STUDIES ON

THESIS FOR T

MARY HE

1

Mercado Com
Pretado

STUDIES
ON HIGH PROTEIN DIETS

STUDIES ON HIGH PROTEIN DIETS

THESIS

Submitted to the Faculty of Michigan State College in partial fulfillment of the requirements for the degree of Master of Science.

BY

MARY HELEN FERGUSON

1930

ACKNOWLEDGMENTS - -

The author of this thesis wishes to acknowledge the valuable assistance of Dr. C. A. Hoppert, Associate Professor of Chemistry, for his aid in directing this experiment, and his kindly criticism of the manuscript.

The author also wishes to express her gratitude to Dr. L. B. Sholl, Assistant Animal Pathologist of the Experimental Station, for his kindness in making histological studies and to Mr. J. F. Witter, Assistant in Animal Pathology, for his kindness in making the sections.

TABLE OF CONTENTS

I Introduction Page 1

A. Review of Literature 2

II Experimental Work 8

A. Table I: Percentage Constituents of Diets 9

III Procedure 10

A. Animals Used 10

B. Care of Animals 10

C. Observations 10

IV Results 12

A. Growth 12

B. Table II: Average Gain of Animals in Series I - IV over a Period of Sixteen Weeks 12

C. Table III: Average Gain of Animals in Series V and VI Over a Period of Twelve Weeks 13

D. Bone development 13

E. Table IV: Percentage Ash in Femurs and Humuri of Rats on Various Rations 13

F. Condition of Teeth 14

G. Reaction of the Digestive Tract 15

H. Table VI: Average P_h Values of Five Parts of the Digestive Tract 15

I. Bacteria Found in Digestive Tract 16

J. Condition of Kidneys 18

TABLE OF CONTENTS

K. Table V: Relationships Between Body Weight and Kidney Weight of Rats — Page 19

V Discussion of Results — 23

VI Summary — 27

VII Bibliography — 30

INTRODUCTION

Proteins are a necessary constituent of plant and animal cells. Animals, unlike plants, cannot build their protein from inorganic materials of the air and soil but must obtain them from their food. The cleavage products of proteins, the amino acids, are the "building stones" for construction and maintenance of animal tissue.

Normally, so far as we know, the amino acids resulting from the digestion of protein, pass through the intestinal wall into the blood stream and are carried by the portal vein to the liver and then passed into the general circulation.

The amino acids, supplied in the food in excess of those needed for the various functions of the body and those from the normal breaking down of body protein, are deaminized largely in the liver. Most of the ammonia, thus formed, is converted into urea. Also there are other end products of protein metabolism, chiefly uric acid and creatinine. The latter is apparently independent of ingested protein. The kidneys are the main excretory organs for these products and dispose of a normal amount without suffering any injury. At present there is no direct proof that the kidneys have a limited capacity for eliminating the end products of protein intake.

The wide prevalence of kidney diseases, particularly nephritis, has led many investigators to make experimental

study of the effect of high protein diets with special reference to the condition of the kidneys in the hope that it might throw some light on the cause of human nephritis.

In reviewing the experimental results secured by various investigators, the opinion seems prevalent that with the exception of kidney hypertrophy, no damage was caused to the kidneys of animals fed with diets rich in protein.

(1) Osborne, Mendel, Park, and Darrow reported on the effects of diets, 90 percent or more protein, on various organs of rats grown to a large size. The only striking change found was in the kidneys which were hypertrophied but on microscopical examination showed no change of degenerative or inflammatory nature. This occurred when the protein was of animal or vegetable origin and was rich or poor in phosphorus.

Diets of 20, 45, and 95 parts protein, used by (2) Reader and Drummond, showed typical growth curves of male and female rats. At the 20 percent level growth was normal, at the 45 percent level growth was normal at first but retarded and at the level of 90 percent growth was subnormal, becoming stationary at about a third of the calculated normal weight. Reproduction was normal in the first group, few litters were produced in group two; and there was no reproduction in group three. In a number of animals, killed at about four months of age, the organs appeared normal with the exception of the kidneys which showed increasing hypertrophy with increased protein intake. No explanation for growth failure in high

protein diets was made but Hartwell's theory of a relationship between vitamin B and protein was said to be worthy of study.

It was found by (3) Hartwell that the young of female rats fed on diets of 0.9 to 6.0 grams of protein per day had spasms which increased in severity with the increase in protein. Also there was an increasing mortality and on the 6.0 gram protein diet none of the young survived. A second series was carried out similar to the first except that tomato juice was used as a source of Vitamin B in amounts increasing with the increase in protein. The young from females on 1.2 grams protein per day showed normal growth and development with 6 c.c. tomato juice per day. (4) Nelson corroborated Hartwell's theory that there seems to be a quantitative relationship between protein and vitamin B.

