THE INFLUENCE OF MAGNESIUM ON THE GROWTH OF CARCINOMA, SARCOMA AND MELANOMA IN ANIMALS

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Many attempts have been made to prove that certain chemical elements have some effect on the prevalence of cancer. Minerals of various kinds, such as sodium, potassium, and calcium, which are essential for growth and maintenance of the life of experimental animals, are also essential for the growth of tumors. It seems a logical possibility that some inorganic substance which is found in the animal body may inhibit or stimulate malignant cell activity.

Experimental evidence which would indicate that magnesium is an essential element for growth of malignant tissues is lacking. The present study was planned to obtain this information. It is possible that the results of this study may throw light upon the recent claim that deficiency of magnesium in the diet is one of the factors in the production of cancer.

MATERIALS AND METHODS

In order to study the problem adequately, it was necessary to prepare a diet complete in every respect, but as nearly as possible free from magnesium. McCollum, Kruse, and Orent, in their memorable papers on the effects of deprivation of magnesium in the animal body, have shown that their purified diet contained only 0.00018 per cent (1.8 parts per million) of magnesium. When young rats were deprived of magnesium but were given adequate amounts of other dietary essentials, they showed a spectacular series of symptoms terminating in early death. Rats upon a similar diet but containing 0.0538 per cent of magnesium showed normal health and growth.

The composition of this magnesium-low diet is as follows: casein, 20.0 per cent; starch, 56.1 per cent; salts, 5.9 per cent; butter fat, 8.0 per cent. A 50 per cent alcoholic yeast extract, 100 c.c. in 100 gm. of ration, and viosterol, 15 drops per kilo of ration, are used to provide vitamins B and G. The composition of the salt mixture is as follows: CaCO₃, 1.5 gm.; KCl, 1.0 gm.; NaHCO₃, 0.7 gm.; Fe₂(SO₄)₃(NH₄)₂SO₄·24H₂O, 0.508 gm.; KH₂PO₄, 1.7 gm.; and NaCl, 0.5 gm.

Casein (commercial, washed) and starch (Argo) were purified according to McCollum. The salt mixture was prepared from C.P. chemicals (Eimer and Amend). The yeast extract was prepared from

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Fleishmann's pure dry yeast. Butter fat was prepared from unsalted butter.

All animals were confined in metal cages with screen bottoms, and distilled water was given in unlimited amount. They received iodized water once weekly.

Preliminary feeding experiments were carried out with young rats from twenty-seven to thirty-two days old, weighing 30 to 49 gm., to determine the reproducibility of the characteristic symptom complex with our magnesium-deficient diet. Three groups of experiments involved a total of 27 rats. As a control, the same number of rats of nearly the same age were fed with the magnesium-low diet plus 0.545 gm. of MgSO₄·7H₂O to every 100 gm. of diet.

The results of these experiments may be summarized as follows. Young rats fed upon the magnesium-low diet passed through a spectacular series of reactions leading to an early and acute death, as McCollum, Kruse, and Orent pointed out in their papers. Within four days all the exposed skin areas, especially the ears, became vividly red from vasodilatation and hyperemia. This redness became increasingly intense until about the seventh day, after which it slowly subsided and was succeeded by marked pallor. The animals showed hyperirritability and hyperexcitability on about the seventh day, as evidenced by the readiness with which they were startled by slight noises or shadows. The first convulsive attack appeared as early as the thirteenth day though in some animals it occurred later. While still lying on their sides, the animals showed continuous spasms of the feet; there was frothing at the mouth; the jaws were fixed. This stage was succeeded by tonic and clonic convulsions. Within a few minutes some animals became normal again. Some survived several attacks. Some animals died as early as the fifteenth day, while 7 out of 27 animals remained alive eight weeks. In young rats fed upon the magnesium-control diet, not only was the characteristic symptom complex absent, but these animals presented a healthy appearance comparable to that of animals fed upon a common diet of wheat bread, milk, and lettuce.

On the basis of our findings in these experiments, it is evident that our diet was deficient enough in magnesium to be considered adequate for the purposes of our experiments. The rations were not submitted to spectrographic analysis for magnesium.

The nutrition of the young animals on these synthetic diets may be summarized briefly. Young rats on the magnesium-low diet gained an average of 0.7 gm. daily during the experimental feeding period of six weeks. Upon the magnesium-normal diet, which contained 0.05377 gm. of magnesium per 100 gm. of ration, the animals grew nearly normally, gaining about 1.3 gm. daily during the experimental feeding period of eight weeks and maintaining excellent health. In both cases, each rat consumed from 5 to 7 grams of the synthetic ration daily. With a common diet of wheat bread, milk, and lettuce, the rate of growth of young rats was slightly higher than on the magnesium-normal diet, the average daily gain being 1.8 gm.
The Flexner-Jobling rat carcinoma was selected for use in these experiments on account of the regularity and high percentage of successful takes. The subcutaneous inoculations of the tumor fragments (about 6 mg. pieces) into rats were done by the trocar method, and the tumors were generally allowed to grow for a period of six weeks.

