The problem of cancer is but one aspect of the broader problem of growth. Just as the young organism is distinguished from the adult by its power to grow, so the cells of the tumor differ primarily from those of the host in their capacity for rapid, and apparently unlimited, increase. Unless proved to the contrary, it must be assumed that the same agencies which are responsible for growth in the young organism are also concerned in the growth of the tumor. Whether the various factors concerned are of equal importance in the two processes, whether each bears the same relation to growth under the two conditions, is at least partly open to experimental investigation at the present time.

Within recent years, the researches of Hopkins, Mendel and Osborne, McCollum, and Funk, together with a number of other investigators, have opened a new era in the science of nutrition. Diets which were formerly believed to be sufficient to maintain life and growth have been found to be wholly inadequate to do either. According to recent conceptions, we may regard diets as "deficient" in any one or more of four ways. Firstly, a diet may be deficient in protein, in inorganic salts, or in water; secondly, it may be deficient in content of calories, i.e., it may not yield enough energy for the requirements of the organism, through a quantitative deficiency in carbohydrates, or fats, or both. Thirdly, it may be deficient in certain fundamentally necessary amino acids, which the organism cannot synthesize. An example of a diet of this type would be one properly bal-
anced in regard to protein, fat, carbohydrate, inorganic salts, accessories, and water, from a quantitative standpoint, but in which the sole source of nitrogen is gelatin or zein. Certain amino acids which the organism cannot synthesize are lacking in such a diet, and the animal will perish after a more or less brief period if they are not furnished. Lastly, the diet may be complete in regard to the essential ordinary food-stuffs while lacking in the so-called accessory food substances (the "water-soluble A, and the fat-soluble B" of McCollum, the "vitamines" of Funk). If such "accessory" food materials are not supplied, the death of the organism will surely ensue. No one has succeeded in maintaining the life of an animal over a reasonably long period upon any diet of chemically purified food-stuffs.

It has been planned in this laboratory to undertake a systematic study of the relationships between the growth of normal and of tumor tissue, with especial reference to the fundamental factors which are at present known to influence normal growth in young animals.

Studies along this line have already been reported by certain investigators, both from this laboratory and from others. These will be referred to at the points where our work touches that of previous investigators.

The present paper is preliminary in nature, but we feel that its publication will be justified if it serves only to call attention to the inherent difficulties in this field of investigation. In addition to this, however, we hope to present some facts which are of interest in relation to the general question of nutrition, and which may have their proper application to the problem of cancer.

The first question which we have taken for study is whether tumor cells, like somatic cells, are dependent upon a supply of vitamines from outside sources, or whether tumor cells possess a power of synthesis of vitamines. If this latter supposition should be correct, then we should have a fundamental difference between tumor and body cells, a difference as great in this respect as between plant and animals cells. The former of these synthesize vitamines; the latter have never been shown to do
so. If tumor cells possess the power of vitamine synthesis, then they have indeed a unique and tremendous advantage over ordinary tissue cells. Obviously this question is an important one, and worthy of detailed study. Investigations planned to deal with this problem have already been reported. Funk (1) found a higher percentage of "takes" and better growth for the Rous sarcoma when inoculated into chickens on normal diets than when the chickens were fed on unpolished rice with or without addition to this diet of yeast (rich in vitamins) or of sarcoma extract. With chickens on polished rice (vitamine-free) Funk failed to get "takes" with the Rous sarcoma. From the vitamine standpoint these results are somewhat contradictory, since the tumor grew better in the birds upon a "normal" diet than in one known to be rich in vitamins (unpolished rice plus yeast). Chickens are not, however, well adapted for an experiment investigating the relation of vitamins to tumor growth, since the agencies determining normal growth in chickens have not been at all adequately studied. Funk (2) has reported investigations along this line, but the results so far do little more than show that the growth-controlling factors in chickens are quite different from those in the mammals so far studied. Indeed from Funk's findings we are convinced that at present no study having any real significance in relation to the question of whether tumor growth differs essentially from normal growth can be successfully carried out upon chickens.

