GROWTH AND DEVELOPMENT

With Special Reference to Domestic Animals

XXVIII. Decline of Endogenous Nitrogen Excretion per Unit Weight with Increasing Weight in Growing Rats, and Its Relation to the Decline in Basal Metabolism. Decline in Live Weight, Nitrogen and Energy Metabolism with the Advance of the Period of Nitrogen Starvation and the Influence of Live Weight and of Preceding Level of Protein Intake on These Declines and on the Survival Periods.

URAL S. ASHWORTH AND SAMUEL BRODY

UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE

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FOREWORD

ABSTRACT AND SUMMARY

The total creatinine-N coefficient (mg. creatinine N per kilo live weight) in the rat is constant between 20 and 600 days of age (its value is of the order of 15); but the total endogenous urinary-nitrogen coefficient declines with increasing live weight as does the basal-energy metabolism coefficient. The ratio of basal energy metabolism to basal nitrogen excretion is thus constant (about 0.7 Calories per mg. endogenous urinary-N), while the ratio of creatinine-N to endogenous urinary-N increases with increase in size of the animal (this percentage ratio is 5 in a 35-gram rat, and 12 in a 340-gram rat). It is suggested that the intensity of basal energy, as also of basal nitrogen metabolism, is limited by the "active protoplasmic mass" in the body.

A prolonged period on a N-free diet reduces the energy metabolism to the same minimum level as during prolonged absolute starvation, namely 400 Calories per square meter per day.

The level of protein intake preceding the period of nitrogen starvation has a profound influence on the time required to reach the endogenous (minimum) level of nitrogen excretion (it required 8 days to reach the minimum level for "low-protein" rats, and 28 days for "high-protein" rats). The time required to reach the endogenous level of N excretion is thus an unknown function of the level of the preceding protein intake. Live weight is not an important influencing factor, but there must be great individual variations as the animals taken from our regular stock diet required from 4 to 26 days to reach the minimum level.

The fecal nitrogen on a N-free diet is a nearly constant percentage of the total nitrogen excretion (about 20%) with a slight tendency to increase with advance of the period on the N-free diet. The ratio of fecal-N to food intake increases very markedly with the advance of the period of nitrogen starvation.

Depending on the initial live weights and ages of the animals, rats may survive up to six months on N-free (but otherwise complete) diets, losing during this time about 60% of their live weight, which is the same order of percentage loss as was previously found for an absolute fast. The time curve of live weight on the N-free diet shows staircase phenomena suggesting the successive use of tissues of successively greater stability. These staircase phenomena are also exhibited in the time curves of nitrogen excretion.

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INTRODUCTION AND GENERAL DISCUSSION

It is generally known that the basal energy metabolism per unit weight declines with increasing weight of animals. Little, however, is known concerning the manner of change of basal nitrogen metabolism per unit weight with increasing weight, and of the ration between basal energy and basal nitrogen metabolism. The major object of this research is to contribute some data and discussion towards the solution of these problems.

We have demonstrated with considerable definiteness that the total creatinine-N excretion per unit live weight in the rat is relatively constant between the age limits of 22 and 600 days and weight limits of 50 and 350 grams (Missouri Res. Bul. 189). The basal energy metabolism per unit weight, on the other hand, declines between these limits from about 200 to about 90 Calories per kilo live weight (Missouri Res. Buls. 166 and 176). The obvious conclusions, therefore, is that there is no direct proportionality between energy metabolism and creatinine excretion; or that the functional relation between basal energy metabolism and creatinine excretion is the same as between basal energy metabolism and body weight.

These results on the rat were substantiated on dairy cattle (Missouri Res. Buls. 166, 176, and 191).While the absolute values of the creatinine coefficients in cattle differ from those in rats (the total creatinine-N coefficient in male rats is of the order of 15, while in female cattle the total creatinine-N coefficient is of the order of

17, and the preformed creatinine-N coefficient is of the order of 9.5), they appear to remain constant at all ages and weights (if the conditions of fatness remain the same), and they are not influenced by the dietary protein level (at least not in the rat), while the basal energy metabolism coefficients decline rapidly with increasing weight.

If, as is generally believed, the creatinine-N is an index of total endogenous-N excretion, then the endogenous-N excretion per unit live weight should likewise remain constant at all ages and weight. This generalization would seem to be substantiated by Mitchell's compilation (Bul. Nat. Res. Council, No. 67, Washington, 1929) indicating that the urinary endogenous-N is of the order of 25-35 mg. N. per kilo live weight for such unrelated species as pigs, cattle, sheep, humans.

We have attempted to find out whether or not this constancy for the endogenous urinary-N excretion holds true for rats varying widely in live weight; that is, whether or not the creatinine-N is a constant fraction of the total urinary endogenous-N at various live weights. The results were reported in Missouri Res. Bul. 189, but they were not satisfactory, because other difficulties came up, as for example the question concerning the enormous variations of the time required to reach the minimum coefficient, and the factors causing these variations. The preceding report, thus, while solving the total creatinine coefficient problem, leaving no doubt that it is constant—that is, it is not affected by live weight, age, or dietary protein level—, has left the total endogenous-N problem, and its relation to basal energy metabolism in an unsatisfactory state.

Mitchell and Hamilton in their *Biochemistry of Amino Acids* (New York, 1929), while they published a table giving the endogenous nitrogen excretion in rats of different ages, clearly indicating a decline in the endogenous nitrogen excretion per unit weight with increasing weight, did not discuss the relation of this decline to the decline in basal energy metabolism per unit weight with increasing weight. This monograph seems to give the impression also that the creatinine-N is a constant fraction of the total endogenous nitrogen.

