THE THRESHOLD OF KETOGENESIS.*

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It is recognized that diets so unbalanced as to contain greatly disproportionate amounts of fat lead to the formation and excretion of acetoacetic acid, β-hydroxybutyric acid, and acetone. The accumulation of these substances depends on the ratio existing between the ketogenic substances, particularly fatty acids and the glucose of the metabolizing foods. That ratio at which significant ketosis first appears may be called the threshold of ketogenesis. Accurate knowledge of the value of this threshold is a matter of importance in gauging the safety of diets high in fat such as may be used in diabetes. Zeller (19), Lusk (4), Ringer (7), Woodyatt (17, 18), Shaffer (8, 9), Palmer (6), Hubbard (2), Hubbard and Wright (3), and others have contributed data to the subject. Woodyatt (18) has discussed the problem with great lucidity and Shaffer (8, 9) has reported investigations which have stimulated thought and study.

With mixtures of varying proportions of acetoacetic acid and glucose in alkaline hydrogen peroxide, Shaffer (8, 9) beheld in the test-tube what he considered to be an *in vitro* analogy to the action of glucose in abolishing or preventing the formation of acetoacetic acid in man. When the proportion of acetoacetic acid to glucose in such mixtures was that of 1 (or possibly 2) molecules of acetoacetic acid to 1 of glucose, the former substance was completely oxidized. When the proportion of glucose was less, a considerable fraction of acetoacetic acid escaped oxidation. In these papers, Shaffer calculated the number of molecules of ketogenic material and the number of molecules of glucose in the determined metabolism of several human subjects and concluded

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that the maximal ratio compatible with the oxidation of the ketogenic compounds was reached when the number of ketogenic molecules just equalled the number of glucose molecules, a ratio of 1:1. He subsequently modified this conclusion (11), and now considers that each glucose molecule is ketolytic for 2 molecules of acetoacetic acid, a 2:1 ratio. Woodyatt (18) and likewise Hubbard and Wright (3) have based working formulas for calculating diets on a 1:1 ratio.

Our interest in the ketogenic threshold was stimulated by Shaffer's first paper and we were encouraged to pursue the study because at the time we were trying, for diabetic patients, diets relatively high in fat such as had been proposed by Newburgh and Marsh (5). We found that we were feeding mixtures of food in which the ratios of ketogenic molecules to glucose molecules, as calculated by Shaffer's method, were considerably higher than 1:1. Despite this we were not provoking any appreciable ketosis. This was true at least under the conditions observed. The protein in the diets did not exceed 1 gm. for each kilo of the subject's body weight and the total calories of the diets were not above the caloric needs of the patients. We recognized, however, that these food mixtures did not necessarily represent the composition of the mixtures which were actually burning and the next step was an endeavor to determine the value of the ratio between the fatty acid molecules and the glucose molecules in the burning mixture. Our first thought was that the non-protein respiratory quotient and the Zunz-Schumburg (4) tables would give us this information, but in this we were disappointed. We depended, for our respiratory metabolic data, on methods involving indirect calorimetry and short period observations; respiratory quotients obtained under these circumstances cannot mirror the relative proportions of fat and carbohydrate engaged in the total metabolism of 24 hours. This has been discussed in another communication (16) and is in agreement with conclusions reached by Shaffer (10). A more reliable method of determining the composition of the burning mixture is the following:

The basal metabolic calories may be accurately measured by short period observations, and if the patient is confined to bed, the extra energy exchange due to the specific dynamic action of food and that resulting from movements in bed may be estimated as 20 per cent of the basal calories with a reasonable degree

of correctness. An approximation of the total metabolism for 24 hour periods may thus be reached. The nitrogen elimination for 24 hour periods being also determinable, the calories from the protein metabolism are known, each gram of urinary nitrogen representing 26.51 calories. If a low carbohydrate quota (60 gm. or less each day) is maintained for several days, it is reasonably safe to assume that all of the carbohydrate included in the diet is burned. The calories from the fat metabolism are obtained by subtracting the sum of the calories of the protein and the carbohydrate metabolism from the total calories of the day.

