Several mammalian, chicken, and mosquito cells grown in vitro take up tritiated dolichol supplied to the incubation medium. The extent of labelling varied markedly between different cell cultures. After 20 h incubation most of the dolichol taken up was unchanged and the major product of metabolism of dolichol was identified as its fatty acid esters. Green-monkey kidney cells were tested with 8 fully unsaturated and 6 $\alpha$-saturated polyprenols ranging from $C_{35}$ to $C_{105}$. In general the uptake of $\alpha$-saturated polyprenols (dolichol type) was higher. Considerable differences were found between the uptake of polyprenols of differing chain lengths. Less than 1% of the polyprenols taken up was converted into more polar product, mainly polyprenyl phosphates and polyprenyl phosphate sugars. The short-chain polyprenols, from $C_{35}$ to $C_{65}$, were metabolized more rapidly than the long-chain polyprenols, as judged from the amount of polar products and fatty acid esters of polyprenols.

The specific requirement for polyprenols in the process of the assembly and transfer of oligosaccharides in the biosynthesis of animal glycoproteins (cf. Hemming, 1974) points to the crucial role of this class of lipids in metabolism and various biological phenomena in the living cell. The knowledge of the occurrence and type of polyprenols (Hemming, 1974) indicates that both the quantity and quality (the length of the chain and extent of unsaturation) of cellular polyprenols show species- and tissue-specific differences. It is not clear whether this may cause differences in glycosylation processes in various animal cells. The in vitro studies on the enzyme systems from rat liver and yeast cells (Mankowski et al., 1977; Radomska-Pyrek et al., 1979a; Palamarczyk et al., 1980), artificially rearranged into a detergent-micellar system, pointed to specificity towards the type of polyprenyl radical. One cannot, however, relate these findings to the function of the natural membrane-bound system. The possibility of introducing atypical polyprenols into living cells could provide valuable information concerning the role of polyprenols as rate-limiting factors in lipid-mediated glycosylations. One should also gain information on the degradation pathway of these compounds. The aim of the present study...
paper is to present the results of studies on the uptake of various types of polyprenols by various cells cultured in vitro and to present preliminary results of research on their metabolism.

Materials and Methods

Cell cultures

The following cell cultures were kindly provided by the Institute of Virology, Justus Liebig University, Giessen, F.R.G.: MDBK, highly differentiated epithelial-like bovine kidney cells, and MDCK, highly differentiated epithelial-like canine kidney cells, both grown in 0.5% lactalbumin hydrolysate in Earle's BSS supplemented with 5% newborn-calf serum; NIL, hamster-kidney fibroblast-like cells, and BHK-21, baby-hamster-kidney fibroblast-like cells, both grown in Glasgow modification of Eagle's medium supplemented with 10% calf serum; chicken-embryo fibroblasts, chicken-embryo fibroblasts transformed with Rous sarcoma virus (strain PxA), chicken-embryo fibroblasts infected with non-transforming strain RAV-3 of Rous sarcoma virus, chicken-embryo fibroblasts infected with fowl-plague virus (Dutch strain), all grown in Dulbecco modified Eagle's medium supplemented with 5% calf serum and 10% tryptose phosphate broth; and mosquito (Aedes albopictus) larva cells grown in Dulbecco modified Eagle's medium supplemented with 10% fetal calf serum. All the above cells were grown in plastic Petri dishes (Greiner, F.R.G.) in an atmosphere of 5% CO₂ in air at 37°C except the mosquito cells, which were grown and incubated with radioactive substrates at 27°C.

A suspension of Cercopithecus aethiops (African green monkey) kidney cells was obtained from the Central Laboratory of Sera and Vaccines, Lublin, Poland. Cells were grown in 0.5% lactalbumin hydrolysate in Hank's solution supplemented with 4% calf serum.

A Vero cell line initiated from the kidney of Cercopithecus aethiops was obtained from the State Institute of Hygiene, Warsaw, Poland, and cultured in minimal Eagle's medium supplemented with 5% calf serum. Both types of monkey cells were grown at 37°C in tightly closed glass flasks (No. 75, Flow Laboratories, Solna, Sweden).

