

Studies of Polyarthrititis Induced in Rats by Injection of Mycobacterial Adjuvant

VI. Effects of Dietary Alterations

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IN laboratory rats a single intracutaneous injection of Freund's adjuvant, containing either whole acid-fast bacilli or a lipid extract therefrom, is regularly followed in ten to fifteen days by a syndrome which includes polyarthrititis and sometimes iritis, dermatitis and balanitis.¹ The technic for producing this "adjuvant disease" and its gross and histologic features has been described previously.¹⁻⁴ The underlying mechanism in the production of adjuvant disease is not known, but it seems quite likely that it is the result of a delayed sensitivity reaction to an as yet undetermined antigen.⁴ The reproducibility of adjuvant disease and its gross similarity to certain aspects of arthritis in man made it seem worthwhile to use this experimental device to test the possibility that diet alters the course of the illness.

Various experiments, too numerous to be

reviewed here, have demonstrated that nutritional factors change the susceptibility of animals to different types of illness, including anaphylactoid shock,⁵ infections,⁶ experimental allergic encephalomyelitis^{7,8} and drug intoxications.^{9,10} The work of Dubos and Schaedler proved that dietary increments of balanced proteins tended to protect mice from several types of bacterial infections¹¹; the mechanism of this protection seemed to lie in the animals' resistance to the toxic effects of the infection rather than in the ability to destroy the organisms.¹²

The experiments reported herein suggest the possibility that high protein feeding tends to protect rats from adjuvant disease, presumably through some effect upon a delayed hypersensitivity reaction.

METHODS

In all the experiments young Long-Evans rats were started immediately after weaning (about twenty-one days of age) and maintained on various experimental or control diets for variable periods of time prior to and after the intracutaneous injection of 0.5 ml. of Freund's water-in-oil adjuvant, containing as the active ingredient the Wax D fraction isolated from killed *Mycobacterium tuberculosis*,¶ as previously described.¹ The severity of the resulting arthritis was numerically graded on succeeding days according to a rising scale from 0 to 20, each of the four limbs and the tail having a possible high score of 4.

The diets employed were all adequate in protein,

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fat, minerals and vitamins and unlimited in amount.⁹ The constituents of the diets are listed in Table I. The variation is in (1) the addition of protein (lactalbumin) beyond usual maintenance levels, (2) the use of corn oil as the only source of calories apart from protein, or (3) the use of dextrose as the main source of calories. The number of animals and their sex in each experimental group are listed in Table II.

In comparing the results the following factors were considered: (1) the type of diet, (2) the number of days the diet was used, (3) the sex of the animals, (4) the body weights of the animals at the time of inoculation, and (5) the "arthritis score," which was simply the sum of the three highest (i.e., most severe) arthritis scores for each animal. The weights of the animals were so close to one another at the outset of the experiments that the differences in ultimate weights were assumed to be due to the diets. The course of the arthritis in these animals is most frequently represented by a smooth curve from onset to peak reaction and back to recovery. Hence the arthritis score reflected not only the magnitude of the reaction but the duration as well, and the three highest scores for each animal were usually consecutive measurements.

RESULTS

The effect of the diets upon body weights of the rats is summarized in Table III, and the mean arthritis scores are in Table IV.

Although the experiments were carried out with design variable not in a balanced design, it was decided to use an analysis of variance technic for evaluation of the results. The method used is called the "General Linear Hypothesis."^{13,14} Since the computations are voluminous and involved (requiring a digital computer), whereas the significant results are few and simple, only the latter will be stated here as follows:

(1) The increase in severity of the arthritis in female rats as compared with male rats was highly significant (Fig. 1). Among groups consuming the various diets, arthritis tended to vary inversely with the body weight at the time of inoculation. Thus, since the weight gain in male rats was generally greater than in female rats, the difference in severity of the arthritis between male and female rats became even more marked when the variables of sex and weight were used in the same computations.

(2) Significant diminution in the severity

TABLE I
Percentage of Total Calories Supplied by Principal Nutrients in Diets*

Diet	Dextrose	Corn Oil	Lactalbumin
Protein A	25	25	50
Protein B	10	10	80
Protein C	0	2	98
Fat	0	85	15
Carbohydrate	83	2	15
Control	43	42	15

* In addition, each diet contained per 10,000 calories, the following: 45 gm. of vitamin B mixture (Lederplex®), 30 drops of oleum percomorphum, 300 gm. of bulk (alpacel) and 120 gm. of Osborne-Mendel salt mixture "W."

