HARING 196

S.A. TYDSKRIF VIR GENEESKUNDE

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STUDIES OF THE MODE OF ACTION OF ASBESTOS AS A CARCINOGEN*

J. S. HARINGTON, Cancer Research Unit of the National Cancer Association of South Africa, SAIMR, Johannesburg, F. J. C. ROE AND M. WALTERS, Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, London, UK

'Asbestos' is the generic name given to a class of fibrous mineral silicates which may vary considerably in physical and chemical composition.¹ The demonstration of differences in the carcinogenicity between different types of asbestos, either in epidemiological or experimental studies, would clarify greatly the mode of action of each type of fibre.

ASBESTOS AS A CARCINOGEN

Two types of malignancy in man are associated with exposure to asbestos; carcinoma of the lung and mesothelioma of the pleura and peritoneum.

There is a very strong association between asbestosis and carcinoma of the lung. The percentage of cases with asbestosis dying of lung cancer ranges from 14% to 20%² and may even rise to 50%.^{3,4} Thus in persons with asbestosis the death rate for cancer at this one site exceeds that for cancers of all sites in those not exposed to asbestos.

In the case of mesothelioma little is known of the incidence in the general population, or in groups with different degrees of exposure to asbestos. When total exposed populations were taken into account, Harington⁵ in his study of the prevention of mesothelioma found that the percentage of deaths due to this cancer of the total deaths of those exposed to asbestos ranged from 0.2% to 27%. Thus, the incidence of mesothelioma is much lower than that of bronchogenic carcinoma in the same population exposed to the same extent.

Although there is suggestive evidence that the risk of developing mesothelioma might increase with exposure to asbestos, the subject awaits further investigation.

Malignant neoplasms of the digestive organs appear to be 2-3 times as frequent in persons exposed to asbestos as in those not exposed.⁵

Epidemiological Considerations

The epidemiological considerations relating to asbestos malignancy in man have recently been discussed by Harington and Roe⁶ and by Harington.⁵ Exposure to crocidolite in the north-western Cape carries with it a distinct risk of developing malignant disease. There is a three-fold excess of deaths from lung cancer as compared with adjoining districts, and as compared with control districts of similar population and degree of urbanization.⁴

Similar figures for cancer mortality in the eastern and

north-eastern areas, where chrysotile and amosite asbestos are mined and milled, showed no excess mortality from any form of cancer. Wagner and his colleagues⁸ found that crocidolite only was associated with pleural mesoihelioma, and no cases have been reported in persons exposed only to amosite." This is not due to differences in the commercial production of these two fibres^{10,11} or to other factors studied.12

Chrysotile asbestos seems to have been implicated in the production of mesotheliomas^{9,13} and in the USA this type of asbestos may be actively involved as an aetiological agent."

In general, it would appear that exposure to crocidolite is a greater cancer hazard for man than exposure to amosite and chrysotile (at least in South Africa) although it would be certainly premature to decide until further investigation of the populations actually exposed have been carried out.

Experimental Considerations

Results of animal experimentation so far available suggest that crocidolite and chrysotile are more active in inducing mesotheliomas than amosite.⁶ Wagner,¹⁵ in 1962, induced mesotheliomas by inoculating various dusts directly into the pleural cavity of rats. Pleural tumours were found in a few animals inoculated with crocidolite and chrysotile asbestos and none in animals which received amosite. In a study still in progress, Wagner¹⁶ has so far found (in individual groups of 100 injected animals) the following numbers of pleural mesotheliomas; 12 from crocidolite, 18 from chrysotile and 2 from amosite. In recent work at the Chester Beatty Research Institute, we failed to obtain pleural mesotheliomas in rats after the animals had been inoculated with amosite asbestos.¹⁷

POSSIBLE MODES OF ACTION OF ASBESTOS AS A CARCINOGEN At present the following possible mechanisms of carcinogenesis are under consideration:

1. That carcinogenesis is due entirely, or in part, to organic materials associated with the fibre, either naturally or as a result of contamination or treatment of the asbestos during processing.

