Reprinted from

FOOD AND COSMETICS TOXICOLOGY

PERGAMON PRESS

NEW YORK · OXFORD · LONDON · PARIS

1965

Fd Cosmet. Toxicol. Vol. 3, pp. 277–280. Pergamon Press 1965. Printed in Great Britain

Effects of Oral Administration of Two Tin Compounds to Rats over Prolonged Periods

F. J. C. ROE, E. BOYLAND and K. MILLICAN

Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, Fulham Road, London SW3, England

(*Received* 28 *November* 1964)

Abstract—Three malignant tumours were seen in 30 August rats (13 male and 17 female) which survived for 1 yr or more on a diet containing 2% sodium chlorostannate. One tumour was an adenocarcinoma of mammary origin, another a pleomorphic sarcoma arising in the uterus and the third, an adenocarcinoma arising in the region of the jaw. In 27 rats (11 males and 16 females) which survived for 1 yr or more on a diet containing at first 1% then 0·5% stannous 2-ethyl hexoate, no neoplasms were seen. In 33 rats (16 males and 17 females) fed on a control diet for the same period no malignant tumours were seen. Chronic murine pneumonia with complicating bronchiectasis was present in most rats at death. The difference in tumour incidence between the 3 groups is regarded as being probably without significance, but a further larger experiment would be necessary to establish this with certainty.

INTRODUCTION

The reasons for undertaking this experimental work have been given and the background of previous studies against which it was undertaken in the previous paper (Walters & Roe, 1965).

EXPERIMENTAL

Materials. Sodium chlorostannate was obtained from the British Drug Houses Ltd., Poole, Dorset and stannous 2-ethyl hexoate from Theodore St. Just & Co., Whitfield, Manchester.

Animals. Inbred August hooded rats of both sexes were used. Mothers were fed the diets from the day their litters were born. The young were weaned at 4 weeks and transferred to fresh cages with 8–10 males or females per cage. Cages made of zinc were used, both for breeding and for subsequent housing. Water was provided *ad lib.* throughout the experiment.

Basic diet. A powdered diet was supplied by Messrs Dixon, Ware, Herts. It was supplemented by the addition of arachis oil (5%) and certain vitamins and minerals. The formula of the resulting 20% protein basic diet is given by Walters & Roe (1965).

Test diets. Sodium chlorostannate was dissolved in water and the solution thoroughly mixed with the powdered diet in an electric mixer for a period of 10 min to make a dough. Stannous 2-ethyl hexoate was added to the diet essentially in a similar manner, except that it was dissolved in arachis oil instead of water before being mixed in. A rounded mass of the dough was placed in each cage of rats, the mass being in excess of their daily requirement. Diets were made up in dough form twice weekly but fed daily during the week. A double quantity was provided on Saturdays, but none on Sundays.

Conduct of experiment. Animals were examined cursorily each day except Sundays, and weighed and thoroughly examined each week. Sick animals were killed and examined *post-mortem* for tumour development and other changes.

Experimental design. Pregnant August rats were divided at random into 3 treatment groups. From the day of birth of litters onwards, groups 1 and 2 were fed for up to 80 weeks on a 20% protein diet containing 2% sodium chlorostannate and 1% stannous 2-ethyl hexoate, respectively; group 3 rats were fed on an untreated 20% protein diet for the same period. According to the initial plan of the experiment, the young raised by mothers fed on the 3 different diets were, at the time of weaning, to be separated by sex and thereafter fed the same diets continuously until death. The numbers of animals in the 3 groups at weaning were: group 1—37 (19 males and 18 females), group 2—37 (17 males and 20 females), group 3—40 (20 males and 20 females).

RESULTS

Unfortunately, the plan of the experiment could not be followed absolutely. Rats of group 2 were severely underweight and macroscopically obviously anaemic at the time of weaning. However, the diet containing 1% stannous 2-ethyl hexoate was still fed to them. By week 8 the average body weight of males of group 2 was 64 g and of females, 74 g. At this time control males in group 3 averaged 146 g and control females 116 g. Rats in group 1, on the other hand, showed no ill effects and were almost as sturdy as the controls, the males averaging 135 g and the females, 105 g.