TABLE II
Number of Rats in Each Dietary Group, Sex and Number of Days on Diet Before Injection of Freund's Adjuvant

Experiment No.	Diet	Rats (no.)		No. Days on Diet Prior to Inoculation
		Male	Female	
1	98% protein	18	2	0
	Fat	18	2	0
	Control	11	9	0
2	98% protein	0	10	14
	Fat	0	10	14
	Control	0	10	14
3	98% protein	5	5	40
	80% protein	5	5	40
	50% protein	5	5	40
	Fat	5	5	40
	CHO	5	5	40
	Control	10	0	40
4	98% protein	0	11	65
	80% protein	0	11	65
	50% protein	0	11	65
	Fat	0	10	65
	CHO	0	10	65
	Control	0	10	65
5	80% protein	0	8	84
	Fat	0	9	84
	CHO	0	9	84
	Control	0	10	84
	Lab Chow	0	10	84

TABLE III
Effect of Diets Upon Body Weights of Rats

Days on Diet Before Inoculations	Diet	Average Weight of Male Rats (gm.)		Average Weight of Female Rats (gm.)	
		At Start of Diet	At Time of Inoculation	At Start of Diet	At time of Inoculation
0	98% protein	180.0	180.0	224.0	224.0
	Fat	163.1	163.1	231.5	231.5
	Control	200.3	200.3	157.2	157.2
14	98% protein	(adults, not weighed)	199.4
	Fat		214.1
	Control		224.0
40	98% protein	79.0	100.4	62.2	79.2
	80% protein	60.4	131.5	60.0	101.8
	50% protein	74.0	195.0	56.8	131.4
	Fat	60.2	124.1	65.2	124.0
	CHO	57.2	120.2	59.0	120.8
	Control	61.6	134.9
65	98% protein	63.0	129.7
	80% protein	71.0	160.0
	50% protein	53.1	186.0
	Fat	52.4	180.4
	CHO	53.4	165.0
	Control	63.4	179.6
84	80% protein	73.0	161.6
	Fat	54.1	175.2
	CHO	76.8	188.1
	Control	49.0	178.0
	Lab Chow	50.4	203.3

TABLE IV
Average of the Sum of Three Highest Arthritis Scores for Each Rat in All Diet Groups

Diet	Days on Diet						
	0		14	40		65	84
	Male	Female	Female	Male	Female	Female	Female
50% protein.....	27.4	36.2	24.4	...
80% protein.....	7.0	9.6	36.2	17.4
98% protein.....	19.2	...	17.5	0.0	0.0	9.6	...
High carbohydrate.....	7.6	30.8	23.7	39.1
High fat.....	2.2	...	19.1	22.5	23.2	44.6	42.0
Control.....	19.2	41.0	17.4	12.5	...	34.3	39.2
Lab Chow.....	46.9

