sweating in man. These studies, performed in a room designed for close control of temperature and humidity, indicate that an environmental temperature of 34.4°C and relative humidity of about 50% are essentially the threshold level for sweating in normal man resting in bed. Exercise and increased rate of heat production are associated with a proportionate lowering of the threshold.

## 16361

## Utilization of Glutamic Acid in the Presence of High Levels of Pteroylglutamic Acid.

A. L. FRANKLIN, M. REGAN, D. LEWIS, E. L. R. STOKSTAD, AND T. H. JUKES.

*From the Lederle Laboratories Division, American Cyanamid Company, Pearl River, N.Y.*

It has been recently suggested that pteroylglutamic acid (PGA) may function as a metabolic antagonist for glutamic acid.[1,2] The statement was made that "folic acid may competitively interfere with the nutrition of the spinal cord just as certain vitamin deficiencies in experimental animals may be caused by closely related chemicals. Thiamine deficiency, for example, may be induced by the administration of pyrithiamine, and pantothenic acid deficiency by pantoyltaurine." It was further suggested that PGA could interfere with the metabolism of the central nervous system *in vivo* or *in vitro*.[1]

The analogy between folic acid and such "anti-vitamin" compounds as pyrithiamine appears to be inappropriate. Folic acid is a member of the vitamin B complex, and is itself inhibited by "anti-vitamin" compounds which are comparable to pyrithiamine and pantoyltaurine. These compounds include "methyl folic acid,"[3,4] pteroylaspartic acid,[5] 4-amino pteroylglutamic acid,[6] certain pteridines,[7] a sulfonyl-substituted benzimidazole analogue of PGA,[8] and $N^{10}$-methyl pteroic acid.[9]

Since representative species of bacteria require both folic acid and glutamic acid, it is obvious that folic acid does not competitively interfere with the metabolism of glutamic acid by these bacteria. However, because the response to glutamic acid obtained with *L. casei* and *S. faecalis* R is so quantitative and sensitive, an experiment was made to determine whether massive amounts of PGA would slow up growth on suboptimal levels of glutamic acid. A culture medium deficient in glutamic acid[10] was used and PGA was added at levels of 0.01 μg, 1 μg, 10 μg, to 3 series of tubes containing varying amounts of glutamic acid. The results are illustrated in Fig. 1.

The growth curves indicate that increasing the level of PGA 1000-fold had no inhibitory

[1] Anonymous, *New England J. Med.*, Editorial, 1947, **237**, 713.

[2] Ross, J. F., Belding, H., and Paegel, B. L., *Blood*, 1948, **3**, 68.

[3] Martin, G. J., Tolman, L., and Moss, J., *Arch. Biochem.*, 1947, **12**, 318.

[4] Franklin, A. L., Stokstad, E. L. R., Belt, M., and Jukes, T. H., *J. Biol. Chem.*, 1947, **169**, 427; Franklin, A. L., Stokstad, E. L. R., and Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1947, **65**, 368.

[5] Hutchings, B. L., Mowat, J. H., Oleson, J. J., Stokstad, E. L. R., Boothe, J. H., Waller, C. W., Angier, R. B., Semb, J., and SubbaRow, Y., *J. Biol. Chem.*, 1947, **170**, 323.

[6] Seeger, D. R., Smith, J. M., Jr., and Hultquist, M. E., *J. Am. Chem. Soc.*, 1947, **69**, 2567.

[7] Daniel, L. J., Norris, L. C., Scott, M. L., and Heuser, G. F., *J. Biol. Chem.*, 1947, **169**, 689.

[8] Edwards, P. C., Starling, D., Mattocks, A. M., and Skipper, H. E., *Science*, 1948, **107**, 119.

[9] Smith, J. M., Jr., and Cosulich, D. B., *J. Am. Chem. Soc.*, in press.

[10] Dunn, M. S., Camien, M. N., Rockland, L. B., Shankman, S., and Goldberg, S. C., *J. Biol. Chem.*, 1944, **155**, 591.

