DNA Synthesis in Cell Cultures Following Repeated Exposure to Fresh Cigarette Smoke

Recent reports from LEUCHTENBERGER et al.[1,2] have demonstrated marked changes in the DNA content of cells exposed to fresh cigarette smoke in vitro. Employing autoradiographic and Feulgen-microspectrographic techniques, they showed that outgrowths of human lung explants exhibited an early reduction in DNA content, prior to a large increase following longer exposure times. In this study we have exposed murine peritoneal macrophages and murine embryonic fibroblasts to fresh cigarette smoke for up to 2 weeks in culture, and measured ^{3}H-protein, ^{3}H-RNA and ^{3}H-DNA synthetic rates.

Materials and methods. Peritoneal macrophages were collected from the peritoneal cavity of C57 Black inbred mice in chilled culture medium[3]. Macrophages were cultured in Eagle's minimal essential medium (MEM) supplemented with 10% calf serum (CAS), in 35 mm plastic petri dishes (Falcon Plastics, USA) containing stainless steel squares[3]. Cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2 for 48–72 h before experimentation. The primary cultures of fibroblasts were obtained from whole C57 Black mouse embryos by trypsinization, grown in Dulbecco's modification of Eagle's minimal essential medium (DEM) with 10% CAS and stored in liquid nitrogen. After thawing, secondary cultures of fibroblasts were grown to confluency in DEM + 10% CAS (48–72 h) and then transferred to medium containing 2.5% CAS.

The radiotracer technique for the measurement of ^{3}H-protein synthesis was similar to that previously reported[3]. Cell cultures on steel squares were transferred to pre-warmed medium containing ^{3}H-leucine, at a final concentration of 1.7 μCi/ml for fibroblasts cultures of 3.3 μCi/ml for peritoneal macrophage cultures. After a 40 min pulse with the radioactive ^{3}H-leucine at 37 °C, the cultures were washed sequentially in phosphate buffered saline (PBS) containing 0.05 *M* leucine, 5% TCA and finally ethanol:acetone, 3:1. After a final wash in acetone and air drying, they were placed on aluminium planchets and their radioactivity was measured in a Nuclear Chicago gas-flow detector (Model D-47). The technique for measuring ^{3}H-RNA synthesis differed from the above in that ^{3}H-uridine (0.85 μCi/ml for both cell types) was substituted for ^{3}H-leucine, and uridine (0.05 *M*) substituted for leucine in the washing solutions. In assaying ^{3}H-DNA synthetic rates, radiolabelling was performed employing a ^{3}H-thymidine (0.80 μCi/ml) solution, and a pulse time of 3 h. 0.05 *M* thymidine was used in all washing solutions. ^{3}H-5-uridine (spec. act. 5.0 Ci/mmole), uniformly labelled ^{3}H-L-leucine (spec. act. 250 mCi/mmole) and ^{3}H-6-thymidine (spec. act. 5.0 Ci/mmole) were obtained from the Radiochemical Centre, Amersham, Bucks., U.K.

Cell cultures were exposed to fresh cigarette smoke in a verticle perspex chamber as previously described[4]. The chamber was designed to expose stainless steel squares in culture dishes from which medium had been removed, to fresh smoke from 1 cigarette mixed with air in a ratio of 1:7 for 2 sec, followed by a 58 sec exposure to fresh air (dosage 1.0). An 0.5 dose rate indicates a 2-sec exposure to smoke/air 1:14; and a dose rate of 2.0 indicates 2 × 2-sec exposure to 1:7 mixture, interspersed with 2 × 58-sec exposures to fresh air. In most experiments, cultures were exposed to cigarette smoke on week days for 1 week prior to labelling.

Results and discussion. The Figure shows the changes in ^{3}H-protein, ^{3}H-RNA and ^{3}H-DNA synthetic rates in cultures of murine peritoneal macrophages and embryonic fibroblasts in response to varying dosages of fresh cigarette smoke.

At low dosages (0.5), macrophages exhibited a striking stimulation in both ^{3}H-protein and ^{3}H-RNA synthesis. As the dosage increased the degree of metabolic stimulation decreased together with the viability of the cultures. These results confirm our earlier observations[5]. Rates of ^{3}H-DNA synthesis in the macrophages showed only minor alterations at low smoke dosages, but at higher dose regimes (dose rate 2.0) a small stimulation (50%) was seen. Attempts to continue the exposure of macrophage cultures over longer periods did not produce consistent results, as most cultures showed a markedly decreased viability after 1 week's exposure in vitro. Furthermore, histological examination of these cultures suggested the presence of an increasing proportion of fibroblastic-like cells at this time. These cells may have

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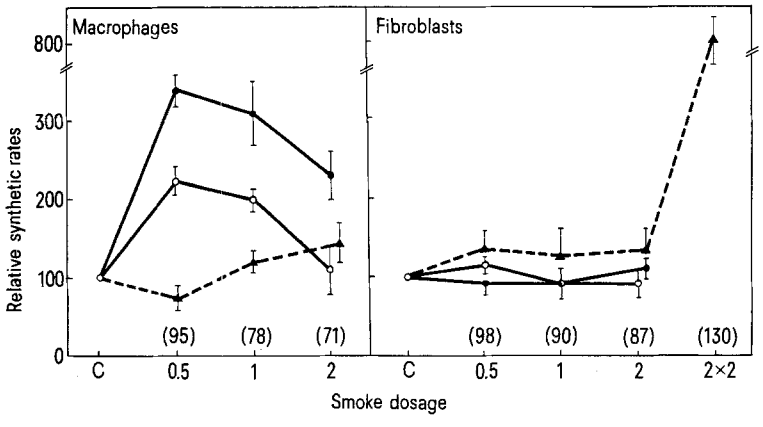