Rats fed on diets of approximately the same number of calories but of a larger amount of protein in some cases were found by (5) Jackson to grow normally and they showed no abnormalities in the urine and no clinical signs of nephritis. On autopsy the kidneys were found to be considerably hypertrophied but showed no lesions in the glomeruli, tubules, or interstitial tissue.

(6) Miller fed rats on diets rich in protein and from one animal in each group a kidney was removed to double the load on the remaining one. Kidneys of animals on high protein diets showed hypertrophy amounting in case of the nephrectomized animals to an increase in weight averaging 0.54 grams or 85 percent of the average weight of the right kidneys

of the controls. Blood analysis and microscopic examinations of kidneys showed no evidence of kidney damage.

The effect of high protein diets fed to white mice was studied by (7) Beard. He found that 75 to 80 percent protein diets gave subnormal growth but no marked differences in weight of kidneys and liver resulted after periods as long as 107 days.

Diets containing excess protein were used on rats by (8) Addis and E. M. and L. L. MacKay. The period of observation was made sufficiently long to cover what would correspond to thirty years of a man's life. The diets were made adequate for growth and maintenance. The rats on the high protein diet did not reach the weight of the controls. On post mortem examination the principal cause of this was found to be due to an almost complete absence of fat in the animals. No microscopic differences were found in the kidneys of the different groups but the weight of the kidneys was higher and of the liver lower in the high protein diets. This would seem to indicate that rats can live for a third of their life span on a diet very high in protein (supplied as casein) and yet escape any kidney damages. Moreover, this would tend to refute the hypothesis that a large consumption of protein is in and by itself necessarily harmful to the kidneys.

(9) Osborne, Mendel, Park, and Winternitz reviewed some previous experiments with rats on high protein diets, some of which had two thirds or more of the entire caloric intake

of protein, and found that the rats were capable of growing to adult size. The rats became adjusted to high protein diets and it is thought that the failure of other investigators to secure good growth on high protein diets was due to vitamin inadequacy of the rations. There was evidence of kidney disturbances and, as noticed before, hypertrophy of the kidney was a characteristic outcome of the high protein diet, the change taking place rapidly and with all proteins tested.

Contrary to these observations (10) Newburgh, and (11) Maynard and Bender have found actual injury of the kidneys of animals fed on high protein diets. Maynard and Bender made studies on the kidneys of three rats from a 50 percent protein ration, two from an 18 percent protein level and two from stock diet. The kidneys from the high protein group showed marked enlargements and considerable degeneration. A large amount of hyaline material was found distending the convoluting and collecting tubules and also a multiplication of connective tissue with a decreased number of tubules. Coagulum was present in the lumen of the tubules. In contrast the kidneys from the lower protein rations showed little or no hyaline and no connective tissue but did show a degeneration of tubules but of a lesser degree. Whether this is dangerous or not remains to be seen. At the date this was published a rat had been placed on a high protein diet and after the tenth lactation one kidney was removed. The animal had recovered, was in good health and pregnant thirty days after the operation.

According to Newburgh the degree of kidney injury is determined by the type of protein, its concentration, and the length of feeding. The character of the protein is more important than the other two factors. When the diet contained 25 percent protein there was no evidence of injury in the first period of eight months. After more than a year an abnormally large number of casts were found in the urine. Very little injury was caused in eight months by diets containing as high as 37 percent protein, but, after another eight months there was evidence of marked injury. The differences in the number of casts caused by different kinds of protein, fed at two different time intervals, are very striking. Casein, beef, and liver were fed at 75 percent levels for 240 and 480 days respectively. At the end of 240 days, the number of casts found in the urine from the casein diet was 1100, from the beef diet were 3200 casts, and that from the liver diet showed 15000 casts. At the end of 480 days there were 2400 and 16000 casts from the casein and beef diets respectively, and the animal from the liver diet was dead.

Diets of 75 percent dried liver caused granular kidneys in less than a year but the same amount of casein, fed 16 months, caused only moderate tubular injury. The effect of a similar amount of beef muscle was intermediate between these two. The kidneys of a rat receiving a diet of about a third of its weight in protein showed injuries of the tubules everywhere and atrophy of the tubular membrane but no evidence of injury of the glomerulus. A kidney from a rat on the same

diet but for a longer time showed marked injury of the tubules and glomeruli. It was shown that on a diet of liver protein rats would be killed in less than a year with chronic nephritis. The kidneys from a rat receiving 75 percent beef protein suggested an effect of chronic nephritis. Microscopic sections showed numerous areas in which seriously injured glomeruli and necrotic tubules were imbedded in fibrotic tissue.