The table in Mitchell and Hamilton's book is based on data obtained incidentally by Mitchell and Carman in connection with a research on biological values of proteins. They assumed that four days on a N-free diet sufficed to reach the endogenous level of urinary-N excretion. We, in this work, kept the rats on a N-

free diet until the time curves of N-excretion showed an irreducible minimum.

More recently Smuts, under Mitchell's direction (Smuts, An Abstract of a Thesis, Urbana, Ill., 1932), studied this problem in mature animals of different species (mouse, rat, guinea pig, rabbit, pig). The following quotations from Smuts' abstract summarize his findings:

"The average creatinine coefficients for the different species are: for male rats, 41; for female rats, 40; for guinea pigs, 34; for mice, 81; for rabbits, 46; and for pigs, 19. The tendency is, in the smaller animals, for the creatinine nitrogen to represent a smaller percentage of the total endogenous nitrogen. The average percentage of creatinine nitrogen in terms of total endogenous nitrogen is for mice, 4.9 per cent; for male rats, 7.3 per cent; for female rats, 6.7 per cent; for guinea pigs, 7.2 per cent; for rabbits, 14.3 per cent; and for pigs, 18.2 per cent.

"A constant relationship exists between the basal metabolism and the total endogenous nitrogen of mature individuals of different species, the average ratio being 1.99 milligrams per Calorie of basal heat for the species studied."

Smuts' results thus contradict the conclusion that might be drawn from Mitchell's compilation (Bul. 67, Nat. Res. Council) that the endogenous nitrogen coefficient is the same in the several species there listed.

Smuts' conclusion that "a constant relationship exists between the basal metabolism and the total endogenous nitrogen of mature individuals of different species" is of course of very great theoretical interest, and if true needs to be extended to include members of the same species of different live weight and ages, and this is the major object of research presented in this bulletin. Smuts concludes that "the validity of the surface area as the best unit of reference both for the basal metabolism and for endogenous nitrogen, is substantiated by these data." We believe, on the other hand, that these data substantiate the "active protoplasmic mass" theory of Benedict and of Schaeffer and Le Breton, and we feel that our N-fasting data presented in this bulletin support our belief.

The general plan of this research was to place on N-free but otherwise complete diets rats ranging in age from 30 to about 600 days, keep them on this diet until death, while measuring the nitrogen and energy metabolism. Preceding the placement on the N-free diet, it was planned to feed some of the rats with a very high-protein stock diet (100 mg. N per gm.), and others on a low-protein stock diet (10 mg. N per gm.) in order to determine the influence of the preceding dietary protein level on the time required to reach the endogenous, or basal, level of nitrogen excretion. Since this research, unfortunately, had to be abbreviated, some modifications had to be introduced as described in the detailed discussion below.

RESULTS

The results are given in Tables 1 and 2, and in a series of charts. Further details are given in the following discussions. Methods of urine collection and analysis, as also the detailed literature, are given in the preceding report (Missouri Res. Bul. 189).

TABLE 1.—THE INFLUENCE OF TIME ON PRACTICALLY N-FREE DIET ON THE AVERAGE LIVE WEIGHT, DAILY FEED CONSUMPTION, URINARY-N EXCRETION, TOTAL BODY-N LOSSES OF LARGE RATS (Nos. 2116, 2213, 3287, 3289) AND SMALL RATS (Nos. 7528, 7529, 7530, 7531, 7532, 7533, 7534, 7535, 7536, 7537)

