inhibitor is independent of the substrate[11,12], a similar difference should be mantained with NA.
In a previous paper, we reported that the 2 forms of MAO, type A and type B coexist in the cat superior cervical ganglion and nictitating membrane and that clorgyline has a greater potency for inhibiting type A in the nerve terminals than in the cell body[13]. Since TM is a substrate not allowing separate assay of the activity of both types of MAO, we also used NA. Under these conditions, d-amphetamine also showed a greater potency for inhibiting deamination in the superior cervical ganglion.
Although the present results do not explain the differences in the metabolism of the transmitter released by d-amphetamine from the superior cervical ganglion and from the nictitating membrane, they point to further differences between the MAO of the cell body and the nerve endings.

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Effects of polycyclic hydrocarbons on the induction of chromosomal aberrations in absence of an exogenous metabolic activation system in cultured hamster cells[1]

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Summary. The effect of carcinogenic polycyclic hydrocarbons on the chromosomes of cultured Chinese hamster cells was investigated. Contrary to earlier reports it was observed that benz(a)anthracene, benzo(a)pyrene, 7,12-dimethylbenz-(a)anthracene and 3-methylcholanthrene were effective in causing chromosomal aberrations without any exogenous metabolic activation. Duration of incubation with these agents may be the cause of difference in results. Importance of prolonged treatment period is discussed.

Investigating the effect of chemicals in causing chromosomal aberrations is a useful tool for rapid screening of mutagens and carcinogens in the environment. Recent studies on cultured mammalian cells indicate that certain carcinogenic polycyclic hydrocarbons (CPH) require metabolic activation to produce a clastogenic effect[2-4]. The activation system utilized in these studies consisted of rat liver microsomal fraction (S_9 or S_{15}) or irradiated feeder layer of Syrian hamster cells. The present investigation describes the production of chromosomal abnormalities by treatment with polycyclic hydrocarbons without any exogenous activation in cultured Chinese hamster cells.
V_{79} Chinese hamster cells used in this study were routinely cultured in 75 cm^2 Corning plastic tissue culture flasks in 10 ml of Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum and antibiotics (penicillin-G, 100 units/ml and streptomycin 100 µg/ml). The cells were grown at 37 °C in an atmosphere of 95% air and 5% CO_2. To test the effects of CPH about 2×10^5 cells were plated in 25 cm^2 plastic flasks in 5 ml of growth medium. After cell attachment the cultures were exposed to test compound dissolved in acetone and suspended in culture medium for 24 h. The CPH namely benz(a)anthracene (BaA), benzo(a)pyrene (BaP), 7,12-dimethylbenz-(a)anthracene (DMBA), and 3-methylcholanthrene (MC) were obtained from Eastman Kodak Co., Rochester, N.Y. The concentrations of the compounds used were 0.6, 1.25, 2.5 and 5.0 µg/ml. The control cultures contained 0.1% acetone and were incubated under identical conditions.
For chromosome analysis, the cells were harvested immediately after the end of treatment period. 4 h prior to harvest, colcemid (0.1 µg/ml) was added to each flask. The growth medium was then discarded, the cultures washed, trypsinized, centrifuged (800–1000 rpm, 10 min) and the supernatant aspirated. The pellet was suspended in 1 ml of 0.075 M KCl (37 °C) for 10 min; an equal amount of fixative (1 part glacial acetic acid : 3 parts methanol) added to it and centrifuged (800–1000 rpm, 10 min). The cells were then washed in fixative; centrifuged and resuspended in 1 ml of fresh fixative for 10 min. The concentrated cell suspension was dropped on chilled wet slides and allowed to dry overnight. The slides were stained with 2% Giemsa solution. The type and number of chromosomal abnormalities were assessed by examining 100 well spread metaphase plates from each treatment. The experiments were repeated twice.
The results of clastogenic effects induced by polycyclic hydrocarbons on V_{79} Chinese hamster cells are summarized in the table. It was noticed that chromosomal gaps were the most common abnormality 24 h after treatment with these compounds, accounting for approximately 25–50% of the induced aberrations. The interpretation of gaps has been a matter of controversy. In present study the gaps were considered to be small, discrete, achromatic bands on one or both chromatids. Most of the gaps were confined to one of the sister chromatids that make up the chromosome arm but occasionally they were found on both chromatids at the same location on the arm. Chromosomal gaps were observed in each concentration of hydrocarbons used as well as the controls, however, the frequency was much more in the former. For instance, only 3% of the control cells showed such gaps compared to 27% of the cells treated with 5 µg/ml of BaP. Chromosome rings, while not that common were seen in metaphase which also showed small, diffuse, double chromatin elements interpreted as acentric fragments. These acentric fragments are considered to be