A normal kidney was shown to be perfectly smooth but the kidney of a rat fed on a liver diet was rough and granular, filled with scars and masses of connective tissue, showing the gross appearance of "chronic nephritis".

EXPERIMENTAL WORK

In view of the fact that there is such conflicting evidence on the effects of high protein diets, it seemed desirable to do further work with high protein diets, using diets that simulated practical human diets and special reference made as to their effects on the kidneys. An attempt was made also to answer certain questions which suggested themselves in reviewing literature on this subject. In the event of kidney injury, is it simply a strain due to the elimination of large quantities of the normal end products of protein metabolism? Is it due to an abnormal intestinal flora that may be encouraged by a high protein diet or is it due to the increased elimination of ammonium salts of the fixed acids that result from the ingestion of large amounts of proteins which are acid forming?

Experimental work included observations on the kidneys, the intestinal flora, the reaction along the digestive tract, the growth of the animals, their bone development, and the condition of their teeth.

In this work a high protein diet with various modifications, intended to throw some light on the causes of kidney injury, was compared with a normal diet composed of similar ingredients. In all cases, the proteins used were of both animal and vegetable origin . As a control animals were fed the regular laboratory stock ration. The make up of these diets is given in Table I.

Table I: Percentage Constituents of Diets

Diet	1	2	High Protein 3a	3b	4a	4b	5	6	7	8a	8b	9a	9b	10a	10b
Corn meal	60	40	35	25	30	20	34	34.5	34	20	10	30	20	34	24
Whole milk powder	30	15								15	15				
Gelatine		10	20	25	20	25	20	20	20	20	25	20	25	20	25
Gluten		10	20	25	20	25	20	20	20	20	25	20	25	20	25
Sugar		10	10	10	10	10	10	10	10	10	10	10	10	10	10
Butter		5	5	5	5	5	5	5	5	5	5	5	5	5	5
Alfalfa	3	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Casein		5	5	5	5	5	5	5	5	5	5	5	5	5	5
Lactose					5	5									
$CaCO_3$							1								
$NaHCO_3$								.5	1						
Yeast												5	5		
Codliver oil														1	1
Oil meal	6														
NaCl	1														

spleen and digestive tract through the cecum were removed. The femurs and humuri were also removed for ashing and the teeth examined for dental caries.

The kidneys were closely examined microscopically, weighed accurately, and placed in Zenker's solution with the spleen and liver for histological examination. This study was kindly performed by Dr. L. B. Sholl of the Pathology Department.

The digestive tract, after removal, was divided into five parts, the stomach, three equal sections of the small intestine, and the cecum. The contents of each part were aseptically removed, by stripping, into 10 c.c. of sterile distilled water, thoroughly mixed and allowed to stand for approximately one minute. A small amount of each solution was removed for bacteriological examination; the P_h of the remaining solution was determined by the Quinhydrone Electrode method. After the first two series, I and II, a bacteriological examination was made only of the fecal material obtained by defaecation of the animal at the time of etherizing.

PROCEDURE

Animals Used

The animals used were of the laboratory albino and piebald stock.

Care of Animals

The rats were kept in round wire cages with raised wire bottoms. Each cage was placed in a granite pan with paper in the bottom which could easily be removed for cleaning purposes. The cages were cleaned twice a week.

The animals were watered daily by means of drinking tubes and fed once a day by placing the ration in feeding cups within the cage. For rations, see Table I.

The weights of the animals were taken once a week and recorded.

Six series of animals were placed on experiment. In each series, excepting VI, were fourteen animals, two in each cage on a single diet. As nearly as possible, the rats were distributed equally with respect to weight and sex. In each series two animals were fed stock ration and two were fed the "normal" ration. As indicated the series were numbered I through VI respectively. The animals were termed L_1, L_2, L_3 etc.

The animals were placed on experiment at the age of about four weeks with the exception of series IV, in which the animals were about five weeks of age.

Observations

As stated before, the animals were weighed each week and the weights recorded. They were kept on experiment for thirteen to twenty four weeks and were killed by etherizing after

weighing for the last time.

When the rat was dead it was opened and the kidneys, liver, spleen and digestive tract through the cecum were removed. The femurs and humuri were also removed for ashing and the teeth examined for dental caries.

The kidneys were closely examined microscopically, weighed accurately, and placed in Zenker's solution with the spleen and liver for histological examination. This study was kindly performed by Dr. L. B. Sholl of the Pathology Department.

