

EFFECT OF VARIOUS GOITER-PRODUCING DIETS ON THE GROWTH OF CARCINOMA, SARCOMA, AND MELANOMA IN ANIMALS

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It is said that in Switzerland, where goiter is prevalent, the incidence of cancer is much higher than in the south of Europe. Although certain authorities maintain that no relation exists between the incidence of cancer and goiter, the fact is known that the metabolism of the cancer cell is more active than that of the normal cell, and that the marked cellular hyperplasia of severe Graves' disease equals that of some malignant tumors (1).

The present study was undertaken for the purpose of determining the influence of a diet either deficient in iodine or rich in iodine, upon the growth of tumors in animals.

EXPERIMENTAL

In recent feeding experiments on albino rats, carried out by Levine, Remington, and von Kolnitz (2), the ingestion by young animals of a diet consisting of yellow corn 76 parts, wheat gluten 20 parts, calcium carbonate 3.0 parts, sodium chloride 1.0 part, and 0.2 gm. of irradiated yeast to each 100 gm. of ration was followed in the short period of thirty-five days by enlarged thyroid glands. Histologic examination of the enlarged thyroids showed marked hyperplasia together with little or no iodine-containing colloid. This goiter-producing diet contained about $15 \gamma^1$ iodine per kilo. The same authors found that approximately 4γ of iodine per rat per day prevented the thyroid enlargement, the thyroids then showing a normal histologic structure with an abundance of colloid.

Preliminary feeding experiments were carried out with young albino rats from twenty-eight to thirty-five days old, weighing 41 to 50 gm., to confirm the goitrogenic activity of this diet. Yellow corn meal (Quaker Oats Co.), wheat gluten (Eimer and Amend), calcium carbonate and sodium chloride (C. P. chemicals, Eimer and Amend), and yeast (Fleishmann's irradiated pure dry yeast) were used in preparing the ration. All animals were confined in metal cages with screen bottom, and distilled water was given in unlimited amount. As a control, young rats were fed either with the non-goiter-producing diet of Levine, Remington and von Kolnitz, consisting of whole wheat (ground) 59.8 parts, whole milk powder (Klim) 29.9 parts, Swift's meat scrap 9.1 parts, and so-

¹ The Greek letter *gamma* (γ) is used for 0.001 milligram.

dium chloride 1.2 parts, or with our common diet of wheat bread, milk, and lettuce. These diets are designated in the present study as a normal diet.

The results of these experiments may be summarized briefly. Young rats fed upon the goitrogenic diet lost weight continuously until death. The average length of life of a group of 24 rats was forty-six days; some, however, died as early as the twenty-fifth day, while others lived sixty-five days on the ration. The general condition of these animals was very poor. Each rat consumed about 2 gm. of the diet daily. With the non-goiter-producing diet of Levine, Remington, and von

TABLE I: Results of Transplanting Flexner-Jobling Rat Carcinoma in Rats Receiving a Goiter-Producing Diet

Experiment Number	Number of Animals Used	Diet	Percentage of Tumor Takes *	Remarks
1	12	Normal	75	3 tumors did not grow; 1 grew slowly; others grew rapidly
	12	Iodine-low	100	10 tumors grew very slowly; 2 grew slowly
2	12	Normal	75	3 tumors did not grow; 2 regressed after growing for 3 weeks; others grew rapidly
	12	Iodine-low	100	Very slow growths
3	10	Normal	90	1 tumor did not grow; 2 regressed after growing for 2 weeks; 1 grew slowly; others grew rapidly
	10	Iodine-low	90	1 tumor did not grow; 4 grew very slowly; 5 grew slowly
4	12	Normal	83	2 tumors did not grow; 1 regressed after growing for 4 weeks; others grew rapidly
	12	Iodine-low	100	5 tumors grew very slowly; others grew slowly

* Some transplants proliferated in the animals for several days, and then regressed and were absorbed. If they were completely absorbed within two weeks they were considered as negative transplants.

Kolnitz, or our common diet, the animals grew rapidly, the average daily gain being 1.8 gm.

At the end of the experimental feeding period of thirty-five to forty days, some of the rats were killed by ether. Some of the thyroids of rats receiving the goitrogenic diet were larger than those of rats fed upon the control diets and were of dark red color. They contained less colloid, and the acini were somewhat smaller and more closely packed. The cells were more cuboidal.

