THE EFFECT OF NUTRITION ON THE TUMOR RESPONSE IN ROUS CHICKEN SARCOMA

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INTRODUCTION

NUMEROUS observations have confirmed the fact that nutrition may influence the genesis and growth of tumors in rats and mice. The search for requirements in the nutrition of malignant tissue should reveal findings that are valid regardless of the nature of the tumor studied or the kind of animal used, as well as more limited findings. Such information might well include the facts that adequate caloric intake has been shown necessary for tumor growth[1] and that vitamins such as pantothenic acid and riboflavin have been found stimulative,[2] whereas choline may be inhibitory.[3]

EFFECT OF NUTRITION IN ROUS SARCOMA

Little and co-workers[4] showed that folic acid has a marked effect on the tumor response in Rous chicken sarcoma. The effectiveness of folic acid free diets in preventing the tumor response of baby chicks and the rapidity and regularity with which the response occurred in adequately nourished chicks led us to our present study of the effect of each of the different constituents in a synthetic diet for chickens.

The data to be presented confirm observations made on rats and mice to the effect that malignant tissue may utilize vitamins such as pantothenic acid and riboflavin for growth. Our results also indicate that nicotinamide and cholic acid may be stimulative. However, folic acid appears to be the only nutrient required to the extent that tumor response is prevented by its absence.

Method for Demonstrating the Effect of Nutrition.—In our tests, the extent to which each nutrient influenced tumor response was determined by comparing the incidence of tumors in groups of chicks fed the complete diet with the incidence of tumors in groups fed the same diet without the nutrient. By comparing observations made on the eighth, tenth, twelfth, fourteenth, and sixteenth day, the effect of nutrition was evaluated for all stages from the usual time of first appearance of tumors to the time when 90 to 100 per cent of control birds showed tumors. The response with and without nutrient was calculated from the combined results of different tests where more than one test was carried out. The percentage of birds showing tumors when fed diets with the nutrient was divided by the percentage showing tumors when fed the same diet without the nutrient. The nutrients which showed no stimulative effect gave values of approximately 1.0.

Relation of Size of Inoculum to Tumor Response.—An even distribution of tumor responses over the eight days selected for observation of results was readily obtained by controlling the size of the inoculum. As shown in Table I, there is a direct correlation between the size of inoculum and the length of the latent period.

We used homogeneous samples of frozen virus, prepared by blending fresh tumor tissue in a Waring mixer and weighing 2 Gm. amounts into sterile Petri plates to be stored in a dry

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TABLE I. TUMOR RESPONSE OBTAINED WITH HOMOGENEOUS PREPARATION OF INOCULUM KEPT FROZEN FOR VARIOUS PERIODS

AMOUNT OF INOCULUM USED (0.25 ML. DOSE)	INOCULUM KEPT FROZEN (DAYS)	TOTAL NUMBER OF CHICKS USED	NUMBER OF CHICKS THAT GREW TUMORS	PERCENTAGE OF TAKES	AVERAGE LATENT PERIOD (DAYS)
10 mg.	Fresh	21	21	100.0	7.90
	16	16	15	93.7	7.93
1 mg.	Fresh	25	25	100.0	9.56
	16	16	15	93.7	9.66
	24	190	173	90.1	9.53
0.1 mg.	Fresh	25	23	92.0	12.04
	16	17	16	94.1	11.25
	24	190	157	82.6	11.08
0.01 mg.	Fresh	23	13	56.5	12.84
	16	20	8	40.0	14.00

ice chest until used. Preliminary suspensions containing 10 mg. in a 0.25 ml. dose were prepared by adding 50 ml. of 2 per cent peptone solution to 2 Gm. of tumor tissue. A Ten Brock grinder was employed, and further dilutions were made in peptone solution.

In our experiments the desired length of latent period was obtained with 1 mg. of inoculum. The frozen virus was found to be stable for at least a month. It is of interest that Reinhard and co-workers[5] found a similar relationship between the size of inoculum and the length of the latent period for growth of a transplantable mouse adenocarcinoma.

