shown that by the addition of adequate amino acid synergists the effectiveness of natural anti-oxidants, which are present in the fats in appreciable quantities, may be greatly increased. This stabilization of the fat may be an important factor in reducing vitamin A and carotene destruction.

Summary. The addition of urea to a diet low in protein did not increase the rate of gain of rabbits. From the data it is apparent that this species cannot use urea in the diet as a replacement of part of the protein. Rabbits fed diets containing 10% of casein as the sole source of protein or 10% casein plus 3.43% urea developed typical vitamin A deficiency symptoms as compared with the control group fed a diet similar except that it contained 20% of casein. The results suggest that protein has a protective action on the oxidation of vitamin A in the diets or that it has a sparing action on the vitamin A requirements of the rabbit.

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Pantothenic Acid and the Metabolism of Amino Acids by Bacteria.*

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Dorfman, Berkman and Koser,[1] and Hills[2] first observed that pantothenic acid functions in the pyruvic acid metabolism of *Proteus morganii*. More recently, Lipmann, *et al.*,[3] and Novelli and Lipmann[4] have found that pantothenate is a part of a coenzyme which is concerned with an acetylation system in tissues and with the breakdown of pyruvate to acetate and other products. They isolated this coenzyme, which they termed "coenzyme A", and were able to recover pantothenic acid from it. They also found that the amount of pantothenate bound in the cells closely correlated with the increased stimulation of pyruvate oxidation, and devised a method for the assay of coenzyme A.[5]

The present study was undertaken to determine if pantothenic acid has any effect on the metabolism of amino acids or other related compounds by bacteria.

Technic. The chemically-defined medium employed in this study for growing the bacteria has already been described.[6] The organism used was *Proteus morganii*. Pantothenate deficient cells were prepared as follows: A small amount of growth was removed from a 6-hour agar slant culture by means of a sterile needle and cultured in 10 ml of a chemically-defined medium for 24 hours at 37°C. One ml of this culture was then used to inoculate larger quantities of the same basal medium containing in this case only 0.01 μg of Ca-pantothenate per ml. The "pantothenate-deficient" cells from 4 liters of a 24-hour culture were then harvested by means of a Sharples centrifuge, and washed twice with 50 ml of sterile saline by the ordinary centrifuge technics. From this quantity of medium it was possible to obtain yields of cells ranging in dry weight from 150

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1 Dorfman, A., Berkman, S., and Koser, S. A., *J. Biol. Chem.*, 1942, **144**, 393.

2 Hills, G. M., *Biochem. J.*, 1943, **37**, 418.

3 Lipmann, F., Kaplan, N. O., Novelli, G. D., Tuttle, L. C., and Guirard, B. M., *J. Biol. Chem.*, 1947, **167**, 869.

4 Novelli, G. D., and Lipmann, F., *Arch. Biochem.*, 1947, **14**, 23.

5 Kaplan, N. O., and Lipmann, F., *J. Biol. Chem.*, 1948, **174**, 37.

6 Pelczar, M. J., Jr., and Porter, J. R., *J. Biol. Chem.*, 1941, **139**, 111.

TABLE I.
Effect of Pantothenate on Ammonia Liberation and on Oxidation of Amino Acids by *Proteus morganii*.

Amino acid	μg NH_3 produced in 90 min.*		μl O_2-uptake in 90 min.*	
	Without pantothenate	With pantothenate	Without pantothenate	With pantothenate
Glycine	10	12	3	5
dl-Alanine	13	23	7	10
dl Valine	7	16	22	26
dl-Threonine	30	43	8	17
dl-Phenylalanine	5	8	0	5
dl-Methionine	14	14	0	6
dl-Isoleucine	13	20	25	29
dl-Citrulline	0	2	13	15
l-Tryptophane	4	6	0	0
l-Proline	5	5	3	5
dl-Norleucine	20	25	6	13
dl-Lysine. HCl	2	4	6	8
l-Histidine. HCl	4	6	0	5
l-Cystine	11	15	15	25
d-Arginine. HCl	5	8	13	19
dl-Tyrosine	8	14	8	10
dl-Serine	74	79	85	94
dl-Norvaline	27	35	13	14
l-Hydroxyproline	0	0	0	4
dl-Glutamic acid	20	48	23	172
dl-Aspartic acid	16	30	12	50
dl-Leucine	30	38	12	13

* Average values for several determinations; all data corrected for controls which were 3-5 μg of NH_3 and 10-15 μl O_2.

to 200 mg. After the final washing and centrifugation the cells were resuspended in saline to give a final concentration of 10 mg of cells per ml. The substrates employed in this study consisted of the 22 amino acids and certain organic acids shown in the tables. These compounds were all prepared in concentrated aqueous solutions, and used as M/450 final concentrations.

