water-soluble compounds were dissolved in water, insoluble compounds were formulated as wettable prowders. The concentrations used were 4000 and 1000 ppm of the active ingredients.

Of the 7 compounds tested, 4-allyl-4-(3,7-dimethyloctyl) morpholinium bromide and 1-allyl-1-(3,7-dimethyl-2,6-octadienyl)piperidinium bromide demonstrated little or no growth retardant effect (Table I), whereas 1-allyl-1-(3,7-dimethyloctyl)piperidinium bromide, the corresponding chloride, and 1-propyl-1-(3,7-dimethyloctyl)piperidinium iodide showed excellent growth retardant activities. The 1-allyl-1-(3,7-dimethylnonyl)piperidinium bromide and the 1-ethyl-1-(3,7-dimethyloctyl)piperidinium bromide were slightly less active .The growth retardation effect could be antagonized by indole-3-acetic acid and gibberellic acid.

As the 1-allyl-1-(3,7-dimethyloctyl)piperidinium bromide (ISO approved common name: piproctanylium bromide) was the most active of the 7 compounds, its activity was further investigated in the greenhouse on 6 additional species: *Vitis vinifera* L. cv. Riesling × Sylvaner, *Euphorbia pulcherrima* Wild. cv. Paul Mikkelsen, *Pachistachys lutea* Nes. and *Brassica napus* L. cv. Rapol. Heights were recorded 4 weeks after treatment, except with *Euphorbia pulcherrima* where the assessment was carried out 10 weeks after application. The effect on fruit ripening was tested on *Lycopersicon esculentum* cv. Tiny Tim. The plants were sprayed when the first fruits turned red. The number of ripe fruits was counted 3 weeks later. The latex-flow stimulation activity was investigated on *Ficus elastica* Roxb.

The wide range of plant growth regulatory activity of piproctanyliumbromide is demonstrated in Table II. Growth retardant effects could be observed on *Vitis vinifera*, *Euphorbia pulcherrima*, *Pachistachys lutea* and *Brassica napus*. The compound accelerated fruit ripening on *Lycopersicon esculentum* and stimulated latex-flow on *Ficus elastica*. Both these effects were reported as being associated with the plant hormone ethylene by WANG et al.[5].

Of the quaternary ammonium derivatives which have been synthesized and tested in our laboratories, a number of compounds have shown interesting plant growth responses, but so far 1-allyl-1-(3,7-dimethyloctyl)piperidinium bromide is the most promising. Therefore large scale greenhouse trials were initiated in commercial nurseries, especially with pot varieties of *Chrysanthemum morifolium*. The results obtained with a single application of 100–200 ppm compared favourably with those obtained from 2–3 applications of 3400 ppm of daminozide. There fore piproctanylium bromide will shortly be made available, as ALDEN, for this use. Other uses, based on the results presented above, are currently under investigation.

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Adenovirus Type 12 Infection of Defined Mouse-Human Hybrid Cell Clones

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Summary. Human adenovirus type 12 does not multiply in mouse cells; only viral T-antigen is detected. Mouse-human cell hybrid clones containing human chromosomes A3, B5, C7, C11, C12, D14, E17, F19 and F20, support synthesis of adenovirus DNA and capsid antigens.

Recent advances in mammalian somatic cell hybridization have allowed the analysis of the mechanisms of host cell restriction to viral infections. The infection of mouse-human cell hybrids with poliovirus has demonstrated that the permissiveness for virus infection can be associated with the human chromosome F19 coding for a cell surface receptor[1].

Oncogenic adenovirus type 12 multiplies in human cells but not in hamster or mouse cells. In hamster cells, an abortive cycle is induced. The infected cells synthesize T-antigen[2] and adenovirus-specific mRNA is transcribed, but synthesis of viral DNA, late mRNA, or viral capsid proteins cannot be detected[3]. When heterokaryocytes of hamster and human cells were infected with Ad12, the synthesis of Ad12 DNA and late viral capsid proteins was demonstrated in nuclei of hamster origin that had been nonpermissive prior to cell fusion[4]. Mouse cells have been reported to be totally nonpermissive to adenovirus type 12 infection and unable to support synthesis of either T-antigen or viral capsid proteins[5].