Employing the values of fat, protein, and carbohydrate secured in this manner, we calculated the ketogenic ratios of a group of patients on high fat diets. The results in one such patient have been reported elsewhere (15, 16). This patient was a young woman with diabetes of the acute, progressive type, and on two occasions the dextrose-nitrogen ratio of the urine reached 3.65:1 and continued at this height for 4 consecutive days. On both occasions, acetone bodies accumulated in the blood and coma threatened. The rising acidosis was checked by a diet in which the proportion of fat was very high, and an analysis of the mixture actually metabolized after acidosis was controlled revealed ratios of ketogenic molecules to glucose molecules of 1.5:1 and 1.8:1, respectively.

The present report is concerned with the determined composition of metabolizing food mixtures in sixteen other patients. Three of these patients had epilepsy but were, in other respects, apparently normal. Thirteen had diabetes; two of these suffered from mild, acute infections. None of the epileptic patients had convulsions and the diabetic patients were without glycosuria at the time of the investigation. The patients were confined to their rooms during the 3 days preceding the test; on the day before the test and on the day of the test, they remained in bed. During this period, each received a constant diet. The food consisted of rice, soy bean bread enriched with fat, butter, and cream; its composition was calculated from the Atwater-Bryant tables. The daily food allowance did not exceed in caloric value the energy needs of the patient and the protein quota was less than 1 gm. for each kilo of body weight. Precautions were taken to secure accurate collections of urine. Daily determinations of urinary nitrogen were made by means of the Kjeldahl method.

Van Slyke's methods were followed for acetone bodies of the urine and blood (Van Slyke and Fitz, 14), and for the carbon dioxide-combining power of the plasma. The basal respiratory metabolism and respiratory quotients were secured by the gasometer method as described by Boothby and Sandiford. The calculations of metabolizing mixtures and the ratios of ketogenic molecules to glucose molecules in these mixtures are based on assumptions, which, for the sake of clarity will be restated.

Assumptions Involved in Calculating the Metabolizing Mixture.

1. The total energy exchange, or total metabolism, is accurately represented by the basal calories for 24 hours plus 10 per cent for the specific dynamic action of food and 10 per cent for movements. This assumption is justified if the patient is confined to bed during the test day and for 1 or more preceding days, as was the case in these experiments.

2. All glucose derived from the food (carbohydrate, protein, and fat) is burned. This assumption is justified, provided the subject has received relatively little carbohydrate and protein for several days preceding the test, and provided the diet does not exceed the energy and nitrogen requirements of the subject, as was the case in these experiments. The total amount of carbohydrate in the diet of a patient with diabetes must not exceed the tolerance for carbohydrate if this assumption is to hold. Furthermore, carbohydrate starvation may very well increase the avidity with which glucose is stored as glycogen, so that less than the amount of glucose derivable from the food will actually burn. Errors of this kind would tend to give erroneously low values for calculated ketogenic ratios.

3. No carbohydrate from endogenous sources (glycogen) is burned. Such an assumption is naturally precarious. It is presumed, however, that the glycogen stores of patients who are on a régime very low in carbohydrate are retained, tenaciously. An error from this source would tend to give erroneously high values for calculated ketogenic ratios.

4. The nitrogen in grams in a 24 hour specimen of urine multiplied by 26.51 is the number of calories from the protein metabolized.

Given: the total metabolism M, the grams of carbohydrate of the food C, and the grams of nitrogen of the urine N. The amount

of fat F in the metabolizing mixture is calculated from the equation:

$$F = \frac{M - (C \times 4.1) + (N \times 26.51)}{9.3}$$

The values for carbohydrate and fat thus obtained are used in the calculation of the ratio between the ketogenic molecules and the glucose molecules as follows:

Assumptions Involved in Calculating the Ratio of Ketogenic Molecules to Glucose Molecules, (the K : G ratio)[1] *(Shaffer 8, 9).*

1. 1 gm. of fat, molecular weight 874 = $\frac{1}{874} \times 3 = 0.00343$ gm. molecules of ketogenic fatty acid. Let a = grams of fat $\times$ 0.00343.

2. 1 gm. of urinary nitrogen = 0.01 gm. molecules of ketogenic substance. Let b = grams of nitrogen of 24 hour urine $\times$ 0.01.

This is based on Osborne's analysis of ox muscle as modified by Lusk (4) and is derived as follows:

Ketogenic Amino-Acids in 100 Gm. of Muscle Protein.

