Choriocarcinoma *in Vitro*: Evidence for Exchange of Enzymes Between Culture Medium and Cells (36874)

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The capacity to synthesize steroid and protein hormones is retained by choriocarcinoma, a neoplasm of the human placenta. In this investigation we compare the specific activities and isoenzyme patterns of β-glucuronidase and *N*-acetyl-β-glucosaminidase (hexosaminidase) in term placentas and in a clone of choriocarcinoma cells grown in culture. Also, we give evidence that β-glucuronidase from the medium is sequestered by the cells and that the cells liberate hexosaminidase into the medium.

Materials and Methods. The JEG-3 clone used in this investigation was established in culture (1) from a human choriocarcinoma adapted to growth in the hamster cheek pouch (2). The cells have been propagated in continuous culture since 1968 (1). They were cultivated at 37° in monolayer in 32 oz glass bottles. Routinely the cells were grown in Ham's F10 medium (3) supplemented with 13% horse serum (HS), 3.3% fetal-bovine serum (FBS), and 50 U penicillin and 50 μg streptomycin per ml; on two occasions the cells were grown in Ham's F10 medium supplemented only with 13% FBS and the antibiotics. A minimum of 2.5 g of packed cells was harvested at a time. After passage, the cells reached the log phase of growth by 48 hr and became confluent by 120 hr. In passage No. 40, cells were harvested at 120 and 144 hr; in passage No. 44, at 48, 72, 120, and 144 hr; and in passage No. 52, at 120 hr. On the appropriate harvest day, cells were removed with 0.5% trypsin-0.2% EDTA solution, dispersed by vigorous pipetting, and washed three times with 100 ml, 50 ml, and 50 ml of 0.85% sodium chloride.

Extracts of the washed choriocarcinoma cells were prepared within 1 hr of harvest. The packed cells were weighed, resuspended to 25% (w/v) with 0.05 *M* Tris-HCl, pH 7.5, homogenized in a Virtis 45 homogenizer for 3.5 min at medium speed, and centrifuged at 20,000*g* for 20 min. All steps were carried out at 0° except the centrifugation, which was at 4°.

Normal-term placentas were obtained at delivery and brought quickly to the laboratory on cracked ice. Multiple small portions were excised, washed in cold tap water, blotted, and admixed to obtain a representative sample of 2.5 g. Similarly, a 2.5 g sample of minced tissue from excised cheek pouches of 4 hamsters was obtained.

The samples of placenta and cheek pouch were extracted in a manner identical to that for the cultured cells.

All extracts were assayed concurrently for β-glucuronidase (4) and hexosaminidase (4) on the day of preparation. Protein was measured according to Lowry *et al.* (5); specific activities were calculated as International Units per mg protein (IU/mg).

The extracts were stored at −20° until the following day when zymograms of β-glucuronidase (6) and hexosaminidase (6) were prepared.

For preparation of some zymograms of the culture medium or its components, some of the materials were concentrated by pressure dialysis.

Results. Both β-glucuronidase and hexosaminidase were present in cell extracts of all passages examined. The variation in the specific activities is shown in Table I. The mean specific activity of each of the enzymes in the choriocarcinoma does not vary greatly from that of the corresponding enzyme in term placenta (Table II). Similar levels of

TABLE I. β-Glucuronidase and Hexosaminidase Specific Activities (× 10^3) of Extracts of Choriocarcinoma.[a]

Enzyme	Passage no. 40		Passage no. 44				Passage no. 52
	120 hr	144 hr	48 hr	72 hr	120 hr	144 hr	120 hr
β-Glucuronidase	1.0	1.2	0.7	0.8	1.3	1.2	1.4
Hexosaminidase	62	59	75	72	87	83	67

[a] Each value is the average of duplicate assays.

β-glucuronidase and hexosaminidase in term placentas were obtained in a previous study (4).

The β-glucuronidase zymogram of choriocarcinoma (Fig. 1c) cultivated in medium supplemented with HS and FBS exhibited two major isoenzymes that were also present in term placenta (Fig. 1e); an additional isoenzyme with increased mobility was also present in the choriocarcinoma. This additional isoenzyme was present in cells from all such routine passages and from the log phase as well as the confluent phase of the growth cycle. Several experiments were designed to determine the origin of this additional β-glucuronidase component.

