23. L. D. Lytle, presented at the meeting of the Eastern Psychological Association, April 1972; M. J. Zigmond, T. G. Heffner, E. M. Stricker, in preparation.
24. R. Laverty and A. Robertson, *Circ. Res.* **20–21** (Suppl. 3), 127 (1967).
25. Pargyline and 6-HDA treatment produced a 96 ± 2 percent depletion of telencephalic norepinephrine and a 94 ± 2 percent depletion of striatal dopamine. Similar depletions are observed in whole brain. After electrolytic lateral hypothalamic lesion, telencephalic norepinephrine concentration ranged from 52 to 103 percent of control, while striatal dopamine ranged from 29 to 49 percent of control. Brainstem catecholamines were unaffected. The endogenous concentration of norepinephrine in the telencephalon was 0.21 ± 0.01 μg/g; striatal dopamine concentration was 8.68 ± 0.37 μg/g.
26. In addition, Oltmans and Harvey (*9*) have observed aphagia and adipsia in rats following bilateral electrolytic lesions of the ventrolateral hypothalamus that produced a mean depletion of 68 percent of striatal dopamine, although 19 of 24 rats resumed spontaneous feeding and drinking without being given special palatable foods, and none required intragastric feeding for body weight maintenance.
27. D. A. Booth, *Science* **153**, 515 (1967); J. A. Slangen and N. E. Miller, *Physiol. Behav.* **4**, 543 (1969); B. D. Berger, C. D. Wise, L. Stein, *Science* **172**, 281 (1971); K. D. Evetts, J. T. Fitzsimons, P. E. Setler, *J. Physiol. London* **223**, 35 (1972); J. S. Richardson and D. M. Jacobowitz, *Fed. Proc.* **31**, 529 (1972).
28. N. J. Uretsky, M. A. Simmonds, L. L. Iversen, *J. Pharmacol.* **176**, 489 (1971); F. E. Bloom, S. Algeri, A. Groppetti, A. Revuelta, E. Costa, *Science* **166**, 1284 (1969).
29. U. Ungerstedt, *Acta Physiol. Scand. Suppl.* **367**, 69 (1971); N. J. Uretsky and R. I. Schoenfeld, *Nature New Biol.* **234**, 157 (1971); R. I. Schoenfeld and N. J. Uretsky, *Eur. J. Pharmacol.* **19**, 115 (1972).
30. R. Katzman, A. Bjorklund, C. Owman, U. Stenevi, K. A. West, *Brain Res.* **25**, 579 (1971); L.-G. Nygren, L. Olson, A. Seiger, *Histochemie* **28**, 1 (1971).
31. We thank M. Lococo, J. Marincic, D. Rubinstein, C. Stewart, S. Wuerthele, and J. Yen for their assistance. Supported by NIH grant MH-20620 and NSF grant GB-28830.

19 April 1973

Pentobarbital: Selective Depression of Excitatory Postsynaptic Potentials

Abstract. *The effects of pentobarbital (Nembutal) on synaptic transmission and postsynaptic potentials were studied by the use of several invertebrate preparations. Pentobarbital selectively and reversibly depressed both excitatory postsynaptic potentials and sodium-dependent postsynaptic responses to putative excitatory transmitters without affecting either inhibitory postsynaptic potentials or chloride- and potassium-dependent postsynaptic responses to putative transmitters. A selective depression of postsynaptic excitatory events was also observed with other central nervous system depressants (ethanol, chloroform, chloralose, diphenylhydantoin, and urethane). The results suggest that central and peripheral depression observed during general anesthesia is due to a selective depression of excitatory synaptic events.*

Theories of general anesthesia have been divided into those which account for depression of synaptic transmission in terms of a blockade of axonal conduction (*1*) and those which account for the phenomenon in terms of a disturbance in the mechanism of synaptic transmission (*2–10*). Since general anesthetics depress synaptic transmission at concentrations which do not block axonal conduction (*3, 6, 9*), it is more likely that the depression is occurring at the synaptic site. Many investigations have demonstrated that general anesthetics depress excitatory postsynaptic potentials (*3–7, 10*), while others have shown that inhibitory postsynaptic potentials are preserved under the same conditions (*4, 8*). The synaptic mechanisms which have been proposed to account for general anesthesia include (i) a decrease in the presynaptic release of excitatory transmitter (*2, 3*), (ii) an increase in the presynaptic release of inhibitory transmitter (*4*), (iii) a decrease in the postsynaptic chemosensitivity to excitatory transmitter (*6, 10, 11*), and (iv) a stabilization of the postsynaptic membrane to inhibit action potential generation (*7*). In this report we present evidence to support the hypothesis that the action of general anesthetics (and central nervous system depressants) is primarily postsynaptic in nature and involves a *selective* depression of excitatory postsynaptic events without change in inhibitory postsynaptic events.

