AN ABSTRACT OF THE THESIS OF

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Title: THE EFFECT OF SELENIUM ON CHEMICAL CARCINOGENICITY IN THE RAT

Abstract approved: Redacted for privacy

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One hundred and fifty-one weanling rats of both sexes were fed control diets or diets containing 160 ppm diethylnitrosamine (DEN) or 150 ppm N-2-fluorenyl-acetamide (FAA). The carcinogen level was calculated so that the LD_{50} should be achieved at about 17 weeks. Each carcinogen treatment was incorporated with three different diet levels of supplemental selenium (0, 0.2 and 2 ppm) in order to evaluate the protective potential of selenium against carcinogenicity. The two control diets contained 0 ppm and 2 ppm selenium and served not only as a control but also to evaluate the function of selenium as an essential trace element involved in growth. Weekly weights and feed consumption were recorded and the data compared among the eight treatment groups.

The rats were necropsied upon death and liver, lung and tumors were excised and subjected to histological evaluation for neoplasia.

In an attempt at a status evaluation control several rats were killed from each control group and from each group containing the various selenium levels for that particular carcinogen. This exercise was performed when half of the rats died from a carcinogen group not

containing selenium in the diet. This procedure was repeated when all the animals of a group died (0 Se/DEN only).

The DEN diets were more palatable than the FAA diets. The level of selenium in the respective diets had no real influence upon consumption, except for the control diets. The consequence of this was that the rats on DEN were exposed to more carcinogen than those on FAA.

All DEN animals and all FAA males were essentially free of peripheral carcinomas. Mammary carcinomas were common in FAA females with a metastatic state the rule.

Selenium appeared to provide protection against tumors when included in the DEN groups. Incidence was reduced at least 20 per cent with selenium; the reduction was most apparent in the females. The protective action of selenium did not present itself in those groups exposed to FAA.

The Effect of Selenium on Chemical Carcinogenicity in the Rat

by

Weldon Kearney Johnston

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Typed by La Rea Dennis for Weldon Kearney Johnston

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INTRODUCTION

Since selenium has been implicated as a carcinogenic agent by some researchers (12, 16, 17, 29); and, as providing a protective effect or as having no deleterious effect by other scientists (6, 7, 17, 18, 19, 20, 21, 31), it was considered important to continue the investigation of the evaluation of selenium's role in diagnosis and treatment of cancer.

Within the past decade the pros and cons of implicating selenium as a prime influence in carcinogenicity have been presented to the scientific community (3, 6, 8, 11, 16, 27). Cumulative research summaries are contained in Selenium in Nutrition (11) and Selenium in Cancer (8). An exhaustive review of pertinent literature on selenium is included in Underwood, chapter 12 (28). The lack of complete agreement among the researchers appeared to depend upon several factors: the form (organic or inorganic) of the selenium presented to the test animal, the age of the animal, the sex of the animal, response difference among strains and among species and in some cases the relative level of protein in the diet.

Volgarev and Techerkes (29), utilizing sodium selenate in a casein diet, demonstrated a tumor incidence of 8.5 per cent. However, these researchers were unable to reproduce this tumor incidence two years later with rats of a different strain. Such studies suggested some genetic predisposition to neoplastic development and/or genetic

variabilities in sensitivity to carcinogenic agents.

Schroeder and Mitchener (17) reported no significant influence of selenium, nor the form of selenium given, on the incidence of neoplasts and malignant tumors in mice. However, these investigators did find that the form of selenium administered was significant in tumor incidence in rats (16).

Tinsley et al. (27) and Harr et al. (6) fed 34 different casein based and commercial diets with various levels of selenate and selenite. They also included two diets with the carcinogen, N-2-fluorenyl-acetamide (FAA). Six per cent of the rats developed neoplasts of some form. Of this six per cent, sixty-eight per cent of the neoplastic rats were from the two diets containing the carcinogen. The balance of the neoplasts were randomly distributed throughout the different diets and could not be attributed solely to the inclusion of selenium in the diet.

Shamberger and Willis (21) found a significant difference in total cancer death rates of human males and females living in low selenium areas as compared to those living in medium and high selenium areas. Their area selenium values were determined by the selenium content in forage crops of the particular area. Blood selenium levels of humans living in the three areas reflected the relative selenium level as predicted by the area forage analysis. Normal patients showed 34 per cent higher levels of blood selenium than did cancer patients. Another study by Shamberger et al. (23) confirmed the earlier work and it could be concluded that one of two situations

developed: (a) the cancer removed and concentrated the available blood selenium from the system; or, (b) the lower blood levels of selenium reflected an inadequate supply of available selenium to combat the development of carcinomas. Shamberger et al. (22) reported a lower incidence of carcinomas throughout the human male gastrointestinal tract in subjects from 17 high selenium areas as compared to males from 17 paired low selenium areas. They also advanced the suggestion that the biochemical protective action of selenium might be by decreasing the attachment of the carcinogen to desoxyribonucleic acid (DNA).

Wedderburn (30) reported that oral administration of selenium to sheep reduced the incidence of intestinal cancer from an impressive level to near zero.

Harr et al. (7) found that the addition of selenium (as sodium selenite) to vitamin E supplemented, low selenium, torula yeast rations decreased the effect of cancer induction by FAA in O.S.U. brown rats. FAA was fed at a level of 150 ppm added to the torula-vitamin E diet. These researchers employed graded levels of selenium of from 0.02 to 2.50 ppm.

Whanger et al. (33) demonstrated that there was some carry over of selenium by the ewe from one pregnancy to the next. McCoy and Weswig (10) verified that this effect applied as well to rats. It was therefore required that second generation rats from selenium depleted dams be utilized for studies evaluating the influence of selenium.

Weisburger and Weisburger (32) reported FAA as evoking neoplasia in many different organs depending in part on species and strain.

However, neoplasia is never produced at the point of application, suggesting that the compound itself is not the active agent but is metabolized to one. In male rats the liver is the prime target; in females, the mammary gland. Organs other than these two are affected more slowly. Manipulation of the hormone balance by castration and introduction of estrogenic compounds, by hypophysectomy, or by adrenalectomy has demonstrated direct or indirect hormonal influence in the development of cancer in several organs of rats such as the liver and mammary gland. The observation of Rajewsky et al. (14) applies to the administration of FAA. That is, the cumulative dosage is more important than the size of independent doses.

Diethylnitrosamine (DEN) continously administered to rats at sub-toxic levels induces multicentral hepatocellular carcinomata in the majority of the treated animals. According to Weisburger and Weisburger (32) the nitrosamines are more potent carcinogens than most aromatic amines or azo dyes; and, that despite the fact that organisms can excrete the major part of a dose very rapidly, several of these compounds have caused cancer in rats after a single exposure. DEN generally affects the liver first. Kidney is also susceptible with bladder and esophagus secondary. In addition to being cancer causing in mice, rats and hamsters this class of carcinogens also produce hepatomas in less sensitive larger species such as rabbits, dogs, guinea pigs, and even monkeys. There appears to be no record of hepatomas being evoked in the guinea pig or monkey with azo dyes or aromatic amines. Rajewsky et al. (14) reported that results from continuous administration of a variety of carcinogens, including DEN,

prompted the conclusion that within a low toxicity dose range the effect is independent of the size of individual doses and is essentially a function of the cumulative dose.

Oxidative damage to red blood cells has been demonstrated in rats maintained on a selenium deficient diet. This damage related to reduced activity of an enzyme, glutathione peroxidase. This enzyme inhibits injury by hydrogen peroxide to hemoglobin. It is believed that glutathione peroxidase may contain some form of selenium acting as an integral part of the functional enzyme molecule (15).

Magee and Barnes (9) described numerous enzyme studies involving DEN. Some enzyme levels were lowered while others were elevated. Poirier et al. (13) indicated that dietary protein also affected the level of activity of some of these enzymes; therefore, the extent to which the DEN affected the animal's food intake would present a factor for consideration.

Smith (24) found that the toxicity of naturally occurring food selenium is largely determined by dietary factors. A selenium level which is highly toxic when fed with a low protein, high carbohydrate diet was only slightly toxic when fed with a diet high in protein and low in carbohydrate.

Franke and Painter (2) reported that female rats are more responsive to selenium levels than are males and that concentrations of less than 5 ppm selenium in diets will prevent normal growth. They also indicated that there was no relationship between the absolute amount of selenium consumed per day and the observed effects. The effect depends more on the the concentration of selenium in the

diet than on the quantity of selenium consumed per day.

Clayton and Baumann (1) indicated that rats fed azo dyes develop tumors at rates that depend upon the diet fed and that tumor incidence decreased as a result of adding 5 ppm selenium to the diet.

Thompson and Scott (25) in a study with selenium depleted chicks concluded that selenium is not a substitute for vitamin E. They found, however, that the lower the vitamin E, the higher the selenium requirement. Thompson and Scott (26) in another study with selenium depleted chicks concluded that selenium is an essential trace nutrient. They also found that selenium deficiency reduced the absorption of vitamin E, resulted in poor growth, poor feathers, and atrophy of the pancreas.

This thesis project was designed to evaluate the protective action of graded sub-toxic levels of ingested selenium (as sodium selenite) on the incidence of carcinogenicity induced in the rat as a consequence of feeding fixed levels of either FAA or DEN for a calculated time to provide a cumulative 50 per cent lethal dose (LD_{50}).

MATERIALS AND METHODS

One hundred fifty-one brown rats (O.S.U. strain) of both sexes were weaned at 30 days of age, divided into eight groups of 17-20 rats each, and subjected to diets as shown in Table I. Every attempt was made to equally disperse each litter, by sex, among as many of the diets as possible. These rats were from the first generation of parents maintained on the low selenium (0.02 ppm) diet of McCoy and Weswig (Table II). For the first 30 days post-partum, rats were fed, ad libitum, the low selenium diet of McCoy and Weswig in addition to the mother's milk. Rats receiving selenium in their diet were maintained in a room separate from the rats on selenium depleted diets. All rats received distilled water ad libitum. Each rat was individually housed in a suspended cage. Rats were weighed and feed consumption recorded on a weekly basis. All rats were observed daily.

This study was designed to evaluate the protective action of graded sub-toxic levels of ingested selenium (Se) on the incidence of carcinogenicity induced in the rat as a consequence of feeding fixed levels of either of two well established chemical carcinogenic agents: N-2-fluorenyl-acetimide (FAA) at 150 ppm or diethylnitrosamine (DEN) at 160 ppm. Selenium (as sodium selenite, Na_2SeO_3) was added to the diet as an aqueous solution. FAA was added to the diet from a stock mixture of the carcinogen in torula yeast. DEN was added to the diet as an aqueous solution in the same manner as selenium. The level of each carcinogen employed was such that the LD_{50} should be demonstrated at 14-17 weeks on diet. The carcinogens were removed from the diets

after 17 weeks exposure and the rats then fed for an additional 14 weeks on the identical diet less carcinogen.

Rats were necropsied upon death and liver, lung and tumors were excised and preserved in 10 per cent formalin. Tissues were imbedded in paraplast, sectioned at 6 μ, stained with hematoxalin and eosin, and examined microscopically for neoplasia. These slides were then submitted to pathological evaluation. Histological evaluation of the tissue related as follows:

Hepatoma: a malignant tumor of hepatic cells.

Carcinoma: a malignant growth made up of epithelial cells tending to give rise to metastasis.

Metastatic: the transfer of disease from one organ (or part) to another not directly connected with it.

Malignant: a virulent, or fatal, growth.

When one-half of the rats on a carcinogen diet, without selenium, died, three rats each were killed from both control groups and from each selenium fortified diet containing that particular carcinogen. Also, in furthering the status evaluation, when the terminal rat on a carcinogen diet, without selenium died, representative animals from each control group and each selenium fortified group containing that particular carcinogen were killed.

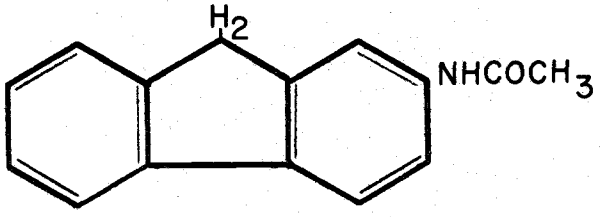
Data reduction and statistical analysis were done utilizing OS-3 systems programs. Regression analysis of growth data was performed with the aid of *SIMLIN (34) or the REGRESS subsystem of *SIPS (5). *EZPLOT (4) was employed to plot data on an IBM model 1627-II plotter.

TABLE I. IDENTIFICATION OF GROUPS BY DIET.

Group 1	(0 Se/Control)	Basal *
Group 2	(2 Se/Control)	Basal + 2 ppm Se
Group 3	(2 Se/FAA)	Basal + 2 ppm Se + 150 ppm FAA
Group 4	(2 Se/DEN)	Basal + 2 ppm Se + 160 ppm DEN
Group 5	(0.2 Se/FAA)	Basal + 0.2 ppm Se + 150 ppm FAA
Group 6	(0.2 Se/DEN)	Basal + 0.2 ppm Se + 160 ppm DEN
Group 7	(0 Se/FAA)	Basal + 150 ppm FAA
Group 8	(0 Se/DEN)	Basal + 160 ppm DEN

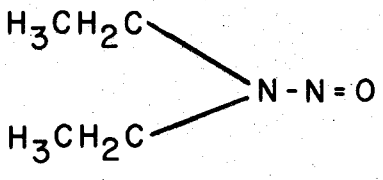
* Basal contains 0.02 ppm inherent selenium.

FAA



2-N-FLUORENYLACETAMIDE

DEN



DIETHYLNITROSAMINE

TABLE II. COMPOSITION OF McCOY-WESWIG LOW SELENIUM BASAL RATIONS (10).

Torula yeast[1]	40.0 per cent
Sucrose	41.5 per cent
Vegetable oil[2]	5.0 per cent
HMW salt mixture[3]	5.0 per cent
Vitamin mixture[4]	1.0 per cent
Cellulose[5]	7.5 per cent
(Selenium content of basal diet:	0.020 ppm)

[1] Lake State Yeast, Rhinelander, Wisc.

[2] Wesson Oil, refined cottonseed oil, Wesson Sales Company Fullerton, Calif.

[3] Hubbell, R. B., L. B. Mendel and A. J. Wakeman. 1937. A new salt mixture for use in experimental diets. J. Nutr., 14:273.

[4] Vitamin mixture contained: (in milligrams) thiamine·HCL, 40; Ca D-pantothenate, 200; menadione, 10; folic acid, 20; riboflavin, 25; pyridoxine·HCL, 20; biotin, 10; and (in grams) vitamin B_{12}, (1% trituration), 1; niacin, 1; choline chloride, 10; and lactose to make 100 g. Vitamin A acetate, 10 mg; d-a-tocopheryl acetate, 60 mg; and Vitamin D_2, 100 μg were supplied in 95% ethanol/kg of diet.

