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SITES OF ESTERASE ACTIVITY IN THE GUINEA PIG EMBRYO

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Our knowledge of the localization and evolution of esterases in the embryo, begins with the papers of *Hunter* [1951], in the mouse, and *Buño and González-Mariño* [1952] in the chick. The former described the distribution of esterase in pancreas, liver, bronchial epithelium, the thyroid gland and the epithelium lining the nasal cavity. No esterases were found in the kidney. The reaction in the pancreas, is positive in 10 to 14 mm embryos; before that age no esterases are demonstrable.

In 1952, *Buño and González-Mariño* published the first comprehensive paper on "lipase" in the chick embryo, as demonstrated by using the Gomori's technique [1945] with Tweens as substrates. In that paper they described a strong reaction in the yolk sac epithelium, demonstrable after 48 hours of incubation. After that age esterases appear in the hepatic anlage and in the foregut. On the 4th day of incubation the reaction appears in the pancreatic bud. A positive reaction was described in some scattered mesenchymal cells. A slight reaction is limited to the white matter of the basal region of the neural tube. The reaction was positive in the tubes of the mesonephros; in the metanephros it was weak and inconstant.

Later, *Yoneda* [1956] published his results on esterases in the chick embryo. Applying both the naphthyl-acetate and the Tweens technique, he was unable to show any esterase in the chick embryo until the 6th day of incubation (in the mesonephros and liver) and only around the 12th day in the other organs. *Zacks* [1954] with a more elaborate technique studied the localization of esterase, cholinesterase and acetylcholinesterase in the duck embryo, limiting his researches to the first 4 days of incubation. In a general way he confirmed and completed our previous studies in the chick. By using different substrates the problem was restudied by *Buño and Dal-Monte* [1962] confirming all the former findings of *Buño and González-Mariño*.

In mammals, besides *Hunter's* paper we must mention the investigations of *Rossi* [1954] and *McKay, Adams, Hertig and Danziger* [1956] on the localization of different enzymes, and, among them esterases, in the human embryo. Although these authors have made important contributions to our knowledge of the embryonic histochemistry, the difficulty in obtaining enough human material in good condition and adequately dated and processed, hinders the study of the appearance

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and distribution of an enzyme in man. In the rat, the localization of esterases was studied in some organs by *Verne and Hébert* [1953].

We have examined the distribution of aliesterases in the guinea pig embryo, focusing attention specially on the first sites of esterase activity and the evolution of this activity through embryonic development till birth.

Material and Methods

We used embryos of guinea pig of known age. Pregnant females were placed with males; it is known that the females are mated the first day after parturition. Vaginal smears were made the morning after the parturition and the finding of spermatozoa determined the 0 day of pregnancy.

Table I. Distribution of esterases in different organs of guinea pig embryos at different ages.

Organs	24 days	27 days	34 days
Tongue	—	++	+++
Oesophagus	—	++	+++
Stomach	—	++ (1)	+++
Intestines	—	++	+++
Liver	+	++	+++
Gall-bladder	—	+ (1)	++
Pancreas	—	—	+++
Nasal Mucosa	—	+	+++
Trachea	—	+	++
Lung buds	—	—	++ (2)
Heart	—	+ (3)	+ (3)
Aorta	—	—	+
Adrenal	—	—	++
Thyroid	—	—	+
Mesonephros	—	—	—
Metanephros	—	—	—
Gonad	—	—	—
Spinal cord	—	—	+++ (4)
Ganglia	—	—	—
Eye	—	++ (5)	++ (5)
Mesenchyme	—	++ (6)	+++ (6)
Muscle	—	—	—
Epidermis	—	+	++

(1) Epithelium and content. (2) In the stem bronchi. (3) Subendocardial. (4) Motor neurons in the anterior horn. (5) In the "hyaloid cells" and anterior chamber epithelium. (6) Some cells only (macrophages). + Weak reaction. ++ Medium reaction. +++ Strong reaction.

