tributaries separately, we have found the lymph formation occurs at about the same rate, when it is expressed as flow per unit weight, in all these different tissues. A better estimate of the mean rise in the concentration of VIP in the intestinal extracellular fluid in response to vagal stimulation may therefore be obtained by correcting for estimated dilution by hepatic, splenic and pancreatic lymph. The peak incremental value (34±7 pmoles/l at 30 min) would then become 34.0 (84.7/65.4)= 51 pmoles/l, which is much greater than the observed rise in the concentration of the peptide in the arterial plasma. We conclude that the rise in VIP concentration in the blood which occurred in these experiments, in response to vagal stimulation, was due to release of the peptide from the alimentary tract.

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2 O.B. Schaffalitsky de Muckadell, J. Fahrenkrug and J.J. Holst, Gastroenterology *72*, 373 (1977).
3 M.G. Bryant, S.R. Bloom, J.M. Polak, R.H. Albuquerque, I. Modlin and A.G.E. Pearse, Lancet *I*, 991 (1976).
4 F. Sundler, J. Alumets, R. Häkanson, S. Ingemansson, J. Fahrenkrug and O. Schaffalitsky de Muchadell, Gastroenterology *72*, 1375 (1976).
5 L.I. Larsson, J. Fahrenkrug, O. Schaffalitsky de Muchadell, F. Sundler, R. Häkanson and J.F. Rehfeld, Proc. nat. Acad. Sci. *73*, 3197 (1976).
6 S.R. Bloom, J.M. Polak and A.G.E. Pearse, Lancet *II*, 14 (1973).

Response of neonatal mouse lung in organ culture to silica

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Summary. Short term organ cultures of lung fragments from neonatal mice maintain their normal architecture. Cultures exposed to silica flour in varying concentrations show effects resembling those seen in chronic silicosis produced by inoculation and inhalation methods.

Although the effects of silica on lungs in vivo and in cultures of macrophages have been reported[1-3], the effects of silica on organ cultures of lung have not been documented. Animal experiments using intrapleural inoculation and inhalation methods are time consuming. The purpose of this study was to determine if an organ culture method could be used to reproduce the in vivo picture of chronic silicosis in a short period of time.

Materials and methods. Fragments of lung from neonatal (2-3 day) mice of strain NMR/BOM were cultured by the watch glass method[4] in medium TC 199[5] supplemented with 25% mouse plasma. Sterile silica flour containing 99.8% SiO_2 with a pH of 6.9 was dissolved in concentrations of 10%, 50% and 75% in distilled water. Control cultures received 15 drops of culture medium plus 3 drops of distilled water. Experimental explants received 15 drops of culture medium plus 3 drops of silica solution. Incubation took place in a Thelco incubator supplemented with 21% O_2, 8% CO_2, and 71% N_2. The culture medium was changed once during the 4-day incubation period.

Results. Control explants showed normal lung architecture comparable to lungs of mice of 2-3 days of age. Explants incubated with 10% silica flour for 4 days displayed pyknotic cells, migration of fibroblasts and macrophages and a decrease in size of alveolar spaces. Thin fibres and patches of eosinophilic material were evident in the alveolar spaces. The effects were progressively more severe with higher doses of silica. In fragments incubated with 75% silica flour the alveolar spaces were insignificant. Thin fibres were also present. The eosinophilic material was masked by the intense cellular infiltration.

Discussion. The results obtained in this study are similar to those reported in animal experiments[1-6]. The amorphous eosinophilic material found in the alveolar spaces have been reported in silica induced alveolar lipoproteinosis[7]. The migrating cells should be macrophages, the key cellular element in the fibrosis reaction[8-9]. Perhaps the macrophages are converted to fibroblasts as observed in aging lung cultures from adult guinea-pigs[10]. Short term organ cultures utilizing asbestos have been reported[11,12]. The experiments reported here with silica flour show that the in vivo picture of silicosis may be reasonably reproduced in a short incubation period.

1 B. Goldstein and L. Webster, Br. J. ind. Med. *23*, 71 (1966).
2 E.J. King, G.P. Mohanty, C.V. Harrison and G. Nagelschmidt, Br. J. ind. Med. *10*, 76 (1953).
3 J. Marks and G. Nagelschmidt, Archs ind. Health *20*, 383 (1959).
4 J.M. Chen, Exp. Cell Res. *7*, 518 (1954).
5 J.F. Morgan, H.J. Morton and R.C. Parker, Proc. Soc. exp. Biol. Med. *73*, 1 (1950).
6 A.G. Heppleston, Br. Med. Bull. *25*, 282 (1969).
7 A.G. Heppleston, N.A. Wright and J.A. Stewart, J. Path. *101*, 293 (1970).
8 A.C. Allison, J.S. Harington and M. Birbeck, J. exp. Med. *124*, 141 (1961).
9 J.S. Harington, S. Afr. med. J. *37*, 451 (1963).
10 J.M.G. Davis, Br. J. exp. Path. *48*, 371 (1967).
11 K.J. Rajan, J.C. Wagner and P.H. Evans, Nature *238*, 346 (1972).
12 J.M.G. Davis, Br. J. exp. Path. *48*, 379 (1967).