DIET STUDIES IN TRANSPLANTABLE TUMORS.*

I. THE EFFECT OF NON-CARBOHYDRATE DIET UPON THE GROWTH OF TRANSPLANTABLE SARCOMA IN RATS.

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It has long been a popular belief that the character of the diet of a group or race of people bears an important relationship to the incidence of malignant growths. A striking example of this attitude is seen in the book entitled: Preventable Cancer,[1] by Rollo Russell, in which the evidence for this belief is ingeniously and, to some degree, convincingly arranged. It is well known, however, that vital statistics in many of the communities which he has investigated are unreliable. In fact, it must be recognized that there is no field of cancer research at the present time which needs more careful handling than that of vital statistics. Particularly in this country it is necessary to make vast improvement if any reliable conclusions are to be drawn. The paper recently presented by Prof. W. F. Wilcox of Cornell at the Sixth Annual Meeting of the American Association for Cancer Research at Washington may be pointed out as a striking example of how a careful analysis of statistics may controvert popular belief. Nevertheless, one cannot read Russell's book without being to some degree influenced by it and impressed with the suggestion that the dietetic habits of a group of people may bear some relation to the number of cases of cancer which develop therein as well as have some influence upon the character and progress of the disease.

The notable work of Mendel and Osborne[2] which has demonstrated that the normal growth and development of an animal may be controlled by the character of the food stuff points to the possibility that the pathological growth of tumors

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may be similarly conditioned. In fact, Sweet, Corson-White, and Saxon [3] have already made a preliminary report on a study aimed to test this hypothesis. These investigators made use of a diet of glutinin and gliadin fed to white mice. They found that such a diet would effectively retard the growth of the animals. One series of fifty mice, twenty-five on a control diet, and twenty-five on the experimental diet, when planted with a tumor obtained from Dr. Rous, gave twenty-three takes on the normal diet and four on the vegetable protein diet, three of which later disappeared. In a second similar series eighteen out of twenty-five on the control diet took the tumors as against three on the vegetable protein diet. A third series gave fifteen out of twenty-five on the normal diet, as compared with seven out of twenty-five on the special diet. Seventy-five per cent of the seventy-five mice took the tumor if fed on normal diet, while nineteen per cent took the tumor if fed on the vegetable protein diet and the tumors in the specially-fed lot grew no more in thirty days than the control animals did in ten days. They state that the most probable conclusion is that the tumor cell is subject to the same laws of growth as is the case with the normal somatic cells. The validity of such a conclusion is, however, open to some question in view of the results of our experiments reported herein.

Many peculiarities in the transplantability of tumors have been noted, and Haaland [4] has cautiously explained his observation that Berlin mice transferred to Christiania become refractory to the Erlich's (Berlin) virulent sarcoma on the ground of a change in diet. In Berlin they were fed chiefly on fat and animal protein (milk), while in Norway the diet was largely carbohydrates. However, he has given no experimental evidence for such a conclusion, and unless the mouse sarcoma has entirely different growth behavior from the rat sarcoma, we should expect the Norway diet to be the most favorable.

Robertson and Burnett [5] have recently published two papers on the influence of cholesterol upon the growth and incidence of rat carcinoma. These experimenters studied the

Flexner-Jobling carcinoma and found that if cholesterol was injected directly into the tumor there was a marked acceleration of both the primary and metastatic growths. Proceeding upon the basis that cholesterol in animal tissues is derived from the food and is not synthesized by the animal, they planned an experiment to determine whether or not rats fed upon a diet poor in cholesterol would be less susceptible to tumor implantation than rats fed on a diet richer in this substance. To quote from their paper: "Accordingly twenty-two white rats, about two months old, were divided, without exercising any selection, into two lots: one lot of fifteen was fed upon a diet composed exclusively of milk, the remainder were fed upon a mixed diet of oats and meat. Both lots of animals thrived well, the milk-fed animals presenting an especially well nourished appearance." At the end of two months' feeding both lots were inoculated with portions of the same tumor, the animals continuing on the same diets as before planting. At the end of twenty days, ten out of fifteen (or 67 per cent) of the animals on the milk diet showed growth, while seven out of seven (or 100 per cent) of the mixed diet showed growth. At the same time another batch of half-grown animals obtained from the same dealer and having been fed on a mixed diet were planted with the result that fifty-five out of sixty-four (or 86 per cent), showed growth. Although they admit that the difference between the milk-fed and the mixed diet rats was not as marked as might be expected if cholesterol is to be considered an important factor in determining the incidence of carcinoma, they state their belief that more conclusive results may be obtained if the animals are fed on a cholesterol free diet from birth. They believe that the conservation of cholesterol in the animal body explains the larger amount of this substance found in older animals and that this, in turn, is the reason for increase in the incidence of carcinoma with advancing age.