2. That it is due to the presence of certain carcinogenic metals or metal-complexes in asbestos.

3. That it is an 'Oppenheimer effect', that is to say, that cancer arises as a result of prolonged residence in the tissues of a chemically-inert material which cannot be removed or can only be slowly removed by phagocytosis.18

800

26 Augustus 1967

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26 August 1967

S.A. MEDICAL JOURNAL

TABLE I. RESULTS OF EXPERIMENTS INVOLVING INTRAPLEURAL INJECTION OF NATIVE AND EXHAUSTIVELY-EXTRACTED ASBESTOS INTO RATS¹

No. rats No. rats No. rats Treatment Other with thick-(25 mg. amosite/0.4 ml. saline with No. rats No. rats Survival with mesothemalignant lesions ening of (months after injection) injected once into right pleural at start of examined pleura lioma lymphoma experiment postmortem cavity) 5, 11, 11, 11, 12, 12, 12, 12, 12, 13, 13, 13, 13, 14, 14, 14, 14, 14, 15, 16, 16, 22, 22, 22, 22 Ave. = $14 \cdot 3$ 5, 6, 8, 11, 11, 13, 13, 13, 14, 14. 0 7 0 24 24 Amosite 5, 6, 8, 11, 11, 13, 13, 13, 14, 14, 14, 15, 16, 17, 19, 21, 22, 22, 23, 0 1 adenomatous 3 1 24 20 Extracted amosite ... hyperplasia of lung 23 Ave. = 158, 12, 12, 13, 13, 13, 13, 13, 13, 16, 16, 16, 17, 18, 19, 20, 20, 20, 21, 21, 22, 22, 23, 24, 24 0 1 23 0 24 Saline only ..

Ave. = 17.2

Oils and Other Organic Materials Associated with Asbestos

Three types of asbestos are used industrially; crocidolite, amosite and chrysotile. The first two of these contain appreciable amounts of natural (primary) oils which may be removed to a large extent by extraction with suitable solvents.^{1,19} Crocidolite, amosite and chrysotile may also contain secondary oils as a result of the addition of oil to the asbestos during processing, or as a result of contamination. An account of the natural and secondary oils which may be found in asbestos has been given by Harington.1

Biological Tests for Carcinogenicity

In order to assess the carcinogenic role of asbestos oils and other extractable material, the following experiments have been undertaken:

- (a) Tests of native and extracted asbestos for carcinogenicity in subcutaneous tissues of rats and mice.
- Tests of native and extracted amosite inoculated (b) intrapleurally in rats.
- Tests for carcinogenic and co-carcinogenic activity of (c) oils associated with asbestos: (i) tests of amosite oil and crocidolite oil for initiating activity by application to the skin of mice (followed by croton oil applications), and (ii) tests of jute oils for carcinogenicity and tumour-promoting activity on mouse skin.

(a) Tests of native and extracted asbestos for carcinogenicity in subcutaneous tissues of rats and mice. These experiments are still in progress. To date, a small number of injection-site sarcomas and mesotheliomas have been found in rats and mice injected with native and extracted amosite and with native and extracted crocidolite. The injection of chrysotile has so far failed to produce either type of the above tumours in rats, but has produced one injection-site sarcoma and a mesothe-lioma in the mice (but see Addendum).

(b) Tests of native and extracted amosite inoculated intrapleurally in rais. After the association between exposure to asbestos and mesothelioma had been recognized,^{*} attention was diawn to the occurrence of 4 cases of lung cancer in was drawn to the occurrence of 4 cases of hing cancer in persons apparently exposed to amosite asbestos in a factory.³⁰ Samples of the actual asbestos fibre to which the patients had been exposed gave an oil yield of 0.76% fibre.¹ In the biologi-cal tests undertaken by us, the carcinogenic activity of native amosite fibre and amosite exhaustively extracted with a series of solvents' were compared."

Two groups of 25 rats each were injected intrapleurally with 25 mg. of each sample. No mesotheliomas were found in either group at the conclusion of the experiment which ran to a maximum length of time of 17 months (Table I). This experiment indicates that under the conditions in which it was conducted neither sample of amosite was care

which it was conducted, neither sample of amosite was carci-

nogenic. On the other hand, the failure to get tumours may be due to the poor survival rate of the animals, 14-17 months (Table I). Wagner¹⁶ obtained a number of pleural mesothe-liomas in rats within 18 months, using different types of asbestos, including amosite.

