EFFECTS OF STARVATION AND THIRST ON THE CHEMICAL COMPOSITION OF RATS OF VARIOUS AGES

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INTRODUCTION

The purpose of this investigation was to determine the comparative effects of the lethal deprivations of food, of water, and of both food and water on the water, fat, mineral and protein contents of rats of various ages, to determine the effect of temperature on these factors, and to calculate the growth of the irreducible or minimum vital fraction and of the reducible or variable fraction of these substances.

A distinction has been made by a number of investigators (Mayer, Schaeffer (1), Terroine (2), and others) between the irreducible fraction, élément constant, and the variable fraction, élément variable, of fat. Fat is present in the tissues of animals which have died from starvation, and the amount of fat left seems to be fairly constant for a given species. It is believed that a certain amount of fat, especially of the phosopholipid fraction, is an essential component of protoplasm, and it is to this amount that the term élément constant has been designated. The reducible or storage fat is variable in amount, depending upon the state of nutrition of the animal, and has been called the élément variable by the aforementioned workers.

In view of the fact that a well-hourished animal readily maintains nitrogen equilibrium on a moderate intake of protein, it has been held that there is ordinarily no storage of protein. However, recent experimental evidence indicates that an animal holds in reserve a considerable amount of protein which is depleted when the supply is removed. Calculations from the results of Addis, Poo, and Lew (3) show that approximately 11 per cent of the original protein content of rats is lost during a seven day fast. It is thus evident that when the body is forced to depend on its own tissues for the supply of energy the amount of protein may be greatly reduced.

During dehydration or starvation there is a continued loss of mineral by excretion in the urine and feces. This is especially true of calcium and phosphorus which are utilized in the maintainance of acid-base equilibrium and of osmotic relations. These minerals may be mobilized from the calcium and phosphorus deposits in the bones when their assimilation is inadequate to meet the needs of the body.

There is a continued loss of water from the body during starvation and dehydration. Calculations from the results of Chanutin (4), show a loss of approximately 35 per cent of the original water content of rats which were starved to an average body weight loss or 42.2 per cent.

It is evident that there is a reducible or variable fraction of the fat, protein, mineral and water contents of the body. It follows that there must be an irreducible fraction of each of these constituents, since it is impossible to imagine a higher organism that does not contain definite smounts of these substances.

This irreducible part will be called the minimum vital fraction. It is not to be confused with the élément constant nor with the active protoplasmic mass. The element constant is an essential component of the protoplasm and the minimum vital fraction is not necessarily so. It represents simply that fraction of a substance beyond which amount further reduction cannot take place without resulting in death. It undoubtedly includes the protoplasmic part of that substance which remains in the animal at the point of death, but it also includes any vital non-protoplasmic part which may be present (intercellular substances, etc.). It is believed by the author that protoplasmic breakdown and reabsorption occur in higher as well as in lower organisms during periods of inanition, and for this reason, as well as because of the possible inclusion of vital non-protoplasmic substances, the minimum vital fraction is not considered to be a measure of the active protoplasmic mass. It may, indeed, be larger or smaller depending upon the amount of non-protoplasmic substance it contains and upon the extent to which autolysis of protoplasm has taken place.

It is the general opinion that most living organisms live longer during food deprivation than during water deprivation, the shortest length of life resulting from the removal of both food and water. However, Underhill and Roth (5), report that fasting rabbits with water freely supplied die in about the same length of time as rabbits deprived of water.

The degree of weight change during inanition is closely associated with the age and size of the rat since young or small rats starve more quickly and in addition show a smaller maximal weight loss at death than adult or large rats. Barlow (6), found that young rats (50 to 90 grams) usually die from four to six days after the beginning of a fast, medium sized rats (125 to 150 grams) from six to nine days, adult rats (175 to 225 grams or larger) from ten to thirteen days, and large rats (larger than 300 grams) from fourteen to fifteen days.

The basal metabolism of the rat is increased approximately 5 per cent for each degree (C.) fall in environmental temperature (Goto (7), Terroine and Trautman (8), and Benedict and MacLeod (9). It would be expected, therefore, that within the biokinetic temperature range the body resources of the rat would be more quickly exhausted by a fast the lower the temperature. This deduction is supported by the work of Horst, Mendel, and Benedict (6) who starved female rats at temperatures of 26° and 16°C. and found that the rats at 26°C. lived on the average for 16½ days and lost 49 per cent of their initial body weight, while the rats at 16°C. lived on the average for only 11 days and lost only 44 per cent of their initial body weight.

Very few investigations have been made on the chemical composition of rats which have undergone inanition to the lethal point. Chanutin (4) found losses of 89.5 per cent of body fat, 52.2 per cent of total solids, 35.3 per cent of proteins, and 15.1 per cent of ash, in rats starved to an average body weight loss of 42.2 per cent. His results show increases in the concentration of ash and of protein from 3.81 per cent to 5.64 per cent and from 21.2 per cent to 24.0 per cent of total body weight, respectively, and decreases in the concentration of total solids and of fat from 38.4 per cent to 32.1 per cent and from 13.4 per cent to 2.44 per cent respectively.

METHODS

The experiments were carried on at two temperatures, $28^{\circ} \pm 3^{\circ}C.$ and $18^{\circ} \pm 2^{\circ}C.$, and under three feeding conditions, food deprivation, water deprivation, and food and water deprivation.

Seven sets of male rats were used, all derived from pure inbred Wistar stock. Fifteen rats were placed in each of the three sets maintained at a temperature of $28^{\circ} \pm 3^{\circ}C.$ The rats in these sets ranged from approximately two weeks to three months in age and from approximately 20 to 300 grams in weight. They were selected so that each set would be comparable in age and weight. One set was killed for analysis, the second set was placed on a food deprivation diet, and the third set was placed on a food and water deprivation diet. The gastro-intestinal contents were carefully removed upon death and the carcasses weighed.

Four sets were run at a temperature of $18^{\circ} \pm 2^{\circ}C.$ Four rats ranging in age from approximately three weeks to four months and in weight from approximately 20 to 190 grams, were placed in each set. They were also selected to make each set comparable in age and weight. One set was killed for analysis, the second set was placed on a food deprivation diet, the third set was placed on a food and water deprivation diet, and the fourth set was placed on a water deprivation diet.

These rats were prepared for analysis in the same manner as the rats maintained at $28^{\circ} \pm 3^{\circ}C$.

A normal dry food consisting of:

Cornmeal	4 parts
Rolled oats	1 part
Whole Wheat	1 part
Dried skim milk	1 part
Tankage	1 part
Cod liver oil	

was placed freely before the rats on the water deprivation diet, while tap water was available at all times to the rats on the food deprivation diet. The rats on the starvation diet had access to neither food nor water.