One-day-old New Hampshire Red chicks which were the progeny of a selected flock (as described in our previous report[4]) were injected in the right breast with 0.25 ml. of the suspension containing the 1 mg. dose. The feathers were removed from the breast area before inoculation. Groups of ten birds were placed on diets with and without each nutrient tested. Nutrients which were of interest because of possible stimulative effect were retested several times. The chicks were maintained in electric brooders at 90° F.; water and food were supplied ad libitum. Approximately 400 chicks (ten to twenty per group) were used in each experiment.

The basal diet contained 53 per cent Cerelose,* 22 per cent alcohol extracted casein, 4.3 per cent salt mixture, 3 per cent calcium gluconate, 8 per cent gelatin, 4 per cent Ruffex,† 5 per cent soybean oil, 0.25 per cent cholic acid, 0.45 per cent cystine, 200 mg. per cent choline chloride, 3 mg. per cent calcium pantothenate, 3 mg. per cent nicotinamide, 0.5 mg. per cent pyridoxine, 0.3 mg. per cent thiamin chloride, 0.03 mg. per cent biotin, 0.5 mg. per cent riboflavin, 100 mg. per cent inositol, 5 mg. per cent para-aminobenzoic acid, 0.2 mg. per cent folic acid, 5 mg. per cent vitamin E, 0.2 mg. per cent vitamin K, 3,500 units per cent vitamin A, and 200 units per cent vitamin D. The diet with nutrient was usually the complete diet. A commercial chick starter gave the same tumor response as the complete synthetic diet. In studies of substitutes for soybean oil, the diet with nutrient contained the substitute and the basal diet was soybean oil free. In these tests the vitamins A, D, E, and K were added in propylene glycol.

The chicks were observed daily beginning with the eighth day and continuing through the sixteenth day at which time adequately nourished groups showed 90 to 100 per cent tumors. Wing tags applied on the sixth or seventh day were used to identify individual birds. The increasing sizes of tumor were recorded (as previously described) for evidence as to the accuracy of first observations. Tumors recorded as questionable were counted in determining the response whenever this observation was confirmed by subsequent findings.

*Glucose monohydrate. Fisher Scientific Co., Pittsburgh, Pa.

†Purified cellulose containing 70 per cent α cellulose and 30 per cent other celluloses. Fisher Scientific Co., Pittsburgh, Pa.

EXPERIMENTAL RESULTS

Three distinct types of results have been obtained with nutrients which demonstrated a definite stimulative action on tumor growth. While with and without values of approximately 1.0 were obtained in tests on nutrients which did not stimulate tumor growth, (A) constant values of approximately 3.0 were obtained in tests of riboflavin, (B) descending values of 13.0, 6.2, 4.6, 2.6, 2.1 were obtained in tests of nicotinamide, and (C) ascending values of 13.0, 40.0, 63.0, 89.0, 93.0 were obtained in tests of folic acid. While other nutrients gave results similar to those illustrated in A and B, folic acid was the only nutrient giving the result C.

The Effect of Vitamins From Liver.—Table II shows the effect of nine water-soluble vitamins on the tumor response of chicks to Rous sarcoma virus. Folic acid produced the greatest effect and para-aminobenzoic acid and biotin produced the least effect. Nicotinamide and calcium pantothenate influenced the rate of growth more than the final incidence; riboflavin did not change the rate of growth but did influence the incidence at all stages.

