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CONTENTS

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SOME EFFECTS OF VARYING CALCIUM AND PHOSPHORUS INTAKE ON THE ESTRUS CYCLE AND REPRODUCTION IN THE RAT[1]

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INTRODUCTION

Normal functioning of the genital tract of our domestic animals is of great economic importance. The regularity of the estrus cycle has been shown by Evans and Bishop[(1)] to be a more delicate index of a sound physiology than is the growth curve alone. If the genital tract is to function normally, the food intake probably must be complete in all dietary essentials except for the limited period of time during which an animal may draw upon its reserves. The same factors are also involved in the rate of growth and of fattening on range feed of our food animals. Growth, fattening, and rate of reproduction vary greatly in different sections. The quantity and quality of the natural feed supply depends to a considerable extent upon the composition of the soil on which it is grown. Moisture and weather conditions and curing of grasses on the ground, with shattering of the seeds and processes involved in drying, bleaching, and possibly leaching, all have a bearing on the quality of the feed at the time it is utilized by the animal. The most efficient utilization of the vast amount of natural forage in the western range area is therefore largely a problem involving the proper supplementing of this feed in such

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a way as to keep the animal supplied at all times with a diet adequate in all essentials. In order to hope to approach this ideal situation we must bring to bear on range livestock feeding all of the recent discoveries in the field of nutrition, together with the results of researches now being conducted, even though at first thought they appear very abstract and with no possible application. Many of the original investigations in this field have been carried out on the rat, and in the experiments reported here we have used this animal. Although evidence obtained from experiments with one species of mammalia may not necessarily apply to another species, indications at the present time are to the effect that all mammalia require the same essential nutrients. Differences may result from variation in quantitative requirements or from the fact, as in the case of particular vitamins, that requirements of the species may be synthesized within the body.

Studies in this and other countries which have been compiled by Orr[2] have shown minerals, particularly calcium and phosphorus, frequently to be limiting factors. Furthermore, the dried grasses native to California ranges are known to be low in protein. Animals subsisting on natural vegetation may therefore be subjected to qualitative as well as quantitative undernutrition. It was the purpose of this study to extend our general knowledge of the relative influences of some of these factors on the estrus cycle and reproduction in the rat, in the hope of aiding in the interpretation of data now being obtained from analyses of range grasses and from observations on range cattle.

METHODS

The rats used in this work were kept in metallic cages, built thirty to the unit. One to three rats were maintained in each cage. During the course of the experiments coprophagy was observed in some of the animals. All of the cages were then supplied with false screen bottoms containing three meshes to the inch and raised 2 inches above the true bottom, which was sufficient to prevent the animals reaching through and obtaining fecal pellets. The general procedure was to select rats at weaning time and divide them into groups on the basis of weight. Rats weighing under 35 grams at 21 days of age were discarded. As far as possible litter-mates were equally divided between groups. Except in experiment 5 the rats were then fed a normal diet (diet 1) until sexual maturity, when they were placed upon the experimental diets. By this means it was possible to study the effect of the various diets upon the functioning of ovaries which had been allowed to develop on a normal diet.

The stage of the estrus cycle was determined by the microscopic examination of the vaginal smear and recorded daily. The weights of the rats were taken at seven-day intervals and later changed to five-day intervals, and recorded on the individual record cards. The animals were given tap water from glass bottles provided with copper tubes and attached to the front of the cages. The feed was weighed into feed cups attached to the rear of the cages. They were fed daily, the amount of feed placed in the cups being slightly in excess of anticipated consumption, in order to minimize waste. The rats were maintained on experimental diets for varying periods up to eight months. During these experiments practically no losses of animals occurred from intercurrent disease; individual variations will be mentioned. At the conclusion of the experiments all the rats were killed and post mortem examinations made. In some cases the animals were killed by bleeding from the throat to collect blood from which serum could be separated for calcium and phosphorus determination. Some difficulty was experienced in getting sufficient blood from individual animals for this work.

EXPERIMENTS

The diets used in experiments 1, 2, and 3 are given in table 1.

TABLE 1

COMPOSITION OF DIETS USED IN EXPERIMENTS 1, 2, AND 3

Ingredients	Diet 1	Diet 3	Diet 4	Diet 5	Diet 6
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Casein	15.0	4.0	4.0	4.0	15.0
Whole milk powder	10.0	3.0	3.0	3.0	10.0
Ground wheat	67.5	25.0	25.0	25.0	69.0
Butter	5.2	5.2	5.2	5.2	5.2
NaCl	0.8	0.8	0.8	0.8	0.8
$CaCO_3$	1.5	1.5	1.5	.....	.....
KH_2PO_4	.....	1.3	.....	.....	.....
Dextrin	.....	39.2	40.5	42.0	.....
Gelatin	.....	20.0	20.0	20.0	.....
Milligrams calcium per 100 grams	820	752	739	140	220
Milligrams phosphorus per 100 grams	500	531	178	178	500

Diet 1 is the McCollum normal stock diet. Diet 3 was made by reducing the ingredients of diet 1, which were rich in phosphorus, and replacing them with dextrin and gelatin. The phosphorus was maintained at approximately the level of diet 1 by the addition of KH_2PO_4. Diet 4 was similar to diet 3 except that the KH_2PO_4 was

replaced with dextrin, thus making the ration low in phosphorus. Both the KH_2PO_4 and $CaCO_3$ were replaced by dextrin in diet 5, thus making it low in both calcium and phosphorus. Diet 6 was a low calcium diet, differing from diet 1 only by the replacement of $CaCO_3$ with ground wheat.