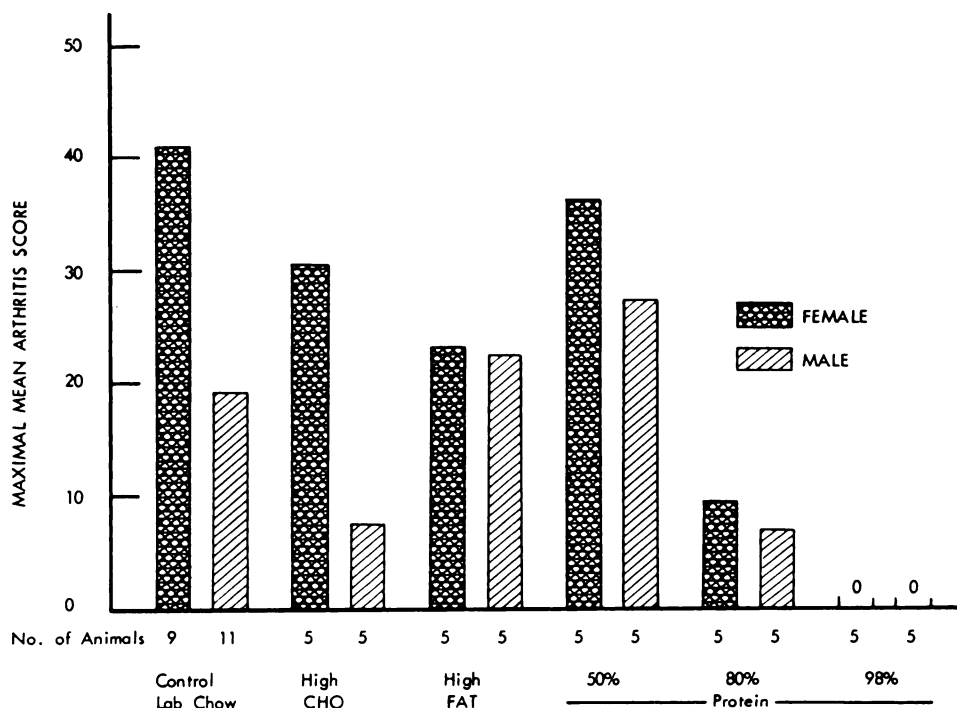


FIG. 1. Effect of diets given forty days on severity of arthritis in male and female rats. Control group ate Lab Chow before injection of adjuvant and "control" diet as in Table 1 thereafter.

of the arthritis was found in the animals fed either 80 or 98 per cent protein. No other significant differences were noted among the groups consuming other diets. There was a linear inverse relationship between the amount of protein in the diet and the severity of the arthritis (Fig. 2). The differences between the

rats fed high protein diets and the others tested (Table 1) were absent in animals fed the experimental diet for zero or fourteen days before inoculation, and were progressively more marked when the diet was fed for forty to eighty-four days.

(3) Weight gain in the animals on the high protein diets was less than in the rats on the other diets. When the data were adjusted for differences in body weight, however, the differences between the groups were no longer significant.

COMMENTS

These experiments confirm the earlier observation of the increased susceptibility of female rats to adjuvant arthritis.

The arthritis was apparently not influenced by feeding the animals high carbohydrate or high fat diets. The protective effect of high protein feeding was significant, however, and varied directly with the percentage of protein in the diet and with the length of time the diet was administered. It thus appears certain that, under the conditions of these experi-

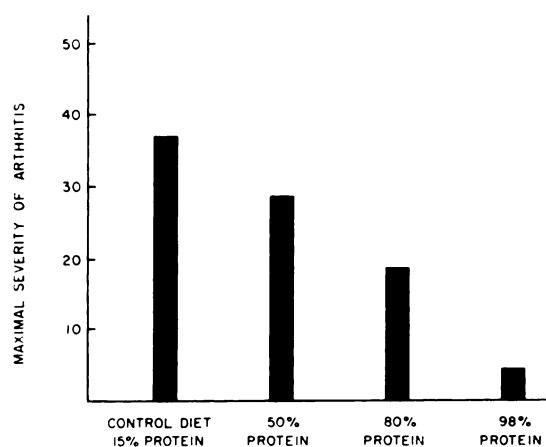


FIG. 2. Beneficial effect of an increasing protein content in the diet on arthritis in rats. Diet fed for forty to eighty-four days.

ments, a high protein diet tends to protect the animals from adjuvant arthritis, but it is not clear whether the protection is nonspecifically dependent upon retarded weight gain with growth, the percentage of protein in the diet *per se* (which happens incidentally to diminish weight gain) or upon some combination of these factors. The separation of weight control and percentage of dietary protein as factors in the severity of the arthritis are being evaluated in additional experiments.

The failure in these experiments to demonstrate any significant effect of high carbohydrate or high fat feeding does not necessarily mean that such effects would not be found in experiments designed differently.

SUMMARY

Long-Evans rats were kept for various periods of time on diets adequate in essential nutrients. The experimental diets were either (1) very high in fat, low in carbohydrate and adequate in protein; (2) very high in carbohydrate, very low in fat and adequate in protein; or (3) high in protein, with 50 to 98 per cent of the calories supplied by lactalbumin, as compared with 15 per cent in the control diets.

Female rats were significantly more susceptible to adjuvant arthritis than male rats regardless of the type or duration of the diet.

No significant diet-induced differences were observed in the severity of the arthritis except in comparing control animals with the animals fed 80 or 90 per cent protein. These high protein diets were significantly protective. There was a linear inverse relationship between the percentage of protein in the diet and the severity of the arthritis. Moreover, the longer the diet was fed prior to inoculation with the adjuvant the more marked was the amelioration of the arthritis.

Rats fed the high protein diets did not gain as much weight as did the control rats; whether the protection was due to the protein *per se* or to a nonspecific effect of diminished weight gain is not known from these experiments.

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