	Grams.	Molecular weight.	Gm. molecules.
Leucine	14.3	131	0.109
Phenylalanine	4.5	165	0.027
Tyrosine	4.4	181	0.024
Sum for 16 gm. of nitrogen			0.160
Sum for 1 gm. of nitrogen			0.010

3. 1 gm. of glucose (carbohydrate), molecular weight 180 = $\frac{1}{180}$ or 0.00556 gm. molecules of glucose. Let c = grams of carbohydrate $\times$ 0.00556.

4. 1 gm. of nitrogen of the urine = $\frac{1}{180} \times 3.65$ or 0.02 gm. molecules of glucose. This is based on the assumption that 3.65 gm. of glucose are liberated from protein with each gram of nitrogen. Let d = grams of nitrogen of 24 hour urine $\times$ 0.02.

5. 1 gm. of fat, molecular weight 874 = $\frac{1}{874}$ or 0.00114 gm. molecules of glycerol $\div$ 2 = 0.00057 gm. molecules of glucose. Let e = grams of fat $\times$ 0.00057.

$$\text{The K:G ratio} = \frac{a + b}{c + d + e}$$

[1] Recently Shaffer (11) has revised some of the assumptions involved in these calculations, but inasmuch as his original assumptions are in closer agreement with those generally accepted, we have preferred to use them at this time. The results of the calculations do not differ materially from what they would be if his revised assumptions were adopted.

TABLE I—*Ratio of Ketogenic Molecules to Glu*

Case No.	Date.	Age.	Sex.	Height.	Weight, naked.	Diagnosis.*	Food, 3 preceding days and test day.				Nitrogen of urine, 24 hours.	Metabolism 24 hour
							Carbohydrate.	Protein.	Fat.	Calories.		Basal, calories.
							C				*N*	
	1921	*yrs.*		*cm.*	*kg.*		*gm.*	*gm.*	*gm.*		*gm.*	
A366687	Sept. 10	44	M	162	58	E	0	0	0	0	6.62	1,335
A373176	Oct. 28	53	"	168	110	D	60	60	100	1,422	8.92	1,885
A374395	" 19	15	"	157	34	D	17	40	83	1,006	9.03	1,084
A374229	Nov. 14	63	"	169	72	D	25	54	172	1,923	11.53	1,625
A378133	Dec. 13	43	"	183	79	D	33	52	200	2,216	10.00	1,768
A377198	" 6	31	"	173	56	D	23	37	140	1,548	6.72	1,243
A368030	Sept. 5	14	"	164	51	E	35	35	213	2,268	6.23	1,561
A377653	Dec. 6	51	"	162	46	D	21	29	120	1,321	4.87	1,094
A377262	Nov. 30	53	"	183	81	D	22	60	166	1,880	11.19	1,807
A371214	Sept. 14	30	"	174	66	E	34	45	196	2,147	7.56	1,793
A374275	Oct. 26	37	"	171	52	D	20	30	140	1,507	9.42	1,521
A376588†	Nov. 16	48	F	170	88	D	30	60	149	1,755	8.96	1,805
A375561‡	" 9	31	"	163	39	D	14	10	86	902	5.45	1,025
A365349	Aug. 12	16	"	169	42	D	20	30	119	1,313	4.40	1,152
A370281	Sept. 13	14	M	157	33	D	14	10	87	903	3.90	962
A367133	" 13	23	"	170	42	D	14	10	87	903	4.37	1,080

* E = epilepsy; D = diabetes.

† Mild infection as a complication; an acute afebrile nasopharyngitis.

The important data obtained in these experiments are presented in tabular form (Table I). The first case in the series was a patient with epilepsy, who had been fasting for 11 days. Although he received no food, a knowledge of the total metabolism and nitrogen excretion permitted the calculation of the metabolizing mixture. The ketogenic ratio of this mixture was 2.8:1 and accompanying this high ratio, acetone bodies appeared in appreciable quantities in both blood and urine. In the other

Molecules in Metabolizing Food Mixtures.

