tions. This observation is in agreement with that seen for hepatic drug-metabolizing enzymes in vivo (*14*). Thus, at one or more steps in the sequence of events occurring during aryl hydrocarbon hydroxylase induction in fetal liver cell cultures, at least two distinct processes must be involved. Either PB or a polycyclic hydrocarbon induce δ-aminolevulinic acid synthetase activity in adult male rat liver and combination of the two compounds does not cause an additive effect (*15*), suggesting that the synthesis of microsomal hemoproteins occurs by the same or closely related mechanisms during treatment with either type of microsomal enzyme inducer.

In Table 2 we have summarized the effects of MC, BA, PB, and *p,p'*-DDT on hydroxylase activity. Either MC or BA alone, or together, induced the enzyme system between six- and sevenfold in 24 hours. Induction of hydroxylase activity by *p,p'*-DDT was approximately twofold and by PB about sixfold; MC plus *p,p'*-DDT and MC combined with PB stimulated the hydroxylase system about 8- and 11-fold, respectively; thus, the effects were approximately additive. Similarly, when BA was added to either *p,p'*-DDT or PB, the oxidase activity was equal to the activity of BA alone plus that of added compound alone. But when PB was added to *p,p'*-DDT, the oxidase activity was not greater than that of one of the compounds alone. In some experiments (as shown here) where the optimum inducing concentration of both *p,p'*-DDT and PB was used, the level of enzyme activity induced was slightly less than that stimulated by PB alone. Both 2 m*M* PB and 100 μ*M* *p,p'*-DDT are concentrations approaching saturation in the growth medium. Thus, the high levels of these inducers in combination are probably causing cytotoxicity or some other nonspecific metabolic effect in the hepatocyte, which in turn affects hydroxylase induction. In contrast, the optimum inducing concentrations of MC and BA are considerably less than that (between 50 and 60 μ*M*) causing saturation in the growth medium. The data in Table 2 further demonstrate that at least two different mechanisms of microsomal enzyme induction exist in cultured fetal hepatocytes.

In conclusion, our experimental model consists of fetal liver cells in culture in which a mixed-function oxygenase inducible by polycyclic hydrocarbons, drugs, or insecticides can be examined. Further studies (*11*) with this model system should elucidate certain differences in the mechanism of action between distinct classes of these inducers; these differences would be difficult to examine in the intact animal. Also, in such a system where the general effects due to nutritional, hormonal, and environmental factors can be strictly controlled, one can study specific effects on cellular metabolism elicited by the various microsomal enzyme inducers.

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Glycopeptides from the Surface of Control and Virus-Transformed Cells

Abstract. *Glycopeptides were removed by trypsin digestion from the surface of control cells and cells transformed by Rous sarcoma virus, murine sarcoma virus, or polyoma virus. After digestion with pronase, the glycopeptides were analyzed by gel filtration. The elution profiles suggest that there are differences in the glycopeptides from the surface of control cells and those from transformed cells.*

A comparative study of the glycoproteins from the surface of BHK_{21}/C_{13} cells and from the same cell line transformed with two strains of Rous sarcoma virus (RSV) has revealed differences in glycopeptides (*1*). The surface membranes and the material removed from the surface of the transformed cells by trypsin digestion were enriched in glycopeptides of higher molecular weight when compared to the glycopeptides from similar fractions of control cells. The question arose as to whether differences could be detected in the glycopeptides from other transformed cells. In order to examine the generality of this finding, we have made similar studies of the glycopeptides from other pairs of control and virus-transformed cells.

The cells used in this study were BHK_{21}/C_{13} (*2*) and BHK_{21}/C_{13} cells transformed by polyoma virus (PyY) (*3*), Balb/3T3 (*4*) and Balb/3T3 cells transformed by murine sarcoma virus (MSV-3T3) or by RSV (RSV-3T3) (*5*), and chick embryo fibroblasts (CEF) and CEF transformed with the Schmidt-Ruppin strain of RSV (SR-CEF) (*6*). All cells were grown as monolayers for 72 hours in the presence of L-[^{3}H]fucose (general label; specific activity, 4 c/mmole; New England Nuclear Corp.) L-[1-^{14}C]fucose (specific activity, 0.5 mc/mmole; Calbiochem). The monolayers were washed and digested with purified trypsin for 15 minutes at 37°C. The material removed from the cells by trypsin was digested exhaustively with pronase and prepared for gel filtration

