STUDIES OF HUMAN DIETS BY LONG-TIME FEEDING EXPERIMENTS WITH ANIMALS

by

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INTRODUCTION

At the present time much attention is being given to diets which vary quite widely from the usual or customary. With the increased interest being manifest in such diets and the number of observations and short experiments reported concerning them, it seems of interest to plan more extensive investigations of the effects of such diets on both the adult and the growing young. The purpose of this study was to show whether or not three diets which had been considered adequate would lead to equally good nutritive condition of animals measured in a number of ways by long time feeding experiments.

REVIEW OF LITERATURE

There have been many reports in the literature of the meat diet of Stefansson. Following Lieb's (1) clinical survey of Stefansson, an experiment was conducted in which Stefansson and Anderson, two arctic explorers of middle age lived for one year on a diet which consisted exclusively of lean and fat meat. Lieb (2) reported that the subjects were in good condition and showed no ill effects from the diet. McClellan, Tolstoi, and others (3, 4, 5, 6) published observations on ketosis, excretion of Acetone bodies, carbohydrate tolerance, chemical constituents of the blood and various

other conditions. McClellan, Rupp, and Toscani (7) reported that these subjects, on a calcium intake of from 0.05 to 0.15 grams per day, were in negative calcium balance throughout the experiment, excreting from 0.44 to 0.47 grams daily. McClellan and DuBois (8) did not find evidence of kidney injury by the diet. Mark Falcon - Lesses (9) said, "So far as I can see, the only conclusion properly deducible from these observations is that an average intake of meat protein of from 100 to 140 grams daily for one year causes no detectable kidney damage. What might happen after two or more years on such a diet remains unknown."

There is much disagreement in the literature concerning the effects of high protein diets. Newburgh et al (10, 11, 12, 13, 14) have found serious kidney injury from the use of diets high in protein. Drummond and his co-workers (15, 16) stated that the excretion of large amounts of nitrogen did not seem to damage the kidney, but that the high protein had a marked effect on reproduction.

Peet, Nelson, et al (17, 18, 19) conducted experiments with animals using a diet containing a high percentage of meat protein. They found that the diet was suitable for growth and reproduction but not to meet the needs of lactation. Variations of the diet were tried with somewhat better results than the basal diet alone. Since these diets contained other foods than meat, they are not exactly com-

parable with those composed of meat alone.

Lane and Bosshardt (20) compared the effects on growth and general appearance of children of a mixed and a vegetable diet. Children from 7 to 15 years of age were used and kept on the diet for ten weeks. The following conclusions were given:

"From the results of this short feeding test there appears no reason to conclude that a scientifically balanced milk diet, including one and one-half pints to one quart of milk a day per child, produces greater growth or better health in growing children from the ages of 7 to 15 years old than a scientifically chosen vegetable diet furnishing a smaller amount of calcium. If there is a greater calcium storage on the milk diet than on the vegetable diet, a supposition which does not appear justified by the gains in height and weight or the other data here presented, there still would seem to be no reason for assuming such excessive storage advantageous for the general health of growing children. Excessive calcium storage may possibly obscure a clear preception of the function of other factors in the diet, whether it be that of the calorie producing nutrients, the various minerals and vitamins or the acid-base equilibrium of the blood.

"A diet of vegetable foods carefully selected with special reference to the quality and quantity of the protein,

low in fat, comparatively high in carbohydrate, with adequate amounts of various minerals and high in vitamins produced in 10 weeks as satisfactory increase in all physical measurements observed in children from 7 to 15 years old, and in most cases greater increases, than a similarly carefully planned milk and meat containing diet."

It is generally recognized that long time feeding experiments often yield results not shown by short periods. Sherman et al (21, 22, 23) have studied the effects on growth, reproduction, and lactation of a simplified food supply. They found that an increased rate of growth and improved reproduction and lactation regularly followed the increase of the proportion of milk in the diet.

Sherman and Campbell (24) conducted an extensive experiment comparing an adequate diet consisting of one-sixth whole milk powder, five-sixths whole wheat and sodium chloride to two per cent of the wheat, with a similar diet differing only in containing one-third whole milk powder and two-thirds whole wheat. Food records were available for the entire experiment which covered at least 16 generations of rats. One of the outstanding findings was that greater gain in growth per 100 calories of food consumed was made by the animals receiving the better diet. The authors stated that "there is not only a line to be drawn but a wide zone to be explored between adequate and optimum nutrition."

Sherman and Booher (25) have stated that individuals otherwise well-nourished may grow up with calcium poor bodies. From their experiment, they conclude that "the body at birth has a low calcium content and its normal development involves a large increase not only in amount but also in the per cent of calcium which it contains and so requires a liberal supply of calcium in the food. Otherwise the growing body remains calcium poor, even though it shows every external appearance of being well-nourished."

The calcium content of the body in relation to age, growth and food was reported by Sherman and MacLeod (26). They based the percentage of calcium upon the net body weight. In general, the results of extensive investigations showed that the animals on a diet containing a higher proportion of milk had larger percentages of calcium in their bodies.

Sherman and Hawley (27) have shown that children do not seem to utilize the calcium of vegetables as efficiently as they do that of milk. The calcium balance was always more variable and less favorable when vegetables replaced about half of the milk as a source of calcium.

METHOD

The experiments here described were planned to compare and contrast, by long time feeding experiments with animals, three diets which have been used by human subjects. The diets chosen were those used by Lane and Bosshardt (20) and the Stefansson meat diet (7, 8). These have been designated as the mixed, vegetable, and meat diets and are as follows:

Mixed Diet

Breakfast Food	Cal.	Protein gms.	Ca. gms.
Milk in postum	100	4.75	.174
Oatmeal	50	2.10	.008
Milk on cereal	50	2.37	.087
Butter	50	0.06	.001
Stewed peaches	100	1.70	.038
Toast	100	3.95	.020
Sugar	33	0.00	.000
Lunch			
Milk	140	6.65	.243
Bread	100	3.95	.020
Butter	50	0.06	.001
Vegetable soup	100	2.27	.030
Apple	100	0.64	.012
Dinner			
Braised beef	165	12.12	.006
Baked potato	100	2.65	.016
Bread	100	3.95	.020
Butter	50	0.06	.001
Cabbage and carrot salad	75	1.10	.067
Milk	140	6.65	.243
Banana	100	1.32	.009
Total	1,703	56.35	.996

Vegetable Diet

Breakfast Food	Cal.	Protein gms.	Ca. gms.
Orange juice	100	1.44	.067
Oatmeal with wheat germ uncooked	77	3.90	.012
Ground almonds	40	1.30	.014
Honey	66	0.08	.000
Stewed peaches	100	1.70	.038
Whole wheat toast	100	3.98	.025
Butter	50	0.06	.001
Lunch			
Orange juice	100	1.44	.061
Whole wheat bread	100	3.98	.025
Butter	50	0.06	.001
Vegetable soup	100	2.27	.030
Apple	100	0.64	.012
Almond	80	2.60	.028
Dinner			
Lentil loaf	150	11.03	.046
Baked potato	100	2.65	.016
Whole wheat bread	100	3.98	.025
Butter	50	0.06	.001
Cabbage and carrot salad	75	1.10	.067
Orange juice	100	1.44	.090
Figs	205	6.61	.312
Total	1,843	50.32	.632

Meat Diet

Food	No. samples	Gms. per 100 gms. wet weight N	Fat
Beef muscle (well trimmed)	27	3.24	5.02
Beef muscle (not trimmed)	9	3.18	11.78
Beef tongue	17	3.15	24.24
Beef liver	14	2.97	5.85
Beef kidney	2	2.35	2.60
Beef brain	1	1.68	9.30
Beef fat	18	0.22	90.20
Beef bone marrow	8	0.20	89.10
Veal	2	2.88	15.31
Lamb	4	2.42	26.85
Bacon	5	1.23	59.50

Average for periods.

	Protein	Fat	Carbo-hydrate	% Ca. from protein
Period I. 110 days	124.1	221.1	9.1	19.6
Period II. 40 days	117.1	175.8	8.2	22.3

In planning the diets for the animals, there was the problem of securing uniform mixtures of a consistency that could be conveniently handled. Since it was impractical to mix the diets in small quantities as needed, some method of storage had to be provided. It was decided to grind all of the foods together, pack in waxed cartons (butter cartons) and keep in cold storage until used.

The formulae which were worked out and used in these experiments are:

Mixed Diet

Article	Amt.in gms.
Oatmeal	12.6
Butter	19.5
Peaches	36.0
Bread	115.2
Carrots	60.0
Cabbage	30.0
Potato	120.0
Vegetable soup	95.0
Apples	212.3
Banana	101.4
Beef	81.0
Sugar	8.3
Milk	84.7
Salt	3.0

Vegetable Diet

Article	Amt.in gms.
Oatmeal	12.6
Butter	19.5
Peaches	36.0
Bread	115.2
Carrots	60.0
Cabbage	30.0
Potato	120.0
Vegetable soup	95.0
Apples	212.3
Figs	64.8
Lentils	43.1
Honey	20.1
Califorange	142.0
Salt	3.0
Wheat germ	7.1
Almonds	18.6

Meat Diet

Article	Amt.in gms.
Beef muscle (well trimmed)	108
Beef muscle (not trimmed)	36
Beef tongue	68
Beef liver	56
Beef kidney	8
Beef brain	4
Beef fat	40
Beef bone marrow	24
Veal	8
Lamb	16
Bacon	18
Salt	

Ratio of N : fat = 10.7129

In calculating the amounts, the foods were used in the same proportions as given in the menus. With the aid of figures given by Rose (28) the weights of the various foods were computed from the calories given. Since the exact compositions of some of the foods were not known, the foods were added in the amounts necessary to furnish the same calories, protein, and calcium given in the original. An equivalent weight of lentils was substituted for the lentil loaf; a mixture of two-thirds cabbage and one-third carrot for the salad; and a commercial brand of canned soup for the vegetable soup. It seemed impossible to use fresh milk and orange juice and at the same time obtain a mixture of the desired consistency. Various investigations, including those of Sherman and his co-workers (21, 22, 23, 24, 25, 26) have indicated that dried milk is well utilized by experimental animals, including the rat. Therefore, an equivalent weight of dried milk was substituted for the fresh. Califorange was substituted for orange juice in the vegetable diet. This is a commercial product, a highly concentrated form of orange juice. It is said by the workers to contain nothing but orange juice and has been used with apparent satisfaction in many places. The caloric value of Califorange as determined by the bomb calorimeter was found to be 2.11 or 4.9 times that of the fresh juice. From this the equivalent amount was calculated. These products were also

more uniform than fresh milk or fresh juice prepared at various times.