The digestive tract, after removal, was divided into five parts, the stomach, three equal sections of the small intestine, and the cecum. The contents of each part were aseptically removed, by stripping, into 10 c.c. of sterile distilled water, thoroughly mixed and allowed to stand for approximately one minute. A small amount of each solution was removed for bacteriological examination; the P_h of the remaining solution was determined by the Quinhydrone Electrode method. After the first two series, I and II, a bacteriological examination was made only of the fecal material obtained by defaecation of the animal at the time of etherizing.

RESULTS

Growth

The differences in the rate of growth of animals on these various diets were very striking. The growth of the animals on the "normal" ration was consistently good and compared very favorably with that of the animals on control stock ration.

From the first, it was readily noticeable that the rate of growth of the animals on high protein was much less than that of the animals on "normal" and stock rations; this was true of all modifications of the high protein diets. For the first two or three weeks, the animals seemed just able to maintain themselves. From all indications, there was a period of adjustment to these diets, after which the animals began to grow. The only exceptions were the animals on high protein diets with milk and yeast added respectively. These animals gained weight from the first week but not to the extent of those of "normal" and stock control animals.

In no case did the final weight of the animals on high protein reach that of the normal and stock ration animals. Table II shows the average gain of the animals in Series I through Series IV over a period of six weeks and Table III shows the average gain of the animals in Series V and VI over a period of twelve weeks.

Table II: Average Gain of Animals in Series I - IV over a Period of Sixteen Weeks

Diet	Males	Females
1	231	141
2	227	141
3a	142	68
8a	153	131
4a	96	70
5	82	54

Table III: Average Gain of Animals in Series V and VI Over a Period of Twelve Weeks

Diet	Males	Females
1	219	
2	215	
3b	65	75
8b	171	81
9b	99	88
4b	140	117
10b	119	101

The number of animals on each diet can be found on Table V and the composition of the diets are in Table I. On Diet 8 in Table II there were no male animals and Diets 1 and 2 in Table III had no females.

Bone development

Table IV shows the averages of the results of ashing of the femurs and humuri of the animals in Series I through Series IV. The bones from the animals in Series V and VI were also ashed and the results, from these animals remaining on the diets for thirteen weeks, corresponded very closely to those in Table IV.

Table IV: Percent Ash in Femurs and Humuri of Rats on Various Rations

Diet	Average percent ash of femurs		Average percent ash of humuri	
	Males	Females	Males	Females
1	59.53	58.85	60.74	60.87
2	58.58	57.84	59.43	58.95
3a	55.38	57.45	55.19	56.26
8a	58.48	59.05	57.12	61.04
4a	57.43	55.91	57.52	58.30
5	54.85	55.77	56.46	57.58
7	55.57	56.96	58.8	56.98
9a	58.73	56.76	60.76	58.50
10a		58.43		60.51

There was not a great deal of difference in the ash analyses of the bones of these animals kept on the different diets. Bone development apparently was normal in all cases.

Condition of the teeth

Inasmuch as dental caries seemed prevalent among the laboratory animals observations were made on the teeth of all animals used in this experiment.

The incisors of all the animals were apparently normal, the enamel was hard and smooth, and there was no evidence of brittleness in the teeth. However, the prevalence of caries in the lower molars of the majority of animals was very astounding. This occurred in the "normal" and stock diets as well as in the high protein diets.

No attempt was made to carry out a detailed study of this condition as work is being done in this department on the effects of diet on teeth. Quite a number of skulls of animals from this experiment were removed and turned over to the department for future study.

One of the most striking results obtained from notations on the appearance of the teeth of the animals used in this experiment, was that dental caries seemed to first make their appearance in the teeth of "normal" and stock ration animals. Animals on high protein diets, with the addition of lactose, showed very bad cases of caries similar to that found in the cases of Diet 1 and Diet 2. In several cases there were two and three teeth missing from the lower molars of animals on these diets.

Carious teeth were found in animals on high protein diets.

The addition of milk, yeast, and cod liver oil to these high protein diets seemed to have some effect in retarding the appearance of caries in the teeth. However, they eventually became carious although to a somewhat lesser degree than the others.

An interesting fact presented itself in the case of four animals in Series III, receiving sodium bicarbonate with their high protein diets. These animals had the best teeth of the series although they were kept on the rations for twenty four weeks. Two of the animals had good teeth and two had only small cavities in their teeth. This lends itself to the suggestion that sodium bicarbonate probably neutralizes acid which might be instrumental in causing dental caries.

