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Enzyme Histochemical Studies on Hydatidiform moles and Choriocarcinomas in Ceylonese

by

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INTRODUCTION

Enzyme histochemical studies have been carried out on the trophoblast of normal placentae by Wislocki and Dempsey (1945), Wachstein, Meagher and Oritz (1963), Jeacock and Morris (1963) and Curzen (1965). There are a few reports of similar investigations on hydatidiform moles and choriocarcinomas. Among these are the papers of (a) Bur, Hertig, Makay and Adams (1962) who looked for phosphatases, non-specific esterase and 5-nucleotidase (b) Jirasek and Votja (1965) who investigated lactic dehydrogenase and (c) Mckay (1966) who detected hydroxysteroid dehydrogenases in hydatidiform moles. There are no reports of attempts to demonstrate leucine aminopeptidase histochemically in the trophoblastic elements of hydatidiform moles or choriocarcinomas and no enzyme histochemical studies have been done on trophoblastic material recovered from Ceylonese. As such in this preliminary investigation an attempt was made to look for acid phosphatase, non-specific esterase and leucine aminopeptidase in normal full term placentae, hydatidiform moles, and choriocarcinomas.

MATERIALS AND METHODS

The material examined consisted of the following (a) 10 hydatidiform moles (b) 4 choriocarcinomas (c) 4 full term placentae. Four out of the 10 hydatidiform moles were simple moles in which the villous pattern was well preserved, trophoblastic proliferation was minimal and there was no tendency for the trophoblast to invade the uterine tissues. The remaining 6 hydatidiform moles were proliferating moles, in which the villous pattern was preserved, there was a marked proliferation of trophoblast and the proliferating trophoblast invaded uterine muscle. Both hydatidiform moles and choriocarcinomas were obtained from surgically removed specimens. Two of the 4 full term placentae examined were taken from normal deliveries, the remainder were obtained at caeserian section.

As a routine a paraffin block was made from each specimen and sections from this were stained with Ehrlich's haematoxylin and eosin. Enzyme studies were done on fresh tissue frozen in liquid nitrogen from which sections were cut at - 20° C using a cryostat.

The methods described by Barka and Anderson (1965) were used in order to demonstrate acid phosphatase, non-specific esterase and leucine aminopeptidase. The substrate for acid phosphatase consisted of the monosodium salt of alphanaphthyl phosphate and hexazonium pararosanilin, whereas that for non specific esterase consisted of alpha naphthyl acetate and hexazonium pararosanilin. 4 methoxy-beta naphthylamide hydrochloride and the diazonium salt fast red formed the substrate for leucine aminopeptidase.

RESULTS

Normal placenta :— Both cytotrophoblast and syncitiotrophoblast show considerable activities of acid phosphatase (Figs. 1 and 7) and non-specific esterase (Fig. 4). Leucine aminopeptidase could not be demonstrated histochemically in the trophoblast of normal full term placentae.

Simple Hydatidiform Moles :— The enzymic pattern of the trophoblast in simple moles resembles that observed in normal full term placentae.

Proliferating Moles :— The enzymic pattern of the proliferating trophoblast differs significantly from that seen in simple moles and normal full term placentae. Acid phosphatase (Figs. 2 and 8) and non specific esterase (Fig. 5) are almost absent in cytotrophoblastic cells whilst being strongly positive in the syncitiotrophoblast. Leucine aminopeptidase activity which is not demonstrable in the trophoblast of normal placentae, appears in both the cytotrophoblast and syncitiotrophoblast in proliferating moles (Fig. 6).

Choriocarcinomas :— Acid phosphatase and non-specific esterase could not be demonstrated in significant amounts in the cytotrophoblastic elements of choriocarcinomas. However, unlike in proliferating moles, choriocarcinoma show a diminution of acid phosphatase in its syncitial elements (Figs. 3. and 9). Although both elements of the trophoblast show leucine aminopeptidase activity in choriocarcinomas, it is not as intense as the activity seen in the trophoblast of proliferating moles.

Table 1 summarizes the enzyme reactions seen in the trophoblast in normal full term placentae, simple moles, proliferating moles and choriocarcinoma.

TABLE 1

Enzyme	Trophoblast cell	Normal full term placentae	Simple moles	Proliferating moles	Choriocarcinomas
Acid phosphatase	cyto	++	++	almost absent	almost absent
	syncitio	++	++	++	+ (weak)
Non-specific esterase	cyto	++	++	almost absent	almost absent
	syncitio	++	++	++	++
Leucine amino-peptidase	cyto	absent	absent	++	+
	syncitio	absent	absent	++	+