We decided to study the infection of specific clones of mouse-human cell hybrids to determine whether the presence of certain human chromosomes in hybrid cells would make them permissive for adenovirus replication. Contrary to previous reports[5] we observed that when mouse cells (3T3 or L) are infected with adenovirus type 12, the adenovirus-specific T-antigen can be demonstrated in the nuclei of infected cells (Figure 1). No virus-specific DNA and late viral capsid proteins were detected, but the infected cells were killed by the infection (Figure 2). The fraction of cells killed was in good correlation with the frequency of T-antigen positive cells. As in the hamster cell system[2], when stationary 3T3 cells were infected, adenovirus type 12 induced a round of cellular DNA synthesis (Table I).

Hybrid cells were made by hybridization of C1-1D mouse cells deficient in thymidine kinase to either KOP-2 human fibroblasts[6] or a line of SV40-transformed human cells derived from patients with the Lesch-Nyhan syndrome[7]. Mass cultures of hybrid cells were cloned on

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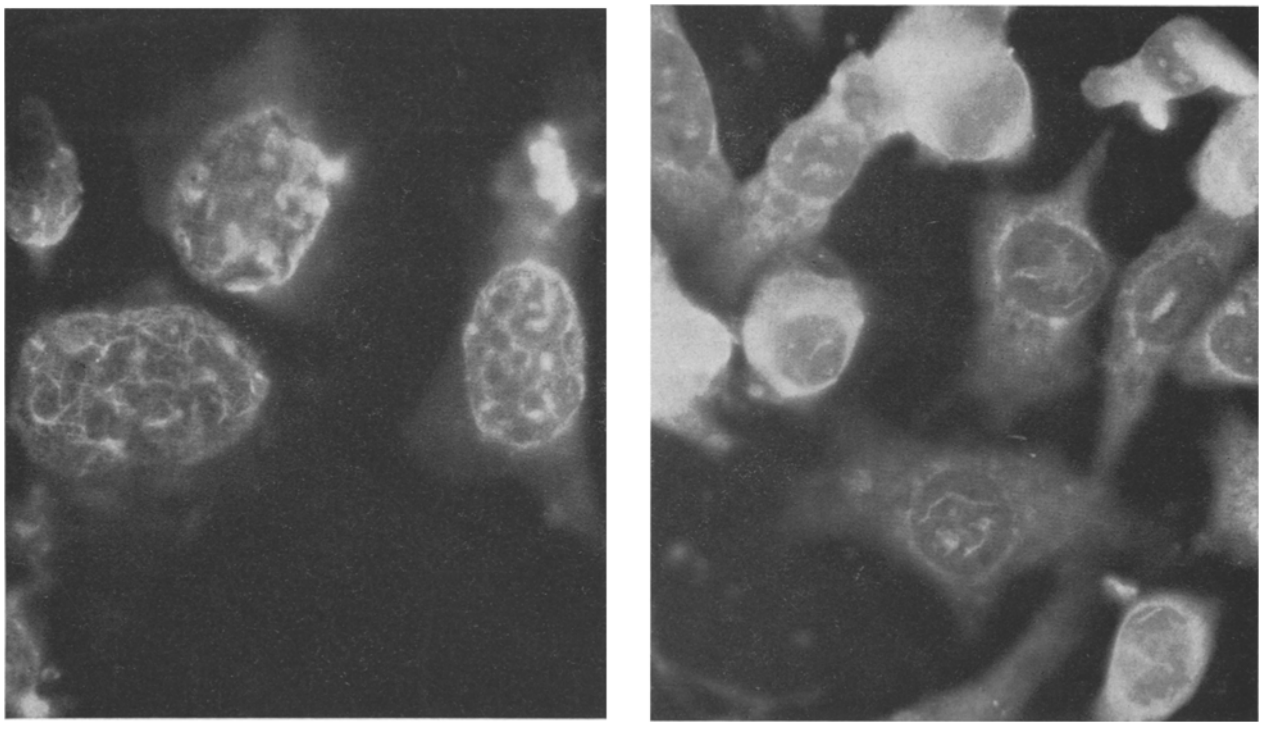

Fig. 1. Ad12 T-antigen in mouse cells. Coverslips were fixed at 32 h after infection with 100 PFU/cell of Ad12 and stained by the indirect immunofluorescence procedure with serum from Ad12 tumorbearing hamsters followed by fluorescein-labeled rabbit anti-hamster globulin[2]. a) Infected 3T3 cells; b) infected L cells.

