Table II. Effects of cyclic nucleotides on distribution of AIB-^{14}C in bovine mesenteric arteries

Nucleotide	Control	Drug	Difference
Cyclic AMP (10^{-3} M) ($n = 9$)	106.0 ± 6.0	105.6 ± 5.5	− 0.4 ± 1.7
Cyclic GMP (10^{-3} M) ($n = 12$)	113.4 ± 5.1	117.8 ± 5.3	4.3 ± 1.6[a]

The distribution in percent was calculated from the quotient $=$ $\frac{\text{content in wet tissue (cpm/mg)}}{\text{medium concentration (cpm/}\mu\text{l)}} \times 100$. Significance as in Table I.

to study the influence of cyclic AMP and cyclic GMP on the incorporation of ^{14}C-leucine in the proteins of vascular smooth muscle – bovine mesenteric artery.

The method has been described in detail by ARNQVIST[6] as the method to investigate accumulation of α-aminoisobutyric acid-^{14}C (AIB-^{14}C). In short, about 20 cm of the mesenteric artery was removed 15–20 min after the slaughter of the animal, rinsed and put in a thermosflask with oxygenated Krebs-Henseleit bicarbonate buffer solution at 37 °C and then transported to the laboratory. The preparation was cleaned from adventitial tissue, cut open along its length and divided into pieces with a length of about 15 mm and breadth of 10–12 mm. The weight of the pieces was about 200 mg. Adjacent tissue samples were used as test and control preparations. The preparations were incubated in 4 ml oxygenated (95% O_2 + 5% CO_2) Krebs bicarbonate buffer for 180 min with 1×10^{-5} M L-leucine containing 0.4 μCi-leucine and 5.6 mM glucose. After incubation for 180 min, the tissue was homogenized in 10% TCA and the protein was purified, dried, weighed and dissolved in 1 ml Soluene. The radioactivity incorporated into protein was measured in a Packard Tri Carb scintillator detector.

Cyclic AMP in a concentration of 1×10^{-3} M reduced significantly the ^{14}C-leucine incorporation, whereas 1×10^{-4} cyclic AMP was ineffective (Table I). In a concentration of 5×10^{-4} the nucleotide was still effective, indicating that the threshold concentration for the cyclic AMP effect may be about between 1 and 5×10^{-4} M. 5′AMP (1×10^{-3} M) had no effect (Table I).

Both theophylline (1×10^{-3} M) and papaverine (5×10^{-5} g/ml), drugs which increase the cyclic AMP level of the muscle by inhibiting the phosphodiesterase activity[7], reduced significantly the leucine incorporation; the effect of papaverine being most marked (Table I). In combination cyclic AMP (5×10^{-4} M) and papaverine had an additive effect but theophylline (1×10^{-3} M) did not increase the action of cyclic AMP (Table I). Cyclic GMP (1×10^{-3} M) in contrast to cyclic AMP stimulated the ^{14}C-leucine incorporation; a concentration of 1×10^{-5} M being ineffective (Table I).

Regarding the mechanism by which the cyclic nucleotides influence ^{14}C-leucine incorporation, both an influence on the transport of the aminoacid through the cell membrane and its incorporation into the proteins may be considered. To study the first of these reactions, the effects of the nucleotides on the AIB-^{14}C transport was investigated. From Table II it is evident that cyclic GMP very moderately stimulated the AIB-accumulation, whereas cyclic AMP had no effect.

The physiological or pathophysiological role of the cyclic nucleotides in protein synthesis is unclear, but the nucleotides may have a role both in metabolic changes observed in atherosclerotic and hypertensive vessels. In aortas from hypertensive rats, the proteins of the vascular wall was found to be increased[8] and the level of cyclic AMP to be decreased[9].

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The Short-Term Effects of Ingested Chrysotile Asbestos on DNA Synthesis in the Pancreas and Other Organs of a Primate[1]

S. S. EPSTEIN and MARIE VARNES

Environmental Health Programs, Case Western Reserve University School of Medicine, Cleveland (Ohio 44106, USA),
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Summary. Single oral administration of chrysotile asbestos to monkeys resulted 9 days later in the stimulation of DNA synthesis in the pancreas as evidenced by increased incorporation of tritiated thymidine.

Asbestos is now a ubiquitous environmental contaminant, particularly in industrialized communities[2]. There is growing recognition of the exposure of general populations to low levels of asbestos in drugs, food, and drinking water, besides in air[3–8]. Nevertheless, there are no valid toxicological or epidemiological data on the carcinogenic effects of orally administered asbestos. The possibility that asbestos, alone or in some interactive combination with other chemical carcinogens, may be a causal factor in the induction of gastrointestinal (GI) tract cancer, besides possibly cancers at other sites, is attracting increasing interest. It should be noted that GI cancers are one of the commonest internal human cancers, the etiology of which is still largely unknown; approximately 100,000 cases are diagnosed annually in the USA.