DISCUSSION

In our material, both elements of the trophoblast of full term placentae show up considerable acid phosphatase activity. Curzen (1964) demonstrated acid phosphatase histochemically in the trophoblast of 44 placentae recovered from cases of normal and abnormal pregnancy. We have earlier stated that acid phosphatase activity in the trophoblast of proliferating moles and choriocarcinomas differs considerably from that seen in normal full term placentae. In 1962 Bur *et al.* investigated phosphatase in hydatidiform moles and choriocarcinomas and detected small amounts of acid phosphatase in the cytotrophoblast and varying amounts of it in the syncitiotrophoblast, of simple and proliferating moles. They further observed marked acid phosphatase activity in the syncitial elements of choriocarcinomas. Our findings differ from these observations in the following manner (a) in our cases of simple moles acid phosphatase activity was strongly positive in both elements of the trophoblast whereas in proliferating moles it was absent in the cytotrophoblast and strongly positive in the syncitial elements and (b) acid phosphatase activity was almost absent in the cytotrophoblast and weakly positive in the syncitial elements in our cases of choriocarcinomas. We cannot explain why our findings in respect of acid phosphatase in hydatidiform moles and choriocarcinomas differ from those of Bur *et al.* (1962). We suggest that it is possible that alterations in the activity of the lysosomal enzyme acid phosphatase seen in proliferating moles and choriocarcinomas, both by us and Bur *et al.* (1962) may reflect changes in ultra structure at lysosomal level.

When we looked for non-specific esterase activity in the trophoblast, its localisation in different varieties of hydatidiform moles resembled that of acid phosphatase, but in choriocarcinomas it was found that non-specific esterase activity was almost absent in the cytotrophoblast and was intensely positive in its syncitial elements. These observations also differ from those of Bur *et al.* (1962) who detected weak non-specific esterase activity in both varieties of hydatidiform moles and only a trace of it in the cells of choriocarcinomas. It is possible that because Bur *et al.* (1962) looked for non-specific esterase in acetone fixed paraffin sections a considerable amounts of the enzyme could have been inactivated in the process of making their histochemical preparations.

Leucine aminopeptidase could not be demonstrated histochemically in the trophoblastic epithelium of normal placentae and simple moles. Similar observations in respect of human placentae at term have been made by Kraurer and Ludwig (1968). In our material both elements of the trophoblast showed up a considerable degree of aminopeptidase activity in proliferating moles, but the activity of this enzyme in the tumour cells of choriocarcinomas was less than that seen in the trophoblast of proliferating moles. We cannot offer any definite explanation to account for the significance of the presence of leucine aminopeptidase in these trophoblastic elements. It is likely that leucine aminopeptidase may contribute to the local infiltrative tendencies of proliferating moles and choriocarcinomas because Holmberg (1961) has pointed out that release of enzymes particularly proteinases forms the physiological basis for the destructive activity of tumour cells at the invasion frontier.

In conclusion our findings indicate that the trophoblastic elements of hydatidiform moles and choriocarcinomas differ enzymically from that of normal full term placentae. Although it has not been possible to establish a definite enzymic pattern diagnostic of choriocarcinomas it appears that cytotrophoblastic cells show greater enzymic alterations than syncitiotrophoblastic cells in proliferating moles and choriocarcinomas. It is likely that such enzymic alterations may reflect changes in ultrastructure and may also lead to greater metabolic derangements in these cells.

SUMMARY

Acid phosphatase, non-specific esterase and leucine aminopeptidase were looked for in the trophoblast in normal placentae, hydatidiform moles and choriocarcinomas. Differences in the activities of these enzymes in the material listed above are pointed out. It is suggested that alterations in acid phosphatase activity in the trophoblast of proliferating moles and choriocarcinomas possibly reflect changes in ultrastructure at lysosomal level. It is also suggested that the presence of leucine aminopeptidase in the trophoblast of proliferating moles and choriocarcinomas may contribute to their local infiltrative tendencies. Enzymic alterations in the trophoblast may lead to metabolic derangements in these cells.

EXPLANATION OF PLATES

PLATE I

FIG. 1.—Acid phosphatase (red), strongly positive in both elements of trophoblast in normal full term placenta. × 400

FIG. 2.—Proliferating hydatidiform mole showing acid phosphatase (red) strongly positive in the syncitiotrophoblast but almost absent in the cytotrophoblast. × 1000

FIG. 3.—Choriocarcinoma showing reduced acid phosphatase activity in cytotrophoblast and syncitiotrophoblastic elements (small dark red cells are macrophages which are rich in acid phosphatase). × 1000

FIG. 4.—Non specific esterase (brown) strongly positive in both elements of trophoblast in normal full term placenta. × 400

FIG. 5.—Proliferating hydatidiform mole showing non specific esterase strongly positive in the syncitiotrophoblast but almost absent in the cytotrophoblast. × 1000

FIG. 6.—Leucine aminopeptidase activity, strongly posirive in both elements of the trophoblast in proliferating hydatidiform moles. × 400

PLATE II

FIG. 7.—Acid phosphatase (black) strongly positive in both elements of the trophoblast in normal full term placenta. (black and white picture compare with Figure 1). × 400

FIG. 8.—Proliferating mole showing acid phosphatase (black) strongly positive in the syncitiotrophoblast and almost absent in the cytotrophoblast. (black and white picture compare with Figure 2). × 800

FIG. 9.—Choriocarcinoma showing reduced acid phosphatase activity in the syncitiotrophoblastic and cytotrophoblastic elements. Small dark black cells are macrophages which are rich in acid phosphatase. (black and white picture compare with Figure 3). × 1000

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