As no definite menu was given for the meat diet, the proportions of the various meat products had to be planned to conform as nearly as possible to the data which were given. The figures for the average protein and fat intake for two periods of 110 days and 40 days were taken and a weighted average made. This gave an average ratio of 209.1 grams fat to 19.5 grams of nitrogen or 10.723. Governed largely by this ratio and taking the number of samples analyzed as an index of the frequency with which the various foods appeared in the diet, the above formula was worked out.

Variation I was the vegetable diet plus an amount of calcium carbonate sufficient to bring the calcium content up to the same amount as in the mixed diet.

The necessary amount of each food was accurately weighed on a solution and soil balance. The foods which required cooking were prepared in the manner suggested in the menus. The potatoes were baked and used without removing the skins. The oatmeal was cooked in a double boiler using only a small amount of water to avoid unnecessary moisture. The lentils were soaked for several hours and cooked until tender. The bread was toasted so that it could be more easily ground. The other foods were used raw. All of the products in the meat diet were used raw. The weighed

and cooked foods were put through an electric grinder, using an attachment with small holes. The foods were fed into the grinder alternately so that the resulting mixture would be as nearly uniform as possible. After grinding, it was thoroughly mixed by hand, ground a second time, remixed and ground the third time. The resulting product was a fairly homogeneous mixture of a pasty consistency. As the formula was mixed and packed in the cartons, portions were retained for sample, placed in a separate carton, and kept for analysis. Determinations were secured of the moisture, protein, fat, carbohydrate, fiber, ash, and calcium content, as shown in Table I.

The animals used were Albino rats of Wistar Institute stock bred in this laboratory and started on experiment at various age levels. Before being placed on experiment, the animals were all living on a uniform diet, the Sherman stock diet (24) and had been kept under the same conditions. Rats were taken for experiment at various age levels as shown in Tables II and III. The various age levels were selected to study the effects of the diets on both the adult and growing animal of different ages. All of the animals were kept under identical conditions and treated in the same way. They were fed the diets for varying lengths of time ranging from 28 to 140 days. At the close of these periods they were killed for determination of calcium.

Table I. Analyses of Diets

Diet	% Gross Moisture	% on "Air dried" Sample						% Ca. on Moist. Wt.
		Moisture	Protein	Fat	CHO	Fiber	Ash	Ca.	
Mixed	43.46	2.96	15.38	12.25	62.92	1.27	5.22	0.26	0.15
Meat	56.95	1.80	36.75	55.57	0.99	2.15	2.74	0.11	0.05
Veg.	53.82	2.86	10.06	7.23	73.48	2.85	3.85	0.11	0.05

Table II. Distribution of Experimental Animals

Age	Lot I	Lot II	Lot III	Lot IV
	Animals taken from stock diet and placed on experiment at ages indicated below.			Young reared by animals of Lots I, II and III.
Birth				Some reared. Others killed for analyses
4 wks.	Put on each diet			Some killed for analyses. Photographs males & females kept together
8 wks.		Put on each diet		
12 wks.	Some killed for analyses Others mated Photographs	Mated	At 12 & older put on each diet	
24 wks.	All survivors killed for analyses			All survivors killed for analyses Photographs
28 wks.		All survivors killed for analyses	After 16 wks. all survivors killed for analyses. 2 kil. after 8 weeks.	

Table III. Count of Animals taken from Stock or kept on Diet on which Born.

		Mixed	Meat	Veg.	Veg. + Ca.
Lot I	Males	7	8	6	2
	Females	7	4	8	4
Lot II	Males	1	1	2	-
	Females	2	1	2	-
Lot III	Males	2	1	2	-
	Females	3	4	4	-
Lot IV	Males	3	1*	0*	-
	Females	5	2*	3*	-

* No more animals available.

The animals were kept in all-metal cages, with removable metal trays. Several layers of newspapers were laid on the tray and a wire screen of half-inch mesh was placed over them. This enabled the worker to keep the cages clean with a very little expenditure of time and energy, since the trays could be removed and fresh paper and wire racks substituted for the dirty ones. Pint jars of stoneware were used for food and water containers.

Only shredded paper was used for the bedding of young and this was not used after the young were three weeks old. All of the cages were cleaned six times each week. At this

time the trays were removed and fresh ones substituted. The food and water jars were also changed and trays, screens and jars were sterilized in the sterilizer at a temperature of 212°F. The cages were changed and sterilized weekly. The food for each cage was weighed to the nearest gram on a torsion balance and placed in a stone jar in the cage, record being kept of the amount given and that left each day. Fresh food was given six times a week and distilled water ad libitum. Precautions were taken so that at no time were the animals without food and water.

The animals were kept in separate cages until 12 weeks of age and were then mated. For those of the second generation, males and females were kept in the same cages. Females were removed to separate cages before the birth of young. The young were weighed in a group at the ages of 7 days, 14 days and 21 days. They were separated from the mothers at a uniform weaning age of four weeks at which time the mothers were returned to the breeding cages. For brevity in tabulation the following symbol was used: 9 y 7 to mean nine young born and seven reared to four weeks of age. K was used to indicate an animal which was killed and D for one which died.

The animals were weighed weekly. For weighing, they were placed in a closed container which was balanced by a weight on the other pan of the scale and weighed to the

nearest gram.

In order that a quantitative comparison be made, the following records were kept: (a) weekly weight records in order that the rate of growth be shown by gain in weight, (b) dates upon which all litters were born, (c) the number of young born and of young reared and the weights of litters at weekly intervals during the suckling period, (d) all unusual conditions such as coughing or wheezing, or any abnormal appearance, (e) and the food consumption. In addition to these, data on the quantitative determination of calcium in animals of different ages on all diets were kept.

Rats were taken for analysis after being kept on the diets for definite periods. They were killed in a manner very like that of Sherman and MacLeod (26). In the cases of those taken for analysis at birth, sexes were not determined and the young of a litter were analyzed together. In all other cases, the sexes were determined and each animal analyzed separately. For ashing the entire bodies, the animals were killed with ether, the alimentary tracts removed, and the bodies then ashed in silica dishes. The body weights were obtained by weighing the alimentary tracts and subtracting the weight from the live weight of the rat, both weighings being made to the tenth of a gram. The ash was dissolved in hydrochloric acid and the solution filtered and brought up to 250 cc. The calcium was determined by

McCrudden's (29) modified Volumetric method. The solutions are being retained for determination of phosphorus.

DISCUSSION

Table IV shows the gains made by the males and females of Lot I during the first eight weeks. The animals on the mixed diet made the best gains in every case. The gains made exceeded those of the control group and the normal growth curves of King as given by Donaldson (30). The total gain made by the males on the mixed diet during the entire period did not greatly exceed that made by the males on the meat diet but was about eight times that of the animals on the vegetable and vegetable plus calcium carbonate diets. The animals on the vegetable and vegetable plus calcium carbonate diets gained very slowly, the two groups making approximately equal gains. Results for the females of this group were much the same as for the males. The gain made by the females on the mixed diet exceeded that made by the females on the meat diet to a somewhat greater extent than in the case of the males. The females on the mixed diet gained about eight times as much as the vegetable group and about six times as much as the vegetable plus calcium carbonate group.

Table IV. Average Weights of Rats of Lot I Compared at Various Ages

Age	Mixed	Meat	Veg.	Veg.+ $CaCO_3$	Con-trol
			Males		
28	46.6	51.9	52.9	40.0	45.0
35	61.7	78.0	52.0	34.5	58.2
42	80.0	110.1	54.3	39.0	70.2
49	104.3	133.3	58.5	40.5	90.6
56	120.9	145.3	64.8	44.0	104.8
63	137.6	158.9	65.3	48.0	113.6
70	159.3	162.4	68.5	51.0	121.2
77	176.3	164.0	71.1	56.5	132.1
84	185.0	178.3	71.1	57.0	140.3
Total gain	138.4	126.4	18.2	17.0	95.3
			Females		
28	45.4	45.5	47.4	44.8	52.5
35	57.7	79.0	47.8	39.5	69.5
42	80.4	100.5	49.9	42.5	96.0
49	99.3	114.0	51.3	43.8	113.5
56	117.4	123.5	52.9	48.3	129.0
63	129.1	129.5	55.4	51.3	133.0
70	142.0	135.7	58.5	55.0	145.0
77	154.4	134.3	60.1	59.8	151.0
84	162.0	138.8	62.5	64.0	154.5
Total gain	116.6	93.3	15.1	19.2	102.0

Figure 1 summarizes the data for the growth of both males and females of Lots I and IV. It may be seen from the curves for Lot IV that the males on the mixed diet grew at a very rapid rate. None of the males on the meat diet lived to 12 weeks of age. The females on the mixed diet also made very rapid gains, reaching at 12 weeks an average weight equal to three times that of the meat group and four times that of the vegetable group.

