Biochemical Parameters on the Nutritional Diet of the Marmoset with a Manufactured Food Source

by

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ABSTRACT

The common marmoset (*Callithrix jacchus*) was used in a nutritonal study of a commercial food source. The marmosets were divided into two groups. Group I was fed a new dry food produced by Purina. Group 2, the control group, was fed a Zu/Preem marmoset diet. Blood analyses were performed on each group to determine RBC, Hgb, PCV and indices. Several biochemical analyses (BUN, Glucose, Total Serum Protein, Creatinine and Albumin) were also performed. The experimental values were compared to normal values, and the values determined for each group were then compared. The results showed little difference in the biochemical parameters of the two groups. However, there was less weight loss with the new dry food diet fed to Group I.

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TABLE OF CONTENTS

LIST OF TABLES

Biochemical Parameters on the Nutritional Diet of the Marmoset with a Manufactured Food Source

I. Introduction

Callithrix jacchus, the common marmoset, is an extremely important laboratory non-human primate (1). The marmoset is so suitable as a laboratory animal for biomedical and biochemical research that it may in time replace the dog and the rodent as a laboratory animal (8).

One of the major problems in a breeding marmoset colony is the nutritional stability of the marmoset. The marmosets are prone to contracting a "wasting disease" characterized by chronic weight loss, muscle atrophy, and haemolytic anaemia (1). The cause of the wasting syndrome is not yet known, but it may have a nutritional basis.

In order to provide proper nutrients for the marmoset diet, commercial feeds have been developed. The validity of each feed in providing the proper amount of nutrients for the development of ahealthy marmoset is extremely important in establishing a colony and avoiding the wasting syndrome.

In this experiment the hypothesis is that a new commercial diet contains an improved nutritional value

over the commercial diet currently being fed to the marmosets. A comparison between a control group on the previously established Zu/Preem diet and a group on the new Purina diet should provide information about the nutritional aspects of each diet. Biochemical parameters for which normal values have been established will be used as a reference to the health status of the marmoset and the acceptability of each diet.

II. Review of Literature

The genus Callithrix is almost entirely of Brazilian nature (11). Callithrix jacchus, the common marmoset, is a commonly used primate in research due to its similarities to humans (6), Its suitability for biomedical and biochemical research stems from the fact that it is large enough for surgery and to obtain blood, but small enough for easy handling and a low maintanance cost is also applicable (2,7,8). The marmoset also has a good reproductive effeciency in the laboratory (2,7). The normal rectal temperature for Callitrichiae is between 38.5^{0}C and 40.0^{0}C (3). The average weight of the marmoset is approximately 336 grams (8).

The nutritional status of the marmoset is extremely important for a successful breeding. In 1975, an indoor-outdoor caging facility for two marmoset species was established at Texas A&M (9). The marmosets have been fed on a commercial diet, Zu/Preem. In order to determine the blood chemical parameters of the marmosets, several biochemical parameters of the blood were examined (9).

The amount of red blood cells are recorded in RBC times 10^{6} per cubic millimeter area and determines if sufficient oxygen is in the blood stream. The red

blood cells or erythrocytes are rich in hemoglobin (Hgb) which carry oxygen and also contain iron. The amount of hemoglobin is measured in grams of hemoglobin per deciliter of whole blood. The percent packed corpuscular volume (PCV) or hematocrit determines the fraction of whole blood which is occupied by the red blood cells (4).

MCV

The mean corpuscular volume (MCV) is the mean or average volume of a single red blood cell. The MCV in cubic meters is determined by dividing the volume of packed red blood cells (PCV) by the number of red blood cells (RBC) (10).

MCH

The mean corpuscular hemoglobin (MCH) is the average hemoglobin by weight of a single red blood cell. The MCH in picograms is determined by dividing the amount of hemoglobin (Hgb) by the number of red blood cells (RBC) (10).

MCHC

The mean corpuscular hemoglobin concentration (MCHC) is the proportion of hemoglobin contained in the average red blood cell. The percent MCHC may be calculated by dividing the amount of hemoglobin (Hgb) by the volume of packed cells (PCV) (10).

The three indices stated above (MCV,MCH,MCHC) should fall within an established range if the values calculated for the RBC, PCV and Hgb are normal values for the marmoset. The indices are primarily used as indicators of errors in the measurements of Hgb, RBC and PCV.

Several tests are performed on blood serum (BUN, glucose, creatinine, total serum protein and albumin). The results of each test is then compared to normal values in order to determine the nutritional status of marmoset.