Reaction of the digestive tract

Table VI shows a comparison of the reactions along the digestive tract of the rats on diets 1, 2, 3a, 4a, 5, 6, 8a, 9a and 10a respectively of the first four series of animals. The numbers under P_h represent the following: 1, the stomach, 2, the first section of the small intestine, 3, the second section of the small intestine, 4, the third section of the small intestine and 5, the cecum.

Table VI: Average P_h Values of Five Parts of the Digestive Tract of Rats on Various Diets

P_h	Stock Diet	Normal	High Protein	H.P.& Milk	H.P.& Lactose	H.P.& $CaCO_3$	H.P.& $NaHCO_3$	H.P.& Yeast	H.P.& C.L.O
1	3.52	4.445	3.935	4.73	4.12	5.06	4.51	3.6	3.64
2	6.48	6.133	6.345	6.50	6.49	6.32	6.02	6.46	6.47
3	6.72	6.476	6.503	6.501	6.65	6.70	6.80	6.605	6.56
4	6.97	6.817	6.80	6.88	7.02	7.24	7.03	6.64	6.86
5	7.00	6.696	6.81	6.98	6.898	7.09	7.11	6.64	6.98

The P_h values of the digestive tract of animals on stock diet increase steadily from acid to the neutral point in the cecum. The last part of the small intestine is nearly neutral.

The average P_h value of the digestive tract of animals on normal ration increases through the last section of the small intestines which approaches the neutral point but does not reach it. There is a drop in the Ph of the cecum. The values for normal ration were slightly lower than those of the high protein rations.

The high protein diet seemed to cause an increase in the P_h of the digestive tract but the neutral point was not reached. The addition of lactose, calcium carbonate, and sodium bicarbonate caused a greater increase in P_h values. The last part of the small intestines of the animals receiving lactose and sodium bicarbonate was neutral and that of those receiving calcium carbonate was slightly alkaline. The reaction of the cecum dropped to neutral in the case of animals getting calcium carbonate and to nearly neutral (but on acid side) in animals receiving lactose; the cecum of the rats receiving sodium bicarbonate was slightly alkaline and of a value higher than that of any in the cecum of animals in these series. The addition of yeast caused a decided drop in the reaction of the digestive tract. The reactions were acid and of the same value in the cecum of the animals. Cod liver oil addition gave an increase in P_h values reaching very nearly the neutral point in the cecum.

Bacteria found in the digestive tracts

One cubic centimeter of the solution from each P_h determination was removed, aseptically, and plated on whey agar

which was found to be a very good medium for the bacterial growth. The plates were incubated at 37°C for 48 hours and examined with respect to the different types of colonies and the relative numbers. The different types were isolated and grown on different laboratory media. Note was made of the morphological and cultural characteristics.

In the first two series (Series I and II) it was found that the number of organisms increased along the digestive tract. The largest number of organisms was found in the cecum, in each case and in only one case of fourteen animals were bacteria found in the stomach. This was a single colony found in the stomach of a rat on the "normal" ration. On examination it was found to be a gram positive coccus and the characteristics of its morphology and growth on media indicate that it was probably a staphylococcus.

Coccus and bacillus forms were found in the digestive tract and fecal material of the animals studied. The number of bacillus forms increased and the number of coccus forms decreased along the digestive tract. In the fecal material bacillus forms were most prevalent. It was interesting to find that gram positive forms predominated in most of the cases. One type alone was gram negative; it was a short, plump rod resembling B. Coli in most ways but gave acid with no gas when grown on lactose broth. This organism completely peptonized litmus milk and formed a heavy white pellicle on it, it gave acid and gas when grown on dextrose, maltose and sucrose, and gave a distinct putrefactive odor when grown on whey agar.

Other organisms found were staphylococci and streptococci. The predominating forms found were rather large gram positive rods of varying sizes and characteristics, but, in general, resembling each other. A large, bipolarly stained, plump rod which completely peptonized milk, liquified gelatine with a pellicle formation, gave acid on all sugar broths but lactose was found in a number of cases in the cecum. A type of bacillus resembling B Acidophilus was quite prevalent in the digestive tracts of the rats. A smaller type of bacillus, also gram positive, was very common.

It was interesting to note that in the high protein diets there were more gram negative organisms than in the other diets but gram positive rods predominated. In cases of animals receiving lactose very few gram negative organisms were found. In the fecal material studied, large numbers of dead organisms were noticed.

Condition of Kidneys

One of the outstanding effects of high protein diets was the distinct hypertrophy of the kidneys. Table V shows the relationship between the body and kidney weight of all the rats used in this experiment.