(c) Tests for carcinogenic and co-carcinogenic activity of oils associated with asbestos - (i) tests of amosite oil and crocidolite oil for initiating activity by application to the skin of mice (followed by croton oil applications). It has already been pointed out' that considerable batch-to-batch variation in the amount of extractable organic and oily materials present in asbestos is to be expected. There is also no way at present known of distinguishing between natural and contaminating oils associated with asbestos fibres. Since wide variation in quantity and quality is to be expected, it follows that the results obtained from experiments using asbestos oils must needs be related to one batch of asbestos only, i.e. that which

needs be related to one batch of assestos only, i.e. that which provided the oil for the actual experiment. In the experiment described below, between 150 ml. and 200 ml. of oil were extracted from about 450 kg. of mixtures of specially milled finely-ground virgin and commercial fibres of both amosite and crocidolite, according to the procedure previously described.²¹ Significantly more polycyclic hydro-carbons were detected in amosite oil than in crocidolite oil,¹ although in both cases the amounts of these compounds were so low that their contribution to any total effect of the oils could be regarded as very slight.

could be regarded as very slight. For the test for initiating effects by skin painting, stock mice were randomly divided into 3 groups of 30 each. Group 1 received both 100% crocidolite oil and 0.1% croton oil (in acetone); group 2, 100% amosite oil and 0.1% croton oil; and group 3, acetone and 0.1% croton oil. After a schedule of treatment described elsewhere,²¹ results were obtained as shown in Table II. in Table II.

TABLE II. DEVELOPMENT OF SKIN TUMOURS IN RESPONSE TO TREATMENT WITH CROCIDOLITE AND AMOSITE OILS IN CONJUNCTION WITH CROTON OIL (SUMMARIZED FROM ROE et al.²¹)

Group	Treatment	% of survivors which developed skin tumours	Total skin tumours seen	Total malignant skin tumours
1	Crocidolite +	51.7	36	0
2	Amosite +	66.6	40	4
3	Acetone + croton oil	6.9	2	0

Treatment with croton oil and acetone only evoked, as expected,²² a weak tumour response. Treatment with both crocidolite oil and croton oil (group 1), or both amosite and croton oil (group 2) elicited significantly more papillomas than treatment with croton oil and acetone only (group 3). In group 2, malignant skin tumours were also seen. It is concluded that both test oils possess tumour-initiating activity.

In comparison with other agents, such as 7,12-dimethylbenz-[a]anthracene, the tumour response seen with the two oils is weak.²² It is possible, indeed likely, that the asbestos oils supplied by themselves would elicit tumours though not as many as in combination with croton oil treatment. Unfortunately, it was not possible to test the oils for complete carcinogenicity because of insufficient material.

(ii) Tests of jute oil for carcinogenicity and tumour-promoting activity on mouse skin. Asbestos is normally transported and stored in jute bags and the oil from these bags is absorbed to an appreciable extent (70 - 85%) by crocidolite, amosite and chrysotile.¹ Such heavy contamination of asbestos would only be of significance in relation to carcinogenesis if the oils themselves were shown to have relevant biological activity. Accordingly, samples of each of two types of oil used in jute manufacture were tested for carcinogenicity and when given after a sub-carcinogenic dose of 7,12-dimethylbenz[a]anthracene exhibited marked tumour-promoting activity. Although no malignant tumours were seen, the results indicate that the light mineral oil probably possessed weak carcinogenic activity. The second oil, a 'soluble' oil (an oil-water emulsion), was inactive both as a carcinogen and a promoter.

These findings suggest that the storage of asbestos in jute bags may introduce a significant variable into subsequent determinations of its carcinogenic activity.

Since the oils are quite minor constituents of asbestos, it is difficult to believe that they play a major role in carcinogenesis by asbestos.²¹ Also, although initiating and promoting activity have been satisfactorily demonstrated, this has only been done by very large doses in relation to the amounts naturally present.

But in certain circumstances, the effect of small amounts of carcinogens may be greatly enhanced by the presence of substances of low chemical reactivity. Thus Shabad *et al.*²⁴ induced lung tumours in rats by mixing benzo[a] pyrene with India ink, while with the carcinogen alone they were unable to do so. Also Saffiotti *et al.*²⁴ have induced lung tumours in hamsters by combinations of iron oxide and benzo[a]pyrene, but not by the latter alone.

Harington and Smith²⁵ suggested that asbestos in the oilfree state may have either tumour-initiating activity additional to that possessed by the oils, or tumour-promoting activity in its own right. In the latter case carcinogenicity could be explained by the combined action of the initiating activity of the oils and the promoting activity of the fibre.

