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Chemicals Which Promote Cell Hybridization

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Abstract—*In order to identify new compounds with cell hybridization properties similar to poly(ethylene glycol), the standard method for PEG-induced cell hybridization has been employed as a screening procedure. Of 118 membrane-active agents studied, over 20 compounds were identified which promoted cell hybridization with nearly the same efficiency as PEG. PEG derivatives which retained cell hybridization activity included polymers with branched and charged structures as well as polymers with chemical alterations of the PEG monomer itself. PEG derivatives with hydrophobic moieties were generally inactive. Several chemically modified derivatives of poly(ethylene glycol), which are commercially important in the pharmaceutical and cosmetic industries, were found to be highly efficient cell hybridization agents. The biohazard of such compounds is discussed. A simplified method is presented for the preparation and use of PEG in cell hybridization procedures.*

INTRODUCTION

Cell fusion induced by poly(ethylene glycol) (PEG) has become a standard method in somatic cell genetics. While many viruses (1–3) and chemicals have been demonstrated to fuse mammalian cells, only PEG (6–9), paramyxoviruses (3, 10), and lysolecithin (5, 11) have proved to be effective in producing large numbers of growing somatic cell hybrids. In order to determine the structural requirements of compounds capable of inducing cell hybridization, a wide range of membrane-active agents have been surveyed in order to detect new compounds with cell hybridization properties similar to PEG. Of 118 compounds surveyed, over 20 compounds were detected which were as active as PEG-1000 in promoting cell hybridization. The structural relationships of active cell hybridization agents are described.

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MATERIALS AND METHODS

Preparation of Polyethylene Glycol (PEG) Solutions. Since it was observed that autoclaving of PEG solutions increased the acidity of such solutions, PEG solutions were sterilized by membrane filtration. Filtration was carried out using 0.2-μm filters (Amicon, Lexington, Massachusetts) with either an Antila filtration system (Schleicher and Schull, Keene, New Hampshire) or a vacuum filtration apparatus (model SM 16510, Sartorius Filters, Inc., Hayward, California). PEG was dissolved at the concentrations specified in the text in 0.15 M HEPES, pH 7.55, containing 0.002% phenol red. The pH of the PEG solution was adjusted with either 12 N HCl or 50% NaOH. Care was taken to assure that 50% NaOH had fully dissolved in the viscous PEG solutions prior to a final pH reading.

Preparation of Other Cell Fusion Agents. All other cell fusion agents employed in this study were dissolved in 0.15 M HEPES, pH 7.55, plus 0.002% phenol red and sterilized by membrane filtration. With the precautions indicated above, the pH of such solutions was adjusted to pH 7.55. In certain cases, membrane filtration was not possible due to the high viscosity of the compound being tested. Such viscous solutions were dissolved in sterile 0.15 M HEPES, pH 7.55, in sterile glassware and used without membrane filter sterilization.

Maintenance of Cell Lines. Cells were propagated with Dulbecco's modified Eagle's medium supplemented with 1 mM L-proline, 10% newborn calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. This medium is hereafter referred to as culture medium. Cells were maintained under a 10% CO_2-in-air atmosphere at 36.5°C.

Cell Hybridization Procedure. With the modifications noted below, the procedure described by Davidson and Gerald (6) for PEG-induced cell hybridization was employed as a screening method to identify new compounds with hybridogenic activity.

The cell fusion procedure employed here was devised in order to optimize the yield of hybrids obtained between the mouse cell lines, LM(TK$^-$) (12) and RAG (2). Parameters involved in Sendai virus-induced cell hybridization of the above cell lines have previously been described (2). Modifications of the existing procedure for PEG-induced cell hybridization (6) were made such that each solution used in the cell fusion procedure contained the minimum number of essential additives.

In brief, the cell fusion procedure employed in this study, is as follows: 2.5×10^5 LM(TK−) and 2.5×10^5 RAG cells were mixed together in 5 ml of culture medium and planted in 25-cm^2 culture flasks (Corning No. 25100, Corning Glass Works, Corning, New York). After 16 h, the culture medium was removed and the cell layer was washed twice with Ca^{2+}-free minimal attachment medium (MAM) (13–15). MAM is a minimal salt solution which

