KVALITATIV OG KVANTITATIV KARTLEGGING AV FIBRE

VED

SYDVARANGER A/S

DELRAPPORT III - CELLEFORSØK

HD 849/80

INNHOLD

FORORD

FORORD

Undersøkelsene beskrevet i denne rapporten er basert på et samarbeid mellom A/S Sydvaranger og Yrkeshygienisk Institutt. Yrkeshygienisk Institutt har videre søkt samarbeid med Medical Research Council's, Pneumoconiosis Unit i Penarth, Wales. Undersøkelsene på cellekulturer er utført ved ovennevnte institutt av doktorene R. Davies, R.C. Brown, M. Chamerlain and D.M. Giffiths. Overingeniør B. Gylseth har sammen med Verneavdelingen, A/S Sydvaranger preparert støvprøven. Førstnevnte er ansvarlig for dette prosjektet.

I INNLEDNING

Cellekulturer brukes i utstrakt grad idag for testing av forskjellige støvtypers biologiske aktivitet. Spesielt fiberforming eller fiberholdig støv har vært viet stor oppmerksomhet. Det var av interesse å teste støv fra A/S Sydvaranger på denne måten, og Yrkeshygienisk Institutt tok i denne sammenheng kontakt med Medical Research Council's Pneumoconiosis Unit i Penarth, Wales. Dette instituttet har lang erfaring med testing av støv i cellekulturer. Taconittstøvet ble testet i 3 av deres cellelinjer.

På grunn av at også andre støvtyper utenfor Sydvaranger-prosjektet ble testet og beskrevet i samme rapport, er kun detaljene som angår taconittstøv, trukket ut og vedlagt som rapport.

II MATERIALE

Bulkstøv fra A/S Sydvaranger ble siktet til - 325 mesh (43 µm) og deretter våtsedimentert for fremskaffelse av fraksjonen < 5 µm. 250 mg av denne fraksjonen ble oversendt MRC i Penarth.

III METODER

Støvet ble testet i 3 cellelinjer. For detaljer vises det til vedlagte rapport.

IV RESULTATER

For detaljer vises det til vedlagte rapport.

V SAMMENDRAG (fritt oversatt)

Taconittstøvet viste ingen cellegiftighet i noen av de 3 celletypene det ble testet på. Det kan virke overraskende at støvet som inneholdt 38 ± 5% kvarts ikke hadde noen virkning på makrofagene (eteceller). Imidlertid, kvartsen i taconitt kan opptre som store partikler som ikke fagocyteres (uskadeliggjøres) av makrofager. Disse forsøkene på celler i kultur kan ikke tas som noe entydig bevis for at taconittstøv ikke kan medføre helsefare ved eksponering idet den beste støvtypen for testing av cellegiftighet er respiabelt støv som ikke var tilgjengelig i denne undersøkelsen.

VI VIDERE FORSØK

Det er av interesse å gå videre med disse undersøkelsene, og en foreslår at det fremskaffes respirable støvprøver som er anriket på de aktuelle mineralene. Dette kan muligens gjøres ved at magnetitt fraskilles ved magnetseparering under eller etter prøvetagningen av respirabelt støv.

The In Vitro Cytotoxicities of Tobermorite and Taconite

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In vitro tests are increasingly being used to assess the biological activities of dusts (Brown et al, 1980). At our laboratory we have developed various in vitro screening tests which attempt to predict the pathogenicity of mineral dusts in animal experiments. These tests involve examination of the effects of dusts towards mouse peritoneal macrophages, chinese hamster lung cells (V79 cell line) and human alveolar type II tumour cells (A549 cell line).

There is a correlation between the cytotoxicities of dusts towards macrophages in vitro and the ability of the dusts to cause fibrosis in animal experiments (Marks and Nagelschmidt, 1959; Davies et al, 1980 and Davies, 1980).

Some dusts are cytotoxic towards the V79 cell line and cause the production of giant cells in the A549 cell line. These dusts have a fibrous morphology and contain fibres which are longer than about 8μ and with a diameter of less than 1μ. (Brown et al, 1978; Chamberlain and Brown 1978; Chamberlain et al, 1979). Stanton and Layard (1978) have concluded from a series of 37 mineral dusts implanted in the pleural cavity of rats, that only those dusts containing fibres longer than 8μ and less than 1.5μ in diameter induce pleural tumours. Thus for fibrous dusts there is a good correlation between the in vitro findings and the in vivo observations.