BUN

The blood urea-nitrogen (BUN) is determined in milligrams per deciliter of serum sample. BUN is the most widely use test for the evaluation of kidney function. Serum urea tests are less sensitive than urea clearance tests, and levels may not be abnormal until the urea levels fall below fifty percent of normal levels.

The Structure of urea is: $NH_2-\overset{O}{\overset{\|}{C}}-NH_2$

Urea is synthesized in the liver from ammonia as a result of the deamination of amino acids. This synthetic pathway is the primary means of excreting nitrogen from the body.

The BUN test is performed in conjuction with a

creatinine test. The determination of the two compounds aids in a diagnosis of prerenal, renal and postrenal hyperuremia. Increases in BUN can be caused by three factors:

1) Prerenal causes: cardaic decompensation, water depletion due to decreased water intake or excessive water loss due to increased protein catabolism.

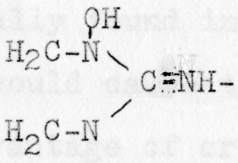
2) Reanl causes: actual malfunction of the kidney such as chronic nephritis, polycystic kidney or tubular necrosis.

3) Postrenal causes: any type of obstruction of the urinary tract (stone, prostrate gland enlargement, tumor) (12).

Creatinine

Creatinine is measured in milligrams creatinine per deciliter of serum sample.

The structure of creatinine is:



Glomerular filtration removes creatinine from the plasma. The creatinine is then excreted in the urine without reabsorption by the tubules to any significant extent. This results in a relatively high clearance

rate for creatinine as compared to urea. When the plasma levels of creatinine increase above normal, the kidney may also excrete creatinine through the tubules. The serum creatinine levels in renal disease usually do not increase until renal function is extremely impaired. An increase in creatinine levels above 2 to 4 mg per 100 ml is descriptive of moderate to severe kidney damage in the presence of normal renal blood flow.

If BUN and creatinine levels are examined together, a normal level of 15/1 to 24/1 (BUN/creatinine) should result. Prerenal causes of high BUN values will bring the ratio to 40/1 especially in the presence of severe intestinal bleeding. A simultaneous increase in BUN and creatinine is caused by the retention of nonprotein nitrogenous compounds due to obstruction of the urinary tract. This condition suppresses the glomerular filtration rate causing an increase in all compounds that are normally found in the glomerular filtrate. Severe cases could cause the ratio to become as low as 10/1. The advantage of creatinine over BUN is the fact that a high protein diet does not effect creatinine. Serum is preffered to whole blood in creatinine tests since whole blood contains a large amount of noncreatinine chromogens that are present in red blood cells (12).

Total Serum Protein

Total serum protein (T.Protein) is measured in grams per deciliter of serum sample. Serum protein can be increased by 10% tol5% in a state of dehydration. Dehydration may be caused by a decrease in water intake, as occurs in actual water deprivation, or from excessive water loss, as in severe vomiting or diarrhea. In the above cases, the actual quantity of serum protein remains the same but the concentration is increased due todecreased volume of solvent water. Hypoproteinemia results when total protein levels fall below 6 gm/100ml which occurs in many unrelated diseaed states. One cause could be Nephrotic syndrome in which large amounts of albumin are lost in the urine due to leakage of albumin molecules through the damaged kidney. Another cause of hypoproteinemia is the salt retention syndrome in which the water is held back to dilute out retained salt. Approximately 7% of plasma consists almost entirely of protein while the other 93% is solvent water (12).

Albumin

Albumin is measured in grams per deciliter of serum sample.

Albumin is a carbohydrate free molecule. It has several functions: the transport of large organic ions

that are normally insoluble in aqeous fluids such as long chain fatty acids, the binding of toxic heavy metal ions, transport of poorly soluble hormones such as cortisol, aldosterone, and thyroxines when their specific binding proteins are not available in a sufficiently large quantity, maintenance of plasma colloidal osmotic pressure, and the provision of a reserve store of protein. The amount of albumin present in the serum does not change due to water dehydration or flooding. Hypoalbuminemia is a common disease caused by four factors which usually act together:

1) An impairment in synthesis such as in malnutrition or liver disease.
2) A increase in catabolism due to acute phase reaction which is caused by a change in serum protein composition following tissue damage as occurs in injury or acute infection.
3) Loss of protein through urine or feces.
4) A change in distribution between intravascular and extravascular components.

Protein loss through the intestinal tract may also account for significant decreases in serum albumin (12).

Glucose

Glucose is measured in milligrams per deciliter of serum sample.