There are abundant epidemiologic data relating occupational respiratory exposure to asbestos with asbestosis, pleural and peritoneal mesotheliomas, and bronchial carcinoma, besides carcinomas at other sites, particularly the GI tract[9–13]. Additionally, there is evidence of a synergistic interaction between asbestos and tobacco smoking in the pathogenesis of both bronchial and esophageal carcinomas[14]. Although asbestos is known to be widely disseminated in the body of experimental animals following its initial primary site of absorption or deposition[15–17], there is still uncertainty as to whether the enhanced incidence of GI cancers in workers exposed to asbestos by inhalation[12] is due to hematogenous or

Incorporation of tritiated thymidine into DNA of various organs at specified intervals following oral administration of chrysotile asbestos to primates

Days following administration of asbestos	Relative mean ^{3}H-thymidine incorporation dpm/μg DNA ± standard error								
	Stomach (fundus)	Small Intestine (jejunum)	Colon (descending)	Liver (middle left lobe)	Kidney (mid-pole)	Pancreas			
						Head	Body	Tail	Mean of 3 segments
Controls	32.1 ± 11.3	47.0 ± 4.9	76.3 ± 11.8	15.9 ± 3.5	15.4 ± 1.1	16.2 ± 2.9	18.1 ± 3.2	20.5 ± 4.9	18.3 ± 1.8
1	45.4 ± 23.0	41.6 ± 10.6	91.0 ± 22.3	9.9 ± 3.9	13.2 ± 2.3	17.0 ± 3.7	13.4 ± 2.3	20.7 ± 9.7	17.0 ± 3.0
3	46.2 ± 8.5	35.2 ± 9.2	93.9 ± 43.8	13.3 ± 3.2	14.4 ± 1.0	14.5 ± 3.6	12.0 ± 3.4	11.9 ± 2.5	13.1 ± 1.2
9	20.1 ± 4.2	41.9 ± 3.8	80.5 ± 26.2	10.4 ± 2.0	16.9 ± 2.5	29.5 ± 8.9	36.2 ± 11.6	40.2 ± 18.2	35.3 ± 2.0
27	26.7	35.4	81.0	8.2	18.3	18.8	2.2	17.8	19.3 ± 1.1

All values are based on arithmetic means of 3 replicate analyses of 3–5 slices from each organ segment. 3 animals were sacrificed at all intervals with exception of the 27-day inverval, the values of which are based on 1 animal.

lymphogenous dissemination of asbestos from the lungs or due to the swallowing of asbestos-laden sputum. In this connection, recent data have demonstrated that inhalation by rats of gamma labelled asbestos is followed by its rapid clearance from the respiratory to the GI tracts[18]. There are no available data on the consequences of continued and progressive exposure of the GI tract to asbestos-contaminated sputum. Similarly, there are no valid data on the possible carcinogenic effects of the continued ingestion of relatively low levels of asbestos in food, beverages, and drinking water[2,3]. Apart from these outstanding public health considerations, there is a striking paucity of data on the basic mechanisms of asbestos carcinogenesis[19–21].

Recent investigations in our laboratories have demonstrated stimulation of DNA synthesis, as evidenced by increased incorporation of tritiated thymidine, in the GI tract of rats following single gavaging with asbestos[22,23]. We report here the results of preliminary experiments demonstrating similar effects in the pancreas of primates, following oral administration of asbestos.

UICC standard reference Rhodesian chrysotile A asbestos was obtained from the National Research Institute for Occupational Diseases, Johannesburg, South Africa. Fibres were washed twice with distilled water and with ethanol, dried overnight in vacuo, and suspended in distilled water immediately prior to dosage.

Thirteen young adult female monkeys, *Macaca mulata*, from Primate Imports, Inc., Port Washington, New York, were used for this study. Prior to gavaging, the monkeys were starved for 24 h and sedated by i.m. injection of ketamine (10 mg/kg) and atropine (1/600 unit). Suspensions of asbestos, in doses of 100 mg/kg in 10 ml of distilled water, were administered to 10 animals via stomach tube with a 10 ml syringe, followed by a wash with 10 ml of distilled water; 3 animals were used as negative controls (Table). Groups of animals, numbering 3,3,3, and 1, were sacrificed at subsequent respective intervals of 1, 3, 9, and 27 days. Animals were first sedated with 2.0 mg/kg i.m. sernylan, injected i.v. with 0.25 μCi/kg ^{3}H-thymidine, and then injected intracardially with 50 mg/kg sodium pentobarbitol. The entire GI tract, liver, pancreas, and kidneys were removed from each animal and placed on wet towels over ice; the GI contents were washed out with normal saline at 4 °C. Organs were divided into segments as follows: stomach – cardia, fundus, and pylorus; small intestine – duodenum, jejunum, and ileum; colon – ascending, transverse, and descending; liver – dorsal, middle, and ventral segments of the left lobe; right kidney – upper, middle, and lower poles; and pancreas – head, body, and tail. Segments of all these organs were then frozen at −20 °C. 3 to 5 slices of each segment, weighing 0.3 to 0.5 g, were subsequently assayed for incorporation of labeled thymidine, according to previously described procedures[23,24]. Thymidine incorporation was expressed as dpm/μg DNA deoxyribose, based on the mean of 3 replicates of a tissue homogenate of each organ segment.