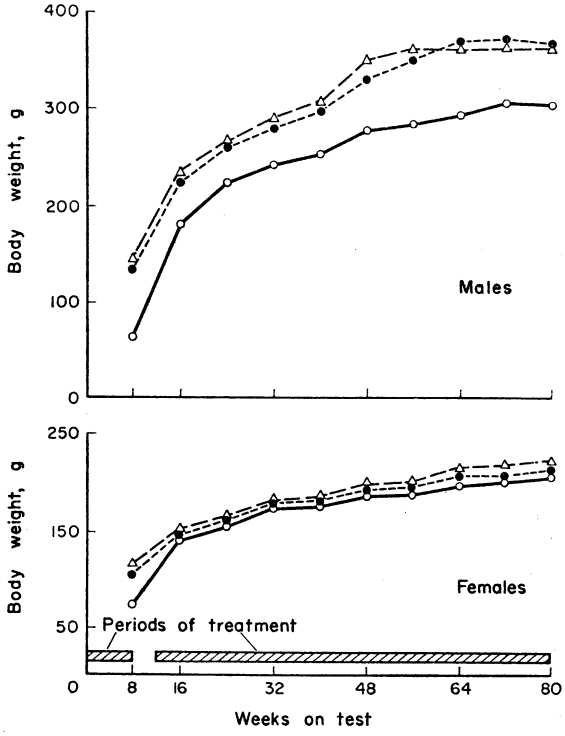

FIG. 1. Body weight gains in males and females of rats receiving 20% protein diets containing 2% sodium chlorostannate (●----●), 1% stannous 2-ethyl hexoate (O——O); or no tin compound, control, (△——△).

Because of the poor condition of rats in group 2, they were given the control diet for 4 weeks after which their condition had greatly improved and their body weights were not much less than the controls. They were then returned to a diet containing 0·5%, instead of 1·0% stannous 2-ethyl-hexoate and remained on this diet until death.

Although there was no evidence of toxic effect, rats of group 1 were also taken off treatment with the test substance from weeks 8–12. However, on resumption of treatment with sodium chlorostannate the level of incorporation into the diet remained, as before, at 2%.

The periods of treatment and the pattern of increase in weight gain in the 3 groups are illustrated in Fig. 1. During the first year of the experiment there were 24 deaths as follows: 7 in group 1 (6 males and 1 female), 10 in group 2 (6 males and 4 females), and 7 in group 3 (4 males and 3 females). Evidence of chronic murine pneumonia was present in most of these animals and was the primary cause of death or reason for being killed, in most cases. No other pathological change or neoplastic lesion was observed.

Chronic respiratory disease was evident in the majority of rats of all groups alive at 1 yr and remained in evidence until the survivors were finally killed at 80 weeks.

The results of the experiment as regards survival beyond week 50, and neoplastic lesions in animals so surviving, are summarized in Table 1. The majority of rats in all 3 groups showed evidence of moderate or severe lung infection with wide cuffs of mononuclear cells around bronchi and vessels, and, in many cases, bronchiectatic changes. Slight degenerative changes were observed microscopically in some of the livers of animals of all 3 groups and were not more frequent in the groups receiving tin. No hyperplastic nor neoplastic lesions were seen in the gastro-intestinal tract. Areas of dilated tubules were seen in the renal cortices of a few rats of each of the 3 groups. There was no other significant pathological finding.

Table 1. *Treatment, survival, and post-mortem findings*

Group	Treatment	No. of survivors at week 8*	52	80	Neoplasms found at necropsy in animals dying during weeks 52—80
1	2% Sodium chlorostannate (5000 ppm Sn) in 20% protein diet from birth till week 8 and from week 12 until death	19M	13M	8M	Adenocarcinoma arising in region of jaw
		18F	17F	16F	Pleomorphic sarcoma of uterus Mammary adenocarcinoma
2	1% Stannous 2-ethyl hexoate (4500 ppm Sn) in 20% protein diet from birth till week 8 then 0·5% (2250 ppm Sn) until death	17M	11M	6M	None
		20F	16F	14F	None
3 (Control)	20% Protein diet only	20M	16M	12M	None
		20F	17F	16F	Lymphosarcoma of lung

M=Male F=Female

*There were no deaths up till week 8.

The 3 malignant tumours seen in group 1 are illustrated in Figs. 2–4.

DISCUSSION

The result of the experiment is, unfortunately, inconclusive. It is possible, perhaps probable, that all 3 malignant tumours in rats of group 1 were spontaneous in origin, and that the high intake of tin played no causative role. This problem could not be settled without the use of much larger groups of animals, and our knowledge and past experience of the August hooded strain used is insufficient to be helpful.

