Alveolar Cells: Depressant Effect of Cigarette Smoke on Protein Synthesis* (33851)

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Cigarette smoke can impair the function of the lung and many of its components (1). It can depress the antibacterial activity of alveolar macrophages *in vitro* (2), and it decreases the growth of cells derived from other tissues (3, 4). In the present study the effect of cigarette smoke on protein synthesis in alveolar macrophages was examined.

Materials and Methods. M. bovis induced alveolar macrophages were obtained from the lungs of New Zealand white rabbits as previously described (5, 6) except that 0.85% sodium chloride was used to lavage the lung. The cells were harvested by centrifuging the lung washings at 1400*g* for 10 min at 0–4° and resuspended in 10 vol (v/wet wt) of Hanks' balanced salt solution (Grand Island Biological Company). For convenience the liquid mixture containing the water-soluble gaseous and particulate elements of cigarette smoke is referred to as smoke solution, as is done by others (7, 8). The system used to prepare the smoke solution consisted of a holder into which a filter could be inserted, a critical flow orifice, and an evacuated flask attached to a vacuum pump (9). A Cambridge CM-113 glass fiber absolute filter (Phipps and Bird, Inc.) was inserted into the holder of the smoking device to separate smoke into gas and particulate phases (10). Cigarettes (85-mm) from a popular brand were employed. Cigarettes were smoked as follows: eight 35-ml puffs were taken, each puff 2-sec in duration, with a 58-sec interval between puffs. Puffs were collected alternately in each of 2 flasks which contained 8 ml of Hanks' medium. After a puff was drawn into a flask, the smoke was mixed with the liquid by manual shaking for 45 sec. The smoke solutions were pooled, kept in stoppered flasks on ice, and used within 1 hr of preparation.

Cell suspensions were incubated at 37° with air as the gas phase, using uniformly labeled L-leucine-^{14}C (sp act 251 mCi/mmole, New England Nuclear Corporation) as the radioactive substrate. Reactions were stopped with cold 20% trichloroacetic acid (TCA). Appearance of radioactivity in the washed TCA-insoluble material of alveolar cells is assumed to represent *de novo* protein synthesis. Protein isolation was performed as previously described (6) except that the steps involving extraction with lipid solvents at 50° and 5% TCA at 90° were omitted after it was shown that less than 3% of the total acid-insoluble radioactivity was removed by these steps. The precipitates were dissolved in 0.5–1.0 ml of 0.2 *M* NaOH. Radioactivity of the NaOH solutions was measured in a Beckman liquid scintillation counter as previously described (6). Because preliminary experiments showed no difference in quenching between samples with and without smoke solution, the data are reported as counts rather than disintegrations per minute per milligram of protein.

Protein was measured on the NaOH solutions (11) using bovine albumin as the standard. The pH was determined directly with a micro pH electrode (Fisher Scientific Co.). Cell viability studies were performed with the eosin dye exclusion test (12). Each set of data is a representative experiment taken from at least three with similar results.

Results and Discussion. Cigarette smoke solution caused a dose–dependent decrease in

* Supported in part by the American Thoracic Society and the American Medical Association Committee for Research on Tobacco and Health.

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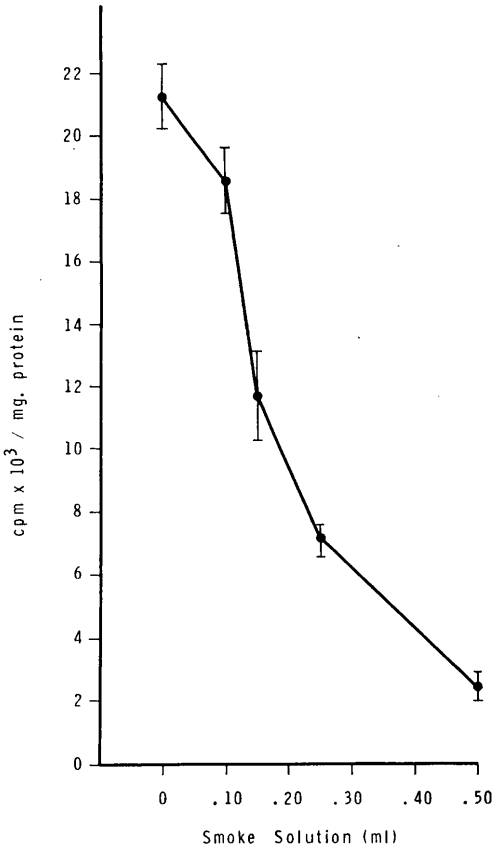