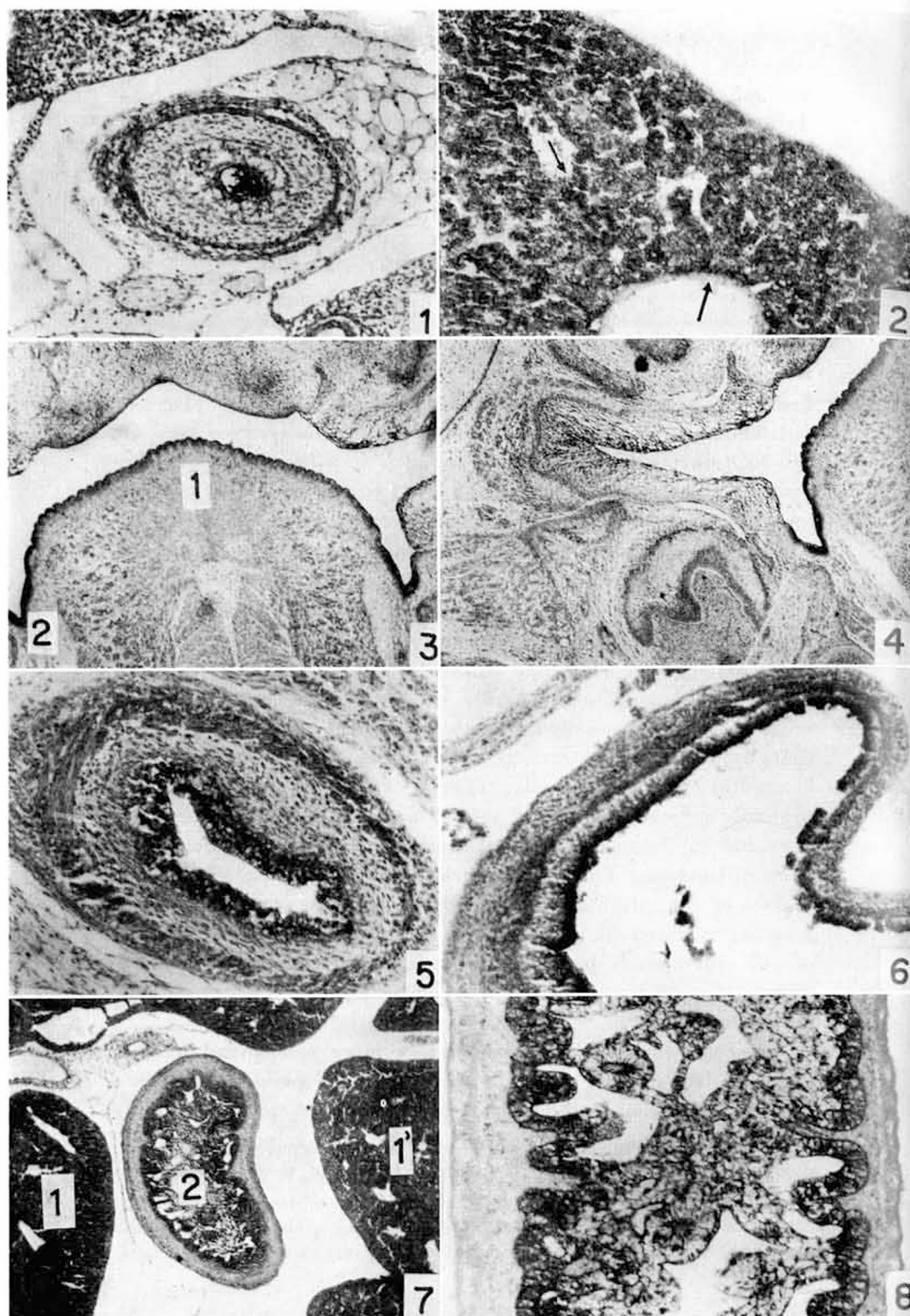
A previous survey demonstrated that no esterases are demonstrable before the 20th day. We have studied embryos from that age onwards. We employed a total of 21 embryos. The technique has been described in a previous paper (Buño and Dal-Monte [1962]). As substrates we used α naphthol-acetate and naphthol-AS-acetate. We also employed sodium taurocholate as activator of the pancreatic lipase and inhibitor of the aliesterases, and prostigmin¹ (0.01 mg per ml) which inhibits specifically acetylcholinesterase. Therefore, our study was mostly directed towards the demonstration of aliesterases and true lipase.