[5] BW 100 Solka Floc purified cellulose, Brown Company, New York, New York.

RESULTS AND DISCUSSION

Feed Consumption and Weight Gain

Feed consumption was recorded for each animal on a weekly basis for the first 17 weeks of the study. Feed intake, carcinogen consumption and Se consumption are given in Appendix Tables 1-8. A summary of these values is given in Table III. Average total weight gain for the initial 17 weeks is given in Table IV. Average values include all animals, even those not surviving 17 weeks.

Group 1 (0 Se/Control) females averaged a higher feed intake than the males, 1,223 g and 1,172 g respectively. The weight gain for the males of this group for the 17 weeks ranged from 131 g to 181 g, with an average for all males in the group of 158.9 g. For the females weight gain ranged from a low of 111 g for the 17 week period to a high of 161 g gained, with an average of 139.1 g.

Group 2 (2 Se/Control) males averaged 771 g of feed and the females averaged 780 g. In weight gain the males averaged 174.3 g with a low of 170 g for the 17 weeks to a high of 211 g (one animal, living just 15 weeks gained only 88 g). For the females the average weight gain was 145.1 g with a range from 121 g to 170 g. In this group the average amount of Se consumed was 1.552 mg, with the females ingesting more than the males (1.560 mg average per animal vs 1.543 mg).

For Group 3 (2 Se/FAA) the average feed intake for males was 531 g and for females it was 474 g. The average weight gain for the males was 110.0 g and ranged from 97 g to 121 g. For the females the

TABLE III. SUMMARY OF AVERAGE FEED, CARCINOGEN AND SELENIUM INTAKE FOR INITIAL 17 WEEKS.

GROUP No.	TREATMENT	Ave. Feed intake (17 weeks) g	Ave. weekly feed intake g	Ave. total carcinogen consumption mg	Ave. weekly carcinogen consumption mg	Ave. total Se consumption mg	Ave. weekly Se consumption mg
1	0 Se/Control						
	MALES	1,172	68.93	none	none	trace	trace
	FEMALES	1,223	71.96	none	none	trace	trace
2	2 Se/Control						
	MALES	771	46.08	none	none	1.543	0.0907
	FEMALES	780	45.87	none	none	1.560	0.0918
3	2 Se/FAA						
	MALES	531	31.22	79.6	4.68	1.061	0.0624
	FEMALES	474	27.89	71.1	4.18	0.948	0.0557
4	2 Se/DEN						
	MALES	744	43.79	119.1	7.01	1.489	0.0876
	FEMALES	721	43.10	115.3	6.86	1.441	0.0862
5	0.2 Se/FAA						
	MALES	550	32.65	82.5	4.90	0.110	0.0065
	FEMALES	568	34.21	85.2	5.01	0.114	0.0068
6	0.2 Se/DEN						
	MALES	777	46.79	124.3	7.49	0.155	0.0094
	FEMALES	732	44.05	117.2	7.05	0.147	0.0088
7	0 Se/FAA						
	MALES	428	27.02	64.1	4.05	trace	trace
	FEMALES	395	23.21	59.2	3.48	trace	trace
8	0 Se/DEN						
	MALES	629	37.35	100.7	5.98	trace	trace
	FEMALES	597	36.48	95.5	5.84	trace	trace

TABLE IV. AVERAGE TOTAL WEIGHT GAIN FOR THE INITIAL 17 WEEKS.

GROUP No.	TREATMENT	Ave. total weight gain g	Range of wt. gain for full 17 week animals g	Mortality <17 weeks wt. gain	wks.
1	0 Se/Control				
	MALES	158.9	131-181	--	--
	FEMALES	139.1	111-161	--	--
2	2 Se/Control				
	MALES	174.3	170-211	88	15
	FEMALES	145.1	121-170	--	--
3	2 Se/FAA				
	MALES	110.0	97-121	--	--
	FEMALES	97.2	66-126	--	--
4	2 Se/DEN				
	MALES	163.7	126-188	--	--
	FEMALES	131.3	119-159	100	15
				131	16
5	0.2 Se/FAA				
	MALES	107.6	101-139	73	16
	FEMALES	100.3	67-131	72	13
6	0.2 Se/DEN				
	MALES	161.2	161-185	105	15
	FEMALES	122.5	104-155	7	8
7	0 Se/FAA				
	MALES	89.0	88-102	68	13
				88	14
	FEMALES	82.3	42-113	--	--
8	0 Se/DEN				
	MALES	129.4	141-152	56	16
	FEMALES	101.8	85-120	67	13
				76	15
				110	15
				119	16

average gain was 97.2 g with a range of 66 g to 126 g. For this group the average carcinogen consumption per animal was 75.4 mg. The males ingested more than the females (79.6 mg vs 71.1 mg). The average Se consumption per animal was 1.005 mg. The males ingested more than the females (1.061 mg vs 0.948 mg).

In Group 4 (2 Se/DEN) the average feed intake was 744 g for the males and 721 g for the females. The weight gain for the males averaged 163.7 g and ranged from 126 g to 188 g. For the females the average was 131.3 g gained, with a low of 119 g for 17 week animals to a high of 159 g (one animal living only 15 weeks gained 100 g). This group consumed an average of 117.2 mg carcinogen per animal over the 17 week test period. The males ingested more than the females (119.1 mg vs 115.3 mg). The average total Se consumption per animal for this group was 1.465 mg; 1.489 for the males and 1.441 mg for the females.

Feed intake for the males of Group 5 (0.2 Se/FAA) averaged 550 g and for the females 586 g. Weight gain for the males averaged 107.6 g (101 g for the low of the 17 week animals to a high of 139 g; one animal that died after 16 weeks gained just 73 g). For the females the average gain was 100.3 g with a range from 67 g to 131 g. This group averaged 83.9 mg of FAA consumed; the females ingested slightly more than the males (85.2 mg vs 82.5 mg). The average total Se consumption per animal was 0.112 mg (0.114 mg for the females and 0.110 mg for the males).

Group 6 (0.2 Se/DEN) males averaged 777 g of feed and the females averaged 732 g of feed. The weight gain for the males of this group

averaged 161.2 g, with a low of 161 g for 17 week animals and a high of 185 g gained (one animal living only 15 weeks gained 105 g). The females averaged 122.5 gained, with a low of 104 g for the 17 week animals and a high of 155 gained. One female lived only 8 weeks and gained just 7 g. This group consumed an average of 120.8 mg of DEN per animal. The males ingested more than the females (124.3 mg vs 117.2 mg). The average Se consumption per animal for the group was 0.151 mg, the average for the males was 0.155 mg and for the females 0.147 mg.

In Group 7 (0 Se/FAA) the average feed intake was 428 g for males and 395 g for females. The weight gain for the males ranged from 88 g for a low for the full 17 weeks to a high of 102 g with an average of 89.0 g gained as one animal lived only 13 weeks and gained just 68 g. For the females weight gain varied from 42 g to 113 g with an 82.3 g gain average. The average amount of carcinogen ingested for the group was 61.7 mg per animal (64.1 mg for the males and 59.2 mg average for the females).

For Group 8 (0 Se/DEN) the males average feed intake was 629 g and for the females it was 597 g. The weight gain for the males of this group ranged from 141 g for a low for the 17 weeks to a high of 152 g, with an average of 129.4 g gained. One animal lived just 16 weeks and gained 56 g. For the females the range was from 85 g for and animal living 17 weeks to a high of 120 g gained; the average was 101.8 g despite four females dying prior to 17 weeks, the low weight gain was 67 g for an animal living only 13 weeks. This group consumed an average of 98.1 mg carcinogen per animal over the 17 week

test period. The males ingested slightly more than the females (100.7 mg vs 95.5 mg).

The foregoing analysis of data demonstrated several facts:

1. With the exception of Groups 1 and 2 (the control groups) and Group 5 (0.2 Se/FAA) the males averaged a higher feed consumption than the females.

2. Males within each group gained more weight than the females.

3. All rats on DEN were exposed to more carcinogenic material than those on FAA.

4. Group 2, the control group with 2 ppm Se, consumed less feed yet gained more weight than rats on the control diet without Se. Although not statistically significant, this would seem to indicate that the basal diet used did not have sufficient Se for optimal feed efficiency.

5. Within a carcinogen series, weight gain increased as the Se content of the diet increased.

6. Group 7 (0 Se/FAA) had the lowest feed consumption and also the least weight gain.

Figures 1 through 8 project the linear weight gain for the eight treatment groups for the first 17 weeks of this study. The slope of the line (plotted by computer) indicated the relative value, or inhibitory effect, of the respective diets.

Figures 1 and 2 reflect the weight gains of Groups 1 and 2 (the control groups). While the slope of Group 2 (2 Se/Control) failed to demonstrate any real difference from the slope of Group 1 (0 Se/Control), Group 2, with 2 ppm Se, utilized less feed in the

process (see Appendix Tables 1 and 2). The slope of both groups exceeds the slopes of the carcinogen groups.

Figures 3, 5, and 7 represent the weight gains for the FAA exposure groups. There was a small positive reflection in the slope as the Se level increased.

Figures 4, 6, and 8 represent the weight gains for the DEN exposure groups. Again, there was a small positive reflection in the slope as the Se level increased.

Statistical evaluation of differences among the curves showed no differences that were significant in terms of the analysis of variance. The range and standard deviation of each curve, of each group logically precluded success in terms of statistical significance.

Figures 9 through 16 represent the terminal period of the test, after all carcinogens had been removed from the diet. As a consequence of very low animal numbers, it was impossible to make a rational evaluation of the effects of Se on weight gain. This evaluation applied to all groups, the apparent increase in the slopes of the higher Se groups being not withstanding. Virtually no degrees of freedom and a high value for standard deviation made statistical evaluation impossible.

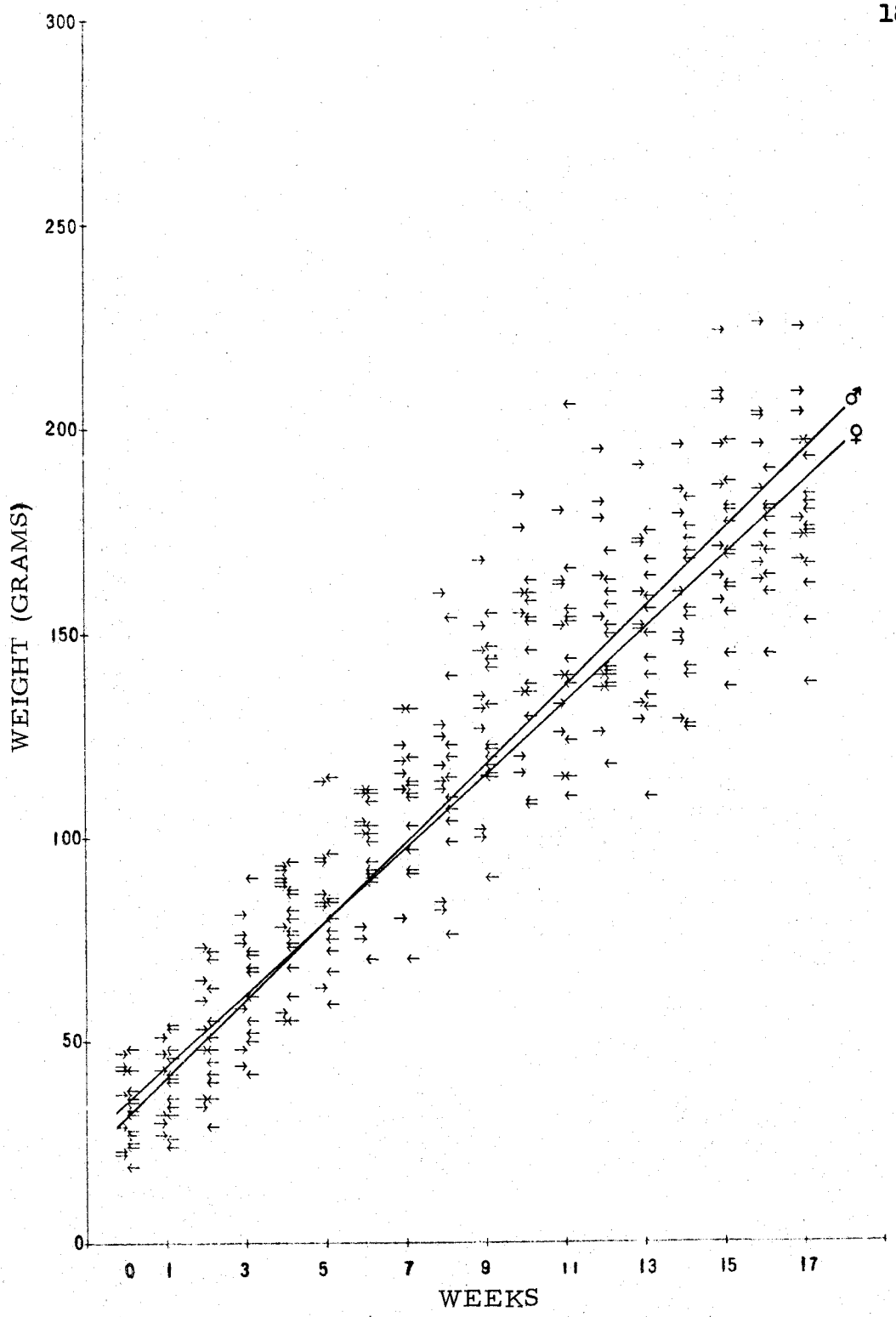


Figure 1. Weight Gain Plots for the 17 Weeks Exposure Period for Group 1 (0 Se/Control).

