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Human Placenta: A Convenient Source of Homologous Material, for Diagnosis and Therapy of Inborn Errors of Metabolism

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Human placenta is an organ, mainly of fetal origin, which grows, differentiates and matures during a genetically programmed life span of 9 months.

To emphasize the current interest in placenta as a research model in biochemistry, cell replication, cancer, immunology and ageing, Peter Beaconsfield and Claude Villee organized, in 1978, a Round Table Discussion, at Bedford College in London, under the deliberately provocative heading "Placenta. A neglected experimental animal".

We will not discuss here placental function in pregnancies of phenylketonuric mothers: our purpose is only to point out, from our experience, some possible interests of human placenta as a source of homologous material for diagnosis and therapy of inborn errors of metabolism. Indeed, the only widely available sources of homologous human material are urine and placental tissue. Blood from donors hardly covers the need of transfusion and blood coagulating factors; autopsy tissues cannot be obtained in good condition. However, placentae can be collected on a large scale: it is a source of human blood and fetal tissue. This tissue can be used: (i) as a source of reagents of human origin for the diagnosis of inborn errors of metabolism, (ii) as a fetal tissue for studying mutants (enzyme polymorphism, onto-fetal development of enzyme and mutant enzymes), (iii) as a source of homologous material for therapy attempts.

PLACENTA: A SOURCE OF HOMOLOGOUS REAGENTS FOR THE DIAGNOSIS OF INBORN ERRORS OF METABOLISM

The placental tissue, the placental blood and their industrial subfractions[1] may be valuable sources of homologous reagents useful in the diagnosis of inborn errors of metabolism.

Source of growth factor(s)

Diagnosis of inborn errors of metabolism largely uses cultured cells which are grown in well-defined media, supplemented with human or animal sera (more commonly calf serum). For the time being, the production of calf serum in the world is not well standardized and is insufficient for the increasing needs of cell cultures. Furthermore, in the diagnosis of metabolic diseases using cultured skin fibroblasts or amniotic fluid cells, calf serum may introduce bias. For all these reasons, more and more work was undertaken to replace calf serum by well-defined growth factors (Gospodarowicz, 1981).

If some growth factors – the primary function of which would be to stimulate cell division – are still present in placentae at term, they would be particularly suitable for cultures of human amniotic fluid cells since they would be obtained from a fetal tissue and would be used for fetal cell cultures.

For this purpose, we have tested the effects of different fractions from frozen placental blood and tissue partially replacing fetal calf serum, using human cultured skin fibroblasts from fetuses and adults, or amniotic fluid cells from early amniocentesis.

The efficiency of these impurified fractions was judged on the basis of stimulation of cell divisions and of DNA synthesis evaluated by incorporating tritiated thymidine into the cells (Khoury, 1983).

A "growth-factor-like" activity was exhibited by one fraction from frozen placental blood and by one acid fraction from frozen placental tissue, both prepared by the Institut Mérieux. The results obtained are summarized in Table 1. The aim of the present work is to further purify the active fractions in order to elucidate, at the final stage, the molecules, probably peptides which are responsible for this "growth-factor-like" activity and their structure.

Source of human antigens to raise antibodies

More usually, placental tissue was used by us and others to purify human proteins, especially enzymes, in order to raise antibodies in animals against these antigens. The following examples emphasize the interest of such procedures in the diagnosis of inborn errors of metabolism.