FIG. 1. Effect of dose of smoke solution on alveolar cell protein synthesis: a 0.5-ml cell suspension was incubated for 30 min with 1.3 μmole of L-leucine-^{14}C and varying amounts of smoke solution in a final volume of 1.0 ml; dot and vertical bar represent mean ± 1 SD of 3 replicate tubes.

the amount of radioactivity appearing in acid-insoluble material (Fig. 1). The time course of this effect is illustrated in Fig. 2. Alveolar cells pulse-labeled with leucine-^{14}C and then incubated with smoke solution showed no more rapid decrease in acid insoluble radioactivity than control cells, indicating that the smoke solution had no effect on turnover of newly synthesized radioactive protein (Fig. 3).

It was found in preliminary experiments that amino acid incorporation into alveolar cell protein has a pH optimum of 7.40. The smoke solution had a pH of 7.00, and the largest volume of smoke solution used in the experiments described in Fig. 1 produced a

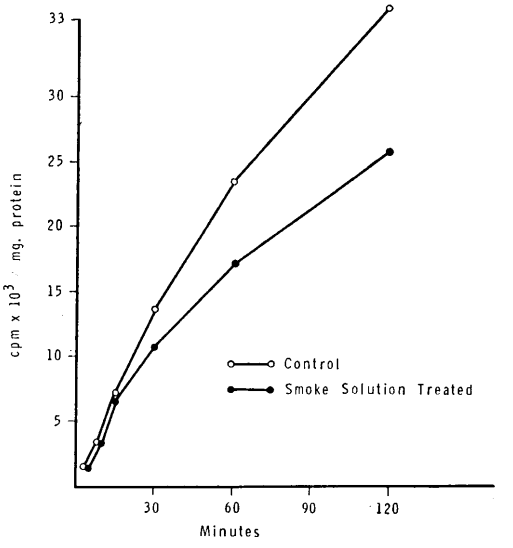

FIG. 2. Time course of smoke solution effect on alveolar cell protein synthesis: 0.45 ml of cell suspension was incubated with 1.0 μmole of L-leucine-^{14}C and 0.15 ml of smoke solution or Hanks' solution for the times indicated; reactions were performed in duplicate.

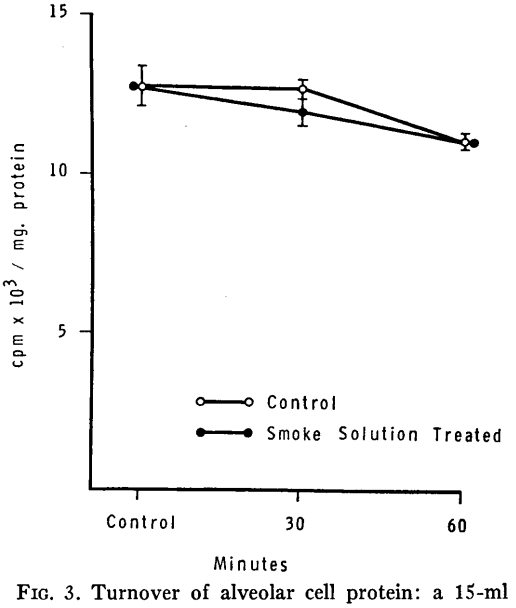

FIG. 3. Turnover of alveolar cell protein: a 15-ml cell suspension was incubated with 30 μmole of L-leucine-^{14}C for 10 min, washed, resuspended in Hanks' medium and incubated with 0.25 ml of smoke solution or Hanks' solution for the times indicated; dot and vertical bar mean ± 1 SD of 3 replicate tubes.

TABLE I. Influence of Reaction Mixture pH on Smoke Depression of Alveolar Cell Protein Synthesis.[a]

Reaction mixture and pH		Protein sp act (cpm × 10^3/mg[b])	Depression (%)
Control	7.40	17.4 ± 1.0	—
Smoke solution treated + Tris	7.40	9.8 ± 0.4	44
— Tris	1.15	8.1 ± 0.1	54

[a] To 0.5 ml of cell suspension was added 1.0 μmole of L-leucine-^{14}C and 0.5 ml of smoke solution or additional medium; 0.01 ml of 0.2 *M* Tris buffer, pH 8.2, or 0.01 ml of 0.9% NaCl was added to each reaction mixture. The mixtures were incubated for 30 min.

