The secretion of the goblet cells is NSE-ase negative.

4. Caecum:

During foetal life the cytoplasm of the epithelial cells shows a slightly positive NSE-ase reaction, which is more intense in the 30 day-old fetus. The reaction reveals homogeneously stained material and granules which are more numerous in the cells at the bases of the glands.

After birth, the cytoplasm of the epithelial cells exhibits a strong NSE-ase reaction with some granules. This reaction is not as strong in the 7 day-old animals but by the 19th day is stronger in the supranuclear cytoplasm. From then on (Fig.4) the reaction increases in strength and includes the *lamina propria* . The brush border is generally NSE-ase negative although it is occasionally positive in the 20 and 43 day-old rabbits. The secretion of the goblet cells is NSE-ase negative.

5. Colon:

By the 26th and 28th day a slight NSE-ase reaction revealed many granules in the cytoplasm of the epithelial cells. These granules sometimes form a line at the bases of the cells of the glands. As in the caecum, on the 30th day there is a more intense NSE-ase reaction in the cytoplasm of the epithelial cells and the glands.

After birth, the colon of the neonatal 1 day-old shows a positive NSE-ase reaction in the cytoplasm of the epithelial cells. This is of variable intensity and appears as homogeneous material which includes granules. The reaction is weaker towards the bases of the cells of the glands.

DISCUSSION

In the present study we found that during the latest stages of foetal life the cytoplasm of the epithelial cells of villi and crypts of the small intestine were NSE-ase positive. However, those of the crypts of the 26 and 28-day-old foetal duodenum were weakly positive and, in some cases, negative. This may be due to a lower rate of hydrolysis of organic esters in these cells.

In the large intestine of the 26- and 28 day- old foetuses the epithelial cells of both caecum and colon show a slight NSE-ase positive reaction, which becomes stronger in the 30day-old foetus.

After birth, the cytoplasm of the epithelial cells of the villi and crypts of the small intestine exhibits a strong reaction, which is most intense in the supranuclear regions of the cells in the duodenum and jejunum.

The reaction of the epithelial cells of the large intestine is strongly positive but in the colon this weakens towards the bases of the glands. From 20 days of age onwards, the reaction becomes even stronger.

These post-natal observations are in general agreement with those of NACHLAS and SELIGMAN (1949), CHESSICK (1953), SHNITKA (1960) and WEIGEL and GEYER (1971). The last authors suggested that the esterase granules, particularly the coarse ones which were also seen in the epithelial cells in the present material, represent lysosomes and that the homogeneous esterolytic reaction in the cytoplasm may be associated with the endoplasmic reticulum. In addition, DEREN (1968) states that the enzyme was observed in high concentrations in adult intestinal tissue.

Our post-natal observations concerning the enzyme distribution in rabbit intestine are in agreement with those observed in the quail and chicken, where it was shown that the intestinal expression of the esterase

patterns became more complex in number and/or staining intensity upon hatching and thereafter (TSITSILONI *et al.*, 1993, VEINI *et al.*, 1986). Similarly, in our study, the enzyme distribution in rabbit intestine was more intense after birth.

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REFERENCES

BOCKING A, VON DEIMLING O., 1982. Dynamics of non-specific esterase during fat resorption in the jejunum of the house mouse, Mus musculus. *Histochemistry, **75**, 377-85.*

CHESSICK R. D., 1953. Histochemical study of the distribution of Esterase. *J. Histochem. Cytochem, **1**, 471-485.*

COATES P. M., MESTRINER M. A., HOPKINSON D. A., 1975. A preliminary interpretation of the esterase isozymes of human tissues. *Ann. Hum. Genet. Lond., **39**, 1-20.* (Cited by TSITSILONI O. E., M. VEINI, A. A. HARITOS., 1993. Esterase in quail (Coturnix coturnix). Physicochemical and developmental aspects. *Comp. Biochem. Physiol., Vol. 106B, No. 4, pp. 1009-1014)*