Polyprenols

The polyprenols listed in Table 1 were isolated from the indicated sources according to published (Lindgren, 1965; Burgos et al., 1963; Stone et al., 1967) or adapted procedures, and the mixtures of polyprenols were separated on a column of Lipidex-5000 (Chojnacki et al., 1975). Polyprenols containing 7, 9, 11, 13, 15, and 17 isoprene units (see column headed 'Symbol' in Table 1) were catalytically hydrogenated and their α-dihydro derivatives (described here according to their content of isoprene units and their saturation, by abbreviations 7H, 9H, 11H, 13H, 15H, and 17H) were isolated from the reaction mixture by column chromatography on Lipidex-5000 (Mankowski et al., 1976). The structure of polyprenols (see Table 1) is described by the number and type of particular isoprene residues.
A natural mixture of pig liver dolichols (C_{85}-C_{105}) was prepared according to Burgos et al. (1963). The preparation of fatty acid esters of dolichols was obtained from pig liver by silica-gel column chromatography (Radominska-Pyrek et al., 1979a).

The labelling of polyprenols with tritium (Keenan & Kruczek, 1975) was performed using NaBTr_{4} (12.2 Ci/mmol; The Radiochemical Centre, Amersham, England). Tritiated polyprenols were purified on small (0.5- x 8-cm) columns of Lichroprep Si-60 (Merck, Darmstadt, F.R.G.) by elution with 10-ml portions of 3, 4, 5, 6, 7, and 8% diethyl ether in light petroleum and stored in toluene at -20°C for up to 2 months.

Before supplying labelled polyprenol to the cells (usually 15 μCi per culture), the required amount of labelled substance was dried in a stream of nitrogen and dissolved in the appropriate amount of ethanol. The ethanolic solution was diluted 1000-fold with serum-free medium, with vigorous mixing on a vortex mixer, and evenly distributed into vessels with cultured cells from which the growing medium had previously been removed.

After 20 h incubation the cells were washed three times with ice-cold phosphate-buffered saline, scraped from the flask surface with a rubber policeman (flasks kept on ice), and extracted according to the method of Folch et al. (1957). The content of protein in the insoluble residue was estimated according to the method of Lowry et al. (1951). Lipid extracts were subjected to t.l.c. in benzene:ethyl acetate, 95:5, by vol. (solvent A); hexane:benzene:ethyl acetate, 60:30:1, by vol. (solvent B); and chloroform:methanol:8 M ammonia:water, 65:35:4:4, by vol. (solvent C) on precoated silica-gel 60 F_{254} plates (0.25 mm thick; Merck, Darmstadt, F.R.G.). The radioactive spots were located using a Berthold II scanner. For quantitative measurements, samples of lipid extracts or radioactive areas scraped off t.l.c. plates were counted in a Beckmann LS 9000 liquid scintillation spectrometer using Bray's scintillation fluid (Bray, 1960).
Table 2. Uptake of 3H-labelled dolichol mixture by animal cells cultured in vitro

Cells were incubated for 20 h with 15 μCi of the tritiated dolichol mixture from pig liver. The contents of unchanged dolichol and of nonpolar products were estimated in lipid extracts by counting the radioactivity of t.l.c. spots (solvent A). Each result is the mean of three experiments.

<table>
<thead>
<tr>
<th>No.</th>
<th>Cells</th>
<th>Uptake (μCi/mg protein)</th>
<th>Unchanged dolichol (%)</th>
<th>Unpolar product (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Chicken-embryo fibroblasts, primary, non-confluent</td>
<td>0.312</td>
<td>74</td>
<td>23</td>
</tr>
<tr>
<td>2.</td>
<td>Chicken-embryo fibroblasts, primary, confluent</td>
<td>1.817</td>
<td>82</td>
<td>16</td>
</tr>
<tr>
<td>3.</td>
<td>Chicken-embryo fibroblasts, transformed (RSV, strain PxA)</td>
<td>1.185</td>
<td>82</td>
<td>15</td>
</tr>
<tr>
<td>4.</td>
<td>Chicken-embryo fibroblasts, infected with non-transforming RSV strain RAV-3</td>
<td>0.173</td>
<td>72</td>
<td>25</td>
</tr>
<tr>
<td>5.</td>
<td>Chicken-embryo fibroblasts, infected with fowl-plague virus, Dutch strain</td>
<td>0.521</td>
<td>85</td>
<td>12</td>
</tr>
<tr>
<td>6.</td>
<td>MDMK, bovine kidney, highly differentiated, epithelial-like</td>
<td>0.086</td>
<td>54</td>
<td>40</td>
</tr>
<tr>
<td>7.</td>
<td>MDCK, canine kidney, highly differentiated, epithelial-like</td>
<td>0.123</td>
<td>78</td>
<td>20</td>
</tr>
<tr>
<td>8.</td>
<td>NIL, hamster kidney, fibroblast-like</td>
<td>0.280</td>
<td>78</td>
<td>20</td>
</tr>
<tr>
<td>9.</td>
<td>BHK-21, hamster kidney, fibroblast-like</td>
<td>0.815</td>
<td>88</td>
<td>9</td>
</tr>
<tr>
<td>10.</td>
<td>Aedes albopictus mosquito</td>
<td>3.113</td>
<td>52</td>
<td>45</td>
</tr>
<tr>
<td>11.</td>
<td>Vero, African green monkey</td>
<td>0.294</td>
<td>86</td>
<td>7</td>
</tr>
<tr>
<td>12.</td>
<td>African green monkey, primary</td>
<td>0.994</td>
<td>75</td>
<td>21</td>
</tr>
</tbody>
</table>