Dr. Gylseth was also interested in our comments on taconite dust which contained some amphibole fibres. Taconite is a low grade iron ore mined in

Norway: The Norwegian Institute of Occupational Health is at present studying the incidence of lung cancer at a taconite mine.

Materials and Methods

1.1. Mineral dusts.

Stocks of UICC crocidolite (Timbrell and Rendall, 1971) are held at MRC Pneumoconiosis Unit.

The taconite sample was dust from a crushing department and sieved to 325 mesh (43μ) and is PU Code No. 85 parent, but was not examined in this study. This material was sedimented in distilled water and a fine fraction collected by Dr. Gylseth, this sample is PU Code No. 85a.

1.2 Macrophage cytotoxicity test.

The detailed methods used have been reported previously (Chamberlain et al, 1979), the main modification being the determinations of enzyme activities which were carried out using the continuous flow fluorimetric method of Morgan et al, (1978). In summary unstimulated mouse peritoneal macrophages (1.5×10^6 cells/35 mm petri dish) were maintained in culture with dust levels (given in the text) for 18 hours. The culture medium (M199) contained 10% acid-treated, heat-inactivated foetal calf serum. At the end of the culture period the cytotoxicity of a dust was assessed by measuring the proportion of the cytoplasmic enzyme lactate dehydrogenase (LDH) released.

The release of a lysosomal enzyme β glucuronidase (βGLU) was also examined.

1.3 Cell lines

The methods have been described in detail elsewhere (Chamberlain and Brown , 1978) . In summary, Chinese hamster lung cells (V79-4) were grown in Eagle's minimal essential medium (MEM) plus 15% foetal calf serum and antibiotics. The cells were cultured with dusts for 6 days, and the cell colonies obtained counted using an automatic colony counter (Micro Measurements Ltd., Cambridge). Cytototoxic dusts reduce the colony numbers.

Human alveolar epithelial type II lung tumour cells (A549) were grown in Dulbecco's MEM plus 10% foetal calf serum and antibiotics. The cells were cultured with dusts for 4 days and the formation of giant cells assessed by their measurement using a digital caliper syste (Lea et al, 1980).

1.4 Dust characterisation

Some information regarding chemical composition and size was provided by Dr. Gylseth. However since the 3 samples under investigation were not respirable dust fractions it was important for us to have some information regarding the size of the particles present in the 3 samples. Dust samples were thus examined by transmission electron microscopy and sized according to the methods used by Brown et al, (1978).

Results and Discussion

1.1 Morphological characterisation of dusts

The taconite samples Fig. 9-12 contained material in the respirable size range but also contained some very large particles (15μ diameter). Some fibrous particles are clearly present.

1.2 Mineralogical characterisation of dust samples In the interpretation of the cytotoxic effects of dusts some information on the contaminating minerals present may be useful. Dr. Gylseth provided us with the following information on the samples under investigation.

The taconite sample contained magnetite and 38 $\pm$ 5% quartz. The fibrous particles present include the amphiboles hornblende and grunerite.

1.3 Effect of dusts on macrophages

The effect of the 3 dusts and UICC crocidolite is shown in Fig. 13. Following the exposure of macrophages to UICC crocidolite there was a rise in the release of LDH from the cells into the culture medium. This indicates that the dust was toxic towards the cells. A similar but larger effect was observed with the fibrous tobermorite (Fig. 13c) indicating that this material is also cytotoxic towards macrophages.

The effect of dusts on the release of βGLU was also studied. It has been suggested that the selective release of this enzyme without concomitant cell death can be caused by many materials which cause chronic inflammation (Davies and Bonney, 1979).

There is a pronounced release of βGLU from crocidolite and fibrous tobermorite at levels significantly higher than the release of LDH, an observation we have made for many pathogenic fibrous dusts. However, strictly speaking the release is not selective since the dusts were also shown to be cytotoxic in so far as they caused the release of LDH.