On the basis of these findings, it is evident that our diet was so deficient in iodine that it may be considered adequate for the purposes of our experiments.

Effect of an Iodine-Low Diet upon the Growth of the Flexner-Jobling Rat Carcinoma

As the first step in the present investigation we determined the effect of the goiter-producing diet upon the growth of transplanted tumors. Young albino rats were maintained upon the goitrogenic diet

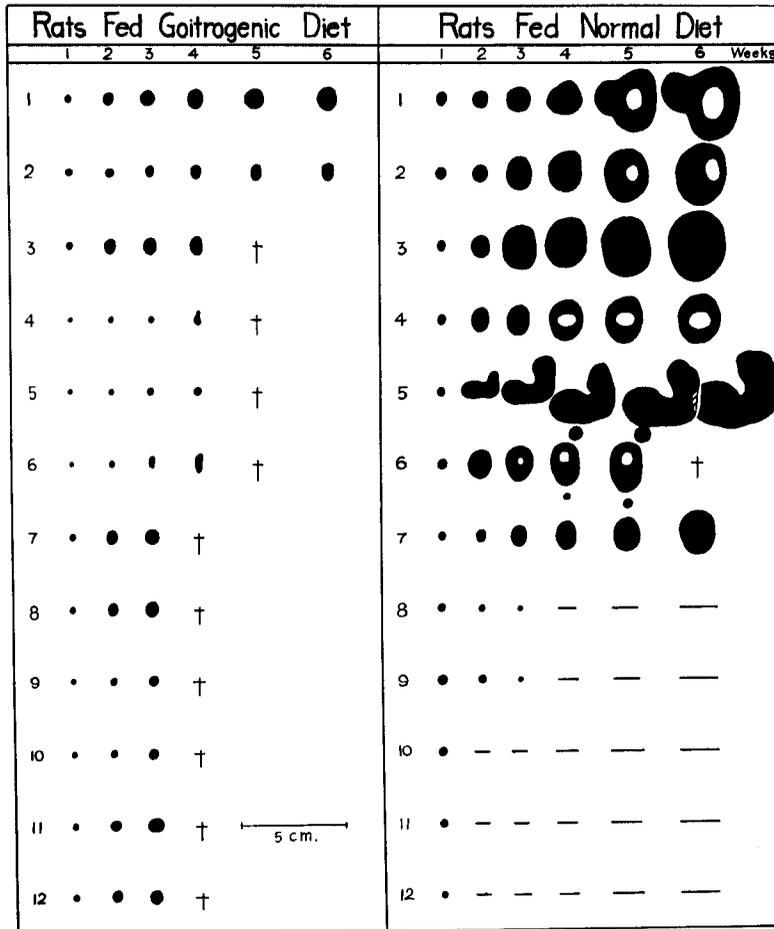


FIG. 1. MARKED RETARDATION OF GROWTH OF THE FLEXNER-JOBLING RAT CARCINOMA IN RATS RECEIVING A GOITER-PRODUCING DIET, CONTRASTED WITH THE RAPID TUMOR GROWTH IN THE CASE OF ANIMALS ON A NORMAL, WELL BALANCED DIET

However, animals on the iodine-deficient diet showed a higher percentage of tumor takes and a lower percentage of regressions than the controls.

for eleven to twenty-three days prior to tumor inoculation. As a control, the same number of rats of nearly the same age were fed with a normal diet and were similarly implanted with grafts of a Flexner-Jobling rat carcinoma. Implants were all made subcutaneously in the region of the axilla. The results are presented in Table I, and a characteristic example is shown graphically in Fig. 1.

As may be seen from the data in Table I and Figure I, the rate of tumor growth in animals fed upon the iodine-deficient diet was markedly diminished. However, the percentage of tumor takes in these animals (average of 98 per cent) was consistently greater than in those fed upon the normal, balanced diet (average of 80 per cent). The tendency of the tumors to regress in the animals on the low-iodine diet was markedly less than in the animals on the normal diet. Whether or not iodine deficiency alone is responsible for this decreased resistance of the body against the neoplasm and the marked retarding influence upon the rate of tumor growth, is uncertain; but it is well known that the growth of transplanted tumors is often affected by the nutritional condition of animals supporting them. This was clearly demonstrated in our earlier studies on the effect of a protein-free diet (3) and of underfeeding (4) upon the growth of transplanted and spontaneous tumors.