There was not only wide variation in the rate of growth of the animals of Lots I and IV on the various diets but many differences in the general appearance of the various groups. All of the animals on the mixed diet had good smooth coats of fur, bright eyes, good appetites and showed all of the other signs of normal, well-nourished and well-kept animals. Some of the animals of Lot I on the meat diet, when judged by size and gain in weight alone, would have been considered normal. They showed, however, many signs of abnormality. Within four weeks after being placed on the diet, all showed distinct signs of weakness in the limbs. Most of them walked in a rather humped-up position. If they were pushed over, they had difficulty in rising. Many of them had swollen joints and would squeal when these were slightly pressed as in handling the animal. This condition usually grew better but appeared again at intervals throughout the experimental period. All of them were very nervous

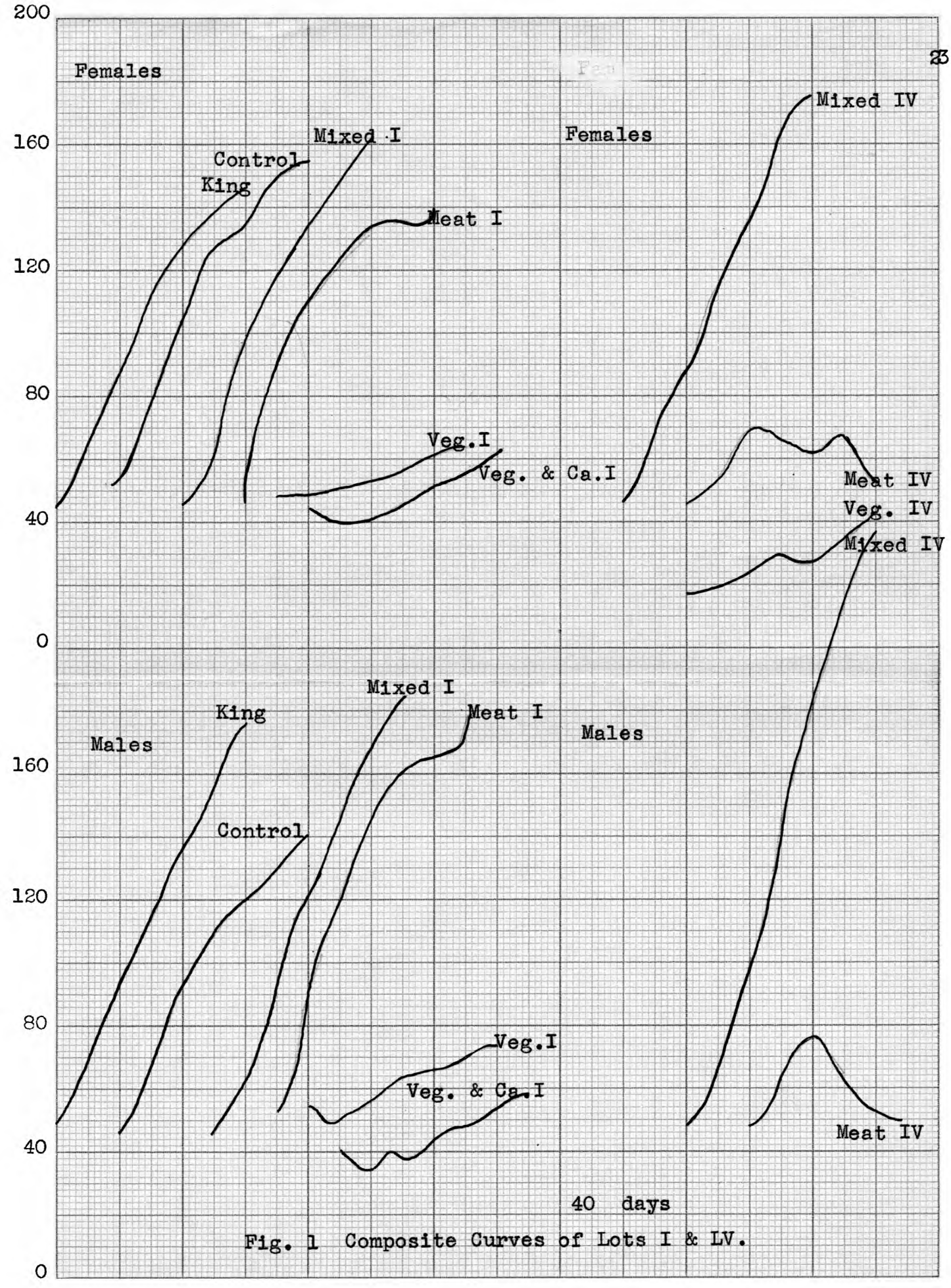

Fig. 1 Composite Curves of Lots I & LV.

and jumped and seemed frightened at even a slight movement of the cage. This condition seemed to grow worse and when the rats were placed together in a cage they squealed and fought at one another, especially if any movement was made in the cage, as placing or removing the food or water jars. Six out of the twelve rats on the diet showed all of these symptoms to a very marked degree. They were wet and dirty most of the time and had diarrhea. When in this condition they had a very offensive odor. Two of this group died before they were 12 weeks old and three others were very sickly-looking and losing rapidly in weight when they were killed. The corresponding group on the vegetable diet showed many signs of abnormality other than failure to make good growth. Their fur soon became rough and shaggy looking and in most cases fell out in spots. These conditions grew worse as the animals grew older. Other symptoms noted were pale eyes and rough scaly tails. Some of these symptoms are similar to those others have noted in Vitamin G deficient animals. The diet however, contains wheat germ, cereals and vegetables all of which contain some of the vitamin. Judging by the frequency with which the animals sneezed, coughed, and developed red watery eyes, their resistance was very low. The animals appeared to be much older than they actually were, but at the same time remained about the size of normal four or five week rats.

Plate I is a photograph of representative animals of Lot I on the mixed and vegetable diets taken when they were 12 weeks of age.

The animals of Lot IV on the mixed diet were normal in every respect. Those on the vegetable diet showed the same signs of abnormality noted in those of Lot I. They appeared much older than either their size or age would indicate. Those of Lot IV on the meat diet were quite abnormal. In addition to a continual loss in weight after the third week, they had very poor use of their hind legs, were inactive, had very frail bodies, pale eyes, bloody noses, and distinct signs of diarrhea. Two of the group died before the sixth week. The other continued to lose weight and was almost dead when it was killed at 12 weeks. Plate II is a photograph of this surviving animal of Lot IV on the meat diet taken at 11 weeks of age. A photograph does not fully present the poor condition of the animal.

Since some of the animals grew more rapidly than others as shown in Table IV, the question next arises as to whether the more rapid growth called for an arithmetically proportion increase in food intake or was more economical as well as more rapid.

Table V shows the food consumed in terms of gain in body weight per 1000 calories. It will be noted that the males on the meat diet gained more per 1000 calories for the first period. Those on the mixed diet gained about

Explanation of Plate I

Animals of Lot I when 12 weeks of age.

Fig. 1 Animal on Vegetable diet.

Fig. 2 Animal on Mixed diet.

Plate I

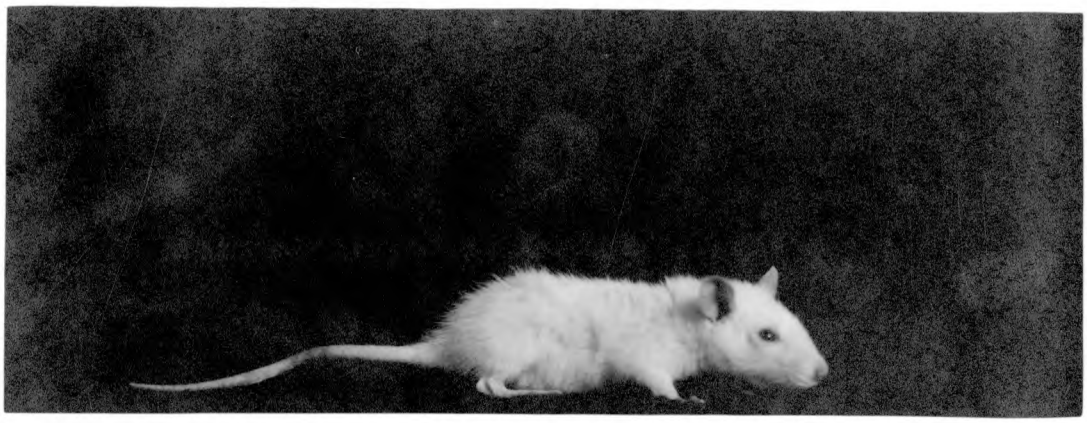

Fig. 1

Fig. 2

Explanation of Plate II

Animal of Lot IV on meat diet taken when 11 weeks of age.

Plate II

Table V. Grams of Gain in Body Weight per 1000 Calories of Food Consumed

Age Period	Mixed	Meat	Veg.	Veg. - Ca
		Males		
5 - 8	47.9	70.6	10.6	9.3
8 - 12	35.5	20.0	8.5	21.4
		Females		
5 - 8	41.9	50.2	6.2	8.1
8 - 12	22.8	12.2	12.7	23.2

two-thirds as much as the meat rats and almost five times that of the groups on the vegetable and vegetable plus calcium carbonate diets. For the second period, the gain of the group on the mixed diet exceeded that of the other groups. There was a marked increase in gain of those in the vegetable plus calcium carbonate diet over their gain in the first period and a marked decrease in the gain of those on the meat diet from that of the first period. The data for the females show very much the same results except that in the case of the vegetable group the gain per 1000 calories during the second period doubled that in the first.

Table VI gives the food consumption in terms of calories per gram of rat per day. These figures show that

Table VI. Food Consumption of Rats in Terms of Calories per Gram of Rat per Day at Different Ages

Age Period	Mixed	Meat	Veg.	Veg. - Ca
		Males		
5 - 8	0.67	0.45	0.71	0.39
8 - 12	0.44	0.24	0.49	0.42
		Females		
5 - 8	0.66	0.58	0.63	0.36
8 - 12	0.43	0.30	0.46	0.38

those on the meat and vegetable plus calcium carbonate diets consumed fewer calories per gram of weight than those on the other diets. There was little difference in the amount consumed by the animals on the mixed and vegetable diets. In each case with the exception of the first period for the females the balance is slightly in favor of the vegetable animals.

In caring for the animals it occurred to the author that the consumption of the vegetable diet might be low on account of a dislike for the very pronounced flavor and odor of orange which permeated this mixture. Accordingly, a formula was made as usual except that Califorange was omitted, peanut butter and carrots being added to furnish

an equivalent amount of calcium. This diet was fed to two animals. The food consumption was much the same as for the regular vegetable diet. If there was a dislike for the diet, it was apparently not due to the orange juice, the only item of distinctive odor and flavor not present in the well-liked mixed diet.