Water content was measured by drying to constant weight at 100°C. and subtracting the dry weight found from the original body weight. The amount of fat was measured by ether extraction. Mineral content was determined by ashing at a red heat until all traces of carbon were removed. The protein content was calculated by subtacting the fat and ash contents from the amount of dry substance. It includes the carhohydrate content of the body, but, since this content is small (0.114 per cent glycogen in rats fasted 48 hours, Cori and Cori (10), and 0.8 per cent to 1.8 per cent glycogen in livers of rats on normal diet, Foster and Benninghoven (11)), it is believed that the error made by this inclusion is not much greater than the error made by the estimation of the amount of protein from the nitrogen content, since the proteins of the body are known to vary in nitrogen concentration from 10 per cent to 18 per cent (Addis, Poo, Lew, Yuen (12)).

RESULTS

In the calculation of the results, the averages for each set were first converted to amounts which are comparable for rats of the same original body weight. These calculated averages are shown in Table V, the actual data obtained being presented in Tables I to IV.

The average weight losses, average percentages of weight lost, and average lengths of life for the different sets are as follows:

Maintained at 28°C.	Wt. Loss Grams	%Wt. Lost	Hr. Lived
Deprived of food and water	69.997	47.6	202.1
Deprived of food	65.546	44.6	196.2

Maintained at 18°C.	Wt. Loss Grams	%Wt. Lost	Hr. Lived
Deprived of food and water	55.632	37.9	141.9
Deprived of food	54.546	37.1	134.5
Deprived of water	62.857	42.8	223.3

The rats deprived of both food and water lived longer than the rats deprived of food alone, and also underwent a greater loss of weight. The rats deprived of water alone (this group being maintained only at the lower temperature) lived longer that the rats of the other groups at either temperature, and their weight loss exceeded the weight losses of the other two groups at 18°C. but was not as great as the weight losses of the two groups maintained at 28°C.

TABLE I

Analyses of Rats on Normal Diet

Age	Weight	Amount Water	% Water Fat-Free Weight	Amount Fat	% Fat Total Weight	Amount Ash	% Ash Fat-Free Weight	Amount Protein	% Protein Fat-Free Weight
13	22.40	17.09	81.0	1.304	5.80	0.542	2.57	3.46	16.0
20	38.24	27.46	78.5	3.433	8.98	1.131	3.25	6.36	18.3
21	20.60	15.35	78.2	1.104	5.36	0.691	3.54	3.55	18.2
26	56.22	39.85	77.3	4.795	8.53	1.796	3.49	9.89	19.2
36	82.05	55.87	75.8	8.655	10.55	2.535	3.45	15.23	20.8
48	91.28	63.67	74.7	7.146	7.83	3.213	3.82	18.08	21.5
50	86.29	59.30	74.3	7.590	8.79	3.181	3.68	17.05	21.7
53	115.50	81.88	74.7	7.425	6.43	4.030	3.73	23.10	21.4
58	135.49	87.89	73.7	17.790	12.13	4.214	3.58	26.75	22.7
63	154.82	104.15	73.5	15.038	9.71	5.376	3.84	31.66	22.7
68	175.88	119.33	73.3	15.328	8.71	6.112	3.82	36.72	22.9
83	118.39	80.63	73.2	10.870	9.19	3.984	3.70	24.86	23.1
83	193.98	132.22	73.1	15.560	8.02	6.728	3.78	41.28	23.2
89	218.14	138.20	72.2	29.394	13.47	6.842	3.62	45.59	24.1
92	236.99	154.88	73.0	27.675	11.68	7.482	3.58	49.05	23.4
93	274.89	184.08	71.7	21.700	7.89	9.741	3.85	61.91	24.5
94	255.93	173.78	73.2	21.769	8.50	8.541	3.65	54.19	23.1
97	296.80	197.72	73.5	31.380	10.67	9.593	3.62	60.76	22.9
119	154.21	103.54	72.7	13.714	8.89	5.510	3.57	32.85	23.4

From these figures it can be seen that the rats at the higher temperature lived longer and underwent a greater loss of weight than the rats on corresponding diets at the lower temperature.

Length of life and loss of body weight increased with the body weight of the rats in all the groups, these factors becoming proportionately larger with increase of body weight.

The average percentages of water and fat losses of the different sets are as follows:

Maintained at 28°C.	% Water Lost	% Fat Lost
Deprived of food and water	46.27	87.52
Deprived of food	42.13	84.36
Maintained at 18°C.		
Deprived of food and water	35.50	91.23
Deprived of food	33.31	89.77
Deprived of water	42.67	91.04

At both temperatures, the rats deprived of both food and water lost a greater per cent of water and fat than the rats deprived of food alone. Although the per cent of fat lost is greater at both temperatures in the rats deprived of both food and water than in the rats deprived of food alone, the per cent of fat in the substance lost is greater at both temperatures in the rats deprived of food alone. (See Table V.) It is thus evident that the greater loss of fat in the rats deprived of food alone is due to their greater total

TABLE II

Analyses of Rats at Death from Food Deprivation

Age	Temp.	% Weight Lost	Orig. Weight	% Water Fat-Free Weight	% Fat Total Weight	% Ash Fat-Free Weight	% Protein Fat-Free Weight	Hours Lived
18	28°C.	18.1	20.03	79.1	1.84	3.30	17.6	57
21	18°C.	23.1	25.59	77.9	1.69	3.47	18.6	51
34	28°C.	28.3	39.11	75.9	2.06	4.42	19.7	78
34	"	25.0	58.97	75.4	1.99	4.44	20.1	86
38	"	39.0	78.02	74.1	1.70	4.47	21.5	129
47	"	45.2	100.96	71.6	1.53	5.00	23.4	117
47	"	50.6	124.05	72.6	1.86	5.22	21.8	204
50	18°C.	35.3	117.47	72.9	2.30	4.24	22.9	106
53	28°C.	47.1	137.97	70.6	1.55	4.93	24.4	231
53	"	41.3	159.12	72.4	1.52	5.14	22.6	189
64	"	41.8	175.96	70.7	1.13	4.85	24.4	213
67	"	40.8	199.10	71.4	5.57	5.32	23.3	211
73	"	45.1	225.97	70.6	0.87	5.33	24.0	270
83	"	49.2	247.10	71.0	0.96	5.36	23.7	336
83	18°C.	35.6	156.58	71.7	11.39	4.98	23.3	354
83	28°C.	44.0	265.04	69.3	1.07	5.12	25.6	124
93	"	49.8	277.02	70.6	0.90	5.11	24.3	357
96	"	47.5	294.08	70.1	0.81	5.83	24.1	376
119	18°C.	41.4	188.62	71.1	1.11	5.60	23.3	166