DAVIS, B. J., ORNSTEIN, L., 1959. High resolution enzyme localization with a new diazo reagent hexazonium pararosaniline. Journal of Histochemistry and Cytochemistry, 7, 297. In: *"Theory and practice of histological techniques', 1977. Eds. J. Bancroft and A.. Stevens, Churchill Livingstone, Edinburgh, London and New York, pp. 296.*

DEREN J. J., 1968. Development of intestinal structure and function. *In: Handbook of Physiology, section 6, alimentation canal III, 1099-1123, American Physiological Society, Washington D.C.*

GOMORI, G., 1952. Histochemistry of esterases. International Review of Cytology, 1, 323. In: *"Theory and practice of histological techniques', 1977. Eds. J. Bancroft and A.. Stevens, Churchill Livingstone, Edinburgh, London and New York, pp. 296.*

HARITOS A. A. SALAMASTRAKIS, S. S., 1982. A comparison of muscle esterases in the fish genus *Trachurus* by vertical gel electrophoresis. *Comp. Biochem. Physiol. **72B**, 477-480.* (Cited by TSITSILONI O. E., M. VEINI, A. A. HARITOS., 1993. Esterase in quail (Coturnix coturnix). Physicochemical and developmental aspects. *Comp. Biochem. Physiol., Vol. 106B, No. 4, pp. 1009-1014.*)

NACHLAS M. M., SELIGMAN A. M. M., 1949. The comparative distribution of esterase in the tissues of five mammals by a histochemical technique. *Anat. Rec., **105**, 677-695.*

SALAMASTRAKIS S. S., HARITOS A. A.., 1988. Physicochemical characterization and tissue distribution of multiple molecular forms of fish (*Trachurus trachurus*) esterases *Comp. Biochem. Physiol., **91B**, 741-750.* (Cited by TSITSILONI O. E., VEINI M., HARITOS A. A.. Esterase in quail (*Coturnix coturnix*). Physicochemical and developmental aspects. Comp. Biochem. Physiol. 106B, No. 4, pp. 1009-1014, 1993).

SHNITKA T. K., 1960. Enzymatic histochemistry of gastrointestinal mucous membrane. *Fed. Proc., **19**, 897-904*

TSITSILONI O. E., M. VEINI, A. A. HARITOS., 1993. Esterase in quail (*Coturnix coturnix*). Physicochemical and developmental aspects. *Comp. Biochem. Physiol., Vol. 106B, No. 4, pp. 1009-1014.*

TZANNETATOU-POLYMERI R., HARITOS A. A., 1989. Physicochemical characterization and tissue distribution of esterases in two Salamandridae species (*Mertensiella luschani* and *Salamandra salamandra*) *Comp. Biochem. Physiol. **92B**, 469-475.* (Cited by TSITSILONI O. E., M. VEINI, A. A. HARITOS., 1993. Esterase in quail (Coturnix coturnix). Physicochemical and developmental aspects. *Comp. Biochem. Physiol., Vol. 106B, No. 4, pp. 1009-1014.*)

VEINI M., TSITSILONI O. E., MARTINI S. M., HARITOS A.A.,1986. Multiple molecular forms of soluble esterases in the digestive system of the developing chicken. *Comp. Biochem. Physiol. **83B**, 775-781.* (Cited by TSITSILONI O. E., M. VEINI, A. A. HARITOS., 1993. Esterase in quail (Coturnix coturnix). Physicochemical and developmental aspects. *Comp. Biochem. Physiol., Vol. 106B, No. 4, pp. 1009-1014.*)

WEIGEL I., GEYER G., 1971. Comparative histochemical study of the distribution of esterases in the small and large intestine. *Z. Mikr. anat. Forsch., **83**,193-204.*

ZUTPHEN van L. F., FOX R. R., BIEMAN M. G., 1983. Genetics of two tissue esterase polymorphisms (Est-4 and Est-5) in the rabbit. *Biochem. Genet., Aug., **21**, 773-80.*