contains only those ions required for cell-substratum adhesion (14). Ca^{2+}-free MAM consists of 116 mM NaCl, 5.4 mM KCl, 5.5 mM glucose, 1 mM $MgCl_2$, and 10 mM HEPES, pH 7.55 (14). Since Ca^{2+} interferes with cell hybridization (16), Ca^{2+} was omitted from fusion solutions. Prior to treatment of a cell monolayer with fusogen, medium was carefully removed in order to avoid dilution of the fusogen. At 21°C, 2 ml of cell fusion agent was added to a washed cell layer for 1 min followed by (a) rapid removal of the cell fusion agent and (b) three washes with 5 ml of Ca^{2+}-free MAM for 10 sec/wash. The fusogen-treated cells were then incubated at 37°C for 30 min in Ca^{2+}-free MAM. Following removal of Ca^{2+}-free MAM, HAT medium (17) was added in order to select for growing somatic cell hybrids. Cultures were fed 3–4 days following fusion. Hybrids appeared between 7 and 9 days postfusion. Hybrid colonies were washed with saline, fixed with neutral buffered formalin, stained with Wright's stain, and enumerated. All experiments employed two types of controls: (a) cultures treated with 50% PEG-1000 (the PEG control) and (b) cultures not treated with fusogen (the HAT control). Under the conditions described above, an average of 65 hybrids per flask was obtained following 50% PEG treatment while an average of less than one hybrid was obtained in the untreated control.

Since PEG is dissolved in serum-free medium in many studies, the effect of tissue culture medium components on PEG-induced cell fusion was investigated. It was found that inclusion of tissue culture medium components in the PEG cell fusion solution reduced the yield of hybrid colonies (Table 1). Thus, only a buffer need be employed, in conjunction with 50% PEG, for optimal yields of hybrid cells. The effect of the pH of the 50% PEG solution on the yield of hybrids is presented in Fig. 1. Hybrids were recovered in the physiological pH range; however, acidic and basic conditions abolished the hybridogenic effect of PEG (Fig. 1). In order to screen for new hybridogenic compounds under the simplest conditions, PEG and other membrane active agents were dissolved in 0.15 M HEPES, pH 7.55.

Table 1. Conditions for Cell Hybridization[a]

Cell fusion solutions	Hybrids/flask
50% PEG-1000 in Dulbecco's medium (serum free)	26
50% PEG-1000 in Ca^{2+}-free minimal attachment medium (MAM)	44
50% PEG-1000 in 0.15 M HEPES, pH 7.55	68
50% PEG-1000 in 0.015 M HEPES, pH 7.55	31
50% PEG-1000 in H_2O (pH 7.5)	13

[a]Cell hybridization was carried out as described in Materials and Methods. The cell fusion solutions given above were exposed to the cell population to be fused for 1 min and then the flask was washed twice with Ca^{2+}-free MAM for 2 min/wash. The number of hybrids/25-cm^2 flask generated by each cell fusion solution is given above.

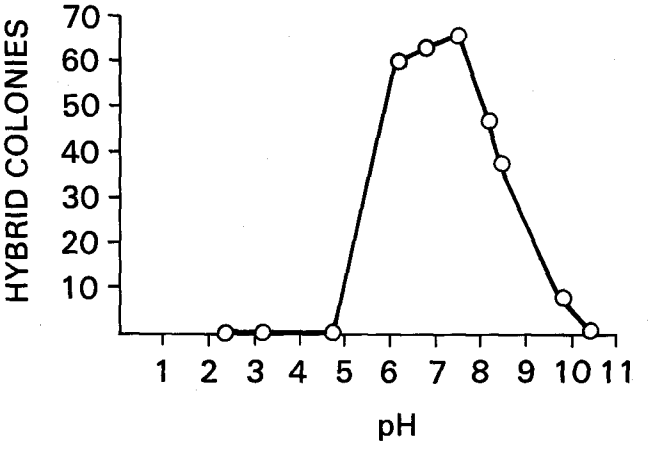


Fig. 1. Effect of pH on yield of hybrid cells. Washed monolayers of LM(TK−) + RAG cell mixtures were treated with 50% PEG-1000 in an 0.15 M buffer and then treated as described in the text. The buffer ions employed to establish a given pH were as follows: glycine (pH 2.35), glycylglycine (pH 3.14), acetic acid (pH 4.76), MES (pH 6.15), PIPES (pH 6.8), HEPES (pH 7.55), tricine (pH 8.15), glycylglycine (pH 8.4), glycine (pH 9.78), carbonate (pH 10.33).

The temperature at which cells were exposed to fusogen had little effect on the yield of hybrids (Table 2) and, hence, exposure to fusogen was carried out at 21°C while all other steps were performed at 37°C.

Reagents. Sources for the compounds tested for fusogenic activity are given in Tables 3 and 4. Organic chemicals were obtained from Sigma (St. Louis, Missouri). All other chemicals were of reagent grade.