Results

No esterases were found in the embryonic body during the first twenty days of development. At this time, only some placental cells and the yolk sac epithelium show a weak positive reaction. On the 24th day the first positive reaction is seen in the cytoplasm of the cells of the hepatic anlage. This reaction is weak and not uniform; some cells show a poor reaction, others none at all. On the 27th day the reaction is present in several organs and tissues. In the gastro-intestinal tract the oesophagus shows a good reaction in its epithelial lining (fig.1). Less intense reaction is visible in the epithelial lining of the stomach and the intestines. The liver shows now a stronger reaction than on the 24th day, still mostly confined to the peri-venous cells (fig.2). In some small sub-endocardial areas in the heart, we observed a weak reaction. We want to stress that, at this age, the strongest reaction seen is most constantly located in two types of cells; (1) cells of dubious origin, but most probably mesenchymal, that appear in the vitreous humour of the eye; and (2) some mesenchymal cells, distributed in different regions of the body. The "hyaloid cells", which are close to the inner surface of the retina and to the posterior aspect of the lens, show a strong reaction for esterase in their cytoplasm. The other type of cell corresponds probably, although not certainly, to macrophages. They are situated in the cephalic, body wall and limb buds mesenchyme.

The 34 days old embryos show a more widespread and, in general, stronger reaction. A very strong and precisely localized reaction, is present in the buccal epithelium. The epithelium lining the tongue is positive (fig.3). The mouth epithelium shows a well defined thickening at the position where the dental lamina is

¹ We wish to thank Hoffmann-La Roche & Co. Ltd. of Uruguay for providing us with the prostigmin used in these experiments.



formed; this epithelium, of very clear cells, exhibits a positive reaction to esterase. On the contrary, neither the enamel organ, nor any other dental structure are positive (fig. 4).

In the gastro-intestinal tract the oesophageal epithelium exhibits a stronger reaction than in previous stages (fig. 5). The gastric wall shows a double line of positive reaction; the internal on the epithelium and the external on the submucosa. The gastric contents show an intense reaction (fig. 6). The intestinal epithelium gives a very strong reaction. The liver exhibits a stronger and more widespread reaction than in the previous stages (figs. 7 and 8). In the gall-bladder, both mucosa and content give a strong reaction. At this stage the pancreatic anlage has appeared, forming tubes and solid buds. In both structures a strong reaction is visible in the form of round dark spots located in the cytoplasm (figs. 11 and 12).

The epithelial cells of the nasal mucosa are positive. The trachea and principal bronchial buds are positive, but the terminal small bronchial channels always lack esterase (figs. 9 and 10). In the heart, we found, as in the 27th day embryo, a positive subendocardial reaction in the atrium. The aorta shows a weak positive reaction in ill defined location on its wall. In the cytoplasm of the proliferating cartilage cells, a weak reaction is present. On the contrary, no reaction is visible, either in the precartilaginous blastema, or in the ossification zone. A strong reaction is present in some joints (fig. 19). The metanephros is negative in the corpuscles and in the tubules, but in some of the collecting tubes a weak reaction appears. The

Fig. 1. Oesophagus. 27 day embryo. Positive reaction in the epithelial cells.

Fig. 2. Liver. 27 day embryo. Positive esterase reaction in many, not in all, cells. The cells in the neighbourhood of the big veins (arrows) show a strong reaction.

Fig. 3. Mouth. Reaction positive in the epithelial cells of the tongue 1 and in the thick epithelium related to the dental lamina 2.