For the deamination studies the microdiffusion method of Conway[7] was employed, and the ammonia determinations were made as follows: The substrates (amino acids) plus pantothenate were added to the outside well of the diffusion cell, together with phosphate buffer (pH 8.0) and a 5 mg suspension of bacteria, to give a final quantity of 1.5 ml. One ml of N/150 HCl, containing an indicator, was placed in the center cup of the diffusion cell. Following an incubation period of 90 minutes at 37°C, 1 ml of saturated K_2CO_3 solution was added to the outside well to release the ammonia, and the units were again incubated for one hour. The acid in the center cup was then titrated with N/75 NaOH, and the amount of ammonia liberated from the amino acids calculated.

For the respiration studies, standard manometric methods were employed. The substrates were added so as to give M/450 final concentrations; 0.1 ml of N/10 HCl was placed in the side bulb to absorb any ammonia given off by the 1.0 ml bacterial cell suspensions; and 0.3 ml of a 20% KOH solution was placed in the center well of the Warburg vessels. In every case the final volume of the vessels was brought to 3.0 ml with phosphate buffer (pH 7.3). The flasks were then equilibrated for 10 minutes in the water bath before readings were started.

Experimental and results. From the data in Table I it will be seen that serine was the most readily deaminated amino acid. However, since this reaction was not stimulated by pantothenate it does not appear to be involved in the process. Alanine, threonine, norleucine, norvaline, glutamic acid, aspartic

[7] Conway, E. J., *Micro-diffusion Methods and Volumetric Error*, 1940, D. Van Nostrand Co., New York.

acid, and leucine were also deaminated in varying degrees, and in certain of these cases the presence of pantothenate appeared to stimulate the process. This was especially true in the case of glutamic and aspartic acids. Few of the other amino acids were attacked by this organism, nor did the presence of pantothenate stimulate ammonia liberation.

Since it is generally accepted that deamination is one of the first steps in the breakdown of amino acids, especially at the pH values where the above experiments were conducted, an attempt was made to determine the role played by pantothenate in the metabolism of certain of these substances. It was found after a few preliminary experiments that the respiration of "pantothenate-deficient" cells of *Proteus morganii*, using amino acids as substrates, showed a definite lag phase during the first half hour or so of the experiment. Since it has been reported by McIlwain[8] that a time factor is involved when deficient cells bind pantothenate, it was decided to divide the cell suspensions into two equal portions after the final washing, and add 1.0 μg per ml of pantothenate to one portion of the cells. When the cells were treated in this manner, followed by incubation at 37°C for about 2 hours, little or no lag was noted in their early respiration period.

From the oxygen uptake values (Table I) it will be seen that serine gave the highest values for deficient cells and likewise quite high values for non-deficient cells. As in the case of the deamination studies, however, pantothenate did not stimulate the oxidation of serine. On the other hand, when such amino acids as threonine, norleucine, cystine, and especially glutamic and aspartic acids were used as substrates, pantothenate did stimulate their oxidation in varying degrees. The fact that the oxidation of these amino acids is stimulated by pantothenate would seem to indicate that pyruvate, which is known to be stimulated, is the key intermediate in their metabolism. With this in mind glutamic acid and aspartic acid were chosen as representative amino acids for further metabolic studies. In these experiments glutamic acid and aspartic acid were studied together with their known, or postulated, intermediates in an attempt to discover at which point, or points, in their metabolism pantothenic acid functions. The intermediates studied were α-ketoglutarate, succinate, fumarate, oxalacetate and pyruvate, and these were kept on an equal molar basis