Whilst the taconite had little effect on enzyme release the platy tobermorite causes the selective release of βGLU. We have also observed this before for synthetic calcium silicates, and more recently for wollastonite (A fibrous calcium silicate). The interpretation of these results must be made with caution as in our hands inert materials like chalk and gypsum also cause a selective release of βGLU.

1.4 Effect of dusts on V79-4 cell line

The effects of the 3 dusts and UICC crocidolite is shown in Fig. 14. UICC crocidolite is cytotoxic towards these cells. On the other hand, platy tobermorite and taconite were without any effect.

1.5 Effect of dusts on A549 cell line

The effect of the 3 dusts and UICC crocidolite is shown in Table 2. Whilst UICC crocidolite produced a singificant proportion of giant A549 cells the 3 dusts under investigation were without any demonstrable activity.

General Discussion

The taconite sample had no cytotoxic effects on any of the 3 cell types used in this report. It may seem surprising that a material containing 38 ± 5% quartz was without any effect towards macrophages. However, the quartz present in the taconite sample may be present as large particles whi may not have been phagocytosed by macrophages. These in vitro results cannot be taken as a clean bill of health for taconite, as the best sample for cytotoxic examination - a respirable dust sample was not available for the study. It is possible that a respirable fraction may be enriched in t mineral fibres which were seen by electron microscopy. Fine quartz parti may also be present in a respirable dust sample.

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TABLE 2

Percentage of A549 cells greater than 25 μm in diameter

Treatment	Dust dose μgs/ml 0	100	200
Control	0 (0 - 1.9)		
UICC crocidolite		19.0 (14.2 -25.0)	18.7 (13.9 -24.7)
Taconite PU No. 85a		2.5 (1. 1 - 5.7)	3.0 (1.4 - 6.4)

The 95% confidence limits are given in brackets.