Recent studies by Miller et al.26 suggest that some type of combined carcinogen-co-carcinogen reaction of the type mentioned above²⁵ may be a property of certain types of asbestos and not of others. Such considerations may help to explain the more potent activity of crocidolite than that of amosite. It was found by Miller et al.26 that intratracheal injections of amosite did not increase the yield of tracheobronchial tumours induced in hamsters by benzo[a]pyrene. This points to a weak or negligible promoting property for this type of asbestos. There are indications" that amosite, either native or extracted, is not a strong carcinogen and that this type of asbestos possesses weak or negligible promoting (and initiating) activity. Clearly, in this particular situation, amosite has little modifying effect on any initiating activity which its oil may have possessed,²¹ that is, within the conditions of this particular experiment. This is important to stress because Wagner's experiments in progress show²⁷ that 29 out of 96

rats injected intrapleurally with amosite developed mesotheliomas compared with 51 out of 96 for crocidolite and 58 out of 96 for chrysotile. Thus, amosite, although a weaker carcinogen than crocidolite and chrysotile, may not be insignificant in activity.

The relatively weak promoting and initiating activity of amosite, however, differs from that found in chrysotile. Miller and his colleagues²⁶ found that this form of asbestos apparently promoted benzo[a]pyrene carcinogenesis but too few animals were used for the results to be significant. More extensive studies²⁶ have shown that chrysotile does promote the carcinogenic effect of benzo[a]pyrene. Crocidolite has not yet been tested in this type of experiment.

To conclude, an important experiment to be carried out concerns the determination of any promoting activity which may be possessed by 'oil-free' crocidolite asbestos when this is injected with benzo[a]pyrene, on the lines of the work done with amosite and chrysotile by Miller et $al.^{26}$

General Conclusions: Asbestos Carcinogenesis and Organic Matter

The following provisional conclusions can be drawn with regard to asbestos carcinogenesis in so far as this is affected by associated oils and other organic matter:

- 1. Single samples of both crocidolite and amosite oils have been found to have tumour-initiating activity.
- 2. A sample of jute oil exhibited marked tumour-promoting activity and was also weakly carcinogenic. (Jute oils may contaminate asbestos to a considerable extent during storage and transport.)
- 3. Amosite appears to possess weak or negligible initiating and promoting activity. This is weak in respect of the action of amosite on its contaminating oils¹⁷ and on benzo[a]pyrene.²⁶ Also, amosite is generally a weak carcinogen.^{5,15-17,27}
- 4. Both crocidolite and chrysotile appear to be more potent carcinogens than amosite. Chrysotile apparently promotes benzo[a]pyrene carcinogenesis;⁵⁶ for crocidolite this is not yet known.
- 5. It may be suggested that the use of oils isolated from asbestos may not give unequivocal results in tests for carcinogenesis because of batch-to-batch variation in content and composition. It is possible that more meaningful results may be obtained with the use of 'oil-free' (i.e. exhaustively-extracted) asbestos in conjunction with a single 'classical' carcinogen, e.g. benzo[a]pyrene. In this way the extent to which individual components of the various types of asbestos possess complete or incomplete carcinogenic (i.e. initiating or promoting) activity could be more easily assessed.

Metal Carcinogenesis and Asbestos

Asbestos as a Metal Complex

Quite apart from any contribution which may be made by organic matter in asbestos, the possibility that metals may be involved in asbestos carcinogenesis seems to merit further consideration.^{1,6}

Many metals have been shown to be capable of inducing cancer.²⁹ Among the more notable ones are arsenic, chromium, nickel, cobalt, cadmium, lead and beryllium.

802

26 August 1967

In addition, several other metals, though not apparently carcinogenic in the simple ionic state, become so when combined in certain macromolecular complexes. The best-known example of this is iron, which as ferrous sulphate or gluconate shows no carcinogenic activity but which in the forms of iron-dextran, iron-dextrin, saccharated iron oxide or ferrous glutamate is markedly active.³⁰⁻³² Aluminium is another example in so far as aluminium dextran is a carcinogen.³⁰

Against this background it is interesting to consider the metal contents of the three types of asbestos.

The amphibole asbestos types, crocidolite and amosite, are fibrous in nature and are complex silicates containing iron, magnesium, calcium and sodium in varying proportions. Crocidolite is a sodium ferroso-ferrisilicate containing 40% iron (as oxides), fairly equally distributed as ferric and ferrous forms. Amosite is a ferrous magnesium silicate and contains the same amount of total iron as crocidolite but mostly in the ferrous form.