TABLE III

Analyses of Rats at Death from Deprivation of Both Food and Water

Age	Temp.	% Weight Lost	Orig. Weight	% Water Fat-Free Weight	% Fat Total Weight	% Ash Fat-Free Weight	% Protein Fat-Free Weight	Hours Lived
18	28°C.	19.5	22.02	79.1	1.78	3.41	17.5	60
21	18°C.	25.0	25.00	77.9	1.71	3.64	18.5	52
34	28°C.	29.6	40.52	76.0	1.81	4.49	19.6	72
34	"	36.4	61.51	74.7	1.94	4.77	20.5	102
38	"	35.3	80.05	74.9	1.24	4.51	20.6	105
47	"	44.5	103.04	71.8	1.86	5.43	22.8	134
47	"	47.7	127.00	70.8	1.36	5.08	24.1	120
50	18°C.	33.7	99.99	71.3	1.19	5.14	23.7	95
53	28°C.	41.0	141.54	69.8	1.06	5.26	24.9	177
53	"	42.8	166.05	71.3	1.15	5.01	23.7	204
64	"	39.7	177.53	69.2	1.14	5.12	25.7	198
67	"	56.3	204.03	69.8	1.27	5.90	24.3	431
73	"	55.1	226.92	68.9	1.72	6.13	25.0	334
83	"	60.4	245.00	67.4	0.93	6.14	26.5	429
83	"	45.0	261.51	68.0	1.24	5.21	26.1	275
83	18°C.	43.9	147.58	69.9	3.07	5.43	24.6	167
93	28°C.	49.3	279.02	67.7	7.96	5.69	26.6	345
96	"	48.2	305.01	67.9	1.09	6.00	26.1	369
119	18°C.	37.0	176.88	69.2	1.76	5.17	25.6	120

TABLE IV

Analyses of Rats at Death from Water Deprivation

Age	Temp.	% Weight Lost	Orig. Weight	% Water Fat-Free Weight	% Fat Total Weight	% Ash Fat-Free Weight	% Protein Fat-Free Weight	Hours Lived
21	18°C.	27.1	25.13	76.64	1.38	3.93	19.32	73
50	"	41.0	100.64	69.50	1.79	5.18	25.33	193
83	"	45.4	149.90	68.87	1.32	5.72	25.41	236
119	"	43.7	178.91	65.18	1.17	5.30	29.52	190

loss of weight. It may be seen from these figures that a smaller per cent of water and a larger per cent of fat is lost at the lower temperature.

The average percentages of mineral losses and the average percentages of minerals in the lost substance of the different sets are as follows:

Maintained at 28°C.	% Mineral Lost	% Mineral in Lost Substance
Deprived of food and water	14.74	1.03
Deprived of food	16.37	1.22
Maintained at 18°C.		
Deprived of food and water	10.47	0.99
Deprived of food	13.33	1.27
Deprived of water	14.90	1.23

There is a greater amount of minerals lost at both temperatures in the rats deprived of food alone than in the rats deprived of both food and water. The rats deprived of water alone (18°C.) underwent a greater loss of minerals than the other groups at this temperature. These figures show a greater loss of minerals at the higher temperature for rats on corresponding diets. That this is due to the greater loss of body weight in the rats at 28°C. is indicated by the fact that there is approximately the same per cent of minerals in the substance lost in groups on corresponding diets.

The per cents of water and of fat were lower and the per cents of protein and minerals were higher in the rats at death is the following order of diet: normal, food deprivation, food and water deprivation, and water deprivation. Differences in the per cents of these substances between the groups of rats on different diets were less pronounced at the lower temperature.

The per cents of water and fat at the time of death of the rats on the deprivation diets, decrease with body weight, while the per cents of protein and minerals at the time of death of the rats on the deprivation diets increase with body weight.

All of the constituents in both control and in deprived rats give straight lines when plotted against total original body weight on a double logarithmic scale, (Graphs 1-4). The following equations where x is the constituent and y is the original body have been derived to show the relationship between the constituents and the original body weight:

Water and original body weight, normal rats - $\log x = .9504 \log y - .0673$

Water and original body weight, rats deprived of water, food or both - $\log x = .7944 \log y + .0427$

Fat and original body weight, normal rats - $\log x = 1.1316 \log y - 1.3252$

Fat and original body weight, rats deprived of water, food or both - $\log x = .7971 \log y - 1.6076$

Minerals and original body weight, normal rats - $\log x = 1.0446 \log y - 1.5756$

Minerals and original body weight, rats deprived of water, food or both - $\log x = 1.0030 \log y - 1.5483$

Protein and original body weight, normal rats - $\log x = 1.0913 \log y - .8979$

Protein and original body weight, rats deprived of water, food or both - $\log x = .9743 \log y - .8229$

The equations for the constituents of the rats deprived of water, food or goth were obtained by plotting all the results for the analysis for the particular constituent regardless of temperature or diet. The amounts of the reducible fractions of the various substances were obtained from these equations and are expressed by the following equations:

Water - $\log x = 1.3064 \log y - 1.2465$

Fat - $\log x = 1.1836 \log y - 1.4852$

Mineral - $\log x = 1.4304 \log y - 3.29966$

Protein - $\log x = 1.4372 \log y - 2.1368$

A much greater variation occurs in the amounts of the substances in the deprived rats due to the individual variation in length of life of rats on deprivation diets and the consequent variation is the amount of weight lost. Considerable variation exists in the original amount of fat which is due to differences in the variable fraction of this substance. There is also a large variation in the final amount of fat which is due to individual differences in ability to catabolize fat during starvation.

