

Med. Lavoro, 4, 394-407, 1982

Clinica del Lavoro "L. Devoto"

**La medicina
del lavoro**

Via San Barnaba 8, Milano, Italy

THE RELEVANCE
OF THE EXPERIMENTAL APPROACH
IN THE ASSESSMENT OF THE ONCOGENIC RISKS
FROM FIBROUS AND NON-FIBROUS PARTICLES.
THE ONGOING PROJECT
OF THE BOLOGNA INSTITUTE OF ONCOLOGY

C. Maltoni, F. Minardi and L. Morisi

«L'importanza del metodo sperimentale nella valutazione dei rischi oncogeni da particelle fibrose e non fibrose. Progetto in corso dell'Istituto di Oncologia di Bologna». - È stato presentato il progetto di saggi di cancerogenicità a lungo termine di particelle fibrose e non fibrose, organiche e inorganiche, naturali e artificiali, in atto presso l'Istituto di Oncologia di Bologna. I dati preliminari dimostrano che: 1) l'erionite è l'agente cancerogeno sperimentale più potente che si conosca per il mesotelio pleurico del ratto; 2) sul ratto l'erionite è più cancerogena per la pleura, e l'asbesto più cancerogeno per il peritoneo; e 3) asbesti di diverso tipo ed origine mostrano nette differenze nella loro capacità di produrre mesoteliomi del peritoneo, nel ratto. Il contributo dell'approccio sperimentale nell'identificazione e quantificazione dei rischi oncogeni di materiali particolati è stato brevemente discusso.

In our opinion, there is an urgent and growing need for the oncogenic risk assessment of the inorganic and organic, non fibrous (amorphous and crystalline) and fibrous, natural and man-made particles, present in the work-place and in the general human environment.

The available data are far from corresponding to the diffusion, the industrial and commercial impact and the growing utilisation of the above-cited materials.

The problem can be faced both from an experimental and, when possible, from an epidemiological approach.

Institute of Oncology - Viale Ercolani, 4/2 - Bologna - Italy.
Supported in part by EEC, contract No. ENV/347/L(S).

Long-term experimental bioassays, in the present situation, may provide different types of important information, i.e. they can:

- identify new potential carcinogenic risks;
- assess, in quantitative terms, the level of risk of a given agent, and compare the risks represented by different agents (assessment of the relative carcinogenic risks);
- evaluate the possibility of additive and multiplicative effects of simultaneous or random exposures to other agents;
- help to predict the target organs;
- define the role of the physical and chemical characteristics of the test-compounds, and of different biological and experimental factors which affect neo-

plastic response and, consequently, shed some light on the pathogenesis of the possible oncogenic effects; and finally, — help to reconstruct the natural history of the tumors which may be induced by the test-compounds.

A vast and systematic project of interrelated long-term bioassays on several types of particles, has been started in the experimental Bentivoglio (BT) Laboratories of the Institute of Oncology of Bologna.

At present, the project includes the study of several types of asbestos (Exp. BT 2101), of kaolin, talc, crystalline silica, amorphous silica, alumina, bentonite and carbon fibers (Exp. BT 2102), of several sedimentary natural zeolites (Exp. BT 2103), of several hydrothermal natural zeolites (Exp. BT 2104 and BT 2105).

Each compound is always tested by intraperitoneal injection, and often by intrapleural and subcutaneous ones, on groups of 40, 60 or 80 animals, of both sexes. The animals are treated by a single 25 mg injection of the test-compound, in 1 ml of H₂O. Only carbon fibers have been tested by subcutaneous route alone (implantation of discs of « preg-preg », 2 cm in diameter). Animals treated with H₂O serve as controls.

The procedures of the experiment are highly standardized. The animals are kept under observation until spontaneous death, and periodically weighed and controlled during all the experiments. A complete autopsy is carried out on each dead animal, and systematic histopathology is performed.

The plan of the started experiments is shown in tables 1-5.

TABLE 1 - Long-term carcinogenicity bioassays of asbestos of different types and origins, by intraperitoneal, intrapleural and subcutaneous injection, on 8 weeks old Sprague-Dawley rats.

Size of the experimental groups: 40 rats (20 M and 20 F).