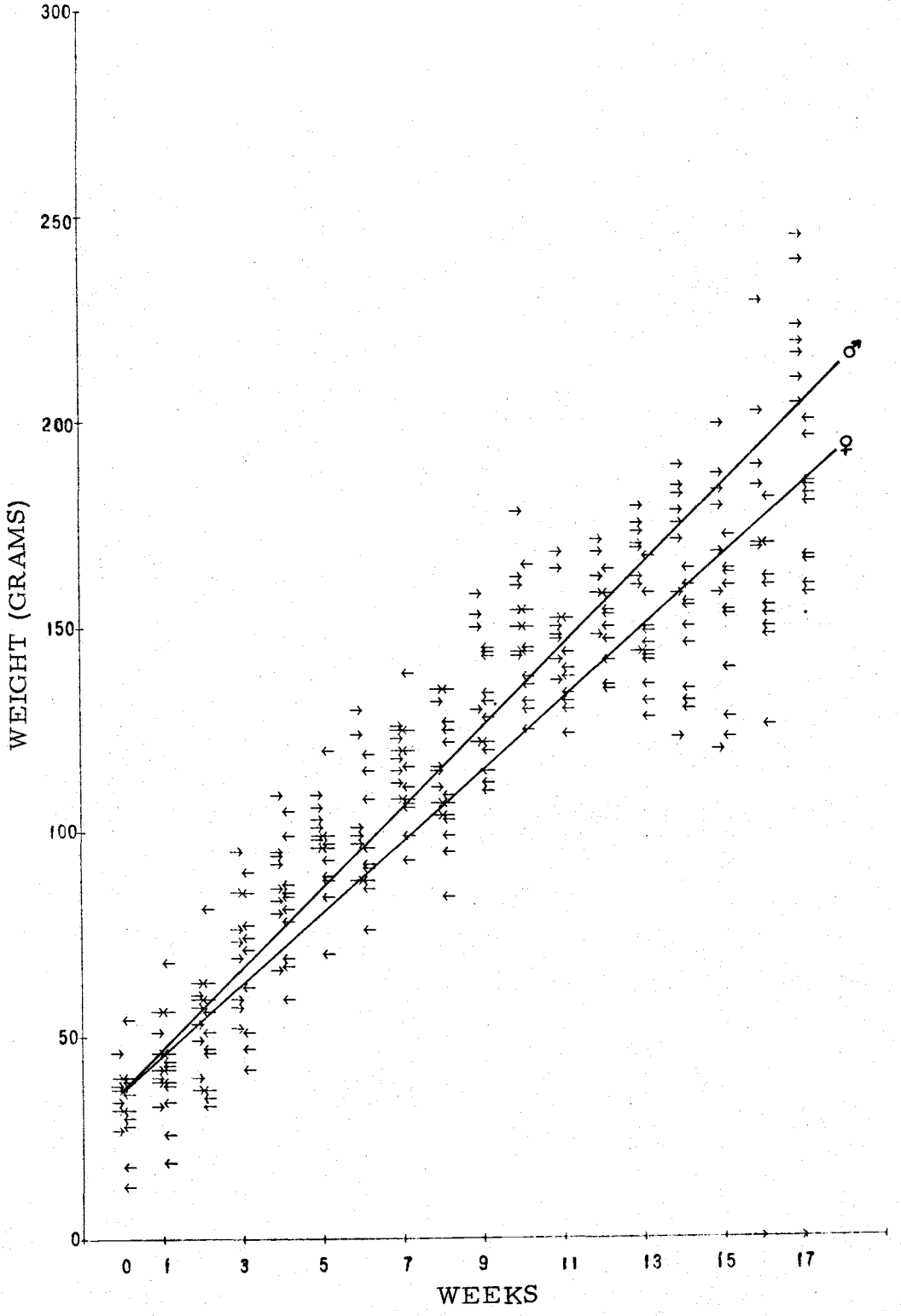


Figure 2. Weight Gain Plots for the 17 Weeks Exposure Period for Group 2 (2 Se/Control).

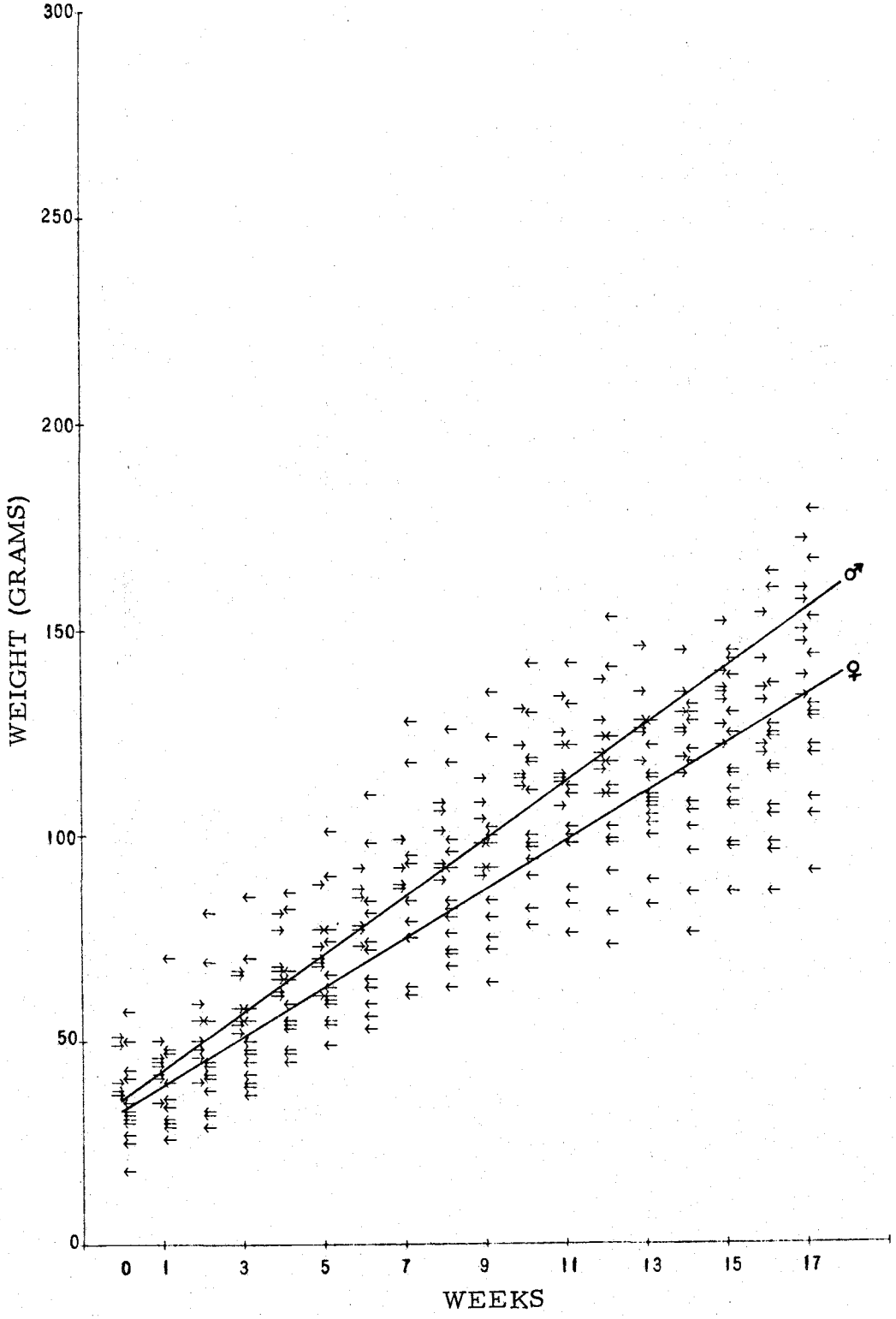


Figure 3. Weight Gain Plots for the 17 Weeks Exposure Period for Group 3 (2 Se/FAA).

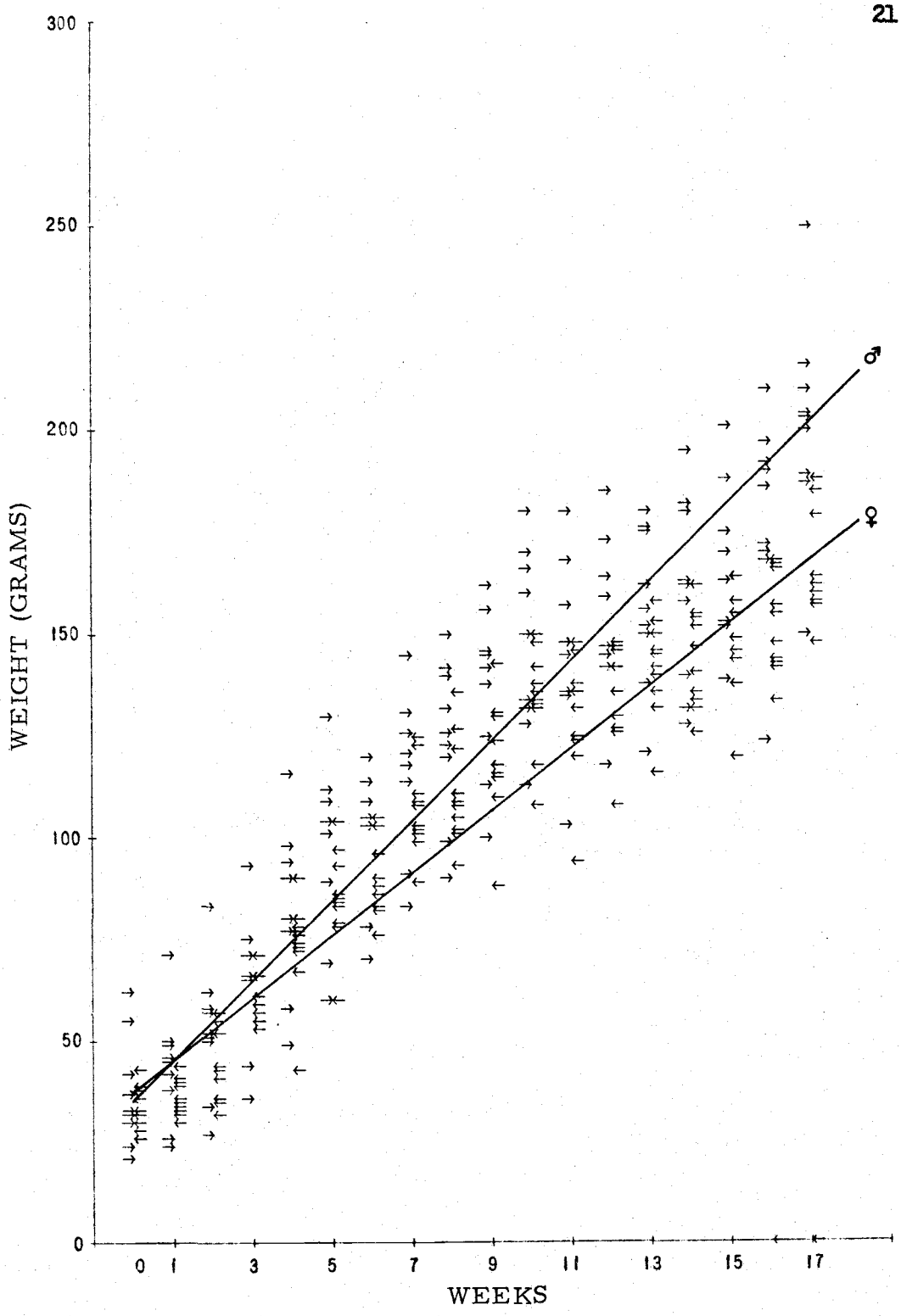


Figure 4. Weight Gain Plots for the 17 Weeks Exposure Period for Group 4 (2 Se/DEN).

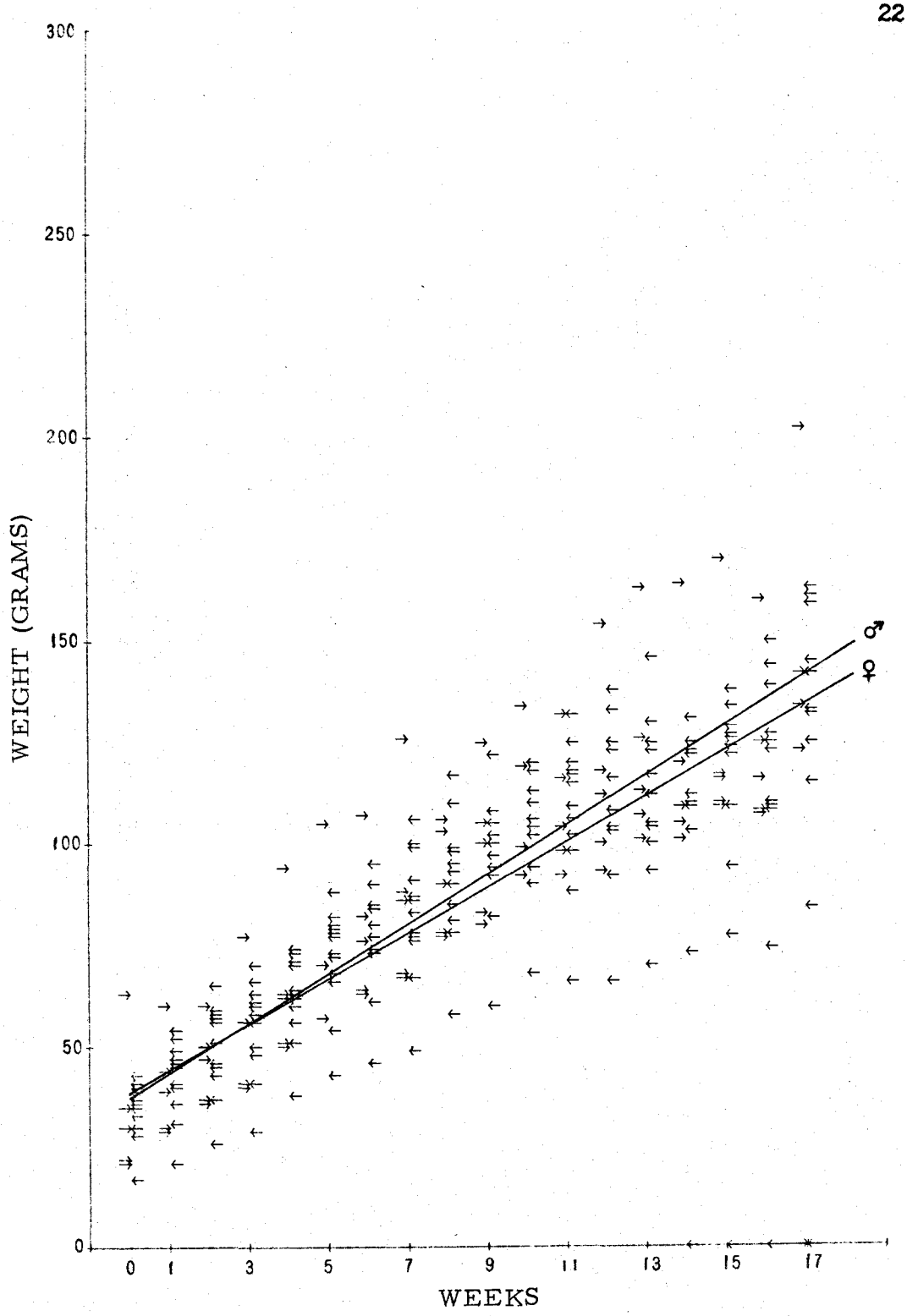


Figure 5. Weight Gain Plots for the 17 Weeks Exposure Period for Group 5 (0.2 Se/FAA).

