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PHARMACOLOGY AND TOXICOLOGY OF URANIUM COMPOUNDS

Chronic Inhalation and Other Studies

Edited by

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First Edition

New York · Toronto · London

McGRAW-HILL BOOK COMPANY, INC.

1953

PHARMACOLOGY AND TOXICOLOGY

OF URANIUM COMPOUNDS

Printed in the United States of America

Lithoprinted
by
Edwards Brothers, Incorporated
Ann Arbor, Michigan

(i) Summary. 1. Groups of male and female rats were given intraperitoneal injections of an aqueous solution of uranyl nitrate hexahydrate (50 mg/ml). The doses were chosen to give a high mortality in adult rats. Male rats received 128 mg/kg; female rats received 200 mg/kg. The 24- and 48-hr mortalities were recorded.

2. The rats selected varied in age from 21 days to 6 months. For the most part groups of 50 males and 50 females were studied.

3. The youngest rats were more susceptible than those 1 to 3 months of age. The older rats were more susceptible than the weanlings.

4. Although the variation in age in the 24-hr mortality is qualitatively similar to the variation of mortality with age in the feeding experiment described in Sec. 2.1, the mechanisms involved are probably different. The reason for the resistance of the 1-month-old rat is not known.

3. EFFECT OF A SINGLE DOSE OF URANYL NITRATE

By W. L. Downs, Elliott A. Maynard, and Harold C. Hodge

3.1 Short-term Feeding Test. From the careful feeding studies of rats given a diet containing 2 per cent uranyl nitrate hexahydrate (reported in this volume[1]), it was apparent that simultaneously with the fall in body weight there was a sharp voluntary reduction in food intake. This reduction in appetite almost without exception began on the 2d day after the rats were given the uranium-containing diet, i.e., the rats ate the customary amount of food on the 1st day they were given the 2 per cent uranyl nitrate hexahydrate diet. Thereafter, for a period of several days, sometimes for more than a week, the intake was one-third to one-fifteenth of their customary intake. At the end of this period of voluntary restriction, when the appetites increased, the body weights, after a lag of a day or two, also began to increase. Since the deaths, if any, occurred on the 4th to the 14th days, i.e., during the period of restricted dietary intake, it was evident that the largest dose of uranium had been received on the 1st day.

To test the importance of this 1st day's uranium dose, a series of feeding experiments was conducted in which rats were given the diet containing 2 per cent uranyl nitrate hexahydrate for only 1 day. Thereafter they were returned to the stock ration (Purina Fox Chow with meat scraps) but were observed for body weight and mortality during the succeeding month. Rats of four ages have been studied in this fashion, namely, 1-, 2-, 3-, and 6-month-old rats. The reason for selection of these age groups was that the 30-day-old rat had been shown to be highly resistant to uranium poisoning, the 6-month-old

rat highly susceptible, and the 2- and 3-month-old rats neither highly resistant nor highly susceptible, although more like the 6-month-old young adult.

(a) Aged 30 Days. Two experiments were carried out with 30-day-old rats. In each case the experiments were planned to include observations of effect on reproduction; therefore the rats were placed in cages in pairs of one male and one female each. Fifty males and 50 females were mated in each control and experimental group; thus a total of 100 male and 100 female rats were observed. As may be seen from Table 20.9, the mortality in these animals was so low as

Table 20.9—Summary of Growth Gains after 1 Week and Mortality in Control and Experimental Rats Given a Single Dose of Uranyl Nitrate Hexahydrate and Then Observed for 1 Month

	Av. weight gain, g				Av. mortality			
	Male		Female		Male		Female	
Age	Control	Expti.	Control	Expti.	Control	Expti.	Control	Expti.
30 days								
Group 1	32	24	28	20	0/50	1/50	0/50	1/50
Group 2	29	4	24	16	2/50	0/50	0/50	2/50
2 months (9 weeks)	22	1	13	−10	0/25	2/25	0/25	1/25
3 months (96 days)	9	−30	4	−7	1/24	3/25	0/25	1/25
6 months	7	−28	3	−22	0/25	2/25	0/25	4/25

to be negligible. In the first group no control rats, one male experimental rat, and one female experimental rat died. In the second group two control male rats and two experimental female rats died. It is difficult to attach any significance to such a low mortality, especially when it is compared to the higher values (12 to 21 per cent) observed when rats were given the 2 per cent uranyl nitrate hexahydrate diet for the entire month.