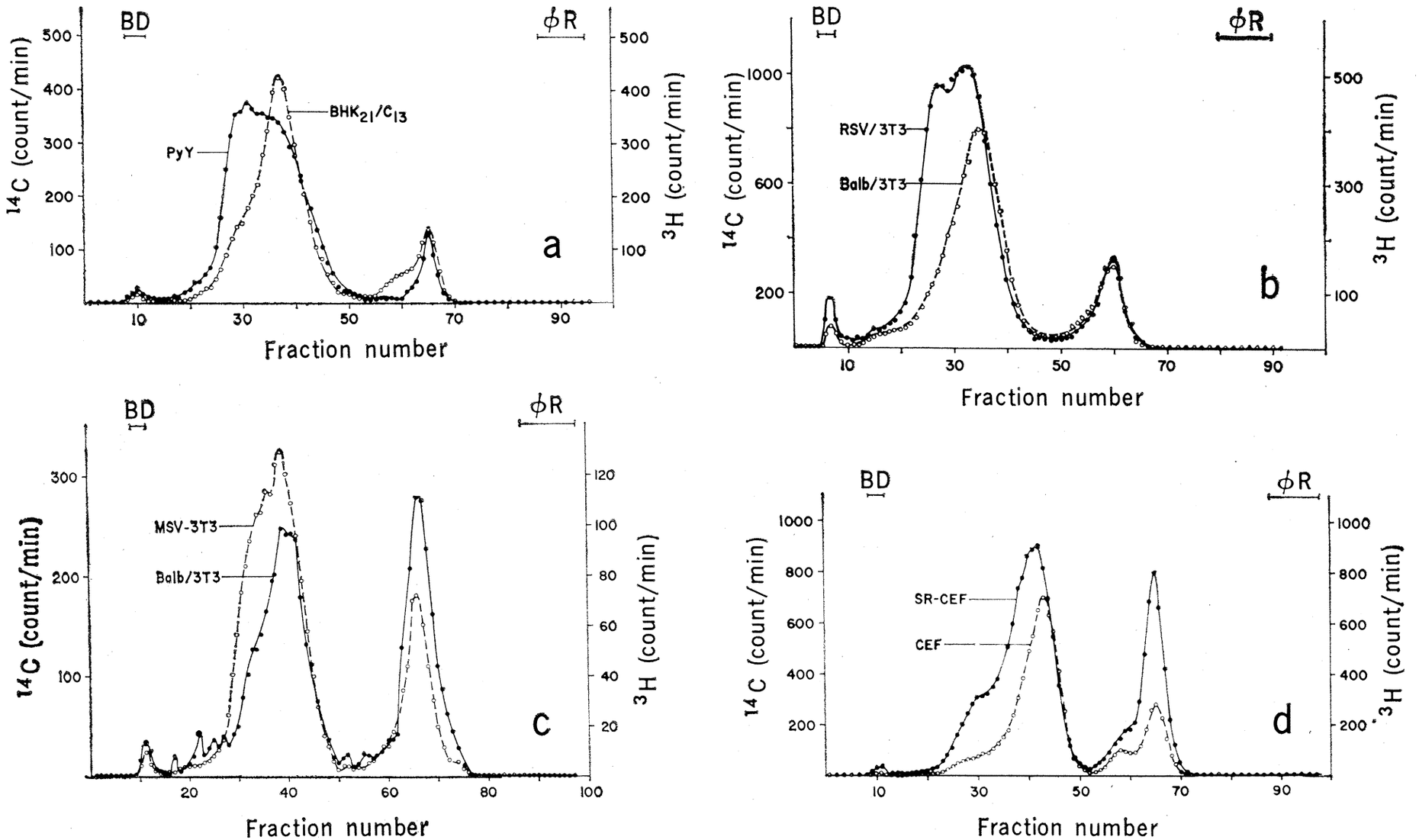

Fig. 1. Cochromatography on Sephadex G-50 of pronase-digested glycopeptides removed from the surface of control and transformed cells by trypsin digestion. Cells were grown as monolayers for 72 hours in the presence of 50 μc L-[^{3}H]fucose (●——●) or L-[^{14}C]fucose (○ — — ○). Monolayers were washed and the cells were digested with purified trypsin (1 mg per milliliter of tris-buffered saline, *p*H 7.5) for 15 minutes at 37°C. Trypsin inhibitor was added and the cells were removed from the tryptic digest by centrifugation. The tryptic digests ("trypsinates") to be compared were pooled and digested with pronase (0.1 mg per milliliter of digest) for 5 days at 37°C in the presence of toluene. The digests were cochromatographed on Sephadex G-50 columns 0.8 by 100 cm. Columns were developed in buffer containing tris-acetate (*p*H 9.0), 0.1 percent sodium dodecylsulfate, 0.01*M* ethylenediaminetetraacetate, and 0.1 percent mercaptoethanol. Fractions of 0.7 ml were collected, and radioactivity was determined by scintillation counting in Aquasol (New England Nuclear Corp.). (a) "Trypsinates" from BHK_{21}/C_{13} cells and BHK_{21}/C_{13} cells transformed with polyoma virus (PyY); (b) "trypsinates" from Balb/3T3 cells and Balb/3T3 cells transformed with RSV (RSV-3T3); (c) "trypsinates" from Balb/3T3 cells and Balb/3T3 cells transformed by MSV (MSV-3T3); and (d) "trypsinates" from chick embryo fibroblasts (CEF) and CEF transformed by SR-RSV (SR-CEF). *BD*, Fractions in which blue dextran 2000 was eluted; *φR*, fractions in which phenol red was eluted.

on Sephadex G-50. Detailed procedures have been described (*1*).