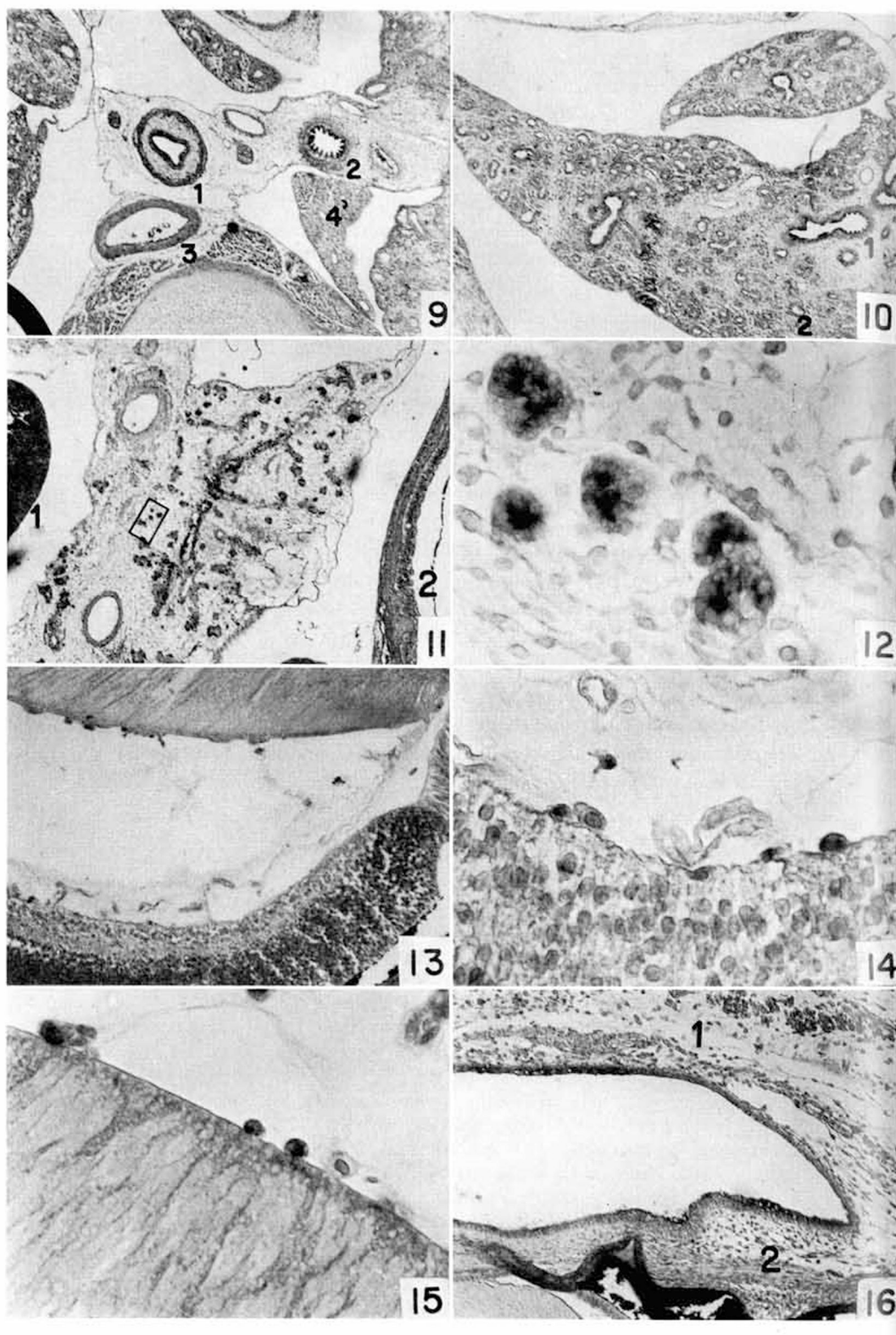
Fig. 4. Dental lamina related epithelium with positive reaction. No reaction in any tissue of the developing tooth.