ig. 9-12 Taconite 1 cm = 1.6 μ

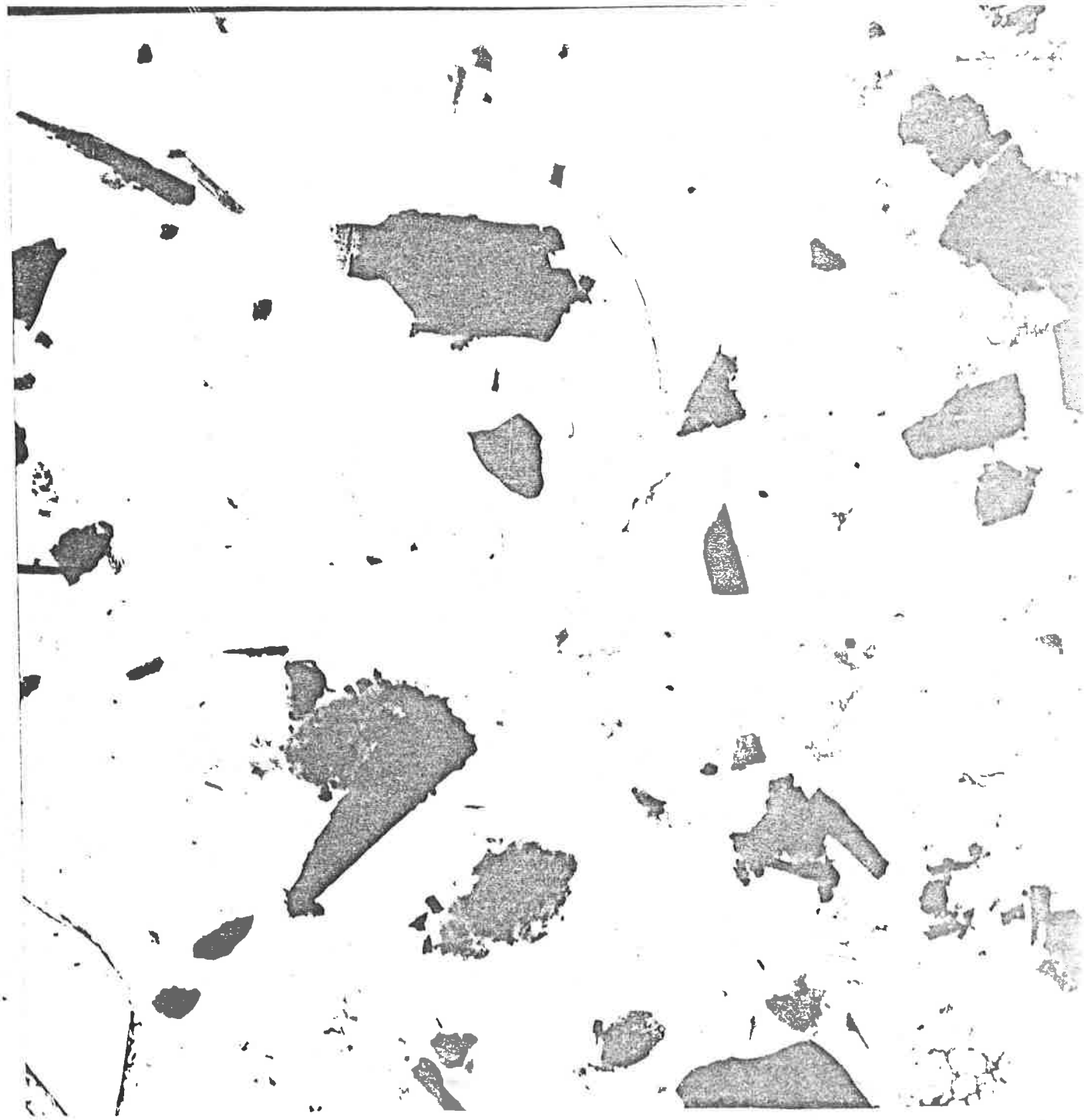

Fig. 9-12 Taconite 1 cm = 1.6 μ

