showed no departure from the modal $2n = 43$ typical of the hypo-diploid tumour line maintained in this laboratory.

These findings confirm previous reports of the viability of Ehrlich ascites tumour cells incubated with $Na_2{}^{51}CrO_4$ even at high concentration. Grown in the more sensitive ascitic phase, a direct comparison of labelled and unlabelled cells in terms of weight gain, transplantability and—to a limited extent—chromosome complement has been possible.

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Experimental Production of Mesothelial Tumours of the Pleura by Implantation of Dusts in Laboratory Animals

In view of the possible association between the development of mesotheliomas of the pleura and exposure to asbestos dust in people living in the Cape asbestos fields[1,2], a preliminary experiment was undertaken to see if it was possible to produce mesotheliomas, in animals, by injecting various forms of asbestos into the pleural cavities. 110 young rats, mice, and guinea pigs were used. The experiment has not yet been concluded as 45 of the guinea pigs, and four of the rats, are still alive thirty months after the start of the experiment. This is a preliminary report of our positive findings in 106 rats which have either died or have been killed.

The rats used were pure-bred Wistar rats, from the South African Institute for Medical Research stock; they were six weeks old at the time of inoculation. These animals were divided into eleven groups of ten animals; five groups were male, and six female.

The following dusts were used: (1) three samples of crocidolite dust prepared from virgin fibre obtained from mines in the southern and northern regions of the Cape asbestos fields and from a mine in the Pietersburg district of the Transvaal; (2) three samples of dust obtained from the mills of crocidolite mines in the same geographical distribution as those described in (1); (3) two samples of chrysotile dust prepared from virgin fibre—the first obtained from a mine in which the asbestiform serpentine occurred as an ore-body in basic rock, and the other dust from a deposit where the serpentine was found in dolomite; (4) a sample of amosite prepared from virgin fibre; (5) a sample of 99·9 per cent pure silica dust; (6) a sample of pure carbon black. (5) and (6) were used as controls.

The dusts were ground to a fine powder, and were suspended in normal saline at a concentration of 50 mg/ml. These materials were injected into the pleural cavities of the rats. The details of the preparation of the dusts and the method of inoculation will be described in a further publication. Dr. J. S. Harington analysed all these samples for the presence of oils and 3 : 4-benzpyrene[3]. These substances were found in all the crocidolite samples, very slight amounts in the amosite sample, but none in the chrysotile, silica or carbon black materials.

Bronchiectasis, a common feature among laboratory rats after the age of 6 months, was found in almost every animal. Indeed, this condition, with associated abscess formation or empyema, was the most usual cause of death. Seven animals with breast adenomata were all killed at a stage when these tumours had become so large that they were causing the animals marked discomfort. Eight animals developed reticulum-celled sarcomas and four mesotheliomas. These rats appeared to have died of these malignancies.

Two female rats died of large pelvic abscesses, and in one small group of animals no obvious cause of death could be found.

Pleural mesothelial tumours were observed in two animals inoculated with crocidolite fibres; one sample of the crocidolite being obtained from the Pietersburg area and the other from the northern portion of the Cape asbestos fields. Of the other two, one occurred in an animal inoculated with chrysotile fibre from the ore body, and one among the rats receiving the pure silica.

So far as can be ascertained from the literature[4], tumours of this type do not occur naturally in rats of the Wistar strain.

A further rat, a survivor of a dusting experiment in which animals were exposed to a fine chrysotile dust for 120 days, died 450 days after removal from the dust room. At necropsy, this animal was found to have a pleural mesothelioma. Macroscopically and histologically, the tumours in these animals were similar to those seen in human cases. In addition, hyaluronic acid secretion was demonstrated histochemically in four of these tumours, while in one animal, Dr. Harington was able to determine its presence chemically in the pleural fluid. The presence of hyaluronic acid has been an aid to the diagnosis of the tumours in human cases[5].

Fibro-adenomata of the breast have been frequently observed among female laboratory rats, and Ratcliffe[6] observed them to be the commonest tumour among stock Wistar rats. Reticulum-celled sarcomas were also not infrequently observed among these animals.

Table 1. Occurrence of Tumours in Rats after Intra-pleural Inoculation of 25 mg of Dust

	Type of dust					
	Crocidolite fibre	Crocidolite ore	Chrysotile	Amosite	Silica	Carbon	Total
No. of rats	30	30	20	10	10	10	110
Mesotheliomas	2		1		1		4
(Months after inoculation)	17 and 23		15		17		
Adenomas of the breast	2	4	1				7
(Months after inoculation)	11 and 18	10, 21, 23, 23	22				
Reticulum-cell sarcoma	3	3	1	1			8
(Months after inoculation)	10, 17, 17	10, 16, 22	16	22			
Sex of rats	20 F	30 F	10 F	M	M	M	
	10 M		10 M				
Survival time of group following inoculation (months)	11–25	7–30 (2 survivors)	11–30 (1 survivor)	14–30 (1 survivor)	11–24	12–22	

In view of these findings it was, therefore, accepted that the occurrence of these tumours was not the result of the experimental procedures.