Days on N-free diet	Av. live wt., gms.		Feed consumption gms.-kg.-day		Urinary-N excretion mg.-kg.-day		Total-N excretion mg.-kg.-day		Body-N losses mg.-kg.-day		Creatinine-N Urine-N %	%
	Large	Small	Large	Small	Large	Small	Large	Small	Large	Small	Large	Small
0	363	57	35	104	883	1927	1040	2450	----	----	1.71	0.8
2	355	50	35	105	308	1140	412	1463	394	1158	4.90	1.3
4	354	52	30	84	249	390	300	482	284	389	6.06	3.9
6	352	50	31	77	219	391	270	470	254	373	6.89	3.9
8	350	48	32	65	199	371	248	451	231	379	7.59	4.1
10	348	46	34	62	166	373	208	445	190	371	9.10	4.0
12	345	45	35	60	179	350	220	425	201	361	8.44	4.3
14	344	44	35	62	164	327	213	413	195	337	9.21	4.6
16	344	43	34	62	145	325	182	406	165	336	10.41	4.6
18	348	43	35	57	110	303	144	382	126	305	13.7	5.0
20	339	42	31	58	130	319	164	399	147	322	11.6	4.7
22	338	42	30	54	128	294	164	378	147	301	11.8	5.1
24	333	41	27	59	132	293	175	368	159	289	11.4	5.2
26	332	41	25	61	129	292	160	369	145	290	11.7	5.2
28	325	40	21	57	147	314	182	401	168	325	10.3	4.8
30	321	40	23	61	142	297	170	365	156	295	10.6	5.1
32	315	39	22	54	159	326	188	409	174	343	9.5	4.6
34	312	38	24	55	145	324	170	411	155	346	10.4	4.7
36	307	37	23	58	149	333	172	422	157	359	10.1	4.5
38	300	37	----	57	158	353	182	445	170	385	9.6	4.3
40	295	36	17	59	161	342	188	440	175	382	9.4	4.4
42	294	36	22	52	135	364	176	452	145	406	11.2	4.1
44	291	36	26	49	123	325	160	403	121	362	12.3	4.6
46	290	37	30	47	125	289	157	363	117	327	12.1	5.2
48	292	40	33	50	115	299	149	392	109	338	13.1	5.1
50	291	39	34	43	121	274	154	368	113	320	12.5	5.5
52	292	40	30	46	112	260	153	366	113	311	13.5	5.8
54	290	40	27	42	126	276	164	389	125	335	12.0	5.5
56	288	39	24	45	128	265	162	361	124	309	11.8	5.7
58	282	38	24	43	149	289	186	382	148	334	10.1	5.2
60	278	38	23	43	151	263	194	336	155	294	10.0	5.7
62	273		23		148		185		162		10.2	
64	271		24		137		174		150		11.0	
66	264		16		157		188		166		9.6	
68	258		18		158		178		139		9.6	
70	262		21		161		212		172		9.4	
72	257		23		143		187		136		10.6	
74	255		----		174		224		192		8.7	
76	251		21		165		220		183		9.2	
78	251		22		153		222		185		9.9	
80	247		21		153		194		152		9.9	
82	243		20		158		213		171		9.6	
84	240		22		174		197		154		8.7	
86	238		21		160		198		142		9.4	
88	235		20		184		231		182		8.2	
90	231		22		160		209		161		9.4	
92	230		25		152		206		186		9.9	
94	229		19		151		198		181		10.0	
96	226		20		165		194		176		9.2	
98	222		22		167		226		188		9.0	
100	220		18		171		217		180		8.8	
102	214		23		174		224		184		8.7	
104	212		25		178		230		209		8.5	
106	212		29		164		210		187		9.2	
108	209		24		159		206		193		9.5	
110	206		21		168		217		198		9.0	
112	206		21		182		237		217		8.3	
114	203		22		189		250		229		8.0	
116	196		26		161		220		197		9.4	
118	194		24		198		238		217		7.6	
120	190		21		199		254		233		7.6	
122	188		19		208		263		243		7.3	
124	186		24		179		246		223		8.4	
126	184		26		176		217		194		8.6	
128	182		25		183				204		8.3	
130	181		24									

TABLE 2.—STATISTICAL DATA, INDIVIDUAL RATS

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)	(24)
					Time to reach minimum N. coefficient	Weight at minimum N. coefficient	N. excreted during period			N-intake during period	Body-N. lost (Urin.-N+Fe N less Feed N)	Body-N content	N. in body of dead rats	Total food consumed during period	Weight of dead rat	Body weight lost	Ratios						Basal Metabolism Min. Ur.-N. excretion
Rat No.	Initial weight	Initial age	Survival period	Minimum Urinary-N coefficient			Uri-nary-N	Fecal-N	Urin.-N + Fecal N.								$\frac{\text{Fecal-N}}{\text{Total-N}}$ %	$\frac{\text{Fecal-N}}{\text{Food Intake}}$ Mg/Gm	$\frac{\text{Urinary-N}}{\text{Food Intake}}$ Mg/Gm	$\frac{\text{Body-N Lost}}{\text{Food Intake}}$	$\frac{\text{Body-N Lost}}{\text{Body Weight Lost}}$	Urinary-N Body wt. lost Mg/Gm	
	gms.	days	days	Mg-N/Kg	days	gms.	mgs.	mgs.	mgs.	mgs.	mgs.	Mg/Gm	mgs.	gms.	gms.	%							Cal/Mg-N
7529	31	26	44	320	20	27	480	139	619	132	487	29.6	610	85	20.6	33.5	22.5	1.64	5.65	5.73	46.8	46.2	.61
7530	37	29	46	300	26	32	609	146	755	127	628	27.0	643	81	23.8	35.7	19.4	1.80	7.52	7.75	47.6	46.1	.64
7528	41	31	46	340	26	25	631	145	776	95	681	28.3	584	105	20.6	49.8	15.3	1.38	6.01	6.49	33.4	30.9	.57
7534	41	26	66	275	14	39	558	155	713	132	581	27.1	767	101	28.4	30.7	21.7	1.53	5.52	5.75	46.1	44.3	.69
7533	48	29	60	265	24	39	840	221	1061	140	921	----	---	146	----	----	20.8	1.51	5.75	6.31	----	----	.71
7532	53	29	74	250	22	45	934	227	1161	185	976	26.8	840	173	31.4	40.8	19.5	1.31	5.40	5.64	45.2	43.2	.74
7531	54	34	58	280	26	44	976	227	1203	160	1043	28.5	890	139	31.3	42.0	18.9	1.63	7.02	7.50	45.9	43.0	.67
7537	59	38	50	285	12	50	741	215	956	136	820	27.0	780	133	29.0	50.8	22.5	1.62	5.57	6.17	27.3	24.7	.66
7536	61	34	70	250	40	50	1217	298	1515	195	1320	25.0	1050	181	41.9	31.3	19.7	1.65	6.72	7.29	69.1	63.7	.76
A-4	66	35	52	282	22	44	946	201	1147	199	948	25.2	1130	159	44.9	32.0	17.5	1.26	5.95	5.96	44.9	44.8	.66
A-3	70	35	64	260	24	48	1081	232	1313	242	1071	25.0	930	194	37.0	47.1	17.7	1.20	5.57	5.52	32.5	32.8	.71
7535	85	38	60	285	20	59	1045	273	1318	173	1145	29.2	1040	179	35.6	58.1	20.7	1.53	5.84	6.40	23.2	21.2	.63
B-4	95	47	92	240	14	78	1784	401	2185	258	1927	28.6	1170	301	41.0	56.9	18.3	1.33	5.93	6.40	35.7	30.0	.71
B-3	101	47	86	245	14	86	1843	430	2273	230	2043	29.0	1430	276	49.0	51.5	18.9	1.56	6.68	7.40	39.3	35.4	.69
2205	235	739	84	132	4	220	2929	1142	4071	514	3557	33.4	4700	506	140.7	40.1	28.1	2.26	5.79	7.03	37.7	31.1	.91
2206	270	739	86	129	4	263	3506	1232	4738	553	4185	35.4	5100	551	144.2	46.6	26.0	2.24	6.36	7.60	33.3	27.9	.84
2116	355	766	148	145	26	315	6265	1753	8018	914	7104	30.0	4650	935	155.0	56.3	21.9	1.87	6.70	7.60	35.5	31.3	.65
3289	355	551	160	122	42	284	6461	1699	8160	1069	7091	32.0	4320	931	135.0	62.0	20.8	1.82	6.94	7.62	32.2	29.4	.84
3287	360	551	184	120	20	340	6720	1949	8669	1164	7505	28.1	4130	1119	147.0	59.1	22.5	1.74	6.01	6.71	35.3	31.6	.74
2213	382	740	176	115	22	357	6690	1893	8589	1089	7500	29.0	4900	1135	169.0	55.8	22.0	1.67	5.90	6.61	35.2	31.5	.74