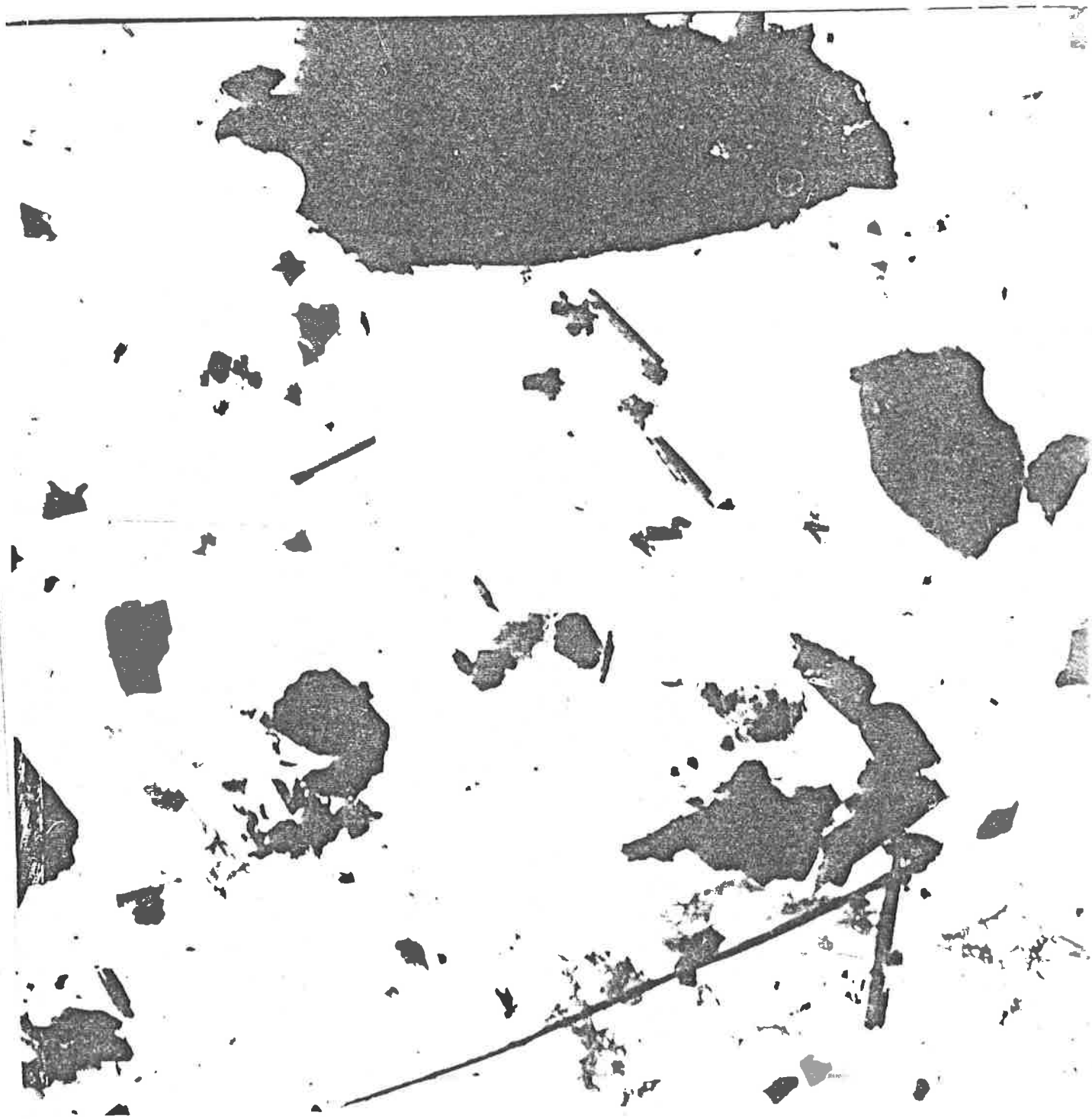

Fig. 9-12 Taconite 1 cm = 1.6 μ

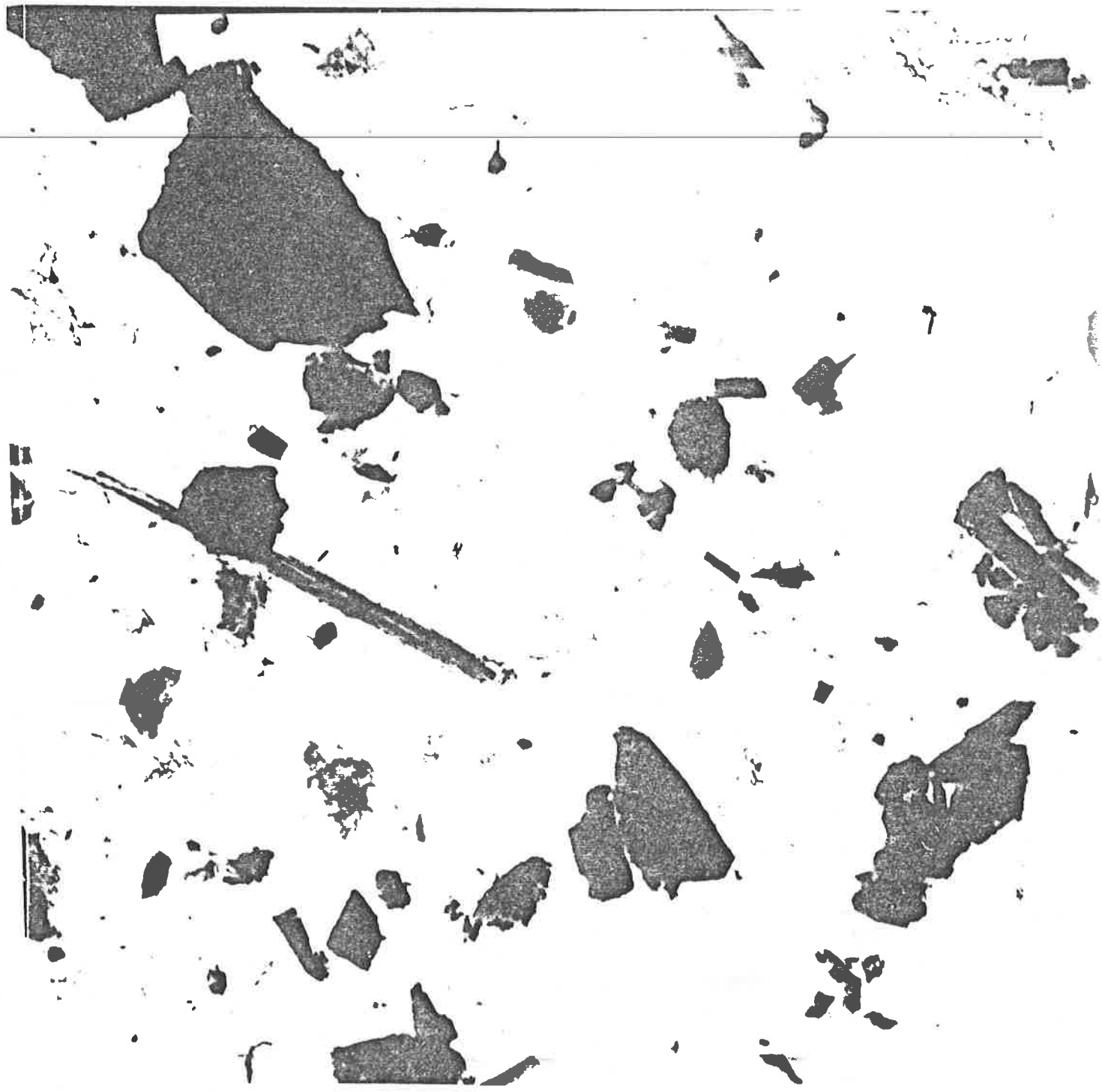

Fig. 9-12 Taconite 1 cm = 1.6 μ