Treatment: 1 injection of 25 mg of the test compounds in 1 ml of H₂O.

Duration of the experiments: life-span.

Start of experiments: 77 weeks ago (*).

55 weeks ago (**).

Exp. BT 2101

Compounds	Intraperitoneal	Routes	
		Intrapleural	Subcutaneous
Crocidolite (UICC) (*)	+	+	+
Chrysotile (Canada) (UICC) (*)	—	+	+
Chrysotile (Rhodesia) (UICC) (*)	+		
Chrysotile (California) (*)	+		
Amosite (*)	+		
Anthophyllite (*)	+		
Asbestos-Cement (**)	+	+	+
H ₂ O (Controls) (*)	+	+	+
H ₂ O (Controls) (**)	+		

Total No. of animals: 680.

40 other samples of asbestos of different types and origins will be tested.

TABLE 4 - Long-term carcinogenicity bioassays of various hydrothermal natural zeolites, by intraperitoneal, intrapleural and subcutaneous injection, on 8 weeks old Sprague-Dawley rats.

Size of the experimental groups: 40 rats (20 M and 20 F).

Treatment: 1 injection of 25 mg of the test compounds in 1 ml of H₂O.

Duration of the experiments: life-span.

Start of experiments: 45 weeks ago.

Exp. BT 2103

Compounds	Intraperitoneal	Routes Intrapleural	Subcutaneous
Mordenite	+	+	+
Heulandite	+		+
Mesolite	+		+
Natrolite	+		+
Scolecite	+		+
Stilbite	+		+
Thompsonite	+		+
H ₂ O (Controls)	+		

Total No. of animals: 640.

Several other samples of various hydrothermal natural zeolites of different types and origins will be tested.

TABLE 5 - Long-term carcinogenicity bioassays of industrial zeolites, by intraperitoneal, intrapleural and subcutaneous injection, on 8 weeks old Sprague-Dawley rats.

Size of the experimental groups: 40 (*) or 60 (**) rats (20 or 30 M and 20 or 30 F).

Treatment: 1 injection of 25 mg of the test compounds in 1 ml of H₂O.

Duration of the experiments: life-span.

Start of experiments: 55 weeks ago (*).
just begun (**).

Exp. BT 2104 - Exp. BT 2105

Compounds	Intraperitoneal	Routes Intrapleural	Subcutaneous
MS 4A (*)	+	+	+
MS 5A (*)	+	+	+
MS 13X (*)	+	+	+
Zs and correlated compounds (correlated compounds (17 samples) (**))	+	+	+
H ₂ O (Controls) (*)	+		
H ₂ O (Controls) (**)	+	+	+

Total No. of animals: 3,640.

Other samples of industrial zeolites of different types and origins will be tested.

The project has utilized 6,580 animals, so far.

The study will be extended in the near future to other types of asbestos, of natural and industrial zeolites, and of carbon fibers, and to other inorganic and organic particles (glass fibers, plastic fibers, vegetal fibers, etc.). In particular, a series of 40 samples of asbestos of different types and origins will be tested. The samples are being collected by gently shaking the natural minerals in order to avoid the possible physical and chemical modifications produced by rough manipulation, which is the common procedure when obtaining sufficient amounts of short fibers for bioassays.

After 15 months (67 weeks) three sets of results seems particularly important and prove the utility of the experimental approach in helping to clarify some areas of the problem of carcinogenesis from particles.

1. CARCINOGENICITY OF ERIONITE

Erionite has been suspected of being a/the causative agent of environmental pleural mesotheliomas in Turkey (Baris et al., 1979).

Under our experimental conditions, the

compound has produced an high onset of pleural mesotheliomas following intrapleural injection. 21 out of the 22 dead animals (over 40 treated ones) within 67 weeks died from pleural mesotheliomas, i.e. 52.5% of all treated animals (table 6).

The induced pleural mesotheliomas are equivalent to the most frequent sub-istotypes of the human ones. Characteristic pictures are shown in figures 1-7.

These results show that erionite is the most potent known experimental carcinogenic agent for the pleural mesothelium.