Rous (3), and Sweet, Corson-White, and Saxon (4) have studied the effects of underfeeding and of certain deficient diets upon the growth of tumors in rats and mice. The diets they employed were probably deficient only in one or more specific amino acids. Under such conditions it seems probable that the tumor tissue may take the amino acid necessary for its own nutrition from the circulating proteins in the blood, where it probably is present in abundance so long as the animal remains alive. The results of Rous, and of Sweet, Corson-White, and Saxon are not, however, directly related to the question which we are now discussing, since the effects of vitamins were not
studied in their investigations. Reference to some of their results will be made later.

In arranging an experiment to study whether tumor tissue in rats is dependent upon vitamine from outside sources for its growth, it would seem that the ideal condition to be fulfilled would be to give the young animal a vitamine-free diet which would keep it in good general condition, but upon which it was unable to grow at all. Inoculation of such an animal with virulent tumor tissue would then be made, and account taken of the number of "takes," and rate of growth as compared with controls upon a diet containing vitamine. Such a procedure is impossible with the present knowledge of diets. Upon a vitamine-free diet a young animal will rapidly decline in weight and will soon die. Even if this were not the case, the experiment would still be open to objection. The results obtained by Funk with pigeons render it highly probable that the tissues of all animals, so long as they live, contain very appreciable quantities of vitamines no matter how free the diet may have been from vitamine for a considerable period. Funk found that an extract of the tissues of pigeons fed upon polished rice until they died of beri-beri, would still readily cure other pigeons which had developed this disease upon similar diets.

Obviously, then, simply to take account of the "takes" and rate of growth of tumors inoculated into animals which are on a vitamine-free or a vitamine-restricted diet, will afford no answer whatever to the question which we are proposing to study. The tumor growth may be retarded simply because of the generally poor nutritive condition found within the host, or it may be retarded because of specific lack of vitamines. Or, conversely, the tumor might grow moderately well, due to its own synthesis of vitamine and greater avidity over the cells of the host for such circulating nutriment as is available. Or, it might be wholly unable to synthesize vitamine, but still might grow moderately well for a considerable period, owing to its greater avidity for both vitamines and food-stuffs available from the tissues and fluids of the animal. Under such conditions the host would probably die before very long, but it is quite conceivable that
it could live for some weeks while the tumor was growing rapidly.

We believe that the objections above enumerated apply to practically all experiments involving studies of a deficient diet (whatever the specific deficiency of the diet may be) where the whole interpretation of the results of the experiment is made to depend upon counting the number of "takes" and measuring the size of the tumor at stated intervals. Such experiments may disclose a general effect of a diet upon the growth of a tumor, and at the same time give no indication whatever of the mechanism of the action. In the great majority of cases no definite effect may be noted upon the tumor growth simply because the tumor cells are able to make use of essentials for growth which are stored in the host's tissues and which may be wholly lacking in the animal's diet. It is quite possible that the slow progress in reaching a solution of the question of the relationship of nutritive factors to tumor growth has been partly due to the common practice of considering nothing but the relative size of the tumor as an element of importance.\(^1\)

In planning our experiments, we have therefore attempted to avoid using the relative size or rate of growth of the tumors as the determining factor in interpreting our experiments. To do this we have assumed at the outset that a given quantity of normal tissue must have an approximately definite quantity of vitamine in order to attain a certain definite growth. In other words, \(x\) grams of tissue must use a definite amount \((y\) grams\) of vitamine to increase to \(2x\) grams of tissue. If tumor tissue is also dependent upon vitamine from outside sources, then it too must fall in line with the above formula. We do not mean, nor need, to assume that the formula above given would hold more than approximately for different tissues, but we do believe that the general proposition that an increase in weight of normal tissue can be accomplished only in the presence of an in-

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\(^1\) In their second paper, Sweet, Corson-White, and Saxon recorded curves giving the weights of their experimental animals plus tumors. The data offered cannot be interpreted from the point of view which we are advocating, nor do the authors attempt any such interpretation.
creased amount of vitamines utilized is in accord with recent observations, and that it will hold also for tumor tissue, if this latter tissue is dependent upon vitamines from outside sources.

With this point of view in mind, the following experiment should throw light upon the question which we are trying to answer. Starting with a young and vigorous rat which is very far from having attained its complete growth (so that the tissues have a constant power to use a maximal quantity of vitamine), we must first determine, as exactly as may be possible, the amount of a particular material known to be very rich in vitamine which will, when fed daily, keep the animal alive in apparently good nutritive condition, but which will permit no appreciable increase in weight over a period of at least some weeks. Having thus determined the minimum quantity which will keep the animal in good condition, and at the same time permit no growth, the rat is inoculated with tumor tissue, and the results of this inoculation followed over a period which suffices to permit the tumor to reach a good size, providing it will grow at all. During this period the same food and the same quantity of vitamine are fed, and the weight of the animal is carefully recorded from day to day during the course of the growth of the tumor.