Experiment 1.—The object of this experiment was to study the influence of a low phosphorus diet (diet 4) upon the regularity of the estrus cycle. A total of 24 rats were used. They were fed diet 1 from weaning time until sexual maturity. At the time they were placed upon the experimental diets, the rats varied from 48 to 57 days of age and all had had from one to three periods of estrus. They were divided into three groups of 6, 6, and 12. Group 1 was continued on diet 1 and was used as a check on the adequacy of diet 3.

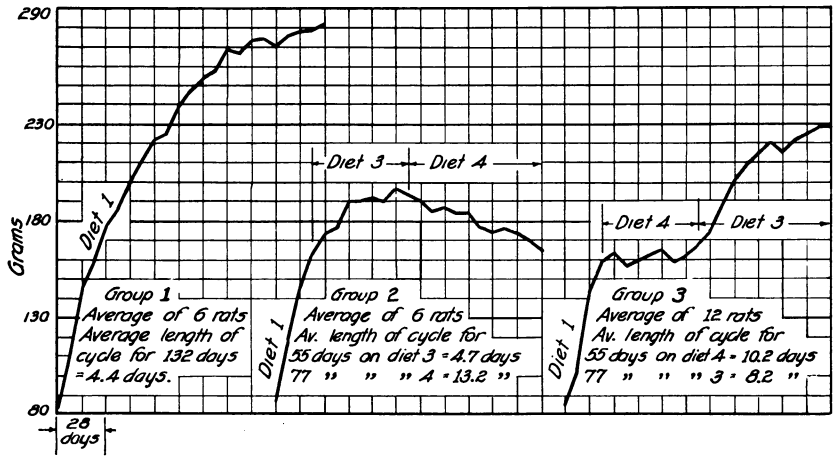

Fig. 1. Estrus cycle data and average growth curves of rats in experiment 1.

Group 2 was fed diet 3 and was a control for group 3, which received diet 4, the low phosphorus diet. The first period covered 55 days, at the end of which time the diets of groups 2 and 3 were reversed. At the termination of the second period, which covered 77 days, the animals were killed, and autopsied. The rats were not in screen bottom cages during this experiment. Coprophagy developed in some cases, and eating of feces had evidently improved their condition, as those individuals transferred to screen bottom cages rapidly decreased in weight. The estrus cycle was regular for the entire period in all rats on diet 1, and averaged 4.4 days per cycle during the entire 132 days.

The average growth curves and the average data on the length of the estrus cycle are given in figure 1. The growth curves show that diet 3 was not fully adequate for normal growth. Phosphorus was shown to be an important limiting factor in diet 4, as evidenced by the stopping of growth in group 3 and the falling off of the growth curve in group 2 when changed to this diet at the expiration of 55 days on diet 3. There was also a marked increase in growth of group 3 when changed from diet 4 to diet 3.

The estrus cycle of group 2 during the first 55 days on diet 3 was normal. Their general physical condition was much better at the end of this period than the animals in group 3. Eight of the twelve animals in group 3 were in poor physical condition. Six ceased having estrus cycles after a period of two to three weeks and two were irregular. The remaining four continued to have regular cycles and were in better condition than the rest of the group. It was observed that they were eating feces. They were then transferred to screen bottom cages when their physical condition declined like the rest of the group and estrus either ceased or became irregular.

In the second period, six of the rats in group 3, then on diet 3, developed estrus cycles and became regular within one to three weeks. The remainder did not become regular, perhaps because of their poor physical condition, possibly complicated by kidney involvment. Half of the animals in group 2, receiving diet 4, ceased having estrus periods in from one to three weeks, while the remainder were irregular. The kidneys of all of the rats in groups 2 and 3 were affected in varying degrees, characterized by pitted and irregular outline of the cortex and a mottled appearance. Calculi were, in several cases, found in the bladder.

Experiment 2.—The object of this experiment was to study the effect of a low calcium diet upon estrus cycle. Two groups of 12 rats each were used. Group 1 was fed diet 1, as controls. Group 2 was fed diet 6, which differed from diet 1 only in that $CaCO_3$ was replaced by ground wheat, thus lowering the calcium from 0.82 per cent to 0.22 per cent of the diet. Both groups were confined in screen bottom cages.

The rats on diet 6 had normal estrus cycles from sexual maturity to 100 days of age, and the growth was comparable to that of animals on diet 1. Both groups were bred. All of the rats on diet 6 gave birth to normal litters. The number of young in each litter was reduced to 6, which were carried through the lactation period. The weaning weights at 21 days varied from 40 to 57 grams. The mothers were

rebred in from 2 to 11 days after weaning the young, and the second litters averaged about the same as the first. The breeding and lactation records of these rats surpassed those of the equal number of controls in this particular case.

Experiment 3.—The object of this experiment was to study the effects of varying the calcium and phosphorus intake on the fertility of males. Three groups of six male rats each were started on experiment at the same time as the female groups in experiment 1, and carried on diet 1 for the same length of time. The first group then received diet 3; the second, diet 5 (low Ca and P); and the third, diet 4 (low P).