(4) Survival period on N-free diet. Note that large animals lived much longer.

(5) The minimum level of urinary nitrogen excreted on the days in column (6).

(7) Body weight of rat at this period.

(8), (9), (10) Sum of excretions of nitrogen until death from N-starvation.

(11) Sum of total N from vitamine supplement and "N-free" diet.

(12) Column (10) minus column (11).

(13) Gained by analysis of the whole carcass.

(14) Column (13) times column (16).

(18), (19), and (20) These ratios seem to be not influenced by size of animal.

(22) Note that the body-N lost per unit weight is much greater than the nitrogen content (13) of the dead rat.

(24) This indicates a direct relationship between basal metabolism and endogenous nitrogen excretion.

DETAILED DISCUSSION OF RESULTS

Each of the results is discussed in the order of its importance.
Basal (Endogenous) Nitrogen Excretion as Function of Body Weight.—To simplify matters, the data were divided into two groups, one representing the average of the small rats, and the other representing the large rats. In Fig. 1 the average nitrogen ex-
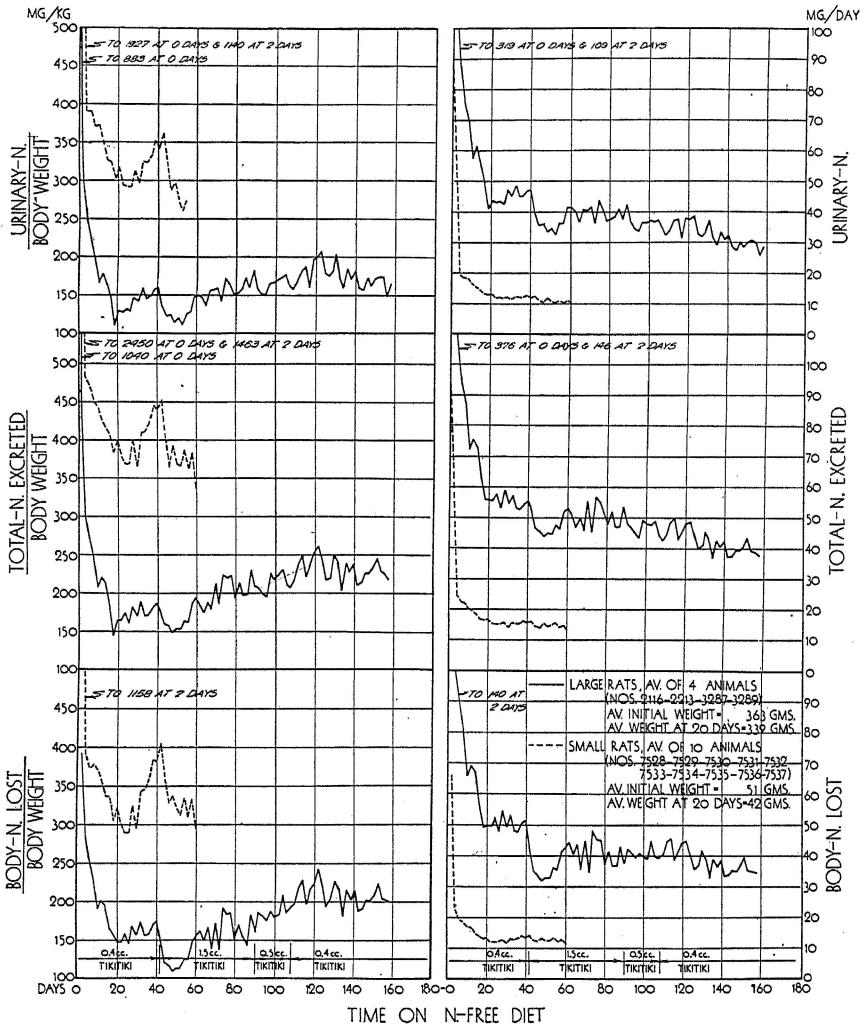

