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Effect of Vitamin Deficiencies on Basal Metabolism and Respiratory Quotient in Rats.*

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It is now definitely established[1] that at least 3 of the vitamins in the B complex function in enzyme systems that control cellular respiration. One might expect, therefore, to find certain changes in the total metabolism of animals suffering from specific vitamin deficiencies. Early studies by Stare and Elvehjem[2] on the total oxygen uptake of tissues *in vitro* indicated that there was little difference in the rate of oxygen consumption between the tissues of normal and vitamin deficient animals. The more recent work which has demonstrated direct relationships such as thiamine to cocarboxylase, riboflavin to d-amino acid oxidase, xanthine oxidase, etc., and nicotinic acid to cozymase was carried out on individual enzyme systems. Thus it appears that any significant decrease in respiration is incompatible with life and that if a specific system is impaired the animal succumbs unless the energy can be obtained through other systems. Nevertheless, we felt that it was worth while to measure the basal metabolism and the respiratory quotient on rats suffering from uncomplicated B vitamin deficiencies. DuBois[3] has reviewed basal metabolism in disease, but as far as the authors are aware, no carefully controlled experiments have been carried out on animals having B vitamin deficiency diseases. In this paper we wish to report the results obtained in riboflavin, pyridoxine and pantothenic acid deficiencies. A total of 56 male rats have been used in these studies. The diets used for the production of each of the individual deficiencies are given in Table I.

The rations used for the production of riboflavin deficiency are based on the one described by Wagner, Axelrod, Lipton and Elvehjem.[4] Since Mannering, Lipton and Elvehjem[5] showed that a higher fat content of the diet produced a more severe riboflavin deficiency, the basal ration was modified to include varying amounts of fat. One-half of the animals were placed on the 4 modified basal rations and a similar number of rats was placed on these basal rations plus added amounts of riboflavin. The control rats received 12 γ of riboflavin per day.

The vitamin B_6 deficiency was produced on a diet described by Conger and Elvehjem.[6] The basal ration 613 was identical with the one described by the above workers and the other basal ration contained 60% of protein in place of the regular 18%. The control rats were fed 25 γ of vitamin B_6 per day.

The pantothenic acid deficiency was produced by feeding a synthetic ration containing all of the known B vitamins except pan-

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2 Stare, F. J., and Elvehjem, C. A., *Am. J. Physiol.*, 1933, **105**, 655.

3 DuBois, E. F., *J. Nutr.*, 1930, **3**, 331.

4 Wagner, J. R., Axelrod, A. E., Lipton, M. A., and Elvehjem, C. A., *J. Biol. Chem.*, 1940, **136**, 357.

5 Mannering, G. J., Lipton, M. A., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 100.

6 Conger, T. E., and Elvehjem, C. A., *J. Biol. Chem.*, 1941, **138**, 555.

TABLE I.
Composition of Rations.

	Riboflavin				Pyridoxine		Pantothenic acid	
	119	120	121	122	613	613A	789	789A
Dextrin	71	21.5	37.5	37.5				
Sucrose					75	33	73	
Cerelose								71
Casein (Labco)	18	18	18	18	18	60	18	20
Lard		22	14					
Crisco				14				
Butter fat	3	3	3	3				
Corn oil	2	2	2	2	3	3	5	5
Salts 4	4	4	4	4	4	4	4	4
Liver extract preparation	4	4	4	4				
Thiamine, γ	200	200	200	200	200	300	200	150
Riboflavin, γ					300	500	300	200
Nicotinic acid, mg	5	5	5	5	2.5	2.5	2.5	
Pyridoxine, γ	400	400	400	400			200	150
Pantothenic acid	500 γ	500 γ	500 γ	500 γ	2 mg	2 mg		
Choline, mg	100	100	100	100	100	200	100	100
Cod liver oil	2	2	2	2				

tothenic acid (Henderson, McIntire, Waisman and Elvehjem).[7] Two basal rations were used, one which contained sucrose and one in which glucose (cerelose) was the carbohydrate. The control animals received 100 or 500 γ of calcium pantothenate per day.

All the control animals received the particular vitamin by mouth. In the case of the riboflavin an alcoholic solution was used and the negative controls in this case were given an equivalent amount of alcohol without the riboflavin. All the animals received two drops of halibut liver oil by mouth per week. The basal metabolism determinations were made when the animals on the basal ration showed marked evidences of avitaminosis.