To test the effects of anesthetics on synaptic transmission and postsynaptic sensitivity we used several molluscan and crustacean preparations with readily obtainable excitatory and inhibitory postsynaptic potentials. The preparations studied included identified nerve cells in *Aplysia californica* (sea hare) and *Otala lactea* (a land snail) and walking leg neuromuscular junctions in crayfish (*Orconectes virulis*) and lobster (*Homarus americanus*). They were placed in appropriate chambers and perfused with artificial salines (*12*), and one or two KCl-filled glass micropipettes were inserted into the neurons or muscle fibers for the purpose of recording membrane potentials and passing current across the membrane. Potentials were measured with conventional techniques and were displayed on an oscilloscope and recorded on a pen-recorder. Drugs were dissolved in the salines just prior to perfusion (*13*).

Pentobarbital, in concentrations present under conditions of surgical anesthesia ($2 \times 10^{-4}M$), reversibly antagonized the excitatory postsynaptic potentials in lobster muscle fibers, but did not affect the inhibitory postsynaptic potentials (Fig. 1A). Under control conditions the excitatory postsynaptic potentials were approximately 5 to 7.5 mv, while in the presence of $2 \times 10^{-4}M$ pentobarbital these had decreased to 2

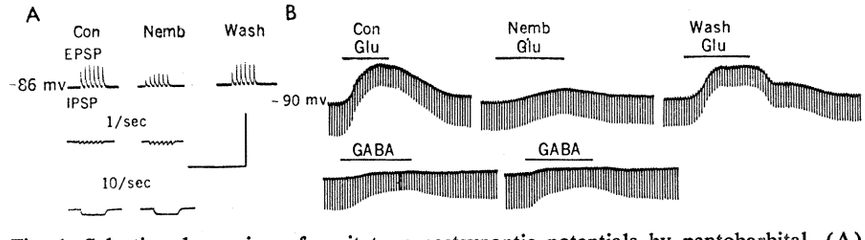

Fig. 1. Selective depression of excitatory postsynaptic potentials by pentobarbital. (A) Membrane potential traces showing excitatory and inhibitory postsynaptic potentials (*EPSP* and *IPSP*) recorded in a lobster muscle. The IPSP's have been evoked at frequencies of one and ten stimuli per second. Pentobarbital (*Nemb*) ($2 \times 10^{-4}M$) reduces the amplitude of the EPSP's without altering the size of the IPSP's. Washing (*Wash*) with drug-free saline restores the EPSP's to their original size. (B) Membrane responses of lobster muscle [from a different preparation than that used in (A)] to the perfusion of putative transmitters glutamate (*Glu*) ($10^{-4}M$) and GABA ($10^{-5}M$) (during the period indicated by the horizontal bars). The downward deflections in the records are voltage responses to constant current pulses. The excitatory transmitter glutamate causes a depolarization and decrease in membrane resistance. The glutamate response is depressed by $10^{-4}M$ pentobarbital. The inhibitory transmitter GABA also produces a depolarization in these experiments, since the muscle fibers were hyperpolarized in K^+-free salines [see (*12*)], and hence the equilibrium potential of the IPSP (or E_{Cl}) was shifted in a depolarizing direction relative to the resting potential. The depolarization and decrease in membrane resistance in response to GABA remain unchanged. Washing with drug-free saline restores the glutamate depolarization. Calibration: 15 mv, 12 seconds in (A), 2 minutes in (B). *Con*, control.

to 3.5 mv, without an alteration in the inhibitory postsynaptic potentials. Washing for 10 minutes restored the excitatory postsynaptic potentials to their original amplitude. In complementary fashion pentobarbital ($10^{-4}M$) selectively and reversibly antagonized the sodium-dependent depolarization of lobster muscle fibers by the putative transmitter, glutamate (*14*), but did not affect the chloride-dependent action of the putative transmitter, γ-aminobutyric acid (GABA) (*15*) (Fig. 1B). The depression of the glutamate response by pentobarbital was apparent over the entire range of glutamate doses tested (10^{-5} to $6 \times 10^{-4}M$). These results were observed in five different lobster neuromuscular junction preparations. Similar results were obtained in experiments on the crayfish neuromuscular juncture.