The lack of any apparent changes in the gastro-intestinal tract, and the lack of an observable difference between the livers and kidneys of treated and control groups is encouraging, since these are the sites most likely to be affected by a dietary carcinogen. The lack of any evidence of carcinogenicity of tin in mice (Walters & Roe, 1965) is similarly encouraging.

Although pulmonary disease was rife in all 3 groups and reduced survival to 80 weeks there is no reason to think that the presence of this disease interferes with the response of animals to carcinogenic agents to the extent of changing a positive result to a negative one, or *vice versa*.

Acknowledgements—We are grateful to Mr. W. H. Percival, Mr. M. Kipling, Mr. G. Pearce, Mr. J. Edmunds and Mrs. Christine Hall for skilled technical assistance, to Dr. C. E Dukes and Dr N. F. C. Gowing for their opinions on the uterine malignancy, and to Mr. K. Moreman and Mr. E. A. Sykes for preparing the photomicrographs and line figures, respectively. Mrs. Kay Foster and Miss Marjorie Butt kindly helped in the preparation of the manuscript. This investigation has been supported by grants from the Medical Research Council, the British Empire Cancer Campaign for Research and by the Public Health Service Research Grant No. CA-03188-08 from the National Cancer Institute, US Public Health Service.

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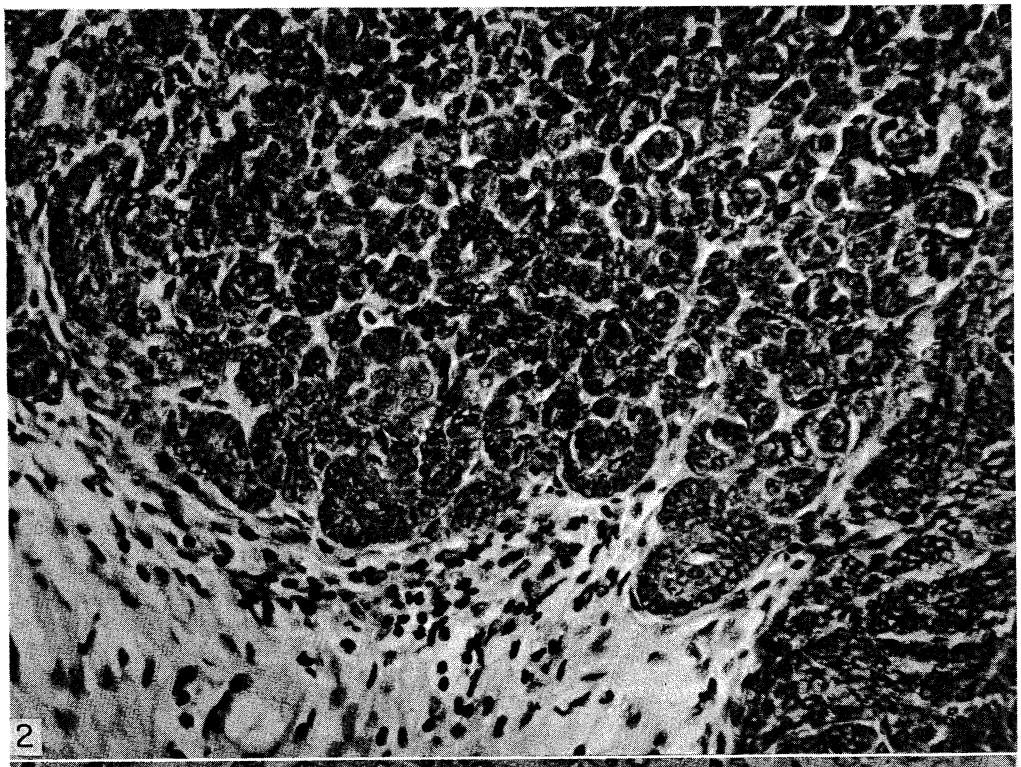
Effets de l'Administration Orale de Deux Composés de l'Étain à des Rats Pendant des Périodes Prolongées

Résumé—Trois tumeurs malignes apparurent chez 30 rats nés au mois d'août (13 mâles et 17 femelles) qui supportèrent pendant un an ou plus un régime contenant 2% de chlorostannate de sodium. L'une des tumeurs était un adénocarcinome d'origine mammaire, une autre, un sarcome pléomorphique de l'utérus, et la troisième, un adénocarcinome de la région de la mâchoire. Sur 27 rats (11 mâles et 16 femelles) qui survécurent un an ou plus avec un régime contenant d'abord 1% puis 0,5% de 2-éthyl hexoate stanneux, on ne constata aucun néoplasme. Sur 33 rats (16 mâles et 17 femelles) gardés sous contrôle alimentaire pendant la même période, on ne détecta aucune tumeur maligne. Il existait de la pneumonie murine chronique compliquée de bronchectasie chez la plupart des rats, à la mort. La différence dans le nombre des tumeurs entre les trois groupes est considérée comme sans signification probable, mais on ne pourrait conclure avec certitude qu'après des expériences à plus grande échelle.