Fig. 1.—The decline of nitrogen excretion with advance of the period on the N-free diet. Right side excretion per animal per day; left side, excretion per kilo live weight per day. Total N refers to urinary-N plus fecal-N; body-N lost refers to urinary-N plus fecal-N less dietary-N.

cretion of each of these two groups of rats is plotted against the time on N-free diet. On the left side the data are represented in terms of mg. N excreted by kilo live weight (coefficients); on the right side, the excretion per animal. The same data are given in numerical form in Table 1. The total nitrogen is the sum of urinary plus fecal N; body N lost is the sum of the total nitrogen less food nitrogen. The indicated changes for vitamin supplements (tikitiki plus Armour's liver extract) relate to the large rats only, the small rats were given a uniform dose of 0.4 cc. of this supplement per day. The curves showing something of a "staircase" phenomenon may be due in part, in the case of the larger rats to the changes in vitamin supplement and food intake. This will be discussed in greater detail in connection with the time curves of losses in body weight.

The absolute amount of nitrogen excreted per day is of course greater for the large than for the small rats.

But as regards the nitrogen excreted per kilo live weight (N-coefficient), it is decidedly greater for the small than for the large animals. The average minimum urinary-N excretion per kilo live weight is seen to be 110 mg. for the large rats as compared to 292 for the small rats; for the fecal-N (in Fig. 2) this is seen to be about 75 mg. for the small rat as compared to 25 for the large rats; the total nitrogen excretion is about 370 mg. for the small rats as compared to 150 for the large rats. The average minimum total N-loss by the body (N of urine plus N of feces less food N) is about 290 mg. for the small rats as compared to 150 (or possibly 113) for the large rats.

It is thus evident that unlike in the case of creatinine excretion, the endogenous urinary and total N excretion per unit live weight decrease with the increase in size of the animals.

Decline in Total Nitrogen Excretion, Food Intake, and Ratios of Fecal Nitrogen to Food Intake.—These are illustrated in Fig. 2. The food intake per unit live weight declines more rapidly in the small than in the large rats. This may explain in part the more rapid decline in live weight of the small than large rats to be discussed presently. The fecal nitrogen, which is, of course, almost entirely of body origin (since the diet was practically N-free), tends to follow the same course as the urinary nitrogen. The upper-right curves show plainly that the ratio of fecal to total nitrogen is nearly constant during the entire period; about 20% (±7%) of

the total endogenous nitrogen excretion is by way of the intestine with a tendency to increase with the time on the N-free diet. The ratio of fecal nitrogen to food intake is seen to increase decidedly with the time on the N-free diet.
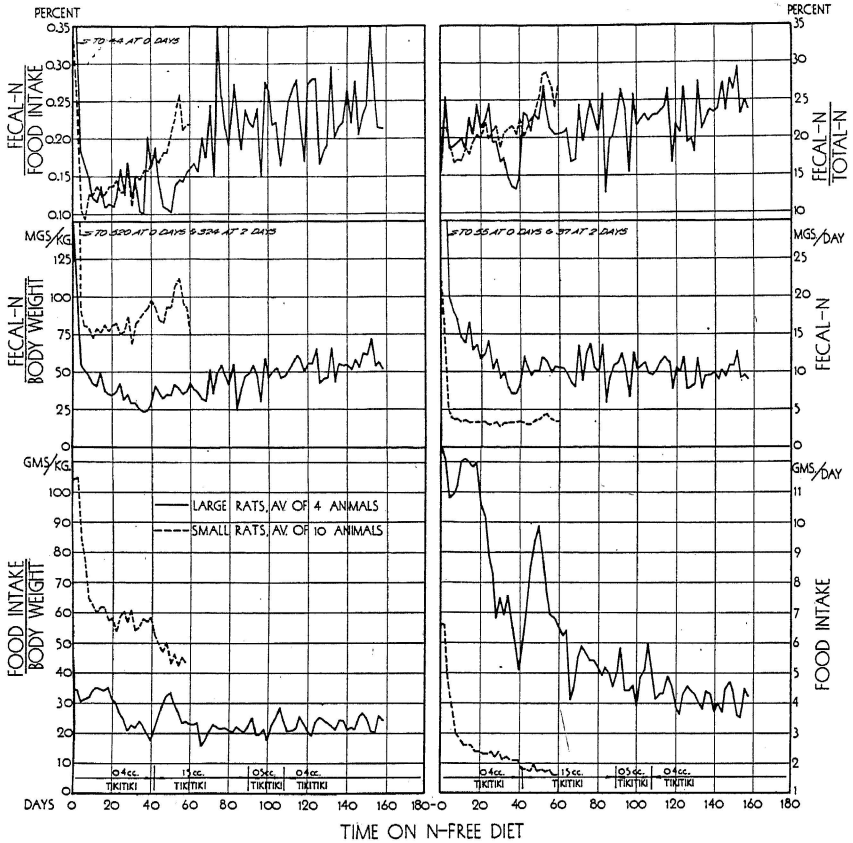

Fig. 2.—Food intake, fecal-N, and ratios of fecal-N to body weight, food intake and total-N excretion with advance of period of N starvation.

