1 that there are two populations of MEPP's—slow small and fast large. The large, rapidly rising potentials are probably generated near the recording site, and the smaller, slowly rising ones probably originate at some distance from it. This interpretation implies that if the electrode could be focally located with respect to each of the junctional sites, all MEPP amplitudes should be distributed about the 1.31-mv mean.

Because many parameters of MEPP's in vivo are strikingly different from those in vitro, we determined whether the anesthetic, α-chloralose, caused the differences. Data for animals anesthetized by cutting the spinal cord are given in Table 1, which also gives measurements obtained before and after the sciatic nerve was cut in cats with spinal section. No significant differences in resting membrane potential or MEPP frequency amplitude, duration, or rate of rise could be discerned (Duncan's new multiple range test). Thus, neither α-chloralose nor section of the sciatic nerve affects MEPP's recorded by our method. Thus, the activity we observed is probably an accurate representation of the physiological response.

Our results show that spontaneous MEPP's recorded in vivo are similar in frequency and randomness of occurrence to those observed in vitro. On the other hand, they have substantially larger amplitudes, higher rates of rise, and shorter durations, and the in vivo resting membrane potential is larger and more stable than that customarily observed in vitro. These differences are probably related to the intact circulation, which assures better oxygenation and osmotic milieu than those in vitro, but the magnitude of these differences is striking. Furthermore, since the resting membrane potential is 10 to 15 mv larger than that in vitro, the profile of the muscle action potential as well as that of the end plate potential may differ substantially from that observed in excised tissue. It may be necessary to reevaluate some of the conclusions related to transmitter release and quantal composition of the end plate potential which are based on data from in vitro records.

Our study demonstrated that the in vivo neuromuscular preparation offers a stable, highly sensitive system for examining the physiological events at the neuromuscular junction. The results raise some fundamental questions regarding the quantitative analysis of synaptic processes. Further use of in vivo recording should provide new insights into the nature of neuromuscular transmission. In addition, this preparation offers a unique opportunity for examining the pharmacological properties of the neuromuscular junction by administering drugs by routes identical to those used clinically.

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Carcinogenicity of Methylchrysenes

Abstract. *Methylchrysenes are present in tobacco smoke and are suspected to contribute to the tumorigenicity of this inhalant. Chrysene and the six isomeric methylchrysenes were obtained in high purity (>99.9 percent); they were tested on mouse skin for tumor initiating activity and carcinogenicity. The 3- and 6-methylchrysenes are strong tumor initiators, whereas the other five chrysenes have moderate initiating activity. 5-Methylchrysene is a strong carcinogen; the other chrysenes are inactive or weak carcinogens.*

Tobacco smoke contains relatively high concentrations of alkylchrysenes in comparison to gasoline engine exhaust and other urban pollutants (*1*). Unsubstituted and alkylated chrysenes, benz[*a*]anthracenes, and benzo[*a*]pyrenes have been identified in neutral subfractions that amount to about 0.05

Table 1. Female, Swiss albino mice (Ha/ICR/Mil) were used. The tumor initiator dose for chrysene and the methylchrysenes was ten applications of 0.1 mg in 1 ml of acetone, and that for benzo[*a*]pyrene was ten applications of 0.0005 mg in 1 ml of acetone. The tumor promotor dose was 2.5 μg of TPA, given three times weekly. The acetone controls were negative.

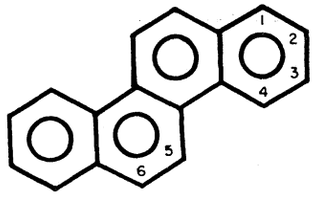
Promotor application (weeks)	No. mice with tumors	Total tumors	Survivors	Promotor application (weeks)	No. mice with tumors	Total tumors	Survivors
	Chrysene				*4-Methylchrysene*		
1	0	0	20	1	0	0	20
10	0	0	19	10	0	0	20
12	1	1	18	12	2	2	20
14	3	6	18	14	2	2	20
16	6	9	18	16	3	3	20
18	8	15	18	18	7	9	20
20	11	19	18	20	7	9	20
	1-Methylchrysene				*5-Methylchrysene*		
1	0	0	20	1	0	0	20
10	0	0	20	10	3	3	19
12	0	0	20	12	6	9	19
14	2	2	19	14	12	31	19
16	2	2	19	16	13	49	19
18	5	5	19	18	17	92	18
20	6	6	19	20	17	96	18
	2-Methylchrysene				*6-Methylchrysene*		
1	0	0	20	1	0	0	20
10	0	0	19	10	0	0	19
12	0	0	19	12	1	1	19
14	2	2	19	14	1	1	19
16	6	9	19	16	5	8	19
18	8	13	19	18	7	11	19
20	8	13	19	20	7	11	19
	3-Methylchrysene				*Benzo*[a]*pyrene*		
1	0	0	20	1	0	0	20
10	0	0	20	10	0	0	20
12	1	1	20	12	1	1	20
14	3	5	20	14	4	5	20
16	6	11	20	16	6	9	20
18	11	21	20	18	6	10	20
20	14	26	20	20	6	10	20

Table 2. Carcinogenic activity of the 3-, 5-, and 6-methylchrysenes. The methylchrysenes were given thrice weekly in doses of 0.1 mg in 0.1 ml of acetone; benzo[*a*]pyrene (BaP) was given in a dose of 0.005 mg in 0.2 ml of acetone. Under the conditions described, chrysene, 1-methylchrysene, 2-methylchrysene, and 4-methylchrysene showed no activity.