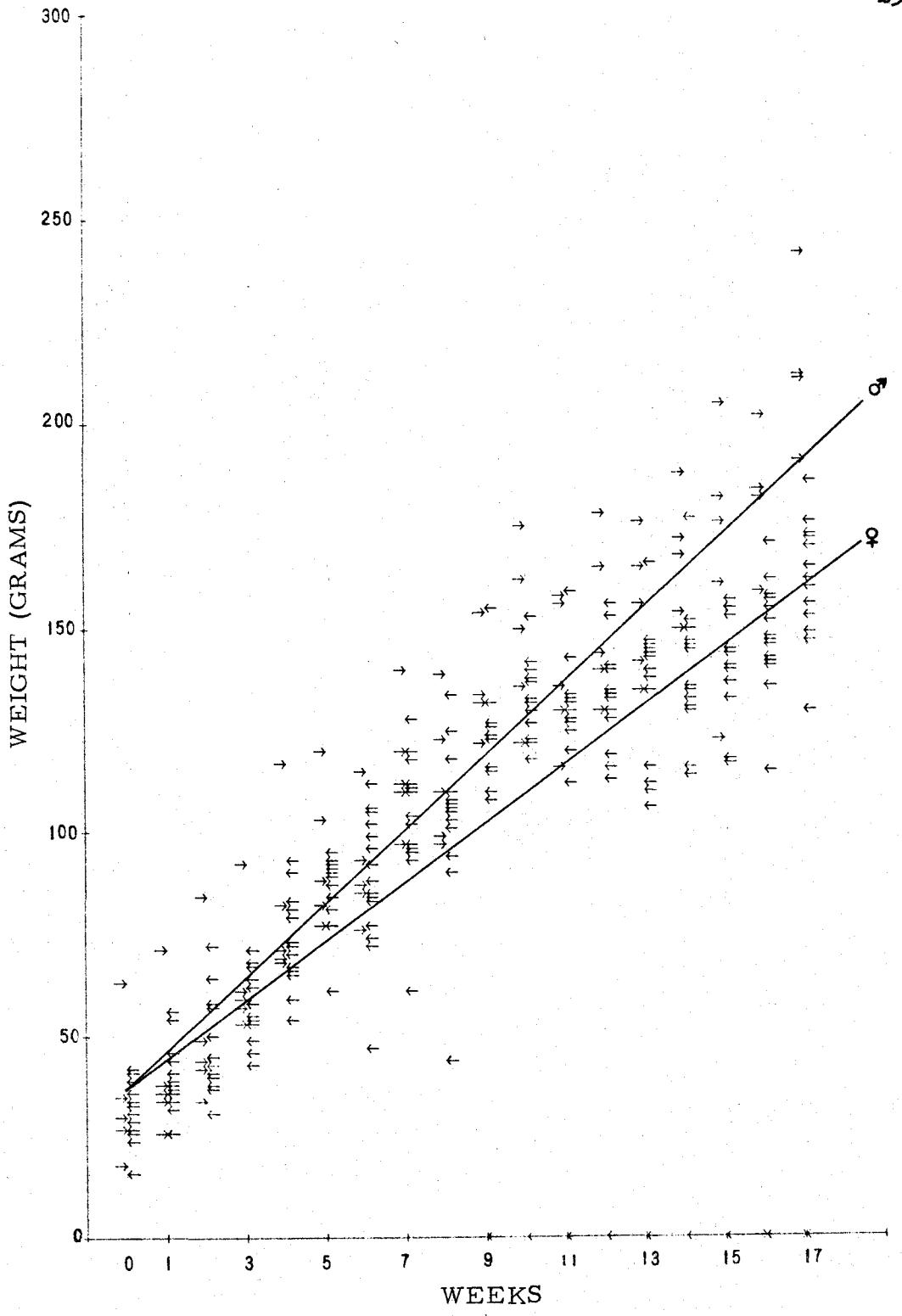


Figure 6. Weight Gain Plots for the 17 Weeks Exposure Period for Group 6 (0.2 Se/DEN).

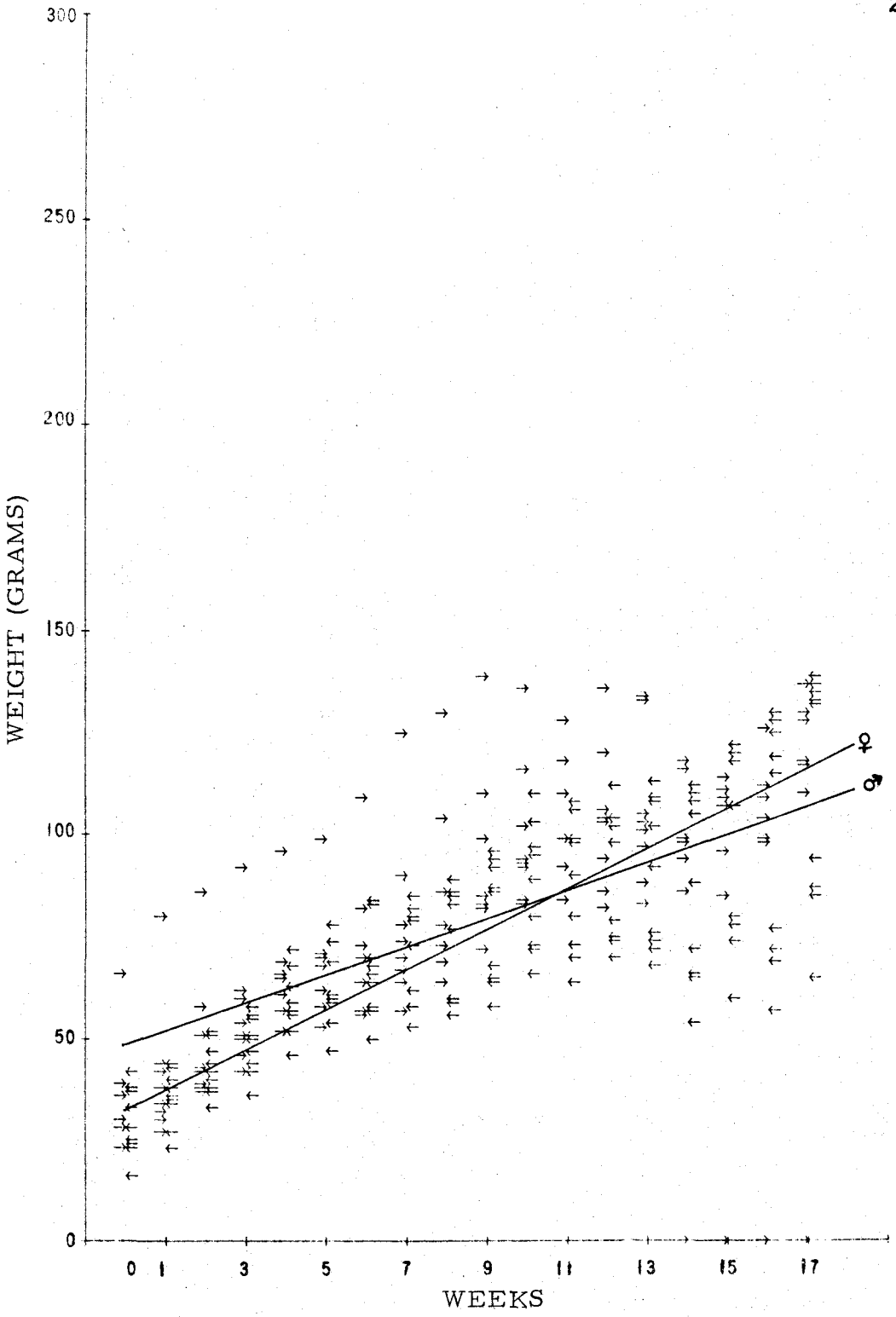


Figure 7. Weight Gain Plots for the 17 Weeks Exposure Period for Group 7 (0 Se/FAA).

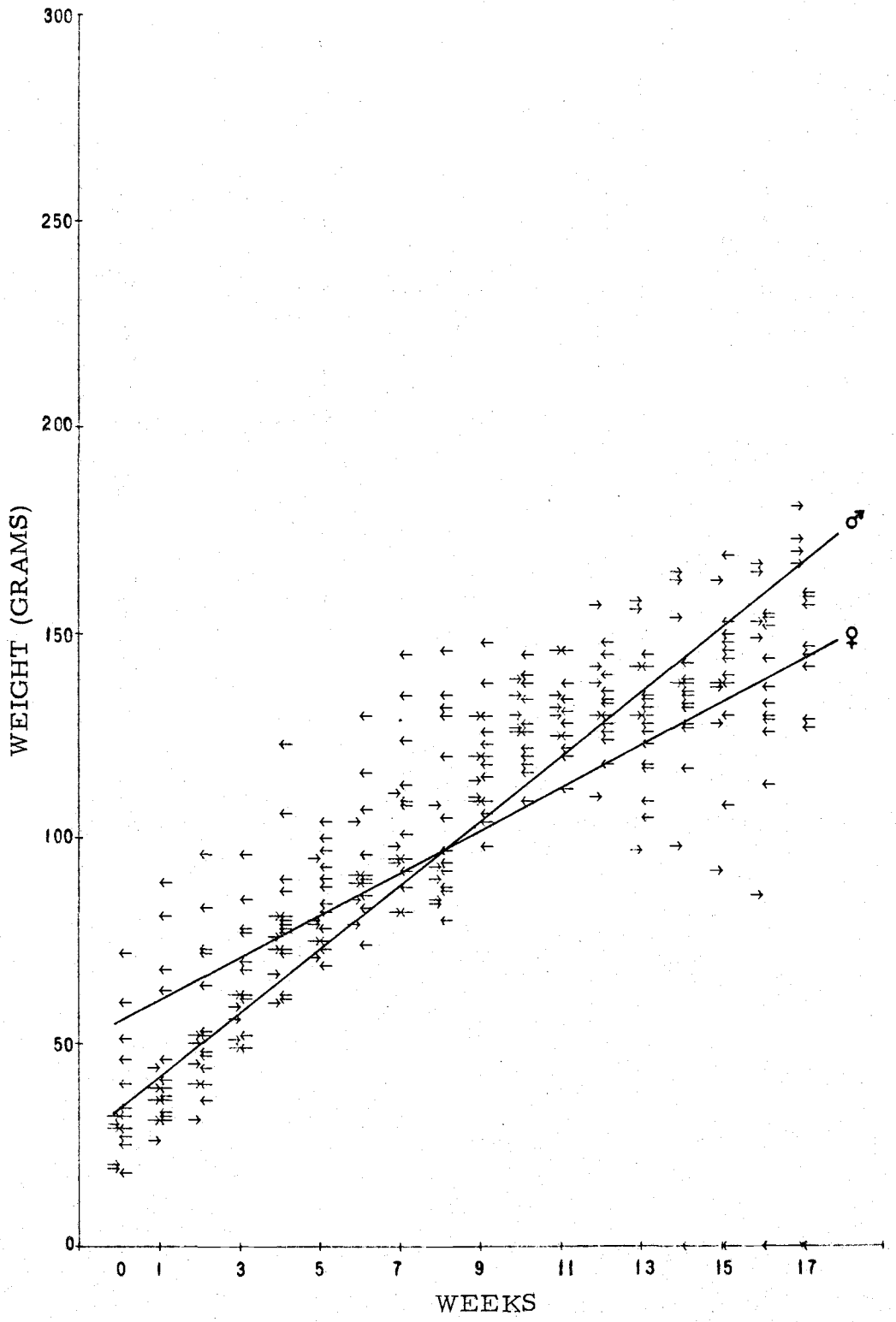


Figure 8. Weight Gain Plots for the 17 Weeks Exposure Period for Group 8 (0 Se/DEN).

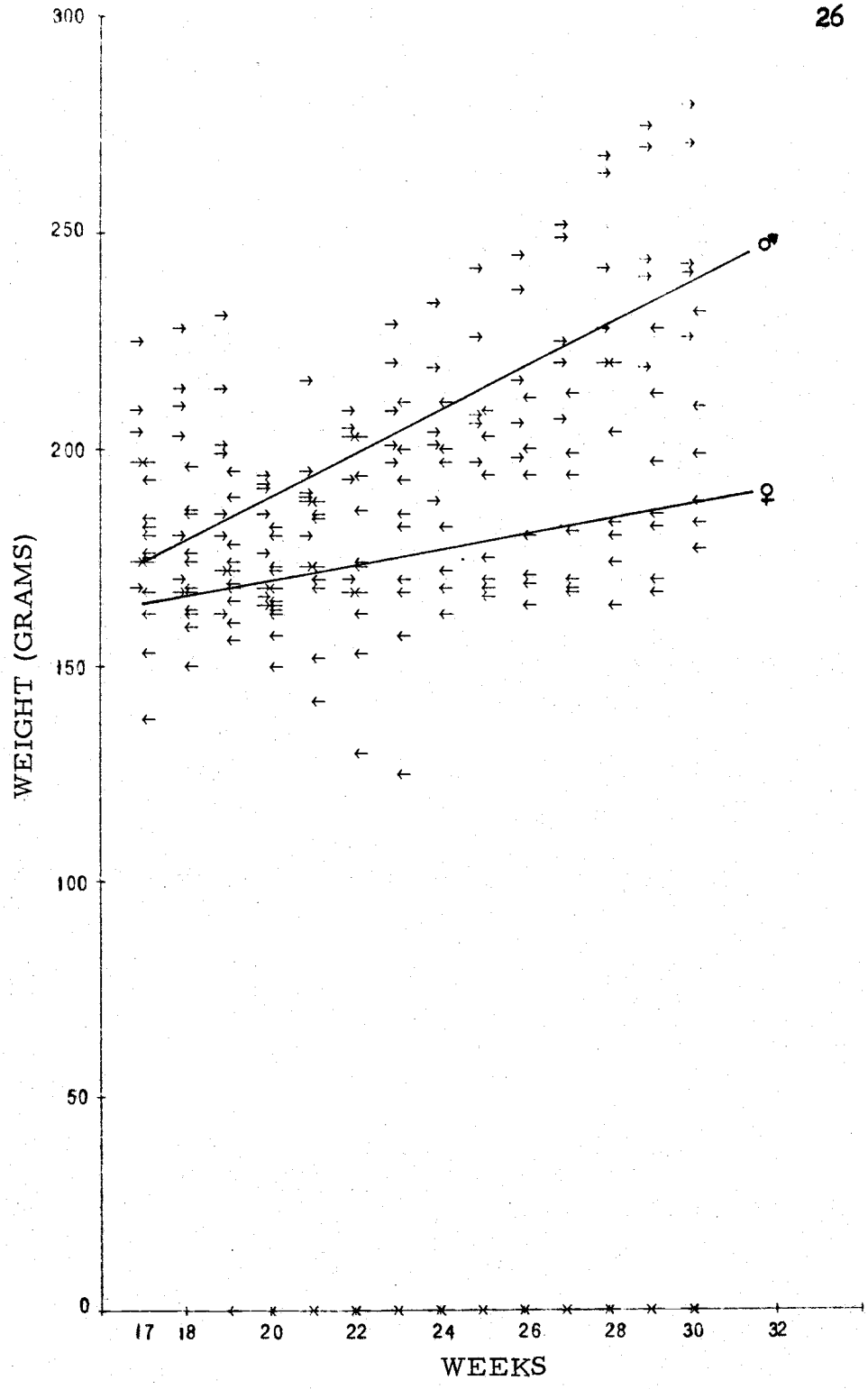


Figure 9. Weight Gain Plots for Group 1 (0 Se/Control).