2. ASSESSMENT OF THE RELATIVE ONCOGENIC RISK OF ASBESTOS OF DIFFERENT TYPES AND ORIGINS

Asbestos of different types and origins, when injected intraperitoneally, show sharp differences in their capability of producing peritoneal mesotheliomas (table 7).

These results stress the need for further studies in order to have a more precise, quantitative risk assessment in the area of asbestos carcinogenesis, and for comparative quantitative data on the possible carcinogenic potential of proposed asbestos substitutes.

TABLE 6 - Incidence of pleural mesotheliomas in Sprague-Dawley rats treated by a single intrapleural injection of 25 mg of sedimentary erionite in 1 ml of H₂O, and then kept under observation until spontaneous death.

Results after 67 weeks.

Exp. BT 2103

Compounds	Sex	Animals (Sprague-Dawley rats, 8 weeks old at start)		Tumours at the site of injection		
		No. at start	Survivors	No.	%	Latency time (weeks)
Erionite (sedimentary)	M	20	7	12	60.0	56
	F	20	11	9	45.0	57
	M+F	40	18	21	52.5	56
H ₂ O (Controls)	M	20	20	0	—	—
	F	20	20	0	—	—
	M+F	40	40	0	—	—

The incidence of mesotheliomas over untreated Sprague-Dawley rats of the Institute of Oncology of Bologna, kept under observation until spontaneous death (Historical controls) was the following:

Peritoneal mesotheliomas: 3 out of 1,179 males and 0 out of 1,202 females

Pleural mesotheliomas: 0 out of 1,179 males and 1 out of 1,202 females

Pericardial mesotheliomas: 1 out of 1,179 males and 0 out of 1,202 females

TABLE 7 - Incidence of peritoneal mesotheliomas in Sprague-Dawley rats treated by a single intraperitoneal injection of 25 mg of asbestos of different types and origins in 1 ml of H₂O, and then kept under observation until spontaneous death.

Results after 67 weeks.

Exp. BT 2101

Compounds	Sex	Animals (Sprague-Dawley rats, 8 weeks old at start)		Tumours at the site of injection		
		No. at start	Survivors	No.	%	Latency time (weeks)
Crocidolite	M	20	5	14	70.0	50
	F	20	8	12	60.0	56
	M+F	40	13	26	65.0	53
Amosite	M	20	9	11	55.0	59
	F	20	10	10	50.0	56
	M+F	40	19	21	52.5	58
Anthrophyllite	M	20	11	7	35.0	53
	F	20	16	4	20.0	55
	M+F	40	27	11	27.5	54
Chrysotile (California)	M	20	13	5	25.0	55
	F	20	16	2	10.0	51
	M+F	40	29	7	17.5	54
Chrysotile (Rhodesia)	M	20	18	0	—	—
	F	20	18	1	5.0	49
	M+F	40	36	1	2.5	49
Chrysotile (Canada)	M	20	18	1	5.0	65
	F	20	19	0	—	—
	M+F	40	37	1	2.5	65
H ₂ O (Controls)	M	20	20	0	—	—
	F	20	20	0	—	—
	M+F	40	40	0	—	—

The incidence of mesotheliomas over untreated Sprague-Dawley rats of the Institute of Oncology of Bologna, kept under observation until spontaneous death (Historical controls) was the following:

Peritoneal mesotheliomas: 3 out of 1,179 males and 0 out of 1,202 females

Pleural mesotheliomas: 0 out of 1,179 males and 1 out of 1,202 females

Pericardial mesotheliomas: 1 out of 1,179 males and 0 out of 1,202 females

3. IMPORTANCE OF THE PHYSICAL-CHEMICAL CHARACTERISTICS OF THE TEST-COMPOUNDS AND OF THE BIOLOGICAL AND EXPERIMENTAL FACTORS

The data reported in table 7 point out the primary role of the physical-chemical characteristics of the test-compounds in the determination of their carcinogenic potency.

They also underline the need to avoid rough manipulation of test materials which may change their physical-chemical characteristics.