Results and Discussion

Uptake of dolichol by various cultured cells

Several types of cells grown in vitro were tested for the capacity to take up the 3H-labelled dolichol mixture (Table 2). All the cells studied acquired tritium label during the 20-h incubation. The extent of labelling expressed in μCi/mg of protein varied markedly between different cultured cells used in comparable amounts. The highest uptake was observed in the case of the mosquito A. albopictus cell line. In the case of chicken-embryo fibroblasts (both primary cultures and established cell lines), monkey kidney cells, and baby-hamster kidney cells, the uptake was moderate. Chicken embryo cells during intensive growth (non-confluent) acquired much less dolichol than did non-dividing (confluent) cells. The uptake was considerably lower with all the other cells studied. It was observed that virus infection causes a decrease of labelling. The lowest uptake was observed with highly differentiated epithelial cells such as the MDCK and MDBK lines. The
differences in the extent of labelling with polyrenols of various cultured cells are in accord with the data reported by Keenan et al. (1977) on the redistribution of tritiated dolichols between various tissues of the rat following intravenous administration. They found 40 h after injection - at a state of equilibrium - that liver acquired 86% and spleen 3.3% of the radioactivity, while other tissues were labelled only negligibly.

In all the cultured cells, the main radioactive compound was shown by t.l.c. to be unchanged dolichol (Table 2). T.l.c. in solvents A and B has revealed that a distinct proportion of radioactive dolichol was converted into a less polar compound with the chromatographic mobility of a fatty acid ester of dolichol. The labelled, less polar material was susceptible to saponification (Burgos et al., 1963), giving rise to the formation of original 3H-labelled dolichol mixture. The highest proportions of the dolichol fatty acid ester (45% and 40%) were observed in cultured mosquito cells and in MDBK cells. In t.l.c. in solvent A, the spots of unchanged dolichol and of dolichyl fatty acid ester represented more than 95% of the radioactivity present in the organic phase obtained upon extracting cells by the procedure of Folch et al. (1957), and no radioactivity was found in the aqueous phase.

The data on the formation of dolichyl esters with fatty acids are in accord with the results of the studies on the in vivo formation of these compounds (Keenan et al., 1977) and on the enzymic system of dolichol esterification in rat liver (Keenan & Kruczek, 1976). In our studies the rate of esterification was not related to the extent of the uptake of dolichols, i.e. it was equally high both in low- and high-labelled cultured cells, as shown in the case of MDBK cells and A. albopictus cells (Table 2). This was also the case in the studies of Keenan et al. (1977), where no such relationship was found in tissues of the rat.

Uptake of different polyrenols by primary cultures of Cerco-pithecus aethiops kidney cells

Radioactive polyrenols and α-dihydro-polyrenols of various chain lengths (from C_{35} to C_{105}) were supplied to primary monkey-kidney-cell cultures. The uptake of labelled compounds was measured after 20 h incubation (Fig. 1). With the series of fully unsaturated polyrenols there were evident differences in the uptake of different prenologues. The highest uptake was observed with C_{55}-polyrenol, C_{65}-polyrenol, C_{95}-polyrenol, and C_{105}-polyrenol, and the lowest with C_{35}-polyrenol and C_{85}-polyrenol. The uptake of dolichol-like, α-dihydro-polyrenols was higher than that of fully unsaturated compounds, except in the case of C_{105}-α-dihydro-polyrenol, which was taken up to a lesser extent. Differences were also observed in the uptake depending on the chain length. The highest labelling was observed with C_{55}-α-dihydro-polyrenol and with C_{95}-α-dihydro-polyrenol. High labelling of cells was also obtained with the natural mixture of tritiated dolichols.