If the tumor does not grow at all, or grows very poorly in practically all such animals, we might assume at once that the tumor tissue was dependent upon vitamines for its growth, and, further, that it was unable, through increased "avidity" to draw vitamine from the tissues of its host—provided, of course, that the virulence of the tumor tissue was properly established. If, on the contrary, the tumor grows fairly well, or even very well, it is only necessary to note whether there is an increase in weight of the animal at least commensurate with that which we should expect from the tumor itself. If the animal plus the tumor increases in weight approximately in accordance with the weight of the tumor, it is obvious that this latter tissue is independent of an outside source of vitamine so far as its growth is concerned, since a quantity of vitamines is supplied in the food which will suffice only for the maintenance of the animal's origi-
nal tissues. If, on the contrary, the tumor grows, but the total weight of the animal plus the tumor remains unaltered, then we should be justified in concluding that the tumor is dependent upon vitamine from outside sources, and that, through increased avidity, it is able to appropriate the vitamines of the host's tissues to its own uses, so that a given quantity of the host's tissue is practically transformed into tumor tissue. In this event there would be no appreciable change in weight in the animal, though the tumor might conceivably grow to a very considerable size before the death of the host. Another possibility would be that during the growth of the tumor, the weight of the animal would increase more rapidly than could be accounted for by the growth of the tumor. In this instance we should have to assume that the tumor produced a growth-promoting substance which escaped into the circulation and affected the growth of the host.

In order to meet the experimental conditions which have been outlined, it is necessary first to do some preliminary work to answer certain questions which arise at the outset of such experiments.

The diet which we adopted to constitute the vitamine-free ration in our experiments is one which has been studied in considerable detail by Funk and Macallum (5). This diet, as suggested by these authors, and employed by us, has the following composition: Casein\(^2\) 22 per cent, cane sugar 10 per cent, starch 33 per cent, butter (ordinary) 30 per cent, agar 2 per cent, salt mixture (Osborne and Mendel (6)) 3 per cent. Funk and Macallum found that young rats fed on this diet lost in weight and died after about thirty to forty days. If 2 or 6 per cent of dried brewer's yeast was added to the ration, the rats grew normally for a period of at least two or three weeks. In their later paper these authors report experiments in which their animals grew normally for from forty to sixty days on the diet containing dried yeast (and in which lard partially replaced the butter), and in which good growth was maintained for about

\[^2\) The casein is purified from the commercial product by repeated extraction with boiling 95 per cent alcohol.\]
one hundred days when the diet was changed occasionally, substituting moist yeast for dry, or changing the percentage of yeast fed.

In studying the suitability of the Funk-Macallum diet for our experiments, it was necessary to ascertain: (1) whether we could essentially duplicate their earlier results; (2) whether the diet plus yeast is adequate for growth over a considerable period, and (3) whether yeast fed separately, i.e., not mixed with food, but given in a separate container, would be as effective as the yeast when mixed with the food. This latter point is of especial significance in connection with our work, in which we wish to control closely the vitamine intake. This obviously cannot be done where the food contains vitamines, since under such conditions the food intake controls the vitamine intake. The point is furthermore of importance in answering the more general question as to whether the stimulating effect of such a strongly smelling and tasting substance as yeast might be due to rendering the food more agreeable (through taste and odor) to the animals, so that they eat more, and hence grow more; or whether it has a specific effect within the organism.

An experiment to study these points over a considerable period of time has been carried out. Forty-two young rats, weighing approximately 25 grams each, were divided into one experimental group and one control group, of twenty-one rats each. The animals were kept in separate cages which were thoroughly cleaned at frequent intervals. The control rats were fed white bread and water ad libitum with the addition of cabbage or kale every second or third day. Such a diet is, we believe, quite commonly employed with animals kept in laboratories for experimental tumor work. The experimental animals received the Funk-Macallum diet above mentioned, with water, ad libitum, and in addition, varying quantities of dried brewer's yeast over different periods of the experiment. Where the quantity of yeast fed was below 1 gram per day, the yeast was given in the form of a thorough mixture with butter. By varying

As mentioned above, this second question is really settled in the later paper of Funk and Macallum, which had not appeared when our work was started.
the percentage of yeast in the butter, it is possible to feed very small quantities of yeast per day with comparative accuracy and with ease of manipulation. Since the diet contained a high percentage (30 per cent) of butter, there is no reason to believe that the small additional quantity of butter fed with the yeast can have any special effect.