Moreover, the data obtained at present indicate that the tumour response (production of mesotheliomas) depends both on the type of the compound and on the exposed serosa (table 8). Erionite, which is the most effica-

Compounds	Intrapertitoneal injection			Intrapleural injection		
	Animals	No.	%	Animals	No.	%
Crocidolite	M	20	70.0	M	20	0
	F	20	60.0	F	20	0
	M+F	40	65.0	M+F	40	0
Amosite	M	20	55.0			
	F	20	50.0			
	M+F	40	52.5			
Anthrophyllite	M	20	35.0			
	F	20	20.0			
	M+F	40	27.5			
Chrysotile (California)	M	20	25.0			
	F	20	10.0			
	M+F	40	17.5			
Chrysotile (Rhodesia)	M	20	—			
	F	20	5.0			
	M+F	40	2.5			
Chrysotile (Canada)	M	20	5.0	M	20	0
	F	20	—	F	20	0
	M+F	40	2.5	M+F	40	0
Erionite	M	20	—	M	20	12
	F	20	5.0	F	20	9
	M+F	40	2.5	M+F	40	21
H ₂ O (Controls)	M	20	—	M	20	0
	F	20	—	F	20	0
	M+F	40	—	M+F	40	0

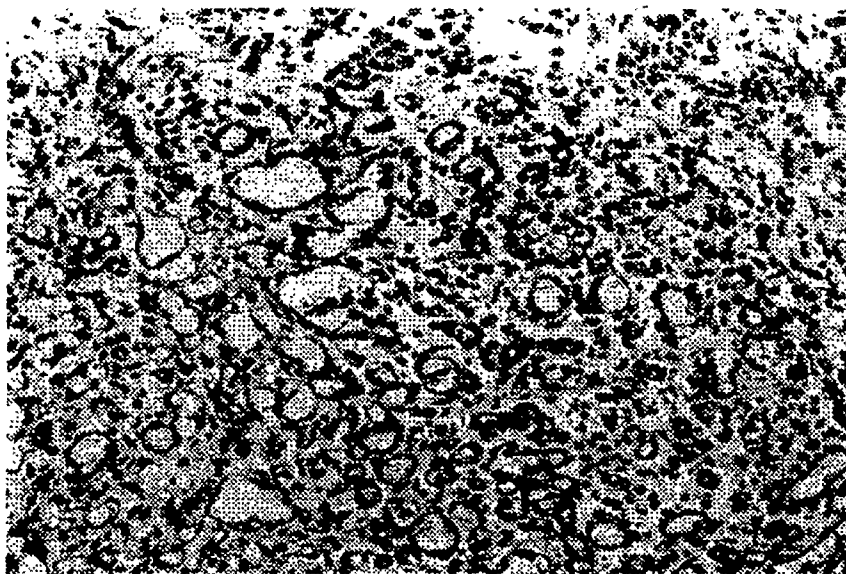


Fig. 1 - Pleural mesothelioma from erionite: tubular pattern.
H. - E. x 200

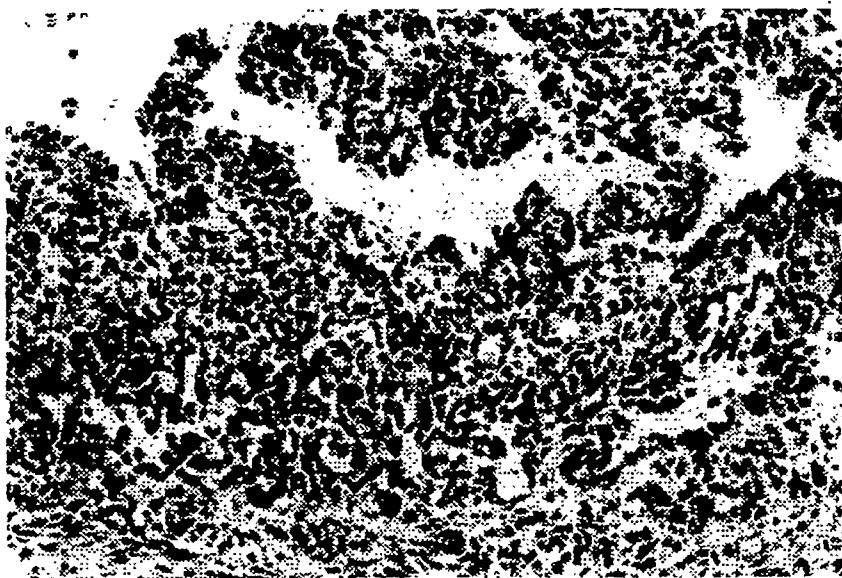


Fig. 2 - Pleural mesothelioma from erionite: tubular-papillary pattern.
H. - E. x 200