The results of these comparisons are shown in Fig. 1. In every case, the elution profiles of glycopeptides from the transformed cells were different from those of the control cells. There was a consistent tendency of material from the transformed cells to elute ahead of material from the control cells. The results were similar for all pairs of cells regardless of the species of origin, that is, hamster (Fig. 1a), mouse (Fig. 1, b and c), or chicken (Fig. 1d). In addition, it appeared to make no difference whether the cells were transformed with DNA-containing (Fig. 1a) or RNA-containing (Fig. 1, b–d) viruses.

Several observations suggest that these differences in the radioactive profiles of material from control and transformed cells reflect an actual difference in the glycoproteins of the cell surfaces. (i) We have found that elution patterns of pronase-digested 'trypsinate" from Sephadex G-50 are similar to the profiles of pronase-digested surface membranes (*1*). (ii) We have previously reported (*1*) that 90 percent of the radioactivity recovered from hamster cells grown for 3 days in the presence of radioactive L-fucose was chromatographically identifiable as fucose. Similar results were obtained by Strauss *et al.* (*7*), who used chick and hamster cells. (iii) Interchanging the isotopes (that is, the use of L-[^{14}C]fucose rather than L-[^{3}H]fucose), increasing the times of trypsinization, or growing the cells for different periods of time or to different cell densities in the presence of radioactive L-fucose did not alter the results. (iv) We have shown (*1*) that pronase digestion as carried out here is complete for material from control and transformed cells. Burge *et al.* (*8*) have found that similar pronase treatment of glycoproteins resulted in digestion of material from viruses grown on either chick or hamster cells. (v) With hamster cells, we have obtained similar results after growing the cells in the presence of radioactive D-glucosamine (*1*) or D-glucose (*9*). (vi) The specific activities of radioactive L-fucose from a pair of control and transformed cells were similar (*1*).

Material from transformed cells was different in the various cell lines. The profiles shown in Fig. 1, a and b, resemble those reported for BHK_{21}/C_{13} cells transformed by the Bryan strains of RSV (*1*). The Balb/3T3 cells transformed by murine sarcoma virus and chick cells transformed by the Schmidt-Ruppin strain of RSV showed less marked, but reproducible, increases in the higher molecular weight glycopeptides.

The material eluting later from the columns (fractions 50 to 75) was found in all cells. The amount of this material was not always the same, even in preparations from the same cell line. Its presence could not be correlated with the presence or absence of glycopeptides in other regions of the profiles.

It will be impossible to know whether the higher molecular weight glycopeptides found in the different virus-transformed cells represent the same molecules until these materials are purified further and characterized chemically. The profiles of surface material from transformed cells shown in Fig. 1 suggest that there may be differences among the glycopeptides from the various transformed cells. The fact that control cells contain small amounts of higher molecular weight glycopeptides that migrate with a large amount of glycopeptides from the transformed cells might suggest that transformation results in a change in the normal glycoproteins or the glycolipids, or both, on the cell surface. Other investigators (*10–12*) have shown that transformation results in the unmasking of regions found in normal cells. The relation of the changes observed here to the unmasking of sites on the cell surface, especially those capable of binding the plant agglutinins, remains to be determined.

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Latent Meiotic Anomalies Related to an Ancestral Exposure to a Mutagenic Agent

Abstract. *When urethan is administered to cytologically normal F_2 progeny descended from grandsires exposed to ethyl methanesulfonate, meiotic anomalies in the forms of site-specific X-chromosome deletions and bivalent associations are noted in spermatocytes of male Armenian hamsters examined 6 and 8 days after treatment, respectively. These latent anomalies are initiated and retained in a premutated state for two generations after the ancestral exposure to ethyl methanesulfonate, the additional impetus required to complete the mutational processes being supplied by urethan.*

Chemical mutagens, such as nitrogen mustards, ethylenimines, and nitroso compounds induce both complete and fractional (mosaic) mutations in drosophilas (*1*). In several instances where expression of the mutation was delayed until the second and third generations, it was postulated that those mutagens having low energy yields may act by elevating the involved gene locus or a segment of chromosome to a labile, premutated state where it remains until additional energy is available to complete the mutational process (*2*). This report deals with a similar phenomenon encountered in cytologically normal F_2 generation males of the Armenian hamster, *Cricetulus migratorius* ($2n = 22$), whose grandsires were treated with a single dose of ethyl methanesulfonate (EMS). Subsequent treatment of the cytologically normal F_2 generation males with an equivalent dose of urethan (ethyl carbamate), an unrelated and virtually ineffective chromosomal mutagen for mammals (*3*), is interpreted as having provided the additional energy necessary for the latent expressions of two highly specific forms of meiotic chromosomal anomalies.