Glucose is a carbohydrate that provides a major source of food supply and energy for animals. Fifty to ninety percent of carbohydrates come from grain, starchy vegetables or legumes. Only a small amount of carbohydrate is stored in the body with one gram of carbohydrate yielding four calories of energy. When blood glucose levels are low such as in a fasting state, glycogen stores in the liver are drawn upon to replenish the lost glucose. Skeleton muscle also contains stores of glycogen, but the enzyme is lacking that will directly change glycogen to glucose. As blood glucose levels in increase by absorption of carbohydrates from the intestine, excess blood glucose is converted into liver and muscle glycogen. Hyperglucosemia can cause impaired metabolism of proteins and fats, and secondary changes in fat metabolism leading eventually to ketosis and possible diabetic coma. A decrease in blood glucose (hypoglucosemia) can lead to muscular spasm and loss of consciousness.

Seveaal hormones are important in regulating the levels of blood glucose:

1) Insulin: decreases blood glucose by increasing the cells permeability to glucose.

2) Growth Hormone and Adrenocorticotrophic Hormone (ACTH): acts antagonistic to insulin and increases blood glucose levels.

3) Hydrocortisone and the other oxysteroids: secreted by the adrenal cortex and stimulate gluconeogenesis.

4) Epinephrine: secreted by the adrenal medulla and stimulates gluconeogenesis with a resultant increase in blood glucose levels.

5) Glucagon: secreted by alpha-cells of the pancreas and increases blood glucose levels by stimulating hepatic glycogenolysis.

6) Thyroxine: secreted by the thyroid gland and stimulates glycogenolysis. It also increases the rate of glucose absorption from the intestine.(12).

III. Procedure

Two groups of marmosets were fed two different commercial diets (Table 10). Each group consisted of five marmosets (3 males and 2 females).

Group I was the experimental group on the dry Purina pellets diet. This group was allowed free choice of the pellets twenty-four hours a day.

Group 2 was the control group. This group was on the Hill's Zu/Preem diet. The group received the Zu/Preem diet in the afternoons with a fresh food supplement.

Bleeding

Prior to bleeding, the marmosets were anesthetized with a 0,05 ml intramuscular injection of ketamine HCl. The dosage is 10 to 15 kg per gm body weight. Since the marmosets weighs approximately 300gms, one-third or 5 mg of ketamine HCl was neede per marmoset which is a 0.05ml injection.

Three milliliters of blood was collected from the femoral vein of each marmoset. Two mls of the blood was centrifuged and the serum extracted to test for BUN, creatinine, glucose, albumin, and total protein. The one ml left was treated with 10% EDTA due to the hyper-coagulability of marmoset blood. The whole blood was

tested for RBC, Hgb, PCV and indices.

Biochemical Tests

All whole blood measurements (RBC,Hgb,PCV,MCV,MCH, MCHC) were completed by a Coulter S Cell Counter. The serum tests (BUN,glucose, albumin, creatinine, total protein) were tested by the Gilford Diagnostics Computer Methods.*

* The Gilford Procedures were obtained from the Gilford Diagnostics System 4 Procedure Manual. Gilford Diagnostics 16035 Industrial Parkway S.W. Cleveland, Ohio 44135.

IV. Results and Discussion

Weights and Temperatures (Tables 1,2,5,6)

The marmosets in both Group I and 2 had normal temperatures on both test dates. The marmosets in GroupI were all fairly close to the normal weight value on December 3. On the second bleeding , Group I marmosets lost weight on the whole. One marmoset did gain 36 gms, but the others lost from five to nine gms with one marmoset losing up to 19 gms. The Group 2 marmosets were also close to the normal weight value on December 3. On January 29, all of the marmosets had lost some weight with one male losing 50 gms. One female became pregnant and gained 52 gms.

Blood Tests (Table9)

The RBC counts for Group I and 2 showed a slight increase from December 3 to January 29, but there was a decrease in the amount of hemoglobin. All of the indices' values decreased for both groups (MCV,MCH, MCHC) which was expected from the decrease in hemoglobin. The PCV decreased for Group 2, but increased slightly for Group I with a subsequent increase in SEM for the measurement which makes the increase insignificant.

Serum Tests (Table 9)

All the serum tests were within the normal values for both groups. There was an increase in BUN for both groups, but since BUN is subject to the water intake of the marmoset the slight increase is negligible. The Group 2 marmosets had a higher blood glucose than Group I which was due to the fresh fruit given to Group 2 as a vitamin C supplement. The amount of T. Protein decreased slightly for each group, as did the albumin content. Creatinine increased for Group 2, but decreased for Group I. However the ratio of BUN to creatinine stayed approximately the same.

