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THE EFFECT OF ZINC ON 9, 10-DIMETHYL-1, 2-BENZANTHRACENE (DMBA) INDUCED SALIVARY GLAND TUMOURS IN THE ALBINO RAT—A PRELIMINARY STUDY

by

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Human tissues contain a variety of trace elements. Eight of these are considered physiologically essential for the well-being and growth of different species [Hadjimarkos, 1968]. Five of these elements namely copper, zinc, chromium, selenium and manganese are essential for mammals. The activity of metalloenzymes such as carbonic anhydrase of the human erythrocyte is dependent on zinc and removal of the metal leads to loss of enzyme activity [Sandstead *et al*, 1970]. Bergman *et al.* [1968] studied the distribution patterns of radioactive ^{65}Zn and found that accumulation of zinc occurred in a large number of organs and tissues including the squamous epithelium of the nasal and oral cavities and oesophagus. Ronaghy *et al.* [1969] described the effects of zinc deficiency on the growth and development of adolescent boys in Iran. The zinc-responsive syndrome consists of dwarfism and sexual infantilism.

The beneficial effect of dietary zinc sulphate supplements on the healing of experimental extraction wounds in hamsters have been well documented [Mesrobian *et al.*, 1969; McCray *et al.*, 1972]. In both these studies the greatest acceleration of repair was observed during the first postoperative week. Similarly, accelerated healing of incised abdominal wounds and excised back wounds were observed in rats given additional zinc intravenously [Savlov *et al.*, 1962]. Clinical studies by Pories *et al.* [1967] demonstrated the need for zinc in the optimal and accelerated healing of granulation tissue wounds caused by excision of pilonidal-sinus tracts in young airmen who were given zinc sulphate by mouth during the period of repair.

On the other hand, reports of the effects of dietary zinc on carcinogenesis have been controversial. Davies *et al.* [1968] reported decreased plasma levels of zinc in patients with cancer. Zinc is required for DNA synthesis (Swenerton *et al.*, 1969; Sandstead *et al.*, 1969] and since tumours often have a high rate of DNA synthesis, decreased plasma levels in the human host may reflect high zinc requirements of a tumour. De Wys *et al.* [1970], on evaluating the growth of transplanted Walker 256 carcinosarcoma in rats on a zinc deficient diet, showed a marked reduction in tumour growth and a striking increase of survival time in these experimental animals. Poswillo *et al.* [1971] however, reported diametrically opposed results. These authors studied the effect of dietary zinc intake on carcinogenesis in the hamster cheek pouch induced by means of a 0.5% solution of 9, 10-dimethyl-1, 2-benzanthracene (DMBA) in liquid paraffin. They reported that the administration of zinc as a dietary supplement exerted an inhibitory effect on tumour formation.

This paper is a preliminary report. The object of this study was to evaluate the effects of the administration of drinking water containing variable amounts of zinc on DMBA induced submandibular salivary gland tumours in the albino rat. The zinc concentration in the solid diet was kept constant.

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METHODS

Twenty Wistar strain albino rats of four to five months of age were used in this experiment. The animals were divided into four groups of five each. The first group was given deionised drinking water and the other three groups drinking water containing 50, 100 and 250 p.p.m. zinc respectively. Analar zinc sulphate was used to make up the solutions. The five rats in each group were caged together and given Epol mice cubes *ad libitum*. The zinc concentration of the mice cubes was determined by means of atomic absorption spectrophotometry and found to be 79 p.p.m. The weight of each animal was recorded at the start of the experiment.

The technique used for tumour induction in the submandibular salivary glands was a modification of that described by Cataldo *et al.* [1964]. The carcinogenic agent employed was DMBA in the pure form. Standardized DMBA pellets, weighing approximately 5 mg. each, were prepared, the rats anaesthetised with thalamonal given intraperitoneally and the pellets implanted in the right submandibular glands two weeks after the start of the experiment (Fig. 1).

The rats were examined at weekly intervals paying particular attention to tumour growth and ulceration. Three months after implantation of the pellets, the animals were weighed, sacrificed and the right submandibular glands carefully dissected and weighed.

The glands were fixed in buffered 10% formol saline and processed in the usual way. Serial sections, 7 μm thick, were cut and stained with haematoxylin and eosin.