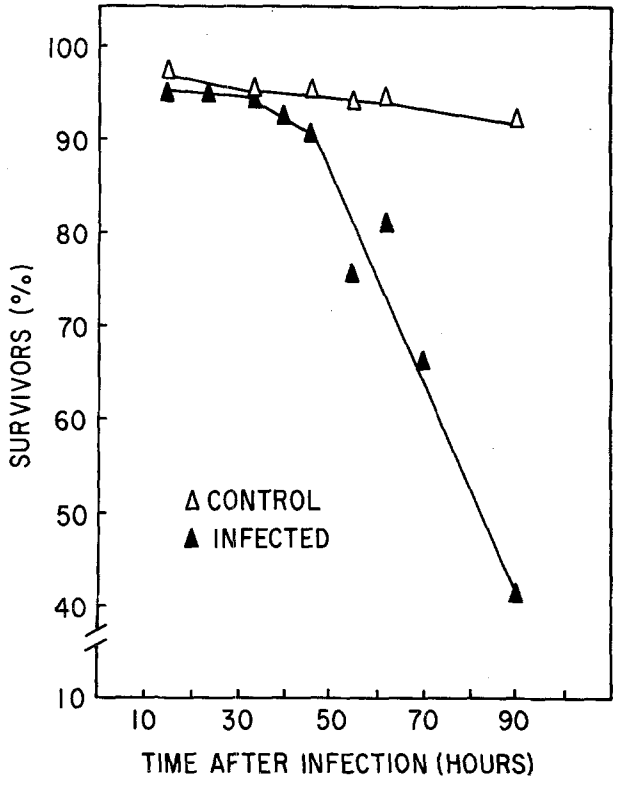

Fig. 2. Killing of mouse cells by Adl2. The cells were infected with 100 PFU/cell of Adl2 and at times indicated the viability of cells was tested by trypan blue exclusion test. T-antigen was induced in 60% of infected cells.

Table I. Induction of DNA synthesis in stationary 3T3 cells by adenovirus type 12 infection[a]

Time (h after infection)	Incorporation of ^{3}H-thymidine/culture (cpm)	
	Controls	Infected[b]
4	406	280
12	310	234
15	342	4,289
18	344	4,760
21	392	3,906
24	468	3,339
27	452	2,321
30	297	1,332
33	283	607

[a] Confluent cultures of 3T3 cells (6×10^5 cells/dish) were switched to medium supplemented with fetal calf serum reduced to 0.5%. 48 h later the cells were infected with Adl2 at input m.o.i. of 100 PFU/cell or mock-infected (controls). At times indicated the cultures were pulse-labeled with ^{3}H-thymidine (1 μCi/ml) for 60 min and then harvested, solubilized with SDS (0.5%), and precipitated with ice-cold trichloroacetic acid. Acid precipitable material was collected on Millipore filters, and radioactivity was determined in liquid scintillation spectrometer. Data represent the averages of 2 cultures.

[b] Hybridization of 120,000 cpm of DNA isolated from cells labeled from 18 to 22 h after infection to filters containing 10 μg of Adl2 DNA failed to detect any radioactivity remaining on filters over that observed with 100,000 cpm of DNA isolated from rapidly growing uninfected cells; the host origin of the DNA is thus apparent.

Table II. Infection of mouse cells and clones of mouse-human cell hybrids with adenovirus type 12[a]

Cell clone	Human chromosomes present	Frequency of T-antigen positive cells[d] (%)	Ad12 DNA synthesis[e]		Frequency of V-antigen positive cells[f] (%)
			Input DNA (cpm)	Fraction in hybrid (%)	
C1-1 D	None	65–82	88,141	< 0.05	< 0.1
Hybrid C1 8[b]	B5, C6, C7, C11, C12, D14, E17, F19, F20	67–80	13,869	< 0.1	< 0.1
Hybrid C1 84[c]	A3, B5, C7, C11, C12, E17, F19, F20	65–80	78,826	< 0.05	< 0.1
Hybrid C1 82	A3, B5, C7, C11, C12, D14, E17, F19, F20, G22	84–92	12,377	9.37	30
Hybrid C1 15 BUDR	A3, B5, C7, C11, C12, D14, F19	70–84	26,829	< 0.1	< 0.1
Hybrid C1 75	A3, B5, C7, C11, C12, D14, D15, E17, F19, F20	61–72	14,280	10.97	15–20
Hybrid C1 21	B5, C7, D14, E17, F19, G21	65–85	10,857	< 0.1	< 0.1
Hybrid C1 16	A3, C7, C11, C12, E17, F19, F20	65–80	23,566	< 0.1	< 0.1
Hybrid C1 91	C7, D15, E17	65–80	15,600	< 0.1	< 0.1