Table I

Weight, Gender, and Temperature
for Group I - Purina Diet
December 3,1984

Eartag	Gender	Weight(gm)	Temperature(^{0}C)
32	M	309	39.4
37	M	331	38.7
38	F	365	39.3
39	M	346	38.7
50	F	340	39.4
Avg. ± SEM		338 ± 20.5	39.1 ± 0.37

Table 2

Weight, Gender, and Temperature

for Group 2 - Zu/Preem Diet

December 3, 1984

Eartag	Gender	Weight(gm)	Temperature(^{0}C)
55	F	340	38.8
56	M	347	39.1
57	F	348	39.1
64	M	310	38.3
73	M	280	39.1
Avg. ± SEM		326 ± 26.4	38.9 ± 0.35

Table 3

Biochemical Parameters
for Group I - Purina Diet
December 3, 1984

Eartag	32	37	38	39	50
RBC(10^6/mm^3)	6.33	5.99	6.45	5.90	5.57
Hgb(gm/dl)	15.2	13.9	13.6	11.5	15.5
MCV(m^3)	73	72	67	61	80
MCH(pg)	23.8	23.2	21.0	19.4	27.6
PCV(%)	46.9	43.7	44.1	36.5	44.9
MCHC(%)	32.2	31.8	30.8	31.3	34.3
BUN(mg/dl)	21.2	12.5	17.7	22.3	23.4
Glucose(mg/dl)	110.7	176.5	126.3	93.7	118.6
Creatinine(mg/dl)	0.48	0.70	0.44	0.66	0.35
T. Protein(gm/dl)	7.4	6.0	7.6	9.4	7.2
Albumin(gm/dl)	5.2	3.7	5.2	4.1	4.5

Table 4

Biochemical Parameters

for Group 2 - Zu/Preem Diet

December 3, 1984

Eartag	55	56	57	64	73
RBC(10^6/mm^3)	5.66	6.92	5.25	5.30	5.76
Hgb(gm/dl)	12.3	16.2	12.6	13.6	14.4
MCV(m^3)	69	73	74	77	75
MCH(pg)	21.6	23.3	23.8	25.5	24.9
PCV(%)	39.6	51.1	39.2	41.5	43.7
MCHC(%)	31.0	31.6	31.9	32.6	32.8
BUN(mg/dl)	16.2	26.6	21.0	23.4	17.2
Glucose(mg/dl)	120.8	176.1	141.1	164.9	163.6
Creatinine(mg/dl)	0.40	0.48	0.75	0.40	0.48
T. Protein(gm/dl)	6.8	7.2	7.0	7.1	7.0
Albumin(gm/dl)	4.1	4.8	5.1	5.1	4.9

Table 5

Weight, Gender, and Temperature
for Group I - Purina Diet
January 29, 1985

Eartag	Gender	Weight(gm)	Temperature(^{O}C)
32	M	345	38.6
37	M	323	38.5
38	F	358	38.6
39	M	327	38.1
50	F	335	38.9
Avg. ± SEM		338 ± 14.2	38.5 ± o.29

Table 6

Weight, Gender, and Temperature
for Group 2 - Zu/Preem Diet
January 29, 1985

Eartag	Gender	Weight(gm)	Temperature(^{0}C)
55	F	322	38.5
56	M	340	39.2
57*	F	400	38.5
64	M	260	38.0
73	M	279	38.8
Avg. ± SEM		320 ± 54.3	38.6 ± 0.44

* Pregnant

Table 7

Biochemical Parameters

for Group I - Purina Diet

January 29, 1985

Eartag	32	37	38	39	50
RBC(10^6/mm^3)	7.69	5.11	6.78	6.38	5.12
Hgb(gm/dl)	17.3	11.2	13.2	11.6	13.7
MCV(m^3)	73	71	63	60	83
MCH(pgm)	22.4	21.7	19.3	18.0	26.5
PCV(%)	56.9	36.5	43.7	38.9	43.0
MCHC(%)	30.4	30.5	30.1	29.6	31.6
BUN(mg/dl)	21.63	25.54	22.82	23.03	23.40
Glucose(mg/dl)	144.3	149.2	132.8	103.4	118.6
Creatinine(mg/dl)	0.57	0.18	0.93	0.49	0.35
T. Protein(gm/dl)	6.8	5.6	7.3	9.0	7.2
Albumin(gm/dl)	4.1	2.7	5.0	4.0	4.5