The animals were kept in an air-conditioned and humidity controlled room. One day before the BMR determinations were made the animals were removed to the room where the apparatus was kept and the food was removed from the cages. The determinations were made in a room kept at a constant temperature of 80°F.

The basal metabolism was determined in an apparatus made available to us by Dr. P. H. Phillips and Mr. Paul Boyer. This apparatus was patterned after that described by Schwabe and Griffith.[8] The technic used has been described by Schwabe, Emery, Griffith[9] and by Orsini.[10] Two or more determinations were made on each animal and only those results were used which showed good agreement in two consecutive runs. The BMR was expressed both as calories per square meter of body surface per 24 hr and as calories per kg body weight per 24 hr. The Diack[11] formula $SA = 7.42 \times W^{2/3}$ was used for calculation of the surface area.

The values for the BMR and RQ for all the animals are given in Table II.

Discussion. It is obvious that there are no significant differences in the BMR of the animals receiving the riboflavin deficient diets and those receiving the same diet plus riboflavin when the rate is expressed as calories per square meter. The values obtained are very similar to those which have been reported in the literature for normal rats. Definite differences are observed when the rate is based on the body weight of the animals. This difference is undoubtedly due

[7] Henderson, L. M., McIntire, J. M., Waisman, H. A., and Elvehjem, C. A., *J. Nutr.*, 1942, **23**, 47.

[8] Schwabe, E. L., and Griffith, F. R., Jr., *J. Nutr.*, 1938, **15**, 187.

[9] Schwabe, E. L., Emery, F. E., and Griffith, F. R., *J. Nutr.*, 1938, **15**, 199.

[10] Orsini, D., *An. Fac. Med. Univ. S. Paulo*, XVI (tones I), page 81, 1940.

[11] Diack, S. L., *J. Nutr.*, 1930, **3**, 289.

TABLE II.
Effect of Vitamin Deficiencies on B. M. R. and R. Q.

Ration	Supplement	Amt per day, γ	No. of rats	Weeks on experiment	Avg wt	B.M.R. Cal/M²/24 hrs	B.M.R. Cal/K/24 hrs	R.Q.
119	0		3	8	66	1,079	182	0.86
119	Riboflavin	12	3	8	197	1,126	149	0.84
120	0		3	8	57	1,073	214	1.10
120	Riboflavin	12	3	8	128	1,041	154	0.84
121	0		3	8	73	1,154	223	0.93
121	Riboflavin	12	3	8	150	1,107	156	0.84
122	0		3	8	53	1,041	196	1.17
122	Riboflavin	12	3	8	152	1,024	137	0.87
613	0		3	17	78	830	147	1.07
613	Pyridoxine	25	2	17	230	1,122	137	0.80
613A	0		6	10	62	773	146	0.91
613A	Pyridoxine	50	3	10	169	1,056	143	0.81
789	0		5	8	83	1,088	188	0.90
789	Ca pantothenate	500	2	8	123	1,154	164	0.82
789A	0		7	9	71	1,071	198	1.02
789A	Ca pantothenate	100	4	9	209	1,095	140	0.84

to the fact that the animals receiving riboflavin were much larger and contained more fatty tissue, which has little or no metabolism. The values obtained for the RQ of the deficient animals were higher in all cases than those for the control animals. It is interesting to note that the least difference was obtained on ration 119 which was low in fat and did not produce as severe riboflavin deficiency as the other rations.

The rats on the pyridoxine-deficient rations show a definite decrease in BMR when expressed on the basis of surface area. The fact that the BMR expressed on body weight is practically the same for the deficient and control rats lends greater importance to the decrease in BMR when expressed on surface area because one would expect that the lighter weight animal would have a disproportionately higher metabolism for each kg of tissue than the heavier animal. Expressed in another way, figures for BMR calculated on the basis of body weight normally show a slightly higher value for lighter animals. Since the values in pyridoxine-deficient animals (lower weight) are not higher than that of normal animals, the BMR is lowered even more than indicated by the figures expressed on the basis of surface area. The RQ is increased in pyridoxine deficiency.

Pantothenic acid-deficient rats showed a BMR identical with the control animals when expressed on the basis of surface area. In the case of body weight the same relationship was obtained as in the case of the riboflavin-deficient rats. The pantothenic acid-deficient rats showed an increased RQ similar to that observed in riboflavin and pyridoxine deficiency.