There was a small transient weight loss (see Figs. 20.11 and 20.12). The maximum weight loss of experimental male rats was 16 g, and that of the females was 8 g; but at the end of the 4-week period the males actually outweighed their controls by 6 g, whereas the female rats weighed only 3 g less than their controls. The growth-depressing effect, not of large order at any time, had disappeared by the end of the 4-week period of observation. This recovery is in marked contrast to the severe weight loss at 30 days when rats were kept on the

2 per cent uranyl nitrate hexahydrate diets: Males weighed 40 to 60 g less and females 30 g less than the respective control groups.

(b) Aged 2 Months. Two groups of male and female rats, 25 in each group, at the age of 9 weeks were given the diet containing 2 per cent

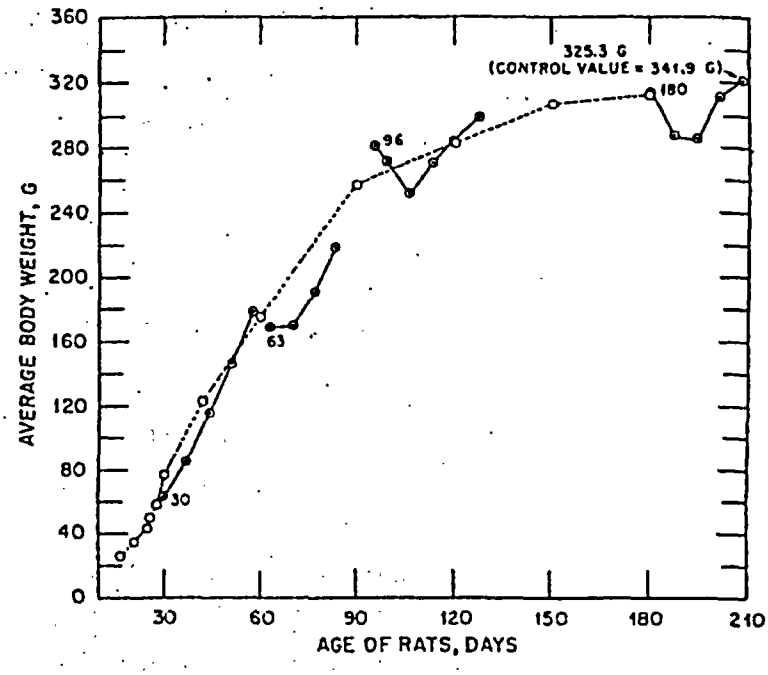

Fig. 20.11—Growth curves for male rats of various ages fed a dietary level of 2 per cent uranyl nitrate hexahydrate for 1 day. One hundred 30-day-old rats and 25 each of 63-, 96-, and 180-day-old rats were used. O, control rats; ⊙, experimental rats.

uranyl nitrate hexahydrate for 24 hr. During the following month none of the control rats died; two of the males and one of the females in the experimental groups died. This mortality was considerably less than had occurred when rats of this age were maintained on the 2 per cent uranyl nitrate hexahydrate diet for 1 month but is comparable with the mortalities in the preceding experiment on 1-month-old rats.

Body weights decreased a little in the first week, but the weight was mostly regained in the succeeding period of 3 weeks during which the animals were observed. Maximum weight depression compared to the controls was 12 g for the males and 24 g for the females. At the

end of the 3-week period the male rats still weighed 12 g less than the corresponding controls, and the female rats weighed 5 g less than their respective control group.

(c) Aged 3 Months. Groups of 25 male and 25 female rats 98 days old were given the experimental diet for 1 day and were observed for