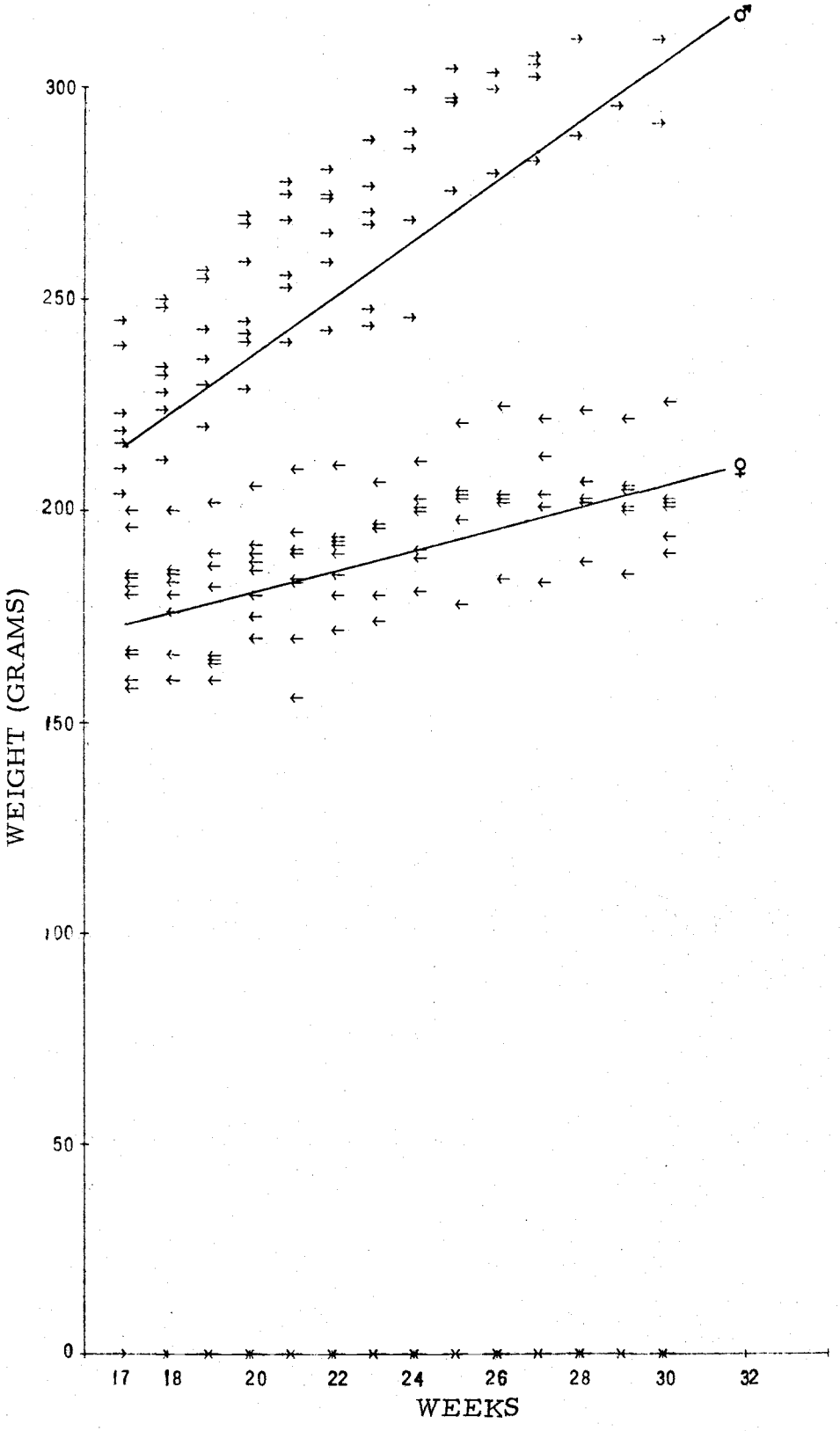


Figure 10. Weight Gain Plots for Group 2 (2 Se/Control).

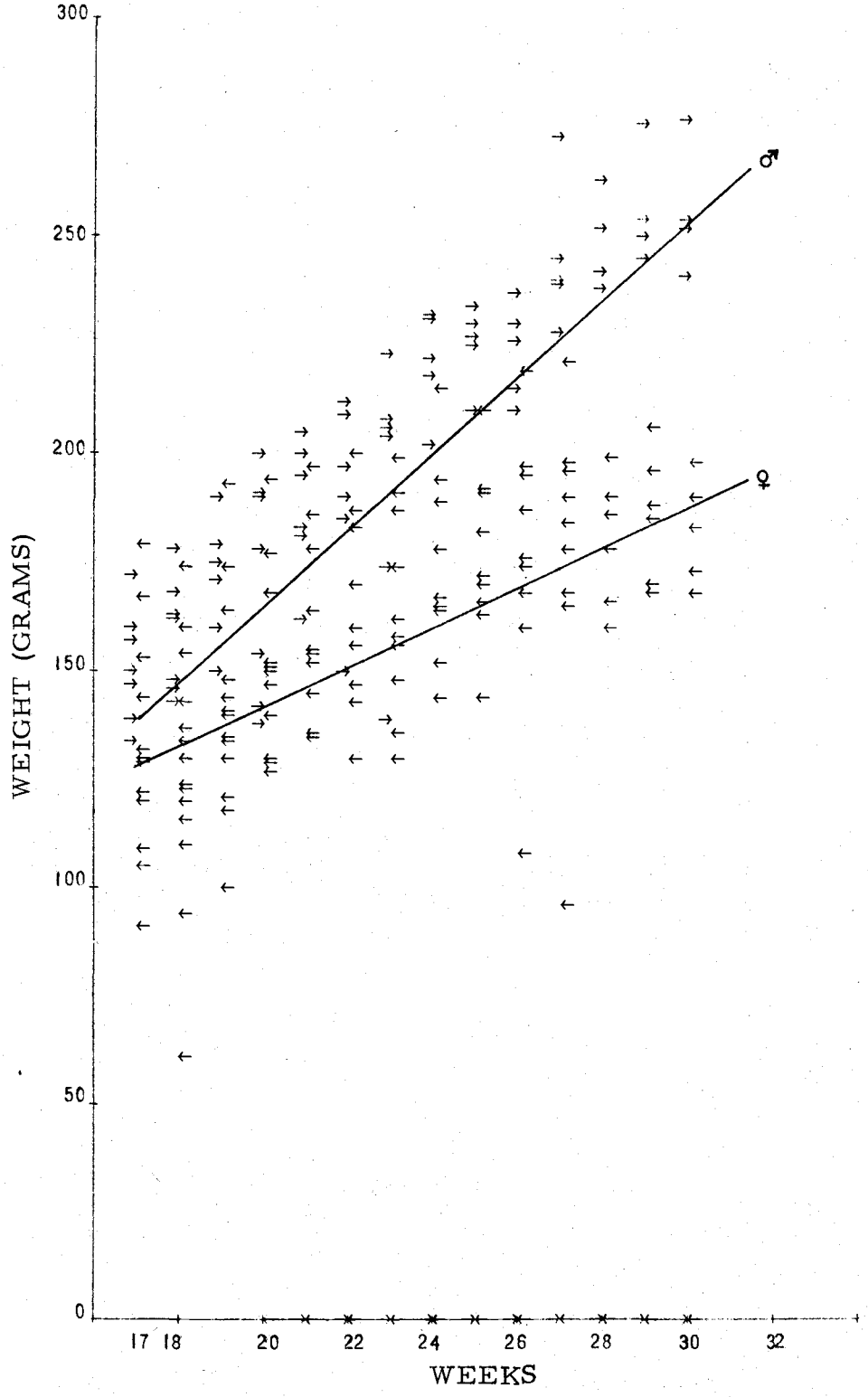


Figure 11. Weight Gain Plots After Removal of the Carcinogen for Group 3 (2 Se/FAA).

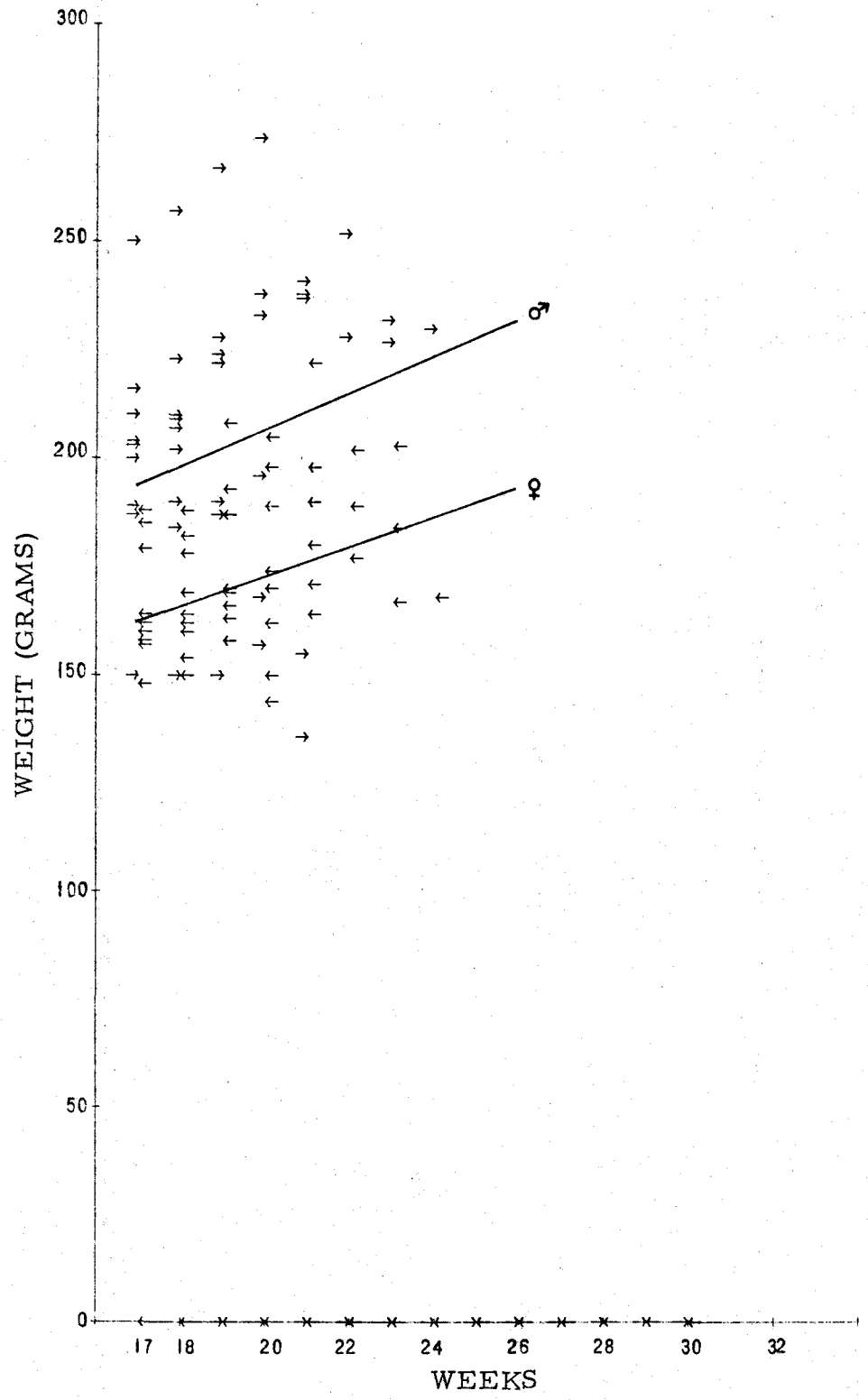


Figure 12. Weight Gain Plots After Removal of the Carcinogen for Group 4 (2 Se/DEN).