[a] Yield of infectious virus above backrround was not detected in any clone so far tested. [b] Clones C1 8, C1 15, C1 16, C1 21, C1 75, and C1 91 are hybrids of LN-SV human cells with C1-1 D mouse cells. [c] Clones C1 82 and C1 84 are hybrids of KOP-2 normal human fibroblasts with C1-1D mouse cells. [d] The cells were stained for adenovirus type 12 specific T-antigen at 32 h after infection as previously described[2]. The ranges correspond to results obtained in 3 experiments. [e] The cells were labeled from 18 to 22 h after infection with either ^{3}H-thymidine (10 μCi/ml) or ^{32}P-phosphate (500 μCi/ml). The DNA was isolated from infected and control cells, denatured, sonicated, and hybridized to purified Ad12 DNA immobilized on nitrocellulose filters under previously described conditions[10]. Radioactivity bound to filters not containing Ad12 DNA was subtracted as background. Each hybridization was performed with a minimum of 2 filters containing 5 μg of Ad12 DNA. [f] The cells were stained for V antigen at 42 and 48 h after infection by the previously described indirect immunofluorescent method[3]. Results in 2 independent experiments are presented.

hypoxanthine-aminopterin-thymidine (HAT) medium or in Eagle's medium containing bromodeoxyuridine[8]. At least 25 metaphases of each clone population were analyzed as previously described[9]. Only low passage clones were used for experiments.

The mouse cells, the human cells (WI-38), and the mouse-human cell hybrids were infected with adenovirus type 12 at input multiplicity of infection of 100 plaque-forming cells (PFU) per cell. At 32 h after infection, the cells were stained for adenovirus-specific T-antigen that was detected in 60–80% of mouse cells, in 90–95% of human cells, and in 61–92% of different hybrid cells. At 42, 48 and 52 h after infection, the cells were stained for adenovirus capsid proteins. No virus capsid proteins were detected in mouse cells; positive staining was detected in 95% of human cells. The presence of virus-specific capsid proteins was observed in 2 clones of hybrid cells, C1 82 and C1 75, but not in 6 other hybrids tested (Table II).

To determine whether Ad12 DNA was synthesized in infected cells, the mouse and hybrid cells were pulse-labeled with ^{3}H-thymidine or ^{32}P-phosphate from 18 to 22 h p.i., the DNA was isolated, purified, and hybridized to adenovirus type 12 DNA. Newly synthesized virus-specific DNA was detected only in clones C1 82 and C1 75, i.e., those which also contained viral capsid antigens. Both clones contained the human chromosomes A3, B5, C7, C11, C12, D14, E17, F19 and F20, derived from either normal human fibroblasts or SV40-transformed Lesch-Nyhan cells. The remaining hybrids were not able to support replication of viral DNA or synthesis of viral capsid proteins (Table II). No increase of infectious virus above background was detected in any of the hybrids.

The detailed study of molecular events during abortive infection of mouse cells and different mouse-human cell hybrids with Ad12 is in progress[11].

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Effects of Dibutyryl Cyclic AMP on Cultured Brain Cells from Chick Embryos of Different Ages

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Summary. The effect of dibutyryl cyclic AMP on cultivated astroblasts from 8-, 12- and 15-day-old chick embryo brain has been studied. The results show that these cells must have reached some degree of maturation in ovo to become morphologically differentiated by the cyclic nucleotide.

It has been shown that cultures from 17–21-day-old rat embryos and from newborn rats are composed predominantly of undifferentiated glial cells (astroblasts). Several authors have reported that the addition of monobutyryl[3] or dibutyryl cyclic AMP[4,5] induces morphological alterations, converting these cells towards a more differentiated state. Electron microscopic studies of this material have demonstrated the presence of an abundant cytoplasmic fibrillar network which is a characteristic of differentiated astrocytes[6]. To study the influence of