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ASBESTOSIS RESEARCH COUNCIL

ELEVENTH ANNUAL REPORT FOR THE TWELVE MONTHS BEGINNING 1st OCTOBER, 1967

1. INTRODUCTION

The Asbestosis Research Council was established on the 1st October, 1957. This report details the activities sponsored by the Council during the eleventh year of its operation.

2. CONSTITUTION

The Constitution was revised in August 1965 to extend the field of activities to asbestos operations other than textile and to provide for the admission of Associate Members as well as Full Members.

3. MEMBERSHIP

The Membership of the Council during the year was as follows :-

Full Members

B.B.A. Group Limited.
The Cape Asbestos Company Limited.
Turner & Newall Limited.

Home Associate Members

The Atlas Stone Company Limited.
Bell's Asbestos and Engineering Limited.
T.B. Ford Limited. (In place of Birfield Limited).
Richard Klinger Limited.

The Universal Asbestos Manufacturing Company Limited ceased to be an Associate Member on becoming part of the Cape Asbestos Company Limited.

Overseas Associate Members

James Hardie & Coy. Pty. Ltd.
Johns-Manville Corporation.
The Quebec Asbestos Mining Association.
Griqualand Exploration & Finance Co. Ltd.

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(c) Dust Control Committee

Chairman :	Mr. J.M. McFarlane	- The Cape Asbestos Co. Ltd.
	Mr. E. Boyes	- B.B.A. Group Ltd.
	Mr. T.W. Anderson	- The Cape Asbestos Co. Ltd.
	Mr. P.E. Bugge	- Turner & Newall Ltd.
	Mr. R.H. Wilson	- Turner & Newall Ltd.
	Mr. W.P. Bamblin	- Turner & Newall Ltd.
Secretary :	Dr. S. Holmes	- Turner & Newall Ltd.

This Committee was re-constituted in July, 1968, as the 'Environmental Control Committee' under the Chairmanship of Mr. A.A. Cross. It held its first meeting on 22nd August, 1968.

Dust Control Committee meetings were held as follows :-

Roohdale	- 24th January, 1968
Hebden Bridge	- 5th June, 1968

5. FINANCE

(a) Banking Arrangements

The finances of the Council have continued to be administered through accounts operated by the Roohdale Branch of the District Bank.

(b) Budget and Expenditure

Council Budget	£23,500
Expenditure	£19,130

6. LIAISON WITH OTHER INTERESTED BODIES

Contact has been maintained with H.M. Factory Inspectorate and the Panel convened by the Senior Medical Inspector of Factories, on which two of the Council's Medical Officers served, has published its Report entitled 'Problems arising from the use of Asbestos'.

Liaison has continued with the Pneumoconiosis Research Unit of the Medical Research Council. The opportunity was taken during the meeting of the Research Committee at Cardiff in May to have a joint one-day meeting with the Unit to discuss common problems. Arrangements have been put in hand to organise a joint ARC/PRU Conference in April, 1970 on 'The Tissue Reaction of Asbestos'.

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The Sub-Committee set up by the Hygiene Committee of the British Occupational Hygiene Society, on which members of the Council served, published a Report in April 1968, entitled 'Hygiene Standards for Chrysotile Asbestos Dust'.

Liaison has also continued with the U.S. Public Health Service and with the Air Hygiene Committee of the Asbestos Textile Institute.

7. RESEARCH PROGRAMME

The research work of the Council was further expanded during the year by Grants to Dr. Margaret Turner-Warwick of the London Hospital of Diseases of the Chest and Dr. Margaret Swinburne of St. James' Hospital Leeds.

A summary of the year's work is given below. Fuller reports appear in Appendices 1 to 5.

(i) The long term animal experiments involving the inhalation of the four main types of asbestos have been completed during the year, but many of the sections are still to be examined. The studies on the mechanism of asbestos body formation have also been completed. Collaboration with the Institut für Lufthygiene in Düsseldorf has continued.

(ii) The collection of biopsy material from suspected cases of human mesothelioma has continued and the production of mesothelial tumours in animals has been further studied. Work on the pathogenicity of minerals other than asbestos has been started. Collaboration has continued with Dr. Gross of the U.S. Industrial Hygiene Foundation in Pittsburgh.