It may be pointed out that they fail to record the relative size of the tumors in the two lots of animals, the relative rate of growth, or the ultimate fate of the animals. On the basis

of fifteen rats put upon an experimental diet, ten of which took the tumor, so significant a conclusion seems hardly justified. A possible explanation of the difference may be found in the fact that the milk-fed animals were, as they state, especially well nourished. Another possibility is in the unfavorable effect of the casein in the milk, an effect recently observed by Leo Loeb.[6] Although these authors state that milk contains .02 per cent cholesterol and meat from .07 to .08 per cent, the total amount of cholesterol eaten by the two sets of rats during the experiment is not given, and one may only infer but not conclude that the milk-fed animals had less of this supposedly important element than the controls.

The experiments which are herein reported may be divided into two classes:

1st. Those experiments in which one group of animals was kept upon a special diet for from three weeks to two months before being planted with the tumor, and,

2d. Experiments in which the special feeding of the animals was begun on the same day that they were planted.

Class I., Experiment No. 1. — A group of thirty-four white rats of uniform age and weight was divided, without exercising special selection, into two groups of seventeen rats each. One group was placed immediately upon the special non-carbohydrate diet which consisted of casein and lard. The second group was continued upon the ordinary laboratory diet, consisting of bread. This feeding was continued for a period of six weeks, at which time the animals were all planted from the same seed. In this experiment the tumor was a Buffalo sarcoma. A large mass of the tumor was removed, finely pulverized and suspended in salt solution. Of the emulsion thus prepared each rat received one cubic centimeter by subcutaneous injection. The animals of both sets were in good physical condition at the time of planting the tumor. This experiment was to some degree a preliminary one, so that the careful measurements made of the growths in the later experiments were not recorded in this group. The results of this experiment may be tabulated as follows:

Non-carbohydrate diet:
17 rats planted:
4 takes: { 2 retrogressed.
1 killed by the tumor.
1 died from unknown causes.

Regular diet:
17 rats planted:

7 takes: { 4 retrogressed. / 2 killed by the tumor. / 1 died from unknown causes.

In this experiment there is a much larger percentage of positive growths shown by the rats fed on the diet containing carbohydrate. However, it is well known that there are marked variations in the virulence of the tumor and susceptibility of the host, so that a single experiment of this type can scarcely be considered of great significance.

Class I., Experiment No. 2. — In this experiment there was a total of twenty-seven rats, divided into two lots, thirteen being placed on the regular diet, and fourteen upon the non-carbohydrate diet. The same diet was used as in Experiment No. 1, and the animals were fed for six weeks before being planted. The results of the experiment are shown in the following table. The methods of planting these rats was the same as that followed in Class I., Experiment 1, a freshly removed tumor serving as the seed for all of the animals:

Non-carbohydrate diet:
14 rats planted:

1 take: { 1 rat took the tumor which grew to large size and killed the animal.

Regular diet:
13 rats planted:

7 takes: { 3 died from unknown causes. / 4 killed by the tumor.

The results in this experiment are so striking that one can scarcely avoid the conclusion that the preparation of one group of animals by feeding on a non-carbohydrate diet has enabled the animals to resist the tumor.

Class I., Experiment No. 3. — In this experiment thirteen rats were fed on the special diet of casein and lard for one month and twenty-seven days; sixteen rats were fed on the regular diet for one month and twenty-seven days. There was one hundred per cent of takes in each set, the trochar method being used for planting the tumor. At this period the virulence of the tumor (Buffalo sarcoma) had greatly increased so that in the rats kept for special experiment as well as those used to maintain the tumor strain there was one hundred per cent growth. The effect of the

diet in this series was not shown in a resistance to tumor implantation, but the subsequent fate of the tumors in the two series was so different as to leave no doubt that the diet had effectively conditioned the tumor growth. In this series of tumors careful measurements were made each week of the actual size of the tumors and from these figures the cubic contents were calculated. In the table given below are shown the figures for the two series for a period of three weeks subsequent to the planting. The results are also expressed in the form of a curve showing the rate of growth in the two series of animals. These measurements cannot be successively carried on for a longer period than three weeks, for the reason that accidental factors such as the death of the animals from the growth of the tumors break up the series:

TABLE SHOWING GROWTH OF TUMORS.