Since the choriocarcinoma had been serially transplanted 387 times in the hamster cheek pouch prior to cultivation, we considered the possibility that hamster genetic information may have been introduced into the choriocarcinoma cell and that the new electrophoretic component was a hamster isoenzyme in an interspecific hamster–human hybrid cell. Interestingly, the β-glucuronidase zymogram of hamster cheek pouch tissues (Fig. 1a) revealed a single component at the same position on the gel as the extra isoenzyme in the choriocarcinoma (Fig. 1c). In spite of this finding, we could not disregard the possibility that isoenzymes in components of the culture medium may have contributed the additional isoenzyme of the choriocarcinoma extract. To evaluate this possibility we analyzed by assay and by zymogram all culture fluid added to and removed from the culture bottles during a complete passage. We found by analysis of the individual components that a total of 2.4 units of β-glucuronidase (present in horse serum) was added to the culture medium. This quantity is approximately 4-fold greater than the amount of β-glucuronidase extracted from the cells. The β-glucuronidase assay of the medium removed from the cultures was useless because of the interference of the pH indicator, phenol red, present in culture medium. Nevertheless, the β-glucuronidase zymogram of culture medium supplemented with HS and FBS exhibited a single, discrete, barely preceptible isoenzyme which corresponded in position to the hamster cheek pouch isoenzyme and to the additional isoenzyme in the choriocarcinoma extract. This isoenzyme was also observed in the zymogram of HS (Fig. 1b) and of 30-fold concentrated culture medium but not in the zymogram of 3-fold concentrated FBS. When the

TABLE II. β-Glucuronidase and Hexosaminidase Specific Activities (× 10^3) of Extracts of Choriocarcinoma and Placenta.[a]

Source	β-Glucuronidase		Hexosaminidase	
	Mean	Range	Mean	Range
Choriocarcinoma	1.2	1.0–1.4	72	59–87
Placenta	2.1	1.8–2.5	62	48–82

[a] The data are for 5 extracts of choriocarcinoma in the confluent phase (120 or 144 hr) and 6 placental extracts, all assayed in duplicate.

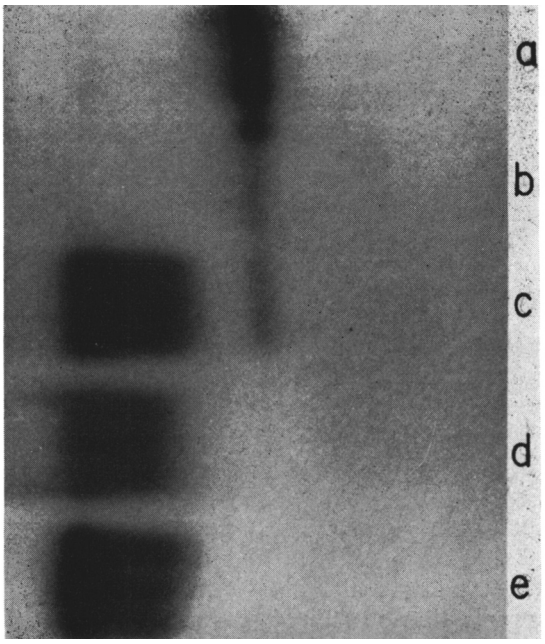

FIG. 1. β-Glucuronidase zymogram of hamster cheek pouch (a), horse serum (b), choriocarcinoma cultivated in medium supplemented with both fetal-bovine serum and horse serum (c), choriocarcinoma cultivated in medium supplemented with fetal-bovine serum (d), and term placenta (e). Note that the fast component in specimen *c* has the same electrophoretic mobility as the components seen in the extract of hamster cheek pouch and in horse serum. The origin is at the left, and anodal migration is to the right.

cells were cultivated in medium supplemented with FBS but not with HS, the additional β-glucuronidase isoenzyme was not observed (Fig. 1d).

The hexosaminidase zymogram of term placenta (Fig. 2a) resembled the zymograms of choriocarcinoma cultivated either in medium supplemented with both HS and FBS (Fig. 2b) or in medium supplemented with FBS (Fig. 2c). The zymogram of 3-fold concentrated FBS (Fig. 2d) showed a single broadly staining zone of weak hexosaminidase activity which coincided in mobility with the slow cellular isoenzyme; 3-fold concentrated HS showed no activity.

In a previous study (6) of hexosaminidase isoenzymes in placenta, we observed a zone of weakly staining, closely spaced isoenzymes located near the origin. This zone was not observed in the present work; its absence was probably due to the use of less concentrated extract in the current study.