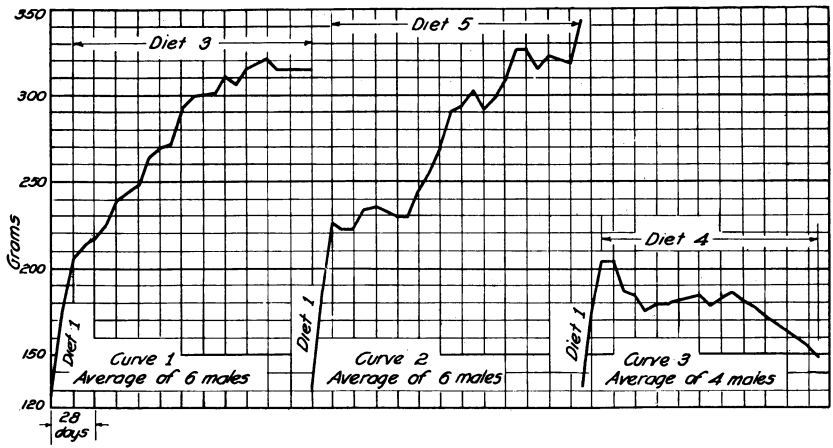

Fig. 2. Average growth curves, showing the effect of varying the calcium and phosphorus intake on male rats.

The average growth curves of the groups on diets 3 and 5, respectively, and of four of the rats on diet 4 are shown in figure 2. Group 1 made fairly regular gains, reaching an average weight of about 320 grams. The individual final weight varied from 261 to 400 grams. The lightest rat, B 12, revealed on post mortem examination a pale yellowish color of the liver, mottled appearance of the right kidney, and several small calculi in the urinary bladder.

Rat G 23 in this group died from stoppage of the urethra by calculi. The abdominal and thoracic cavities were filled with fluid; the bladder was distended and contained calculi and flocculent material. The kidneys had pitted areas in the cortex. Rat B 19 also had yellowish and pitted areas in the kidneys. The organs of the remaining three rats appeared normal.

The rats on diet 5 appeared to adjust themselves after a time and eventually reached a weight similar to those on diet 3. On post mortem examination the organs appeared normal in all the rats on this diet.

The rats on diet 4 lost weight immediately after the change from diet 1. One died at the end of nine weeks. Another was killed at the end of seventeen weeks. The others died shortly after the period represented by the weight curve. A post mortem examination of one of these showed the kidneys to be irregular in outline with yellowish areas. Calculi were found in the bladder.

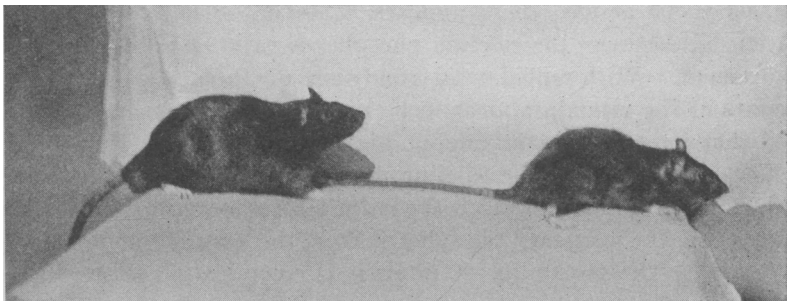

Fig. 3. Rats from diets 5 and 4, respectively, illustrating the result of varying the calcium to phosphorus ratio from 0.8:1 to 4:1. The phosphorus in both cases was maintained at the low level of 0.18 per cent.

Figure 3 shows the emaciated condition of the rat on the right, fed diet 4, as compared to the good condition of the rat on the left receiving the same diet, less 1.5 per cent calcium carbonate (diet 5), which reduced the calcium to phosphorus ratio to a more favorable relationship.

After 120 days on the diet these males were tested for fertility with normal females on diet 1. The results of the breeding tests are given in table 2. Each male was placed overnight with a female in proper stage for breeding. On examination of the vaginal smear the following morning, if no sperms were found, it was designated a negative mating; when sperms were found, a positive mating.

The diet 4 rats were in very poor condition when the mating tests were made. In fact, it was surprising that animals in such a cachectic condition would mate at all. The data were too meager for any inferences to be drawn, although in the case of the two positive matings of diet 4 rats, the sperms did not appear normal. One of these resulted in an apparently normal litter which died a few days

after birth. The other female apparently resorbed the fetuses, as a vaginal smear taken on the fourteenth day showed red blood cells.

The growth curves of the females in experiment 1 showed that diet 3 was inadequate for normal growth and that phosphorus was a limiting factor in diet 4 as compared with diet 3, both for growth and for normal functioning of the ovaries. The growth of the males on diet 5 (low calcium and phosphorus), as compared with the growth of the males on diet 4 (low phosphorus), indicated that the ratio of calcium to phosphorus is also involved rather than an absolute deficiency of phosphorus. The ratio of calcium to phosphorus is known to be an important factor in rickets. The vitamin D content of the diets was assumed to be ample; there appears, however, to be a quantitative relationship between the calcium phosphorus ratio and the vitamin D requirement. With unbalanced condition of these elements larger amounts of the vitamin appear to be required. It is generally recognized that improvement in calcium-high, phosphorus-low rickets may be effected by lowering the calcium, increasing the phosphorus, or by administering vitamin D. That the latter may in some way be involved in liberating the necessary phosphorus from the tissues is suggested by Bergeim.[3] The possibility of vitamin D complication is considered in experiment 4.