In general, the animals were maintained upon the special diets for about seven days prior to tumor inoculation, so that the experimental rations might have their full physiological effect both before and after tumor implantation.

**Effect of Magnesium-Low Diets upon the Growth of the Flexner-Jobling Rat Carcinoma**

As a preliminary study, experiments were carried out with a diet containing only 1.8 parts per million of magnesium but otherwise adequate.

*Experiment 1:* Thirty normally fed albino rats, about twenty-eight days old, were divided into three groups. Ten of them were placed upon the magnesium-low diet, and 10 on the magnesium-normal diet. Another lot of 10 rats were continued on the common diet of wheat bread, milk, and lettuce. At the end of the fourteenth day on the special diets, the animals were inoculated with fragments of Flexner-Jobling rat carcinoma, and feeding was continued during the experimental period of six weeks.

The result of this experiment showed that the rate of tumor growth in animals upon the magnesium-deficient diet was markedly diminished, but this ration had no influence upon tumor takes (100 per cent). On the other hand, the growth rate of tumors in animals to whose basal diet MgSO₄·24H₂O had been added was essentially the same as in the controls.

*Experiment 2:* The preceding experiment was repeated using 36 young rats weighing from 30 to 38 grams each. The animals were inoculated with tumor fragments on the seventh day, so that the majority of the animals on the magnesium-low diet would survive more than three weeks after tumor implantation. During the course of our study we had found that by avoiding any unnecessary noises it was possible to prevent some of the convulsive attacks in these animals, thus prolonging the period before death.

The results obtained from this experiment were essentially the same as those of the preceding one, namely, a marked retardation of tumor growth in the case of animals fed upon the magnesium-low diet, and normal tumor growth in the case of animals fed upon magnesium-normal diet. These results are graphically shown in Figure 1. In this and in all other experiments, graphical presentation of tumor growth in rats fed upon our common diet of wheat bread, milk, and lettuce was omitted, since tumor takes and the rate of tumor growth in these rats were nearly the same as in rats on the magnesium-normal diet.

*Experiment 3:* Twelve young rats of 31 to 37 grams initial weight
were placed on the magnesium-low diet. On the fourth day, when they reached the stage of maximum vasodilatation and hyperemia, the animals were inoculated with small pieces of Flexner-Jobling rat carcinoma. A control series of the same number of rats, of nearly the same age, were fed with the magnesium-normal diet or common diet, and were inoculated with the same tumor tissue at the same time.

It was found that the magnesium-deficient diet caused a distinct inhibition of tumor growth. On the other hand, the magnesium-normal diet failed to show any inhibiting or accelerating influence upon tumor growth. In both cases the number of tumor takes was 83 per cent.

**Experiment 4:** Twenty-four young, healthy rats, the average weight of which was 36 gm., were divided into two groups. One group of 12 animals was placed on the magnesium-low diet and the remaining 12 were placed on the magnesium-normal diet. Four days later tumor fragments were inoculated into these animals. At the end of the third week the tumor-bearing animals were killed by means of ether. The tumors were then removed, and weighed and measured immediately. It was found that animals on the magnesium-low diet grew subnormally throughout the experimental period of twenty-five days, the average daily gain in body weight being 0.51 gm. Grafts of the Flexner-Jobling
rat carcinoma in these animals exhibited a very slow rate of development. The average weight of the ten tumors (two animals died) was 0.070 gm., and the average dimensions $5.2 \times 4.8 \times 3.8$ mm. On the other hand, the animals which had been fed upon the magnesium-normal diet increased rapidly in weight, the average daily gain in body weight being 1.29 gm. The average weight of the eleven tumors in these rats was 1.80 gm., and the average measurements $15.1 \times 13.0 \times 11.0$ mm. Further analysis of these data reveals that the body growth of rats

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Fig. 2

The rate of growth of the Flexner-Jobling rat carcinoma in rats to whose diet a small amount of magnesium sulphate (0.0108 per cent of magnesium) had been added was decidedly diminished, but this inhibition of tumor growth was not so great as in animals upon a diet practically free from magnesium.

fed upon the magnesium-low diet was about 39 per cent of that of the animals fed upon the magnesium-normal diet. On the other hand, the tumor weight in the specially fed animals at the end of the third week was only about 4 per cent of that in the control series. These results suggest that the requirement of magnesium for body growth is less than that for tumor growth.