RESULTS

One of the rats died during the experiment. Tumours developed in the right submandibular glands of all the remaining animals. The tumours were clearly palpable and visible six weeks after implantation of the carcinogenic agent (Fig. 2). Ulceration of the skin overlying the tumour occurred in all the animals on deionised drinking water and water containing 50 p.p.m. zinc. The ulceration became progressively worse. In the group on water containing 100 p.p.m. zinc milder ulceration was observed in all the animals but the ulcers healed completely in two of the animals eight weeks after implantation of the carcinogen. Slight ulceration occurred in the skin overlying the tumours in the animals on 250 p.p.m. zinc in the drinking water and at sacrifice only small scars were visible in three of the rats.

The animals were sacrificed three months after implantation of the DMBA pellets and their weights recorded. The mean weights of the rats in the four different groups at the start of the experiment and at sacrifice were determined. The mean weight gain and the standard deviation of the weight gain were calculated from the above information. The results are presented in Table I.

TABLE I

Weight gain of animals during experiment

Zn Concentration in drinking water p.p.m.	No. of rats	Mean weight at start of experiment ± S.D. gram	Mean weight at sacrifice ± S.D. gram	Mean weight gain gram	± S.D. Weight gain gram
0	5	279 ± 40	280 ± 43	1	± 59
50	4	364 ± 31	370 ± 28	6	± 42
100	5	279 ± 39	314 ± 45	35	± 60
250	5	241 ± 29	296 ± 39	55	± 49

At sacrifice all the rats on deionised water had developed well circumscribed, nodular tumours of firm consistency (Fig. 3). The animals in the other three groups showed variable tumour morphology, varying from firm, nodular tumours to soft tumours with apparent abscess formation. The affected right submandibular gland of each rat was carefully dissected and the wet weight recorded. The weight of the gland was expressed as a percentage of body weight for each animal. The mean wet weight of the glands and the mean percentage of gland to body weight were calculated for each group (Table II). At the end of the experiment a further five male albino rats of approximately the same age as the experimental animals were weighed and sacrificed. The right submandibular glands were dissected, the weights of these glands recorded and again expressed as a percentage of body weight. The mean values are presented in Table III.

TABLE II
Wet weight of submandibular glands at sacrifice

Zn Concentration in drinking water p.p.m.	No. of rats	Mean wet weight gland ± S.D. gram	Mean % of gland to body weight ± S.D.
0	5	1.44 ± 0.16	0.53 ± 0.14
50	4	1.81 ± 0.54	0.49 ± 0.16
100	5	1.87 ± 0.34	0.61 ± 0.15
250	5	1.08 ± 0.31	0.37 ± 0.10

TABLE III
Normal submandibular glands

No. of rats	Mean weight ± S.D. gram	Mean wet weight submandibular gland ± S.D. gram	Mean % of gland to body weight ± S.D.
5	294 ± 61	0.20 ± 0.03	0.07 ± 0.02

Histological examination revealed that the rats in the group on deionised water had all developed well defined squamous carcinomas in their right submandibular glands (Fig. 4). Remnants of salivary gland tissue are clearly visible. In the group on 50 p.p.m. zinc, squamous carcinomas were present in the glands of all the animals but the epithelium was reduced in amount. Granulation tissue, infiltrated with inflammatory cells was present adjacent to the carcinomatous epithelium (Fig. 5). The glands of the animals on 100 p.p.m. zinc all contained squamous carcinomas but the epithelium was further reduced, and the inflammatory response in these glands was more marked with isolated areas of abscess formation (Fig. 6). Three of the animals in the group on drinking water containing 250 p.p.m. zinc showed no histological evidence of carcinomatous epithelium. Marked abscess formation surrounded by granulation tissue was a prominent histological feature in this group (Fig. 7). In one of the glands masses of keratin embedded in an apparent lymph node was seen (Fig. 8).

DISCUSSION

From the results presented in Table I it would appear that there was a progressive weight gain of the animals as the zinc concentration in the drinking water increased. These results are not statistically significant.

The mean wet weight of the glands and the mean percentage of gland to body weight in the four groups are presented in Table II. Analysis of variance within and between the four groups revealed that the results are significant at the 90% level but not at the 95% level. By determining the contrast between the mean percentage of the weights of the glands related to the body weight in the first three groups on the lower zinc concentration in the drinking water and the fourth group on the higher (250 p.p.m.) zinc intake, results, significant at the 97.5% level were obtained. It would therefore appear that the development of DMBA induced carcinogenic tumours in the submandibular glands of rats is retarded when the zinc concentration in the drinking water is increased to 250 p.p.m.

The mean wet weight of normal submandibular glands and the mean percentage of these glands to body weight are presented in Table III. By comparing these results with those in Table II it is evident that a marked increase occurred in the weights of the glands implanted with DMBA pellets.