The experiment was continued over a period of one hundred and thirty-two days. Each animal was weighed individually and the weight recorded every four days. Early in the experiment a few of the animals on the special diet refused to eat the yeast, and died within the first week or two. No further deaths occurred among this group. In the controls there were no deaths during the experiment. At the end of the one hundred and thirty-two days, there remained alive twenty-one control animals, of which seven were females and fourteen males. Of the experimented animals seventeen remained alive at the end of the experiment, of which eight were females and nine were males.

The results of the experiment are summarized in Chart I. We have averaged together males of each group and females of each group. This is allowable only where (as in the present case) the results are very clear-cut, and where large variations among the members of each group do not occur. Thus out of the fourteen males in the control lot, as compared with nine males in the experimental lot, only one of the former exceeded in weight the lightest of the experimental animals. Among the females the lightest experimental animal weighed as much as the heaviest control animal.

An inspection of Chart I reveals several points of interest. The growth of the control animals is slow and regular throughout the entire period. At the end of the one hundred and thirty-two days the females of the control lot had practically ceased to grow, while the males were still noticeably increasing in weight. In the case of the experimental animals the rate of growth during the early periods appears to parallel the quantity of yeast fed, but only within certain limits. From the 1st to the 14th day each rat received 5 mgm. of yeast. There is a
marked growth of the males during this period, and very little
growth of the females. Probably this difference is accidental.
From the 14th to the 28th day, each animal received 50 mgm.
of yeast. The females show some improvement in growth
during this period, while the males are almost unaffected. From
the 28th to the 38th day, 200 mgm. of yeast were fed to each

animal daily. The effect on the rate of growth is remarkable.
During this ten-day period the animals almost doubled in
weight, and the females caught up with and even surpassed the
males. It is only during this period of ten days out of the entire
experiment that the growth curves of the experimental animals
approximate closely that for normal rats as adopted by McCollum and others. From the 38th day to the end of the experiment, each animal received 1 gram of yeast daily. Many of the animals would not eat the whole of this yeast allotment, particularly toward the end of the experiment.

It will be noted that the increase in yeast from 200 mgm. to 1 gram per day is not accompanied by any increase in the rate of growth. On the contrary, the rapid growth of the preceding period is not long maintained, and falls off markedly for the females very shortly, and for the males a little later. Whether the optimum growth secured with 200 mgm. of yeast daily was only a chance result in this experiment can be definitely decided only by future work. But since the data represents about twenty animals, and since the effects appear to be so definite, we are inclined to accept the view that an "optimum" yeast intake can be found, and that this may be well below the maximum which the animal will eat.

As the result of their attempts to obtain growth over long periods with diets containing yeast, Funk and Macallum came to the conclusion that "it seems also possible that yeast on account of its high content in purines, and perhaps other constituents, is not an ideal addition in experiments of long duration, even in spite of its marked growth-promoting power." It would seem very possible, according to our results, that with careful regulation of the yeast intake the disadvantage of this substance as a growth-promoting agent over long periods of time might be minimized.