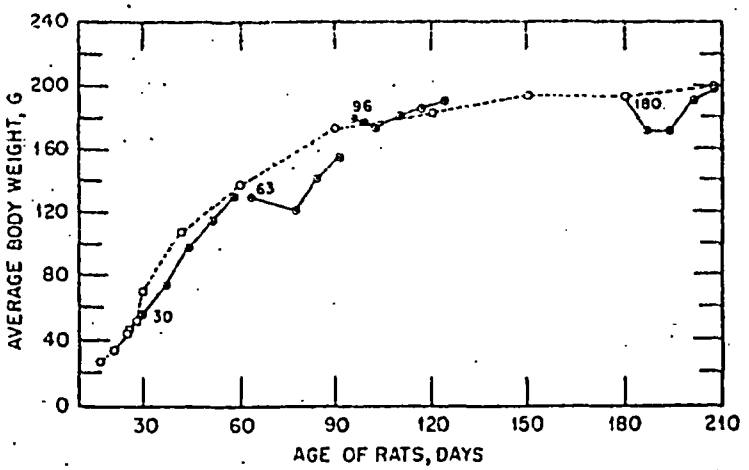

Fig. 20.12—Growth curves for female rats of various ages fed a dietary level of 2 per cent uranyl nitrate hexahydrate for 1 day. One hundred 30-day-old rats and 25 each of 63-, 96-, and 180-day-old rats were used. O, control rats; o, experimental rats.

1 month. None of 25 female and 1 of 24 male control rats died. A small mortality among the experimental rats was observed; 3 of 25 males and 1 of 25 females died. This mortality was much like that of the 2-month-old rats described above.

The loss of body weight was fairly large and was only partly regained in the 1-month period. The weight loss of the male rats was particularly striking (the maximum difference amounted to 42 g), whereas with the females it was only 14 g. At the end of the 4-week period the experimental male rats still weighed 15 g less than the corresponding controls, and the experimental female rats weighed 8 g less than their control group.

(d) Aged 6 Months. Groups of 25 male and 25 female rats, 6 months old, were fed the 2 per cent uranyl nitrate hexahydrate diet for 1 day and were observed for 1 month. The mortality among the control rats was zero; in contrast, 2 of the male and 4 of the 25 female rats died. This may indicate a slight increase in susceptibility in the older animals.

Although the body-weight losses were large (see Figs. 20.11 and 20.12) and more protracted than those of the preceding experiments, most of the lost weight was regained by the end of the 4-week period. The maximum weight loss amounted to 50 g for the male rats and 30 g for the female rats; however, at the end of the 4-week period the males weighed only 16 g less than the controls, and the females weighed only 2 g less than their controls.

The mortalities were uniformly small in these experiments, i.e., less than 10 per cent, except in the case of the 6-month-old females, whose mortality was 16 per cent. This is in sharp contrast with the mortality found (68 to 76 per cent) when the uranyl nitrate was provided during the entire 1-month period for rats of this age. It can hardly be doubted that the extra uranium taken during the period of voluntary dietary restriction sharply increased the mortality, even though the total dose taken after the 1st day was not large. It has been shown that uranium in the blood stream is rapidly excreted via the kidney; therefore, even though the added doses were relatively small, the repetition of insults might have been sufficient to have a major effect on survival.

The growth curves (Figs. 20.11 and 20.12) do not exhibit the regularity or the severity of depression seen in the experiments in which uranyl nitrate was present in the diets during the entire 30-day period (see Figs. 20.8 and 20.9). It is interesting, however, to see how similar the response to a single dose is. The change from practically no effect on body weight in the 30-day-old rats to a slight effect in the 2-month-old rats to an abrupt major depression in the older rats is exactly the same pattern of change that was found when the diet contained 2 per cent uranyl nitrate hexahydrate during the entire month. The effect of a 24-hr feeding reproduces to a surprising extent the whole response seen during a 30-day feeding period on the 2 per cent uranyl nitrate hexahydrate diet.

(e) Summary. 1. A series of feeding experiments was conducted in which rats were given diets containing 2 per cent uranyl nitrate hexahydrate for only 1 day and thereafter were maintained on the stock ration for a period of 1 month.

2. The rats varied in age from 1 to 6 months at the time the 1-month experimental period was begun. For the most part groups of 25 male and 25 female rats were studied.

3. The mortalities observed were uniformly low, but in most cases they were slightly larger in the experimental groups than in the control groups. The mortalities were much lower than observed in the rats of comparable ages maintained for the duration of a 30-day period on diets containing 2 per cent uranyl nitrate hexahydrate (see

Sec. 2.1).[1] Evidently the mortality arises from the extra uranium ingested on the 2d and subsequent days of the experiment.

4. The growth curves of the 1-month-old rats showed only a slight depression as compared to the growth curve of control rats. The older rats exhibited a tendency toward a drastic weight loss over a period of a week or more with a rapid increase in weight in the last 2 weeks of the experiment. This response was similar to that of the rats of the same age maintained on the 2 per cent uranyl nitrate hexahydrate diet but was notably milder in degree.