An immuno-assisted determination of acid α-glucosidase (EC 3.2.1.20) was developed for the diagnosis of typical or atypical glycogenosis type II (McKusick 23230). Acid α-glucosidase was purified from placental tissue. Using this purified antigen, antibodies were raised in rabbits and tested by Ouchterlony double immunodiffusion and inhibition of enzyme activity. Their specificity was tested by comparison in their

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Table 1 DNA synthesis of cells (human fetus skin fibroblasts 3839 E 01) cultivated in the presence of one acid extract of placental tissue, AEP, (Institut Mérieux, Marcy l'Etoile, France)

Medium FCS v/v	*H_3 percentage thymidine incorporation*
0.2%	0
15%	100
0.2% + hormones	19
0.2% + AEP 10 $\mu g\,ml^{-1}$	115
0.2% + AEP 10 $\mu g\,ml^{-1}$ + Hormones	107

FCS: Fetal calf serum
Hormones: insulin 5 $\mu g\,ml^{-1}$ + transferrin 5 $\mu g\,ml^{-1}$ + dexamethasone 50 $ng\,ml^{-1}$
Results expressed as percentage H_3 thymidine incorporation of the maximum control in FCS 15%

inhibition of the activities of either human placenta, or liver or skin fibroblasts acid α-glucosidase. The antibodies obtained were able to inhibit this activity but had no effect on fibroblast neutral glucosidase. This immuno-assisted enzyme assay (Figure 1) is, as a routine, used in our laboratory for the diagnosis of acid α-glucosidase deficiencies (Bienvenu and Mathieu, 1981).

More recently, in a work directed by Joseph Tager[1], an enzyme immuno-blotting technique was developed with one of our colleagues in order to demonstrate the presence or absence of immunologically reacting material in the cultured skin fibroblasts from Pompe's disease (Halwoort *et al.*, 1983). The principle of this enzyme immuno-blotting procedure derives from the work of Muilerman *et al.* (1982). The denatured protein bands from polyacrylamide gel electrophoresis are transferred to a nitrocellulose sheet and incubated with an excess of antibodies raised against a crude or semipurified preparation of acid glucosidase (in this respect placenta may be a good source) giving IgG complexes with one of the two antigen-combining sites of the IgG molecule still available. This site may bind a newly added, active enzyme and the specificity of the detection results from the catalytic activity measurement.

PLACENTA: A FETAL TISSUE FOR STUDYING MUTANTS

The preliminary requirement for the recognition of pathological mutants is the knowledge of enzyme polymorphism. Placentae at term may provide available samples for large scale studies. So the polymorphism of acid glucosidase and of acid phosphatase (EC 3.1.3.2) have been respectively demonstrated by Swallow *et al.* (1975) and Harris and Hopkinson (1976).

Furthermore, human placentae from different stages of the fetal development represent a good model for studying the onto-fetal development of human proteins. The evolution of the diaphorase (EC 1.6.4.3) isoensyme pattern in human placentae was reported by Fisher *et al.* (1977), diaphorase 3 present in early placentae disappears in tissue at term. Such schemes of evolution are known for other enzymes and much work has been undertaken for many years on the similarities of these fetal forms with the neoplasic liver enzymes (Schapira *et al.*, 1970).

In any case, knowledge of the onto-fetal development in the enzyme is a preliminary requirement for the early prenatal diagnosis of metabolic diseases. This pre-requirement becomes more and more important considering the recent increasing interest in new placental biological material available for this purpose: the chorionic villi obtained by biopsy between the 6th and 13th week of pregnancy making earlier prenatal diagnosis possible. Furthermore, we can hope that some enzyme activities which are not easily, or not at all, detectable in amniotic fluid cells could be studied using this tissue. The present main limitation to this approach comes from the problems of reliably obtaining fetal trophoblastic cells free from contamination by maternal decidua.

Nevertheless, prenatal diagnosis is still currently performed by studying enzymes in cultured amniotic fluid cells obtained from amniocentesis at the 16th–17th week of amenorrhoea. When an affected fetus is then diagnosed, among the fetal tissues obtained after therapeutic abortion, placenta is quantitatively one of the most abundant and can be used for studying the mutant enzyme and confirming the diagnosis.

From such a study one of us has been able to confirm the possibility of prenatal diagnosis of mannosidosis (McKusick 24850) (Maire *et al.*, 1976). Such a mutation could be extensively studied after purification of the mutant placental enzyme (Maire, 1978).