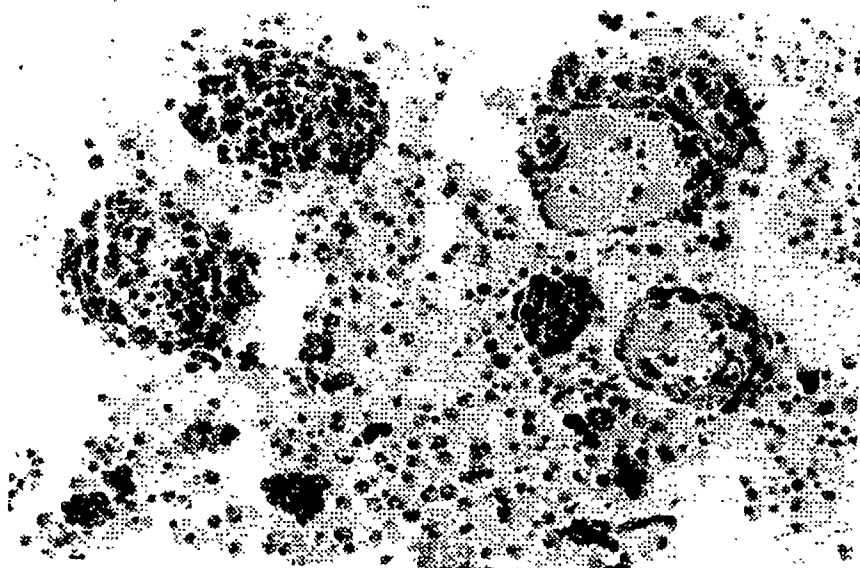


Fig. 3 - Pleural mesothelioma from erionite: papillary pattern (serous effusion).
H. - E. $\times 200$

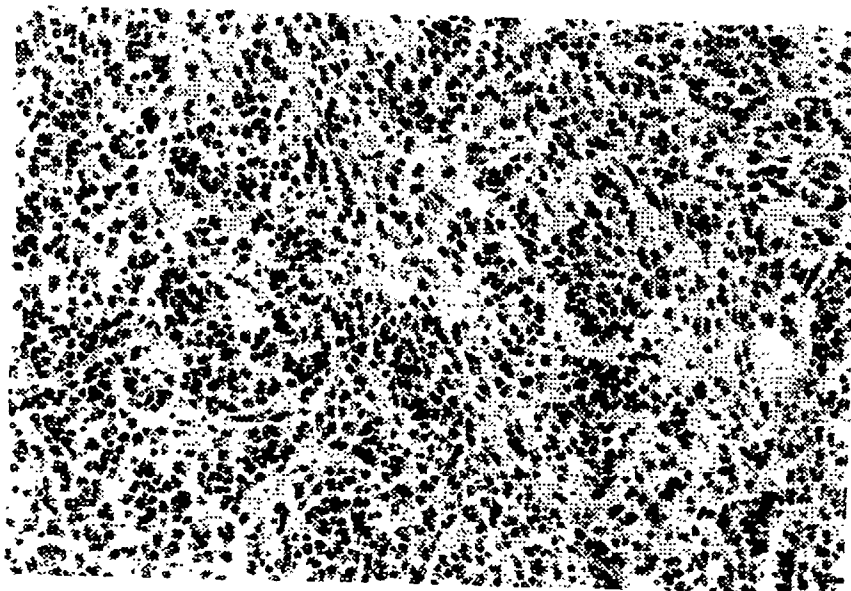


Fig. 4 - Pleural mesothelioma from erionite: solid (trabecular) pattern.
H. - E. $\times 200$



Fig. 5 - Pleural mesothelioma from erionite: spindle cell type with epithelial pattern.
H. - E. $\times 80$