The serpentine asbestos, chrysotile, is a magnesium silicate with 40% magnesium (as oxide) and relatively small amounts of iron (up to 2.5% as oxides), made up ot ferrous and ferric iron replacing magnesium in the structure.

The effect of introducing excess iron in a relatively stable state into tissues and cells has been discussed in detail elsewhere.⁶ In addition, it has also been suggested that quite apart from the absolute amounts of iron present, the ratio of ferric to ferrous iron may be important. Such data as are available suggest that carcinogenicity may be associated with a low ferric: ferrous ratio.⁶ In order to determine whether this association is valid, a large number of minerals with known contents of ferric and ferrous iron are being tested in mice by subcutaneous injection. These experiments are still in progress.

Trace Metals in Asbestos

The aspects so far discussed concern the part played by metals as integral features of the molecular structure of asbestos. Recently, however, spectrochemical trace analyses of different forms of asbestos carried out by Harington' have shown the presence of a number of extramolecular metals in relatively high concentration. Of these, at least three—lead, chromium and nickel—are known to be carcinogenic.³⁰ A single sample of chrysotile was found to contain 5 mg. nickel/G fibre (0.5%) and 1 mg. chromium/ G fibre (0.1%), suggesting that further studies of these metals in this type of asbestos might be profitable. Other metals appearing in relatively high concentration were zirconium, titanium and manganese in all three types of asbestos studied.

Separate experiments¹ have shown that significant amounts of these metals are eluted by serum kept in contact with asbestos for a period of 2 months.

In the case of crocidolite and amosite, the iron content is well in excess of that in certain macromolecular complexes of iron, in particular, iron-dextran, known to be highly carcinogenic in animals. The iron content of chrysotile, though much lower, may still be sufficient to account for carcinogenesis by a mechanism comparable with that of iron-dextran.

The possibility that the ferric: ferrous ratio rather than the absolute content of the iron is important in relation to carcinogenesis requires further study. Of the other metals in asbestos, the high nickel and chromium contents of chrysotile deserve the most attention.

Asbestos Carcinogenesis as an Example of Tumour Induction by Chemically Inert Materials In agreement with Schmähl,³³ we feel it is unlikely that

there is much in common between the mechanism by which asbestos induces cancer and what has been called the 'Oppenheimer effect'. In the first place, asbestos cannot be regarded as chemically inert in the same way as many of the materials studied by the Oppenheimers and their colleagues, and secondly, the essential characteristic of the Oppenheimer effect is that carcinogenesis only occurs when large bodies are implanted-no effect being seen when these materials are introduced in powdered form. Thirdly, and following on from the previous point, in the classical Oppenheimer effect tumours arise only at the site of implantation of objects and not at distant sites. Finally, the differences in carcinogenic activity between amosite and crocidolite, which are very closely related physically and chemically, would be difficult to justify in terms of the Oppenheimer effect. The relationship between cancer induced by chemical agents and the Oppenheimer effect has recently been discussed by Roe.¹⁸

DISCUSSION

The chemical and physical nature of asbestos is complex so that it would not be surprising if in the induction of cancer by asbestos, several different mechanisms were shown to be implicated. The following possible modes of action are worthy of consideration:

1. The asbestos fibre in the pure state (that is, not contaminated with organic matter or trace metals) may act as the carcinogen, possibly as a macromolecular iron or metal complex, in an analogous way to iron-dextran.

The carcinogenic activity could be investigated by the use of asbestos rendered as pure as possible, by solvent extraction and removal of trace metals (if this last is practicable).

2. Asbestos fibres in the impure (contaminated) state may act in conjunction with (a) oils and other organic matter, (b) trace metals known to be carcinogenic, (c) with both of these, or (d) with any radioactivity possessed by the fibres.

Oils (natural and contaminating) in at least one large batch of crocidolite and one of amosite have been found to possess tumour-initiating activity. In this case, it is conceivable that the fibre may act as a promoting agent. Alternatively, the fibre may act as a carrier for carcinogens—in the same way as India ink or hematite acted in experiments of Shabad *et al.*²³ and Saffiotti *et al.*²⁴

Jute oil, which is absorbed to a considerable extent by asbestos during storage, has been found in one instance to have marked tumour-promoting activity; this could act in conjunction with any intrinsic initiating activity possessed by the pure fibre or present in the natural oil.