Pentobarbital ($2 \times 10^{-4}M$) also reversibly depressed excitatory postsynaptic potentials recorded in cells R_2 and R_{15} of the marine mollusk *Aplysia*. The selective nature of the pentobarbital depression was investigated in this and another molluscan preparation by studying the effects of pentobarbital on a biphasic postsynaptic potential (elicited by stimulation of either the left parietal nerve in *Otala* (cell 11, Fig. 2A) or the branchial nerve in *Aplysia* (R_{15}, Fig. 2B). The biphasic postsynaptic potential in these cells is thought to be mediated by dopamine with the depolarizing phase primarily dependent on sodium and the hyperpolarizing phase primarily dependent on potassium (*16, 17*). Pentobarbital reversibly depressed the depolarizing (excitatory) component of the biphasic postsynaptic potential while leaving the hyperpolarizing (inhibitory) component relatively unchanged (*18*). This effect was observed in five different preparations. Thus, the action of pentobarbital appeared to be directed primarily to the synaptically induced *depolarizing* event.

We then examined the effects of pentobarbital on pharmacologically induced conductance and potential changes in neurons by studying the drug's interaction with putative transmitters on the postsynaptic membrane of cell 11 in *Otala*. Pentobarbital reversibly antagonized sodium-dependent, cholinergic depolarizations (Fig. 2C) over a tenfold range of acetylcholine concentration (Fig. 2D) in a dose-dependent manner. Neither the potassium-dependent hyperpolarization of the membrane potential by dopamine (Fig. 2C) nor the potassium- and chloride-dependent hyperpolarization (of the same cell) by glutamate (not illustrated) was affected by pentobarbital. These results were obtained in over 30 different preparations. Similar, *selective* depressions of acetylcholine depolarizations were obtained with chloralose, chloroform, diphenylhydantoin, ethanol, and urethane. The relative potency of these depressants was diphenylhydantoin ≅ pentobarbital > chloralose > chloroform > urethane > ethanol.

The above results indicate that pentobarbital can selectively depress excitatory postsynaptic events without altering inhibitory postsynaptic events in a variety of crustacean and molluscan preparations. Although we cannot exclude the additional involvement of a presynaptic mechanism (*19*), the parallelism between the selective depression of excitatory postsynaptic potentials and the selective depression of pharmacologically induced postsynaptic depolarizations strongly suggests that the depression of these potentials is, at least in part, mediated through a postsynaptic mechanism. The fact that we have observed such selective effects with four different transmitters (acetylcholine, glutamate, GABA, and dopamine) coupled to three different conductances (sodium, potassium, and chloride) indicates that the phenomenon may be generalizable