Fig. 5. Oesophagus of a 34 day embryo. Compare with fig. 1. Epithelium intensely positive.

Fig. 6. Stomach. 34 day embryo. Positive reaction in the epithelium and the submucosa. Also the content is positive.

Fig. 7. Liver 1, 1', and small intestine 2. 34 day embryo. The liver exhibits a much stronger reaction than in former stages (see fig. 2). The small intestine has still no visible lumen, but a strong reaction is present in its epithelium.

Fig. 8. Small intestine (34 day embryo) localization of esterases in the epithelium, more precisely in the cells that will become definitive epithelium.



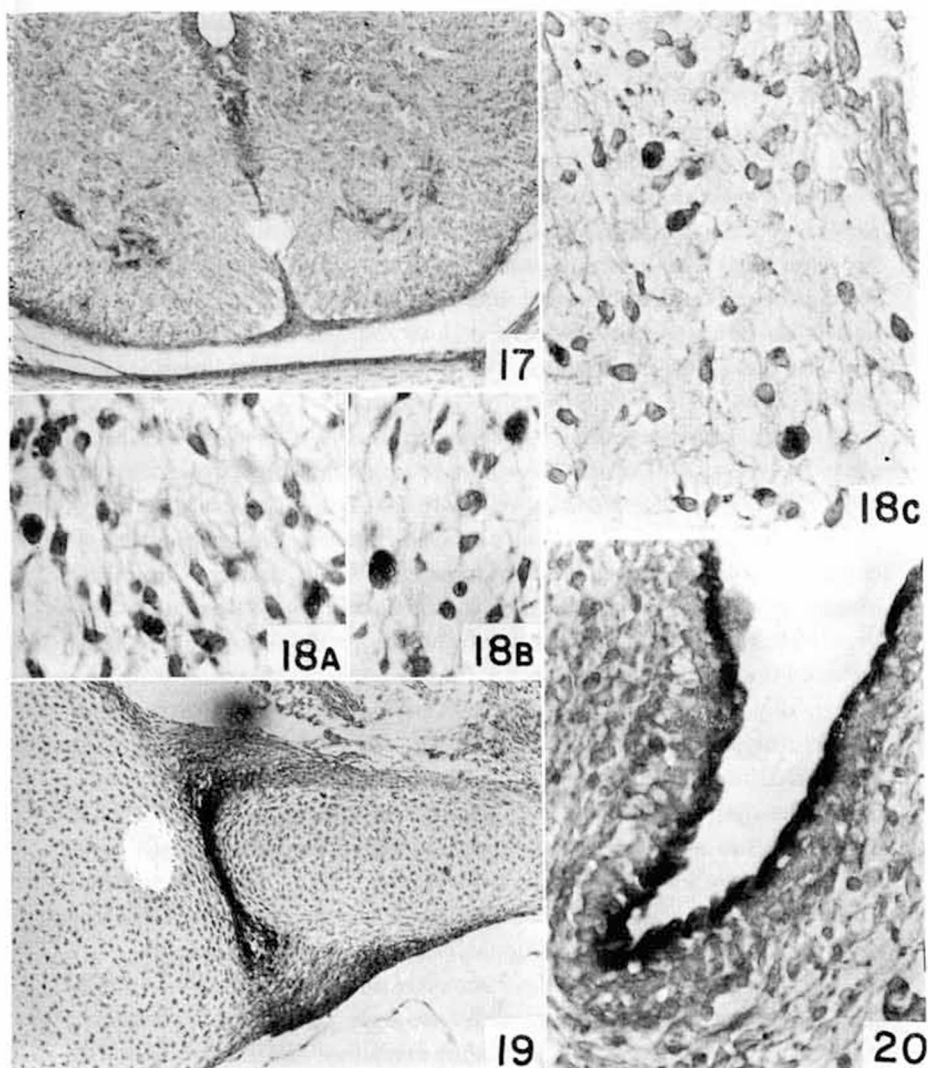


Fig.9. 34 day embryo. Transverse section of the thoracic region. 1 Oesophagus; 2 Trachea; 3 Aorta; 4 and 4a. Lungs.