Carcinogenic trace metals present in asbestos (in particular, chrysotile) may contribute to the final carcinogenic effect of this type of asbestos, although it seems unlikely that they could account for the entire carcinogenic effect.

The values obtained for the radioactive content of crocidolite, amosite and chrysotile' are so low that it seems clear that radioactivity plays no more than a very minor role in the induction of cancer by asbestos.

FUTURE EXPERIMENTATION

The rather complex picture painted above can perhaps be simplified by experiments with pure fibre with and without suspected contributory agents, in particular, organic matter and a pure carcinogen such as benzo[a]pyrene.

For such experimentation, it would seem advisable to extract all fibres exhaustively with a series of organic solvents (see Harington' for procedures) and to use such 'pure' asbestos in experiments on the carcinogenic and co-carcinogenic activity of asbestos, using preferably, intrapleural, intraperitoneal or intratracheal routes of administration.

In this way individual components of the 3 types of asbestos could be examined separately or in combination for complete or incomplete (i.e. initiating or promoting) carcinogenic activity. Where oils and organic matter are to be used in conjunction with pure fibre, they should be derived by extraction from native fibre before any possibility of contamination with extraneous oils or other materials, the extracted fibre serving as the 'pure' material. Organic matter and oils should also be used in amounts equivalent to those present on the actual unextracted fibre.

SUMMARY

Recent epidemiological studies of lung cancer and mesothelioma have revitalized the subject of the mode of action of asbestos as a carcinogen.

In this paper, possible mechanisms of carcinogenesis, and experimental methods of elucidating them, are considered from a biological standpoint. The significance of oils naturally pre-sent in asbestos and of oils purposely or accidentally added to it, and the possible role of metal constituents, either as part of the molecular structure of asbestos or as trace contamiof the molecular structure of asbestos, or as trace contaminants, are discussed in some detail.

ADDENDUM

Later work on the pathological effects of subcutaneous injec-tions of asbestos fibres in mice³⁴ has shown first, the apparently specific transport of asbestos fibres of all three types to the

sub-mesothelial tissues of the thorax and abdomen; and secondly, the development of extensive inflammatory and proliferative changes in these regions culminating, in 10 instances, in the formation of mesotheliomas. Six animals developed sarcomas at the injection site but, in most mice, there was little cellular response to asbestos in the subcutaneous tissues in contrast to the vigorous reactions seen in serosal membranes. All three types of asbestos induced both injection-site tumours and distant mesothelial changes and the removal of oils from amosite and crocidolite did not abolish—though it may have reduced-these manifestations of carcinogenic activity.

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THERAPEUTIC CONSIDERATIONS IN ACQUIRED PURE RED CELL APLASIA IN ADULTS

SIDNEY KRAMER, M.D., M.R.C.P. (EDIN.), LOUIS KAPLAN, M.B., CH.B., AND JACK METZ, M.D., M.C. PATH., From the Haematology Department, South African Institute for Medical Research, Johannesburg, and Boksburg-Benoni Hospital, Boksburg

Pure red cell aplasia is a disease of unknown aetiology characterized by virtual absence of erythropoiesis in the bone marrow while granulopoiesis and platelet genesis are unaffected. It may occur in a transitory form in kwashiorkor^{1,2} and in congenital haemolytic anaemias³ or as a rare disease affecting both children⁴ and adults.⁵ The pathogenesis of cases recorded in the literature has recently been reviewed by Schmid et al.6 and Harvard.7 The disease in adults is frequently associated with a tumour of the thymus,^{1,8} while in many cases without a thymoma there is a history of exposure to drugs or chemicals.^{5,6,9}

Therapy in cases both with and without a thymoma has been disappointing.^{6,7} Success has been reported in a few

cases following thymectomy,^{7,10} splenectomy,^{6,11} or treatment with prednisone,12 testosterone6 and cobaltous chloride,13-15 either singly or in combination. However, in the majority of cases repeated blood transfusions are necessary to maintain life.

In view of the limited success of therapy in pure red cell aplasia we report here the course of two patients who responded to therapy. In the first case, the anaemia was associated with a malignant thymoma, and remitted after radiation therapy and steroids. In the second case, without evidence of thymoma, haematological remission followed cobaltous chloride therapy.

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