Expressed as cubic millimeters in the two series of animals.

	Special Diet.	Regular Diet.
1st week	46.6	181.2
2d "	166.3	636.0
3d "	175.6	804.0

CURVE I.
Showing rate of growth of tumors in Experiment 3.

Some additional observations on this experiment may prove of interest. One month after planting there had been on the special diet six regressions, while on the regular diet there were only three. Prior to five weeks and two days after planting there had been on the special diet one death from unknown causes, while on the regular diet five rats had been killed by the tumor, and three had died from unknown causes. The rats were weighed each week after planting at the time the tumors were measured, and in the table given below are the figures in grams showing the average weight of the rats in the two series for four weeks after planting.

AVERAGE WEIGHT OF RATS DURING TUMOR GROWTH.

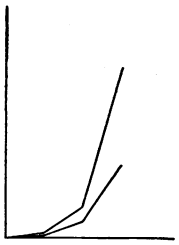
	Special Diet.	Regular Diet.
1st week	72.1	60.4
2d "	76.3	65.1
3d "	77.8	73.6
4th "	78.6	69.2

Class I., Experiment No. 4. — In this experiment a lot of fifty-one young rats was divided into two groups of twenty-five and twenty-six. One group was fed for a period of three weeks on the special diet of casein and lard, while the other was continued on the laboratory diet of bread. In this experiment the planting was done by trochar since we had found this method more satisfactory. Another point worthy of notice is that at this time the virulence of the tumor had increased to such a degree that nearly all of the animals showed positive growth. Attention may be called also to the fact that in this case the period of preparation was three weeks instead of six. Out of the twenty-six rats in the controls there were twenty-two takes and in the twenty-five rats on the special diet there were also twenty-two takes. These figures indicate an apparently identical condition of reaction of the two groups toward tumor implantation. However, the subsequent growth in the two lots was very different. As in the former experiment, careful measurements were made at weekly intervals of the size of the tumors and from these figures the cubic contents in the two sets were calculated. In the table given below the results in cubic millimeters of the two series are contrasted.

TABLE SHOWING GROWTH OF TUMORS.

Expressed as cubic millimeters in the two series of animals.

	Special Diet.	Regular Diet.
1st week	30.9	46.6
2d "	157.4	314.4
3d "	753.6	1815.8



CURVE II.
Showing rate of growth of tumors in Experiment 4.

Forty days after planting examination of the records of the two lots reveals the fact that of those rats on the regular diet fourteen had died from the tumors and three from unknown causes. Of those on the special diet two had died from the tumor and one from unknown causes. This difference is so striking as to leave no room for doubt that the diet was an important factor in enabling the rats to resist the tumor after growth had started.

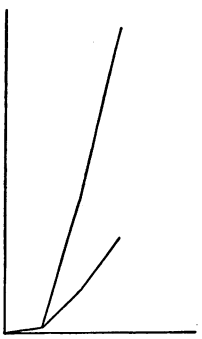
The average weights in grams of the rats in the two series for four weeks following the planting of the tumor are given in the following table:

	Special Diet.	Regular Diet.
1st week	28.7	26.1
2d "	33.7	30.0
3d "	36.8	34.1
4th "	47.6	35.2

Class I., Experiment 5. — In this experiment the diet of the controls was different from that used in the former experiments in the following respect. It will be recalled that the special diet consists of casein and lard, while the regular diet consists chiefly of bread. In order to be certain that the effects noted in the previous experiment were caused by the carbohydrate portion and not by some extraneous factor, the rats in the control series in this experiment were fed upon casein, lard, and pure lactose. The animals were fed their respective diets for one month before

planting. There were twenty-six animals, in each series, the trochar method was followed in planting, and each series showed one hundred per cent takes. The animals were weighed, the tumors measured and charted weekly. The following table shows the rate of growth in the two series, the figures showing the average bulk of the tumor in the two series, expressed in cubic millimeters:

	Special Diet.	Control Diet.
1st week	46.0	46.0
2d "	439.0	1367.0
3d "	1000.0	3421.0



CURVE III.
Showing rate of growth of tumors in Experiment 5.