(iii) Preliminary work on the effect of asbestos on lysosomal cell systems terminated in June, 1968.

(iv) Work on the surface properties of asbestos has continued.

(v) An immunological study has been started but only preliminary results are yet available.

(vi) Animal experiments on the ingestion of asbestos have commenced and it is hoped to have some results in the next twelve months.

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8. DUST SAMPLING AND ANALYSIS

While the Royco Automatic Particle Counter has continued to be used as a routine monitoring instrument, the fibre count by membrane filter has remained the standard method in the industry. The method has been published by the Council as its Technical Note No. 1.

Techniques for assessing the asbestos content in other than industrial environments have been further explored. Interest has been shown in this subject by a number of Government Departments and a meeting of those involved was convened by the Ministry of Technology in May, 1968. Representatives of the Council were present.

During the year, the Council has carried out a survey to determine the level of asbestos dust in completed buildings of various types in which asbestos products have been incorporated in their construction. A report has been prepared for publication.

9. DUST CONTROL

During the first part of the year, the Dust Control Committee continued to work on various problems affecting the manufacturing and applications side of the industry. In July, 1968, however, this Committee was reconstituted with the title of 'Environmental Control Committee' and with wider terms of reference to enable it to deal with all relevant matters likely to affect the industry and its customers under the New Asbestos Regulations.

10. THE NEW ASBESTOS REGULATIONS

In April, 1968, representatives of the Council were called to a meeting at the Department of Employment and Productivity to discuss the Council's comments on the preliminary draft of the New Regulations. It was learnt that the Council's suggestions were likely to be given consideration in framing the Statutory Draft, in particular the replacement of the 'no dust' concept by a more practical approach to the problem.

The Statutory Draft was published in August, 1968, and the Council again felt obliged to make a number of comments, which are still under discussion.

11. CODES OF PRACTICE

During the year the Council issued its fourth Code of Practice - 'Recommended Code of Practice for Handling Consignments of Asbestos Fibre in British Ports'.

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The demand for copies of all Codes of Practice has continued steady.

12. OTHER PUBLICATIONS

"The Mechanism of Formation of Asbestos Bodies"
S.K. Botham and P.F. Holt, J. Path. and Bact. Vol. 96, No. 2, p. 443.

"Interaction of Poly-2-Vinyl Pyridine 1-Oxide and Poly-4-Vinyl Pyridine Oxide with Monosilicic Acid"
P.F. Holt and E.P. Nasrallah, J. Chem. Soc. 1968, p.400.

13. PULMONARY FUNCTION TESTS

The work done by the Member Companies in this field has continued to be reviewed by the Research Committee.

14. VISITS

In November, 1967, Mr. Waddell, Dr. Gaze, Dr. Knox, Dr. Smither, Dr. Beattie, Dr. Davis and Dr. Holmes attended the Second Antigua Conference organised by the Quebec Asbestos Mining Association.

Dr. Addingley, Dr. Knox, Dr. Smither, Dr. Davis, Dr. Holt, Dr. Lewinsohn, Mr. Hunt and Dr. Holmes attended the International Conference on the Biological Effects of Asbestos held in Dresden in April, 1968.

15. GENERAL

Dr. Knox has continued as Medical Consultant to the Council and has attended the meetings of the Management Committee and the Research Committee.

Dr. Beattie has continued as Research Consultant and has attended the meetings of the Research Committee.

APPENDIX .

Report on Research at the University of Reading - Dr. Holt

During this year most of the long term experiments started 2 to 3 years ago, in which guinea pigs and rats inhaled four types of asbestos, have been completed. This has required a heavy load of tissue processing and cutting to provide about 3,000 stained sections. It has been impossible to examine many of these sections while the experimental work was proceeding and consequently few final results have been reported. There is now available, however, a library of sections in which the effects on the lungs and lymph glands of reasonably small doses of inhaled amosite, anthophyllite, chrysotile and crocidolite can be studied. There is also a series of slides in which guinea pigs are compared with rats that have inhaled asbestos. These are unique.