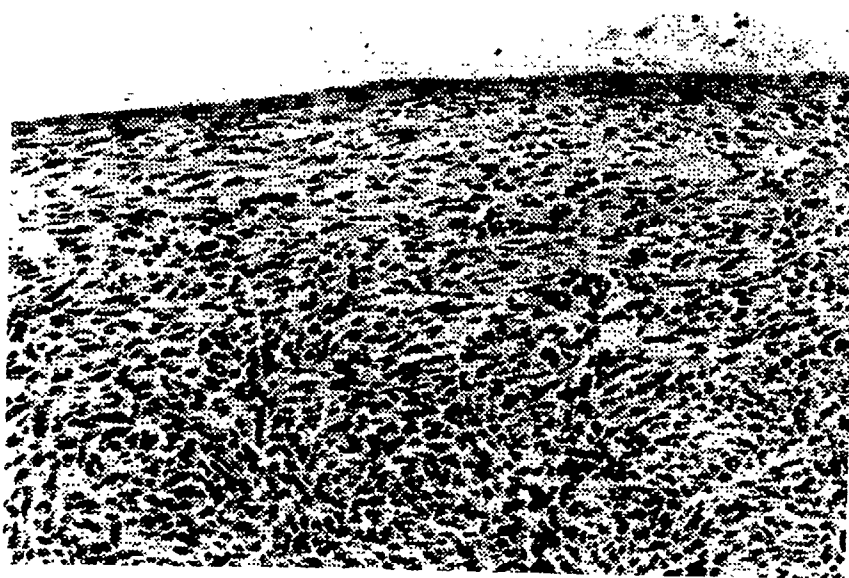


Fig. 6 - A detail of fig. 5.
H. - E. $\times 200$

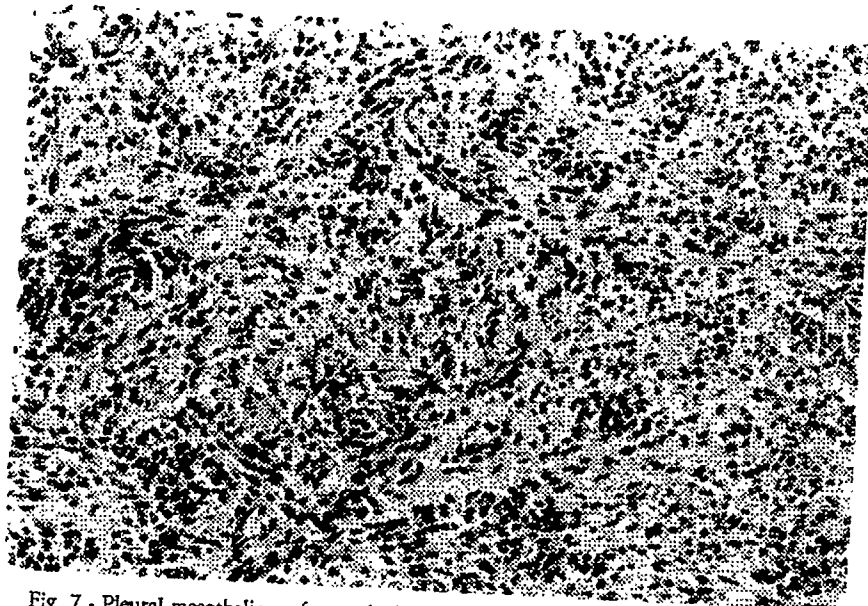


Fig. 7 - Pleural mesothelioma from erionite: spindle cell type with sarcomatous pattern.
H. - E. $\times 200$



Fig. 8 - Pleural tubular mesothelioma associated to fibrous nodules and plaques, from erionite.
H. - E. $\times 80$