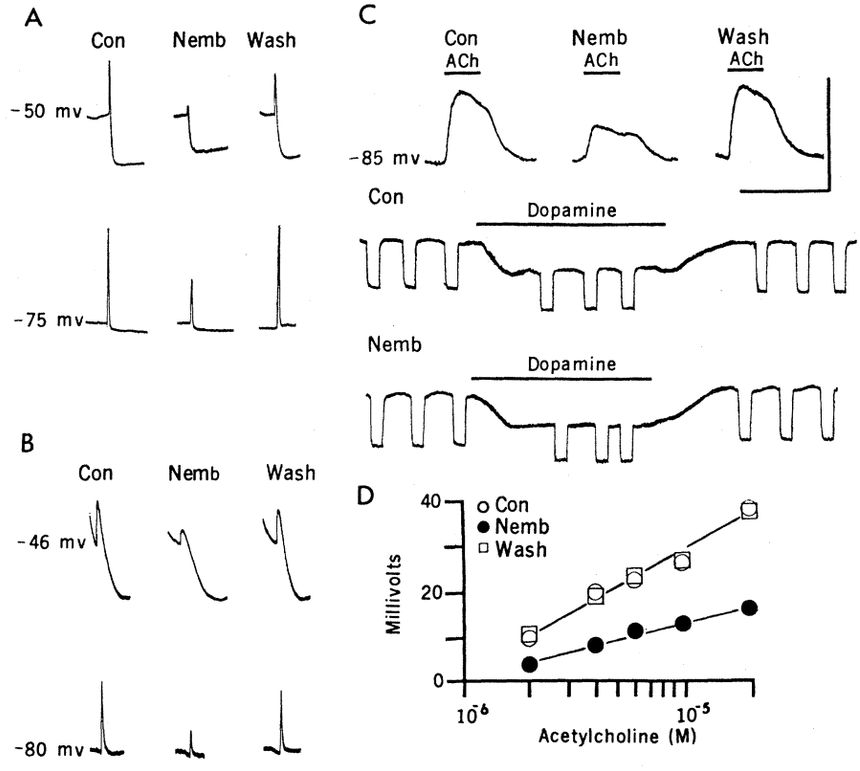

Fig. 2. Pentobarbital selectively depresses postsynaptic excitatory events. (A) Membrane potential traces from neurosecretory cell (No. 11) in the land snail *Otala*. Postsynaptic responses to stimulation of the left parietal nerve were recorded at −50 mv to enhance the inhibitory component and at −75 mv to enhance the excitatory component of the biphasic response. The membrane potential was controlled by passing current through a second intracellular electrode. Bathing for 5 minutes in a saline containing $2 \times 10^{-4}M$ pentobarbital (*Nemb*) greatly reduces the excitatory component with little effect on the inhibitory phase. Washing (*Wash*) with drug-free saline restores the excitatory phase. (B) Membrane potential traces from a bursting pacemaker neuron (R_{15}) in the sea hare *Aplysia*. Postsynaptic potentials were recorded at −46 and −80 mv in response to stimulation of the branchial nerve. The excitatory phase of the biphasic response is selectively reduced by bathing with $2 \times 10^{-4}M$ pentobarbital. (C) Membrane potential traces from the neurosecretory cell (No. 11) in *Otala*. Bath application of acetylcholine (*ACh*) (during the time indicated by horizontal bar above trace) causes a depolarization of the membrane potential, while dopamine ($10^{-5}M$) increases the membrane potential and conductance. Pentobarbital ($10^{-4}M$) depresses the ACh depolarization without altering the dopamine hyperpolarization. Washing with drug-free saline restores the ACh response. (D) Dose-response curves to bath application of ACh in the same cell as illustrated in (C). Pentobarbital ($10^{-4}M$) reversibly depresses the ACh dose-response curve over the entire range of ACh concentrations tested. Calibration: 40 mv in (A), (B), and (C) (ACh), 20 mv in (C) (dopamine); 12 seconds in (A) and (B), 2 minutes in (C). *Con*, control.

to include selective depression of all transmitter-coupled sodium conductances with preservation of all transmitter-coupled chloride and potassium conductances. Furthermore, the close correspondence between the concentration of depressant producing a half-maximal depression of the acetylcholine depolarization and the plasma concentration present under conditions of general anesthesia or anticonvulsive therapy (*9, 20*) suggests that a similar, selective postsynaptic depression of excitatory postsynaptic potentials may be involved in these forms of central nervous system depression. The mechanism underlying this selective depression of the transmitter-coupled sodium ionophore requires investigation.

JEFFERY L. BARKER
HAROLD GAINER
National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20014