3.2 Breeding Experiment. In an extensive experiment reported earlier,[1] two large groups of male and female rats (a control and an experimental group) were maintained on a 2 per cent uranyl nitrate hexahydrate diet for a period of 1 year so that the effects on reproduction could be observed. The experimental diet was provided for a period of 7 months, after which the animals were replaced on the stock ration for the balance of the year. While the animals were on the experimental diet, a notable reduction in growth occurred, and a serious interference with reproduction was found: (1) Fewer experimental females had litters; (2) the litters were smaller in number; (3) the oestrous cycles were irregular.

The 2 per cent uranyl nitrate hexahydrate diet undoubtedly interfered with nutrition and perhaps with appetite; thus loss in fertility should possibly be ascribed partly to malnutrition or undernutrition. Partial inanition has long been known to interfere with and to reduce normal reproduction. Two types of experiments might have been instituted: (1) The growth of the control group might have been kept equal to that of the experimental group using paired feeding techniques; or (2) the administration of uranium might have been controlled so that no depression of growth ensued. The second alternative was chosen. To discover whether uranium administration might influence reproduction in the absence of depression in body weight and in rats whose nutrition was comparable with control rats, an experiment was carried out in which the rats were given access to the 2 per cent uranyl nitrate hexahydrate diet for a single 24-hr period only.

Fifty male and 50 female rats and the same number of litter-mate control animals were selected on the basis of body weight, making groups practically identical in average weight. At weaning the experimental group was given a diet containing 2 per cent uranyl nitrate hexahydrate for a single 24-hr period. Thereafter the stock ration of Purina Fox Chow and water was supplied ad lib. to both control and experimental animals. The rats were kept in pairs, one male and one female in each cage. Male rats were removed from the cages near the end of the gestation period. The litters were removed at birth,

and the rats were remated. Observations were made as to body weights, number of females having litters, number of litters, and number of offspring per litter.

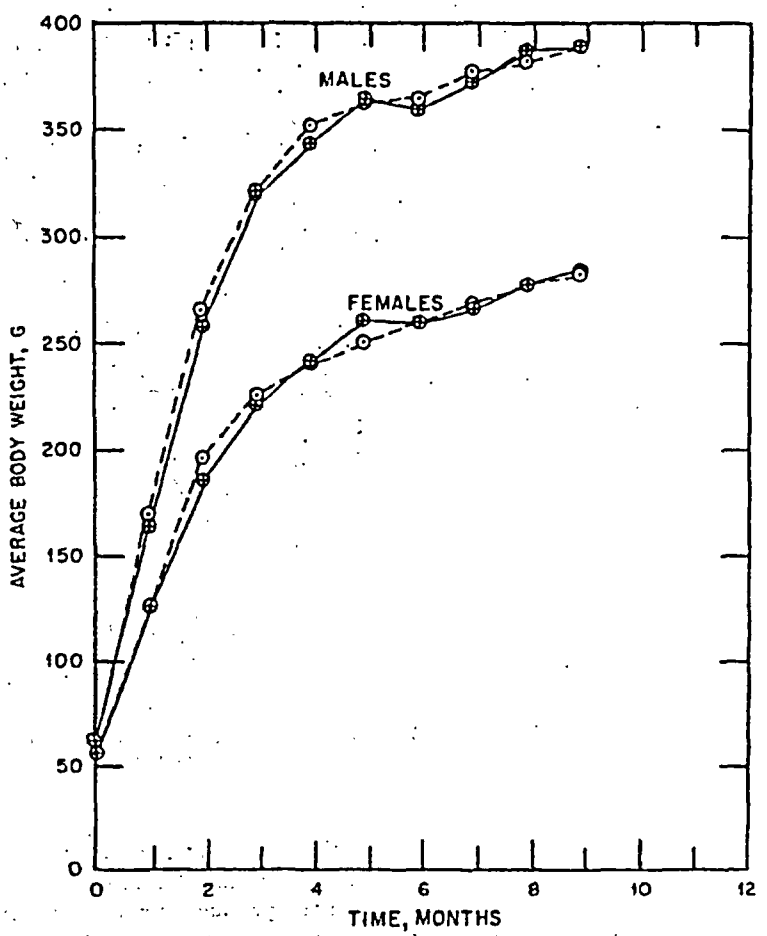

Fig. 20.13—Growth curves of male and female rats (50 per group) following a single 24-hr feeding of uranyl nitrate hexahydrate (2 per cent in diet). ○, control rats; ⊕, experimental rats.