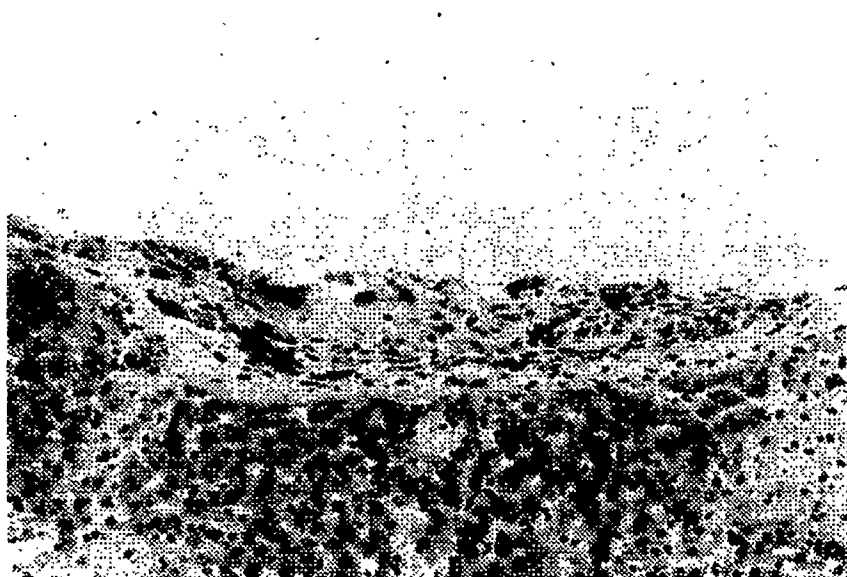


Fig. 9 - Peritoneal fibrous plaque from crocidolite: asbestos deposits can be seen within the plaque.
H. - E. x 200