It has been demonstrated that it is possible to produce tumours which appear to be arising from the mesothelial cells of the pleura by inoculating certain dusts into the pleural cavities of rats. One further tumour was obtained by exposing an animal to an asbestos dust cloud. The fact that these neoplasms were produced by silica and chrysotile asbestos as well as crocidolite fibre complicates the interpretation of these preliminary observations, and indicates the need for further investigations.

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An Early Test for Possible Skin Carcinogens in the Mouse: Effect of Different Doses of 3-Methylcholanthrene in Benzene Solution

An early test for possible skin carcinogens in the mouse has been reported[1,2]. The test is based on a tetrazolium reduction method. It was demonstrated that the test could reveal the carcinogens in a blind test of 21 substances of which 7 were carcinogens.

The present communication gives the values obtained with different doses of 3-methylcholanthrene in benzene solution applied to a circumscribed area of the skin of hairless mice. The results are given in Table 1.

Table 1. Tetrazolium Test with Different Doses of 3-Methylcholanthrene

µg applied	% solution in benzene	No. of mice	Test result
0·4	1/128	16	0·808
0·8	1/64	16	0·792
1·6	1/32	16	0·818
3·1	1/16	16	1·107
6·3	1/8	32	1·168
25·0	1/2	32	1·301
50·0	1/1	32	1·410
		16	

Terracini, Shubik and Della Porta[3] found a step-like increase in the tumour yield after single applications to mouse skin of different doses of DMBA. Studies are in progress at our Institute to find out if such a step exists for hairless mice exposed to single applications of 3-methylcholanthrene. The preliminary results ten months after the single painting are shown in Table 2.

Table 2. Skin Papillomata Occurring Ten Months After a Single Surface Application of 3-Methylcholanthrene in Benzene to Hairless Mice

% solution	No. of mice	Papillomata
1/64	30	
1/32	30	1
1/16	30	1
1/8	30	20
1/1	30	22
		34

These results demonstrate that such a well-known carcinogen as 3-methylcholanthrene gives negative results with the tetrazolium test when very small doses are given. The testing of unknown substances should therefore be performed with relatively strong, but non-ulcerative, doses. The possible specificity of the test, however, is given some further support by the preliminary results reported in Table 2. These seem to be in accordance with the conclusion of Terracini, Shubik and Della Porta[3]: "A critical dose level exists at which single application of carcinogens becomes fully effective".

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Mineral Phase in Osteoporotic Bone

In a survey of the incidence of fractures[1], particularly those of the head and neck of the femur, expressed in terms of population at risk, the distribution found made it quite clear that some must be of pathological origin. Such bones appear osteoporotic, and there has been a widespread assumption that the tissue component at fault is the calcium phosphate.

Should a change occur in the mineral, or in the calcium- and phosphate-level of its surroundings, one would expect to find changes in composition. Traditional methods of chemical analysis for calcium and phosphate cannot be profitably used, because the main need is to distinguish between a number of closely related calcium phosphates which might be present as mixtures. A further complication is that the mineral crystallites in bone have a very large surface area, so that adsorbed ions could produce misleading results. At present the most sensitive method for demonstrating individual components, which can be interpreted unambiguously, is X-ray diffraction.

A satisfactory method for distinguishing the various calcium phosphates involves taking photographs at room temperature, and then after heating to 1,000° C (ref. 2). At room temperature possible compounds are hydroxyapatite, octacalcium phosphate, monotite, brushite and calcium carbonate. After heating: apatite remains as apatite; apatite mixed with the other calcium phosphates, or apatite with phosphate adsorbed on to the crystal surfaces, reacts to form β-tricalcium phosphate, the proportion depending on the amount of excess phosphate present; calcium carbonate is transformed into calcium oxide.

One example where a difference is known in the tissue fluids surrounding the mineral in bone is in infants. These children have a higher serum phosphate than older children and adults. X-ray diffraction examination of bone from infants gave the hydroxyapatite pattern when unheated, and hydroxyapatite mixed with a little β-tricalcium phosphate after heating. Bones from the older age-groups gave hydroxyapatite only. In this case the different ionic concentrations in the surrounding fluids have been mirrored in the mineral composition.

To investigate possible differences in the mineral composition in osteoporotic bone the upper ends of the femur were used. At least three separate sub-