The growth curves were practically identical for the males and for the females (see Fig. 20.13) of the two groups. The rats grew well and gave no indication of abnormality other than the slight and tran-

sient depression described in Sec. 3.1a. The average body weights differed occasionally by a few grams during the 10-month period of observation, but the differences were so small that they are meaningless. It is obvious that the nutrition must have been generally similar for the control and for the experimental groups. Certainly it would

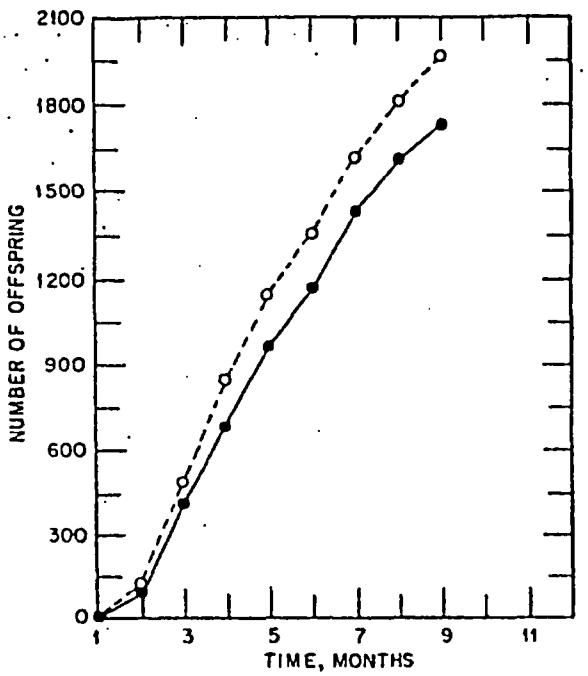

Fig. 20.14—Interference with reproduction following a single 24-hr feeding of uranyl nitrate hexahydrate (2 per cent in diet). O, control rats; ●, experimental rats.

be difficult to say that any real difference in nutrition existed between the control rats and the experimental rats. The program therefore was successful in producing rats at the age of 10 months in which an exposure to uranium had occurred but in which over almost the entire period of experimentation the nutrition had been comparable to that of the control group.

Consequently it is surprising to note the clearly established and increasing interference with reproduction shown in Fig. 20.14, where the number of offspring born to control and to experimental groups is plotted against the time in months. Forty-three of fifty control female

rats had one or more litters; 44 of the 50 experimental rats had one or more litters. Thus the initial 24-hr uranyl nitrate feeding did not reduce the number of litter-bearing females. This is in marked contrast with the earlier breeding experiment[1] in which 43 of 50 control female rats had litters but only 30 of the experimental female rats had litters.

The number of litters shows a small difference; the control female rats had 252 litters up into the 10th month, whereas the experimental female rats had 233 litters in the same period. This difference of 19 litters amounts to a decrease of about 7 per cent in the experimental rats and again is quite small in comparison with the decrease found in the earlier breeding experiment in which the control female rats had 306 litters during 7 months and the experimental rats had only 174. In this case the decrease of 132 litters amounted to a 43 per cent reduction in productivity of the experimental group. The difference in number of litters in the control groups produced per month in the two experiments may be attributed tentatively to differences in the time of year at which these experiments were conducted. The single-dose experiment was carried on in the winter, when the breeding rate is lower than in the summer as illustrated by the earlier experiment.

The number of offspring shows a greater percentage difference between the control and experimental groups than was seen in the number of litters. The control female rats in 9 months had a total of 1958 offspring; the experimental female rats had only 1725 offspring. This difference of 233 offspring represents a reduction of about 12 per cent in the experimental group. Again, these differences seem small when compared with the results of the earlier breeding experiment. The control rats had 2726 offspring at the 9-month period; the experimental rats had only 1407. This was a difference of 1319 offspring and represented a 48 per cent decrease in productivity.

Another comparison which might be made is that of the average litter size during the 7-month experimental period. Table 20.10 shows this figure for the rats that had earlier been fed continuously as well as for the animals given the single 24-hr feeding.