Table 8

Biochemical Parameters

for Group 2 - Zu/Preem Diet

January 29, 1985

Eartag	55	56	57	64	73
RBC(10^6/mm^3)	5.40	7.13	6.53	4.04	5.90
Hgb(gm/dl)	9.8	15.9	14.3	10.0	14.7
MCV(m^3)	63	72	69	75	75
MCH(pg)	18.1	22.1	21.8	24.5	24.7
PCV(%)	34.0	52.1	46.1	30.4	44.6
MCHC(%)	28.8	30.3	31.0	32.8	32.7
BUN(mg/dl)	21.68	24.49	28.51	29.55	24.35
Glucose(mg/dl)	113.5	139.8	127.0	181.5	132.6
Creatinine(mg/dl)	0.31	1.06	0.31	0.62	0.44
T. Protein(gm/dl)	7.3	7.7	7.1	6.8	6.3
Albumin(gm/dl)	3.8	5.0	5.3	4.9	4.6

Table 9

Comparison of Biochemical Parameter Averages

for each Bleeding to Normal Values

Test	GroupI 12/3/84	Group2 12/3/84	GroupI 1/29/84	Group2 1/29/84	Normal Values
RBC($10^6/mm^3$)	6.05±.35	5.78±.68	6.22±1.11	5.8±1.18	6.3±0.1 [a]
Hgb(gm/dl)	13.9±1,58	13.8±1.57	13.4±2.42	12.9±2.84	14.4±0.2 [a]
MCV(m^3)	70.6±7.09	73.6±2.97	70.0±9.06	70.8±5.06	64.0±0.5 [a]
MCH(pg)	23.0±3.11	23.8±1.52	21.6±3.28	22.2±2.67	22.0 [c]
PCV(%)	43.2±3.95	43.0±4.86	43.8±7.90	41.4±8.98	40.0±0.7 [a]
MCHC(%)	32.1±1.35	32.0±0.74	30.4±0.74	31.1±1.69	32.0 [c]
BUN(mg/dl)	19.4±4.42	20.9±4.32	23.3±1.02	25.7±3.25	24.1±0.7 [b]
Glucose(mg/dl)	125.2±31.1	153.3±22.2	129.7±18.8	138.9±25.7	126.0 [c]
Creatinine(mg/dl)	0.53±.15	0.50±.14	0.50±.28	0.55±.30	0.50±.02 [b]
T. Protein(gm/dl)	7.5±1.22	7.0±,15	7.2±1.22	7.0±.53	7.5 [c]
Albumin(gm/dl)	4.5±.67	4.8±.42	4.1±.86	4.7±1.01	4.8±0.1 [b]

[a] (8) [b] (9) [c] (12)

Table 10

Diet Composition for the

Purina Diet and the Zu/Preem Diet

I. Purina Diet Composition

Protein	- 20.0%	Potassium	- .64%
Arginine	- 1.00%	Magnesium	- .21%
Cystinte	- .28%	Sodium	- .28%
Glycine	- .61%	Chlorine	- .41%
Histidine	- .49%	Menadione(Added)	-8.8 PPM
Isoleucine	- 1.02%	Thiamine	- 13.5 PPM
Leucine	- 1.83%	Riboflavin	- 10.0 PPM
Lysine	- 1.09%	Niacin	- 88.7 PPM
Methionine	- .48%	Pantothenic Acid	- 65.9 PPM
Phenylalanine	- .97%	Choline, PM X100	- 18.0
Threonine	- .80%	Folic Acid	- 38.0 PPM
Tryptophan	- .24%	Puridoxine	- 14.3 PPM
Valine	- 1.15%	Biotin	- .3 PPM
Fat	- 9.00%	B-12	- 21.6Mcg/Lb.
Fiber	- 4.7%	Ascorbic Acid	- .75 Mg/Gm
TDN	- 78.0%		
Calcium	- 1.03%		
Phosphorus	- .60%		

II. Zu/Preem Diet Composition

Crude Protein	Min.	8.0%
Crude Fat	Min.	2.5%
Crude Fiber	Min.	1.5%
Moisture	Max.	61.0%
Ash	Max.	3.2%
Calcium	Min.	0.2%
Phosphorus	Min.	0.2%

Vitamin D_3-4,000 U.S.P. Units per LB.

V. Summary and Conclusion

The greatest difference between the Group I and Group 2 marmosets was due to weight loss. The Group 2 marmosets on the average lost more weight than the Group I. The biochemical parameters studied showed no significant difference between the two groups. The marmosets loss less weight on the Purina diet, the diet is in a convinient pellet form and does not need a supplement. The Zu/Preem diet being a wet food was slightly inconvinient to feed, caused a greater amount of weight loss, and did not contain an adequate amount of vitamin C requiring a supplement to be provided. From the data collected in this study, the new Purina diet was more benificial for the marmosets than the original Zu/Preem diet.

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