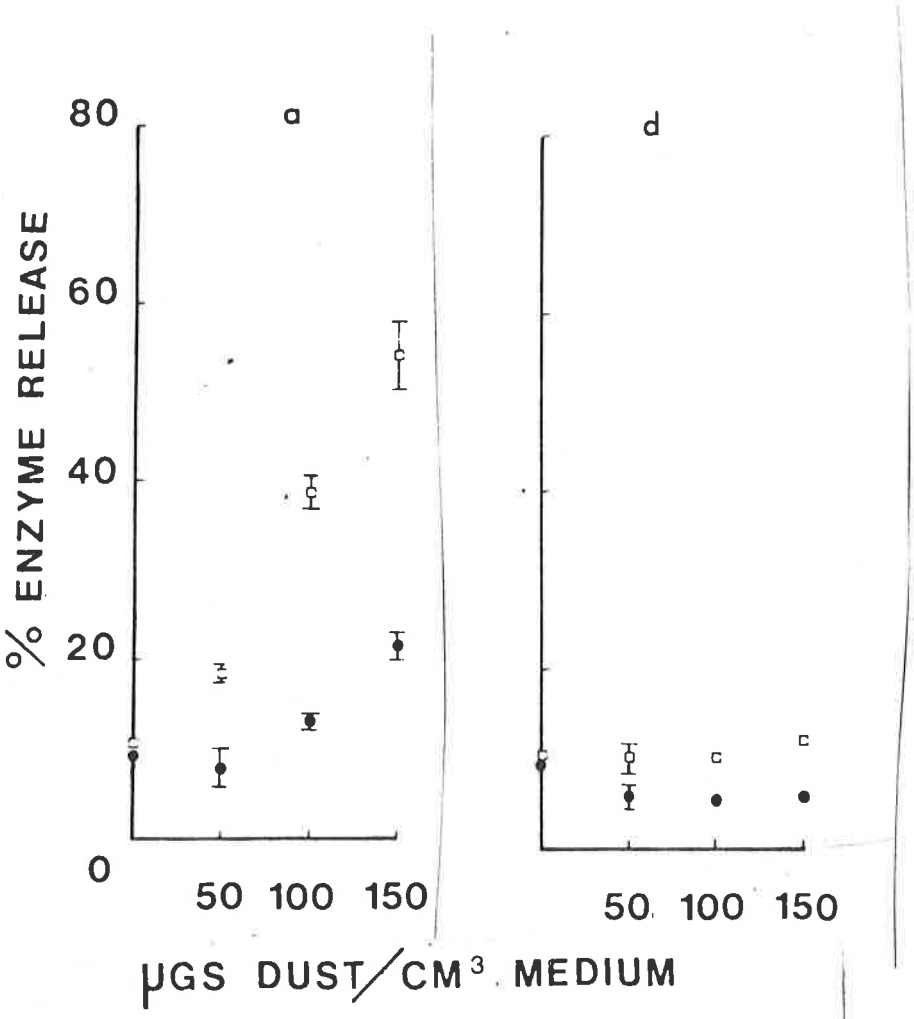

Fig. 13 The effect of dusts on enzyme release from macrophages.