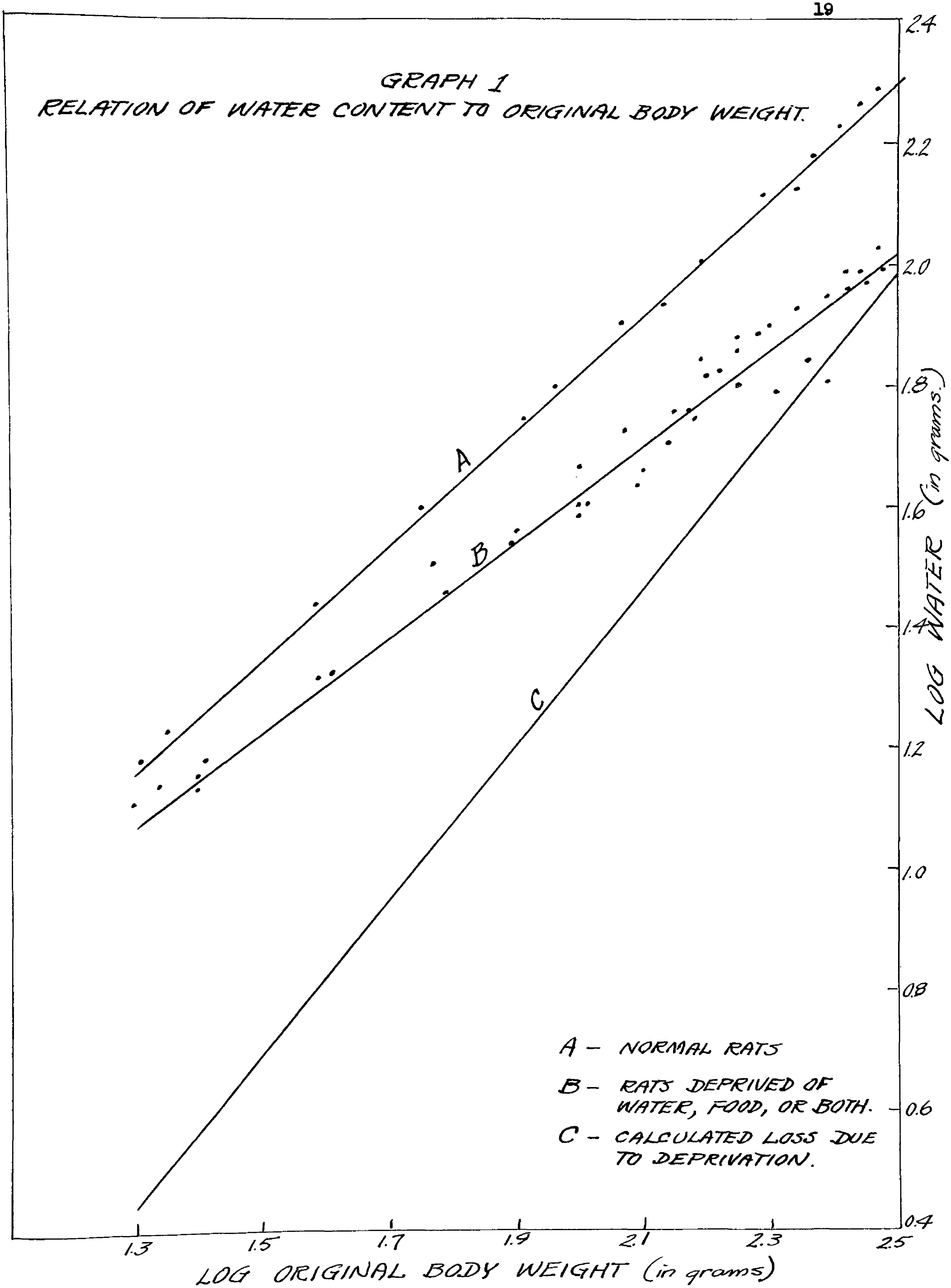
TABLE V

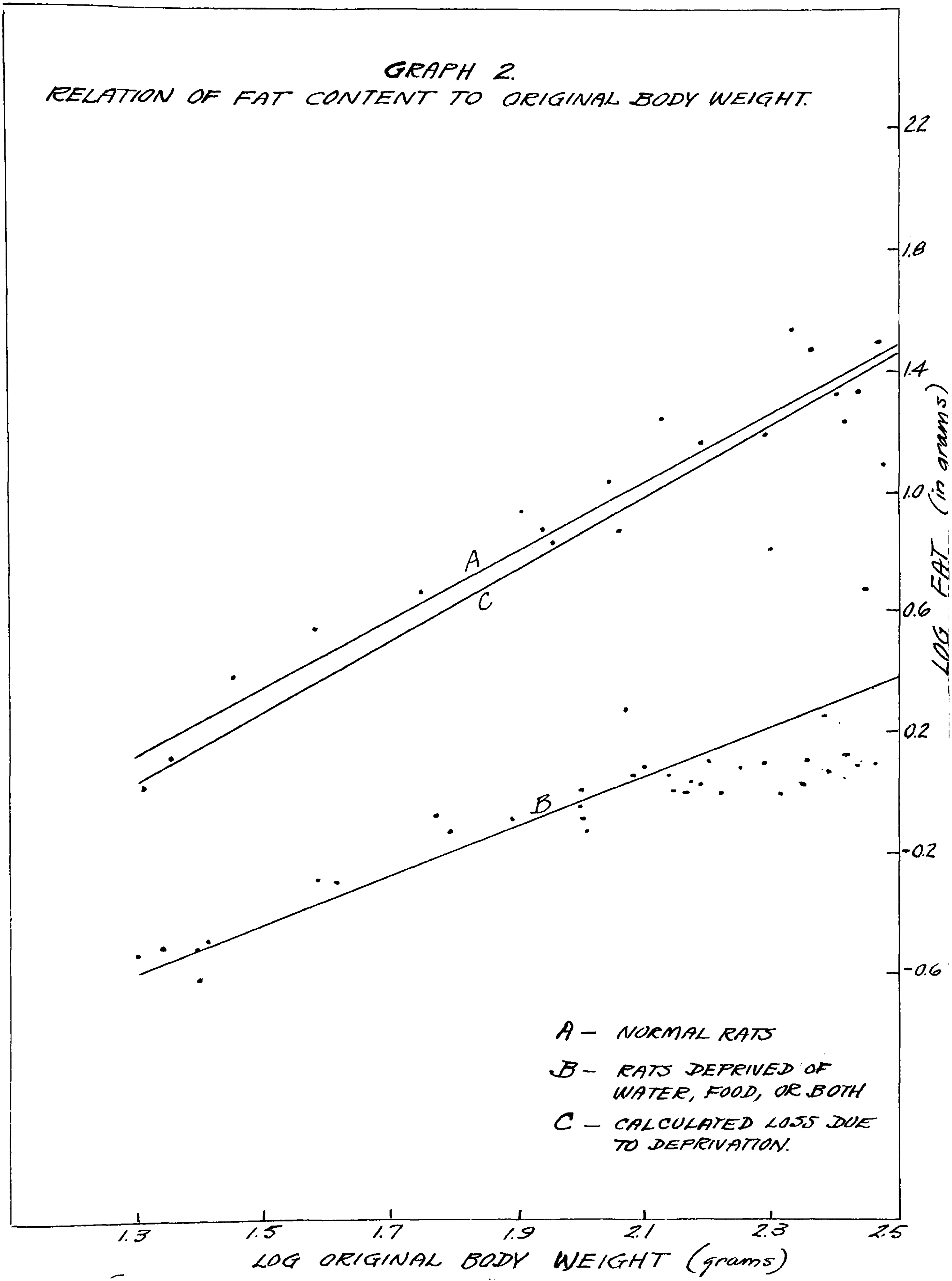
Summary of Analyses of All Groups*

	28°C.			18°C.			
	Normal	With H_2O	Starved	Normal	With H_2O	Starved	With Food
Wt. Lost		65.546	69.997		54.546	55.632	62.857
% Wt. Lost		44.6	47.6		37.1	37.9	42.8
Hr. Lived		196.2	202.1		134.5	141.9	223.3
Am't Fat	14.288	2.233	1.782	12.882	1.319	1.131	1.156
% Fat, Total Weight	9.72	2.82	2.37	8.78	1.43	1.24	1.37
Am't Water	97.490	56.423	52.382	98.556	65.730	63.569	56.496
% H_2O, Fat-Free Wt.	73.51	71.29	69.71	73.53	72.18	70.50	68.13
Am't Ash	4.872	4.075	4.154	5.175	4.485	4.633	4.404
% Ash, Fat-Free Wt.	3.67	5.15	5.53	3.86	4.92	5.14	5.31
Am't Protein	30.282	18.635	18.606	30.321	20.853	21.958	22.021
% Protein, Fat-Free Wt.	22.83	23.55	25.76	22.62	22.86	24.35	26.56
Water Lost		41.067	45.108		32.826	34.987	42.060
% H_2O Lost		42.13	46.27		33.31	35.50	42.67
Fat Lost		12.055	12.506		11.563	11.751	11.726
% Fat Lost		84.35	87.52		89.77	91.23	91.04
Mineral Lost		0.797	0.718		0.690	0.542	0.771