Some data collected from observations of the females on the three diets are presented in Table VII.

Table VII. Average Breeding Records of all the Females of the Various Lots

	Lot	Mixed	Meat	Vege-table
Average age at birth of first young	I	110	No Y	No Y
	II	112	No Y	175
	III	First young reared previously		
	IV	87	No Y	No Y
Total number of young born	I	36	0	0
	II	44	0	11
	III	57	8	30
	IV	40	0	0
Average weight of young at weaning	I	53	-	-
	II	49	-	-
	III	48	43	20
	IV	57	-	-

The females of Lots I and II on the mixed diet bore their first young at approximately the same average age. These animals were mated at 12 weeks and bore young at the

earliest possible date continuing to bear and rear young at a good rate. The animals of Lots I and IV on the meat and vegetable diets did not bear young. Those of Lot II on the vegetable diet bore young at a much later age than those on the mixed diet. No age is given for Lot III since they were mature animals and had reared young before being placed on experiment. It is seen that the mixed group have more young than the others. All of these grew well and reached a good average weight at the time of weaning. Since some of the young were killed for analysis at birth, the percentage of young reared could not be given. All of the young born to the vegetable Lot II died within 24 hours after birth. In the meat Lot III only one litter was born and this two weeks after the mother was placed on the diet. Seven of the eight were reared and were a good average weight at weaning. Several litters were born in the vegetable Lot III but most of the young were dead at birth or died a few hours afterward. No sign of milk could be found in the stomachs of the young. Three rats from a litter of nine born one week after the mother was placed on the diet were reared. At weaning they weighed less than one-half that of the young of the mixed Lot III.

Some individual growth curves of representative animals of Lot I are given in Figure 2. These curves represent the

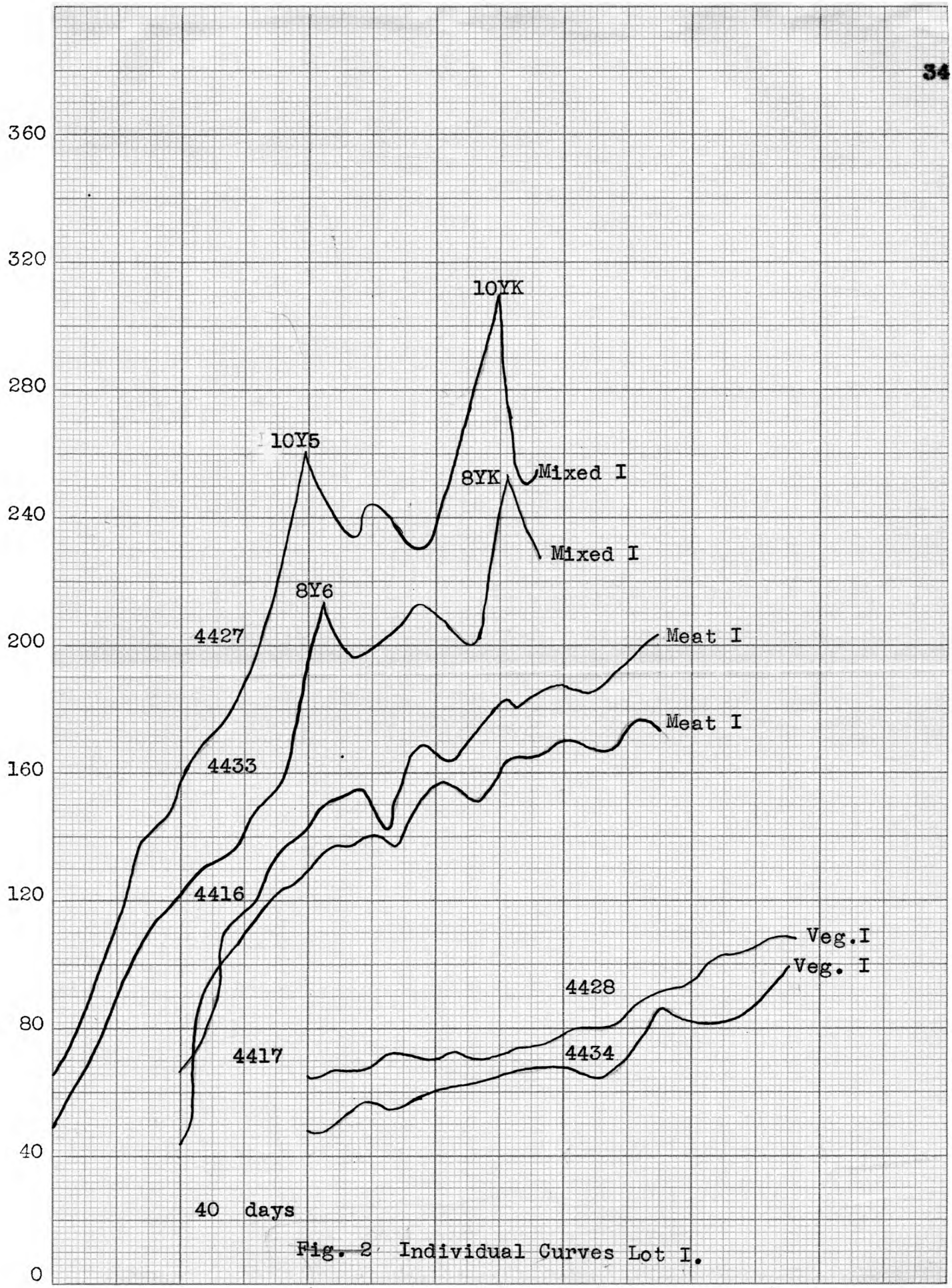

Fig. 2 Individual Curves Lot I.

growth of the animals throughout the experimental period. Those on the mixed diet gained continuously in weight and bore and reared young at a normal rate. As has been noted the rats on the meat diet made normal gains up to the twelfth week. After this time they grew very irregularly as can be seen from the curves. The conditions of the animals noted previously were present in varying degrees throughout the period. As shown by the curves, none of the vegetable group attained a weight approximating that of the normal animal so were not capable of reproduction.

Plate III is a photograph of representative animals of Lot I on the mixed and vegetable diets taken at 20 weeks of age. The condition of the animal on the vegetable diet was much worse than is shown by the picture. All of the fur was off of the neck and shoulders and the remainder of the coat was rough, shaggy, and patchy.

Figure 3 shows some individual curves for representative animals of Lot II. The same indications of good normal growth and reproduction noted for animals of Lot I on the mixed diet are shown in this group. The growth of the meat animals was very irregular after the fourth week. This group did not show all of the signs of abnormality noticed in Lot I but were nervous and easily frightened. Although the gains made by the vegetable group were not so large as those of a normal animal, they grew somewhat better than

Explanation of Plate III

Animals of Lot I when 20 weeks of age.

Fig. 1 Animal on Vegetable diet.

Fig. 2 Animal on Mixed diet.