It is apparent that of the vitamins studied, pyridoxine is the only one that has a specific effect on BMR. However, in all the vitamin deficiencies studied there was produced a definite increase in RQ. One logical explanation for the increased RQ is one of incomplete combustion of the intermediate metabolites. These metabolites are either stored in the tissues or excreted as such thereby increasing the carbon dioxide-oxygen ratio. One may argue that the cause of this increase in RQ was due to inanition in all cases since it is well known that in inanition metabolites accumulate due to an insufficient supply of carbohydrate material. However, most of the animals did not show severe inanition, and most of them consumed almost as much food as the control animals when based on body weight. It is just as logical to suggest that the intermediate compounds accumulated because of faulty carbohydrate metabolism rather than insufficient supply of carbohydrates. Riboflavin deficiency is known to produce a disturbance in the respiratory mechanism, but up to the present time no re-

lationship has been demonstrated in the case of vitamin B_6 and pantothenic acid. Much more work is necessary before we can establish the true relationship between vitamin deficiencies and the respiratory quotient. But these preliminary experiments do indicate that studies along this line may be profitable and lead to a clearer understanding of the relation of vitamin deficiency to energy metabolism.

Summary. Riboflavin and pantothenic acid deficiencies in rats do not alter the BMR. A definite decrease in the BMR was observed in severe vitamin B_6 deficiency. All rats showing a riboflavin, vitamin B_6, and pantothenic acid deficiency had a higher RQ than normal rats. The possible explanation for this increased RQ is given.

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Production of a Diazotizable Substance by *Escherichia coli* during Sulfonamide Bacteriostasis.

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The classical paper of Woods,[1] describing the anti-sulfonamide action of p-aminobenzoic acid, crystallized the concept that sulfonamide bacteriostasis is the result of some interference with the normal metabolism of susceptible bacteria.[2] Woods[1] and Fildes[3] suggested that the p-aminophenyl grouping of the sulfonamides is sufficiently similar to that in the hypothesized metabolite, p-aminobenzoic acid, so that competitive inhibition occurs when the relative concentrations of PAB/Drug approach 1/5000. Our recent studies on the ionization of sulfonamides[4] seem to indicate that in terms of concentration of anions this ratio is 1/1 - 1/6.

Up to the present p-aminobenzoic acid has not been found essential for the normal growth of pathogenic bacteria nor has it been detected in such bacteria. During sulfonamide bacteriostasis, on the other hand, another diazotizable aromatic amine appears in the medium. The circumstances of its appearance are described in this preliminary note.

Synthetic medium* containing bacteriostatic concentrations of sulfonamide drugs[5] was placed in flasks or test tubes and inoculated with *Es. coli* (final dilution = 1 to 5 million per cc). Control samples taken at once were refrigerated and the remainder incubated at 37°C for 18 hr. Turbid cultures were centrifuged and diazo tests† were then done on the supernatant and the control samples. A typical result is as follows:

*Ammonium citrate	5	g
Magnesium sulfate	1.0	"
Sodium carbonate	3.0	"
Potassium dihydrogen phosphate	3.0	"
Sodium chloride	2.0	"
Iron ammonium citrate	.05	"
Nicotinic acid	.005	"
Glucose	.2	"
Acid hydrolysate of gelatin (Knight)	10	cc
Tap water to 1000 cc.		

[1] Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.

[2] (*a*) Mayer, R. L., *Bull. d. l'Acad. d. med.*, 1938, **117**, 727; (*b*) McIntosh, J., and Whitby, L., *Lancet*, 1939, **1**, 431; (*c*) Lockwood, J. S., *J. Immunol.*, 1938, **35**, 155; (*d*) Fox, C. L., Jr., *Am. J. Med. Sci.*, 1940, **199**, 487; (*e*) Mellon, R. R., Locke, A. P., and Shinn, L. E., *Am. J. Med. Sci.*, 1940, **199**, 749.

[3] Fildes, P., *Lancet*, 1940, **1**, 955.

[4] Fox, C. L., Jr., and Rose, H. M., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 142.

[5] Rose, H. M., and Fox, C. L., Jr., *Science*, 1942, **95**, 412.

† The method of Bratton and Marshall was used.[6] This test is not specific for sulfonamides; positive results are obtained with most primary aromatic amino compounds.