Natural polyrenols are a heterogeneous class of compounds. Fully unsaturated, mainly-cis-polyrenols of various chain lengths have been obtained with high yield from various plants (cf. Hemming, 1974). It
Fig. 1. The uptake of various polyprenols by primary culture of green-monkey kidney cells. $^5$H-labelled polyprenols (15 $\mu$Ci) were supplied to each of three monolayer cultures (confluent). After 20 h incubation, cells were extracted with a chloroform:methanol mixture, 2:1, v/v. The uptake is expressed as total radioactivity of lipid extract per mg of protein. The values are means from three independent experiments performed with different batches of cells. Symbols in the bars describe the type of polyprenol (number, number of isoprene residues; $H$, presence of saturated $\alpha$-isoprene residue).

Has previously been accepted that animal cells contain a mixture of polyprenols made up only of $C_{85-110}-\alpha$-dihydropolyprenols (dolichols). However, the presence of medium-chain-length $C_{55}-\alpha$-dihydropolyprenol in pig liver (Mankowski et al., 1976), of fully unsaturated $C_{55}$-polyprenol in bovine pituitary glands (Radomska-Pyre et al., 1979b), and of fully unsaturated $C_{95}$-polyprenol in hen oviduct (Hayes & Lucas, 1980) has now been shown. The use in this paper of various fully unsaturated and $\alpha$-dihydropolyprenols of unusual chain length was therefore justified. Moreover one suspects that a variety of polyprenols of dietary origin enter animal cells (Keenan et al., 1977).
Metabolism of different polyprenols by primary cultures of Cercopithecus aethiops kidney cells

As shown by t.l.c. in solvents A and B (Fig. 2), the main radioactive spot found by chromatography of lipid extracts from cells corresponded to the original polyprenol. A considerable proportion of the label (ca. 30%) was found also in the spot of polypropenyl fatty acid esters in the case of fully unsaturated C_{35}-polyprenol and C_{35}-α-dihydropolyprenol. In the case of all the other polyprenols (C_{45}-C_{105}), the amounts of fatty acid esters did not exceed 10%. The lipid extract from the post-incubational medium contained almost exclusively original polyprenol; only traces of substances migrating near the front or remaining at the origin (t.l.c. in solvent A) were present. Recent in vitro studies (Radominska-Pyrek et al., 1979a) have shown that rat-liver microsomal transacylases were acting on various polyprenols and were rather unspecific towards the extent of saturation and to the chain length of polyprenols.

Fig. 2. Scans of thin-layer chromatograms of the lipid extract from primary culture of green-monkey kidney cells incubated with 3H-labelled dolichol mixture. Aliquots containing 10^5 cpm were chromatographed on 20-cm silica gel 60 plates in the indicated solvents. F, front; 0, origin; D_{mix}, dolichol mixture from pig liver; e, dolichyl fatty acid esters.
As shown in Fig. 2, lipid extract from cells incubated with labelled polyrenol contained also polar metabolites, as evident from the radioactivity remaining at the origin (solvent A). When lipid extracts were subjected to t.l.c. in solvent C, these polar compounds formed two main radioactive spots, of $R_F$ ca. 0.3 and 0.4 (Fig. 3).
Fig. 3 (opposite and above). Scans of thin-layer chromatograms of lipid extracts from a primary culture of green-monkey kidney cells incubated with various $^3$H-labelled polyprenols. Aliquots containing 106 c.p.m. were chromatographed on 20-cm silica gel 60 plates in solvent C. F, front; O, origin; a, polypropenyl phosphates; b, polypropenyl phosphate monosaccharide. Symbols on the left of each scan describe the type of polypropenol used in the experiment (number, number of isoprene residues; H, presence of saturated α-isoprene residue).

The lower spot corresponded to polypropenyl phosphate, and the upper one may represent polypropenyl phosphate sugars. The formation of these compounds was most distinct in experiments with polypropenols ranging from C$_{35}$ to C$_{55}$. The proportion of these metabolites was
rather low and never exceeded 1% of the total lipid radioactivity of the cell. It should be mentioned that in similar experiments with rat hepatocytes, the conversion of exogenous C₂₅₅₀-dihydropolyprenol into phosphorylated derivatives was much higher (Chojnacki et al., 1980).

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References