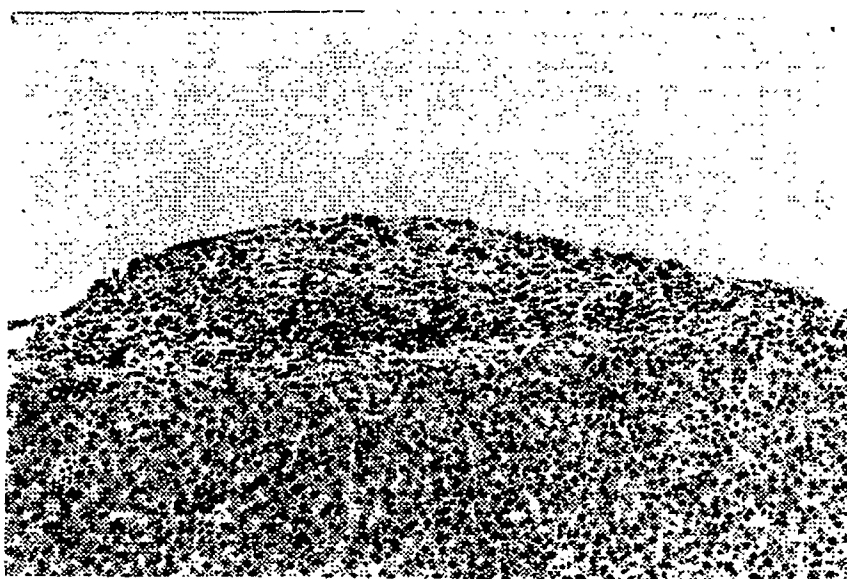


Fig. 10 - Peritoneal cellular (active) plaque from crocidolite.
H. - E. x 80

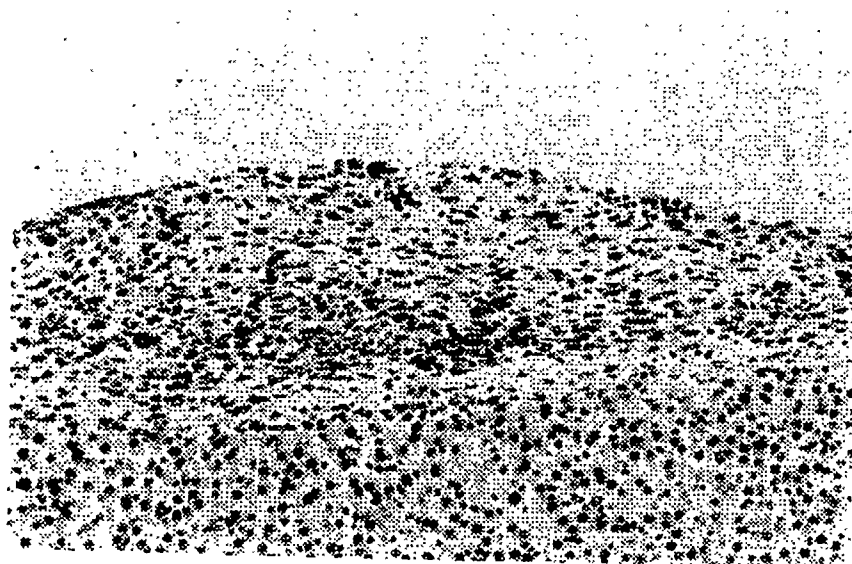


Fig. 11 - A detail of fig. 10.
H. - E. x 200

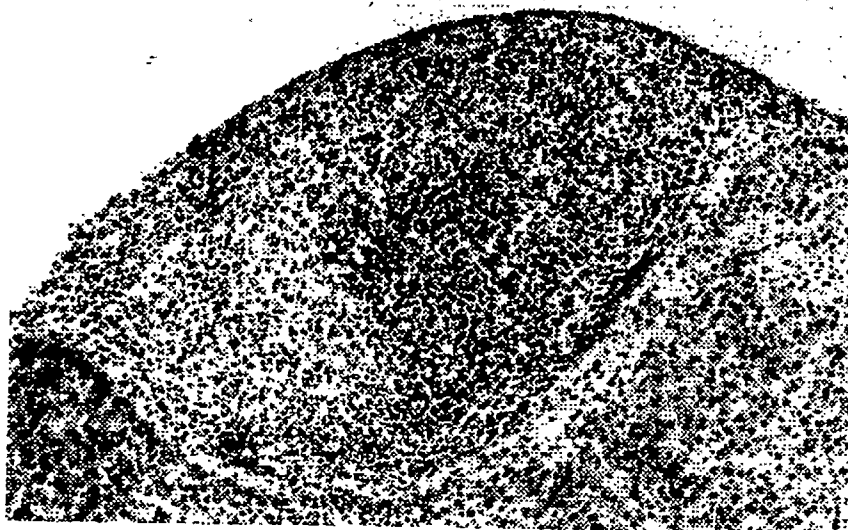


Fig. 12 - Peritoneal mesothelioma from crocidolite arising within an active plaque.
H. - E. x 128

cious in producing pleural mesotheliomas, when injected intrapleurally, has a far lower potency in producing peritoneal mesotheliomas when injected intraperitoneally. The opposite has been observed in the bioassays of different types of the tested asbestos.

4. NATURAL HISTORY OF MESOTHELIOMAS WITH REFERENCE TO PRECURSORS

Some early observations of ours show that mesotheliomas often arise in correspondence with and within pleural and peritoneal localized fibrosis (plaques and nodules) (figures 8-12).

The matter of plaques as precursor lesions of human pleural mesotheliomas is controversial. The experimental data can help to clarify the role of the plaques as a precursor, and the degree of their potential to undergo mesotheliomatous transformation.

We expect that, from the progress and expansion of our project, clarifications may be produced on the carcinogenicity of particles with some direct contribution to the most urgent current public health problems, particularly dealing with asbestos and its substitutes.

Furthermore, we hope that closer, more

productive collaborations may take place among researchers concerned with this problem, working in experimental laboratories and in epidemiological units.

SUMMARY

The project of long-term carcinogenicity bioassays of fibrous and non-fibrous, organic and inorganic, natural and man-made particles, which is going on at the Bologna Institute of Oncology, is presented.

The up-to-date results show that: 1) erionite is the most potent known experimental carcinogenic agent for the pleural mesothelium, in rats; 2) on the rat, erionite is more carcinogenic for the pleura, and asbestos more carcinogenic for the peritoneum; and 3) asbestos of different types and origins show sharp differences in their capability of producing mesotheliomas of the peritoneum, in rats.

The contribute of experimental approach to the identification and quantification of the oncogenic risks of particles is briefly discussed.

REFERENCES

- BARIS Y.I., ARTVINLI M. and SAHIN A.A.: Environmental mesothelioma in Turkey. In: I.J. Selikoff and E.C. Hammond: *Health hazards of asbestos exposure*. Annals N.Y. Acad. Scienc., 330, 423 (1979).