PLACENTA: A SOURCE OF HOMOLOGOUS MATERIAL FOR THERAPY

At the present time, among the attempts for the therapeutic treatment of inborn errors of metabolism, the possibilities of enzyme therapy are investigated in several groups. Three possibilities are offered in this area:

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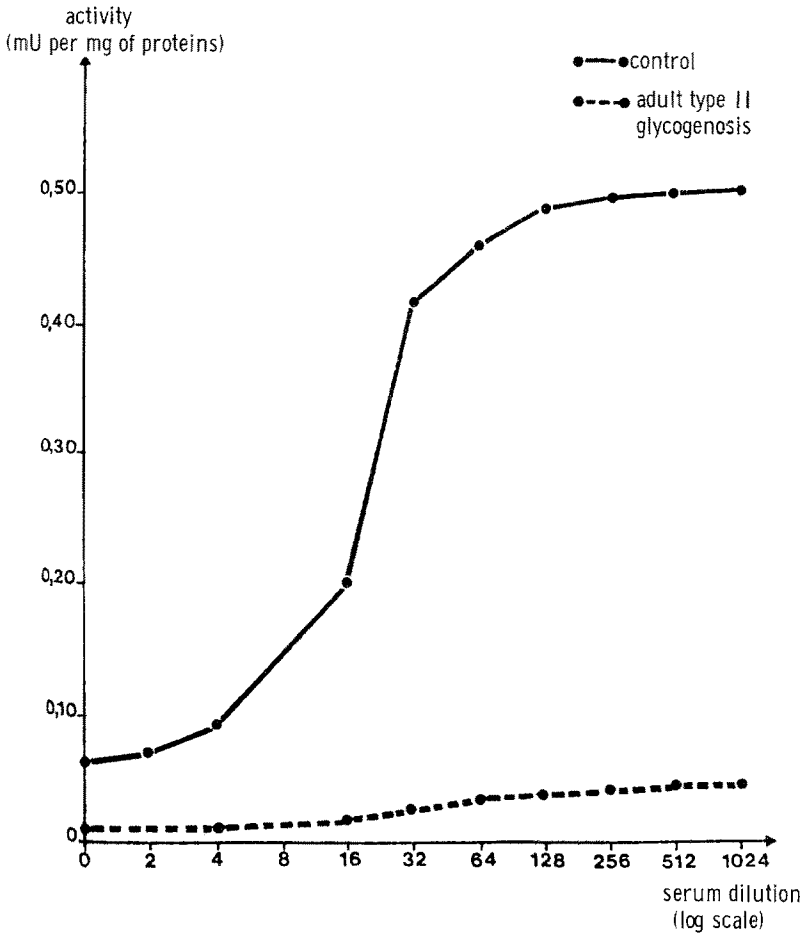

Figure 1 Inhibition of fibroblasts acid α-1,4-glucosidase by antibodies raised in rabbits against human placental enzyme

mutant enzyme induction or modification, cofactor supplementation, and normal enzyme administration using naked, modified or encapsulated enzymes.

With this last possibility, replacement therapy, mainly developed in the field of lysosomal storage diseases, human placental tissue may supply homologous material. The lysosomal storage diseases are particularly well adapted for such attempts since lysosomal enzymes are glycoproteins which can be delivered to the storage site after endocytosis.

Prior to therapy attempts *in vivo*, studies of the possibilities of uptake of naked or modified enzyme *in vitro* by cultured cells are a necessary preliminary step. We have developed such *in vitro* assays in the case of Fabry's disease (McKusick 30150) using purified placental α-galactosidase A (EC 3.2.1.22) and deficient skin fibroblasts: the placental enzyme added to the culture medium was quickly incorporated into deficient fibroblasts and the initial addition to the medium was almost sufficient to reach a maximum level of incorporation for the preparation used, as demonstrated by the use of the unlabelled and ^{125}iodine-radiolabelled enzyme (Veyron *et al.*, 1982). The instability of α-galactosidase A in the culture medium led us to try to stabilize it with Zn^{2+} ions, and we were so able to increase the enzyme uptake by fibroblasts; but the mechanism involved may then be different, possibly phagocytosis, since no saturation could be obtained (Figure 2).