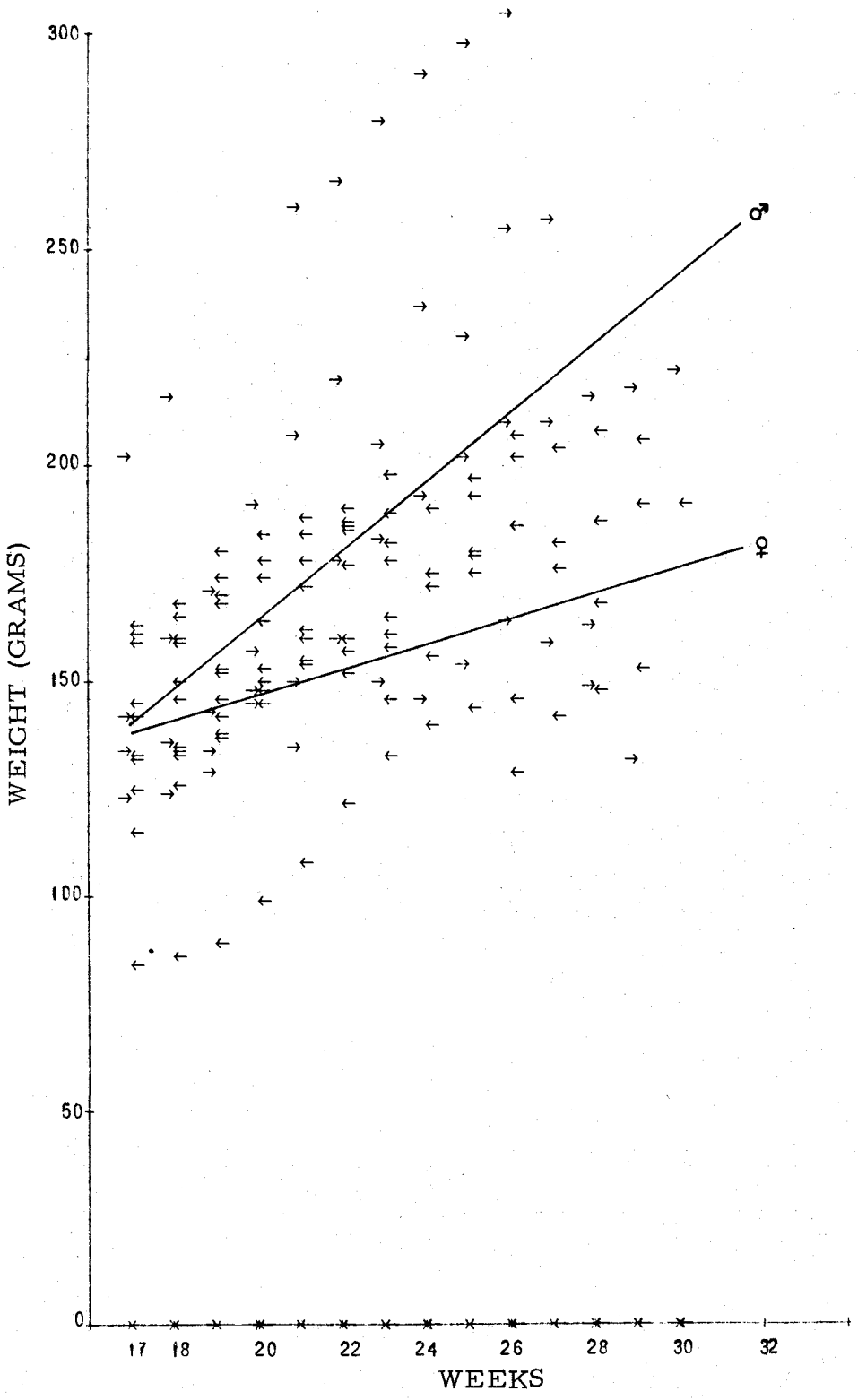


Figure 13. Weight Gain Plots After Removal of the Carcinogen for Group 5 (0.2 Se/FAA).

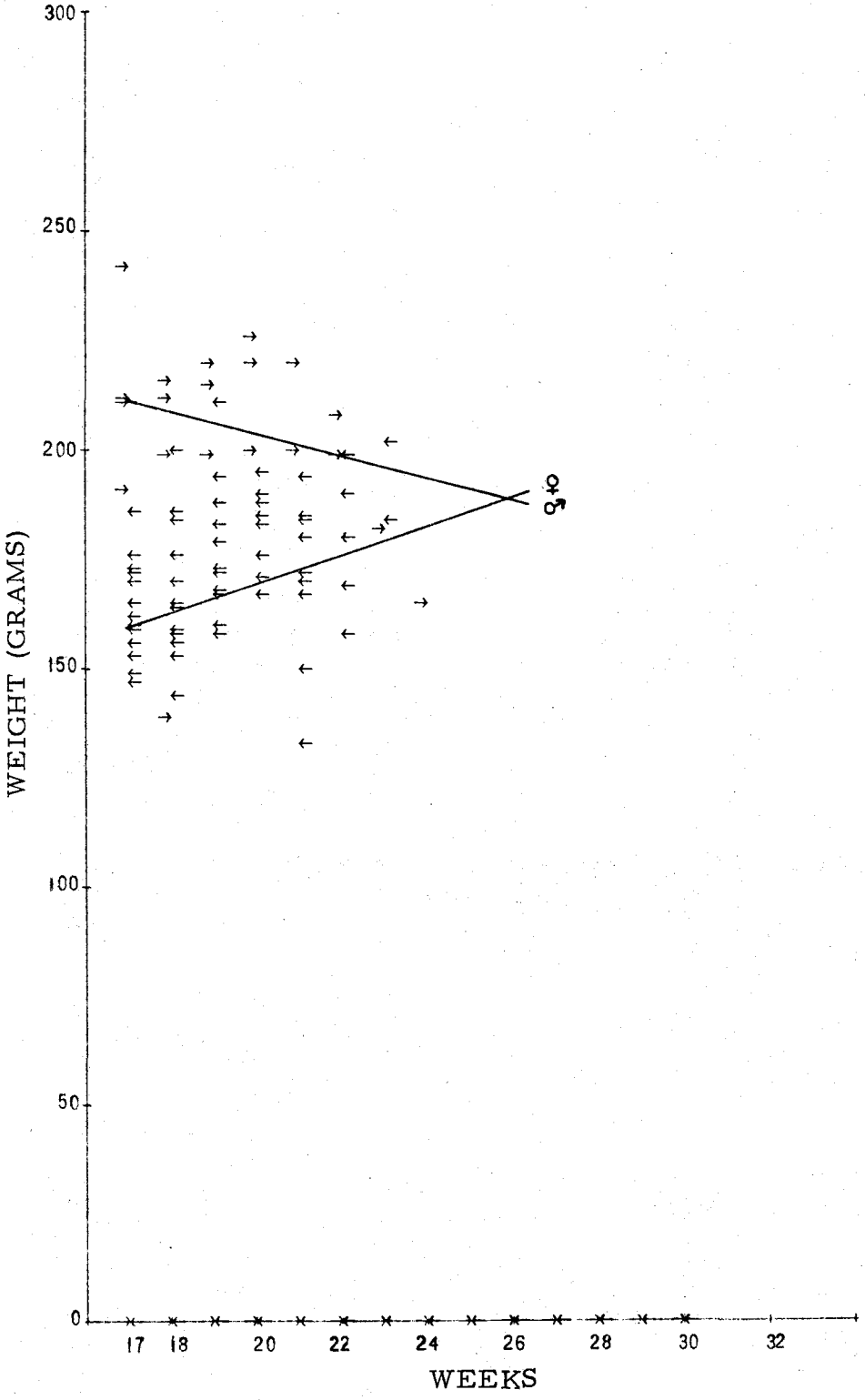


Figure 14. Weight Gain Plots After Removal of the Carcinogen for Group 6 (0.2 Se/DEN).

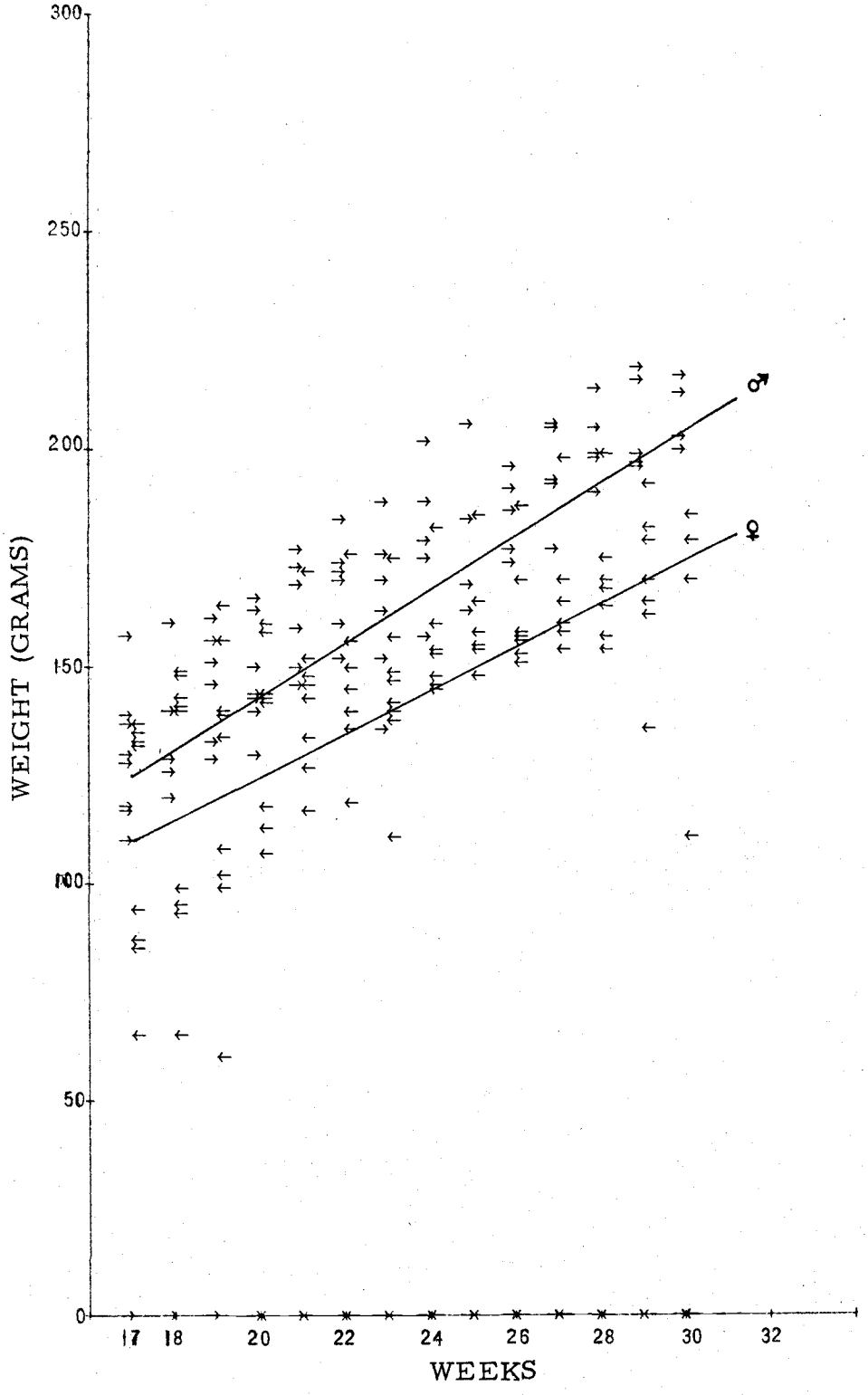


Figure 15. Weight Gain Plots After Removal of the Carcinogen for Group 7 (0 Se/FAA).

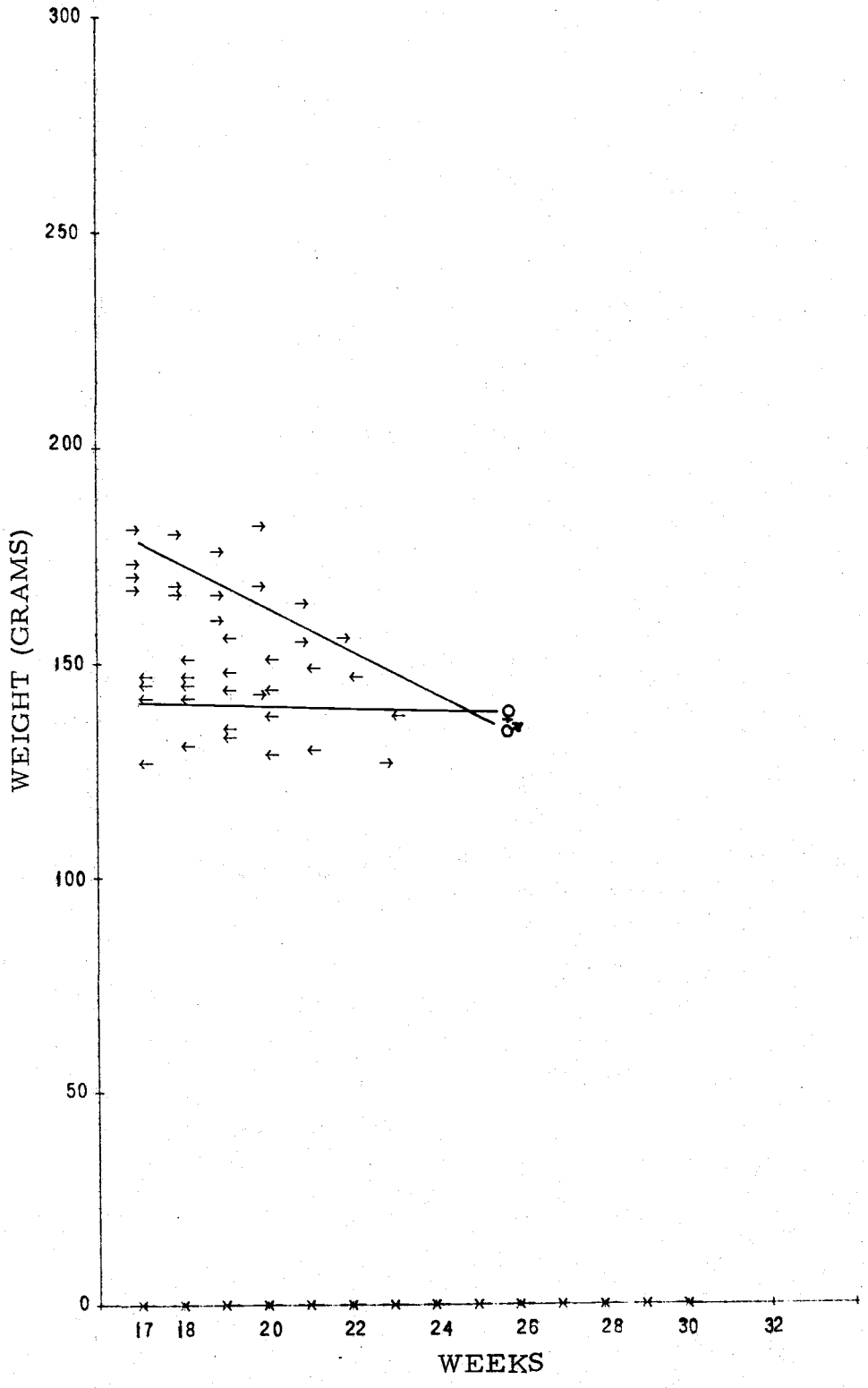


Figure 16. Weight Gain Plots After Removal of the Carcinogen for Group 8 (0 Se/DEN).

Status Evaluation

When one-half of the animals in Group 8 on the 0 Se/DEN diet or one-half of the animals in Group 7 on the 0 Se/FAA diet died, the design of this experiment called for killing three animals from each control group and from all three groups containing that particular carcinogen for histopathological comparison. Similarly, when the terminal animal on either 0 Se/carcinogen diet died representative animals from both control groups and from all related carcinogen groups were to be killed.

Only one-half of Group 7 (0 Se/FAA) died during the experiment; but all Group 8 (0 Se/DEN) animals died. A complete breakdown of information on the animals which died and those that were killed is given in Table V for Group 7 (0 Se/FAA) and in Tables VI and VII for Group 8 (0 Se/DEN).