TABLE II. EFFECT OF WATER-SOLUBLE VITAMINS IN DIET ON ROUS SARCOMA (RATIO OF PER CENT INCIDENCE ON THE COMPLETE DIET TO PER CENT INCIDENCE ON THE DEFICIENT DIET)

VITAMIN	AMOUNT IN DIET	NUMBER OF CHICKS (A/B†)	NUMBER OF TESTS	RESPONSE WITH VITAMIN / RESPONSE WITHOUT VITAMIN				
				8	10	12	14	16
Thiamin*	3 mg./kg.	10/10	1	30.0	1.5	1.3	1.1	1.1
Riboflavin	5 mg./kg.	39/37	4	2.1	3.0	3.1	3.1	3.1
Pyridoxine*	5 mg./kg.	30/30	3	2.0	2.8	1.8	1.4	1.3
Nicotinamide	30 mg./kg.	29/29	3	13.0	6.2	4.6	2.6	2.1
Calcium pantothenate*	30 mg./kg.	29/18	3	24.0	11.0	2.6	2.0	1.8
Inositol	1 Gm./kg.	29/25	3	13.0	1.2	1.1	1.0	1.0
Para-aminobenzoic acid	50 mg./kg.	19/19	2	1.4	1.1	1.0	0.8	0.8
Biotin	0.3 mg./kg.	29/27	3	1.1	1.8	1.2	1.1	1.1
Folic acid	2 mg./kg.	30/32	3	13.0	40.0	63.0	89.0	93.0

*Vitamin deficient chicks were revived with complete diet beginning on the tenth day.

†a/b, Total chicks used to determine effect of diets (a) with and (b) without the vitamin specified.

Since deficiencies of thiamin, pyridoxine, and calcium pantothenate caused serious loss of weight, chicks fed diets without these vitamins were revived with complete diet beginning on the tenth day. In previous work with folic acid it had been shown that the stimulative effect of restoring this vitamin on the tenth day does not become apparent for at least seven days. This practice was adopted in the case of thiamin, pyridoxine, and calcium pantothenate deficiencies to permit survival of the birds for the duration of the test. The responses with and without one of these vitamins for the twelfth, fourteenth, and sixteenth day may or may not be influenced by restoring the vitamin on the tenth day.

Since liver is a rich source of still unidentified vitamins, several fractions of liver were tested in the presence of the nine purified vitamins for possible stimulative effect on the tumor response of chicks to Rous sarcoma virus. None of the liver fractions influenced the rate of growth or incidence of tumor to an extent which would indicate the presence of additional factors for tumor growth.

The Effect of Vitamins A, D, E, and K.—The results of tests of diets with and without vitamins A, D, E, and K are shown in Table III. None of these vitamins stimulated tumor growth when present in the diet. The values 0.8, 0.6, and 0.5 suggest that the oil-soluble vitamins may slightly retard tumor growth. It is of interest that diets with and without the combination of soybean oil and vitamins A, D, E, and K influenced the tumor response to a greater extent than did the diets with and/or without the oil-soluble vitamins alone.

TABLE III. EFFECT OF OIL-SOLUBLE VITAMINS IN DIET ON ROUS SARCOMA (RATIO OF PER CENT INCIDENCE ON THE COMPLETE DIET TO PER CENT INCIDENCE ON THE DEFICIENT DIET)

VITAMIN	AMOUNT IN DIET	NUMBER OF CHICKS (A/B*)	NUMBER OF TESTS	RESPONSE WITH VITAMIN / RESPONSE WITHOUT VITAMIN 8	10	12	14	16
Combined								
A	35,000 I.U./kg.							
D	2,000 I.U./kg.	19/19	2	0.8	1.0	1.0	1.0	1.0
Combined								
A	35,000 I.U./kg.							
D	2,000 I.U./kg.							
E	50 mg./kg.							
K	2 mg./kg.	10/10	1	5.0	1.3	1.1	1.1	1.1
E	50 mg./kg.	10/ 8	1	0.6	1.2	1.4	1.2	1.2
K	2 mg./kg.	10/10	1	0.5	1.0	1.5	1.1	1.0
Combined								
Soybean oil	5 per cent							
A, D, E, K	As above	20/23	2	10.0	2.6	2.3	2.0	1.6

*A/B, Total chicks used to determine effect of diets (A) with and (B) without the vitamin specified.