On the fourth week after planting, eight of the animals on the control diet had been killed by the tumors and there were no regressions among the remaining animals. Of those on the special diet, six had regressed completely and six were regressing, and there were no deaths. On March 1 — five weeks and two days after the planting of the tumors — all of the animals of both series were put upon the laboratory diet. Twenty-five days later the condition of the animals in this experiment is worthy of note: Of the twenty-six in the control series, eighteen had died with large tumors, eight were yet alive, six of these having large ulcerated tumors. Of the twenty-six animals of the special diet,

twenty were alive. There was no tumor tissue in any of them and they were in excellent condition, in spite of the fact that the ordinary laboratory diet containing carbohydrate was now their food.

The final fate of the series is well shown in the following table:

Special Diet.	Control Diet.
26 animals planted:	26 animals planted:
100% takes:	100% takes:
2 died from the tumor;	24 died from the tumor;
4 " " accidental, unknown causes;	2 regressed.
20 regressed.	

The average weights of the two series of animals during the first four weeks after planting are shown in the accompanying table:

	Special Diet.	Control.
1st week	41.7	42.3
2d "	40.5	47.9
3d "	42.1	46.6
4th "	48.0	45.1

There seems to be no reasonable ground for doubt in view of these experiments that a lack of carbohydrate in the diet produces such an influence upon the rats as to make them more resistant to tumor growth. A sufficient number of experiments has been quoted, the experiments have included so large a number of animals in both series as to rule out the accidental factors which occasionally complicate observations of this character. When the diet includes

carbohydrate the tumors grow luxuriantly. When the diet does not include carbohydrate, the animals show a marked resistance not so much to a beginning growth when the tumor is virulent, as to its continued progress. It is possible to theorize to some length upon these observations, but at the present time such theorizing can scarcely be considered a profitable pastime. It is our purpose in this paper to state facts and not theories. It may be pointed out, however, that Dr. Leo Loeb[6] has found that casein exercises some peculiar inhibitory influence upon the growth of tumors in rats and someone may possibly suggest that such an influence was operating in these experiments. The last experiment, however, should effectively controvert such a deduction, for the reason that the rats on both special and control diets had an equal amount of casein. In the control diet a portion of the lard was replaced by an isodynamic amount of crystallized lactose. It would, in fact, appear that the lactose has exercised some specific influence in rendering the animals more susceptible because in no case either in these experiments or in the other records of the laboratory does there appear so large a percentage of the animals planted growing the tumor to the point of being killed by its growth. It is our purpose to repeat the experiment and to compare lactose with other sugars in this effect.

It probably has occurred to the reader who has followed these experiments thus far that it would be of interest to determine whether a non-carbohydrate diet would inhibit the growth of tumors already started, and it need not be supposed that the writers have been oblivious to such a suggestion. In fact we quote in the following pages four experiments in which the special non-carbohydrate diet was begun with one-half the number of animals on the same day that they were planted with the tumor. There was, therefore, no previous preparation of the animals by non-carbohydrate feeding.

Class II., Experiment 1. — A lot of twenty-three rats which had been kept in the laboratory for some days was divided into two lots and

planted by trochar with the Jensen sarcoma. On the day of planting, twelve of the rats were placed upon special diet, consisting of casein and lard and were continued on this diet throughout the experiment. The Jensen tumor was not virulent and caused only a small degree of growth. However, nine rats in each lot showed a positive growth of the tumor. But in no case did the tumors grow to large size in either control or special diet, and the behavior of the control and special-diet group throughout the experiment was in all respects the same. The diet apparently had no influence.

Class II., Experiment 2. — In this experiment the Buffalo sarcoma was planted by trochar into twenty-one rats. Ten of these were immediately placed upon the special diet of casein and lard. All of the rats in both series showed positive growth of tumors. The subsequent fate is indicated in the following table:

Special Diet.	Regular Diet.
10 rats planted:	11 rats planted:
10 showed growth;	11 showed growth;
6 retrogressed;	9 retrogressed;
2 killed by tumors;	1 killed by tumor;
2 with large tumors killed, and tumor material used for other work.	1 died from unknown cause.

This experiment gives small basis for the hope that feeding on the special diet subsequent to tumor implantation would prove effective in rendering the animals resistant. Apparently a period of feeding previous to planting the tumor is necessary to develop that remarkable resistance shown in the first class of experiments.

Class II., Experiment No. 3. — A lot of thirty-six rats was planted by trochar with the Jensen sarcoma, and immediately after planting eighteen of them were put upon the special diet of casein and lard. The table given below shows the subsequent history of the animals:

Special Diet.	Regular Diet.
18 rats planted:	18 rats planted:
6 showed growth;	8 showed growth;
4 retrogressed;	5 retrogressed;
2 died from unknown causes, with very small tumors.	1 with small tumor died from unknown causes;
	2 grew very large tumor.