References and Notes

1. S. Thesleff, *Acta Physiol. Scand.* **37**, 335 (1956); M. P. Blaustein and D. E. Goldman, *Science* **153**, 429 (1966); M. P. Blaustein, *J. Gen. Physiol.* **51**, 293 (1968); P. Seeman, *Pharmacol. Rev.* **24**, 583 (1972).
2. Y. Løyning, T. Oshima, J. Yokota, *J. Neurophysiol.* **27**, 408 (1964); E. K. Mathews and J. P. Quilliam, *Brit. J. Pharmacol.* **22**, 415 (1966); W. D. M. Paton and R. N. Speden, *Brit. Med. Bull.* **21**, 44 (1965); J. N. Weakly, *J. Physiol. London* **204**, 63 (1969); P. Streit, K. Akert, C. Sandri, R. B. Livingston, H. Moor, *Brain Res.* **48**, 11 (1972).
3. C. D. Richards, *J. Physiol. London* **227**, 749 (1972).
4. R. A. Nicoll, *ibid.* **223**, 803 (1972).
5. J. M. Crawford and D. R. Curtis, *ibid.* **186**, 121 (1966).
6. G. G. Somjen, *Anesthesiology* **28**, 135 (1967).
7. C. M. Brooks and J. C. Eccles, *J. Neurophysiol.* **10**, 349 (1947).
8. J. N. Weakly, D. W. Esplin, B. Zablocka, *Arch. Int. Pharmacodyn. Ther.* **171**, 385 (1968); M. D. Larson and M. A. Major, *Brain Res.* **21**, 309 (1970); J. C. Eccles, D. S. Faber, H. Taborikova, *ibid.* **25**, 335 (1971).
9. M. G. Larrabee and J. M. Posternak, *J. Neurophysiol.* **15**, 91 (1952).
10. A. I. Shapovalov, *Farmakol. Toksikol.* **26**, 150 (1963).
11. J. H. Karis, A. J. Gissen, W. L. Nastuk, *Anesthesiology* **27**, 42 (1967).
12. *Aplysia* saline consisted (in millimoles) of 500 NaCl, 10 KCl, 50 $MgCl_2$, 10 $CaCl_2$, and 10 tris-Cl buffered to *p*H 7.8. Snail saline (in millimoles) was 100 NaCl, 10 $CaCl_2$, 10 tris-Cl buffered to *p*H 7.8, 4 KCl, and 5 $MgCl_2$. Crayfish saline (in millimoles) was 160 NaCl, 15 $CaCl_2$, 10 $MgCl_2$, 5 KCl, and 5 tris-Cl buffered to *p*H 7.8. Lobster saline (in millimoles) was 450 NaCl, 20 $CaCl_2$, 10 KCl, and 10 tris-Cl buffered to *p*H 7.8. Potassium was omitted from the lobster saline in the experiments where bath perfusion of putative transmitters was done in order to increase the size of the depolarization. Potassium was also omitted from the snail saline to enlarge both depolarizing and hyperpolarizing drug responses.
13. The following drugs were obtained from the following laboratories: acetylcholine and γ-aminobutyric acid (both from Sigma, St. Louis), L-glutamic acid (Calbiochem, Los Angeles), dopamine (Nutritional Biochemicals, Cleveland), sodium pentobarbital (Abbott Laboratories, North Chicago), sodium 5,5-diphenylhydantoin (K & K Laboratories, Plainview, New York), α-chloralose, chloroform, ethanol, and urethane (all from Fisher Scientific, Fair Lawn, New Jersey).
14. A. Takeuchi and N. Takeuchi, *J. Physiol. London* **170**, 296 (1964); M. Ozeki and H. Grundfest, *Science* **155**, 478 (1967).
15. J. Boistel and P. Fatt, *J. Physiol. London* **144**, 176 (1958); H. Grundfest, J. P. Reuben, W. H. Rickles, *J. Gen. Physiol.* **42**, 1301 (1959); A. Takeuchi and N. Takeuchi, *J. Physiol. London* **177**, 225 (1965). Observations on the interaction between pentobarbital and the GABA response have been reported by J. Iravani [*Arch. Exp. Pathol. Pharmakol.* **251**, 265 (1965)]. At concentrations of pentobarbital similar to those used in the present study Iravani reported a 10 percent decrease in the conductance change elicited by GABA in the presence of pentobarbital. However, it is unclear whether the effect of the drug on the GABA response has been corrected for its effects on resting membrane properties, which may account for the slight antagonism observed. We did not see either the change in membrane conductance or the minor decrease in the GABA response.
16. G. A. Kerkut, N. Horn, R. J. Walker, *Comp. Biochem. Physiol.* **30**, 1061 (1969); R. J. Walker, K. L. Ralph, G. N. Woodruff, G. A. Kerkut, *Comp. Gen. Physiol.* **2**, 15 (1971); P. Ascher, *J. Physiol. London* **225**, 173 (1972).
17. H. M. Gerschenfeld, *Physiol. Rev.* **53**, 1 (1973).
18. The low concentrations of pentobarbital used in the present study did not alter the current-voltage relations of the cell over the range of membrane potentials used to observe postsynaptic potential behavior. Previously, Chalazonitis has reported that volatile anesthetics markedly increase membrane potential and conductance, abolishing both excitatory and inhibitory postsynaptic potentials at anesthetic vapor pressures twice those necessary to induce surgical anesthesia in man [N. Chalazonitis, *Anesthesiology* **28**, 111 (1967)]. He concluded that these agents were acting primarily postsynaptically to depress membrane excitability, but a systematic investigation of synaptic transmission at lower vapor pressures was not performed.
19. Recently, biochemical evidence has been presented using a neuromuscular junction preparation to demonstrate that, although three different general anesthetics depress the contractile response to nerve stimulation, they do not alter the release of the transmitter acetylcholine [S. O. Gergis, K. L. Dretchen, M. D. Sokoll, J. P. Long, *Proc. Soc. Exp. Biol. Med.* **141**, 629 (1972)]. These authors conclude from these results that general anesthetics are acting postsynaptically.
20. E. S. Vesell and G. T. Passananti, *Clin. Chem.* **17**, 851 (1971).
21. J.L.B. was supported by NINDS special fellowship No. 5 F11 NS02609-01.

