Detection of pregnancy specific β_1 -glycoprotein in formalin-fixed tissues

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SUMMARY Using an enzyme-bridge immunoperoxidase method, pregnancy specific β_1 -glycoprotein (PS β G) has been demonstrated in the cytoplasm of the trophoblast in several formalin-fixed tissues, namely, implantation sites of ovum, normal placentae, hydatidiform moles, invasive moles, and choriocarcinoma of uterus and testis. It is suggested that this technique may prove helpful in the detection of choriocarcinomatous elements in malignant tumours.

We have recently shown (Horne *et al.*, 1976), by a variety of techniques that the trophoblast produces pregnancy specific β_1 -glycoprotein (PS β G), first described by Bohn (1972) and referred to by him as SP $_1$. Electron microscopic studies of fixed first trimester placentae clearly demonstrated that, at least *in vivo*, the syncytiotrophoblast and not the cytotrophoblast is the source of this protein (Horne *et al.*, 1976). Subsequent to these observations we found that it was possible to demonstrate the presence of PS β G in formalin-fixed paraffin-embedded normal placentae using an established enzyme-bridge immunoperoxidase technique (Mason *et al.*, 1969; Streefkerk, 1972).

In this paper we present evidence for the distribution of PS β G in normal placentae, implantation sites of ovum, hydatidiform moles, invasive moles, and choriocarcinoma, including cases of choriocarcinoma arising in malignant teratoma of testis.

Material and methods

Paraffin-embedded blocks of placental tissue and trophoblastic tumours which had been stored for up to eight years were obtained from our own departmental files. Unstained sections of trophoblastic tumours (10 cases) were also kindly provided by Professor W. W. Park, University Department of Pathology, Dundee from the files of the Registry for Diseases of the Trophoblast.

In addition to 10 normal placentae (12-41 weeks'

gestation), the following specimens were examined: implantation site of ovum (1-3 weeks' gestation)—3 cases; hydatidiform mole—5 cases; invasive mole—4 cases; choriocarcinoma of uterus—5 cases; and malignant teratoma of testis—5 cases.

ENZYME-BRIDGE IMMUNOPEROXIDASE METHOD
Sections (5 μ m) were cut from paraffin embedded blocks of tissue which had been fixed in neutral buffered formalin. After taking the sections to water, partial blocking of the endogenous peroxidase activity was achieved by treating the sections with 0.3% hydrogen peroxide/methanol/0.74% HCl for 30 minutes at room temperature (Streefkerk, 1972; Weir *et al.*, 1974). Thereafter the staining procedure was essentially that of Mason *et al.* (1969).

Test sections were incubated with a 1/40 dilution of rabbit antihuman β_1 SP $_1$ -glycoprotein antiserum (Behringwerke AG). Following titration this dilution gave optimal staining with minimal background reactivity. The rabbit antiserum was shown to be monospecific by immunoelectrophoresis with undiluted pregnancy plasma. Control sections were incubated with 5 mM Tris/saline buffer, pH 7.6, or with normal rabbit serum in place of the specific antiserum. There was no difference between the use of buffer or normal rabbit serum in controls other than a slight increase in the non-specific staining of connective tissue when the normal rabbit serum was used.

Marked diminution but not complete loss of the positive staining was obtained by absorption of the rabbit antiserum with a freeze-dried purified PS β G (kindly provided by Dr Bohn, Behringwerke AG).

Figs 1-5 Sections stained using the enzyme-bridge immunoperoxidase technique described in the text. The test section is shown on the left and the control on the right.

Fig 1 Implanted ovum (gestational age 2-3 weeks) showing positive staining in syncytiotrophoblast (arrowed) ($\times 440$).

Fig 2 Normal placenta showing positive staining of trophoblast ($\times 440$).

Fig 3 Hydatidiform mole showing dense staining of syncytiotrophoblast ($\times 180$).

Fig 4 Invasive mole with positive staining of large trophoblast cells (arrowed) ($\times 440$).

Fig 5 Choriocarcinoma of uterus showing positive staining of syncytiotrophoblast (arrowed). Note absence of staining in the cytotrophoblast (CT) ($\times 440$).

Results

Of three cases of implantation of ovum, two, morphologically aged 2 to 3 weeks, showed evidence of PS β G in the cytoplasm of the syncytiotrophoblast layer in 'test' but not 'control' sections (Fig. 1). The implanted ovum which was negative for PS β G appeared to be less than 2 weeks old. All normal placentae examined contained PS β G in the syncytiotrophoblast (Fig. 2) but not in the cytotrophoblast. Similarly, PS β G was detected in the syncytiotrophoblast of all hydatidiform moles (Fig. 3) and invasive moles (Fig. 4). The degree of staining of the syncytiotrophoblast in the choriocarcinoma of uterus was more variable than that observed in the normal placentae and hydatidiform moles. Figure 5 clearly shows that only the syncytiotrophoblast in this choriocarcinoma contains PS β G, the cytotrophoblast being negative for this protein.

Of the five cases of malignant teratoma of testis, two were known to contain choriocarcinomatous elements. Although, in these cases, the primary tumours were not available for study, metastases from each were examined for the presence of PS β G. These tumours, a metastasis in stomach wall and a metastasis in lung, both contained tumour cells which stained positively for PS β G.

Discussion

Pregnancy specific β_1 -glycoprotein (PS β G) was present in the syncytiotrophoblast of all normal placentae (12-41 weeks gestational age) in agreement with the findings of Bohn (1972) and Towler *et al.* (1976) that this protein is present in maternal serum throughout the second and third trimesters of pregnancy. The demonstration of PS β G in the syncytiotrophoblast of implanted ova with a gestational age of 2-3 weeks clearly indicates that this protein is in fact synthesised very early in the first trimester of pregnancy. By using a sufficiently sensitive assay technique such as radioimmunoassay, it may therefore be possible to detect PS β G in the peripheral blood a few weeks after conception and so provide an alternative diagnostic test for pregnancy.

Our detection of PS β G in the syncytiotrophoblast elements of placentae and trophoblastic tumours is in keeping with our previous observation (Horne *et al.*, 1976) that this protein is synthesised *in vivo* by the syncytiotrophoblast. Although PS β G may be a specific marker for the trophoblast, it is important to note that the other 'pregnancy-specific' proteins, placental lactogen (hPL) and chorionic gonadotrophin (hCG), are produced by a small percentage

of non-trophoblastic tumours (Weintraub and Rosen, 1971; Braunstein *et al*, 1973). Clearly, therefore, a much larger group of malignant tumours should be screened for evidence of PS β G production.

If PS β G is shown to be a truly specific marker for trophoblast, staining for this protein may well be useful in examining tumours thought to contain choriocarcinomatous elements. Such a use is exemplified by our demonstration of trophoblast in metastases arising from malignant teratomata of testis, the primary tumours being unavailable for study.

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