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No mortality or significant weight loss occurred in any animals during the experiment. The effects of asbestos on DNA synthesis in various organs are summarized in the Table; each value represents the mean ± SE of 3 replicate analyses. The data presented for all organs, except the pancreas, are based on only 2 specified segments, as these were representative of values in other segments of the same organ; for the pancreas, data on each individual segment and their composite means are also presented.

Levels of thymidine incorporation in all organs were relatively low, in contrast to previous studies in rodents in which comparable doses of tritiated thymidine were used (Table[23,24]). As can be seen from the standard errors, variability in thymidine incorporation between animals in the same test or control group was relatively high. In general, incorporation was similar in all organ segments of control and test animals (Table). A marked increase, approximately twice control values, in thymidine incorporation was consistently found in all segments, head, body, and tail, of the pancreas 9 days following administration of asbestos. Incorporation in the pancreas at other intervals, and at all intervals in all other segments of all other organs was comparable to control values. Histopathological examination of the pancreas and other organs assayed for thymidine incorporation, failed to reveal any differences between test and control animals.

While the relatively small numbers of animals used in these preliminary studies limits formal statistical analysis of the data, the marked increase in DNA synthesis, which was consistent in all segments of the pancreas, 9 days following asbestos administration is of particular interest. These findings are in contrast with data from the rat, in which asbestos induced elevated thymidine incorporation in DNA of the stomach, duodenum, and jejunum, but not in DNA of the colon or pancreas over a 3-day period following administration of chrysotile in a dose range of 5 to 100 mg/kg[23]; transient increases in DNA synthesis were noted at other intervals in the GI tract of the rat following 100 mg/kg dosage[22].

The small number of primates tested over restricted intervals following oral administration of asbestos limits generalization from these preliminary findings. While the evidence of increased DNA synthesis in the pancreas 9 days following gavage is consistent with data on the widespread dissemination of asbestos fibres in various organs of experimental animals following its ingestion[15–17], failure to demonstrate such effects in the GI tract of primates may be consistent with findings of recent studies in which asbestos fibres could not be identified in the gastric mucosa of baboons following gavage with asbestos[24]; it has been suggested that the high level of gastric mucus in monkeys may limit mucosal penetration of asbestos fibres[25].

Increased synthesis of pancreatic DNA in primates and similar effects in the GI tract of rats at relatively short intervals following the ingestion of asbestos may reflect DNA replication following asbestos-induced cytotoxicity or a direct stimulation of DNA replication. Further study of such early effects of asbestos on DNA synthesis may provide information on basic mechanisms of asbestos carcinogenesis.

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Antischistosomal and Some Toxicological Properties of a Nitrodiphenylaminoisothiocyanate (C 9333-Go/CGP 4540)

E. BUEDING, R. BATZINGER and G. PETTERSON[1].

Department of Pathobiology and Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins University, 615 North Wolfe Street, Baltimore (Maryland 21205, USA), 3 February 1976.

Summary. A procedure to enhance the schistosomicidal effectiveness in vivo of an isothiocyanate derivative and some of its antischistosomal properties are reported. Determinations of the effects of this compound on tissue thiol levels and on highly sensitive bacterial tester strains have indicated that its mutagenic potential is of a low order and that the latter is decreased further after reduction of the host's intestinal bacterial flora.

Studies of STRIEBEL[2] have revealed that 4-isothiocyanato-4′-nitrodiphenylamine (C9333-Go/CGP4540) has high antischistosomal activity when administered as a single oral dose to animals experimentally infected with schistosomes. In order to be effective, a high oral dose (200 to 300 mg/kg) of moderately large particle sizes (average diameter: 30 μm)of this compound was required. The effectiveness of this isothiocyanate derivative was greatly enhanced when its particle size was reduced to an average of 0.5 μm in diameter by ball mill treatment for 14 days of a suspension of the compound in 1% Cremophor EL (BASF) and 25% glycerol. When this formulation (subsequently referred to as 'formulated' compound) was administered to mice infected with *Schistosoma mansoni*, parasitological cures in 23% of the mice were observed with a single dose as low as 5 mg/kg; when this dose was doubled, 95% of the mice were free of worms (Table). On the basis of this steep dose response, there were only slight variations in the susceptibility of the entire worm population to the antischistosomal effects of this compound. There were few, if any, differences in the susceptibility of 4 geographic strains of *S. mansoni* (3 strains from Puerto Rico, 2 strains from Liberia, and 1 strain each from Brazil and St. Lucia); furthermore, a strain resistant to another antischistosomal compound, hycanthone[3,4], proved susceptible to the isothiocyanate derivative. In

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