Metabolizing mixture.									Ketogenic molecules.		Glucose molecules.			Ratio.	Ketosis.			
Carbohydrate.			Protein.			Fat.			K		G			K:G				
Calories, ($C \times 4.1$).	Per cent.	Grams.	Calories ($N \times 26.51$).	Per cent.	Grams ($N \times 6.25$).	Calories $M - (X + Y)$.	Per cent.	Grams ($Z \div 9.3$).	$F \times 0.00343$.	$N \times 0.01$.	$C \times 0.00556$.	$N \times 0.02$.	$F \times 0.00057$.	$\frac{a+b}{c+d+e}$	Acetone of urine, 24 hours.	Acetone of blood, for each 100 cc.	CO_2-combining power of plasma.	Respiratory quotient.
X		C	Y			Z		F	a	b	c	d	e		gm.	mg.	vol. per cent	
0	0	0	176	11	41.4	1,427	89	153.4	0.526	0.066	0	0.132	0.087	2.7	5.7	52	44	0.69
46	11	60	237	11	55.8	1,777	79	191.2	0.656	0.089	0.334	0.178	0.109	1.2	1.0	6	50	0.75
70	5	17	239	18	56.4	993	77	106.7	0.366	0.090	0.095	0.181	0.061	1.4	1.2	16	46	0.78
03	5	25	306	16	72.1	1,541	79	166.0	0.569	0.115	0.139	0.231	0.095	1.5	0.5	1	62	0.73
35	6	33	265	13	62.5	1,722	81	185.2	0.635	0.100	0.183	0.200	0.106	1.5	0.8	11	53	0.73
94	6	23	178	12	42.0	1,220	82	131.2	0.450	0.067	0.128	0.134	0.075	1.5	0	4	52	0.73
44	8	35	165	9	39.0	1,564	83	168.2	0.577	0.062	0.195	0.124	0.096	1.5	1.8	17	56	0.71
86	7	21	129	10	30.4	1,098	83	118.1	0.405	0.049	0.117	0.097	0.067	1.6	0.2	3	58	0.72
90	4	22	297	14	69.9	1,781	82	191.5	0.657	0.112	0.122	0.224	0.109	1.7	1.1	7	55	0.73
39	6	34	200	9	47.3	1,813	85	194.9	0.669	0.076	0.189	0.151	0.111	1.7	0.1	3	57	0.70
82	4	20	249	14	58.9	1,494	82	161.0	0.553	0.094	0.111	0.188	0.092	1.7	0.7	6	51	0.72
23	6	30	238	11	56.0	1,800	83	193.8	0.668	0.090	0.167	0.179	0.110	1.7	6.2	18	44	0.69
57	5	14	145	12	34.1	1,028	83	110.6	0.379	0.055	0.078	0.109	0.063	1.7	5.0	23	44	0.66
82	6	20	117	8	27.5	1,180	84	127.0	0.436	0.044	0.111	0.088	0.072	1.8	0	3		0.76
57	5	14	103	9	24.4	994	86	106.8	0.366	0.039	0.078	0.078	0.061	1.9	0.6	11		0.73
57	4	14	116	9	27.4	1,124	87	121.0	0.415	0.044	0.078	0.088	0.069	2.0	1.2	10		0.74

‡ Mild infection as a complication; an acute afebrile maxillary sinusitis.

fifteen cases the ketogenic ratios lay between 1.2:1 and 2:1. Case A376588, with a ratio of 1.7:1 had an acetone excretion of 6.2 gm. and blood acetone of 18 mg. for each 100 cc. Case A375561, with a ratio of 1.7:1 had an acetone excretion of 5.0 gm. and blood acetone of 23 mg. for each 100 cc. Both of these cases were complicated by afebrile infections. Excepting them, the acetone values accompanying ratios between 1.2:1 and 2:1 are such as are commonly encountered in what are considered to be well controlled cases of diabetes and are so small as to be theoretically unimportant and clinically insignificant.

These results (15) were discussed at the last meeting of the American Society of Biological Chemists. They harmonize with the conclusions reported by Shaffer (11) at that meeting and apparently permit conclusions as follows:

CONCLUSIONS.

1. Certain assumptions, stated herein, are employed in the calculation of the composition of the mixture of food substances engaging in metabolism. Under the conditions of these experiments, provided these assumptions are tenable, the ratio between the ketogenic and the glucose molecules at which a clinically significant ketosis appears has a value of at least 2:1. A ratio of this value implies that every molecule of glucose is ketolytic for 2 molecules of acetoacetic acid.

2. The existence of infection lowers the ketogenic threshold so that significant ketogenesis may occur with lower ratios. Other factors, thus far undetermined, may also lower this threshold. It is advisable, therefore, in planing diets for diabetic patients to allow only such food mixtures as will avoid the 2:1 ratio by a safe margin.

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