Table V: Relationship Between Body Weight and Kidney Weight of Rats

Diet	Number of Animals	Average time on diets	Average Wt. per animal	Average Wt. of Kidneys	Percent
1	3 males	19 weeks	303 gms.	2.0990	.6927
	2 females	24	200	1.5604	.7802
2	8 males	20	294	2.7167	.9240
	5 females	20	203	1.7757	.8747
3a	3 males	19	246	2.8072	1.1409
	4 females	19	143	1.7079	1.1943
3b	2 males	13	128	2.1657	1.6919
	2 females	13	130	2.1820	1.6784
8a	2 males	22	256	3.2420	1.2664
	3 females	22	203	2.3448	1.1550
8b	2 males	13	231	3.1006	1.3422
	2 females	13	132	1.6813	1.2737
4a	5 males	21	220	2.7392	1.2450
	6 females	21	166	2.0938	1.2613
4b	1 male	13	157	2.6765	1.7047
	2 females	13	136	2.4608	1.8094
($CaCO_3$) 5	4 males	23	178	2.3465	1.3182
	2 females	23	132	1.6368	1.2400
($NaHCO_3$) 7	2 males	24	239	2.9251	1.2152
	2 females	24	195	2.3505	1.2053
9a Yeast	1 male	20	288	2.9251	1.0156
	1 female	20	236	2.4950	1.0572
9b Yeast	2 males	13	195	2.8940	1.4841
	2 females	13	178	2.4156	1.3570
C.L.O. 10a	2 females	21	181	2.1226	1.1727
C.L.O 10b	2 males	13	198	2.7453	1.3865
	2 females	13	151	2.0367	1.3488

From the results of this experiment, as shown in Table V, it is very apparent that an increased intake of protein causes an increase in the size of the kidneys. Using animals on stock ration as a standard it seems that the "normal" ration is slightly high in protein. Upon examination of the external appearance of the kidneys of these animals, it was found that there were distinct and marked differences in their gross appearance. The kidneys from the "normal" and stock ration animals had small, smooth, firm, dark kidneys. Those from the animals on high protein diets varied from large, smooth, dark or slightly discolored kidneys to rough, granular, light colored kidneys. The exterior appearance of the kidneys from animals on high protein diets varied with the diets and the different modifications. Some kidneys were merely enlarged with a few cavity-like holes in them and others were large, dull, porous kidneys with so many perforations that it made them have the exterior appearance of a sponge.

The effects of increasing the protein content of the diets is shown in comparing the kidneys of the first four series of animals with those of the last two series. The latter series were on high levels of protein but for a shorter length of time. The size of their kidneys was greater in proportion to their weight and the gross appearance was more outstanding than in the first four series.

The effect of time in feeding high protein diets was clearly shown by animals in the first four series. In cases of the animals killed after being on diets sixteen to twenty weeks, there were no decided changes in the appearance of the kidneys

although there was distinct hypertrophy. The kidneys of animals killed at the end of twenty-two to twenty-four weeks on the various high protein diets showed distinct changes in external appearances.

In general the kidneys of the animals of the first series (I to IV) may be described as follows: On high protein diet they were slightly granular with small perforations and several larger cavities, on high protein with milk added they were somewhat porous and rough with dark lines; on high protein with lactose they were finely granular with a few holes and perforations; on high protein with $CaCO_3$ they were very bad, having a very rough, porous, irregular, granular appearance and in one case part of the kidney appeared infected and eaten away; those on high protein with sodium bicarbonate were slightly granular with a few perforations; those on high protein with yeast were slightly rough but had no holes; and those receiving cod liver oil with high protein were quite smooth but had a few small holes.

The kidneys from animals on the higher protein level showed a fine but distinct granular appearance; those from animals receiving milk were rough and granular; those from animals receiving lactose were granular with several perforations; those from animals receiving yeast were porous and granular; and those from animals receiving cod liver oil were merely granular.

It was impossible for Dr. Sholl to study the histological structure of all the kidneys removed for study, but the descriptions of a few studied were given as follows:

The kidneys of L_{20}, an animal receiving diet 3a (high protein at first level with milk) showed "marked congestion and some cloudy swelling. Several small cavities were present in the cortex, appearing as spaces from which the glomeruli had dropped out"; L_{19}, an animal on the same diet showed "some distension of the tubules and some cloudy swelling. Considerable granular material was present in the tubules."

The kidneys of L_{21}, on Diet 4a receiving lactose, showed "considerable congestion and slight cloudy swelling. Some granular material was present in the tubules and two or three small hemorrhages in the cortex were found in one section."