TABLE 2

FERTILITY TESTS WITH MALES

Diet	Male rat	Number of negative matings	Number of positive matings	Litter
Diet 3	W17	0	1	Normal
	B16	0	1	Normal
	W19	0	1	Normal
	G20	0	1	Normal
	G23	2	1	Normal
	B12	0	1	Normal
	G21	3	0	.....
Diet 4	B13	0	1*	Apparently normal† at birth
	B17	2	1‡	Red blood corpuscles, 14 days; resorption
Diet 5	W21	0	1	Normal
	B19	0	1	Normal
	W16	0	1	Normal
	B18	2	1	Normal
	W18	0	1	Normal
	B15	0	1	Normal

* Sperm did not appear normal; there were many loose sperm heads and tails.
† All died. Mother apparently failed to lactate.
‡ A few sperm heads only were found.

It seemed probable that the deficiency of diet 3 for normal growth resulted from poor quality of protein, since a greater part was furnished by gelatin and since only small quantities of milk and wheat protein were included in the diet.

The general appearance of the kidneys on macroscopic examination was similar to that reported by Jackson, Sommer, and Rose,[4] which they attributed to a deleterious effect of gelatin.

Experiment 4.—This experiment was planned to check the results of experiment 1 and to study further the influence of the calcium to phosphorus ratio on the estrus cycle. Diets 3 and 4 were obviously not entirely satisfactory, since it was desired to study variations in calcium and phosphorus uncomplicated, if possible, by other factors. When this was recognized during the progress of the previous experiments, some preliminary tests were run which demonstrated good growth from weaning time and sexual maturity at an average age of 50 days on a diet in which 10 per cent of egg albumin replaced a similar amount of gelatin in diet 3.

The diets used in experiment 4, with the exception of diet 1 (see table 1), are shown in table 3.

TABLE 3

COMPOSITION OF DIETS USED IN EXPERIMENT 4

Ingredients	Diet 9	Diet 10	Diet 14	Diet 15
	per cent	*per cent*	*per cent*	*per cent*
Casein	4.0	4.0	4.0	4.0
Whole milk powder	3.0	3.0	3.0	3.0
Ground wheat	25.0	25.0	25.0	25.0
Butter	5.2	5.2	5.2	5.2
NaCl	0.8	0.8	0.8	0.8
$CaCO_3$	1.5	1.5	1.5	.....
KH_2PO_4	1.3	.....	1.3	.....
Dextrin	39.2	40.5	37.2	42.0
Gelatin	10.0	10.0	10.0	10.0
Egg albumin	10.0	10.0	10.0	10.0
Yeast	.....	.....	2.0	.....
Milligrams calcium per 100 grams	825	825	825	225
Milligrams phosphorus per 100 grams	557	220	572	220

Thirty-nine rats were placed on experiment on May 15, 1929. During the first 25 days, covering the time from a few days after weaning until sexual maturity, all the animals were fed diet 1 and developed in a normal manner. They were then all placed in screen bottom cages; and during a second period, covering 60 days, 9 remained on diet 1 as controls, 15 were given diet 9, and 15 received

diet 10. The rats on diet 9 consumed about 9.25 grams of feed daily; those on diet 10 about 7.3 grams daily during this period. The average amount of feed consumed per 100 grams live weight for those on diet 9 and diet 10 was approximately 5.4 and 4.7 grams, respectively. The data on food consumption include the food eaten plus a variable amount of waste. Since the amount of feed wasted was greater in the case of the rats on diet 10, the conclusion that these rats ate less per day and less per 100 grams body weight is justified.

The growth curves of all three groups are shown in figure 4, each curve being a composite of three rats kept together in the same cage.

At the end of the second period three animals from each group represented by curves 3, 8, and 13, were killed for post mortem examination, and their blood was collected for phosphorus determinations. Small calculi were found in the bladder of one rat on diet 10. The surface of the kidneys of the other two was granular in appearance, and each kidney had one or two pitted areas. The organs of the rats on diet 9 and diet 1 appeared normal. The inorganic phosphorus content of the blood serum of these rats was as follows:

Diet 1	Milligrams phosphorus per 100 cc	Diet 9	Milligrams phosphorus per 100 cc	Diet 10	Milligrams phosphorus per 100 cc
Rat B109	7.44	Rat B117	7.73	Rat B108	9.74
Rat B115	12.40	Rat B125	9.38	Rat W187	5.56
Rat G134	11.66	Rat B110	12.54	Rat BH 9	4.43

The remaining 30 animals were continued on the experiment.

The rats in group 1 were fed diet 1 in order to afford direct comparison of growth rate and estrus cycle history, and to check any possible environmental conditions in the laboratory other than diet which might influence the estrus cycle. Fairly high temperatures cannot always be avoided during the summer months in this laboratory, and the effect of this condition on rats eating normal diets had not been determined previously. At the end of the second period the three animals represented by curve 2 were changed to diet 10 to ascertain whether or not it would affect the estrus cycle in practically mature rats raised on a normal diet. An obvious break in the growth curve coincides with diet 10 feeding. The estrus cycle was not affected.