We next investigated the influence upon tumor growth exerted by a basal diet containing 0.109 gm. of MgSO$_4$·24H$_2$O in 100 gm. of ration, or 0.0108 per cent of magnesium. Such a diet was found to permit about one third of normal growth in young rats during the experi-
mental period of seven weeks; their general appearance was either good or poor. Some animals showed mild vasodilatation of the ears, but none had convulsive attacks or died as a result of magnesium restriction.

Experiment 5: Thirty-six young rats, about 28 days old, were divided into three groups. Twelve of them were placed on the magnesium-low diet (0.0108 per cent of magnesium), 12 were fed upon the magnesium-normal diet (0.0538 per cent of magnesium), and the remaining 12 were kept on the common diet. Seven days later tumor fragments were inoculated into the animals and the tumors were allowed to grow for a period of six weeks. The results obtained from this experiment are shown in Fig. 2.

It will be seen that the rate of tumor growth in animals to whose diet a small amount of magnesium sulphate had been added was decidedly diminished, but this tumor inhibition was not so great as that observed in animals fed upon a diet practically free from magnesium.

We repeated the preceding experiment with another group of 30 young rats and found that transplanted rat carcinomas in animals on a magnesium-deficient diet grew much more slowly than in control animals. However, the resistance of the animals to tumor regression was definitely increased.

Some attempts have been made to determine the magnesium content of tumor tissue. Beebe analyzed human malignant tumors and found that the amount of magnesium was so exceedingly small that quantitative determinations were impossible. Clowes and Frisbie analyzed Jensen mouse adenocarcinoma and failed to find magnesium in appreciable quantities. Kimura showed that the Flexner-Jobling rat carcinoma contained about the same amount of magnesium as the liver and muscle of tumor-bearing rats. Recently Eichholtz, using a new micro-method for determination of magnesium, showed that rat tumors contained significant amounts of magnesium: 125 mg. magnesium per 100 gm. dry tissue. We have not determined whether ingested magnesium will accumulate in the tumors, nor have we analyzed the normal magnesium content of the Flexner-Jobling rat carcinoma or other transplantable tumors in our laboratory. Investigations upon this point will be carried out later.

Further proof of the inhibitory action of magnesium-low diets upon the growth of tumor grafts is seen from the following experiments.

Experiment 6: Ten tumor-bearing animals fed upon the magnesium-low diet (0.00018 per cent of magnesium) were given the magnesium-normal diet (0.0538 per cent of magnesium) from three to five weeks after tumor implantation. The results are shown in Fig. 3.

It was found that when magnesium in the form of MgSO\textsubscript{4}·7H\textsubscript{2}O was restored to the diet of tumor-bearing animals which had survived for three to five weeks on the magnesium-deficient ration, the tumor nodules grew very rapidly and attained the normal sizes for the fourth, fifth, sixth, and seventh week respectively. Also these animals grew
supernormally for the first few days after MgSO$_4$$\cdot$7H$_2$O was added to the diet, gaining 24 to 33 gm. in seven days.

In two similar experiments, using 24 tumor-bearing rats, we were able to confirm the above findings relative to the marked increase in tumor growth and body growth when the magnesium-deficient diet was replaced by a ration containing a normal amount of magnesium.

When magnesium in the form of magnesium sulphate was restored in adequate amount (0.0538 per cent of magnesium) to the magnesium-low diet (0.00018 per cent of magnesium) of tumor-bearing animals, the tumor nodules grew very rapidly and attained normal size in about seven days.

The following experiments were undertaken to observe what influence a diet extremely poor in magnesium (0.00018 per cent of magnesium) may have on the growth of firmly established malignant tumors in animals.

Experiment 7: Fourteen young rats bearing seven-day-old Flexner-Jobling rat carcinomas were placed on the magnesium-poor diet. The rate of tumor growth in these animals was decidedly diminished, as was the body growth. However, the tumor inhibition was not so marked as in rats receiving the same diet prior to tumor implantation.
Experiment 8: Twelve young adult rats bearing fourteen-day-old Flexner-Jobling rat carcinomas were placed on the magnesium-poor diet. The progress of tumor grafts in these animals was slightly slower than in normally fed animals. There was no tumor regression.

Experiments 7 and 8 show that cancer cells obtain magnesium from the tissues of the host and will continue to grow even when the nutrition of the host has failed because of the lack of magnesium in the food.

Effect of a Magnesium-High Diet upon the Growth of the Flexner-Jobling Rat Carcinoma

In 1922 we showed that oral administration of magnesium carbonate or magnesium chloride had a slight but distinct accelerating effect upon the growth of Flexner-Jobling rat carcinoma. Previous to this only a few studies upon the effect of magnesium salts in cancer had been reported. Since then numerous papers have appeared. The results are contradictory, some authors claiming that magnesium inhibits tumor growth, others that it has a stimulating effect or that it is without influence on tumor growth. Shear has recently published an excellent critical review of the subject, with a complete bibliography.