The effect of cigarette smoke on ^{3}H-protein (○), ^{3}H-RNA (●) and ^{3}H-DNA (▲) synthetic rates in cultures of macrophages and fibro- blasts. Synthetic rates were initially computed as cpm/10^3 cells, and then expressed as 'relative' rates by conversion to percentages of control rates. C represents Control (sham-smoked) cultures (i.e. smoke dosage zero). Each point shown is the mean ± S.D. of at least 5 observations. 2 × 2 represents a dosage level of 2 for a 2-week period. Cell viability counts (expressed as a percentage of that in control cultures) are shown in parentheses for each exposure level.

been transferred over with the original peritoneal cell suspension as a minor percentage of the initial population, and might have adapted to the culture conditions more rapidly than the macrophage population.

The metabolic activity of cultures of fibroblasts showed little variability over the 1-week-exposure period regardless of the smoke dosage employed. Furthermore, the cultures remained extremely healthy over the period and viability counts relative to controls were much higher than those seen in the corresponding macrophage cultures. Consequently, several experiments were performed in which cultures of fibroblasts being exposed to the highest smoke dosage were maintained for a second week in culture, during which the exposure regime was continued (dosage 2×2).

At the end of the experimental period, these cultures contained more viable cells than corresponding control cultures (130%), and ^{3}H-DNA synthetic rates were elevated up to 8 times those seen in the controls. These data confirm the observations of LEUCHTENBERGER et al.[1,2] employing indirect measures of DNA synthesis, and suggest that cigarette smoke exposure may potentially exert dramatic effects on rates of DNA synthesis in susceptible cells. Direct effects of this nature on nucleic acid synthesis may be involved in the development of hyperplastic changes which are invariably seen in cells lining the respiratory tract of smokers, and may also play a part in the eventual development of neoplasia.

Zusammenfassung. Makrophagen- und Fibroblastenkulturen wurden bis zu 2 Wochen lang frischem Zigarettenrauch exponiert. Während die Makrophagen schon bei geringen Rauchkonzentrationen eine erhöhte Protein- und RNS-Synthese zeigten, wurden bei Fibroblasten keine Veränderungen beobachtet. In höheren Rauchkonzentrationen waren bei den Makrophagen die DNS-Synthesewerte leicht bei den Fibroblasten jedoch stark erhöht.

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The Effect of L-Dopa, Noradrenalin and Adrenalin on P-388 Mouse Leukemia, B-16 Mouse Melanoma and E 0771 Mammary Carcinoma

The relationship between levodopa and neoplasia has been discussed by a few authors, contradictory reports having been published as to the effect of the drug upon human and experimental tumors[1–4]. A recurrence of melanoma was reported in 2 patients with Parkinson's disease treated with L-Dopa[5] and a striking temporal relationship was reported between the initiation of L-Dopa therapy and growth of melanoma[4,6]. Levodopa was suspected to enhance tumor growth through an effect of increased growth hormone secretion[7] or by its direct incorporation into melanoma[8]. ROBINSON et al.[1] failed to observe an enhancement of melanoma in L-Dopa treated experimental mice.

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Table I. Effect of L-Dopa, L-Dopa + $CuSO_4$, noradrenalin and adrenalin on the survival time of mice bearing P-388 tumor implanted intraperitoneally

Compound	Dose (mg/kg)	No. of treatments	Route of administration	Mean survival time (days ± S.D.)		Increase[a] or decrease in survival time (%)
				Treated	Controls	
L-Dopa	100	5	i.p.	7.8 ± 0.7	7.9 ± 0.7	0
L-Dopa + $CuSO_4$	100 + 4	5	i.p.	8 ± 1.05	7.9 ± 0.7	0
L-Dopa	150	6	i.p.	7 ± 0.35	8 ± 1.05	13
L-Dopa	250	6	i.p.	7.5 ± 0.4	8 ± 0.7	
L-Dopa	50	9	i.p.	8 ± 0.7	8.5 ± 0.34	6
L-Dopa	50	8	i.p.	9 ± 0.7	9 ± 0.5	0
L-Dopa	50	7	i.p.	9 ± 1.05	9 ± 1	0
Noradrenalin	0.1	5	i.p.	8.2 ± 1.75	7.9 ± 0.7	0.3[a]
Adrenalin	0.1	5	i.p.	7.5 ± 0.7	7.1 ± 0.7	0.7[a]
L-Dopa + $CuSO_4$	100 + 4	5	s.c.	7.2 ± 0.5	7 ± 0.7	0.2[a]
L-Dopa + $CuSO_4$	100	5	i.p.	7.2 ± 0.7	7 ± 1	0.2[a]
	4	5	s.c.			
Noradrenalin	0.1	5	s.c.	8 ± 0.5	7 ± 0.7	14[a]
Adrenalin	0.1	5	s.c.	7.2 ± 0.7	7 ± 0.5	0.2[a]