% Mineral Lost		16.37	14.74		13.33	10.47	14.90
Protein Lost		11.647	11.676		9.468	8.363	8.300
% Protein Lost		38.46	38.56		31.23	27.58	27.37
% Water in Lost Substance		62.65	64.44		60.18	62.89	66.91
% Fat in Lost Substance		18.39	17.87		21.20	21.12	18.65
% Ash in Lost Substance		1.22	1.03		1.27	0.99	1.23
% Protein in Lost Substance		17.77	16.68		17.36	15.03	13.20

* All weights are expressed in grams. For a more accurate comparison the values in this table are corrected for slight differences between the average weights of each group and the average weight of all the rats analyzed. This correction obviously does not affect the % values.

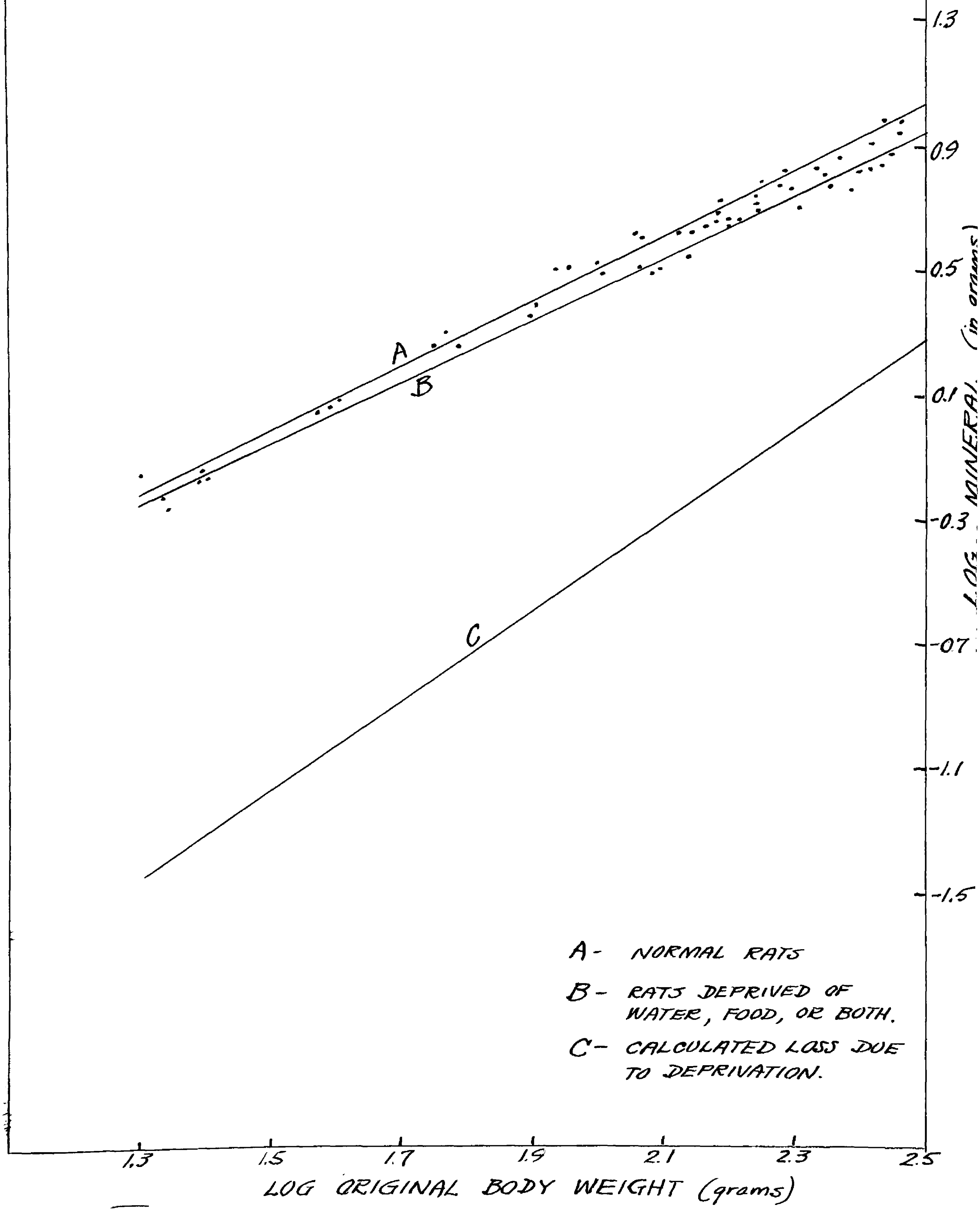
Weight lost, the total reducible fraction, and final weight, the total minimum vital fraction, also give straight lines when plotted logarithmically against original body weight, (Graph 5). The following equations have been derived for these two fractions: final weight - $\log x = .9532 \log y - .1052$, weight lost - $\log x = 1.2548 \log y - .9367$.