RESULTS

Survey of Membrane-Active Agents for Hybridogenic Activity. With the modifications noted in Materials and Methods, the now standard procedure for PEG-induced cell hybridization was employed as a screening method

Table 2. Effect of Temperature on Cell Hybridization[a]

Temperature (°C)	Hybrids/flask
6	57
15	43
21	84
37	58

[a]All solutions employed were equilibrated to the desired temperature. Cell layers were washed free of culture medium with Ca^{2+}-free MAM, and then cultures were treated with 50% PEG-1000 for 1 min after a 5-min period which permitted establishment of the desired temperature. Following washing with CA^{2+}-free MAM to remove PEG, culture flasks were placed at 37° for 30 min before the addition of HAT medium.

Table 3. Hybridogenic Compounds[q]

Name of compound	Number of experiments	Concentrations producing hybrids	Concentration producing most hybrids	Concentrations toxic to cells	Percent of PEG-1000 hybridization (%)
1. PEG 600[a]	2	50–30%	50%		128
2. Glucam ™ E-10[e]	2	60–50%	60%		112
3. PEG methyl ester (MW 350)[i]	2	50–40%	50%	70–60%	105
4. PEG-1000[b]	(>200)	50–40%	50%		100
5. Polyglycol P15-200[h]	2	60–20%	60%		93
6. PEG methyl ester (MW 5000)[i]	2	70–40%	50%		89
7. PEG methyl ester (MW2000)[i]	2	60–40%	50%	70%	89
8. Pluronic F38 Polyol Prilled[g]	2	50–40%	50%		86
9. PEG-6000[b]	2	50–40%	50%		84
10. Glucam ™ E-20[e]	2	50–40%	50%		79
11. PEG-3000[a]	2	50–40%	50%		60
12. PEG-400[b]	2	50–25%	50%		58
13. Decaglycerol[j]	2	60	60%		56
14. Capmul 101 Butyrate[k]	2	70–50%	70%		47
15. Tetronic 304 Polyol[g]	2	60–20%	60%		30
16. Glucam ™ p-10[e]	2	60%	60%		33
17. Triglycerol[k]	2	60–50%	60%	80–70%	29
18. Poly(vinylpyrrolidone)[a] (MW 10,000)	2	50–25%	50%		23
19. Glycerin[b]	3	50–20%	30%		22
20. Pluronic 10R8 Polyol Prilled[g]	3	40%	40%	70–50%	18.6
21. Triton QS-15[a]	3	1%	1%		17.1
22. Caprol 10G10[k]	3	40–30%	30%	60–40%	16
23. Triton CF32[a]	3	5%–0.1%	5%	50%	12.7
24. Retinol[a]	2	0.1–0001%	0.1–0.01%		9
25. Triton CF54[a]	3	0.01%	0.01%	10–0.1%	8.8
26. KCl[a]	2	2.5–0.5 M	2.5 M		8.6

Table 3. Continued

Name of compound	Number of experiments	Concentrations producing hybrids	Concentration producing most hybrids	Concentrations toxic to cells	Percent of PEG-1000 hybridization (%)
27. Chenodesoxycholic Acid[a]	2	0.001–0.0001%	0.001%	0.1%	8.6
28. 12-Crown-4[a]	2	0.5–0.05%	0.5–0.05%	50%	8
29. Glycine (pH 9.8)[a]	2	0.2 M–2 × 10^{-4} M	0.02 M		8
30. Pluronic 25R8, Polyol[g]	2	40%	40%		8
31. Caprol 3G10[k]	2	40%	40%		8
32. Poly(acrylic acid) (90,000 MW)[d]	2	12.5–3.1%	12.5%		8
33. Deoxycholic acid[a]	4	0.01–0.0001%	0.001%	1%	7.7
34. Urea[d]	4	4–1 M	4 M	8 M	6.9
35. Triton DF12[a]	4	0.5–0.05%	0.5%	50–5%	6.8
36. Polyvinyl alcohol (type I)[a]	2	12.5–6.2%	6.2%		6.6
37. Triton B-1956[a]	3	1–0.001%	1%		6.5
38. Poly(ethylene oxide) (100,000)	2	12.5–6.2%	6.2%		6
39. Poly(methacrylic Acid)[m]	2	3.1%	3.1%		6
40. Cholic acid[a]	2	0.1–0.0001%	0.001%		5.7
41. Diglyme	2	10%	10%		5.2
42. Polymixin B sulfate[a]	2	0.01%	0.01%		5.2
43. Poly(ethyleneimine)-600[m]	2	10%	10%		5
44. PEG 20,000	1	40%	40%		4.8
45. Polyethyleneimine (1800MW)[m]	1	25%	25%	75%	4.3
46. Triton X-45[a]	3	0.1–0.01%	0.1%	10–1%	4
47. Poly(vinyl methyl ether)[d]	1	0.05%	0.05%		4
48. Poly(propylene glycol) 425°	2	5–0.5%	5%	50%	4
49. Triton X-67[a]	1	0.5%	0.5%	50–5%	4
50. $NaHCO_3$ (pH 8.5)[a]	1	0.5 M	0.5 M		3.1
51. 18-Crown-6[a]	1	0.05%	0.05%	50%	2.7