In the preceding experiments, the animals were maintained on a modified form of the ricket-producing diet of Steenbock and Black (5), deficient not only in iodine, but also in some vitamins and in necessary amino-acids. Therefore many rats on this special diet died at the end of five weeks, and many of the others were in very poor condition.

In our next experiments a similar goitrogenic diet was used, but containing 20 parts of casein in place of 20 parts of wheat gluten. The latter protein is lacking in tryptophane, indispensable for animal nutrition. The new ration was found to be superior in nutritional quality to the ration containing wheat gluten. At the conclusion of the experiment, the majority of the animals were still living.

The results obtained following inoculation in animals (2 groups of 24 rats) receiving this ration containing casein showed that the rate of tumor growth was decidedly diminished, but the tumor inhibition was not as great as in the first experiment. This indicates that under the conditions of these experiments the growth of transplanted tumors roughly parallels the growth of the animals supporting them. In experiments with certain inorganic salts we have found the contrary to be true (6).

Effect of an Iodine-Normal Diet upon the Growth of the Flexner-Jobling Rat Carcinoma

In recent studies on the relation of diet to goiter (7), it was clearly shown that the minimum iodine requirement of the rat was approximately 1-2 γ per rat per day. Since it is now known that a small amount of iodine is essential for growth and maintenance of the life of experimental animals, the following experiments were undertaken for the purpose of securing further information concerning the rôle of iodine in relation to the growth of transplanted tumors.

Young albino rats were maintained upon the goitrogenic diet, to which iodine (2-3 γ per rat per day) was added. This diet contained 0.52 mg. of potassium iodide per 100 grams of food. Two weeks after

the beginning of the feeding the animals were inoculated with fragments of the Flexner-Jobling rat carcinoma, and the feeding was continued during the experimental period of six weeks.

Animals fed on this iodine-normal diet maintained their body weight after a slight drop during the first two days, but did not grow. At the end of the experiment the general condition of these animals was poor.

TABLE II: *Results of Transplanting Flexner-Jobling Rat Carcinoma in Rats Receiving an Iodine-Normal Diet*

Experiment Number	Number of Animals Used	Diet	Percentage of Tumor Takes	Remarks
1	12	Normal	75	3 tumors did not grow; 2 regressed after growing for 2-5 weeks; others grew rapidly
	12	Iodine-normal	33	8 tumors did not grow; 2 regressed after growing for 2 weeks; others grew slowly
2	12	Normal	100	2 tumors grew slowly; others grew rapidly
	12	Iodine-normal	0	No growth
3	10	Normal	70	3 tumors did not grow; 2 regressed after growing for 3 weeks; others grew rapidly
	10	Iodine-normal	70	3 tumors did not grow; 1 regressed after growing for 2 weeks; 2 grew very slowly; others grew slowly
4	12	Normal	75	3 tumors did not grow; 2 regressed after growing for 3-5 weeks; 2 grew slowly; others grew rapidly
	12	Iodine-normal	50	6 tumors did not grow; 2 regressed after growing for 3-5 weeks; others grew very slowly

The thyroids of rats receiving the goitrogenic ration containing added iodine, having an iodine content of 80 γ per 100 gm. of food, were examined grossly and histologically. They were generally pale pink in color and were non-hyperplastic; in some sections they showed slight hyperplasia without hypertrophy. They contained considerable colloid. The acini were of moderate size.

The results obtained following tumor inoculation in animals fed with an iodine-normal diet are presented in Table II.

The data show clearly that not only was the rate of tumor growth greatly reduced, but the percentage of tumor takes in animals on the diet with a normal iodine content was smaller than in animals receiving a normal, well balanced diet (average of 37 per cent takes against 80 per cent for the controls). A typical example is graphically shown in Fig. 2.

Neither regional and intrathoracic nor visceral metastases occurred

in rats on the special diet, probably because large tumors were rare. However, under normal feeding and conditions, about 20 per cent of the animals showed regional metastases, about 7 per cent intrathoracic metastases, and about 2 per cent lung metastases. Metastases in other viscera seldom occurred.