Fig.10. 34 day old embryo. Lung. The reaction is positive in the primary bronchi 1, and negative in the small bronchi 2.

Fig.11. Pancreatic anlage in the dorsal mesenterium. Both main duct and solid branches are positive. 1 Liver; 2 Stomach (34 day old embryo).

Fig.12. The square is of fig.11 at higher magnification.

Fig.13. Vitreous humour. The "hyaloid cells" are strongly positive. 34 day embryo.

Fig.14. "Hyaloid cells", against the innermost layer of the retina.

Fig.15. "Hyaloid cells" strongly positive, against the posterior aspect of the lens.

Fig.16. Eye. Anterior chamber. 1 Cornea; 2 Iris. The posterior epithelium of the cornea 1. and the anterior epithelium of the iris 2. are positive.

Fig.17. Spinal cord. The motor neurons in the ventral horn are positive.

Fig.18a and 18b. Mesenchymal cells. The macrophages are strongly positive.

Fig.19. Costo-vertebral joint. The joint is strongly positive.

Fig.20. Skin. The epidermal cells in some areas are strongly positive.

gonads are negative. The nervous system is negative with one exception; the big motor neurons of the anterior horn of the spinal cord are strongly positive. No other positive reaction is visible in the nervous system. The sympathetic and spinal ganglia are quite negative. The reaction of the motor neurons is not inhibited by prostigmin. The epidermis exhibits an irregular, positive reaction. At this stage, adrenal cortex and medulla are still separated. The first is positive, and the last, as in the sympathetic system, is negative. In the thyroid gland the cells forming the folliculi are weakly positive.

As in younger embryos, some mesenchymal cells exhibit a very strong cytoplasmic reaction. It is difficult to ascertain the type of cells we are dealing with. Many of them resemble macrophages; they are round free cells located in the meshes of the mesenchymal tissue; others have long processes and, finally, others are identical with the undifferentiated mesenchymal elements. This type of "esterase positive" cell is found in the head and in different areas of the body wall and limb bud mesenchyme. No adipose tissue is yet developed at this stage in the embryo. Adipose tissue is esterase negative in this species, even in the adult. After the 34th day (middle of the pregnancy) the localization of esterases exhibits little change, although the intensity of the reaction varies. In pancreatic acini, liver cells, intestinal epithelium the intensity of the reaction increases. On the contrary, in the gall-bladder epithelium the reaction diminishes and disappears around the 40th day of embryonic development. The salivary gland acini exhibit a good reaction from the 35th day, but always weaker than in the pancreatic acini. The thymus presents many strongly positive cells, localized in the boundary between cortex and medulla. The Hassall's bodies are negative. The positive cells are either reticular cells or invasive macrophages.

From this age onwards the distribution of esterases adopts the pattern of the adult.

Discussion

The first cells exhibiting esterase activity in their cytoplasm are the hepatic cells in 24–25 day embryos. Before that date the reaction is only present in some placental cells. A weak positive reaction is also visible in the epithelial cells lining the yolk sac. Although it is very difficult to compare chronologically the embryonic

development in different species, similar results were reported in human (*McKay et al.* [1956], *Rossi* [1954]); in mouse (*Hunter* [1951]) and in chick embryos (*Buño and González-Mariño* [1952], *Buño and Dal-Monte* [1962]). In the chick embryo, the earliest site in which esterase activity has been shown is in the yolk sac endoderm. It has been suggested by the last authors that the enzyme is present here to act in the lipidic metabolism. This interpretation, which may be suggestive in the chick embryo, is obviously valueless in the guinea pig. In mammals the liver is the first and most important site of esterase activity during earlier embryonic life. The gall-bladder gives in early stages a positive reaction both in its epithelium and contents, but the reaction disappears before the end of pregnancy.