There was no growth in the rats on the special diet comparable to the growth shown by two rats on the regular diet, but the differences in these two sets are so small that one can scarcely conclude that the diet has been effective in causing the differences in growth behavior.

Class II., Experiment No. 4. — Thirty rats were planted with the Buffalo tumor by the trochar method and, immediately after planting, fifteen of the animals were placed upon the special diet of casein and lard. The following table gives the details of the results:

Special Diet.	Regular Diet.
15 animals planted:	15 animals planted:
15 showed growth;	15 showed growth;
8 retrogressed;	7 retrogressed;
5 grew very large tumors:	4 grew very large tumors which were used for other work.
2 died early in the experiment from unknown causes with small tumors.	4 died early in the experiment from unknown causes, with small tumors.

The results in this experiment show that the two sets of animals reacted in almost identical fashion to tumor implantation. The differences are so slight that when taken in conjunction with the other experiments of the same nature outlined above, one is left in no doubt of the ineffectiveness of non-carbohydrate feeding begun at the time of tumor implantation.

The period of preparation is vital to success. The animals fed on carbohydrate free diet for a few weeks prior to planting the tumor are able to a large degree to resist its growth. We do not know how long a period of preparation is necessary, nor whether other proteids and carbohydrates may not have different values than those tested. Such considerations remain for future investigation. The last experiment in Class I. suggests, however, that not all carbohydrates are alike in preparing the soil for tumor growth. Those animals which received pure lactose as a portion of the diet grew the tumor much better than those in the former experiments which were fed on bread. This lactose diet is, indeed, so striking in its effects that it might serve as a means for rescuing a tumor of low virulence, or for producing a great bulk of tumor tissue for experimental purposes. The contents of glycogen in the tumors of the contrasted series has not been determined, nor have we any accurate pictures of the histology of the tumors in the two sets.

From the weights of the rats following tumor implantation as given in the tables quoted in Class I. experiments, it does not appear that there are any considerable differences in the nutrition of the two sets of animals. They had an abundance of food and except for accidental infections throve well. It will be noted, however, that not all of the rats in a set have similar reactions to the tumor. Even in Class I., Experiment 5, where twenty-four out of twenty-six animals produced large tumors to which they succumbed, there were two regressions. Individual peculiarities are not entirely eliminated by diet. Among the animals on carbohydrate free diet we have occasionally had one which in spite of his preparatory feeding grew the tumor luxuriantly.

In view of these results the conclusion of Sweet, Corson-White, and Saxon that tumor cells agree with normal somatic cells with respect to their laws of growth hardly seems justified. Those investigators fed the animals a diet which inhibits growth, while in our experiments both sets of animals were in excellent nutritional condition. Those on the special diet of casein and lard grew quite as well as those upon the normal diet. This is well shown in Experiment 5 in Class I., in which the weights of the animals in the two series are almost identical. And yet twenty-four of the twenty-six animals on the diet of casein, lard, and lactose grew the tumor with fatal results, while on the diet of casein and lard twenty regressed and only two were killed by the tumor.

Further experiments are needed to confirm these observations, but it seems justifiable to conclude that an animal may be in excellent nutritional condition, and yet because of peculiar preparation by diet is unable to grow tumor tissue. Certain it is that the susceptibility to tumor implantation in rats is markedly modified by the character of the food. It is quite possible that Robertson's results mentioned above may be due to the fact that the rats fed on cholesterol containing food also obtained large amounts of carbohydrate in the oats, while the animals on the milk diet had relatively small amounts of this important constituent. It cannot be said, of course, as a result of these experiments that tumor incidence is higher in a lot of animals or race of people eating rich, carbohydrate diet, than on a flesh diet. It may be maintained, however, that susceptibility and immunity to tumor implantation are not entirely independent of the diet.

[The experiments reported in this paper were reported to the Congress of Clinical Surgeons at the meeting in New York November, 1912; and to the American Society for Experimental Therapeutics and Pharmacology at the meeting in Cleveland, Ohio, December, 1912; and to the American Association for Cancer Research at the meeting in Washington during April, 1913.

NOTE. — We are indebted to Prof. Stanley R. Benedict, of Cornell University Medical School, for many fundamental suggestions and valuable advice in the course of this investigation. It was at his suggestion

that this study to determine the effect of non-carbohydrate compared with ordinary diet was initiated. The success of these experiments confirms his judgment.]

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