Plate III

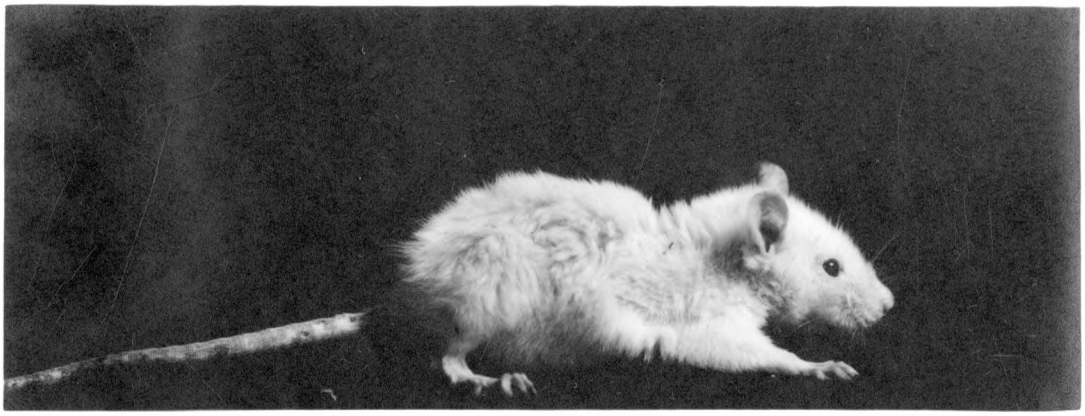

Fig. 1

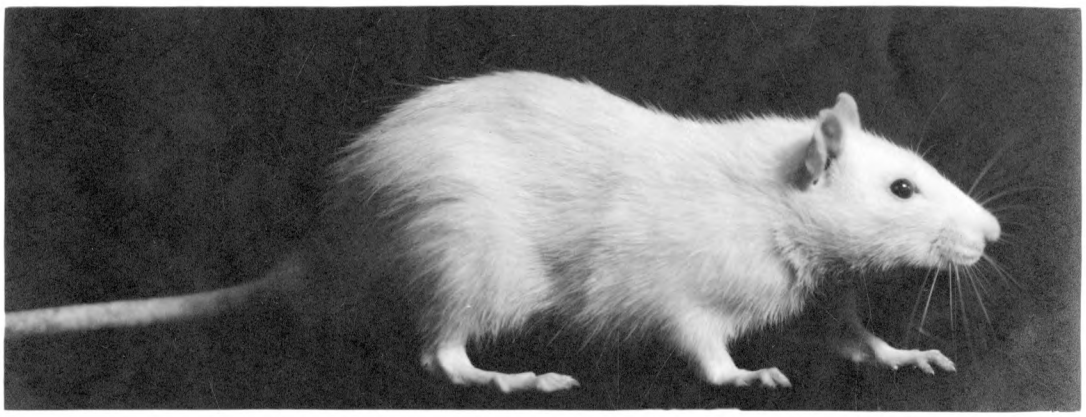

Fig. 2

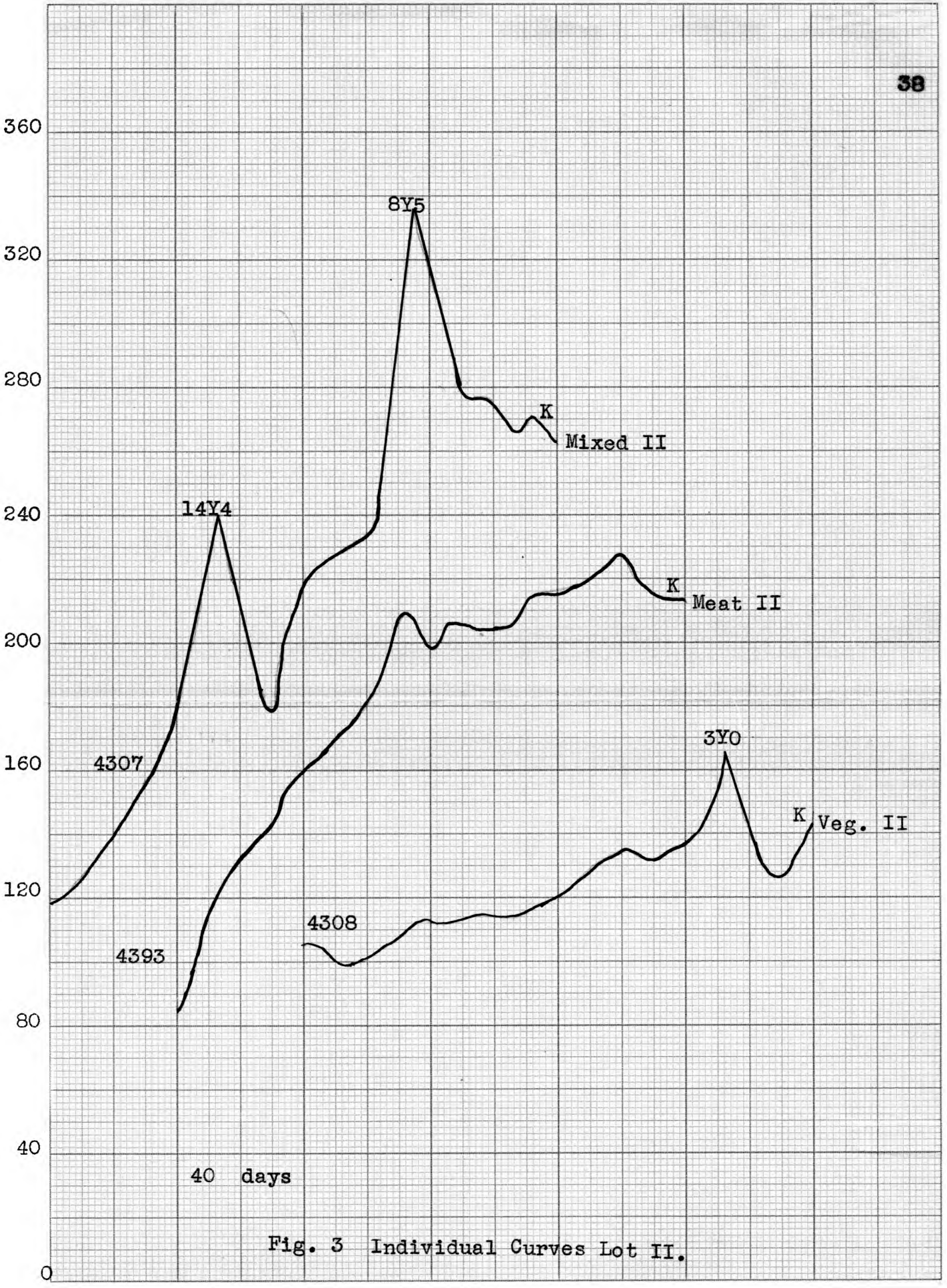

Fig. 3 Individual Curves Lot II.

those of Lot I. Some of them finally attained a weight which approximated that of a normal adult rat. There was a much less total gain before the birth of young than was found in the mixed group. The animals of this group presented a better appearance than those of Lot I but seemed to have lowered resistance and looked older for their age than the normal rat.

Figure 4 gives the weight curves of three females of Lot III on the three diets. The one on the mixed diet gained regularly in weight in addition to bearing and rearing young at the normal rate. Those on the other two diets gained very irregularly. In each case the animal made a comparatively large gain at one point ant then lost rapidly. Apparently no young were born at this point. The comparative size and appearance at weaning of the young of the mixed and vegetable mothers is shown in Plate IV.

The growth of these animals from birth to the end of the experiment and the surviving one on the meat diet, shown on Plate II, is given in Figure 5. The rat on the mixed diet made rapid gains throughout the period. Maturity was reached at an early age and practically all of the young born were reared. The meat rat in this group has been described elsewhere. As is shown, it soon declined in weight and was in very poor condition when killed. The one on the vegetable diet gained slowly and irregularly through-

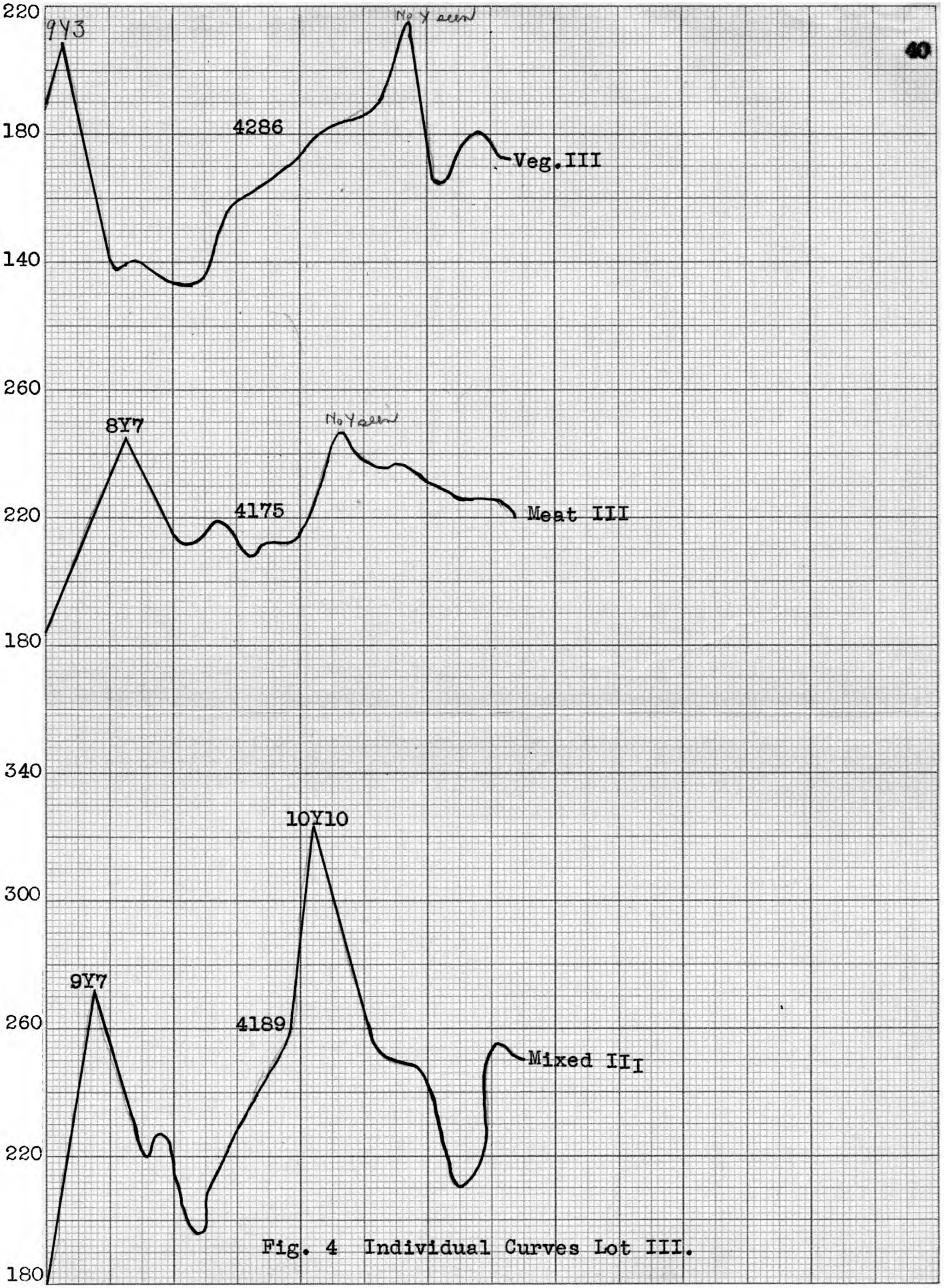

Fig. 4 Individual Curves Lot III.

Explanation of Plate IV

Animals of Lot IV at 4 weeks of age.

Fig. 1 Animal reared on Mixed diet.

Fig. 2 Animal reared on Vegetable diet.