At 13 weeks one animal of Group 8 (0 Se/DEN) died and at 20 weeks one-half of this group was dead (7 females and 3 males). Of the first half of Group 8 (0 Se/DEN) that died, all but one animal (a male) was found to have hepatomas. The three Group 8 (0 Se/DEN) animals which were killed (all females) all had hepatomas. Of the three Group 4 (2 Se/DEN) animals killed (all males) one was negative and two had hepatomas. All Group 6 (0.2 Se/DEN) animals killed at this time (1 male and 2 females) had hepatomas. All control animals (Groups 1 and 2) killed were negative.

At 23 weeks the last Group 8 (0 Se/DEN) animal had died. All five (2 males and 3 females) from this last group were found to have

hepatomas. The Group 4 (2 Se/DEN) animals (1 male and 1 female) and the Group 6 (0.2 Se/DEN) animals (1 male and 2 females) killed all had carcinomas. All control animals (Groups 1 and 2) killed were negative.

At 13 weeks one animal from Group 7 (0 Se/FAA) died, and by 30 weeks one-half of the animals on this diet had died. Of these eight animals, five were females and three were males. All males in this group were negative; three of the females had carcinomas. Two males were killed from the remaining animals of Group 7 (0 Se/FAA) and both were found to have hepatomas. Of the two females and the one male from Group 3 (2 Se/FAA) that were killed the male had a metastatic hepatoma and one female had a mammary carcinoma, the other female was negative. One male and two females from Group 5 (0.2 Se/FAA) were killed; the male was negative, but both females had hepatomas. All six control animals killed were negative.

TABLE V. STATUS EVALUATION OF SELECTED RATS AT THE TIME OF DEATH OF THE FIRST HALF OF GROUP 7 (0 Se/FAA).

Rat. No. Sex	Treatment	Histological evaluation	Wks. in experiment	Wks. on carcinogen	Total carcinogen consumed (in mg)	Total selenium consumed (in mg)	Feed consumed (1st 17 wks) (grams)	Starting wt. (grams)	Wt. at death (grams)
4 F	0 Se/FAA	Mam. C.	29	17	64.8		432	38	165
28 F	0 Se/FAA	Hep./Mam. C.	29	17	61.2		408	37	192
30 M	0 Se/FAA	Neg.	23	17	59.5		399	28	136
37 F	0 Se/FAA	Neg.	19	17	61.1		407	23	60
67 F	0 Se/FAA	Hep.	30	17	49.5		330	29	111
93 F	0 Se/FAA	Neg.	23	17	48.8		325	16	111
152 M	0 Se/FAA	Neg.	14	14	62.1		414	30	118
160 M	0 Se/FAA	Neg.	13	13	80.9		539	66	134
1 M	0 Se/Cont.	Neg.	30				1,059	22	241
2 F	0 Se/Cont.	Neg.	30				1,011	36	188
17 M	0 Se/Cont.	Neg.	30				1,046	37	243
16 F	2 Se/Cont.	Neg.	30			1.476	738	40	203
35 F	2 Se/Cont.	Neg.	30			1.542	771	28	201
39 M	2 Se/Cont.	Neg.	30			1.552	776	40	292
11 M	2 Se/FAA	Met. Hep.	30	17	79.7	1.062	531	49	254
13 F	2 Se/FAA	Neg.	30	17	60.8	0.810	405	50	198
165 F	2 Se/FAA	Mam. C.	27	17	108.2	1.442	721	57	221
9 F	0.2 Se/FAA	Met. Hep.	30	17	64.1	0.085	427	28	191
75 F	0.2 Se/FAA	Hep.	30	17	54.0	0.072	360	17	153
139 M	0.2 Se/FAA	Neg.	28	17	91.1	0.121	607	30	163
65 M	0 Se/FAA	Hep.	30	17	48.2		321	23	200
134 M	0 Se/FAA	Hep.	29	17	64.8		432	28	196

TABLE VI. STATUS EVALUATION OF SELECTED RATS AT THE TIME OF DEATH OF THE FIRST HALF OF GROUP 8 (0 Se/DEN).

Rat No. Sex	Treatment	Histological evaluation	Wks. in experiment	Wks. on carcinogen	Total carcinogen consumed (in mg)	Total selenium consumed (in mg)	Feed consumed (1st 17 wks) (grams)	Starting wt. (grams)	Wt. at death (grams)
5 M	0 Se/DEN	Hep.	20	17	120.8		755	20	143
6 F	0 Se/DEN	Hep.	17	17	97.0		606	40	129
31 M	0 Se/DEN	Hep.	18	17	109.6		685	32	166
59 M	0 Se/DEN	Neg.	16	16	73.3		458	30	86
72 F	0 Se/DEN	Hep.	16	16	99.4		621	18	137
86 F	0 Se/DEN	Hep.	19	17	97.1		607	29	156
113 F	0 Se/DEN	Hep.	15	15	83.5		522	34	144
119 F	0 Se/DEN	Hep.	20	17	105.3		658	27	151
143 F	0 Se/DEN	Hep.	15	15	91.0		569	32	108
164 F	0 Se/DEN	Hep.	13	13	63.7		398	51	118
114 M	0 Se/Cont.	Neg.	20				1,364	29	192
117 F	0 Se/Cont.	Neg.	20				826	25	162
140 F	0 Se/Cont.	Neg.	18				1,445	33	163
103 F	2 Se/Cont.	Neg.	20			1.494	747	18	170
115 M	2 Se/Cont.	Neg.	20			1.516	758	34	240
147 F	2 Se/Cont.	Neg.	19			1.642	821	30	200
127 M	2 Se/DEN	Neg.	19	17	122.6	1.532	766	37	222
138 M	2 Se/DEN	Met. Hep.	18	17	130.4	1.630	815	33	207
153 M	2 Se/DEN	Hep.	18	17	124.0	1.550	775	32	190
79 M	0.2 Se/DEN	Hep.	20	17	123.0	0.154	769	35	226
129 F	0.2 Se/DEN	Hep.	19	17	122.4	0.153	765	31	211
133 F	0.2 Se/DEN	Hep.	19	17	110.6	0.138	691	27	167
105 F	0 Se/DEN	Hep.	20	17	88.2		551	25	138
162 F	0 Se/DEN	Hep.	17	17	110.1		688	60	160
163 F	0 Se/DEN	Hep.	17	17	108.5		678	46	159

TABLE VII. STATUS EVALUATION OF SELECTED RATS AT THE TIME OF DEATH OF THE LAST HALF OF GROUP 8 (0 Se/DEN).

Rat No. Sex	Treatment	Histological evaluation	Wks. in experiment	Wks. on carcinogen	Total carcinogen consumed (in mg)	Total selenium consumed (in mg)	Feed consumed (1st 17 wks) (grams)	Starting wt. (grams)	Wt. at death (grams)
21 F	0 Se/DEN	Hep.	23	17	78.2		489	29	138
32 F	0 Se/DEN	Hep.	21	17	110.4		690	28	130
48 M	0 Se/DEN	Met. Hep.	21	17	109.0		681	29	155
85 M	0 Se/DEN	Hep.	23	17	90.7		567	19	127
161 F	0 Se/DEN	Hep.	20	17	108.6		679	72	130
18 F	0 Se/Cont.	Neg.	23				1,099	38	185
145 M	0 Se/Cont.	Neg.	21				1,422	43	216
156 F	0 Se/Cont.	Neg.	21				1,268	28	170
70 M	2 Se/Cont.	Neg.	24			1.530	765	27	246
71 F	2 Se/Cont.	Neg.	24			1.608	804	32	191
130 M	2 Se/Cont.	Neg.	23			1.618	809	37	271
12 M	2 Se/DEN	Hep.	24	17	109.9	1.374	687	55	230
24 F	2 Se/DEN	Hep.	24	17	117.0	1.462	731	39	168
69 M	0.2 Se/DEN	Met. Hep.	24	17	125.3	0.159	783	30	165
155 F	0.2 Se/DEN	Mam. C.	22	17	129.1	0.161	807	27	180
170 F	0.2 Se/DEN	Hep.	21	17	125.3	0.157	783	41	180

Histological Evaluation

Detailed histological evaluations on individual rats are shown in Appendix Table 9. A condensed histological summary evaluation is given in Table VIII. None of the rats on either control diet (Groups 1 and 2) developed neoplasts. With one exception the males in all FAA groups were free of peripheral neoplasts. The single exception was a male in Group 3 (2 Se/FAA) and the development was related to a metastatic hepatoma with a 5 mm diameter subcutaneous growth just behind the left foreleg. Peripheral neoplasts were common in the females of all FAA groups. The majority of these tumors were classified as mammary carcinomas and a metastatic state was common. There were no peripheral neoplasts observed in the males of the DEN groups and only one observation in the females of these groups. This single tumor was a mammary carcinoma. Except for the two control groups hepatomas were quite common and well distributed among both sexes and among all other groups.

Within the DEN groups Se appeared to provide protection against the tumor induction of the carcinogen. Without Se in the diet 94 per cent of the group developed carcinomas. This was reduced to 80 per cent when the Se level was 0.2 ppm; and was further lowered to 70 per cent when Se was increased to 2 ppm. The results were more pronounced in the females with tumor incidence decreasing from 100 per cent in the females without selenium in their diet to 64 per cent in those fed 2 ppm Se. This protection was not as significant in the males of these groups.

Within the FAA groups Se did not appear to provide protection against the carcinoma incidence. In fact, Se in the diet appeared to adversely affect the males. The higher the Se level in the diet, the greater the incidence of carcinomas. This increase in male incidence ranged from 25 per cent in Group 7 (0 Se/FAA) to 57 per cent in Group 3 (2 Se/FAA). Males receiving 0.2 Se/FAA (Group 5) had an incidence of 40 per cent. The percentage of tumors in both sexes related to the relative quantity of FAA ingested. Group 7 (0 Se/FAA) females consumed an average of 3.48 mg FAA per animal with a tumor incidence of 67 per cent. The Group 5 (0.2 Se/FAA) females ingested an average of 5.01 mg FAA with a tumor incidence of 83 per cent. The Group 3 (2 Se/FAA) females consumed an average of 4.18 mg FAA per animal with an incidence of 54 per cent. The ratio of carcinogen to Se was identical within a group.

TABLE VIII. CONDENSED HISTOLOGICAL EVALUATION OF GROUPS BY TREATMENT.

Treatment	No. of rats in treatment	No. of rats developing carcinomas	Percentage of rats developing carcinomas		
			Total	Males	Females
0 Se/Control	20	none			
2 Se/Control	19	none			
2 Se/FAA	20	11	55	57	54
2 Se/DEN	20	14	70	78	64
0.2 Se/FAA	17	12	71	40	83
0.2 Se/DEN	20	16	80	60	87
0 Se/FAA	17	8	47	25	67
0 Se/DEN	18	17	94	80	100

SUMMARY

Rats of both sexes were fed one of two chemical carcinogens with and without selenium at varying levels for 17 weeks. The carcinogens were: (FAA) N-2-fluorenyl-acetamide (at 150 ppm) and (DEN) diethylnitrosamine (160 ppm); and, selenium was introduced as Na_2SeO_3 (0, 0.2, and 2.0 ppm). All other dietary factors were constant among all groups. After 17 weeks the carcinogen was withdrawn from all diets for the remainder of the experiment.

Weekly weight gains and feed intake were recorded. Histological evaluations were performed on all livers, lungs and tumors. Plots of weight gain were not statistically significant as a result of low degrees of freedom and a very high standard deviation applied to the computer plots.

Selenium demonstrated some measure of protection in both sexes against the carcinogenicity promoted by ingestion of DEN, but failed to protect against FAA.

Since the control group supplemented with 2 ppm selenium gained as well as the control group without selenium --- but at a lower feed consumption --- this lends credence to the essentiality of selenium as a trace nutrient.

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APPENDIX

Appendix Table 1. Total and Average Feed Intake for Group 1 (0 Se/Control)

Group 1 0 Se/Control Rat No.	Feed intake in grams (17 weeks)	Ave. weekly feed intake in grams	Total carcinogen consumption in mg	Ave. weekly carcinogen consumption in mg	Total Se consumption in mg	Ave. weekly Se consumption in mg
Males						
1	1,059	62.29				
17	1,046	61.53				
29	1,194	70.24				
46	1,266	74.47				
64	889	52.29				
98	1,135	66.76				
114	1,364	80.24				
145	1,422	83.65				
Average	1,172	68.93				
Females						
2	1,011	59.47				
18	1,099	64.65				
27	1,322	77.76				
66	1,301	76.53				
80	1,233	72.53				
91	1,314	77.29				
97	916	53.88				
111	1,544	90.82				
117	826	48.59				
140	1,445	85.00				
156	1,268	74.59				
158	1,401	82.41				
Average	1,223	71.96	none	none	trace	trace

Appendix Table 2. Total and Average Feed Intake and Selenium Consumption for Group 2 (2 Se/Control). † = Spontaneous death.

Group 2 2 Se/Control Rat No.	Feed intake in grams (17 weeks)	Ave. weekly feed intake in grams	Total carcinogen consumption in mg	Ave. weekly carcinogen consumption in mg	Total Se consumption in mg	Ave. weekly Se consumption in mg
Males						
15	728	42.82			1.456	0.0856
39	776	45.65			1.552	0.0913
52	777	45.71			1.554	0.0914
58	836	49.18			1.672	0.0984
70	765	45.00			1.530	0.0900
115	758	44.59			1.516	0.0892
130	809	47.59			1.618	0.0952
151	721 (†15 wks)	48.07			1.442	0.0848
Average	771	46.08			1.543	0.0907
Females						
16	738	43.41	none	none	1.476	0.0868
35	771	45.35			1.542	0.0907
45	794	46.71			1.588	0.0934
49	757	44.53			1.514	0.0891
71	804	47.29			1.608	0.0946
83	779	45.82			1.558	0.0916
92	733	43.12			1.466	0.0862
103	747	43.94			1.494	0.0879
131	822	48.35			1.644	0.0967
135	815	47.94			1.630	0.0959
147	821	48.29			1.642	0.0966
Average	780	45.87			1.560	0.0918

Appendix Table 3. Total and Average Feed, Carcinogen, and Selenium Consumption for Group 3 (2 Se/FAA).