In the quantitative study, data were obtained which are consistent with a release into the medium of cellular hexosaminidase. Total hexosaminidase in the medium removed (44.5 units) from the cultures was 40% greater than the total activity of the added medium (31.8 units, all of which was derived from FBS). (The cell extract contained 20.9 units).

Discussion. Several possibilities can be proposed for the origin of the β-glucuronidase isoenzyme seen in extracts of choriocarcinoma but not in extracts of term placentas.

The most attractive consideration is that this isoenzyme is derived from serum components of the culture medium. This thesis is supported by the β-glucuronidase zymograms which show (a) the presence of a fast component both in HS and in choriocarcinoma grown in HS-supplemented medium and (b) the absence of such a component in FBS and in choriocarcinoma grown in FBS-supplemented culture medium.

These findings, however, do not exclude

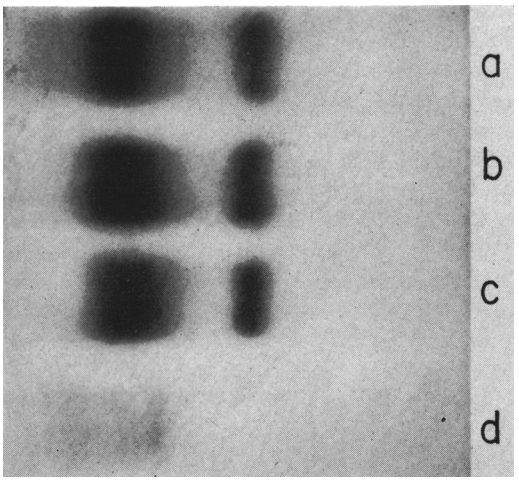

FIG. 2. Hexosaminidase zymogram of term placenta (a), choriocarcinoma cultivated in medium supplemented with both fetal-bovine serum and horse serum (b), choriocarcinoma cultivated in medium supplemented with fetal-bovine serum (c), and fetal-bovine serum (d). The origin is at the left, and anodal migration is to the right.

the alternative possibility that the cell is a hamster–human hybrid, since macromolecules, present in HS but not in FBS, may be required for expression of such a hamster phenotype in culture. Goldenberg *et al.* (7) have recently reported the spontaneous formation *in vivo* of hamster–human hybrid somatic cells. The similarity of hamster and human karyotypes, as well as the extreme heteroploidy of choriocarcinoma, will make difficult a solution to the problem through chromosome studies.

A third consideration is that the additional isoenzyme is a choriocarcinoma-specific enzyme, the production of which is stimulated by HS, but this cannot be resolved with the present data.

Finally, the possibility of simultaneous propagation of human cells and hamster cells in the culture is excluded, since the choriocarcinoma cells are derived by multiple cloning techniques.

If the additional β-glucuronidase isoenzyme is in fact derived from the medium, the cells evidently concentrate this enzyme. On zymograms of even the undiluted HS it stained less intensely than the analogous isoenzyme of the extract, which is prepared from thoroughly washed cells and 3 volumes of diluent. In considering the evidence that the cells take up the isoenzyme from the medium, it should be noted that the amount of β-glucuronidase added to the cultures is approximately 4-fold greater than the total amount extracted and that all of the added activity exists as a single isoenzyme. Thus, it would seem that the medium could supply the requisite amount of the additional isoenzyme. The cells could presumably sequester enzymes from the medium by either surface adsorption or by pinocytosis. Evidence exists for the occurrence of both phenomena in tissue culture (8, 9).

Finally, it should be noted that, in addition to taking up β-glucuronidase from the medium, the cells also liberate hexosaminidase into the medium: The activity of the medium removed from the culture was 40% greater than the activity of the medium added to the culture.

Because of the low hexosaminidase activity in the medium removed from the culture, we were unable to prepare zymograms suitable for determining which of the cellular isoenzymes contributed to the increase in activity of the medium.

Summary. β-Glucuronidase and hexosaminidase isoenzyme patterns of choriocarcinoma in culture are similar to those of placenta. We present evidence for an exchange of enzymes between the culture medium and choriocarcinoma cells. The β-glucuronidase pattern for choriocarcinoma grown in medium supplemented with horse serum has a component, not found in placenta, with electrophoretic mobility identical to that of the single band of horse serum β-glucuronidase. Hexosaminidase activity in the culture medium removed was 40% higher than the medium added to the culture. The suggestion is made that the extra β-glucuronidase isoenzyme is derived from the serum supplement of the culture medium and that the increase in hexosaminidase activity in the medium removed from the culture is consistent with the release of this enzyme from the cells.

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