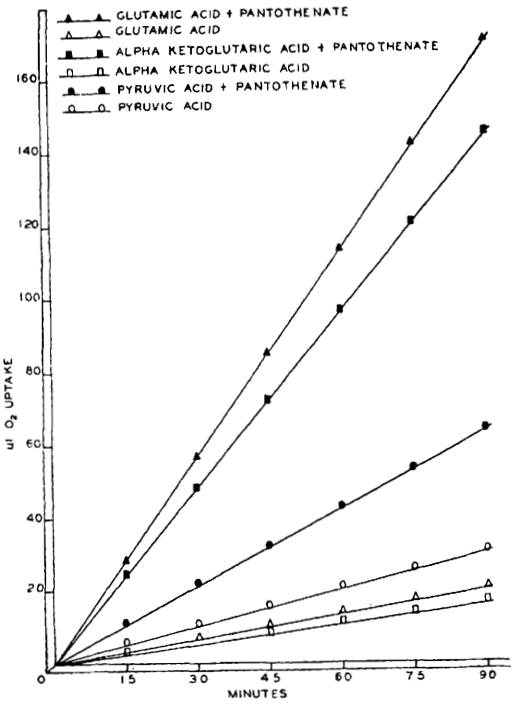

FIG. 1.

Effect of pantothenate on oxidation of glutamic acid, α-ketoglutarate, and pyruvate.

TABLE II.

Effect of Pantothenate on Oxidation of Glutamic Acid, Aspartic Acid, and Their Metabolic Intermediates.

	μl O_2 uptake in 90 min.*	
	Without pantothenate	With pantothenate
Glutamic acid	20	170
α-Ketoglutarate	16	143
Succinate	44	103
Fumarate	10	23
Oxalacetate	29	55
Pyruvate	30	64
Aspartic acid	15	55

* Average values for 4 determinations; all data corrected for controls which gave 5.15 μl O_2 uptake.

8 McIlwain, H., *Biochem. J.*, 1945, **39**, 279.

with glutamic acid. The results of these studies are shown in Table II and Fig. 1. From these data it can be seen that pantothenate stimulated respiration considerably when either α-ketoglutarate or glutamic acid was used as a substrate, but succinate, fumarate, oxalacetate and aspartic acid were oxidized to lesser extent. It will be noted that the stimulation values for most of these substrates are in the same range as was observed with pyruvate. It was also possible to demonstrate pyruvate in the vessels after all of these substrates had been oxidized. Thus it can probably be assumed that pantothenate stimulation of certain amino acids and other substrates is due, in part at least, to their being converted to pyruvate which is in turn stimulated. However, the pantothenate stimulation observed when glutamic acid or α-ketoglutarate was used as substrate cannot be accounted for entirely as being due to pyruvate stimulation alone. Therefore it appears that pantothenic acid also functions in some other way in the oxidation of these substrates. Since the values observed with glutamic acid were very close to those noted with α-ketoglutarate, it would seem to eliminate the possibility of pantothenic acid functioning in the deamination of glutamic acid.

Summary. From this study is can be concluded that the pantothenate stimulation of amino acid oxidation by *Proteus morganii* is limited to those amino acids which can be converted to pyruvate. However, in the case of glutamic acid, and its metabolic intermediate α-ketoglutarate, the pantothenate stimulation appears to be more than can be attributed to pyruvate oxidation.

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Testicular Degeneration as a Result of Microwave Irradiation.

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The mammalian scrotum has been established as being a local thermoregulator for the testes. Moore and Quick[1] found the scrotal temperature of white rats to be from 2° to 8°C lower than the temperature of the abdominal cavity. A sub-abdominal temperature in the scrotum has been shown to be necessary for the continuance of spermatogenesis. Moore[2] confined the testes of guinea pigs in the abdominal cavity for varying periods of time and found that an abdominal retention of seven days resulted in a complete disorganization of the germinal epithelium of the seminiferous tubules. He considered the cause of this degeneration to be due to the higher temperature of the abdominal cavity.

Investigations of Moore[3] showed that testicular degeneration resulted from a single application of heat at approximately 7°C above body temperature for a 15-minute period. The heating devices which were used consisted of hot water baths, electric stoves, electric light bulbs, and hot water pads. Testicular degeneration was visible histologically within four to six days following the heat application, and was entirely similar in type to that resulting from early experimental cryptorchidism.

Fukui[4] exposed the scrotum of rabbits to sunlight and to warm air, and found a definite relationship between the temperature and the time required to cause regressive changes in the germinal cells. The minimum scrotal temperature at which he was able to produce testicular damage was 40°C. The time of required exposure at this temperature was more than one hundred hours.

It was the purpose of this study to deter-

1 Moore, C. R., and Quick, W. J., *Am. J. Physiol.*, 1924, **68**, 70.

2 Moore, C. R., *Am. J. Anat.*, 1924, **34**, 269.

3 Moore, C. R., *Am. J. Anat.*, 1924, **34**, 337.

4 Fukui, N., *Japan M. World*, 1923, **3**, 27.