Group 3 2 Se/FAA Rat No.	Feed intake in grams (17 weeks)	Ave. weekly feed intake in grams	Total carcinogen consumption in mg	Ave. weekly carcinogen consumption in mg	Total Se consumption in mg	Ave. weekly Se consumption in mg
Males						
11	531	34.24	79.7	4.69	1.062	0.0625
23	506	29.76	75.9	4.47	1.012	0.0595
40	588	34.59	88.2	5.19	1.176	0.0692
53	458	26.94	68.7	4.04	0.916	0.0539
60	483	28.41	72.5	4.26	0.966	0.0568
76	591	34.76	88.7	5.22	1.182	0.0695
107	558	32.82	83.7	4.92	1.116	0.0656
Average	531	31.22	79.6	4.68	1.061	0.0624
Females						
13	405	23.82	60.8	3.57	0.810	0.0476
22	422	24.82	63.3	3.72	0.844	0.0496
41	576	33.88	86.4	5.08	1.152	0.0678
50	363	21.35	54.5	3.20	0.726	0.0427
55	354	20.82	53.1	3.12	0.708	0.0416
73	415	24.41	62.3	3.66	0.830	0.0488
87	446	26.24	66.9	3.94	0.892	0.0525
99	547	32.18	82.1	4.83	1.094	0.0644
108	397	23.35	59.6	3.50	0.794	0.0467
120	315	18.53	47.3	2.78	0.630	0.0371
123	529	31.12	79.4	4.67	1.058	0.0622
165	721	42.41	108.2	6.36	1.442	0.0848
166	673	39.59	101.0	5.94	1.346	0.0792
Average	474	27.89	71.1	4.18	0.948	0.0557

Appendix Table 4. Total and Average Feed, Carcinogen, and Selenium Consumption for Group 4 (2 Se/DEN).

Group 4 2 Se/DEN Rat No.	Feed intake in grams (17 weeks)	Ave. weekly feed intake in grams	Total carcinogen consumption in mg	Ave. weekly carcinogen consumption in mg	Total Se consumption in mg	Ave. weekly Se consumption in mg
Males						
12	687	40.41	109.9	6.47	1.374	0.0808
43	822	48.35	131.5	7.74	1.644	0.0967
54	795	46.76	127.2	7.48	1.590	0.0935
61	740	43.53	118.4	6.97	1.480	0.0871
77	788	46.35	126.1	7.42	1.576	0.0927
106	512	30.12	81.9	4.82	1.024	0.0602
127	766	45.06	122.6	7.21	1.532	0.0901
138	815	47.94	130.4	7.67	1.630	0.0959
153	775	45.59	124.0	7.29	1.550	0.0912
Average	744	43.59	119.1	7.01	1.489	0.0876
Females						
14	662 (†15 wks)	44.13	105.9	7.06	1.324	0.0883
24	731	43.00	117.0	6.88	1.462	0.0860
51	666	39.18	106.6	6.27	1.332	0.0784
56	782	46.00	125.1	7.36	1.564	0.0920
74	758	44.59	121.3	7.13	1.516	0.0892
96	581	34.18	93.0	5.47	1.162	0.0684
100	750	44.12	120.0	7.06	1.500	0.0882
121	765	45.00	122.4	7.20	1.530	0.0900
124	720	42.35	115.2	6.77	1.440	0.0847
146	724 (†16 wks)	45.25	115.8	6.81	1.448	0.0905
148	788	46.35	126.1	7.42	1.576	0.0927
Average	721	43.10	115.3	6.86	1.441	0.0862

Appendix Table 5. Total and Average Feed, Carcinogen, and Selenium Consumption for Group 5 (0.2 Se/FAA).

Group 5 0.2 Se/FAA Rat No.	Feed intake in grams (17 weeks)	Ave. weekly feed intake in grams	Total carcinogen consumption in mg	Ave. weekly carcinogen consumption in mg	Total Se consumption in mg	Ave. weekly Se consumption in mg
Males						
62	567	33.35	85.1	5.00	0.1134	0.0067
78	719	42.29	107.9	6.34	0.1438	0.0085
84	433	25.47	65.0	3.82	0.0866	0.0051
128	423 (†16 wks)	26.44	63.5	3.97	0.0846	0.0053
139	607	35.71	91.1	5.36	0.1214	0.0071
Average	550	32.65	82.5	4.90	0.1100	0.0065
Females						
9	427	25.12	64.1	3.77	0.0854	0.0050
25	604	35.53	90.6	5.33	0.1208	0.0071
33	554	32.59	83.1	4.89	0.1108	0.0065
42	561	33.00	84.2	4.95	0.1122	0.0066
75	360	21.18	54.0	3.18	0.0720	0.0042
101	629	37.00	94.4	5.55	0.1258	0.0074
125	547	32.18	82.1	4.83	0.1094	0.0064
132	547	32.18	82.1	4.83	0.1094	0.0064
154	726	42.71	108.9	6.41	0.1452	0.0085
167	666	39.18	99.9	5.88	0.1332	0.0078
168	528 (†13 wks)	40.62	79.2	4.66	0.1056	0.0081
169	668	39.29	100.2	5.89	0.1336	0.0079
Average	568	34.21	85.2	5.01	0.1136	0.0068

Appendix Table 6. Total and Average Feed, Carcinogen, and Selenium Consumption for Group 6 (0.2 Se/DEN).

Group 6 0.2 Se/DEN Rat No.	Feed intake in grams (17 weeks)	Ave. weekly feed intake in grams	Total carcinogen consumption in mg	Ave. weekly carcinogen consumption in mg	Total Se consumption in mg	Ave. weekly Se consumption in mg
Males						
8	697 (†15 wks)	46.47	111.5	7.44	0.1394	0.0093
44	840	49.41	134.4	7.91	0.1680	0.0099
63	795	46.76	127.2	7.48	0.1590	0.0094
69	783	46.06	125.3	7.37	0.1566	0.0092
79	769	45.24	123.0	7.24	0.1538	0.0090
Average	777	46.79	124.3	7.49	0.1554	0.0094
Females						
10	785	46.18	125.6	7.39	0.1570	0.0092
26	223 (†8 wks)	27.88	35.7	4.46	0.0446	0.0056
34	747	43.94	119.5	7.03	0.1494	0.0088
57	817	48.06	130.7	7.69	0.1634	0.0096
82	801	47.12	128.2	7.54	0.1602	0.0094
90	783	46.06	125.3	7.37	0.1566	0.0092
102	728	42.82	116.5	6.85	0.1456	0.0086
110	759	44.65	121.4	7.14	0.1518	0.0089
126	751	44.18	120.2	7.07	0.1502	0.0088
129	765	45.00	122.4	7.20	0.1530	0.0090
133	691	40.65	110.6	6.50	0.1382	0.0081
150	707	41.59	113.1	6.65	0.1414	0.0083
155	807	47.47	129.1	7.60	0.1614	0.0095
170	783	46.06	125.3	7.37	0.1566	0.0092
171	837	49.24	133.9	7.88	0.1674	0.0098
Average	732	44.05	117.2	7.05	0.1465	0.0088

Appendix Table 7. Total and Average Feed, Carcinogen, and Selenium Consumption for Group 7 (0 Se/FAA).

Group 7 0 Se/FAA Rat No.	Feed intake in grams (17 weeks)	Ave. weekly feed intake in grams	Total carcinogen consumption in mg	Ave. weekly carcinogen consumption in mg	Total Se consumption in mg	Ave. weekly Se consumption in mg
Males						
19	409	24.06	61.4	3.61		
30	399	23.47	59.9	3.52		
65	321	18.88	48.2	2.84		
116	468	27.53	70.2	4.13		
122	438	25.76	65.7	3.87		
134	432	25.41	64.8	3.81		
152	414 (†14 wks)	29.57	62.1	4.44		
160	539 (†13 wks)	41.46	80.9	6.22		
Average	428	27.02	64.1	4.05		
Females					trace	trace
4	432	25.41	64.8	3.81		
20	425	25.00	63.8	3.75		
28	408	24.00	61.2	3.60		
37	407	23.94	61.1	3.59		
67	330	19.41	49.5	2.91		
93	325	19.12	48.8	2.87		
118	343	20.18	51.5	3.03		
157	483	28.41	72.5	4.27		
159	398	23.41	59.7	3.51		
Average	395	23.21	59.2	3.48		

Appendix Table 8. Total and Average Feed, Carcinogen, and Selenium Consumption for Group 8 (0 Se/DEN).

Group 8 0 Se/DEN Rat No.	Feed intake in grams (17 weeks)	Ave. weekly feed intake in grams	Total carcinogen consumption in mg	Ave. weekly carcinogen consumption in mg	Total Se consumption in mg	Ave. weekly Se consumption in mg
Males						
5	755	44.41	120.8	7.11		
31	685	40.29	109.6	6.45		
48	681	40.06	109.0	6.41		
59	458 (†16 wks)	28.63	73.3	4.58		
85	567	33.35	90.7	5.34		
Average	629	37.35	100.7	5.98		
Females						
6	606	35.65	97.0	5.70		
21	489	28.76	78.2	4.60		
32	690	40.59	110.4	6.49		
72	621 (†16 wks)	38.81	99.4	6.21		
86	607	35.71	97.1	5.71		
105	551	32.41	88.2	5.19		
113	522 (†15 wks)	34.80	83.5	5.57		
119	658	38.71	105.3	6.19		
143	569 (†15 wks)	37.93	91.0	6.07		
161	679	39.94	108.6	6.39		
162	688	40.47	110.1	6.48		
163	678	39.88	108.5	6.38		
164	398 (†13 wks)	30.62	63.7	4.90		
Average	597	36.48	95.5	5.84	trace	trace

Appendix Table 9. Histological Evaluation for Individual Animals by Treatment Group.

0 Se/Control Rat. No.	Weeks in experiment	Weeks on carcinogen	Histological evaluation
Males			
1	30		Negative
17	30		Negative
29	31		Negative
46	31		Negative
64 †	22		Negative
98	31		Negative
114	20		Negative
145	21		Negative
Females			
2	30		Negative
18	23		Negative
27 †	24		Negative
66	31		Negative
80	31		Negative
91	31		Negative
97	31		Negative
111	31		Negative
117	20		Negative
140	18		Negative
156	21		Negative
158	29		Negative
2 Se/Control			
Males			
15	31		Negative
39	30		Negative
52	31		Negative
58	30		Negative
70	24		Negative
115	20		Negative
130	23		Negative
151 †	15		Negative
Females			
16	30		Negative
35	30		Negative
45	31		Negative
49 †	22		Negative
71	24		Negative
83	31		Negative
92	31		Negative
103	20		Negative
131 †	21		Negative
135	31		Negative
147	19		Negative

Appendix Table 9 -- Continued.

2 Se/FAA Rat No.	Weeks in experiment	Weeks on carcinogen	Histological evaluation
Males			
11	30	17	Metastatic hepatoma
23	23	17	Hepatoma
40 †	20	17	Negative
53	31	17	Negative
60	31	17	Hepatoma
76	31	17	Negative
107	31	17	Hepatoma
Females			
13	30	17	Negative
22	31	17	Hepatoma & mammary carcinoma
41	20	17	Metastatic mammary carcinoma
50	31	17	Hepatoma
55	23	17	Mammary carcinoma
73	31	17	Negative
87 †	21	17	Negative
99	31	17	Negative
108 †	27	17	Negative
120	20	17	Mammary carcinoma
123	31	17	Negative
165	27	17	Mammary carcinoma
166	29	17	Mammary carcinoma
2 Se/DEN			
Males			
12	24	17	Hepatoma
43 †	21	17	Hepatoma
54 †	20	17	Hepatoma
61 †	21	17	Hepatoma
77 †	23	17	Hepatoma
106 †	21	17	Negative
127	19	17	Negative
138	18	17	Metastatic hepatoma
153	18	17	Hepatoma
Females			
14 †	15	15	Negative
24	24	17	Hepatoma
51 †	20	17	Hepatoma
56 †	23	17	Hepatoma
74 †	21	17	Hepatoma
96 †	21	17	Negative
100 †	23	17	Negative
121 †	20	17	Negative
124 †	19	17	Hepatoma
146 †	16	16	Hepatoma
148 †	21	17	Hepatoma

Appendix Table 9 -- Continued.

0.2 Se/FAA Rat No.	Weeks in experiment	Weeks on carcinogen	Histological evaluation
Males			
62	31	17	Metastatic hepatoma
78	31	17	Negative
84	29	17	Hepatoma
128 †	16	16	Negative
139	28	17	Negative
Females			
9	30	17	Metastatic hepatoma
25	23	17	Mammary carcinoma
33	29	17	Mammary carcinoma
42	23	17	Hepatoma & mammary carcinoma
75	30	17	Hepatoma
101 †	27	17	Metastatic mammary carcinoma
125 †	20	17	Negative
132	28	17	Metastatic hepatoma & mammary carcinoma
154	21	17	Mammary carcinoma
167	26	17	Mammary carcinoma
168 †	13	13	Negative
169 †	24	17	Hepatoma & mammary carcinoma
0.2 Se/DEN			
Males			
8 †	15	15	Negative
44 †	18	17	Negative
63 †	22	17	Hepatoma
69	24	17	Metastatic hepatoma
79	20	17	Hepatoma
Females			
10 †	22	17	Hepatoma
26 †	8	8	Negative
34 †	21	17	Hepatoma
57 †	23	17	Metastatic hepatoma
82 †	23	17	Hepatoma
90 †	18	17	Negative
102 †	22	17	Hepatoma
110 †	21	17	Hepatoma
126 †	18	17	Hepatoma
129	19	17	Hepatoma
133	19	17	Hepatoma
150 †	17	17	Hepatoma
155	22	17	Mammary carcinoma
170	21	17	Hepatoma
171 †	21	17	Hepatoma

Appendix Table 9 -- Continued.

0 Se/FAA Rat No.	Weeks in experiment	Weeks on carcinogen	Histological evaluation
Males			
19	31	17	Negative
30 †	23	17	Negative
65	30	17	Hepatoma
116	31	17	Negative
122	31	17	Negative
134	29	17	Hepatoma
152 †	14	14	Negative
160 †	13	13	Negative
Females			
4	29	17	Mammary carcinoma
20	31	17	Hepatoma
28	29	17	Hepatoma & mammary carcinoma
37 †	19	17	Negative
67 †	30	17	Hepatoma
93 †	23	17	Negative
118	31	17	Hepatoma
157	30	17	Mammary carcinoma
159	29	17	Negative
0 Se/DEN			
Males			
5 †	20	17	Hepatoma
31 †	18	17	Hepatoma
48 †	21	17	Metastatic hepatoma
59 †	16	16	Negative
85 †	23	17	Hepatoma
Females			
6 †	17	17	Hepatoma
21 †	23	17	Hepatoma
32 †	21	17	Hepatoma
72 †	16	16	Hepatoma
86 †	19	17	Hepatoma
105	20	17	Hepatoma
113 †	15	15	Hepatoma
119 †	20	17	Hepatoma
143 †	15	15	Hepatoma
161 †	20	17	Hepatoma
162	17	17	Hepatoma
163	17	17	Hepatoma
164 †	13	13	Hepatoma