Plate IV

Fig. 1 Fig. 2

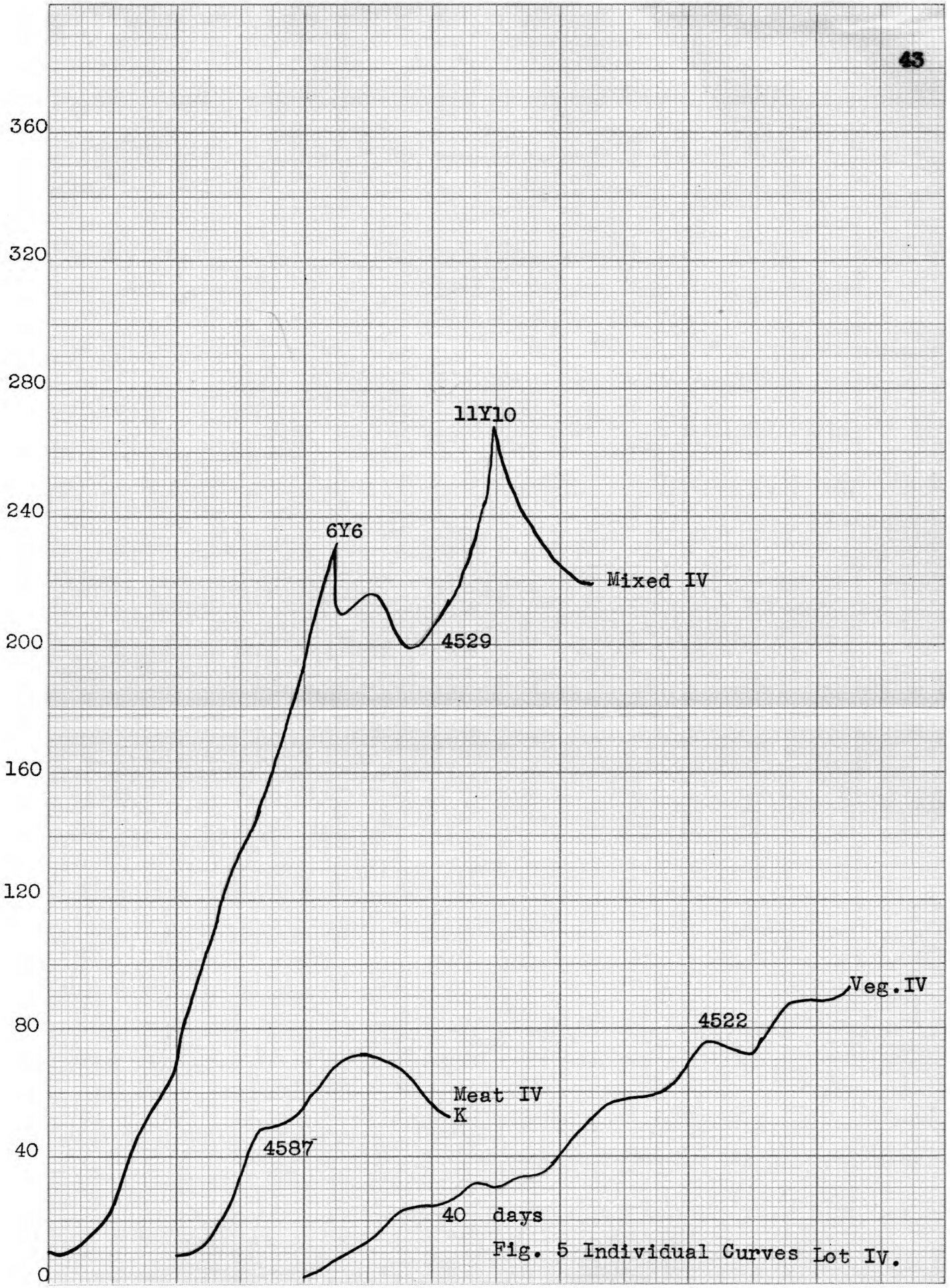

Fig. 5 Individual Curves Lot IV.

out the period, never reaching a weight approaching the normal. The relative size and appearance of the two animals on the mixed and vegetable diets are shown in Plate V, taken at the close of the experimental period when the animals were 24 weeks old.

The results of the calcium determinations made on the whole bodies of the animals are given in Tables VIII to XI. Table VIII gives the results of Lot I killed at 12 weeks. The males on the mixed diet had stored a larger percentage as well as larger amount of calcium than any other group except those on vegetable plus calcium carbonate. The percentage here is somewhat larger but the amount is only about one-third as great. When this and the fact that the animals in the mixed group weighed about four times as much as those on the vegetable plus calcium carbonate is considered, the percentage is not very significant. The animals on the meat diet stored less than half the amount of calcium stored by the other groups. This is rather significant since their weights were about normal. Although the per cent of calcium in the vegetable group is approximately that of the mixed group, if this were calculated on the basis of either age or normal weight, it would show a very marked reduction. There is quite a wide variation in the percentages given for the mixed group. This is, perhaps due to the variations in body weights, some animals being very

Explanation of Plate V

Animals of Lot IV taken at 24 weeks.

Fig. 1 Animal on Vegetable diet.

Fig. 2 Animal on Mixed diet.

Plate V

Fig. 1

Fig. 2

Table VIII. Calcium Determinations of Lot I
Killed at 12 Weeks

Diet Rat No.	Last live Wt.	Net Wt.	Wt.Ash	Wt. Ca. in Ash	% Ca. in Ash	% Ca. on live Wt.	% Ca. on net Wt.
			Males				
Mixed							
4368	259	237	5.4521	1.3710	25.14	0.53	0.58
4375	166	151	6.5423	1.8627	28.47	1.12	1.23
4421	209	185	5.1734	1.5498	29.95	0.74	0.84
Ave.	217	191	5.7226	1.5945	27.85	0.80	0.88
Meat							
4542	158	135	2.9485	0.5954	20.19	0.38	0.44
4543	173	155	3.1561	0.5837	18.49	0.34	0.38
4544	152	133	3.0164	0.6307	20.90	0.42	0.47
Ave.	161	141	3.0403	0.6033	19.83	0.38	0.43
Veg.							
4369	94	79	2.2889	0.5516	24.09	0.59	0.70
4419	86	75	2.2331	0.6435	28.81	0.75	0.86
4431	61	52	1.8866	0.4960	26.29	0.82	0.95
Ave.	80	69	2.1362	0.5637	26.39	0.72	0.84
Veg. + $CaCO_3$							
4633	55	47	1.8291	0.5106	27.91	0.92	1.09
4634	59	49	2.0034	0.5484	27.34	0.94	1.12
Ave.	57	48	1.9162	0.5295	27.62	0.93	1.10
Control							
4435	132	126	4.1290	1.0547	25.54	0.74	0.84
4440	137	120	3.8972	0.9748	25.01	0.71	0.81
4444	142	128	4.2873	1.1475	26.76	0.81	0.89
Ave.	137	125	4.1045	1.0590	25.77	0.75	0.84

Diet Rat No.	Last live Wt.	Net Wt.	Wt. Ash	Wt. Ca. in Ash	% Ca. in Ash	% Ca. on live Wt.	% Ca. on net Wt.
				Females			
Mixed							
4639	171	157	5.3250	1.4271	26.80	0.83	0.91
4640	149	135	4.8351	1.2775	26.42	0.86	0.94
4647	153	140	5.1010	1.4218	27.87	0.93	1.02
Ave.	158	144	5.0870	1.3754	27.03	0.87	0.96
Meat							
4541	105	88	2.1991	0.4704	21.38	0.45	0.53
Veg.							
4644	66	56	1.8941	0.4917	25.96	0.74	0.87
4645	71	62	1.8123	0.4746	26.13	0.67	0.77
4646	57	49	1.6467	0.4244	25.77	0.74	0.86
Ave.	65	56	1.7844	0.4635	25.95	0.72	0.83
Veg. + $CaCO_3$							
4635	64	55	2.1214	0.5755	27.13	0.90	1.05
4636	68	56	2.0977	0.5502	26.23	0.81	0.98
4637	66	55	2.1881	0.5969	27.28	0.90	1.08
4638	50	41	1.7568	0.4843	27.56	0.98	1.19
Ave.	62	52	2.0420	0.5517	27.05	0.90	1.07

large and fat. If the amount is considered, it is seen to be much more constant. Both per cents and amounts are more constant for the other diets where there is less variation in body weight. The data given for the females of this group show very much the same trend. The percentages are a little more constant and in some cases larger than for the males.

Table IX gives figures for Lot I killed at 24 weeks.

Table IX. Calcium Determinations of Lot I Killed at 24 Weeks

Diet Rat No.	Last live Wt.	Net Wt.	Wt.Ash	Wt.Ca. in Ash	% Ca. in Ash	% Ca. on live Wt.	% Ca. on net Wt.
			Males				
Mixed							
4377	259	227	7.4552	2.0238	27.14	0.78	0.89
4383	222	207	7.4730	2.0578	27.53	0.93	0.99
4426	348	332	11.4376	3.2157	28.11	0.93	0.97
4432	331	307	10.2416	2.8495	27.82	0.86	0.93
Ave.	290	268	9.1518	2.5387	27.65	0.87	0.94
Meat							
4403	174	156	3.3464	0.6986	20.87	0.38	0.45
4413	246	232	4.8007	1.1438	23.82	0.46	0.49
4414	247	228	4.6891	0.9765	20.82	0.40	0.43
Ave.	222	205	4.2787	0.9396	21.83	0.42	0.45
Veg.							
4382	86	73	1.9624	0.5285	25.91	0.59	0.69
4420	107	90	3.1756	0.8536	26.87	0.80	0.95
4425	132	119	3.9682	1.0150	25.57	0.77	0.85
Ave.	108	94	3.0321	0.7990	26.12	0.72	0.83
Control							
4370	258	237	8.8948	2.5994	29.22	1.01	1.10
4404	160	142	5.9481	1.7535	29.48	1.10	1.23
4415	277	257	9.2926	2.6561	28.58	0.96	1.03
4436	209	191	6.9238	1.8722	27.03	0.89	0.98
4437	237	215	8.1889	2.3415	28.59	0.99	1.09
4439	241	221	8.4864	2.4653	29.04	1.02	1.12
4445	194	177	6.8234	1.9753	28.94	1.02	1.12
Ave.	225	206	7.7940	2.2361	28.69	1.00	1.09

Diet Rat No.	Last live Wt.	Net Wt.	Wt.Ash	Wt.Ca. in Ash	% Ca. in Ash	% Ca. on live Wt.	% Ca. on net Wt.
			Females				
Mixed							
4387	239	226	8.1615	2.2461	27.52	0.94	0.99
4427	249	230	8.8977	2.5607	28.77	1.03	1.11
4433	226	208	7.3349	2.0501	27.95	0.91	0.99
Ave.	238	221	8.1313	2.2856	28.08	0.96	1.03
Meat							
4407	167	152	3.5130	0.7948	22.62	0.47	0.52
4416	202	188	4.2857	0.9712	22.66	0.48	0.52
4417	173	161	3.5160	0.7414	21.08	0.43	0.46
Ave.	181	167	3.7716	0.8358	22.12	0.46	0.50
Veg.							
4373	107	97	3.3753	0.8922	26.43	0.84	0.92
4379	61	51	1.7119	0.4724	27.59	0.77	0.92
4380	97	86	2.7865	0.8190	29.39	0.84	0.95
4428	109	98	3.4360	0.8830	25.69	0.81	0.90
4434	102	91	3.0308	0.8355	27.56	0.82	0.92
Ave.	95	84	2.8681	0.7804	27.33	0.81	0.92
Control							
4372	183	163	6.7702	2.0135	29.74	1.10	1.24
4429	184	166	7.6540	2.3982	31.33	1.31	1.44
Ave.	183	164	7.2121	2.2058	30.53	1.21	1.34

These show the same general trend as those given in Table VIII. The percentage in the mixed group is higher than at 12 weeks while that of the meat and vegetable groups remains constant. An increase in percentage and a decrease in amount will be noted in the female group as compared with the males. No figure is given for the calcium carbonate

group as they were all killed at 12 weeks.