The rate of growth of rats in group 2 which were fed on diet 9 during the second period compares favorably with that for diet 1, although on the average the curve is slightly less steep. The average length of cycles during this period was 8.6 days as compared with 6.3 days for the controls of group 1.

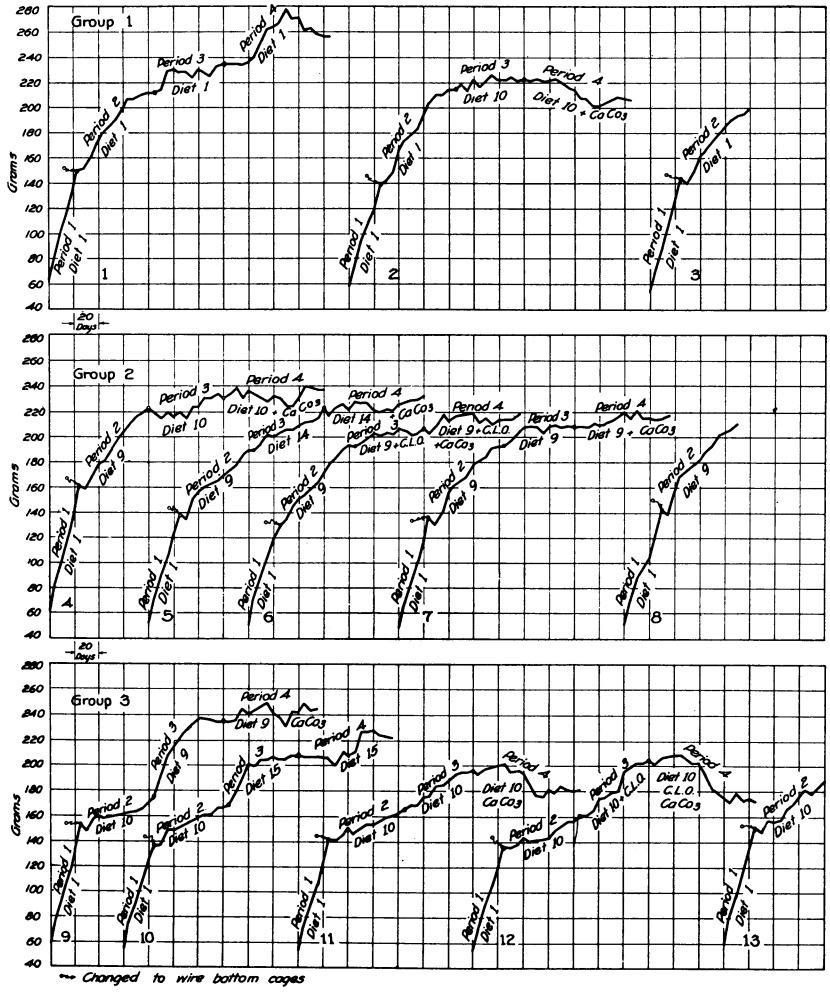

Fig. 4. Effect of varying diets on average growth curves of rats fed over four periods of time.

During the third period the rats represented by curve 4 had normal cycles (4.5 days) similar to group 1, curve 2.

The rats represented by curve 5 were given 2 per cent yeast (diet 14). Whether the continued growth of these rats compared to those represented by curves 6 and 7 in this group resulted from yeast or from individual variation cannot be definitely stated. The average length of the cycle (4 to 6 days) in all these groups is so nearly the same that no favorable influence can be attributed to yeast feeding.

The addition of cod liver oil to the three rats represented by curve 6 had no apparent beneficial effect.

The rats in group 3 were placed on diet 10 during the second period, and 12 of the 15 had no estrus cycles after the first five days. The remaining 3 had one, three, and three cycles, respectively, during the period. The diets of the 12 rats remaining in this group were not identical during the third period. When the 3 represented by curve 9 were changed to diet 9, the growth response was immediate, and estrus cycles started within a few days and averaged 4.5 days for the period.

The rats represented by curve 10 were changed to diet 15 (low calcium and phosphorus) and they likewise showed an immediate growth response. All had cycles within a week after the change of diet and continued regular with five-day average intervals. The initial growth response on lowering the calcium was practically parallel to that of increasing the phosphorus (curve 9). This diet has about the same amount each of calcium and phosphorus, namely 0.22 per cent. Either the level of both was too low to maintain the initial growth rate, or they had reached a point where they would normally plateau.

The rats represented by curve 11 were continued on diet 10 during the third period; and all began to have regular 5-day cycles following the beginning of this period, although they started 4 or 5 days later than the rats represented by the previous two curves. Their growth rate remained about the same. The phosphorus content of the diets was checked and found to be the same as the previous determination. This change occurred when the rats were about 100 days of age and when the growth curves of the diet 9 and diet 1 rats were definitely leveling out. Growth in the diet 10 rats appeared to have been in excess of the increase in weight, and possibly skeletal growth was decreasing at this time.

Sherman and Quinn[5] in a study of the phosphorus content of the body in relation to age, growth, and food, state, "The average percentage of phosphorus in the body of the normal white rat is found to increase from 0.34 per cent at birth to about 0.49 per cent at 15 days; 0.53 to 0.56 per cent at 28 days; 0.57 to 0.65 per cent at 61 days; 0.62 to 0.68 per cent at 3 months; 0.65 to 0.69 per cent at 4 months, and 0.70 to 0.75 per cent in adult life." They also show that in making normal growth from birth to maturity, the original weight is multiplied by about 70, while the original phosphorus must be multiplied by about 150 and the calcium by about 340.