The following tentative conclusions need to be confirmed. The asbestos dust is first deposited in the bronchioles, the longest fibres (up to 70 μ long) in the terminal bronchioles only. Remarkably, some fibres subsequently appear to move into the alveoli which is contrary to what would be expected. No histological difference has yet been observed between the effects of the four types of asbestos but a few pleural adhesions were present in animals that had received amosite and crocidolite but none in those that received anthophyllite, chrysotile or glass fibre. No clear morphological difference has been observed between the asbestos bodies produced on the four types of asbestos; those produced by glass fibre are similar except that, since they form round single fibres rather than fibre bundles, they are usually smaller. In a search of other organs for evidence of transport of asbestos from the respiratory system in a guinea pig, so far one perfectly formed asbestos body has been found in the thyroid gland.

The studies on the mechanism by which asbestos bodies are produced have now been completed. The initial effect of the fibre in the lung is the production of haemorrhage and the resulting iron-containing compounds from the haemoglobin in the red cells are taken up by dust cells and deposited on an asbestos fibre in the same cell. The production of an asbestos body does not therefore depend on the chemical nature of the fibre and we have produced bodies from mica fibres and very fine glass fibres. This last experiment is being extended and some guinea pigs have been made to inhale glass fibre and others glass powder of the same chemical composition.

An experiment has been concluded which was set up to investigate whether the progress of pneumoconiosis due to asbestos could be followed in animals by chemically estimating the collagen formed in the damaged lungs. The results are disappointing; the variation between animals is at least as great as the variation caused by small doses of asbestos.

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Now that the fundamental work with animals is well established attention is being turned to the methods of modifying the pathological effects of asbestos. Studies are continuing on the possible use of polymeric substance such as poly-2-vinylpyridine 1-oxide, which has proved effective in silicosis in animals and on whether this polymer can alter the rate of fragmentation of asbestos bodies. The possible coating of the asbestos with an adsorbed monolayer, either to modify its effects or to prevent its being retained in the lung, is also being investigated. The initial studies on adsorption are now well under way and early results will be reported during next year.

calcified pleural plaques, histologically very similar to those found in human cases. In the mice the pleural fibrosis had often developed to a degree where the pleural cavity was completely full and the lungs encased by masses of fibrous tissue. The macroscopic picture was exactly that of a mesothelioma, but histological examination showed no signs of neoplasia. It would appear that this massive fibrosis is caused by a turnover of dust. When the initial dust granuloma ages the macrophages and giant cells die and are replaced by fibrous tissue. The asbestos dust is left behind among the collagen fibrils and is rephagocytosed by a new generation of macrophages that actively penetrate the fibrs tissue to pick up the dust. This turnover process appears to continue as long as dust is present and very large masses of fibrous tissue eventually resulted. There is one type of fibrous mesothelioma that pathologists find often difficult to distinguish from normal fibrous tissue. These fibrous masses are in fact only considered tumours because they grow to a very large size. From the mouse evidence it is possible that they may not be tumours but normal fibrous tissue constantly stimulated to new growth by a relatively indestructable content of asbestos dust.

Experiment to test the pathogenicity of minerals other than asbestos

During the last twelve months the following minerals have been tested by intra-pleural injection in mice :- Talc, serpentine, brucite, magnetite, pyroxene, olivine, forsterite and chlorite. So far, all these minerals have failed to produce lesions other than the normal foreign body reactions. The results of injection experiments with fine glass fibre (dia. 0.05 μ) and calcium silicate are still under review. Follow-up experiments involving inhalation on all these materials are planned.

Experiments with P204

During the first part of this year experiments continued which were designed to test the effect of P204 on the development of asbestos lesions in guinea pig tissue. With the chrysotile sample used, no difference could be found between animals treated with dust + P204 and those that had received dust only. For this reason, the experiments were terminated in March. During September, however, some experiments were started using samples of synthetic chrysotile injected intraperitoneally into mice. This dust has proved much more toxic than normal asbestos, and it may be worth repeating the P204 experiments with this dust.