Table 3. Continued

Name of compound	Number of experiments	Concentrations producing hybrids	Concentration producing most hybrids	Concentrations toxic to cells	Percent of PEG-1000 hybridization (%)
52. Caprol 6G10[k]	1	40–30%	40–30%	50%	2.5
53. D-Sorbitol[a]	2	50–20%	50%		2.5
54. Ethyl ether[c]	3	10%	1%		2.5
55. SDS (sodium dodecyl sulfate)[a]	2	0.1%	0.1%	10–1%	2.2
56. PEG-200	4	12.5%	50%	75%	2
57. Taurodeoxycholic acid[a]	2	0.001–0.0001%	0.001%		2
58. Dioxane	2	1%	1%		1.7
59. $CaCl_2$ (pH 10.7)[a]	5	1–0.02 M	1 M		1
60. Hexanol	1	0.01%	0.01%		0.8

[a]Sigma Chemical Co., St. Louis, Missouri.
[b]Fisher Scientific Co., Houston, Texas.
[c]J. T. Baker Chemical Co., Phillipsburg, New Jersey.
[d]Aldrich Chemical Co., Milwaukee, Wisconsin.
[e]Amerchol Corp., Edison, New Jersey.
[f]Rohm and Haas Co., Philadelphia, Pennsylvania.
[g]BASF Wyandotte Corp., Wyandotte, Michigan.
[h]Dow Chemical Co., Midland, Michigan.
[i]Armak Co., Chicago, Illinois.
[j]Emery Industries, Inc., Cincinnati, Ohio.
[k]Capitol City Products Co., Columbus, Ohio.
[l]Union Carbide Corp; New York, New York.
[m]Polysciences, Inc., Warrington, Pennsylvania.
[n]Evans Chemetics, Inc., Darien, Connecticut.
[o]Monsanto, Co., St. Louis, Missouri.
[p]Calbiochem-Behring, Corp., San Diego, California.
[q]The compounds listed above were screened for their ability to promote cell hybridization as described in the text. Structures of nearly all of the compounds studied can be found in reference 27. The maximum hybridogenic activity observed for a given compound is expressed as a percent of the activity of the reference compound, PEG-1000.

Table 4. Nonhybridogenic Compounds[q]

Name of compound	Concentration tested
Brij 96[a,r]	10–0.01%
Brij 58[a,r]	10–0.01%
2-(2-*n*-Butoxethyoxy) ethanol[d,r]	50–5%
Ca nitrate (pH 8)	0.2–0.02 M
Capmul 101 acetate[k,r]	80–50%
Caprol 10 GIL[k,r]	60–30%
Caprol 10 G1 caprylate[k,r]	60–30%
Cetyltrimethyl ammonium Br[a,r]	10–0.01%
Dibucaine	10–0.01%
Dicyclohexyl (18-crown-6)[d,r]	50–0.05%
Diiodosalicylic acid[d,r]	0.2–0.02 M
Dimethyl sulfoxide (DMSO)[a]	50–20%
Ethomeen 18/25[i]	40–15%
2-Ethoxyethanol[d,r]	50–5%
2-(2-Ethoxyethyl) ether[d,r]	50–5%
2-Ethoxyethyl ether[d,r]	40–5%
Ethylene glycol[b]	50–20%
Fungizone[r]	10–0.1 mg/ml
Gelvatol 20–30[o]	40–20%
Gelvatol 40–10[o]	50–20%
Glucam ™ P-20[e,r]	60–20%
Glycocholic acid[a]	0.1–0.0001%
$NaCO_3$ (pH 11)[a]	0.75–0.075 M
Octanol[r]	3–0.01%
Poly(acrylic acid) (5000 MW)[d]	25–6.2%
Poly(*N*,*N*-dimethyl-3,5-dimethylpiperidium) chloride[d]	25–5%
Pluronic L64, polyol[g,r]	50–15%
Pluronic F68[g]	33–15%
Pluronic 17R8, Polyol[g]	80–40%
Pluronic L61[g]	80–50%
Pluronic L35[g]	80–40%
Pluronic L31[g]	80–40%
Poly(ethyleneimine, hydroxyethylated)[m]	50–20%
PEG dimercaptoacetate[n,r]	80–50%
PEG Di(3-mercaptopropionate)[n]	80–50%
PEG-600 diglycidyl ether[m]	50–0.005%
PEG-600 Dimethacrylate[n,r]	50–0.005%
PEG-600 Disterate[m]	20–1.5%
PEG 15 oleyl quarterium 4[i,r]	40–10%
Polyvinyl alcohol (type II)[a]	25–6.2%
Polyvinyl sulfate[m]	25%
Procaine[a]	10–0.01%
Propylene glycol[a]	50–20%
Quadrol[g,r]	80–50%
Starch	12.5–3.1%
Taurocholic acid[a]	0.1–0.0001%
Tetrahydrofuran[c]	25–1%
Tetronic 908, Polyol[g]	15–2.5%
Triton X-155[f,r]	60–2%
Triton X-405[a]	10–0.01%
Triton X-100[a,r]	10–0.01%
Triton DN-65[a,r]	50–0.5%