The period during which the rats on the low phosphorus diet failed to ovulate is a period in which the normal rat is increasing not only

in body weight but also in phosphorus in relation to body weight. The time at which these rats began to ovulate coincides with the normal time at which the percentage of phosphorus becomes practically constant with body weight.

The rats were continued in a fourth period during which $CaCO_3$ was added to all the diets except those represented by curve 1 of group 1, and by curve 10 of group 3, to secure additional evidence on the influence of changing the calcium to prosphorus ratio.

At the time this change was made, all of the rats had been having regular 4 to 6 day cycles for the previous 45 days (period 3). The addition of 1 per cent $CaCO_3$ to diets 9 and 10 made the calcium to phosphorus ratio 2.1:1 and 5.5:1, respectively. Twenty-five days on this level produced no marked change in weight or in the regularity of the estrus cycle. At this time 2 per cent $CaCO_3$ was added to the diets, making the calcium to phosphorus ratio practically 3:1 and 8:1, respectively, and continued for 55 days.

The most significant effect of the addition of $CaCO_3$ was on the rats in group 3, curves 11 and 12, which had been on diet 10 continuously from sexual maturity. Four of the six rats had no estrus during the 55 day period, a fifth had one cycle, and the sixth had two cycles. All of these rats ceased ovulating during period 2, returned to normal 4 to 6-day cycles during period 3, but with the addition of 2 per cent $CaCO_3$ again ceased ovulating.

TABLE 4

POST MORTEM EXAMINATIONS AND BLOOD PHOSPHORUS DETERMIATIONS

Curve No.		Post mortem	Milligrams P per 100 cc blood serum
Group 1	1	Organs appeared normal	8.00
	2	Kidneys somewhat enlarged. Kidneys in 2 rats slightly pitted, 1 showing hydronephrosis of right kidney. Two rats had extensive gelatinous infiltration of the lungs	6.00
Group 2	4	Kidneys of 2 rats mildly affected. Kidney of 1 rat much enlarged and surface irregular	6.42
	5	All organs appeared normal	8.20
	6	Kidneys of 1 rat slightly pitted. Others appeared normal	7.70
	7	Organs appeared normal	7.37
	9	Kidneys somewhat enlarged, and in 2 rats there were pitted areas. Atrophy of right kidney in one rat	7.11
	10	Organs appeared normal	6.88
Group 3	11	Kidneys of all rats much enlarged, surface granular. Pale color	5.00
	12	Marked enlargement of kidneys. Surface granular and pale. Hydronephrosis in 2 cases. Ureter plugged at entrance to bladder. No calculi found	5.20

All the rats were then killed for post mortem examination and blood analysis, at which time their average age was 233 days. Blood phosphorus analyses were made by mixing equal quantities of serum from each of the three rats.

The phosphorus content of the blood serum of the rats fed diet 9 plus $CaCO_3$ (curves 5, 6, 7, and 9) and diet 1 plus $CaCO_3$ (curve 1) varied from 7.11 to 8.2 mg phosphorus per 100 cc of serum. The rats which were on diets 9 and 1 during the second period and diet 10 during the third and fourth periods (curves 2 and 4) had a lower content of phosphorus in the blood serum, namely 6.0 and 6.4 mg per 100 cc. The rats represented by curves 11 and 12, which had been on diet 10 continuously, showed a still lower value of 5.0 and 5.2 mg phosphorus per 100 cc, respectively.

The feed records show no significant difference in consumption during the fourth period.

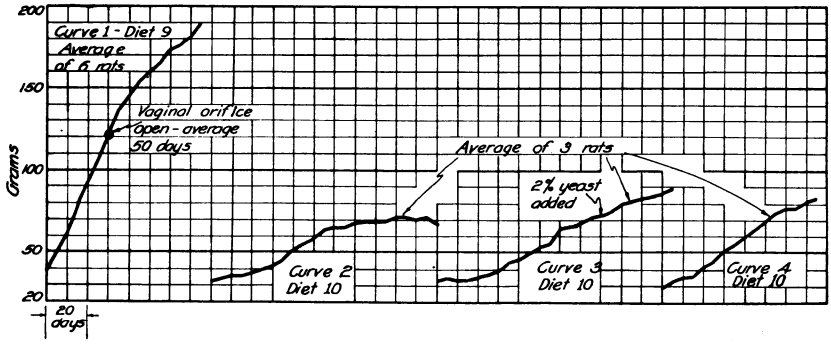

Fig. 5. Average growth curves of rats, showing effect of a low phosphorus diet from weaning time.

Experiment 5.—This was planned to study the influence upon growth and sexual maturity in females placed on a low phosphorus diet at weaning time. Nine female rats from mothers fed from weaning time and during lactation on diet 9 were placed on diet 10 at 21 days of age. Six litter-mate female controls were fed diet 9. The weight curves are shown in figure 5.

The weight of the group on diet 9 (curve 1) at 100 days is somewhat under that of the stock rats, but may be considered fairly satisfactory growth.