Experiments to determine the chemical processes of asbestos body formation

Two studies are at present in progress in this field. These involve the electron microscope examination of very old calcifying

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asbestos lesions and very young lesions in the guinea pig pleural cavity. Normal asbestos bodies are produced intracellularly and perhaps as few as one in one thousand fibres of dust ever become coated. It has been found, however, that in old calcifying lesions, which are acellular and where the dust is now lying free among the collagen fibrils, all asbestos fibres become coated with a layer of material about as thick as the normal asbestos body coating. This coating is much less dense than the 'ferritin' of normal asbestos bodies although some 'ferritin' granules may be present among the other coating material. From a study of the substances known to occur in calcifying tissues it appears most likely that this coating material is a mucoprotein or mucopolysaccharide. Since colloidal iron is a well known stain for mucin, and is effective because the iron selectively impregnates the mucin, it begins to appear likely that asbestos body formation may be a two stage process, an initial coating of mucoprotein or mucoplysaccharide being followed by the impregnation of this material with 'ferritin'. The density of 'ferritin' in normal asbestos bodies might be due to its high content in living granulation tissue, and the low content in the calcifying tissue bodies may be due to lack of 'ferritin' in these dead avascular area. An examination of very early asbestos lesions is now in progress and already some dust fibres have been found in giant cells coated with low density material containing only a few granules of 'ferritin'.

APPENDIX I

Report on Research at the Strangeways Laboratory - Dr. Barrett (Work terminated, June 1968)

Work on Macrophages

It has been found possible to maintain mouse peritoneal macrophages for six days in Medium 199 (Burroughs Wellcome) supplemented with 10% unsuckled calf serum in an atmosphere of 5% CO_2/95% air, on coverslips, but a serious difficulty arose in that the available assay techniques proved not to be sufficiently sensitive to detect the release and synthesis of enzymes by the small number of cells (a few million) that could be isolated and maintained. A more sensitive method has however, only recently been developed and this should prove invaluable in future experiments of this nature.

The effect of Chrysotile on the Stability of Lysosomes

A rat liver was homogenised in 0.25 M sucrose, and a mitochondrial-lysosomal preparation was obtained by differential centrifugation. This was suitably diluted and incubated at 37°, either alone or with a rather dense suspension (10 mg/ml) of finely milled chrysotile asbestos (supplied by Dr. P.F. Holt). Over a period of three hours, the mixtures were gently agitated and samples were removed periodically and centrifuged. Determination of the enzyme activity in the supernatant and sedimented fractions gave a measure of the extent to which the limiting membranes of the lysosomes had broken down, under each treatment to release the enzymes in a free, soluble form. Workers have shown that quartz can act by rupturing lysosomal membranes and it seemed likely that asbestos would have a similar effect, in view of its haemolytic activity, which was comparable with that of silica, but no such effect was detected; parallel release of acid proteinase was obtained in suspensions with or without asbestos. In view of a recent German report on the action of asbestos on lysosomes in vitro further experiments of this type might be justified.

The Cellular Consequences of Phagocytosis

Lysosomes form the digestive system of cells, and in many ways this is comparable with that of higher animals. When a macrophage engulfs a bacterium, it is enclosed in a digestive vacuole, or phagosome, the contents of which are acidified. The lysosomal enzymes, which are active under acid conditions, are then secreted into the vacuole and the bacterium is digested. It is known that the biosynthesis of the lysosomal enzymes is stimulated by the process of phagocytosis in macrophages (and the same may well apply to the macrophages that take up asbestos, if they survive). It is not

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thought that this is solely a characteristic of phagocytic leucocytes since various tissue cell types can show phagocytosis and a lysosomal response, in tissue culture. Generally the lysosomal digestive enzymes produced in response to the phagocytosis are not confined to the interior of the cells, but are released into the culture medium, and in organ cultures of cartilagionous bone rudiments the result is an extensive digestion of the extracellular matrix strongly reminiscent of that seen in rheumatoid arthritis. It is felt that excessive lysosomal activity of this kind may well be involved in the pathology of rheumatoid arthritis and certain other conditions, and investigations have been done on the way in which endocytosis (a general term including pinocytosis and phagocytosis) stimulates the synthesis and release of the lysosomal enzymes.