Table 4. Continued

Name of compound	Concentration tested
Triton DF-16[a,r]	50–0.005%
Triton QS-44[a,r]	10–0.01%
Tween 80[a]	10–0.01%
Tween 20[a,r]	10–0.01%
Tyloxapol[a,r]	10–0.01%
Zwittergeht 314[p,r]	10–0.01%

[a]Sources of the compounds studied are indicated by the superscript letter following the name of a compound (see Table 3 for list of sources).
[r]Indicates cytotoxicity.

to detect additional compounds with cell hybridization activity. Tables 3 and 4 present the results of this survey of 118 membrane-active agents for cell hybridization activity. Cell hybridization activity was calculated from the following equation:

$$\% \text{ Cell hybridization activity} = \frac{\begin{array}{c}(\text{hybrids produced by compound X}) \\ -\ (\text{hybrids in untreated control})\end{array}}{\begin{array}{c}(\text{hybrids produced by 50\% PEG-1000}) \\ -\ (\text{hybrids in untreated control})\end{array}} \times 100$$

The compounds studied are listed in decreasing order of their cell hybridization activity, with PEG-1000 serving as a reference compound. Table 3 indicates that 60 membrane-active agents had some cell hybridization activity while 19 compounds were identified which produced at least 20% of the number of hybrids generated by PEG-1000. Table 3 presents other data concerning (1) the concentration tested which produced the maximum number of hybrids, (2) the concentrations which produced hybrids, and (3) those concentrations which proved to be cytotoxic. The survey was carried out by screening a given compound over a wide range of concentrations and then retesting each compound over a narrower range of concentrations. Thus, each active compound was tested at least twice at generally several concentrations which yielded growing somatic cell hybrids. The highest concentration tested was often determined by the solubility limit of the compound being tested. In other cases, the highest concentration tested was chosen due to prior knowledge of the toxicity of the compound. The cytotoxic concentration of a compound was determined by microscopic observation of cells after treatment. In some cases, cell lysis occurred almost immediately upon contact with a compound; while in other cases, several days were required for cell death and detachment to occur. The range of concentrations which produced

Table 5. Synergism between Hybridogens[a]

	Second Hybridogen	
	Poly(vinyl alcohol)	Poly(glycerol)
0% PEG-1000 + 30% 2nd hydridogen	0	1
25% PEG-1000 + 0% 2nd hybridogen	4.5	4.5
25% PEG-1000 + 10% 2nd hybridogen	18	9
25% PEG-1000 + 20% 2nd hybridogen	17	43
25% PEG-1000 + 25% 2nd hybridogen	12	63
25% PEG-1000 + 30% 2nd hybridogen	0	65

[a]Cell hybridization was carried out as described in the text. Cell fusion solutions consisted of 25% PEG-1000 plus the concentrations indicated above of a second hybridogen. The second hybridogens employed were Gelvatol 40–10 [a 10,0000-molecular-weight poly(vinyl alcohol)] and Decaglycerol (a polymer of ten glycerol subunits). The number of hybrids/flask promoted by each hybridogen solution is entered in the table. The results indicate that PEG acts in a synergistic fashion with both poly(vinyl alcohol) and poly(glycerol).

hybrids and that concentration which produced the maximum yield of hybrids is noted in Table 3. As in the case of PEG itself, many of the most effective hybridogens required a concentration of 50% or more in order to promote cell hybridization. Structural relationships between the hybridogens identified in this survey will be presented in the Discussion.