Application (weeks)	3-Methylchrysene			5-Methylchrysene					6-Methylchrysene			BaP		
	Total mice	Tumors		Total mice	All tumors		Carcinoma		Total mice	All tumors		Total mice	Tumors	
		No. mice	Total No.		No. mice	Total No.	No. mice	Total No.		No. mice	Total No.		No. mice	Total No.
1	20	1	1	20					20			20		
11	20	1	1	20	3	3			20			20		
15	20	1	1	20	7	13			19			20		
20	20	1	1	19	18	64	5	5	19	2	2	20	1	1
25	19	1	1	16	20	85	9	11	19	2	2	20	1	1
30	18	1	1	12	20	99	12*	37	19	2	2	20	3	4

* The eight mice that were killed had carcinomas which were histologically confirmed; two mice had multiple metastases in the lung, one mouse had metastases in the lung and spleen. In the eight mice examined histologically (20 to 30 weeks of treatment), we did not find any lung adenomas or any primary tumors other than skin papillomas and carcinomas.

percent of the total particulate matter and that show high tumor initiating activities (*2*). However, the biological activity of the fractions could not be accounted for by the unsubstituted polynuclear aromatic hydrocarbons alone. This observation and the fact that chrysenes are selectively produced by the pyrolysis of phytosterols (*3, 4*) motivated us to synthesize chrysene and the six isomeric methylchrysenes and to bioassay them for tumor initiating activity and carcinogenic activity.



Chrysene

In previous studies, chrysene and 1-, 4-, 5-, and 6-methylchrysene have been tested for tumorigenicity. Chrysene itself appeared to have marginal carcinogenic activity; 1-, 4-, and 6-methylchrysene were inactive, but 5-methylchrysene showed high sarcomagenic activity in one mouse strain (*5*).

To ensure absolute chemical and isomeric purity, chrysene and the six methylchrysenes were synthesized individually by unambiguous routes (*4, 6*). The purity of the hydrocarbons was ascertained by melting point, elemental analysis, ultraviolet and infrared absorption spectra, mass spectra, nuclear magnetic resonance spectra, and paper and gas chromatography. Final purity was greater than 99.9 percent.

Twenty Swiss albino, female mice (Ha/ICR/Mil) were used to bioassay the tumor initiating activity of each hydrocarbon. Ten doses of 0.1 mg of the test chrysene in 0.1 ml of acetone (total dose, 1.0 mg) were applied on alternate days to the shaved backs of each mouse after the animal had entered the second telogen period of the hair phase. Ten days after the last application of initiator, promotion was begun by applying 2.5 μg of tetradecanoyl phorbol acetate (TPA) (Schuchardt, Munich, Germany) in 0.1 ml of acetone three times a week for 20 weeks (the total TPA dose was 0.15 mg). Benzo[*a*]pyrene (10 doses of 0.005 mg in 0.1 ml of acetone) was used as a positive control and acetone alone (10 doses of 0.1 ml) was used as a negative control.

Each compound was concurrently bioassayed for complete carcinogenicity with 20 mice of the same strain and sex. A dose containing 0.1 mg of each chrysene in 0.1 ml of acetone was applied to the shaved backs of each animal, under the same conditions described above for the initiator test, three times weekly for the duration of the test. Acetone alone and benzo[*a*]pyrene in acetone served as positive and negative controls.

Table 1 summarizes the tumor initiating activity of chrysene and methylchrysenes. Chrysene and the 1-, 2-, 4-, and 6-methylchrysenes have moderate tumor initiating activities, whereas 3- and, especially, 5-methylchrysenes are strong tumor initiators. Since cigarette smoke contains about four to six times more chrysene and methylchrysene than benzo[*a*]pyrenes (*4*), this finding supports the concept that the tumor initiating activity of chrysenes contributes significantly, at least in the mouse skin bioassay, to the overall carcinogenicity of tobacco smoke.

The first 30 weeks of the bioassay for complete carcinogenicity of chrysenes (Table 2) have shown a high carcinogenicity for 5-methylchrysene and no significant activity as yet for chrysene or the other five methylchrysenes. This finding is of great theoretical interest in that it suggests that methylation in the 5-position changes the electron distribution or conformation (or both) of the chrysene ring system to that of a strong carcinogen or procarcinogen.

At present, we are determining the molecular structures of chrysene and 5-methylchrysene by x-ray crystallography. We are also studying the metabolic rates and metabolites of these two hydrocarbons, and synthesizing derivatives of 5-methylchrysene for carcinogenicity tests.

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6. The five chrysene samples purchased from domestic and foreign suppliers contain impurities >1.0 percent; these are mainly methylchrysenes and 5*H*-benzo[*b*]carbazole.
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