Certain other points in connection with the experiment recorded in Chart I are of interest. It is noteworthy that neither the experimental nor the control lot of animals attained nearly the weight to be expected in normal rats fed upon wholly adequate (normal) diets, as reported by McCollum, Mendel and Osborne, and Donaldson. At the termination of our experiment, the "expected" weight for our male animals would have been about 220 grams, of the females about 170 grams. This would seem to imply that both the diets employed are quite deficient as regards growth-promoting substances. Certainly
the diet used for our controls (bread and cabbage) is low in protein, and we should be inclined to ascribe the slow and seemingly incomplete growth of the animals wholly to a protein deficiency, were it not that previous investigators have reported similar results upon diets against which this criticism could not be offered. Thus Sweet, Corson-White, and Saxon, in their second paper criticize the conclusions of Van Alstyne and Beebe (7) regarding the inhibiting effect of deprivation of carbohydrates upon the growth of experimental tumors in rats, upon the ground that neither the experimental nor the control animals employed by Van Alstyne and Beebe grew at all normally—hence these latter investigators were studying simply the relative effects of two diets, each of which was partially deficient for growth. It is true that the animals of Van Alstyne and Beebe did not grow normally, nor anything like normally, but that does not justify the conclusion of Sweet, Corson-White, and Saxon that the diets of these animals were deficient. That further work would be necessary to justify such an assumption is shown by the figures on growth reported by Sweet, Corson-White, and Saxon themselves for some of their own animals. These were presumably upon a diet of bread, oats, wheat, rice, corn, sunflower seed, and water, which must be regarded as complete in all ways. Yet these animals required a period of 21 weeks to increase from a weight of 50 grams to about 110 grams, which is approximately half the rate of growth "expected" for normal animals on an adequate diet. In a further period of six weeks, the animals declined in weight to about 90 grams, when, if normal and on an adequate diet, they would have increased to about 220 grams.4 It is true that the rats of Sweet, Corson-White, and Saxon were abnormal in the respect that they carried tumors, but there is nothing recorded to show that rats developing tumors of considerable size thereby lose the power to grow at an approximately normal rate, even when the weight of the tumor is included. It would seem that some other factor

4 In recording the growth curve of their animals, Sweet, Corson-White, and Saxon make no reference to the sex of their animals.
is concerned in these results and in those which we illustrate in Chart I.

The failure to grow at a normal rate exhibited by the rats of Sweet, Corson-White, and Saxon, and by our own animals, must rest rather upon a difference in the rats themselves than upon a deficiency in diet.

That this is probably the case is in accord with the observations of numerous workers who have carefully studied the growth problem in rats. Thus Funk and Macallum state that in their studies it was necessary to reject 80 per cent of the rats obtained from Toronto dealers because of physical defects which developed after initiation of the experiment; and Funk (8) states that animals furnished by dealers were unsuited to his work, and that the difficulties were overcome by employing rats bred in the laboratory under proper dietary conditions. (Italics ours.)

In view of such results, it is indeed questionable whether animals normal in their capacity for growth as determined by McCollum, Mendel and Osborne, and others, are to be obtained from dealers, or are to be found in many laboratories for the study of transmissible tumors. Apparently such animals must be bred under special conditions of diet. Conversely, it might be pertinent to ask whether the growth curves reported by the investigators for such animals are not maximal rather than normal,—since breeding under ideal nutritive conditions may improve the power of the offspring to grow.

Up to the present we have confined our experiments to animals purchased from standard dealers who supply many laboratories with animals for experimental cancer work. A little later we hope to study the effects of inoculating with tumor tissue animals having maximal growth rates.

Before leaving our first experiment, we wish to refer to the question of the general appearance and well-being of our animals. The control lot appeared to be in excellent condition throughout the experiment, with no detectable signs of inadequate nutrition. Infections were absent among this group, except in one animal which developed an eye infection after some
weeks, and the fur of the animals was abundant, white, and soft.

In the case of the experimental animals, there was complete freedom from infectious disease, and the animals appeared to be in excellent nutritive condition in all respects save one. This point was the appearance and texture of the fur. The hair was abundant in all members of the group, but in every animal upon the special diet it was coarse and stiff, and slightly yellowish in color, as opposed to the soft and snow-white fur of the controls. This difference was so marked that with any rat of the entire two series a hasty inspection sufficed to indicate the diet on which it had been fed.

Thus the special diet, although far better suited for the growth of the rats than was the diet of the controls, was at the same time not so satisfactory in at least one respect.

A discussion of this point would lead us too far into a theoretical consideration of the vitamines. It will be sufficient for the present to point out that the difficulty with the diet is seemingly not connected with any toxicity associated with the yeast. We reach this tentative conclusion from the fact that the coarseness of the hair developed within a week of the beginning of the experiment, when the quantity of yeast fed was very small; also, from the further fact that we have seen it upon the same general diet when the quantity of yeast fed was less than 3 mgm. per day. An explanation of the difficulty with the diet which naturally occurs to one is that it may be associated with the probable absence of cystine. We hope to test this explanation in later experiments.