[b] Mean ± 1 SD of 3 replicate tubes.

pH of 7.15 in the reaction mixture, compared to 7.40 in controls. Smoke solution-treated cell suspensions brought to pH 7.40 with Tris had almost as much depression of incorporation of radioactivity as suspensions not containing Tris (Table I). It thus appears that pH changes alone do not account for the depressant effect of smoke solution on protein synthesis.

Previous investigators have reported that smoke constituents can inhibit growth of cells in culture (3, 4) but at least some of this effect may have resulted from direct killing of cells. The reversibility of the depressant effect was examined by washing alveolar cells with Hanks' medium after they had been incubated with smoke solution. These cells, and appropriately treated controls, were then incubated with L-leucine-^{14}C. Washing the cells eliminated almost half of the smoke solution effect (Table II). Furthermore, there was no difference in viability between control cells and those treated with a volume of smoke solution causing 41% inhibition of protein synthesis. It is thus concluded that cigarette smoke solution is capable of depressing protein synthesis in nonlethal doses and that this effect is partly reversible.

Earlier studies have differed as to whether the gas or particulate phase of smoke is responsible for its cytotoxic effects (3, 7, 8). When the particulate phase was removed from the smoke solution by preparing it with a Cambridge absolute filter in the collection apparatus, the resultant gas phase solution caused almost as much depression of protein synthesis as whole smoke solution (Table III). Identification of the responsible components of the gas phase, and determination of whether or not this is an isolated effect on protein metabolism, awaits further study.

The relevance of these data to *in vivo*

TABLE II. Reversibility of Smoke Solution Effect on Alveolar Cell Protein Synthesis.[a]

	Protein sp act (cpm × 10^3/mg[b])	Depression (%)
Unwashed, control	21.0 ± 1.9	—
Smoke solution treated	5.8 ± 0.2	72
Washed, control	31.0 ± 5.5	—
Smoke solution treated	19.0 ± 4.0	39

[a] The mixtures labeled "unwashed" were set up as follows: To 0.5 ml of cell suspension was added 0.5 ml of smoke solution and 1.3 μmole of L-leucine-^{14}C; these were incubated for 30 min. The mixtures labeled "washed" were prepared as follows: To 2.0 ml of cell suspension was added 2.0 ml of smoke solution or Hanks' solution. These reaction mixtures were maintained at 37° for 30 min; they were then chilled, the cells were washed three times with cold Hanks' solution and resuspended in 4 ml of Hanks' solution. One-ml samples were incubated with 1.3 μmole of L-leucine-^{14}C as indicated above for 30 min.

[b] Mean ± 1 SD of 3 replicate tubes.

TABLE III. Effect of Smoke Fractions on Alveolar Cell Protein Synthesis.[a]

Reaction mixture	Protein sp act (cpm × 10^3/mg[b])	Depression (%)
Control	38.4 ± 3.7	—
Gas phase smoke solution	28.5 ± 0.9	26
Whole smoke solution	26.3 ± 1.1	32

[a] To 0.2 ml of cell suspension was added 1.3 μmole of L-leucine-^{14}C and 0.2 ml of gas phase smoke solution or additional medium. The reaction mixtures were incubated for 30 min.

[b] Mean ± 1 SD of 3 replicate tubes.

conditions is not clear, since the concentrations of smoke achieved in alveolar cells in the intact organism are unknown. It seems likely, however, that if protein synthesis were depressed in these cells for extended periods of time, it would interfere with phagocytosis and killing of microorganisms by interfering with synthesis of membranes and of hydrolytic enzymes. This mechanism may be of importance in the increased incidence of respiratory infections found in human subjects who smoke (13, 14).

Summary. The water soluble constituents of cigarette smoke depress protein synthesis in rabbit alveolar cells *in vitro*. This effect is dose dependent, partly reversible, and greater than can be accounted for by the increase in acidity caused by addition of smoke solution to cell suspensions. It occurs at concentrations of smoke solution which do not affect cell viability or protein turnover. The major portion of this depressant activity is in the gas phase.

The author thanks Dr. Donald Massaro of the Washington V. A. Hospital and Drs. W. W. Bates and V. Norman and Mrs. Rebecca Vinson of the Liggett and Myers Tobacco Company, Durham, North Carolina for their help.

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Received Dec. 26, 1968. P.S.E.B.M., 1969, Vol. 131.