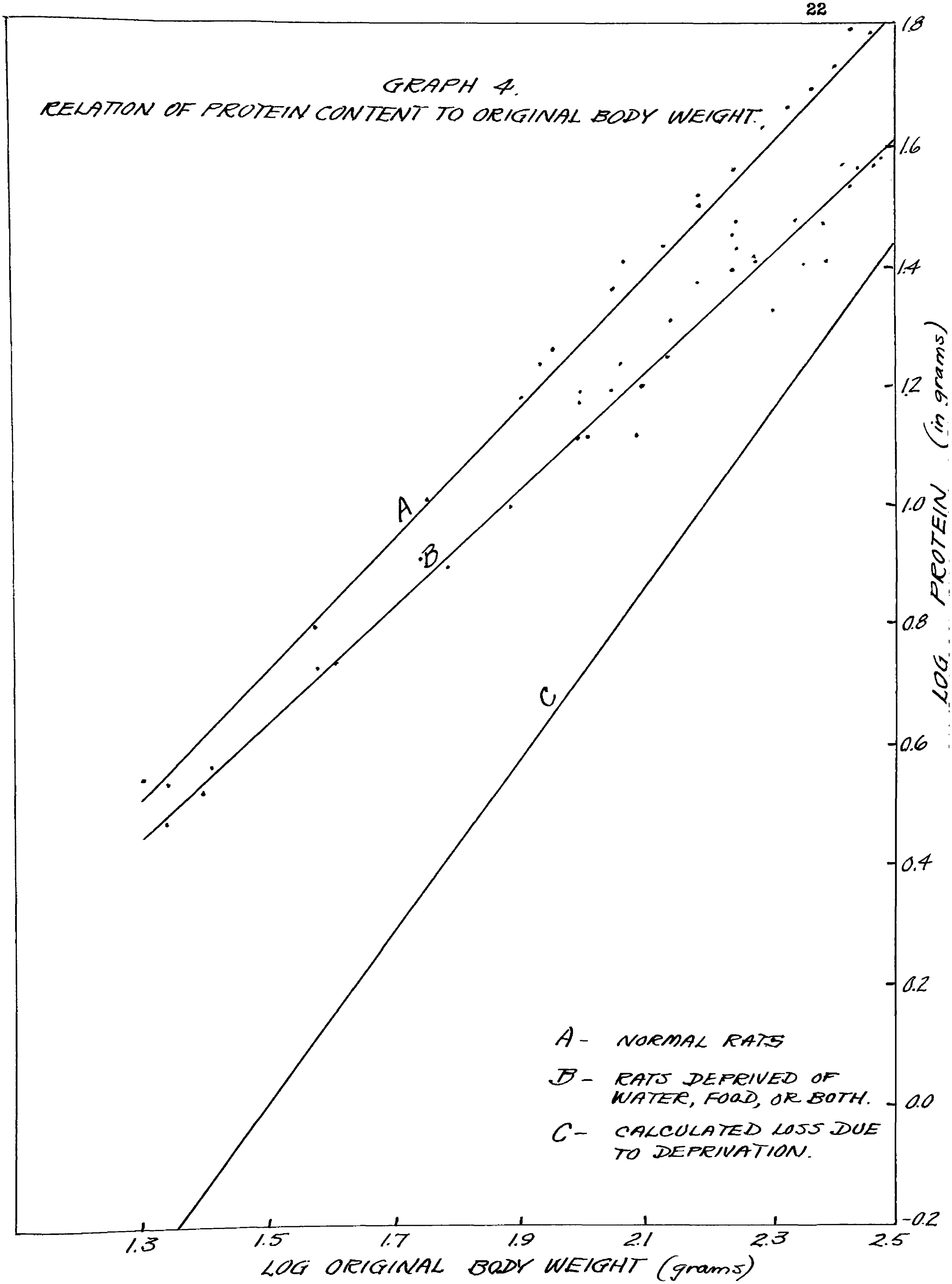

GRAPH 1
RELATION OF WATER CONTENT TO ORIGINAL BODY WEIGHT.
A
B
C
A – NORMAL RATS
B – RATS DEPRIVED OF WATER, FOOD, OR BOTH.
C – CALCULATED LOSS DUE TO DEPRIVATION.
LOG WATER (in grams)
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
1.3
1.5
1.7
1.9
2.1
2.3
2.5
LOG ORIGINAL BODY WEIGHT (in grams)