The two further experiments which we wish to report in the present paper were more definitely directed toward the question of vitamine relationship to tumor growth than the one we have been discussing. In these experiments we have employed the same diets as described for the preceding experiment, but in the case of our experimental animals we have attempted to reduce the yeast intake to the lowest level compatible with the general well-being of the animal, and one upon which it would not grow appreciably.
Chart II illustrates an experiment which is, we believe, the first on record designed to determine the minimal intake of a vitamine-rich constituent, essential to the growth and to the maintenance of young rats. The animals, twenty-four in number with an average weight of about 30 grams, were first upon a bread diet for a few days. They began to grow rapidly, and were then changed to the diet of casein, butter, and salt mixture described under the first experiment reported in this paper. The progress of the animals can be followed in Chart II, which represents an average of the twenty-four animals. With as large a group of small animals as this, a variation of a gram or two in weight for the average is of distinct significance. There was no separate charting of males and females because the rate of growth was not to be especially considered in these experiments.
It will be noted from the chart that the first three days the rats continue to grow without any yeast in the diet—apparently an after-effect of the bread diet. From the third to the fourth day, however, there is a sharp drop in weight which is promptly checked, and soon wholly recovered from when 25 mgm. of yeast (in mixture with butter) was fed once a day, separately from the rest of the food. In spite of the decreasing amounts of yeast fed, there is a definite tendency to grow until a minimum of 2.5 mgm. of yeast is reached, when the weight is constant, and the animals remain in good condition for at least a week.

The animals were then planted (by trocar) with the Buffalo sarcoma, and were divided into two lots of twelve animals each. Lot I (the experimental lot) was continued on the same diet as was previously given, while Lot II (controls) was placed upon a diet of bread and cabbage.

Difficulties were encountered in the handling of the experimental lot as regards the quantity of yeast necessary to keep the animals alive. Apparently there is a definite drain upon the animals as the tumor cells begin to grow which is especially notable for the lighter weight animals. In spite of an increased allowance of yeast on certain days, four out of the twelve animals died within the first three weeks after planting. The average weight of the remaining eight animals at the time of

[Funk and Macallum report experiments in which their animals remained alive, and at almost constant weight for two or three weeks on this diet with no yeast at all. Apparently their animals were far stronger and of greater vitality than ours, as we have been unable to obtain similar results in a number of attempts which we have made. From a reference to our discussion earlier in this paper, it will be seen that Funk and Macallum certainly worked with quite a different class of rats from those which we have employed. In duplicating this experiment, we have found that the quantity of yeast necessary for maintenance varies both with the animals employed and with the sample of yeast used. The chart represents minimal figures so far as our results go up to the present time. In some experiments the animals almost wholly refuse to eat the yeast, while in others (as in the one illustrated in Chart II) all the animals eat the yeast quantitatively, practically as soon as it is placed in the cage. The yeast is always prepared in the same way (by pressing, drying, and grinding fresh brewer's yeast) but seemingly different samples have different growth-promoting powers.]
planting was 48 grams. At the end of three weeks all eight animals had tumors ranging in size from a trace to 1600 cmm. in content with an average size of 850 cmm. The average weight of the animals (plus the tumors) at the end of these three weeks was 47 grams (as compared to an average weight at the time of planting of 48 grams).

The control animals, fed upon bread and cabbage after being planted, increased rapidly in weight, and grew tumors of very fair size within the three-week period. Nine of the twelve animals planted remained alive at the end of three weeks after inoculation: these were eight "takes," and two regressions, leaving six tumors. These varied in size from 480 cmm. content to about 11,500 cmm., with an average size of about 6000 cmm. The weight of these animals (plus tumors) increased from an average of 44 grams at the time of planting to an average of 77 grams three weeks after inoculation and the initiation of the bread and cabbage diet. This is fully up to the normal rate of growth for white rats over this three-week period.

It is obvious that this experiment would warrant no conclusion concerning the necessity of vitamine for tumor growth. It is true that the difference in size of the tumors in the two lots is striking. The tumors of the controls were between seven and eight times as large as those of the experimental animal, as will be seen from the following table.