L_{25}, an animal on Diet 5, receiving calcium carbonate showed "one small band of cellular infiltration and fibrosis. One rather marked focus of infection with polymorphoneuclear exudate was present. There was some cloudy swelling and pyknosis". L_{26}, an animal on the same diet showed "congestion and some slightly cloudy swelling of the kidneys. Some tubules contained granular material and cell debris. Several small foci in the cortex showed some endothelial cell and lymphocytic infiltration and some fibrosis tubules in these areas were degenerated and many contained hyaline casts."

DISCUSSION OF RESULTS

High protein diets fed to rats showed no striking changes in their bone development as compared with that of normal animals; the essentials for normal bone development evidently were not lacking in these diets. However, the rate of growth of these animals was much less than that of the "normal" and stock ration animals. The addition of milk, yeast, and cod liver oil to these diets caused some increase in the growth rate of animals but it did not approach that of normal animals. The diets were made to contain, as nearly as possible, the essentials for growth and maintenance and the failure of animals to grow was probably not due to a lack of these factors. Two possibilities for explanation of the failure of animals to grow normally on these diets might be (1) the large amounts of amino acids and other end products of protein metabolism may have a toxic effect on the tissues of the animals, or, (2) the increased task of the body to eliminate these end products of metabolism may cause the growth to be retarded. Whether or not palatability was a factor could not be shown as no effort was made to keep records of the food consumed by the animals. Food was kept before the animals continually.

No real explanation is forthcoming for the prevalence of dental caries found in the teeth of the animals on high protein diets. The fact that the teeth of the animals, fed on a high protein diet with the addition of sodium bicarbonate, were not in as bad a condition as those of the animals on the other rations, offers an interesting piece of information as regards the theory that an acid reaction in the mouth may give

rise to carious teeth. It is possible that the sodium bicarbonate might have neutralized acid in the mouths of these animals, thus establishing an environment that was unsuitable for the development of carious teeth.

A great deal of significance is not attached to the P_h values found in the digestive tracts of the animals used in this experiment. The addition of milk, lactose, calcium carbonate, cod liver oil and sodium bicarbonate caused some increases in the P_h values of the digestive tract. No correlation could be formed between the P_h and the bacteria of the digestive tract of these animals. The same types of bacteria were generally found in all the animals on these diets.

The fact that proteolytic bacteria were not found in the digestive tracts of animals, fed high protein rations, in as large numbers as might be expected, finds a possible explanation in the fact that ten percent of sucrose was included in all these diets. Bacteria generally prefer a carbohydrate medium and will act on carbohydrate, if it is present, for their food supply before attacking protein. The addition of lactose to high protein diets seemed to give rise to a greater number of gram positive bacilli; this might be due to a greater increase of carbohydrate for the food supply of the bacteria.

The kidneys from rats fed on high protein diets showed distinct macroscopic differences when compared with the kidneys of normal animals. Hypertrophy was shown in all cases and there were marked differences in the appearances of the kidneys. There was a difference in extent of the changes

in the kidneys of the animals kept on high protein diets. They varied from a finely granular state to a rough, irregularly granular condition; the number of cavities varied in number and size. Some kidneys were uniformly porous and others had only a few cavities. It was noticed that the animals fed on high protein with the addition of sodium bicarbonate showed hypertrophy of the kidneys but the external appearance was normal excepting for a dark line on one of the kidneys and the animals fed high protein diets with calcium carbonate had by far the worst kidneys. No explanation can be given, to account for these results.

The two kidneys of normal rats were found to be very nearly of the same weight and in the cases of hypertrophy the kidneys of each animal were still of about the same weight. This indicates that the work of elimination is equally divided between the two kidneys, as might be expected.

There was no evidence that bacterial infection caused the macroscopic and microscopic changes in the kidneys of rats fed on high protein diets. In only one case was an infection found This animal received calcium carbonate with the high protein diet. The animal had appeared sick for some time before the conclusion of the experiment and an autopsy showed that the side of one kidney appeared eaten away. This kidney weighed a great deal less than the other one. Upon microscopic examination it was found that there was a marked focus of infection with a polymorphonuclear exudate.

The fact that the addition of sodium bicarbonate to a high protein diet seemed to improve the external condition of

the kidneys might find possible explanation in that the sodium bicarbonate might assist in the elimination of the acids that are formed as a result of the ingestion of large amounts of proteins which are usually acid forming.

In a number of kidneys examined microscopically, there was some evidence of degeneration and that the animals were possibly not kept on the diets long enough to show distinct changes. However, in some cases, there were decided microscopic changes and differences in the kidneys of animals kept on high protein diets.

The results of this experiment support the work of Newburgh and others showing that high protein diets cause macroscopic and microscopic changes in the kidneys of animals.