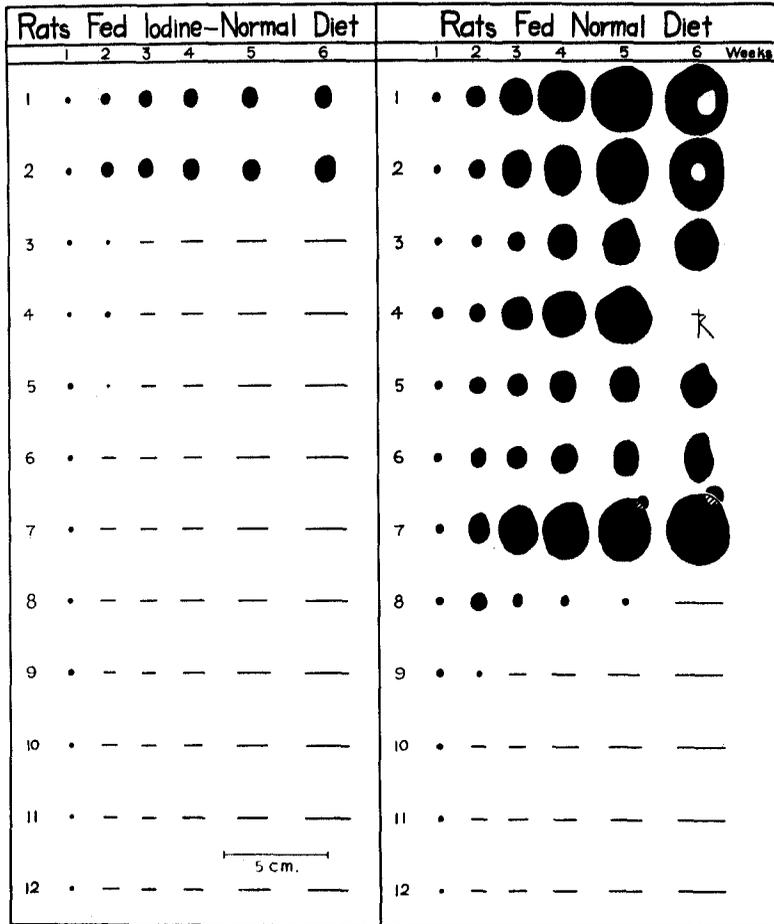


FIG. 2. GROWTH OF FLEXNER-JOBLING RAT CARCINOMA IN ANIMALS ON AN IODINE-NORMAL DIET CONTRASTED WITH ANIMALS ON A NORMAL DIET

When iodine in the form of potassium iodide was restored in adequate amount (2-3 γ per rat per day) to the goitrogenic diet of animals, not only was the rate of tumor growth greatly reduced, but there were a smaller number of tumor takes and a greater number of tumor regressions.

The reduced tumor susceptibility could not be entirely due to the impaired health of the hosts, since our earlier experiments with an iodine-low diet indicated that such a deficiency had an apparent stimulating influence upon the tumor takes. It is possible that the combination of a small amount of iodine and defective diet is important. In order to test further the rôle of iodine in tumor growth, we investigated the effect on the growth of transplanted tumors of diets rich in iodine.

Effect of Iodine-High Diets upon the Growth of Carcinoma, Sarcoma, and Melanoma in Animals

Having found that the addition of a small amount of iodine to a goitrogenic diet exerted an inhibitory effect upon transplanted tumors, preliminary experiments were conducted with a similar diet but containing an amount of iodine greatly above the requirement of the rat.

Young albino rats were fed on the standard goitrogenic diet of Levine, Remington and von Kolnitz containing added iodine, bringing the iodine content to 8,000 γ or 800,000 γ per 100 grams of food. Two weeks later, all the rats were implanted with fragments of a Flexner-Jobling rat carcinoma. Six groups of experiments were conducted, involving a total of 70 rats. As a control, rats of nearly the same age were fed with our normal diet and were inoculated with the same tumor tissue.

The results obtained from these experiments were essentially the same as those with animals fed exclusively upon the goitrogenic diet, namely, a marked retardation of tumor growth in the case of animals fed upon the goitrogenic diet containing excessive amounts of iodine and rapid tumor growth in the case of animals fed upon a normal, balanced diet.