Synergism between Hybridogens. Many of the hybridogens which require 50% or greater concentration to induce cell hybridization fall into four classes: (a) poly(ethylene glycol) derivatives, (b) poly(vinyl alcohols), (c) poly(vinylpyrrolidone), and (d) poly(glycerols). In order to test whether these four classes of hybridogens had a similar mode of action, studies were performed to test whether synergism occurred between various hybridogens. The existence of synergism between hybridogens was assessed by treating cell populations with cell fusion solutions consisting of 25% PEG-1000 plus various concentrations of a second hybridogen (Table 5). Thus 25% PEG-1000 alone promoted little cell hybridization; however, addition of either poly(vinyl alcohol) or poly(glycerol) to 25% PEG-1000 did yield hybrids when the combined hybridogen concentration was 35% or more. It should be noted that poly(glycerol) had a greater synergistic effect than poly(vinyl alcohol). Synergism between poly(ethylene glycol), poly(vinyl alcohol), and poly(glycerol) indicates that these hybridogens act in a similar fashion.

DISCUSSION

By employing the standard procedure for PEG-induced cell hybridization (6,9) as a screening procedure for identifying other hybridogenic compounds, it has been possible to identify 60 compounds with cell hybridization properties similar to PEG. Nineteen of the hybridogens identified by the

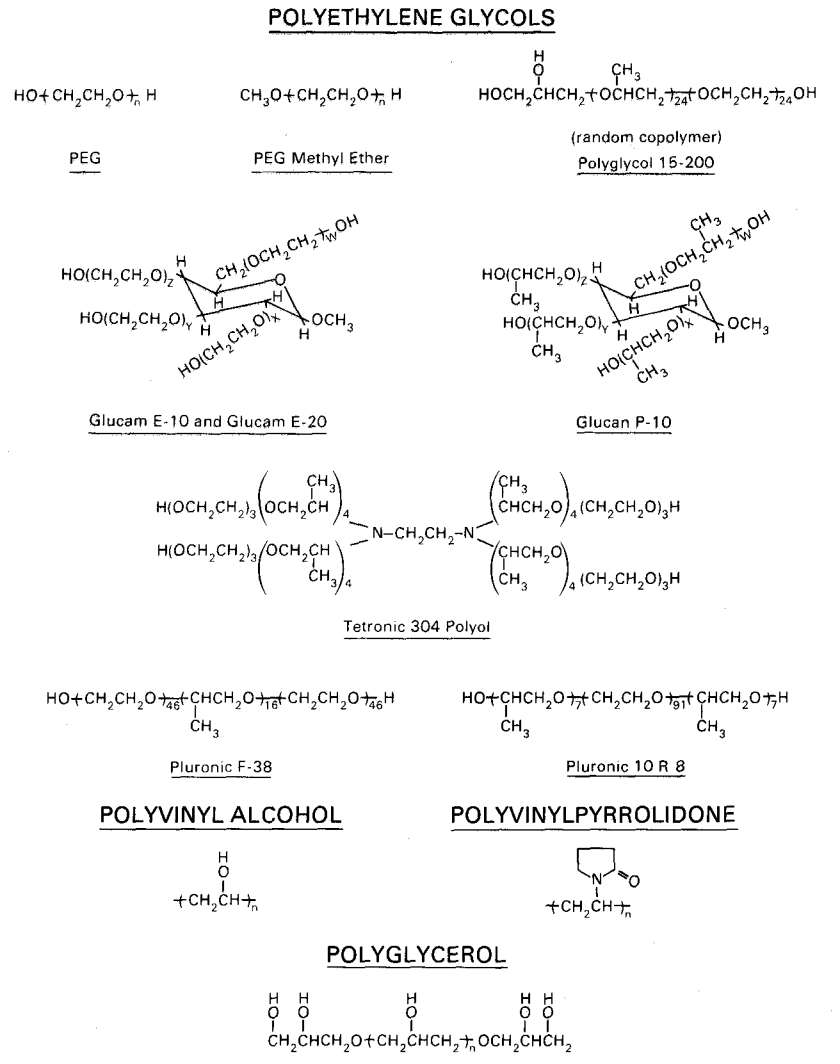
screening procedure had at least 20% of the cell hybridization activity of PEG-1000 (Table 3). Prior to presenting the structural relationships of the hybridogens identified in this study, it should be noted that the screening procedure employed would fail to identify all compounds which fuse cells. Some compounds which readily fuse cells may not be detected by this screening procedure due to cytotoxic effects which prohibit the generation of growing somatic cell hybrids. In most applications, cytotoxic fusogens would be of far less value than the hybridogens identified by the screening procedure employed in this study.