The Effect of Various Fats and Oils.—Table IV shows the results of tests in which twelve different substances were tested in a soybean oil free diet. None of these substances stimulated tumor growth. Soybean lecithin, cod-liver oil, and linoleic acid appeared to retard tumor growth when present in the diet. Most of the other substances in the group showed this effect to some degree.

TABLE IV. EFFECT OF FATS AND OILS IN DIET ON ROUS SARCOMA (RATIO OF PER CENT INCIDENCE ON THE COMPLETE DIET TO PER CENT INCIDENCE ON THE DEFICIENT DIET)

NUTRIENT*	AMOUNT IN DIET (%)	NUMBER OF CHICKS (A/B†)	NUMBER OF TESTS	RESPONSE WITH NUTRIENT / RESPONSE WITHOUT NUTRIENT 8	10	12	14	16
Beef liver fat	5	10/10	1	1.0	1.0	0.7	1.0	1.0
Cholesterol	1	54/54	3	1.0	1.2	0.7	0.7	0.8
Coconut oil	5	30/30	3	0.1	1.3	1.1	1.3	1.3
Cod-liver oil	3	10/10	1	1.0	0.7	0.4	0.5	0.6
Corn oil	5	10/10	1	0.5	0.5	0.8	1.0	1.0
Crisco	5	10/10	1	1.0	1.2	0.5	0.8	0.8
Lanolin	3	10/10	1	1.0	0.7	0.5	0.8	0.8
Lard	5	10/10	1	0.0	0.7	0.5	0.6	0.8
Linoleic acid	3	30/30	3	0.0	0.5	0.7	0.8	0.8
Sodium oleate	3	30/30	3	0.1	1.2	1.0	1.2	1.3
Soybean lecithin	1	20/20	2	0.0	0.8	1.3	1.4	1.3
Soybean lecithin	3	10/10	1	0.0	0.5	0.5	0.6	0.6
Soybean oil	5	20/20	2	2.0	1.3	1.0	1.0	1.0

*The basal diet was oil-free; vitamins A, D, E and K were added in propylene glycol.

†a/b, Total chicks used to determine effect of diets (a) with and (b) without the substance specified.

The Effect of Cholic Acid.—Table V shows the results of tests of diets with and without cholic acid, sodium chloride, gelatin, calcium gluconate, and Ruffex. Diets with and without cholic acid influenced tumor growth in much the same way as did diets with and without riboflavin. Three times as many tumors developed when cholic acid was present in the diet.

TABLE V. EFFECT OF CHOLIC ACID IN DIET ON ROUS SARCOMA (RATIO OF PER CENT INCIDENCE ON THE COMPLETE DIET TO PER CENT INCIDENCE ON THE DEFICIENT DIET)

NUTRIENT	AMOUNT IN DIET (%)	NUMBER OF CHICKS (A/B*)	NUMBER OF TESTS	RESPONSE WITH NUTRIENT / RESPONSE WITHOUT NUTRIENT				
				8	10	12	14	16
Cholic acid	0.25	20/20	2	30.0	3.2	3.8	3.1	3.1
Sodium chloride	1	15/15	1	0.4	0.6	0.9	0.8	0.9
Gelatin	8	10/10	1	3.0	2.3	1.4	1.2	1.2
Combined								
Gelatin	8							
Calcium gluconate	3	10/10	1	3.0	7.0	1.6	1.6	1.4
Ruffex	4	65/65	4	1.6	1.0	0.9	0.8	0.8

*a/b, Total chicks used to determine effect of diets (a) with an (b) without the substance specified.

SUMMARY

The effect of nutrition on the tumor response in Rous chicken sarcoma was determined by comparing observations of chicks fed synthetic diets with and without each nutrient. Tumor response was stimulated by the presence of folic acid, nicotinamide, calcium pantothenate, riboflavin, and cholic acid in the diet. Folic acid was the only nutrient required to the extent that tumor response was prevented by its absence from the diet.

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