TABLE III: *Results of Transplanting Flexner-Jobling Rat Carcinoma in Rats Fed Upon Various Amounts of Iodine*

Diet	Number of Animals	Percentage of Tumor Takes	Percentage of Tumor Regressions	Rate of Tumor Growth
Iodine-low (goitrogenic)	46	98	0	Very slow
Iodine-normal (80 γ per 100 gm.)	46	37	11	Very slow
Iodine-high (8,000 γ per 100 gm.)	34	100	5	Very slow
Iodine-high (800,000 γ per 100 gm.)	36	94	3	Very slow
Normal	162	83	15	Rapid

Although the percentage of tumor takes was normal in the animals fed with the iodine-high diets, the number of tumor regressions was less than where the animals were fed with a normal diet, or a goitrogenic diet containing a normal amount of iodine. Evidently the daily intake of a normal amount of iodine exerts an inhibitory effect on tumor takes, while large amounts of iodine abolish this effect. A composite table showing the effects of various amounts of iodine upon the progress of tumors may illustrate this point more clearly (Table III).

Young rats fed on a goitrogenic diet containing 8000 γ iodine per 100 grams of food thrived as well as those fed on a similar goitrogenic diet containing a normal amount of iodine (80 γ per 100 gm. of food). However, a much higher concentration of iodine in the diet (800,000 γ per 100 gm. of food) produced toxic effects, as shown by the early death of the animals.

Since the preceding experiments were conducted under abnormal

conditions, in which the animals were maintained on a faulty diet, our study was extended by adding potassium iodide to a normal, well balanced diet. The basal diet consisted of a uniform mixture of 32.1 parts by weight of cracker meal and 7.7 parts by weight of whole milk powder (Klim). To each 100 gm. of the ration was added 1.0 gm. of dried yeast. A dough was prepared with 50 parts of the basal diet and an equal amount of the potassium iodide solution (0.1, 1.0, 5.0 or 10.0 per cent).

Preliminary feeding experiments with young rats and mice indicated that the lowest concentration of the potassium iodide solution caused no harmful influence upon the body metabolism, as shown by the normal rate of body growth; while the higher concentrations produced toxic effects. With a 1.0 per cent solution of potassium iodide the animals maintained their body weight, but did not grow; their general appearance was good. With 5.0 per cent solution of potassium iodide, the animals lost weight; some died early, while others appeared to be in poor condition toward the end of the experiment. With a 10 per cent solution of potassium iodide, the animals lost body weight rapidly. Some mice died as early as the second day, while others died within fifteen days; rats died in from thirteen to eighteen days.

Young and young adult animals were maintained upon the special diets for about ten days prior to tumor inoculation, so that the potassium iodide might have its full physiological effect both before and after tumor inoculation.

The Flexner-Jobling rat carcinoma, mouse sarcoma 180, and Passey mouse melanoma were selected for this study. The behavior of these transplantable tumors in the hosts has been reported elsewhere. Forty animals, including normal controls, were used for the first experiment with each type of tumor. This experiment was repeated in each group; a total of 224 animals was used.

The results of the experiments showed that the growth of Flexner-Jobling rat carcinoma in rats and of sarcoma 180 and Passey mouse melanoma in mice was not affected by the daily ingestion of 8 mg. of potassium iodide (0.1 per cent solution of potassium iodide) per rat, or 3 mg. of potassium iodide per mouse. On the other hand, the daily intake of large amounts of potassium iodide (1.0 and 5.0 per cent solutions) had a definite inhibitory effect upon the growth of transplanted carcinoma, sarcoma, and melanoma, slight with the lower concentration and marked with the higher. This retardation of tumor growth, however, we attribute to the impaired health of the hosts. This fact suggests that the treatment of human neoplasms with large amounts of iodine in the diet would be of no value.

SUMMARY

1. In all groups of experimental animals fed on a goitrogenic diet or the same diet containing added iodine (80, 8000 or 800,000 γ per 100 grams of food), the rate of tumor growth was very slow. This was

probably due to the impaired health of the animals produced by the faulty diet irrespective of the iodine content. The percentage of tumor takes and of tumor regressions was found to vary significantly with variation of the iodine content of the diets. The lowest number of takes and the greatest number of tumor regressions occurred in those animals receiving a normal amount of iodine, while animals on either high-iodine or low-iodine diets showed a high percentage of takes and a low percentage of regressions.

2. Prolonged feeding of the iodine-high diets (animals fed on a normal, well balanced diet containing excessive amounts of potassium iodide) had no therapeutic effect on the growth of Flexner-Jobling rat carcinoma, mouse sarcoma 180, and Passey mouse melanoma.

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