In the following discussion, the structural interrelationships between hybridogenic chemicals will be presented. In order to facilitate comparison, Table 6 presents the structures of several key compounds which promote cell hybridization while Table 7 presents the structures of several compounds which were found to be inactive in inducing cell hybridization. Four structurally related classes of compounds were found to be active hybridogens: poly(ethylene glycols) (PEG), poly(vinylpyrrolidone), poly(vinyl alcohol), and poly(glycerols) (Table 6). All of the above compounds must be employed at concentrations ranging from 40% to 70% in order to induce cell hybridization. While poly(ethylene glycols) with average molecular weights ranging from 400 to 3000 were found to be active in producing hybrids, ethylene glycol monomer, PEG-200, and PEG-20,000 were found to be almost inactive in promoting cell hybridization (Table 4 and ref. 10). Thus, factors other than purely chemical structure are involved in determining whether or not a compound will be an active hybridogen.

Table 6 presents selected poly(ethylene glycol) derivatives which possess hybridogenic activity similar to that of PEG-1000. That PEG methylethers (of molecular weight 350–5000) possess hybridogenic activity demonstrates that one of the two terminal hydroxyls of polyethylene glycol may be blocked without loss of hybridogenic activity. The hybridogenic activity of Glucam E-10 (and E-20) and Tetronic 304 Polyol indicates that fusogenic molecules need not be linear in order to promote cell hybridization. Since Tetronic 304 Polyol bears a net positive charge at neutral pH, hybridogenic compounds also need not be electrically neutral.

Table 7 presents the structure of poly(ethylene glycol) derivatives which are either totally inactive as hybridogens or possess greatly reduced hybridogenic activity in comparison to their parent compound. As indicated earlier, extremely high and very low molecular weight poly(ethylene glycols) are inactive as hybridogens. While a wide variety of chemical derivatives of PEG maintain hybridogenic activity (Tables 3 and 6), certain derivatives of PEG are inactive. Poly(propylene glycol), which differs from PEG by the substitution of a methyl group for a hydrogen (Table 7), is completely inactive. At high concentrations, poly(propylene glycol) causes clear cytotoxic effects

Table 6. Hybridogens[a]



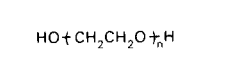
[a]The structures of key compounds with fusogenic activity are presented. Structures of other hybridogens presented in Table 3 can be obtained from reference 27. For Glucam E-10 and P-10 W + X + Y + Z equals 10; for Glucam E-20, W + X + Y + Z equals 20.

(Table 4). Poly(ethylenimine), which contains a nitrogen in place of the oxygen in PEG (Table 7), is also completely inactive as a hybridogen.

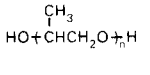
While poly(propylene glycol) (PPG) is inactive as a hybridogen (Tables 4 and 7), several poly(propylene glycol) derivatives were found to be active as

Table 7. Nonhybridogens[a]

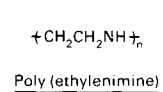
POLYETHYLENE GLYCOLS



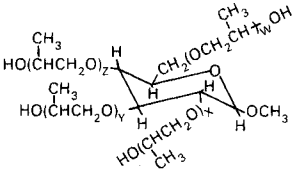
PEG



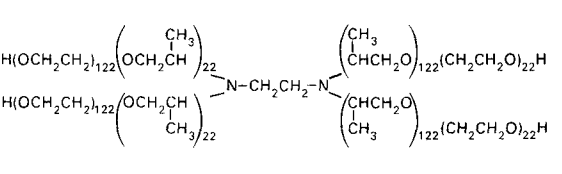
Poly (propylene glycol)



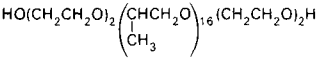
Poly (ethylenimine)



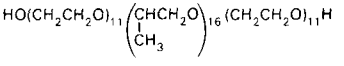
Glucam P-20



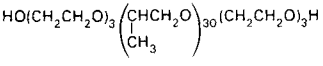
Tetronic 908



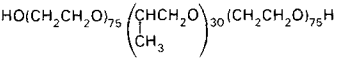
Pluronic L-31



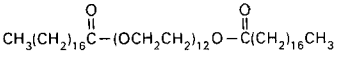
Pluronic L-35



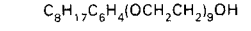
Pluronic L-61



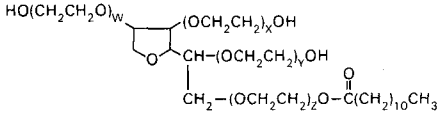
Pluronic F-68



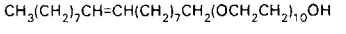
PEG-600 Distearate



Triton X-100



Tween 20



Brij 96

[a]The chemical structures of compounds which either lack hybridogenic activity or possess much less hybridogenic activity than the parent compound are presented. Structures for other compounds in Tables 3 and 4 may be obtained from reference (27). Note that PEG is presented in this table of nonhybridogens since low molecular weight PEGs (n = 1 or 6) and high molecular weight PEG-20,000 (n = 400) retain little of the hybridogen activity of PEGs with molecular weights ranging from 400 to 3000 (Table 3). In the structure of Tween 20, the average number of ethylene glycol monomers is 20 (= W + X + Y + Z).