<table>
<thead>
<tr>
<th>NUMBER OF ANIMAL</th>
<th>SIZE OF TUMOR AT END OF THREE WEEKS</th>
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<tbody>
<tr>
<td></td>
<td>Special diet</td>
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<tr>
<td></td>
<td>cmm.</td>
</tr>
<tr>
<td>1</td>
<td>720</td>
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<tr>
<td>2</td>
<td>160</td>
</tr>
<tr>
<td>3</td>
<td>660</td>
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<tr>
<td>4</td>
<td>1,638</td>
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<tr>
<td>5</td>
<td>800</td>
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<tr>
<td>6</td>
<td>Trace</td>
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<td>7</td>
<td>980</td>
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<td>8</td>
<td>840</td>
</tr>
<tr>
<td>9</td>
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</tr>
</tbody>
</table>

* Computed by measuring growth in three directions and multiplying.
It will be noted that except for one tumor (which was regressing) the smallest growth among the control animals was more than twice as large as the largest one among the controls.

We do not, however, regard these results as of any special significance, since they might be due to any of several factors mentioned earlier in this paper besides the one we are trying to investigate. They tend, however, to indicate that vitamin from outside sources may be of importance for tumor growth. The crucial test of whether there was an increase in weight of the tumor bearing animals without appreciable increase in the vitamin ingested, could not be satisfactorily applied in this experiment, since the tumor growth in the experimental animals was so restricted that it would not, by itself, have caused any appreciable increase in the total weight of the animal.

The experiment is, however, of some interest in connection with the following experiment which is essentially a duplicate of it, but in which diametrically opposite results are obtained as regards the rate of growth of the tumors on the two diets.

The diets and general conditions of this experiment were the same as for the preceding, except that the preliminary period on the special diet covered only six days before the animals were inoculated. There was a higher mortality among the experimental animals, possibly due to the higher virulence of the tumor tissue among these animals, as compared with those in the preceding experiment. Starting with seventeen rats on the special diet, and with a minimal yeast intake, there remained alive eight animals four weeks after inoculation. Eleven out of fifteen "control" animals remained alive four weeks after inoculation.

Of the eight experimental animals, seven had tumors at the end of the four week period. The tumors ranged in size from 600 to 28,000 cmm., with an average size of about 8000 cmm. The average weight of the animals remained constant (within two or three grams) throughout the entire period of the experiment.

Of the eleven control animals, seven had tumors at the end of the experiment, which ranged in size from a trace to about 6000 cmm., with an average size of about 2200 cmm. The aver-
age weight of this series increased from 27 grams at the time of planting to 54 grams during the experimental period.

In this experiment the animals on the vitamine-restricted diet developed tumors of very considerable size within a period of about four weeks. The fact that these tumors were about four times as large as those developed by the controls, would, according to the usual method of interpretation, be taken to indicate that the tumor can grow independently of outside source of vitamine, thus being directly contradictory to the preceding experiment. But when we apply the test proposed at the outset of these experiments it is plain that no such conclusion is justified. In fact the experiment is wholly independent of the controls. If the tumor could synthesize its own vitamine, then the total weight of the animals should have shown an increase in some proportion to the growth of the tumor. In no single case did this occur. The rat developing the largest tumor was the heaviest animal in either lot, and weighed 52 grams at the time of inoculation. This animal showed, it is true, the maximal gain of any of the experimental animals, and gained 6 grams in the first ten days after inoculation, having then a weight of 58 grams, and a small tumor (under 2000 cmm.). At the end of the experiment the animal’s weight (plus the tumor) was still 58 grams, but the tumor had increased to a weight of nearly 20 grams, or about one-third of the weight of animal plus tumor. The weights were even more constant for the other animals which developed tumors of very appreciable size, though not so large as this one.

The experiment serves to bring out, perhaps more clearly than has been done before, the remarkable avidity which tumor cells may show, since a very rapid growth of tumor has been obtained under conditions which completely prevented growth of the body tissue. It also shows that even a very rapidly growing experimental sarcoma does not liberate into the blood-stream any substance which may act as a stimulant to body growth in the absence of the necessary amount of chemical stimulant contained in yeast. We may tentatively assume that the tumor itself is also dependent upon this stimulant from outside sources,
either from the food, or from the tissues of the host. We recog-
nize fully the desirability of confirming these results by further
experiments, and are continuing the work along this and similar
lines. It should be noted however that our experiment as re-
ported above, being independent of the rate of growth of the
tumor, and therefore of controls, can afford positive evidence
much more readily than those based upon the usual procedure
of measuring rate of growth in experimental animal and control.

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