CONCLUSION

In conclusion, human placenta may be a fruitful research tool which has not been widely investigated.

It is a biological sample useful for different purposes. At-term placentae are readily available on a large scale and can be used for studying genetic population markers. Early placentae, and more precisely chorionic villi, from the first trimester pregnancy seem from recent experiences of several groups, to offer the possibility of earlier prenatal diagnosis of inborn errors of metabolism.

Furthermore, it is the most abundant source of homologous material of human origin freely available and it can be collected and stored in good condition on a large scale. For this reason it may be the source of specific reagents and specific molecules for attempted therapy in man.

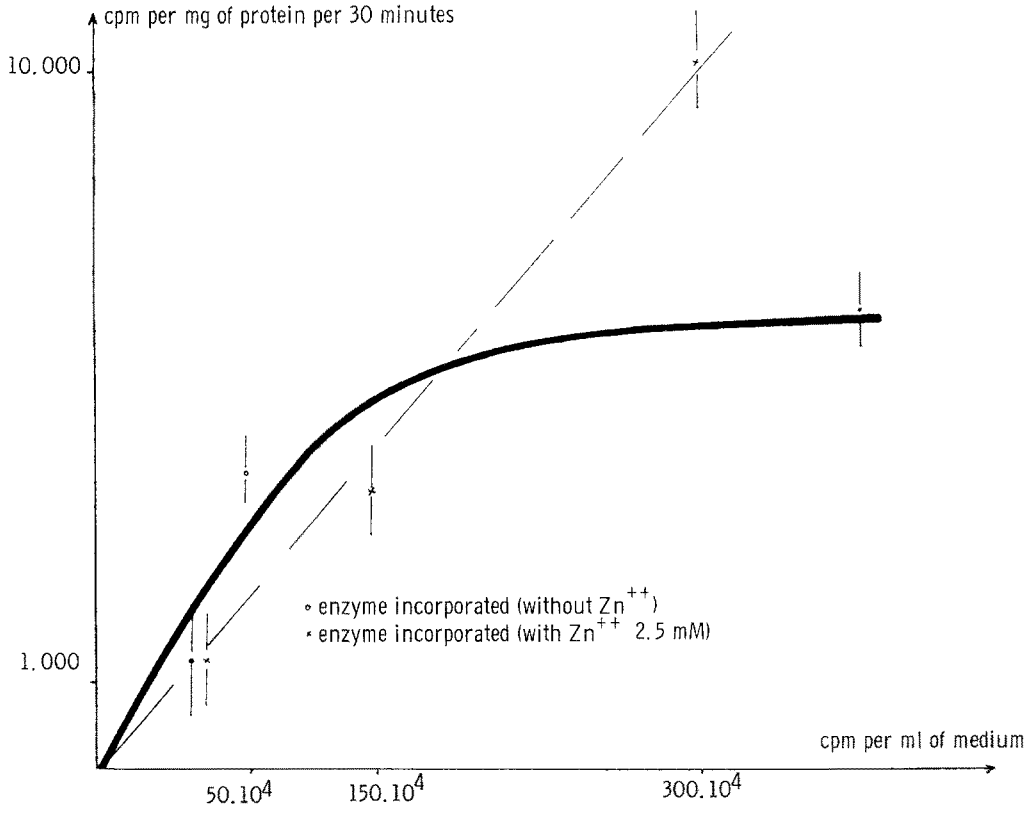

Figure 2 α-Galactosidase A incorporation by deficient fibroblasts: Zn^{2+} influence

Finally, placenta, which possesses a complete spectrum of cellular and biochemical activities and a very short life cycle of 9 months from embryonal tissue to maturity, may be considered as an experimental animal and one which is everyday less and less neglected.

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