The Relation Between Basal Metabolism and Endogenous Urinary Nitrogen Excretion.—This is illustrated in Fig. 3. The broken curves represent the basal energy metabolism per unit live weight. These curves were copied from pp. 72-74, Missouri Res. Bul. 166. The circles represent data on endogenous urinary nitrogen excretion, as obtained on small and large rats (on the left side, data are from Mitchell and Hamilton, **Biochemistry of Amino Acids,** New York, 1929; on the right side, Missouri data). It is seen that

the ratios of basal metabolism to endogenous urinary nitrogen excretion are practically the same for small and large rats.
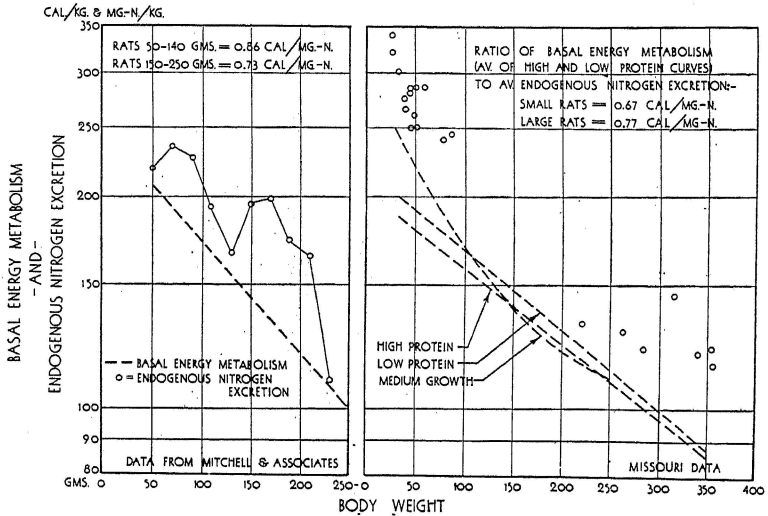
Fig. 3.—Relation between basal energy and basal nitrogen metabolism with increasing live weight of animals.

The Decline in Energy Metabolism on the N-Free Diet.—Fig.

4 shows the course of change of heat production per unit weight and per unit area in one rat with the advance of the period of N
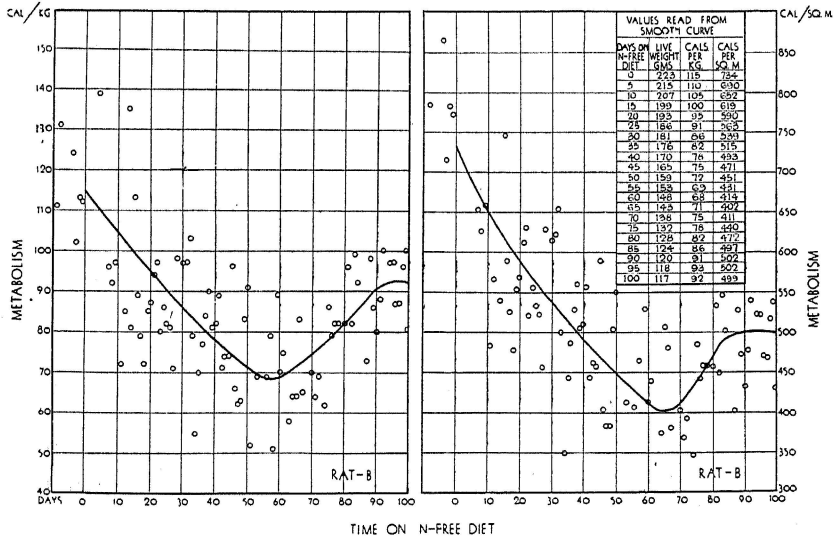
Fig. 4.—Basal metabolism per kilo and per square meter with advance of the period of N starvation.

starvation. The rat was not in post-absorptive condition, having food in front of him at all times. The data were secured by the closed-circuit oxygen consumption method.

The energy metabolism is seen to decline from 850 Cals./sq. meter before placing on the N-free diet to about 400 Calories on the 75th day, then it rises to about 500 on the 100th day.

This rat, on a N-free diet, reached the same level of energy metabolism (400 Calories per sq. meter) as Benedict's rat, which was fasted completely (Benedict, Horst and Mendel, J. Nutrition, 1932, 5, 571; see also addenda on p. 28, Missouri Res. Bul. 180). Is it possible that the same energy-metabolism level was reached by Benedict's and our rats because the "protoplasmic mass," or "active mass" was reduced to the same level by the two kinds of fasting? If so, should not then these results be considered as substantiating the active-protoplasmic mass theory as previously explained, rather than the surface area theory as assumed by Smuts? It would seem reasonable to assume that the intensity of both types of metabolism (energy and nitrogen) are actuated by the same ultimate mechanism, namely the "active protoplasmic mass."

Influence of the Preceding Level of Protein Intake on the Time Required to Reach the Endogenous Level of N Excretion.—The results of this investigation are summarized in Fig. 5. From the upper left-hand quadrant we see that not until after 28 days of specific nitrogen starvation does the urinary nitrogen coefficient of the high protein animals reach a level similar to that which the low protein rats reached after about 8 days. The fecal nitrogen excretion of the high protein rats is also definitely higher, in fact the ratio of fecal-N to urinary-N curves show that the increase is proportional to the urinary nitrogen increase. The food intake for the two groups was approximately the same, but the body weight loss is more rapid for the high protein group. This is no doubt caused by their more intense nitrogen catabolism. From the lower left-hand quadrant one may observe that the high protein rats excrete more fecal nitrogen per unit food intake than the low protein rats.