SUMMARY

1. Studies made on several high protein diets showed that they were not as conducive to growth as were the stock and normal diets.

(a) The addition of milk, yeast, and cod liver oil increased the growth of the animals somewhat but the growths never reached those of normal animals.

(b) The animals seemed to go through a period of adjustment to high protein diets before they started to gain weight.

2. The bone development of animals on these high protein diets was apparently normal.

(a) The addition of milk, yeast, and cod liver oil to the high protein diets had no appreciable effect on the ash content of the bones of these animals.

3. Dental caries were found in the teeth of all animals on high protein diets.

(a) Animals on high protein rations with lactose added, had the worst cases of caries.

(b) Animals on high protein diets receiving sodium bicarbonate had the best teeth.

(c) The addition of milk, yeast, and cod liver oil seemed to retard the appearance of caries but did not prevent the ultimate appearance of carious teeth.

4. The P_h values increased along the digestive tract of animals fed on high protein diets and were fairly uniform.

(a) The addition of milk, lactose, calcium carbonate, cod liver oil and sodium bicarbonate to the high protein diets increased the P_h values of the digestive tract.

(b) The addition of yeast to the high protein diets apparently decreased the P_h values to a certain extent.

(c) Since the P_h values are quite uniform in the digestive tract of the rats fed on high protein diets, a great deal of significance cannot be attached to them.

5. The number of bacteria increased along the digestive tract of rats receiving high protein rations.

(a) The types of organisms found were gram negative and positive cocci and gram negative and positive bacilli.

(b) Gram positive bacilli predominated in the digestive tract. One type resembled B. Acidophilus.

(c) Gram negative bacilli, apparently of only one kind, increased in numbers with an increase in protein ingested, the largest numbers being found in the cecum and fecal material. This type resembled B. Coli.

(d) The variety of organisms found in animals on high protein diets was not strikingly different from that found in normal animals, the only difference being in the relative numbers of different types.

(e) When lactose was added to the high protein ration the number of gram positive organisms were increased.

6. The ingestion of high protein diets caused distinct changes in the kidneys of animals as compared with the kidneys of

normal animals.

(a) Distinct hypertrophy of the kidneys took place when animals were fed on diets rich in protein.

(b) The external appearances of kidneys from animals consuming high protein diets differed from those of normal animals. They varied from fairly smooth kidneys with a few small cavities to irregular granular kidneys with many perforations.

(c) Microscopic changes found in the kidneys of some of the animals on high protein rations included granular material in some of tubules, degeneration of some of the tubules, hyaline casts in some of the tubules, cell debris in some of the tubules, two or three small hemorrhages in the cortex of one section, some fibrosis and endothelial cell and lymphocytic infiltration in several foci in the cortex of one section, some cloudy swellings, and some pyknosis.

(d) The amount of protein ingested and the lengths of the feeding periods were found to be important factors in causing changes in the kidneys of rats.

(e) There was some evidence that the addition of sodium bicarbonate to a high protein diet might have had some effect in preventing serious changes in the kidneys of animals.

BIBLIOGRAPHY

(1) Osborne, T. B., Mendel, L. B., Park, E. A., and Darrow, D., Soc. Expt. Biol. and Med. Proc. 20, No.8, pp. 452-453 (1923)

(2) Reader, V. B., and Drummond, J. C., Jour. Physiol. 59, No.6 pp.472-478 (1925)

(3) Hartwell, G. A., Biochem. Journ., 18, No.5, pp. 785-794 (1924)

(4) Nelson, P. M., Journ. Home Econ., 18, No.7, pp.383-388 (1926)

(5) Jackson, H. Jr., Soc. Expt. Biol. and Med. Proc., 22, pp. 482-483 (1925)

(6) Miller, A. J., Jour. Expt. Med., 42, No.6, pp. 953-957 (1925)

(7) Beard, H. H., Amer. Jour. Physiol. 75, No.3, pp. 645-695 (1926)

(8) Addis, T., MacKay, E.M., and L. L., Jour. Biol. Chem., 71, No.1, pp. 139-166 (1926)

(9) Osborne, T. B., Mendel, L. B., Park, E. A., and Winternitz, M. C., Jour. Biol. Chem., 71, No.2, pp. 317-350 (1927)

(10) -a- Newburgh, L. H., Med. Vol.2, p. 77 (1923)

-b- Newburgh, L. H., Proceedings of the Third Race Betterment Conference, pp. 407-416 (1928)

-c- Newburgh, L. H., and Curtis, A. C., Arch. Int. Med., 42, 801, (1928).

(11) Maynard, L. A., and Bender, R. C., Report Lab. Animal Nutrition Cornell University (1928).