The vaginas opened at from 40 to 55 days of age with an average of 50 days. Estrus cycles were regular at 4 to 6-day intervals during the time indicated by the weight curve.

Curve 2 is an average of three rats on diet 10 up to 130 days of age. The vagina opened in one of these rats at 100 days, but there was no evidence of estrus. The vaginal orifice of the remaining two was still closed at 130 days.

Curve 3 is an average of three rats on diet 10 up to 100 days of age, at which time 2 per cent yeast was added to the diet. At 130 days of age, the vagina had not opened in any of this group.

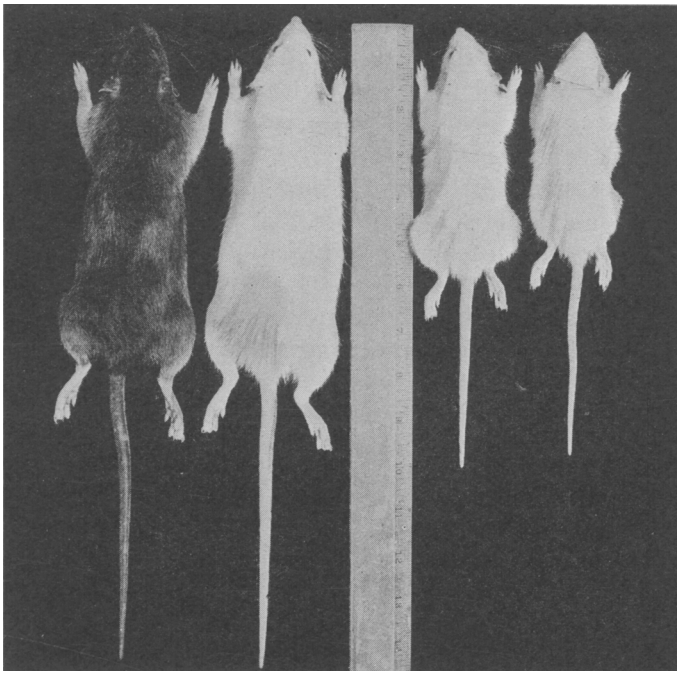

Fig. 6. Rats at 100 days of age, showing retarded growth in those on the right which received a diet containing 0.22 per cent phosphorus with a calcium to phosphorus ratio of 4:1 (diet 10). On the left, controls fed diet 9 are shown.

Curve 4 is an average of three rats on diet 10 to 100 days of age, at which time they were killed. The vagina opened in two of these rats a few days prior to killing. The better growth curve is due largely to one rat which made much more rapid gain than any of the others. All of these rats were in screen bottom cages throughout the experiment.

It is evident that phosphorus fed at a level of .22 per cent with a calcium to phosphorus ratio of approximately 4:1 has a very marked influence upon growth rate, sexual maturity, and ovulation history of rats placed upon this diet at 21 days of age (see fig. 6).

DISCUSSION

The experiments show that the effect of low phosphorus diets on female rats varies with age. A diet containing 0.22 per cent phosphorus with a calcium to phosphorus ratio of approximately 4:1 seriously interfered with growth and sexual maturity of female rats placed upon the diet at weaning time. Diets containing 0.18 to 0.22 per cent phosphorus with a calcium to phosphorus ratio of approximately 4:1, fed to rats from sexual maturity to about 100 days of age, caused ovulation to be irregular or to cease entirely. This level of phosphorus, however, appears adequate to maintain normal estrus cycle after about 110 days of age.

Experiments 2 and 4 have shown that the calcium to phosphorus ratio, as well as the absolute phosphorus intake, is involved in the results obtained from these diets. The widening of the calcium to phosphorus ratio to 8:1, with phosphorus 0.22 per cent of the diet, resulted in cessation of ovulation in practically mature rats which had been fed since sexual maturity on a low phosphorous diet.

There appeared to be a relation between low phosphorus content of the blood serum and irregular ovulation history, but more data will have to be secured before this can be definitely concluded.

Experiment 2 showed that lowering calcium from 0.82 to 0.22 per cent in a diet adequate in other essentials did not limit growth, estrus, gestation, or lactation, at least through two successive lactation periods.

It is recognized that the vitamin B content of the experimental diets with the exception of diet 6, was lower than that of diet 1, the decrease being coincident with the reduction of ingredients high in phosphorus. The difference in response, however, was so definite between the groups in which the only difference in the diets was the addition or withdrawal of KH_2PO_4, or $CaCO_3$, that any direct bearing of vitamin B on the results obtained appears remote.

The regularity with which kidney lesions were found in the experimental animals appeared significant. In no case were they found in animals on diets low both in calcium and phosphorus (diets 5 and 15). Very slight indications of abnormality were observed in the kidneys of a few rats, and definite lesions in two rats on diets 3 and 9. This condition has never been observed in rats fed diet 1. Marked abnormality was always associated with diets in which the phosphorus was low and the calcium to phosphorus ratio 4 or more to 1

(diets 4 and 10). The most striking abnormality was found when the ratio was 8:1 (diet 10, plus 2 per cent $CaCO_3$).