All these observations point to the fact that the previous level of dietary protein has a marked influence on the time necessary to reach the endogenous level of nitrogen excretion in the rat. This may account for the differences observed in the time previously noted at this laboratory and that given by Mitchell.
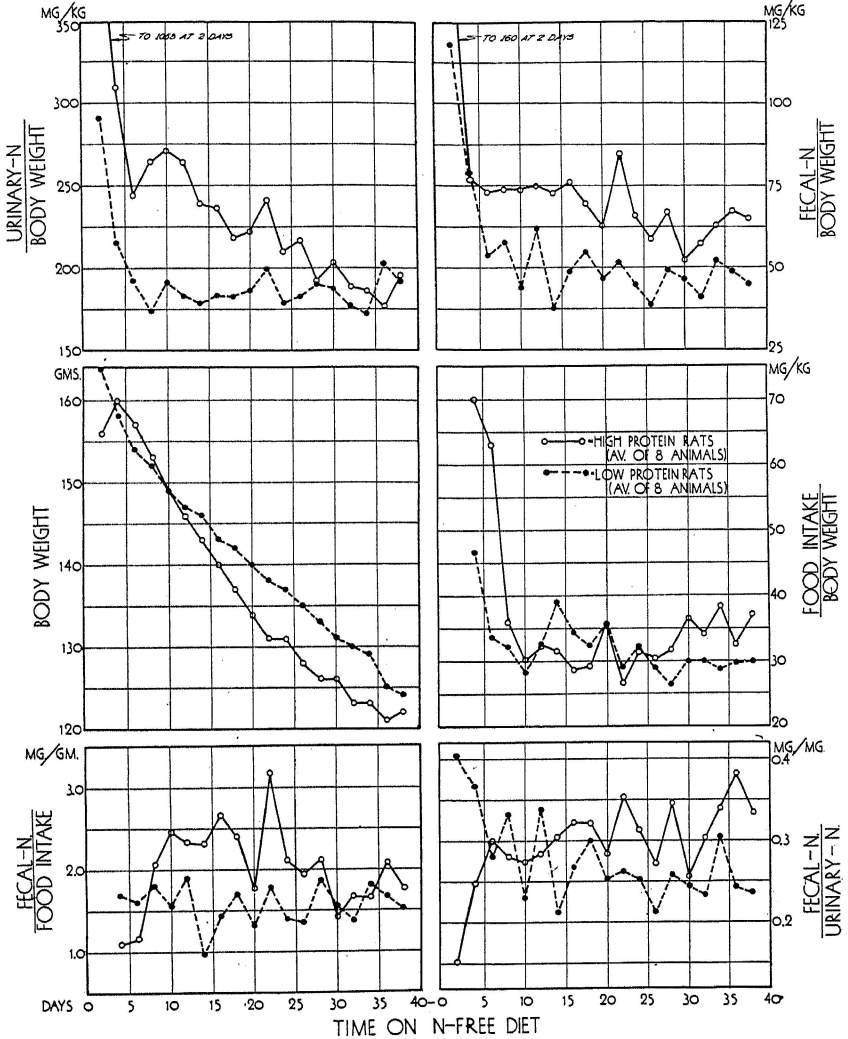
Fig. 5.—The influence of the preceding level of protein intake on the time required to reach the endogenous nitrogen level, etc.

The Decline in Body Weight.—This is illustrated in Fig. 6 for individual rats of various live weights. A striking feature of the curves, especially of the larger animals, is a peculiar staircase effect, especially during the first 60 days. This phenomenon might indicate the presence in the body of various tissues differing in stability or in what might be termed as "lytic potential." Some tissues or constituents, such for example, as what is termed "deposit protein," succumb most easily; this is followed by the "lysis" of more stable tissues, etc. Or this phenomenon (if present) may be limited not so much by the stability of the several tissue systems as by changing enzyme complexes.
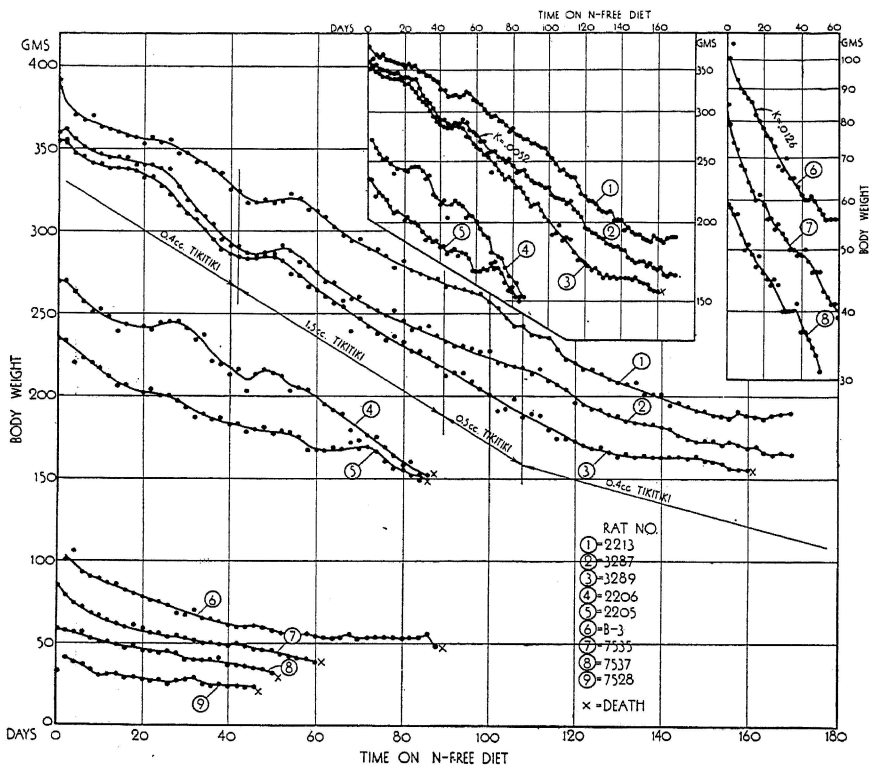
Fig. 6.—Decline in body weight of individual rats with the time on the N-free diet. The insets represent plots on arithlog paper, with the k's representing the slopes of the curves.