Wirkung Zweier Zinnverbindungen bei Ratten bei Längerer Oraler Verabreichung

Zusammenfassung—An 30 Augustratten (13 männlichen und 17 weiblichen), die ein Jahr oder länger die Verabreichung eines Futters mit einem Gehalt von 2% Natriumchlorstannat überlebten, wurden drei maligne Tumoren festgestellt. Ein Tumor war ein Adenocarcinom mammaren Ursprungs der zweite ein pleomorphes Sarkom im Uterus und der dritte ein Adenocarcinom im Kieferbereich. Bei 27 Ratten (11 männlichen und 16 weiblichen), die ein Jahr oder länger die Verabreichung eines Futters mit einem Gehalt von zuerst 1%, dann 0,5% Stanno-2-äthylhexoat überlebten, wurden keine Neoplasmen gefunden. Bei 33 Ratten (16 männlichen und 17 weiblichen), die ebenso lange eine Kontrolldiät erhielten, wurden keine malignen Tumoren festgestlelt. Beim Tode wurde bei den meisten Tieren Rattenpneumonie mit Bronchiektasie als Komplikation gefunden. Der Unterschied in der Tumorhäufigkeit bei den drei Gruppen ist wahrscheinlich nicht signifikant, doch wäre eine weitere ausgedehntere Untersuchung erforderlich, um dies mit Sicherheit feststellen zu können.



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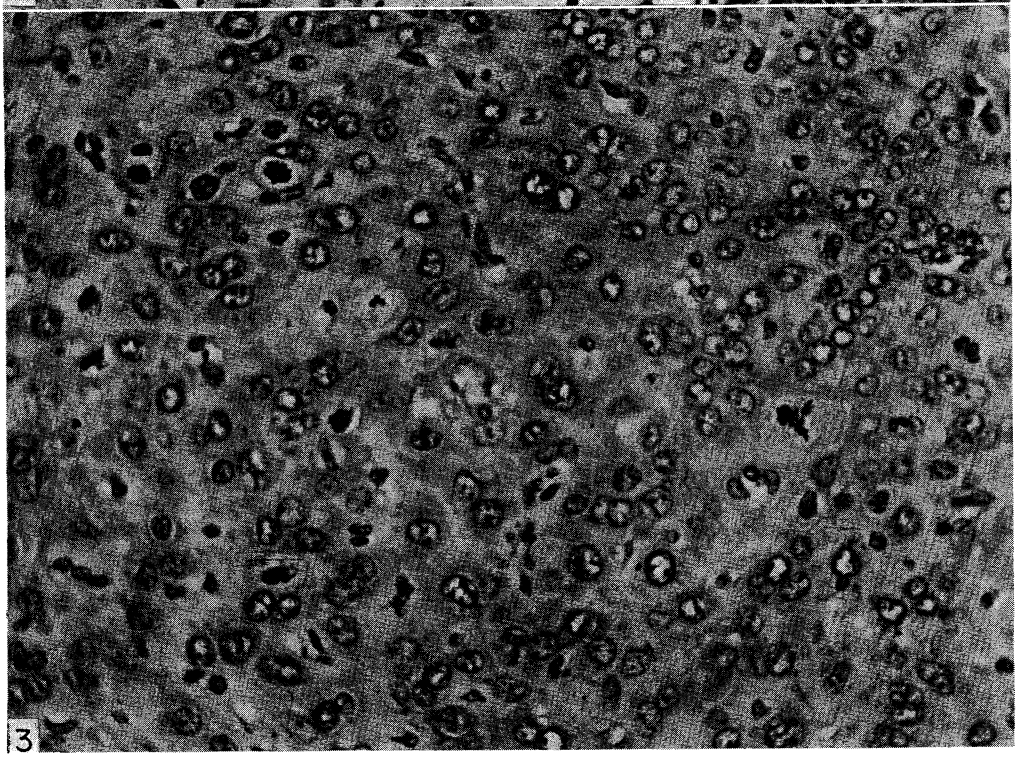

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FIG. 2. Adenocarcinoma from jaw region of a male rat of group 1 (2% sodium chlorostannate) killed during week 80 of the experiment. The tumour was thought to have arisen in one of the adnexal glands of the skin. Haematoxylin and eosin × 400.