hybridogens (Glucam P-10, Pluronic F-38, Pluronic 10 R8, and Tetronic 304) while other PPG derivatives displayed no hybridogenic activity (Tetronic 908 and several Pluronic series compounds). Hybridogenic poly(propylene glycol) derivatives contain a high ratio of hydrophilic PEG moieties, while inactive derivatives contain a low ratio of PEG moieties and are cytotoxic.

While several detergents have been shown to induce cell fusion (4,5), such detergents were found to be inactive as hybridogens. While the bulk of the molecular weight of the Triton, Tween, and Brij series of detergents consists of PEG monomers, such molecules are inactive as hybridogens due to their high toxicity at low concentration (Table 4). Thus, the introduction of long-chain fatty acids or aromatic groups into poly(ethylene glycol) renders such molecules hydrophobic, toxic, and nonhybridogenic. In a similar fashion, addition of hydrophobic groups to poly(glycerol) renders such derivatives inactive as hybridogens (see Caprol 3 G10, Caprol 6 G10, Caprol 10 G1L, and Caprol 10 G1) (Table 4). Of the numerous detergents surveyed, only Triton QS-15, proved to have appreciable hybridogenic activity (Table 3).

For optimum effect in promoting cell hybridization, the 20 most active hybridogens identified in this survey all must be employed at a percent solution of greater than 40%. Hybrids were produced at concentrations ranging from 40% to 60% with hybridogens of varied chemical structure and molecular weight (Table 3). Even though the molecular weight of the most effective hybridogens varied by more than 20-fold, a percent solution of 40% or more was required to promote cell hybridization. In a prior study of PEGs of various molecular weights (18), it was also found that the factor determining the optimum yield of hybrids was the percent solution of fusogen employed rather than the molar concentration of hybridogen. The requirement for at least a 40% solution for cell hybridization may be accounted for by the suggestion, which has been made by several groups (19,20), that PEG-induced cell fusion may be mediated by an alteration of a critical amount of bulk water at the cell surface. The ability of PEG to decrease the solubility of proteins (21) has also been attributed to a PEG-mediated change in the hydration shell surrounding proteins (22–25).

An important outcome of this study is the finding that many compounds employed in the pharmaceutical and cosmetic industries are potent hybridogens. PEG derivatives currently find applications as vehicles for drugs, creams, lotions, shampoos, hair conditioners, soaps, shaving creams, makeup sticks, hair conditioners, ointments, suppositories, lipsticks, sun-screens, solid antiperspirant sticks, bath oils, depilatories, germicidal skin cleaners, vehicles for edible flavorings, and a host of other uses of industrial importance (26–35). Due to the application of hybridogenic PEG derivatives to skin and other tissues at high concentration, cell fusion and the production of prolifer-

ating somatic cell hybrids may accompany the use of pharmaceuticals and cosmetics which contain PEG derivatives. In vivo cell hybridization may result in several types of genetic damage to an organism. It is now well documented that fusion of different cell types can result in stable changes in the pattern of gene activity (36). Alteration of the developmental potential of a cell may have long-term deleterious consequences of the organism. In the phenomenon of virus rescue by cell hybridization, a virus resident in a latent form may be induced to undergo vegetative growth following fusion with a suitable host cell (37). Hence, in vivo cell hybridization promoted by hybridogenic pharmaceuticals and cosmetics may result in the liberation and spread of viruses. When subjected to conventional toxicological tests, many PEG derivatives prove to be nontoxic. While overt toxicity is not observed, hybridogens utilized by the pharmaceutical and cosmetics industries may represent a unique new class of agents of interest in genetic toxicology.

It is important to note that the majority of hybridogenic compounds identified in this study require a concentration of 40% or more in order to induce cell hybridization. Thus, commercial products which contain 40% or more hybridogen may represent a biohazard to man. The potential biohazard of hybridogens in pharmaceuticals and cosmetics would be reduced or eliminated if the concentration of hybridogens (and synergistic agents) were limited to 30% or less.

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