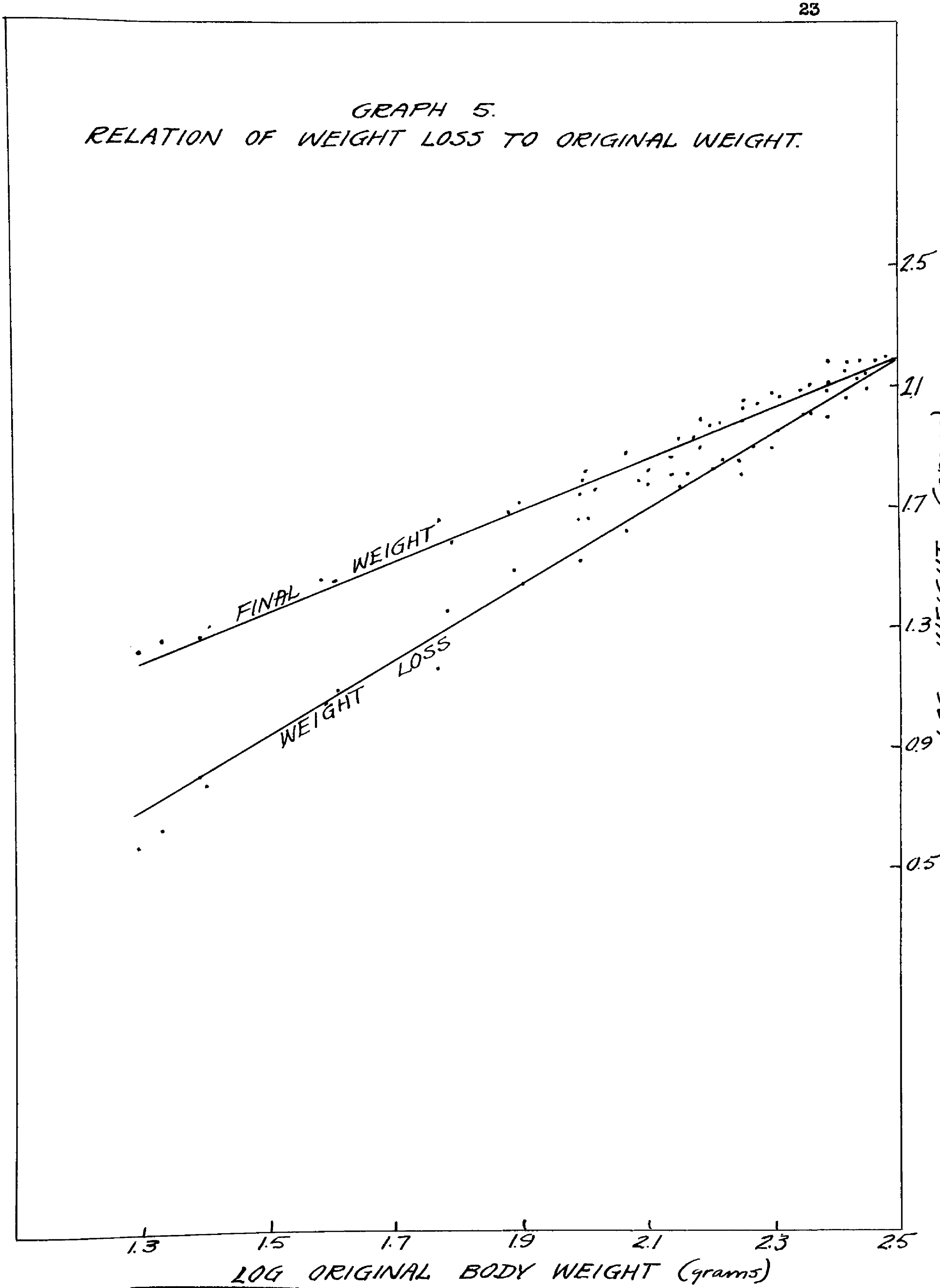

GRAPH 2.
RELATION OF FAT CONTENT TO ORIGINAL BODY WEIGHT.
A
C
B
2.2
1.8
1.4
1.0
0.6
0.2
-0.2
-0.6
LOG FAT (in grams)
1.3
1.5
1.7
1.9
2.1
2.3
2.5
LOG ORIGINAL BODY WEIGHT (grams)
A – NORMAL RATS
B – RATS DEPRIVED OF WATER, FOOD, OR BOTH
C – CALCULATED LOSS DUE TO DEPRIVATION.

GRAPH 3

RELATION OF MINERAL CONTENT TO ORIGINAL BODY WEIGHT




GRAPH 4.
RELATION OF PROTEIN CONTENT TO ORIGINAL BODY WEIGHT.
A
B
C
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
LOG PROTEIN (in grams)
1.3
1.5
1.7
1.9
2.1
2.3
2.5
LOG ORIGINAL BODY WEIGHT (grams)
A - NORMAL RATS
B - RATS DEPRIVED OF WATER, FOOD, OR BOTH.
C - CALCULATED LOSS DUE TO DEPRIVATION.


GRAPH 5.
RELATION OF WEIGHT LOSS TO ORIGINAL WEIGHT.
FINAL WEIGHT
WEIGHT LOSS
2.5
2.1
1.7
1.3
0.9
0.5
LOG WEIGHT (grams)
1.3
1.5
1.7
1.9
2.1
2.3
2.5
LOG ORIGINAL BODY WEIGHT (grams)

DISCUSSION

At 18°C. death in the starved rats ensues before inanition becomes as complete as in the rats with food, which, due to their increased length of life lose more weight than the rats which are starved. The food therefore seems to sustain life a time sufficiently long enough to produce a greater extent of autolysis and a consequently greater loss of weight than that which occurs in the starved rats.

At both temperatures water appears to be toxic to starved rats since death takes place more quickly and a smaller degree of autolysis occurs. The toxity of water to starved rats may be due to the effect it has in decreasing the concentration of the blood, thereby promoting the ill effects of starvation edema. Ordinarily, the consumption of water even in large quantities does not dilute the blood. Underhill and Kapsinow (13) report that water taken by normal dogs does not alter blood concentration, however, they found that water taken by water deprived animals decreases blood concentration to normal. If these findings apply to rats it would be expected that blood concentration is increased in water deprived rats and thus the edema produced by starvation is less pronounced than in the rats which have access to water.

At the lower temperature the length of life on the deprivation diets is shorter and consequently the extent of autolysis is less. The decreased length of life is not directly due to inanition, since the extent of inanition is not as great as it is at the higher temperature. The differences in the effect of the two temperatures on the length of life and the loss of weight may be due to the fact that at the lower temperature the rate of autolysis is speeded up to such an extent that the starved animals are not able to meet the requirements by maintaining a physiological equilibrium. It has been mentioned before that the rats with food not only live longer but suffer a greater loss of weight. This may be due to a slower rate of autolysis which enables them to maintain physiological equilibrium for a longer time. It may also be caused by a facilitated fat catabolism due to carbohydrates in the ingested food.

That there is at times an upset fat metabolism during starvation which reduces the extent to which fat catabolism usually occurrs is shown in four rats out of the thirty maintained at 28°C., two on a food deprivation diet, and two deprived of both food and water, which died with a large amount of fat still present in their bodies.

The amount of water lost is greater in the rats deprived of both food and water than in the rats deprived of water only at both temperatures, as would be expected.

The amount of fat lost is slightly more in the rats deprived of both food and water than in the rats deprived of food only at both temperatures, while the amount of protein lost is less. This may be due to a preferential catabolism of fat to protein since more water is produced by its hydrolysis. The amount of mineral lost is greater in the rats deprived of food only than in the rats deprived of both food and water at both temperatures. This may be due to an increased facilitation of mineral disposal, which may be hampered by the partial dehydration which occurs in the rats deprived of water.

In the group to which food was administered (18°C.) it was found that dehydration proceeded to the greatest extent. This may have been due both to the large amount of total weight lost and to the use of metabolic and stored water for excretory purposes. The fat loss was not as great as in the starved rats, although it exceeded the loss of fat in the rats, which had access to water, the former probably due to the preferential catabolism of ingested food to stored fat, the latter probably due to increased length of life as indicated by a smaller per cent of fat in the substance lost. Of the sets maintained at 18°C. the mineral loss was greatest in the rats with food. This may have been due to the great loss of water in the rats of this group, and the consequent disposal of minerals. The protein loss was less in the group of rats with food than in any of the other groups at either temperature due to the availability of food for energy, and to the more efficient catabolism of body fat because of the carbohydrates in the food.

Huxley (14) in 1924 first considered the relation between the growth of parts of a living organism when the parts were increasing or decreasing in size. The process is described by the equation: $\log x = k \log y - \log h$, which indicates that there is a constant ratio between the relative growth rates of the two factors.