The gastro-intestinal tract epithelium is positive from the 27th day and along its whole length. Particularly interesting is the positive reaction in the buccal epithelium in the tongue, which has also been described in the human by *Rossi* [1954] and more precisely in the neighbourhood of the dental lamina. The reaction is only positive on the surface epithelium and negative in the dental lamina proper, as in any other tooth structure.

The pancreatic bud is positive almost from its beginning, in both human and chick embryos (*Rossi* [1954], *Buño and González-Mariño* [1952]). The reaction is positive both in the ducts and in the primitive acini. These cells are very active in the adult in the production of true lipase. This fact demonstrates that already at its earlier stages of differentiation the pancreatic cells are active in the elaboration of esterases. The respiratory system presents esterase activity in different places: the nasal epithelium is precociously and strongly positive, what has also been noticed in human embryos (*Rossi* [1954]). This localization has never been observed in chick embryos. The epithelial lining of the larynx, the trachea and the stem bronchi all show a strong positive reaction; on the contrary no reaction is visible in the terminal bronchi. These facts agree with the report on the mouse (*Hunter* [1951]) and is not in accordance with the finding in human (*Rossi* [1954]) or rat (*Verne et Hébert* [1954]) embryos. In the localization of esterase in the respiratory system of the embryo there are important and clear cut species differences. In the heart only a weak reaction is demonstrable, immediately underneath the endocardium but as we were not able to determine more precisely its localization, we will not express an opinion about it. Also a positive reaction is visible on the 34th day

in the aortic wall. Of the endocrine glands, only in two of them was a rather weak reaction demonstrated; in the thyroid of the 34 day embryos and in the adrenal cortex of the same age. In the human embryo the reaction is positive in the thyroid cells and negative in the adrenal (Rossi [1954]).

Both the mesonephros and metanephros were negative; in the last organ a weak positive reaction is seen in the epithelium of the collecting tubes. The previous reports of different authors in different species, and even in the same species, are contradictory. In human embryo positive (McKay *et al.* [1956]) or negative results (Rossi [1954]) were reported; negative was also reported in mouse (Hunter [1951]) and rat embryos (Verne and Hébert [1954]). On the contrary, very strong and precisely localized reactions in the tubules of the mesonephros and metanephros was reported in the chick (Buño and González-Mariño [1952]). Also important species differences in the distribution of esterases in the adult kidney were reported (Gomori [1946]); and guinea pig kidney is characterized by the lack of esterase reaction.

Irregularly distributed in different areas of the mesenchyme, among the usual stellate cells, some special type of cells are found that exhibit, in their cytoplasm, a strong and patchy reaction. Many of these cells are undoubtedly macrophages; others are probably undifferentiated mesenchymal cells. The adult adipose cells in the guinea pig do not present esterase activity, which eliminates, we believe, the possibility that the esterase positive mesenchymal cells are adipoblasts. The contrary occurs in the same elements of the adult adipose tissue of the rat and rabbit (Gomori [1946]) and in the chick embryo (Buño and González-Mariño [1952]).

In the nervous system we have only found esterase activity in the cytoplasm of the motor neurons located in the anterior horn of the spinal cord. These cells give also a positive reaction in the human (Rossi [1954]) and the chick embryo (Buño and González-Mariño [1952]). The treatment with prostigmin does not suppress the reaction; therefore it is not due to cholinesterase. The reticular-type cells situated in the vitreous body give a very prominent positive reaction; these cells are located peripherally in the vitreous, against the posterior aspect of the lens or against the internal face of the retina. Its positive reaction is strong and precocious and has never been mentioned before.

The histochemical reaction for esterases in the guinea pig

embryo shows no differences using a naphthol-acetate or AS-naphthol-acetate; in other words with both substrates we have obtained identical results. The use of prostigmin does not inhibit the enzymatic reaction in any cell, not even in the nervous cells; taurocholate does not inhibit the enzymatic reaction in any tissue.

Conclusions

(1) The first tissues that exhibit esterase activity in the guinea pig embryo are the liver anlage and the yolk sac epithelium. This reaction appears about the 20th day of age.