During recent years a considerable interest has developed concerning the role of magnesium in cancer because of the claims of certain authors that the lack of this metal in the diet is significant in the production of cancer, and that administration of magnesium salts is of palliative value in the treatment of cancer.

The following experiments were undertaken for the purpose of securing further information concerning the possible therapeutic value of magnesium in cancer.

Throughout the experiments a magnesium-high diet (magnesium-low diet plus 1.799 gm. of MgSO₄·7H₂O to every 100 gm. of ration) was given to young rats for from seven to fourteen days prior to tumor inoculation. The composition of this magnesium-high diet is as follows: casein, 20.0 per cent; starch, 54.301 per cent; salts, 5.9 per cent; butter fat, 8.0 per cent; MgSO₄·7H₂O, 1.799 per cent; also a 50 per cent alcoholic yeast extract, 100 c.c. in 100 gm. of ration, and viosterol, 15 drops per kilo of ration.

It will be noticed that the amount of magnesium sulphate used in the diet was a little more than three times the normal magnesium requirement of the rat (Kruse, Orent and McCollum). Preliminary feeding experiments with young rats (about thirty days old) indicated that individual animals consumed about 11 mg. of magnesium daily. This amount of magnesium did not disturb the body metabolism, as indicated by the normal rate of body growth and the perfect health of the animals during the experimental period of ten weeks.

This study included four groups of experiments, involving a total of 48 rats. As a control, the same number of rats, of nearly the same age, received the magnesium-normal diet or our common diet, and were inoculated with the same tumor tissue at the same time as the experimental animals.
It was found that daily intake of excessive amounts of magnesium had a demonstrable stimulating effect upon the growth of Flexner-Jobling rat carcinoma. A characteristic example is shown in Fig. 4. Although the percentage of positive tumor takes was normal in the animals fed with the magnesium-high diet, the number of tumor regressions appeared to be greater than where the animals received a normal or inadequate amount of magnesium in the basal diet. However, the percentage of tumor regression in rats fed with an excessive amount of magnesium or common diet was essentially the same. A composite table showing the effects of various amounts of magnesium in diet upon the progress of tumors may demonstrate this point more clearly. See Table I.

**Effect of Magnesium-High Diet upon Tumors Already Firmly Established in the Hosts**

As part of the present study an attempt was made to ascertain whether oral administration of large amounts of magnesium sulphate is capable of exercising a destructive action against transplanted carcinomas, sarcomas, and melanomas.
INFLUENCE OF MAGNESIUM ON GROWTH OF CARCINOMA

As before, fragments of tumor tissue were inoculated into young or young adult animals. When the tumor grafts grew for seven to fourteen days, the normal diet of animals was changed to our synthetic magnesium-high diet (0.1775 percent of magnesium), and this diet was continued for about six weeks. In general, tumor-bearing animals were divided into two groups, one to be fed with the magnesium-high diet and the other to be continued on our common diet.

Experiment 1: Twenty-four young rats bearing seven- to fourteen-day-old Flexner-Jobling rat carcinoma were placed on the magnesium-high diet and feeding was continued for six weeks. No differences could be observed in the growth and regression of the tumor in these animals and controls fed with our common diet.

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<th>Table 1: Results of Transplanting Flexner-Jobling Rat Carcinoma in Rats Fed upon Various Amounts of Magnesium</th>
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<td>Magnesium-low (0.00108 per cent)</td>
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<td>Magnesium-normal (0.0538 per cent)</td>
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<td>Magnesium-high (0.1775 per cent)</td>
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<td>Common diet</td>
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Experiment 2: Twenty young adult mice bearing seven-day-old mouse sarcoma No. 180 were placed on the magnesium-high diet and feeding was continued for four weeks. The daily ingestion of the magnesium-high diet failed to exert any inhibiting effect upon the tumor growth. Furthermore, in many of the animals pressure necrosis developed in the subcutaneous portions of the tumors as early as the third week, subsequently causing death of the animals from toxemia, septicemia and nutritional failure.

Experiment 3: Twenty-two young adult mice bearing fourteen-day-old Passey mouse melanoma were placed on the magnesium-high diet and feeding was continued for ten weeks. The normal growth rate of the transplanted melanoma was not altered by the daily administration of an excessive amount of magnesium (about eight times the normal magnesium requirement of the mouse).

The present results are in accord with our previous findings with oral administration of magnesium carbonate and magnesium chloride, in that no definite improvement could be found in animals with cancer after the ingestion of excessive amounts of magnesium salts.

SUMMARY

1. Transplants of the Flexner-Jobling rat carcinoma in rats fed on a diet containing only 1.8 parts per million of magnesium but otherwise adequate survived more frequently but grew very much more slowly