The biological system used is one in which chick embryonic limb bone rudiments are cultured in a synthetic medium to which sucrose has been added. Surprisingly, the cells are unable to metabolise sucrose, and it causes the formation of persistant large digestive vacuoles within them, but there is no cell mortality and cell division is scarcely inhibited. There is, however, a dramatic increased in the release of lysosomal enzymes. The radiochemical microassay is designed to follow the time-course of the new synthesis with precision, and an inhibitor of protein synthesis, cycloheximide, is being used in an attempt to decide what proportion of the enzyme release represents newly-formed, as opposed to pre-existing molecules.

Further work has led to the preparation of a pure rabbit anti-chicken acid proteinase antiserum with which it is hoped to isolate minute amounts of the enzyme synthesised in culture in the presence of radioactive tracers, as the immunoprecipitate. This would allow changes to be followed in the specific activity of the released enzyme which could give valuable insight into the mechanism of the stimulated release.

Appendix 4

Report on Research at the University of Leeds - Dr. Grimshaw

During the last twelve months period the research programme has been directed mainly to studying the release phenomena of magnesium and silicon from chrysotile under a variety of conditions. A raw Canadian fibre was selected for detailed analysis, but other samples have been shown to possess similar characteristics.

Both magnesium and silicon can be extracted from raw fibre under most pH conditions. The amounts were greater in acid solutions but the ratio of $MgO : SiO_2$ in solution bore no relationship to the stochiometric composition of the parent mineral.

Once the fibre had been ground, the amounts of cation release increased enormously and to a far greater extent than would be expected from simple surface area considerations. Chrysotile which had been ball-milled for a few hours still possessed a typical fibrous habit when examined under the microscope, but it was possible to extract virtually all its structural magnesium. Comminution processes also caused marked changes in X-ray, infra-red and differential thermal patterns.

Perhaps the most significant observation of the release phenomenon in treated chrysotile is the fact that the absorption capacity of the mineral was increased considerably. Not only was water absorbed but also cations other than magnesium and many organic compounds and complexes. Stearic acid and some amino-acids were absorbed and the mechanism involved chemical as well as physical phenomena. It has been shown by infra-red studies that the characteristic spectra of certain organic groups changes markedly when absorbed onto the treated chrysotile fibres.

APPENDIX 5

Report on Immunology Study - Dr. Turner-Warwick

In collaboration with Dr. Parkes of the Pneumoconiosis Medical Panel, patients referred to the Panel for assessment have been examined in person and it is hoped that this special series will form the basis of a long-term follow-up study. In addition blood sera supplied by co-operating chest clinics and industry have also been examined. Included in the study so far have been 53 patients and 116 blood sera samples.

Antinuclear factors and rheumatoid factors have been studied. Using the double-layer immunofluorescent technique, 20% were found to be A.N.F. positive, against an expected figure of 5% in a 'normal' population. Assessing rheumatoid factor by the differential agglutination titre technique (DAT), the percentage DAT positive was 37, against an expected less than 4% in a 'normal' population. Using Latex F11 tests (Latex RA), the percentage positive was 39. Antinuclear factors and rheumatoid factors have therefore been demonstrated more frequently than expected by chance in patients with asbestosis. Furthermore, the incidence of these antibodies is remarkably similar to findings in idiopathic fibrosing alveolitis. The next study is to analyse the clinical, radiographic and physiological features correlated with immunological re-activity.

A search for lung reactive antibody has been undertaken in 24 patients using the antiglobulin consumption test (AGCT). It appears that antilung reactive antibodies are present only in low titre and are unlikely to be an important immunological finding in asbestosis.

Immunofluorescent studies have been carried out on three asbestotic lungs. There was no evidence of antibody nor complement fixed to any structure surrounding asbestos bodies within the lung.

General Conclusion

Circulating non-organ specific tissue antibodies have been found in patients with asbestosis. Moreover the incidence of these is similar to that found in fibrosing alveolitis. These findings suggest that a similar immunological stimulus occurs during the development of both these lung diseases. Whether the immunological response is damaging or protective is unknown. An attempt to get further evidence for or against a pathogenetic role of these antibodies (or some immunological reaction occurring pari passu with them) will now be sought through a study of these antibodies in different stages of evolution of the disorder, using clinical, radiographic and physiological data as the yardsticks to assess the extent and severity of the disease.