The diets used by Jackson, Sommer, and Rose[4] which produced "badly deteriorated kidneys" contained a salt mixture that furnished 212 mg phosphorus per 100 grams of diet when fed at the 4 per cent level. Since gelatin fed at the level of 35 per cent was the sole source of protein and since no other food in the diet supplied phosphorus, the phosphorus content of the diet could not be much more than 0.22 per cent, which is similar to the amount in the low phosphorus diets in these experiments. When they replaced casein with gelatin, the phosphorus was lowered from about 0.37 per cent to about 0.22 per cent. Although the calcium content of their diets was somewhat lower than those used by us (diets 4 and 10), their observations on both the kidney abnormality and the nutritive properties of gelatin were probably complicated by phosphorus deficiency. However, we have obtained growth comparable to rats on diet 9, and much superior to rats on diet 3, on a diet differing from diet 3 only by replacing the gelatin with dextrin, which would tend to confirm their observations that gelatin may be actually detrimental.

The variation of calcium and phosphorus of the diets used in these experiments does not approach that occuring on the range. Samples collected during the past year have varied from 0.04 to 0.56 per cent phosphorus and from 0.17 to 2.90 per cent calcium. The calcium to phosphorus ratio varied from 0.5:1 to 44.5:1. The silica-free ash varied from 1.64 to 14.48 per cent, and the alkalinity from 128 to 2006 cc tenth normal reagent per 100 grams of sample. The protein content of these samples varied from 3.18 to 28.0 per cent, 3.5 to 6.0 per cent being common in the dry feed. It has been shown in a previous publication[6] that there is a decrease in both protein and phosphorus in range forage during the maturing and curing process. The influence of protein intake upon the estrus cycle of the rat is being investigated.

The age and diet relationship brought out in the experiments with rats appears to have a parallel in range cattle. Yearling heifers which have been given a good start in life on mothers' milk and grass will usually breed. In certain areas at least the majority of the heifers which become pregnant fail to breed the following year. Evidence which is accumulating indicates that the blood serum of yearling and two-year-old heifers on dry range feed may be only 50 per cent of that of animals of comparable age under optimum conditions and that these heifers may remain lower in blood phosphorus than mature cows for a time, at least, after new feed is avail-

able, whereas heifers are higher in blood phosphorus than mature cows under optimum conditions. Evidence that phosphorus deficiency interferes with normal ovulation in cattle has been reported by Eckles, Becker, and Palmer,[7] by Theiler, Green, and DuToit,[8] and by DuToit and Bisschop.[9]

In order to understand more fully the relationship of stage of development and feed intake to reproductive processes, it will be necessary to conduct experiments at all stages of growth and development and during repeated pregnancy and lactation periods, with diets in which the level of particular nutrients are varied. Extensive tests of the fertility of males on different diets will also be necessary.

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The titles of the Technical Papers of the California Agricultural Experiment Station, Nos. 1 to 20, which HILGARDIA replaces, and copies of which may be had on application to the Publication Secretary, Agricultural Experiment Station, Berkeley, are as follows:

1. The Removal of Sodium Carbonate from Soils, by Walter P. Kelley and Edward E. Thomas. January, 1923.

4. Effect of Sodium Chlorid and Calcium Chlorid upon the Growth and Composition of Young Orange Trees, by H. S. Reed and A. R. C. Haas. April, 1923.

5. Citrus Blast and Black Pit, by H. S. Fawcett, W. T. Horne, and A. F. Camp. May, 1923.

6. A Study of Deciduous Fruit Tree Rootstocks with Special Reference to Their Identification, by Myer J. Heppner. June, 1923.

7. A Study of the Darkening of Apple Tissue, by E. L. Overholser and W. V. Cruess. June, 1923.

8. Effect of Salts on the Intake of Inorganic Elements and on the Buffer System of the Plant, by D. R. Hoagland and J. C. Martin. July, 1923.

9. Experiments on the Reclamation of Alkali Soils by Leaching with Water and Gypsum, by P. L. Hibbard. August, 1923.

10. The Seasonal Variation of the Soil Moisture in a Walnut Grove in Relation to Hygroscopic Coefficient, by L. D. Batchelor and H. S. Reed. September, 1923.

11. Studies on the Effects of Sodium, Potassium, and Calcium on Young Orange Trees, by H. S. Reed and A. R. C. Haas. October, 1923.

12. The Effect of the Plant on the Reaction of the Culture Solution, by D. R. Hoagland. November, 1923.

14. The Respiration of Potato Tubers in Relation to the Occurrence of Blackheart, by J. P. Bennett and E. T. Bartholomew. January, 1924.

16. The Moisture Equivalent as Influenced by the Amount of Soil Used in its Determination, by F. J. Veihmeyer, O. W. Israelsen and J. P. Conrad. September, 1924.

17. Nutrient and Toxic Effects of Certain Ions on Citrus and Walnut Trees with Especial Reference to the Concentration and Ph of the Medium, by H. S. Reed and A. R. C. Haas. October, 1924.

18. Factors Influencing the Rate of Germination of Seed of Asparagus Officinalis, by H. A. Borthwick. March, 1925.

19. The Relation of the Subcutaneous Administration of Living Bacterium abortum to the Immunity and Carrier Problem of Bovine Infectious Abortion, by George H. Hart and Jacob Traum. April, 1925.

20. A Study of the Conductive Tissues in Shoots of the Bartlett Pear and the Relationship of Food Movement to Dominance of the Apical Buds, by Frank E. Gardner. April, 1925.