According to Shklar [1970], different carcinogenic chemicals, different methods of application and different species of animals could result in different types of tumours induced in salivary glands of experimental animals. Cataldo *et al.* [1964] found that fibrosarcomas only were produced by implanting DMBA pellets in the submandibular glands of Syrian hamsters. Shafer [1962] reported that carcinomas of the epidermoid variety were induced by implanting DMBA in the submandibular glands of rats. The above observations were confirmed by Chaudry *et al.* [1966]. In this study only squamous carcinomas were observed in the DMBA implanted salivary glands in rats.

According to Shklar [1970] the pathogenesis of the lesion resulting from DMBA implantation in the submandibular gland of the rat is understood. The initial lesion is glandular necrosis followed by the development of epidermoid cysts. Epidermoid carcinomas arise from the walls of the latter cysts and the entire process requires about ten weeks. Chaudry *et al.* [1966] decribed the sequence of events that led to the formation of epithelial malignant tumours in the rat submandibular gland. It consisted of metaplasia of ductal epithelium, ductal proliferation, epidermoid cysts, dyskeratosis, carcinoma in situ and finally frank squamous cell carcinoma. The well developed squamous carcinomas observed in this study (Fig. 4) were seen twelve weeks after implantation of the carcinogen. It is therefore not surprising that the initial lesions were not observed in this study particularly in the group on deionised water where characteristic squamous carcinomas had developed.

The histology of the induced tumour followed a definite pattern when related to the concentration of the zinc in the drinking water. The tumours in the group on deionised water were well developed squamous carcinomas within areas of normal gland. As the zinc concentration increased, the squamous epithelium became progressively decreased in amount and the inflammatory response more marked. Large areas of abscess formation were observed at high zinc concentrations obliterating the normal glandular structure and squamous epithelium.

An interesting observation is the increased amount of lymphatic tissue within the tumour at high zinc concentrations. This pronounced lymphatic tissue proliferation in the rat could indicate an immune response to the developing carcinoma. According to Ham [1961] the rejection of a transplant seems to be associated with an increase of lymphocytes beneath and around the transplant. This type of immune response,

it is believed, may be due to the function of the lymphocytes in transporting antibodies from the site of formation to the site where the antigen is located, thus producing a high local concentration of antibody at the site where it would be most effective. By eliminating the immune response accelerated tumour growth has been observed. Giunta *et al.* [1971] studied the effect of systemically injected anti-hamster lymphocytic serum (ALS) on DMBA induced carcinogenesis of the Syrian hamster buccal pouch. They found that there was a higher incidence of carcinomas in the ALS/DMBA group and a suggestion of earlier tumour development. Histologically this group presented a constant tumour pattern with deeper invasion and more anaplasticity than the control group.

The zinc concentration in the solid nutrients of the diet of the experimental animals (79 p.p.m.) was adequate for the nutritional zinc requirements of the animals. We believe that protein or DNA synthesis were not impaired even in the group on deionised water.

The authors are aware of shortcomings in this experiment. The daily zinc intake of each individual animal from solid and liquid sources was not determined in this investigation. Furthermore animals were only sacrificed at the end of the experiment, i.e. three months after implantation of the DMBA pellets. In future studies rats in the different groups should be sacrificed at regular intervals to study the pathological changes that occur with time.

CONCLUSIONS

Tumour growth, induced by 9,10-dimethyl-1, 2-benzanthracene (DMBA) implantation in the submandibular glands of albino rats, was retarded when the zinc concentration in the drinking water reached 250 p.p.m. The pathology of the tumours changed with increased zinc content of the drinking water. In the group on deionised water well differentiated squamous carcinomas developed in the submandibular glands of all the animals. As the zinc concentration in the drinking water increased, the carcinomatous epithelium became progressively less and the inflammatory response more marked. The authors are unable to explain the inhibitory effect and the change in pathogenesis produced by the zinc in the drinking water.

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LEGENDS TO PLATES

PLATE 1

Fig. 1. Implantation of DMBA pellet in submandibular gland.
Fig. 2. Development of tumour six weeks after implantation.
Fig. 3. Macroscopic appearance of induced submandibular salivary gland tumour.
Fig. 4. Stratified squamous carcinoma with remnants of glandular tissue. H and E × 60.

PLATE 2

Fig. 5. Degenerating carcinomatous epithelium with granulation tissue infiltrated with inflammatory cells. H and E × 80.
Fig. 6. Abscess formation in tumour. H and E × 100.
Fig. 7. Well defined abscess and granulation tissue. H and E × 80.
Fig. 8. Mass of keratin embedded in an apparent lymph node. H and E × 80.