The number of offspring per litter was the same for the experimental groups in each case, but the control rats had fewer offspring per litter on the single feeding test so that the difference between control and experimental groups is less in this study.

(a) Pathology. Twenty male rats and 10 females were sacrificed, and tissue sections were taken for histological study. Twenty control male rats were also examined at the same time.

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The most common finding in the kidneys of these rats was mild chronic interstitial nephritis characterized by scattered small interstitial accumulations of chronic inflammatory cells associated in most instances with localized tubular constriction and increase in connective tissue. This type of change was observed in 5 of 20 control rats, 7 of 20 male experimental rats, and 4 of 10 female experimental rats. These changes did not conform to the usual damage seen in uranium poisoning.

Table 20.10—Average Litter Size for 7-month Period

	Average number of offspring per litter		
	Control group	Experimental group	Difference
Rats fed 2% $UO_2(NO_3)_2.6H_2O$ continuously for 195 days	9.2	7.8	1.4
Rats fed 2% $UO_2(NO_3)_2.6H_2O$ for one 24-hr period	8.6	7.9	0.7

A few of the experimental rats showed blue-staining casts (presumably calcium) in the collecting tubules of the medulla. Although these casts showed no apparent relation to the chronic inflammatory changes and although they have not been observed previously in animals exposed to uranium compounds, they may represent calcification of casts collected in those tubules during the acute stage of renal injury early in the experiment.

Atrophic changes were observed in a few cases in both control and experimental rats. The number of tubules involved was very small, and the changes were described as mild.

Some pulmonary disease was observed in two of the control and in two of the male experimental rats. Reticulum-cell sarcomas involving the abdominal lymph nodes were present in one male experimental rat and in two control rats.

(b) Summary. 1. Fifty male and 50 female rats were given access to a diet containing 2 per cent uranyl nitrate hexahydrate for a single 24-hr period and thereafter were maintained for 10 months on the stock ration. Equal-sized groups of control rats were observed during the same period.

2. The rats were kept in pairs, one male and one female in each cage. The male rats were removed from the cage near the end of the gestation period. The litters were removed at birth, and the rats were remated.

3. There was no interference in growth associated with the single dose of uranyl nitrate.

4. A single 24-hr feeding of uranyl nitrate did not reduce the number of females bearing litters. The experimental female rats had a small reduction in the number of litters but a larger reduction in the total number of offspring.

5. It is concluded that, under the circumstances of this experiment, uranium administration adversely affected the reproductive functions in the absence of a severe derangement of nutrition.

4. ONE-YEAR FEEDING TEST: SERIAL STUDY OF URANYL NITRATE

By Elliott A. Maynard, Thomas B. Barnett, W. L. Downs, William F. Neuman, and Harold C. Hodge

4.1 First 3 Months. Groups of male and female rats, 25 in each group, were placed soon after weaning on diets containing 0 (control groups), 0.1, 0.5, and 2.0 per cent uranyl nitrate hexahydrate. As may be seen from Fig. 20.15, the animals on the lower doses grew approximately as well as the control rats. The rats given the 2 per cent diet showed a serious depression of the body weights during the 1st week but thereafter recovered and resumed growth at a rate not greatly different from the growth rate of the control animals. At the end of 3 months the male animals weighed 78 g less than their control group, and the females weighed 22 g less than their control group.

In addition to the rats removed for sacrifice to provide material for histological examination, a few male and female rats died during the 3-month period. All the deaths were confined to the 2 per cent groups and to the first 2 weeks of the feeding period; 2 males and 10 females died (see Tables 20.11 and 20.12).

4.2 Three to Six Months. Other groups of 25 male and 25 female rats were studied for a longer period. In Fig. 20.16 are given the average body-weight curves of the groups of male and female rats fed on diets containing the same percentages of uranyl nitrate hexahydrate as stated before; the test continued for 3 to 6 months. In the case of the male rats fed 0.5 per cent the average body weight was 15 g less than that of the control group at the end of the experiment. Otherwise all the growth curves for rats on the lower dietary percentages are practically coincident. The growth depression of the 2 per cent groups evidenced in the first 3 months was continued throughout the period of 22 weeks, at which time the males weighed about 130 g less than their control group and the females about 17 g less than their control group.