a UICC crocidolite

d Taconite PU No. 85a

● LDH □ BGLU

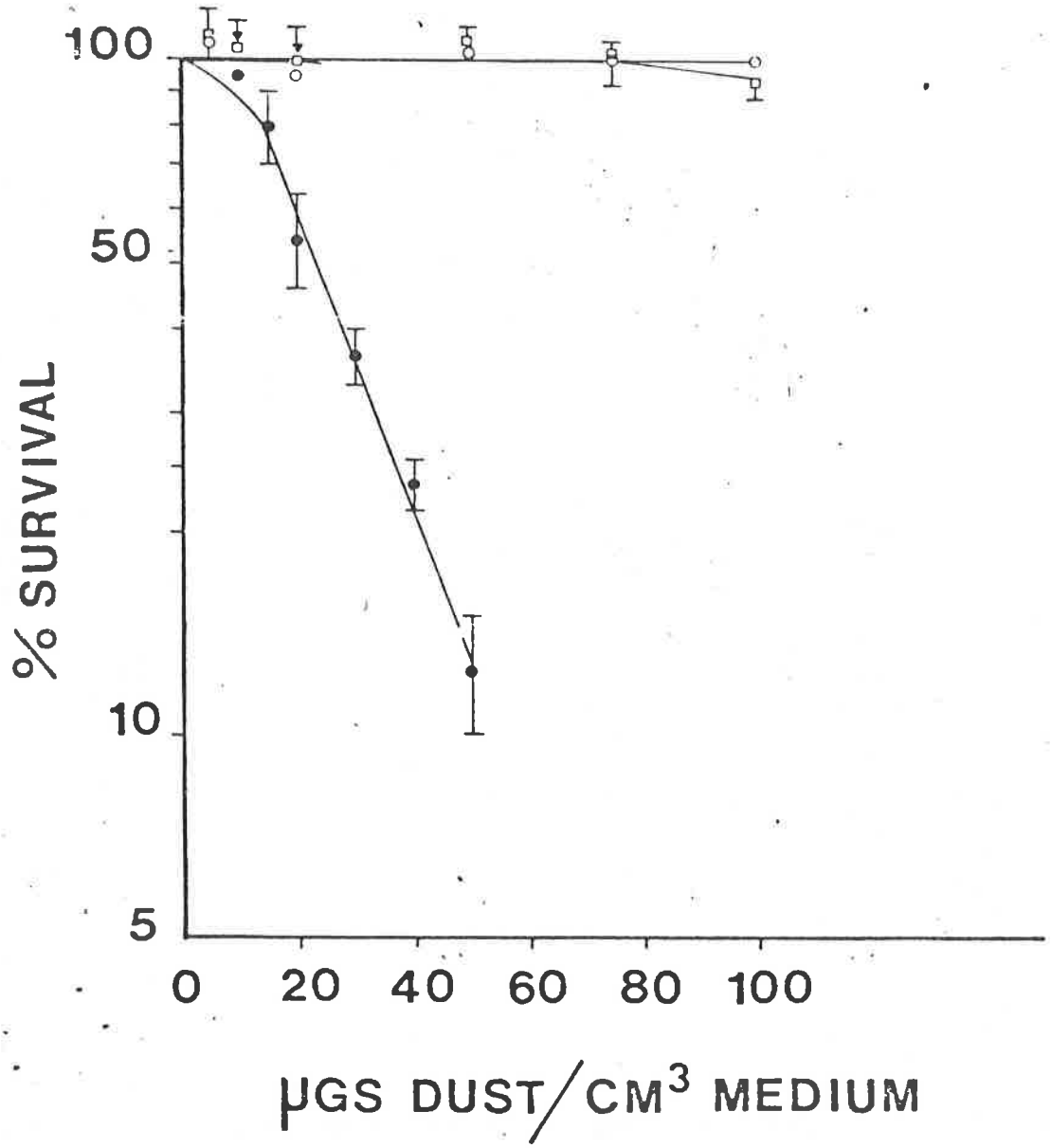

Fig. 14 The effects of dusts on survival of V79-4 cells.

● UICC crocidolite

□ Taconite PU No. 85a