The results for Lots II and III are given in Table X.

Table X. Calcium Determinations of Lots II and III Killed after 16 Weeks

Diet		Ave. Wt.	Net Wt.	Wt. Ash	Wt.Ca. in Ash	% Ca. in Ash	% Ca. on live Wt.	% Ca. on net Wt.
				Lot II				
Mixed	Males	347	327	11.5022	3.3039	28.72	0.95	1.01
	Females	247	229	7.9273	2.2745	28.69	0.92	0.99
Meat	Males	253	239	5.1931	1.1089	21.35	0.44	0.46
	Females	211	198	4.9351	1.1785	23.87	0.56	0.60
Veg.	Males	234	217	6.7413	1.7658	26.45	0.77	0.82
	Females	182	168	5.6826	1.5562	27.31	0.85	0.92
				Lot III				
Mixed	Males	371	351	11.5788	3.4504	29.79	0.94	0.99
	Females	245	223	7.5203	2.2254	29.58	0.91	0.99
Meat	Males	291	274	8.4610	2.4026	28.39	0.83	0.88
	Females	276	261	7.7626	2.1874	28.02	0.78	0.83
Veg.	Males	261	244	8.2244	2.3511	28.69	0.92	0.99
	Females	192	180	6.6703	1.9791	29.68	1.04	1.11
Con-trol	Males	292	271	9.6101	2.7603	28.69	0.95	1.02
	Females	223	205	8.1591	2.4024	29.42	1.07	1.16

These show very much the same results as those found in the other groups, both percentage and amount being higher in the animals on the mixed diet than on either the meat or vege-

table. A slight increase in both percentage and amount of calcium over that found in Lot I will be noted in the case of the animals of Lot II on the meat diet. This increase is even more marked in Lot III. This would indicate that the adult animals were harmed less by the deficient diet than the younger ones. A similar increase in amount is noted in the vegetable group.

Table XI gives the data for Lot IV. In the case of those analyzed at birth, the young on the vegetable diet show a slightly higher amount and per cent of calcium. When the size of the litters and the weights are taken into consideration, however, these figures are of little significance. In the group analyzed at four weeks, the mixed group shows a percentage somewhat lower than the control group and much higher than the meat group. For the animals of this lot killed at 12 weeks, the averages are approximately the same as in Lot I. The per cent for the females on the meat diet is slightly higher than in Lot I, but the amount smaller. This again is due to the smaller body weight. Of those killed at 24 weeks the results are very like those in Lot I. The females of the vegetable group show a slight decrease in both per cent and amount. In cases where no figures are given, no animals were available.

Table XI. Calcium Determinations of Lot IV
Killed at Ages Indicated

Diets		Wt.	Net Wt.	Wt. Ash	Wt. Ca.	% Ca. in Ash	% Ca. on live Wt.	% Ca. on net Wt.
				Birth				
Mixed	10 Y	42		0.6453	0.0962	14.91	0.23	
	13 Y	65		1.0399	0.1539	14.80	0.24	
	11 Y	58		0.9384	0.1417	15.10	0.25	
	Ave.	4.9		0.0766	0.0114	14.94	0.24	
Veg.	3 Y	8		0.1770	0.0417	23.55	0.52	
	4 Y	14		0.2344	0.0385	16.41	0.28	
	Ave.	3.1		0.0588	0.0117	19.98	0.38	
				4 Weeks				
Mixed	Males	47	40	1.1087	0.2658	24.12	0.57	0.66
	Females	44	37	1.0443	0.2529	24.03	0.57	0.68
Meat	Males	46	41	0.8702	0.1703	19.54	0.37	0.42
Con-trol	Males	42	36	1.1222	0.2954	26.33	0.70	0.82
				12 Weeks				
Mixed	Males	233	214	6.6356	1.7243	25.98	0.73	0.81
	Females	168	153	5.1183	1.4004	27.38	0.83	0.92
Meat	Females	52	46	1.1927	0.2673	22.40	0.51	0.58
				24 Weeks				
Mixed	Males	340	321	10.2762	2.9294	28.51	0.86	0.91
	Females	227	196	6.7338	2.1534	32.33	0.95	1.11
Veg.	Females	84	73	2.2225	0.5833	26.24	0.69	0.79

Figure 6 shows the relation between age in days and the per cent of calcium from birth to maturity. Both males and females of the mixed group show a higher percentage than those on either of the other two diets. The meat rats show a much lower per cent than the other groups. Although the per cent of calcium in the vegetable group is higher than that of the meat group, Figure 7 shows that they are very similar in amount. As has been mentioned, the seemingly high percentage in the vegetable group is due to the small body weight.

It is of interest to note the relation of the weight of calcium in the body to the body weight. Figure 8 shows that with the exception of the vegetable group averaging 84 grams, the mixed group show a consistently higher calcium content per gram of weight than either of the others. This is even more striking when considered from the standpoint of age in relation to body weight. It seems safe to assume that the animals on the mixed diet had considerable advantage over those on other diets in that they were able to store good amounts of calcium, comparing favorably with those reported by Sherman and Mac Leod (26) for normal rats.

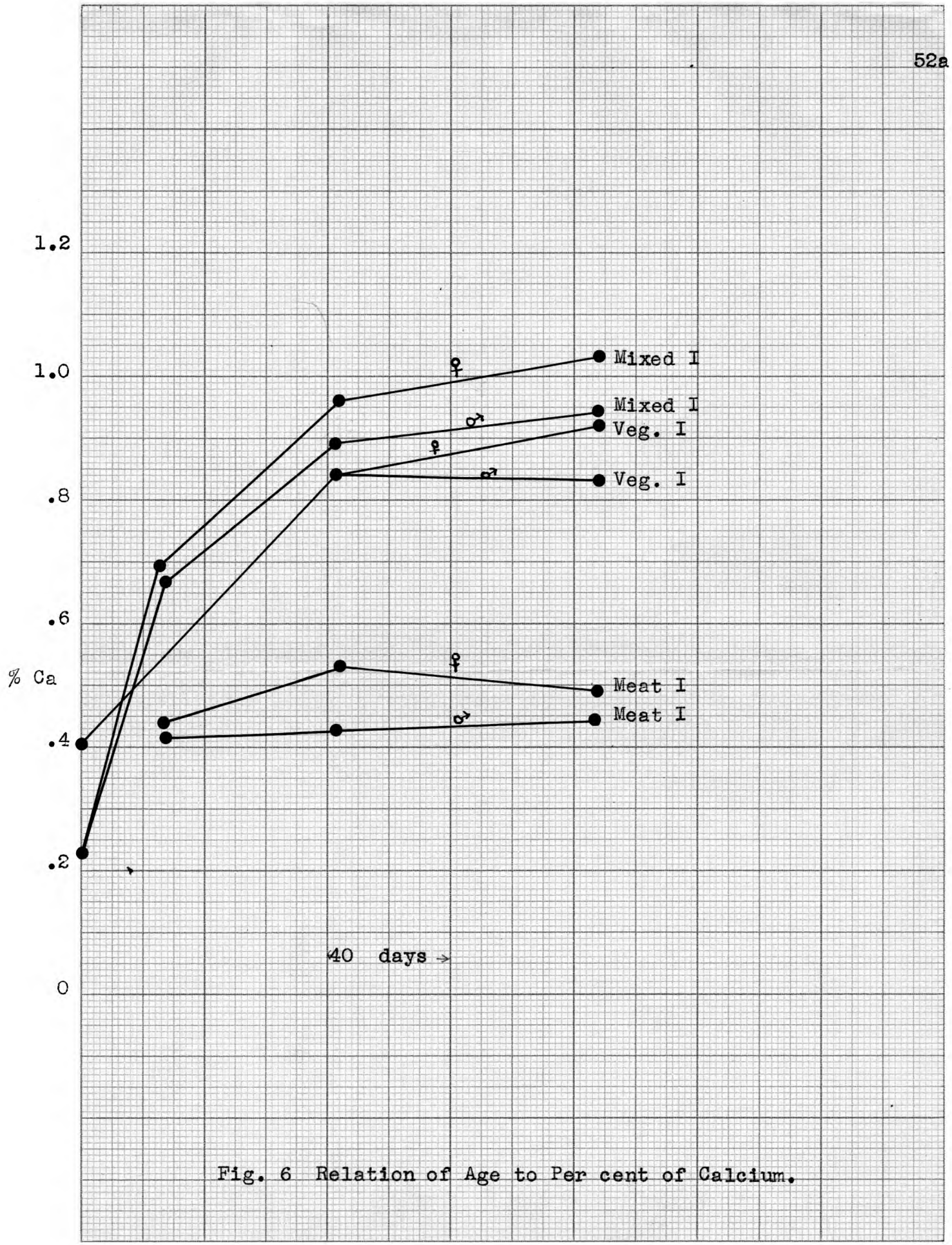

Fig. 6 Relation of Age to Per cent of Calcium.