(2) On the 24th day the reaction is present in the gastro-intestinal epithelium, in the trachea and in some mesenchymal and "hyaloid cells".

(3) On the 34th day the reaction is positive in the digestive tract epithelium, from the mouth to the anus. It is now stronger in the liver cells, and the pancreatic bud exhibits a strong reaction. The epithelium of the trachea and the primary bronchi show a positive reaction, but the secondary and final bronchial branches are negative. The motor neurons in the ventral horn of the spinal cord are negative. No other activity was found in the nervous system. The epidermis is irregularly positive; the adrenal cortex and the thyroid gland are positive.

(4) We wish to stress the strong positive reaction in the hyaloid cells and the macrophages.

All sections were made using the same technique (Buño and Dal-Monte [1962]). Some of them were counterstained, either with haematoxylin or with basic fuchsin.

Summary

The localization of esterases in the guinea pig embryo has been studied using, as substrates, α -naphthol and naphthol-AS-acetate (Gomori [1952]).

Esterase appears only after the 20th day. Liver is the first organ to exhibit a positive reaction on the 21th day. On the 27th day the reaction is positive in the gastro-intestinal tract, the liver, the nasal mucosa, the "hyaloid cells" in the eye and macrophages, disseminated in different regions of the mesenchyme. On the 34th day the reaction is stronger in the aforementioned organs and moreover appears in the pancreatic bud and the motor neurons in the ventral horn of the spinal cord. This last reaction is not inhibited by prostigmin, what excludes the possibility that it be acetylcholinesterase. No differences between the reaction obtained with α -naphthol acetate or the naphthol-AS-acetate as substrates were observed.

Résumé

On a étudié la localisation des estérases chez l'embryon de cobaye à différents stades de développement, en employant la technique de Gomori [1952] à l'acétate de α -naphtol et l'acétate de AS-naphtol comme substrats.

Les estérases font leur apparition chez l'embryon de cobaye après le 20^e jour. On les trouve tout d'abord au niveau du foie qui, au 21^e jour déjà, montre une réaction très nette. Au 27^e jour, la réaction est positive au niveau du tractus digestif, du foie, de la muqueuse de la cavité nasale, des "cellules hyaloïdes" de l'œil et des macrophages distribués dans différentes régions du mésenchyme.

Au 34^e jour, la réaction devient plus intense aux points déjà mentionnés et apparaît dans l'ébauche pancréatique et dans les neurones moteurs de la corne antérieure de la moelle épinière. Cette réaction n'est pas inhibée par la prostigmine, ce qui élimine la possibilité qu'il s'agisse d'acétylcholinestérase. Nous n'avons trouvé aucune différence entre la réaction obtenue avec l'acétate de α -naphtol ou l'acétate de AS-naphtol.

Zusammenfassung

Mittels der Gomori-Technik mit α -Naphtol und AS-Naphtol-Azetat wurde die Lokalisation der Esterasen bei Meerschweinchen-Embryonen verschiedenen Alters untersucht. Die Esterasen treten beim Meerschweinchen-Embryo erst nach dem 20. Tag auf.

Die erste Reaktion tritt in der Leber auf, und zwar schon am 24. Tag. Eine positive Reaktion zeigt sich schon am 27. Tag im gesamten Verdauungskanal, Leber, Nasenschleimhaut, «Hyaloida-Zellen» des Auges und in den Makrophagen, die in verschiedenen Regionen des Mesenchyms verteilt sind.

Am 34. Tag verstärkt sich die Reaktion in den vorgenannten Organen und erscheint überdies kräftig in der Pankreasanlage und in den Moto-Neuronen des Vorderhornes der Medulla spinalis. Die letztgenannte Reaktion wird nicht durch Prostigmin gehemmt, was die Möglichkeit ausschließt, daß es sich um Azetylcholinesterase handeln könnte. Es gibt keinen Unterschied zwischen der mit α -Naphtol und der mit AS-Naphtol-Azetat erhaltenen Reaktion.

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