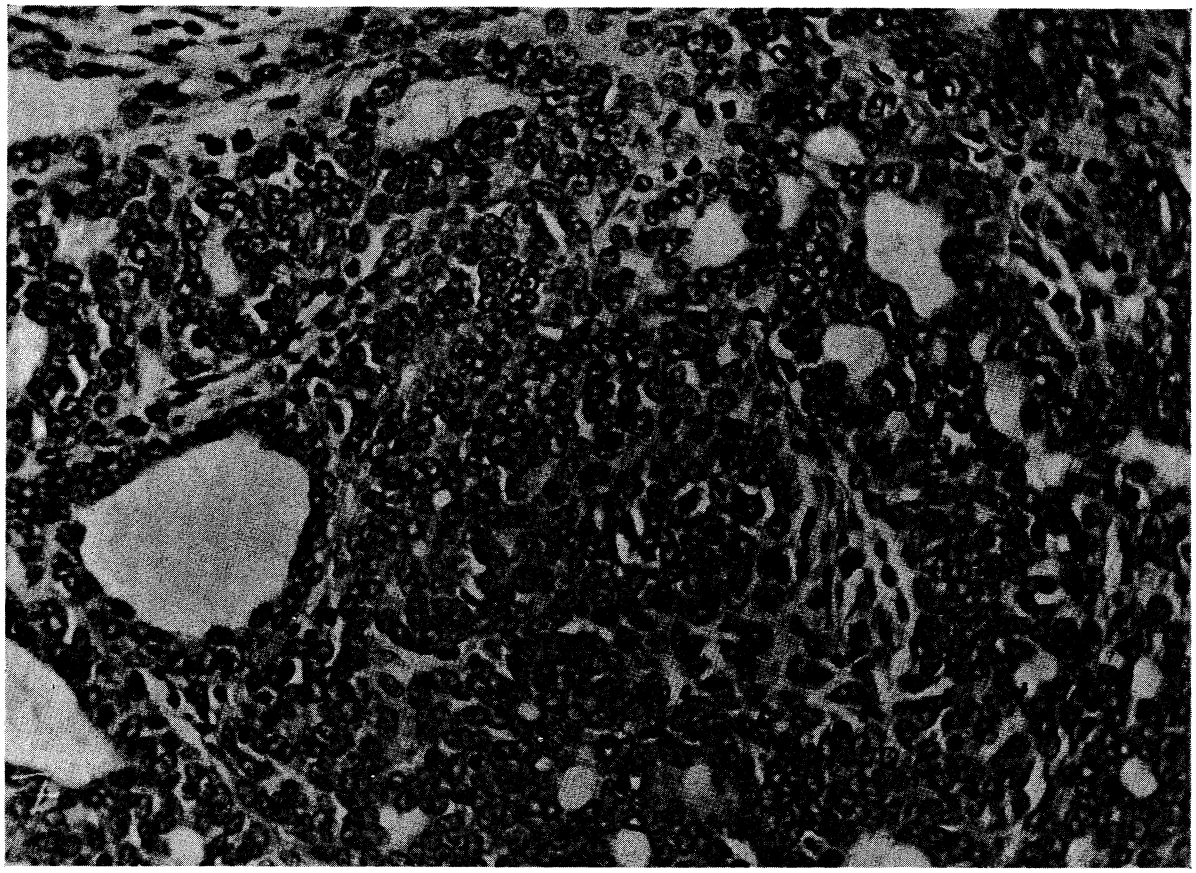

FIG. 4. Adenocarcinoma of mammary gland origin found in a rat of group 1 (2% sodium chlorostannate) killed during week 80 of the experiment. Haematoxylin and eosin × 400.

FIG. 3. Pleomorphic sarcoma of the uterus in a rat of group 1 (2% sodium chlorostannate) killed during week 80 of the experiment. The cells showed marked variation in size, abundant pale eosinophilic cytoplasm and frequent, often abnormal, mitotic figures. Haematoxylin and eosin × 400.