27 June 1973; revised 3 August 1973

Retinyl Acetate: Effect on Cellular Content of RNA in Epidermis in Cell Culture in Chemically Defined Medium

Abstract. *Cell cultures of epidermis from newborn mice were established in chemically defined medium. Additions of retinyl acetate to these cultures caused a significant increase in cellular RNA content. Addition of insulin and hydrocortisone to the cultures potentiated the effect of retinyl acetate on cellular RNA content.*

Although it was established over 50 years ago that vitamin A is required for normal differentiation of epithelial cells in many organs (*1*), the molecular mechanisms whereby vitamin A controls this process are still unknown. The structures of the metabolite or metabolites of vitamin A which control cell differentiation are also unknown; there is a great deal of evidence which suggests that structures other than vitamin A alcohol (retinol) may be involved in this process (*2*). Although there have been numerous reports of the ability of vitamin A to control epithelial cell differentiation in organ culture, particularly of its ability to suppress keratinization and enhance secretory activity (*3*), the molecular mechanisms involved have not been elucidated by organ culture studies. The use of chemically defined media for epithelial cell cultures

Table 1. Changes in levels of RNA, DNA, and protein in mouse epidermal cell cultures 3 days after culture in chemically defined medium supplemented with hormones or retinyl acetate or both. Hormone treatment was crystalline bovine insulin, 5 μg/ml, and hydrocortisone hemisuccinate, 5 μg/ml. Retinyl acetate was the *all-trans* isomer, at either 1.56 or 3.12 μg/ml, dissolved in dimethyl sulfoxide to give a final dimethyl sulfoxide concentration of 1.25 percent. Dimethyl sulfoxide was included in all cultures (final concentration, 1.25 percent). Values for total RNA, DNA, and protein have been normalized with respect to the cultures which received no treatment. Standard deviations from the mean are given for all values.

Supplements to medium	Total RNA per dish	Total DNA per dish	Total protein per dish	RNA/DNA	Protein/DNA
None	1.00*	1.00*	1.00*	0.61 ± .06	17.3 ± 1.9
Hormone	0.97 ± .08	1.00 ± .15	1.10 ± .19	.59 ± .07	19.1 ± 2.1
Retinyl acetate	1.40 ± .19†	1.18 ± .18	1.13 ± .14	.73 ± .03†	16.9 ± 2.1
Hormone and retinyl acetate	2.08 ± .23†§	1.40 ± .21†	1.54 ± .18†§	.92 ± .12†§	19.4 ± 3.2

* The mean values for this treatment were derived from five separate sets of experiments and were as follows: total RNA per dish, 10.1 μg; total DNA per dish, 16.6 μg; and total protein per dish, 291 μg. At the start of the experiment ("zero-time" controls), these mean values were: RNA, 7.3 μg per dish; DNA, 11.2 μg per dish; and protein, 149 μg per dish. † These values differ significantly from the corresponding values from untreated cultures ($P < .001$ by *t*-test) or from cultures treated with hormone alone ($P < .01$ for values for total DNA). § These values differ significantly ($P < .001$) from the corresponding values from cultures treated with retinyl acetate alone.