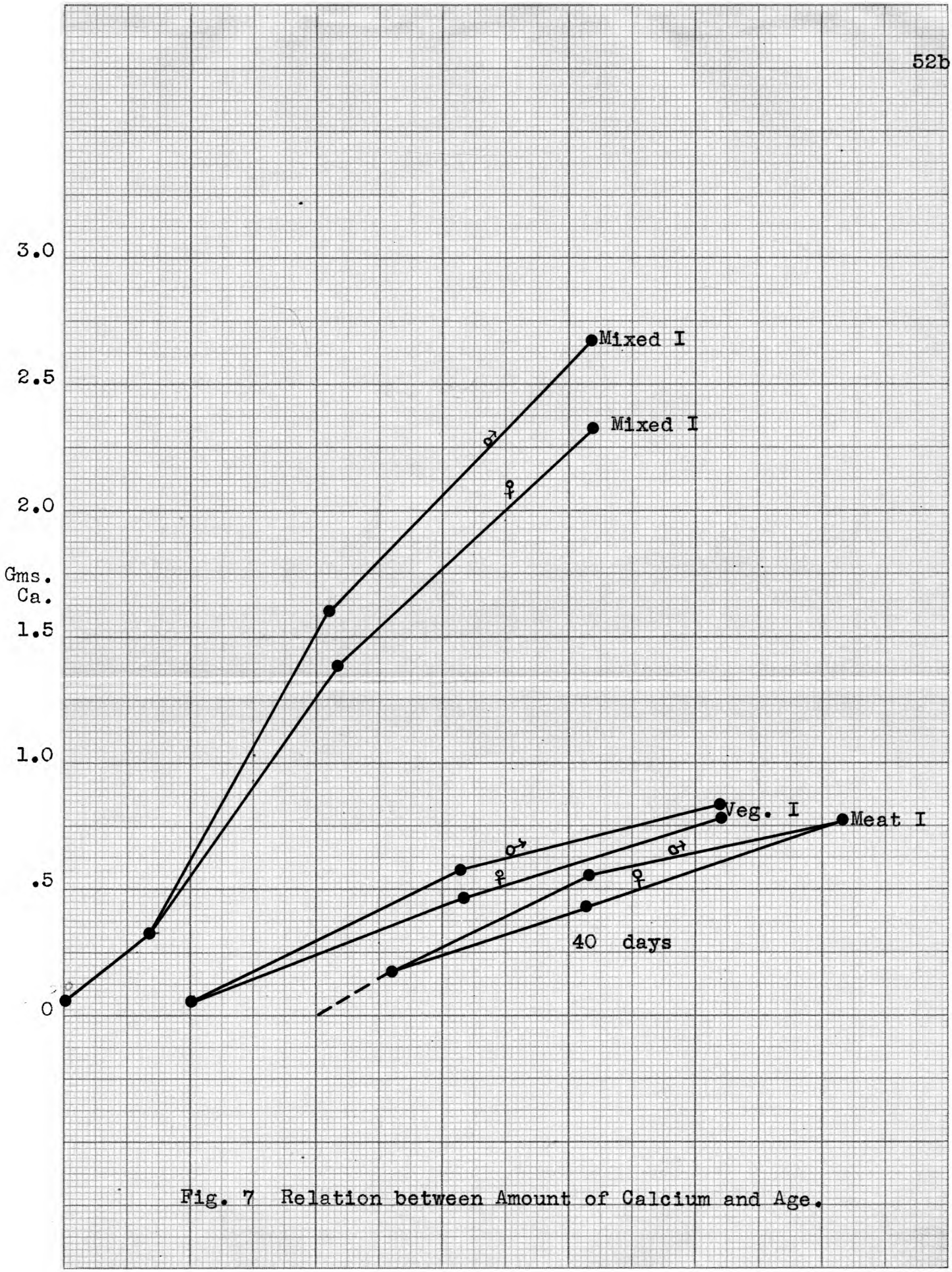

Fig. 7 Relation between Amount of Calcium and Age.

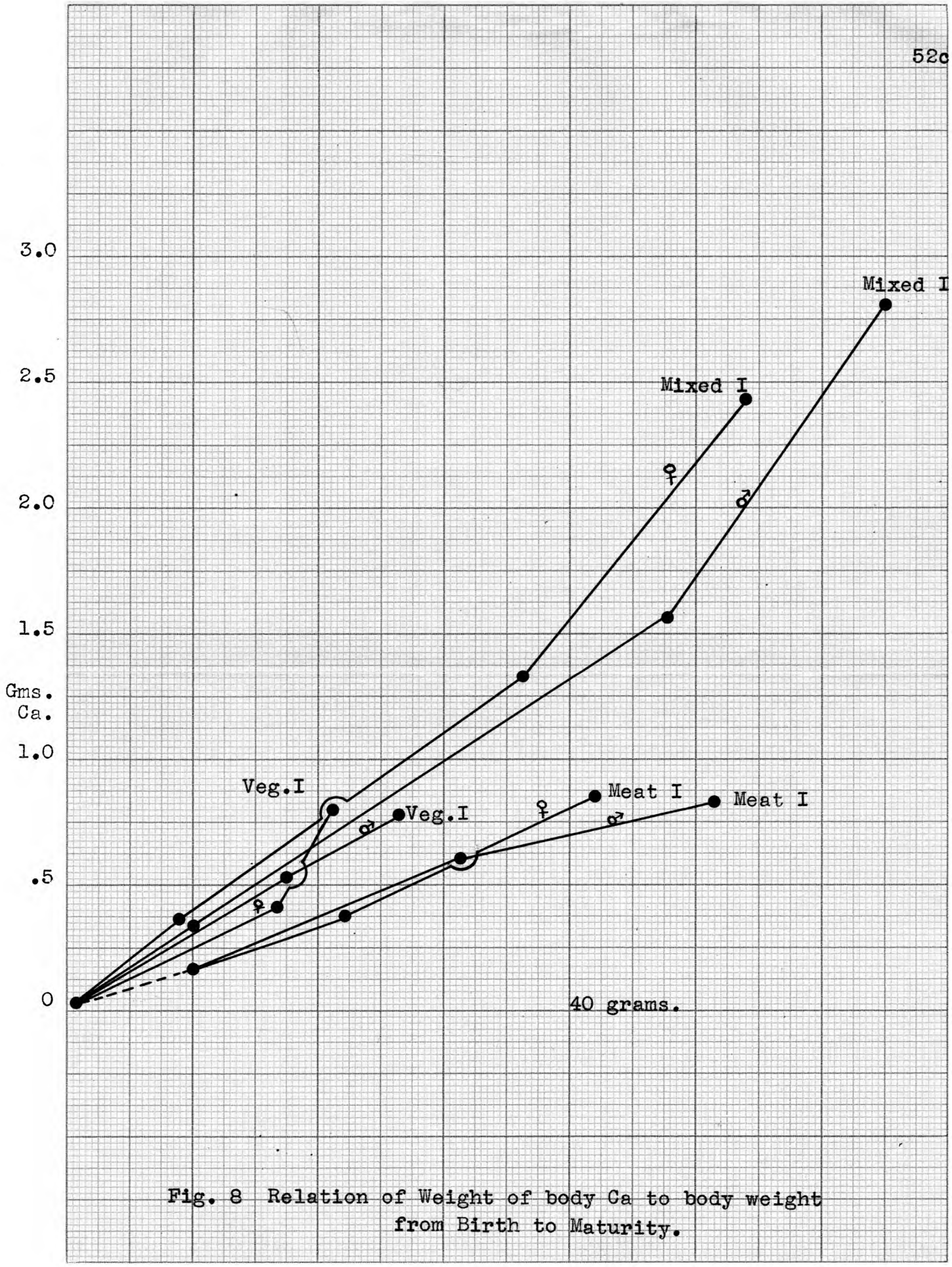

Fig. 8 Relation of Weight of body Ca to body weight from Birth to Maturity.

SUMMARY AND CONCLUSIONS

An investigation to compare three diets, which had been used by human subjects was conducted by means of long time feeding experiments with animals. The diets selected were the mixed and vegetable diets used by Lane and Bosshardt with children for seven weeks and the Stefansson meat diet used by two men for one year. The mixed diet contained a mixture of cereals, vegetables, fruits, meat, milk and butter. The vegetable diet was very similar except that the meat and milk were replaced by additional fruits and vegetables. The meat diet was composed entirely of meat products including some of the organs, bone marrow and fat. A modification of the vegetable diet containing addition calcium in the form of calcium carbonate was also tested for a short period. The experimental animals were albino rats. These were divided into four groups, according to age when placed on the diets:

4 weeks of age - called Lot I
8 weeks of age - called Lot II
12 weeks and older - called Lot III
Young of these lots - called Lot IV

These various age levels were selected in order to study the effect of the diets on both the adult and the growing animal.

From the data collected the following points are outstanding:

1. The rats of Lot I on the mixed diet grew more rapidly and on the average exceeded in weight the animals on the other diets. The meat rats made good average gains but gain was very irregular after the first six weeks. Those on the other two diets grew very slowly and never reached a normal adult weight.

2. In Lots II and III the animals on the mixed and meat diets made normal gains although that made by the meat rats was rather irregular.

3. In Lot IV the growth of the animals on the mixed diet greatly exceeded that of the other two groups. The meat and vegetable animals made very poor gains, most of them dying before 12 weeks of age.

4. Data from food records show that the growth of the animals on the mixed diet was more economical as well as more efficient than on the vegetable diet.

5. The animals on the mixed diet appeared normal in every respect. Many signs of abnormality, such as swollen joints, weak limbs, nervousness, and diarrhea were noted in the animals on the meat diet. Such conditions as rough, shaggy, and patchy coats of fur; rough, scaly tails; and lowered resistance were common among the animals of the vegetable group.

6. Breeding records show that the females on the mixed diet reached sexual maturity at an early age and bore and reared young at a normal rate. No young were reared on the other diets with the exception of one litter on each born soon after the mothers were placed on the diets. The young on the mixed diet were a good average weight at weaning. Those on the vegetable diet weighed only about half as much as those on the mixed diet.

7. Calcium determinations made on the whole bodies of animals on the various diets show that those of the mixed diet had stored more calcium, both as to amount and per cent than those on the other diets. The animals on the meat diet showed the smallest percentage of calcium, but an amount similar to that of the vegetable group.

8. A mixed and a vegetable diet had been used by children for a period of seven weeks with approximately equal success. A meat diet had been used for one year by middle-aged men with seemingly good results. These diets showed many marked and significant differences when tested by means of long time feeding experiments with animals.

ACKNOWLEDGMENT

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