Frequencies of chromosomal aberrations at metaphase produced by treatment with various carcinogenic polycyclic hydrocarbons in V_{79} Chinese hamster cells

Treatment and concentration (μg/ml)	Cells with aberrations (%)	Aberrations/100 cells Gaps	Rings	Chromatid breaks	Chromatid exchanges	Fragmentation	Others
Control	6	3	0	2	1	0	0
Benz(a)anthracene							
0.6	17	8	0	5	4	0	1
1.25	23	7	3	5	6	0	2
2.5	30	12	6	7	9	0	1
5.0	38	13	5	14	12	4	3
7,12-Dimethylbenz(a)anthracene							
0.6	24	12	0	7	7	0	3
1.25	28	10	0	9	6	0	3
2.5	34	14	3	12	8	3	2
5.0	47	20	5	11	9	6	4
Benzo(a)pyrene							
0.6	23	13	1	5	7	0	3
1.25	32	16	4	9	9	2	2
2.5	45	23	5	15	6	7	2
5.0	56	27	7	12	11	8	3
3-Methylcholanthrene							
0.6	21	7	0	9	2	0	3
1.25	22	8	0	10	3	0	1
2.5	28	10	3	12	5	2	2
5.0	37	13	4	11	6	6	2

formed from 2 terminal regions of chromosome beyond the point of exchange.

The aberrations resulting from events taking place following the chromosome replication and scored as chromatid breaks and exchanges were also recovered at each level of CPH used (table). Some metaphases showed incidence of complex exchange structures involving 3 or more chromatids. In extreme cases a large fraction of chromosomal material may be involved in a complex exchange structure. Treatment with higher concentration of hydrocarbons resulted in some metaphases where the chromatin was in punctate form, or otherwise so severely fragmented that chromosome structure was seldom recognized. Such figures were scored as complete fragmentation of chromosomes. In most severely affected cultures, fragmentation was the predominant type of aberration and few normal metaphase plates were found.

Beside the major chromosomal aberrations mentioned above, other anomalies found during the course of investigation were as follows: 1. metaphase in which double or other multiple of the modal chromosome number was present, were referred as polyploid cells. This condition is considered to be the result of endoreduplication of chromosomes; 2. cells with chromosomes in much elongated shape with brief interspersed areas lacking spiralization - an observation similar to one made by Prantera et al.[5]; 3. metaphase plates showing stickiness which made chromosome look dense and sticky due to interchromosomal adnations.

The association of carcinogenic hydrocarbons with chromosomal aberrations has been amply demonstrated both in vivo and vitro[2-9]. Recent studies on cultured Chinese hamster cells conducted by Matsuoka et al.[2] and Nishi et al.[4] show that chromosomal abnormalities were detected only if exposure to CPH was carried out in presence of rat-liver microsomal fraction plus the cofactors (S_9 or S_{15} mix). The data obtained in present investigation suggest that CPH could cause aberrations without exogenous activation and the microsome-mediated system is unnecessary. The discrepancy between these 2 studies and our data may be due to the duration of treatment with CPH. Both Matsuoka et al.[2] and Nishi et al.[4] treated cells for 3 h period in presence of microsomal fraction while in this study the treatment period was 24 h. However, in their report Matsuoka et al.[2] indicate that some chemicals cause chromosomal anomalies only after their exposure to cells for a longer duration. This is evidenced by their studies on isoniazid, a known carcinogen that was ineffective when incubated with cells for 3 h with or without S_9 mix. Nonetheless, aberrations were recovered 48 h after exposure even without S_9 mix.

At this time it is not certain whether these effects are produced by the CPH themselves or mediated through their carcinogenic metabolites as there is no evidence of appreciable levels of CPH metabolizing enzymes in V_{79} cells. However, Pal et al.[10] have recently reported that Chinese hamster ovary cells (CHO) do possess significant activity of mono-oxygenases and epoxide hydratase. This is evidenced by detection of 3-hydroxybenzo(a)pyrene and the 4,5-, 7,8- and 9,10-dihydrodiols formed by the action of these enzymes in medium in which the cells were incubated with BaP for 24 h. This prolonged treatment period needs a careful evaluation as in environment the individual is constantly exposed to low levels of hazardous chemicals rather than for a few hours. Relatively longer duration of treatment may also eliminate laborious procedure of preparing microsomal fractions and feeder layer cells.

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