On the other hand, some of the irregularities may be the result of fluctuations in dietary intake; for, as noted, one of the dietary constituents, the vitamin supplement (mixture of tikitiki and Armours' liver extract), was not kept constant in the diet of the large

rats. It may be seen from the chart that in the case of the large rats, at about 42 days, the tikitiki was increased from 0.4 to 1.5 cc. in order to stimulate the animals to take more of the "N-free" diet. This dietary change coincides with a flattening of the curve. But this does not enter as a factor in change of slope at about 22 days; nor the changes in slopes of the smaller rats which received a constant amount of this supplement.

The insets represent the same data plotted on arithlog paper on a smaller scale. The values of k represent the average slopes of the curves (the relative, or when multiplied by 100 the percentage, decline). A comparison of the numerical values of k shows clearly that the small animals decline in live weight more rapidly than the large ones. Thus, rat 6 declines in live weight about 2.4 times as rapidly as rat 2 (.0126/.0052=2.4) ; and the smaller rats also succumb sooner than the large rats. It would thus appear that since the energy needs were met by the N-free diet, the more rapid decline in live weight of the small animals must be due to their more intense nitrogen metabolism, which is indeed the case as demonstrated by the nitrogen excretion curves in Fig. 1.

Survival Period.—Some of the rats died as result of infection or intestinal disturbances. The greatest survival periods of these nitrogen-starved rats, however, seem to occur when the animals lose about 60% of their live weight, which is the same order of loss in live weight of several species of animals that are starved absolutely (water given), as described in Missouri Res. Bul. 179. The ultimate cause of death may be the same in absolute or nitrogen starvation, namely, the stepping over a critical point in loss of "active protoplasmic mass." A possible difference in the survival periods on the two types of starvation is that in the case of specific nitrogen starvation the "active protoplasmic mass" is protected by the N-free dietary intake. This reasoning, if correct, would seem to furnish further substantiating evidence concerning the limiting nature of the "active protoplasmic mass" on the metabolic processes. It would then be more correct to say that death occurs not when a certain percentage of body weight is lost, but when a certain percentage of "active protoplasmic mass" is lost; or at least when a certain percentage of body nitrogen is lost.

CONCLUSIONS

The principal contribution and conclusion of this research is that the basal (endogenous) nitrogen and the basal energy metab-

olism follow the same course with increasing live weight, age, or conditions of starvation.

The theoretical implications, as they seem to us, are first, that there is a very intimate functional relation between basal energy and endogenous nitrogen metabolism; and second, that this relation is ultimately caused by the same mechanism, namely, by the "active protoplasmic mass" in the body.

It does not seem reasonable to assume that surface area is a limiting factor under conditions of thermal neutrality. The fact that the basal metabolism (energy or nitrogen) tends to be proportional to surface area is probably due to a more or less constant relationship between surface area and "active mass" (see Fig. 1c, p. 8, Missouri Res. Bul. 176). It is the living, continuously active, catabolizing tissues of the body—the "active protoplasmic mass"— that expends energy and nitrogen for its life processes, and not area. It is necessary to specify "active protoplasmic mass" rather than merely "protoplasmic mass" because not all protoplasmic tissues are equally active. Thus, under conditions of absolute rest, the skeletal muscles are relatively inactive and stable in comparison to the visceral organs such as kidney, heart, liver, digestive tract in which catabolic processes are continuous. The skeletal and similar tissues while relatively inactive are yet, of course, not inert; so that the active mass is a term representing in as yet vague manner the statistical average of the catabolic activities of the protoplasmic mass. The vagueness of the concept is in itself no argument against it, and a concrete equivalent may be discovered in due time. Indeed, if a satisfactory method were available for measuring area, then area might be found to be the practical if not concrete equivalent of active protoplasmic mass. Unfortunately, as previously noted (Missouri Res. Bul. 166, p. 11), the measuring of "active" area of living animals offers apparently unsurmountable difficulties. (Professor Max Rubner, indeed, told one of the writers that by area he meant not so much measurable area as "physiological area"; this seems to us to approach in vagueness the "active mass" concept.)

As a corollary of the principal contribution, it follows that creatinine nitrogen is a decreasing function of the total endogenous nitrogen with decreasing live weight of the animal (see the last two columns in Table 1).

A practical implication of the principal contribution is that the dietary protein needs for maintenance per unit live weight probably

decrease with increasing live weight in the same manner as the energy needs.

A number of incidental contributions have been made which are listed in the abstract at the beginning of this report.