By differentiation of the logarithmic equations of the reducible and minimum vital fractions to the original body weight the following differential growth ratios are obtained:

Reducible fractions to original body weight

Reducible fat = 1.184

Reducible mineral = 1.430

Reducible protein = 1.437

Reducible water = 1.306

Total reducible fraction = 1.258

Minimum vital fat = 0.797

Minimum vital mineral = 1.003

Minimum vital protein = 0.974

Minimum vital water = 0.794

Total minimum vital fraction = 0.952

The differential growth ratios show an increase with total growth in the amounts of water, fat, mineral and protein in both the reducible and minimum vital fractions. They also show an increase with total growth in the relative amounts of mineral and protein components, and a decrease with total growth in the relative amounts of the water and fat components of both the reducible and minimum vital fractions. With growth, therefore, both the reducible and minimum vital fractions become increasingly dehydrated, less fatty, and more mineralized, and their protein concentration increases.

The differential growth ratios of total fat, water, mineral and protein to total weight for the 19 rats analyzed are as follows:

Fat = 1.132

Water = 0.950

Mineral = 1.045

Protein = 1.091

These differential growth ratios also show an increase in the relative amounts of protein and minerals and a decrease in the relative amounts of water and fat. Thus, with growth the rat becomes increasingly dehydrated, less fatty, more mineralized, and its protein concentration increases.

It has long been recognized that there is a decrease in the concentration of water and an increase in the concentration of minerals coincident with growth, (see von Bezold (15) in 1857). In respect to fat and protein concentrations, these results compare favorably with those obtained by other investigators. Haiti (16) and Chanutin (4) found a very low fat content in rats at birth which rapidly increased during the suckling period with a definite decrease after weaning. Their results show a decrease in the concentration of fat after weaning.

It is evident that during growth the relative concentrations of water, fat, minerals, and protein vary in a corresponding manner in the reducible and minimum vital fractions with their relative concentrations in the whole animal, namely with a decreasing concentration of water and fat and with an increasing concentration of minerals and protein.

The differential growth ratio of the total reducible fraction to the total weight shows an increase of the total reducible fraction with growth, while the differential growth ratio of the total minimum value fraction to the total weight shows a decrease of the total minimum vital fraction with growth. The value of the total reducible fraction varies from 18.1 per cent to 60.4 per cent of the total body weight and the value of the total minimum vital fraction varies from 39.6 per cent to 81.9 per cent of the total body weight. The average composition of the minimum vital fraction for rats varying in age from two weeks to four months is approximately 69 per cent water, 2 per cent fat, 5 per cent mineral and 24 per cent protein, and the composition of the reducible fraction is approximately 64 per cent water, 20 per cent fat, 1 per cent mineral, and 15 per cent protein.

SUMMARY

Sixty one Wistar strain male rats of various ages on normal, food deprivation, water deprivation, and both food and water deprivation diets at temperatures of 28°C. and 18°C. were analyzed for water, fat, mineral and protein content. The rats varied from approximately two weeks to four months in age, and from approximately 20 to 300 grams in weight. The rats in the various diet and temperature groupings were selected so as to make each group comparable with respect to age and weight.

The averages of the different sets were converted to conform with sets of the same average original body weight. The amounts of the substances lost by the deprived rats were determined by subtracting the amounts of the substances present at death from the amounts of the substances present in the rats on the normal diet. The amounts of the substances lost were considered to be the reducible fractions of the substances, and the amounts of the substances present at death were considered to be the irreducible or minimum vital fractions of the substances. The minimum vital fraction of a substance is defined as that amount of the substance beyond which further reduction cannot take place without resulting in death.

The results were plotted against original body weight on a double logarithmic scale and straight lines with certain variations due to differences in degree of

inanition were obtained. The following differential growth ratios of the reducible and minimum vital fractions to the original body weight were found:

Reducible fractions to original body weight

Reducible fat = 1.184

Reducible mineral = 1.430

Reducible protein = 1.437

Reducible water = 1.306

Total reducible fraction = 1.258

Minimum vital fractions to original body weight

Minimum vital fat = 0.797

Minimum vital mineral = 1.003

Minimum vital protein = 0.974

Minimum vital water = 0.794

Total minimum vital fraction = 0.952

The differential growth ratios of the total constituents to the original body weight were found to be as follows:

Fat = 1.132

Water = 0.950

Mineral = 1.045

Protein = 1.091

From these differential growth ratios it may be seen that during growth the relative concentrations of water, fat, minerals, and protein in the reducible and minimum vital fractions vary in a like manner to their variance in the whole animal, namely with a decreasing concentration of water and fat, and an increasing concentration of minerals and protein. The minimum vital fractions of all the substances with the exception of fat are larger than the reducible fractions of the substances.

The average compositions of the minimum vital fraction and of the reducible fraction were found to be approximately as follows:

	Minimum vital fraction	Reducible fraction
Water	69%	64%
Fat	2%	20%
Mineral	5%	1%
Protein	24%	15%

The differential growth ratio of the total reducible fraction to the total weight shows that the total reducible fraction increases with the growth of the rat, while the differential growth ratio of the total minimum vital fraction to the total weight shows that it decreases with the growth of the rat. The value of the total reducible fraction varied from 18.1 per cent to 60.4 per cent of the total body weight and the value of the total minimum vital fraction varied from 39.6 per cent to 81.9 per cent.

It was concluded that inanition was not the sole cause of death in most cases since varying states of inanition were apparent in the rats depending upon diet and temperature. Variance in the state of inanition at death is contributed to differences in the ability of the animal to maintain a physiological equilibrium during inanition. This ability appears to depend largely upon the rate of autolysis which is taking place, being lessoned by an increased rate and increased by a lowered rate. At the lower temperature (18°C.) the rate of autolysis is increased and a lesser degree of inanition takes place with the exception of fat. In the group of rats which were deprived of water only, the use of food consumed as a preferential source of energy to that derived by the catabolism of body substances permitted a greater degree of autolysis by reducing the rate at which it took place.

Water appeared to have a toxic effect on the rats which were deprived of food, since death ensued before autolysis had occurred to as great an extent as in the rats deprived of both food and water. A decreased concentration of the blood in the rats which had access to water, by promoting the ill effects of starvation edema is suggested as the possible cause.

That there is often an inhibited fat metabolism during starvation is indicated by the unusually large amount of fat still present in the bodies of four of the rats at the time of their death.

There appears to be a preferential catabolism of fat in preference to protein as a source of water in the rats deprived of both food and water, since they lost more fat and less protein than the rats deprived of food only.

Water seems to facilitate the disposal of minerals, which may be hampered by dehydration as indicated by the greater amount of minerals lost in the rats deprived of food only in comparison with the rats deprived of both food and water.

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