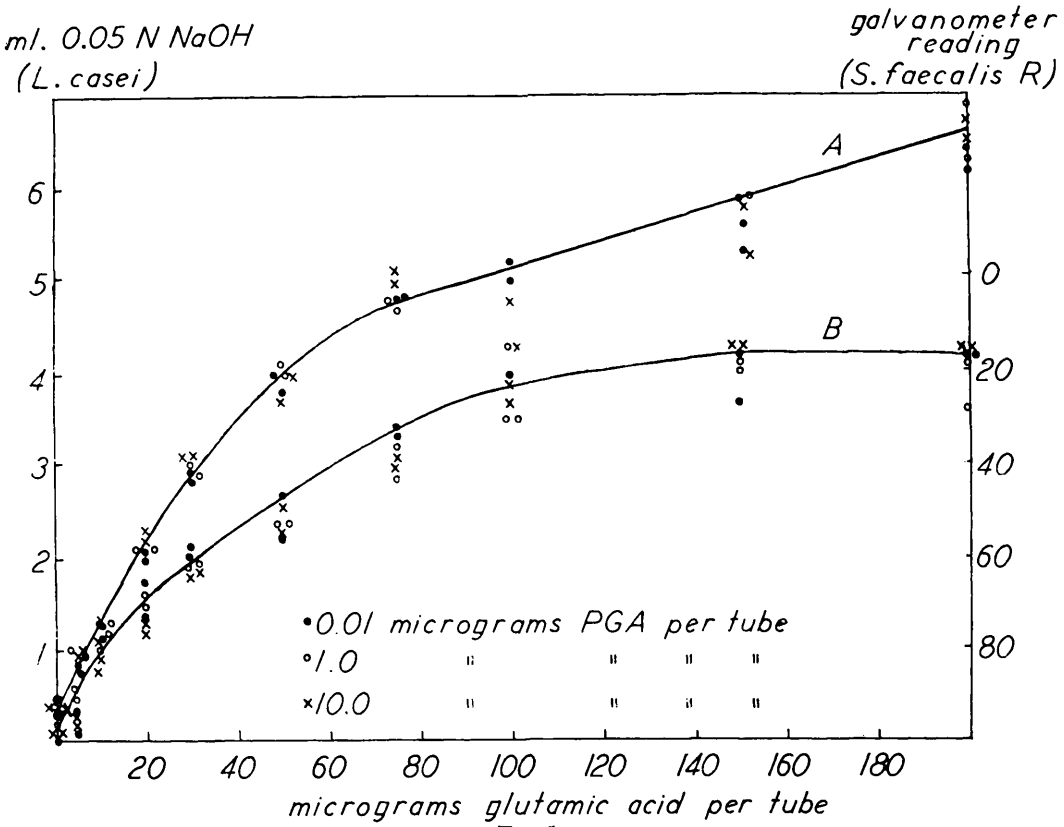


FIG. 1.

Growth response of *Lactobacillus casei* (curve A) and *Streptococcus faecalis* R (curve B) at 72 hours to glutamic acid in the presence of various levels of pteroylglutamic acid (PGA).

effect upon the utilization of glutamic acid by either *L. casei* or *S. faecalis* R. The scattering around the experimental points was within the anticipated limits of the assay method and in no case was the trend dependent upon the level of PGA. The experiment also demonstrates that neither organism is able to use PGA as a substitute for glutamic acid.

In view of the suggestion that PGA interferes with the metabolism of glutamic acid by the central nervous system *in vitro*, further experimental work was done to determine the effect of PGA on the utilization of glutamic acid by brain and kidney slices. The slices were suspended in Ringer-phosphate solution and the rate of oxygen consumption was determined at 37° in the Warburg-Barcroft apparatus. The minimum concentration of glutamic acid ($3 \times 10^{-3}$M) which caused an appreciable stimulation of oxygen consumption, was used, for this concentration would presumably give the most favorable conditions for observing any competitive interference with the utilization of this acid.

The results are given in Table I. $Qo_2$ values (cu mm of oxygen consumed per mg dry tissue per hour) are included for brain slices for both the first hour and the first 3 hours of respiration, for the consumption of oxygen was not a linear function with time. The results can be evaluated by examining the values obtained for increases in $Qo_2$ caused by adding glutamic acid to the medium as summarized in the last 2 columns of Table I. When the concentrations of glutamic acid and PGA were equimolar there was no significant depression in the utilization of glutamic acid by brain or kidney slices. Had the PGA interfered with the utilization of glutamic acid, the addition of PGA should have

TABLE I.
Effect of Addition of Pteroylglutamic Acid to the Medium on Utilization of Glutamic Acid by Surviving Brain and Kidney Tissue Slices of Normal Rats.

| | | $Q_{O_2}$ | | | | | | | | | Increase in $Q_{O_2}$ with addition of glutamic acid* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Brain slices | | | | | | | | | | |
| | | 1st hr | | | 3-hr interval | | | Kidney slices | | | | |
| Exp. | PGA conc. | (a) | (b) | (c) | (a) | (b) | (c) | (a) | (b) | (c) | Brain | Kidney |
| 1 | 0 | 3.4 | 4.6 | 6.1 | 1.9 | 3.4 | 4.7 | 10.4 | 12.8 | 13.3 | 2.8 | 2.9 |
| | $3 \times 10^{-4}$ M | 3.4 | 5.8 | 6.3 | 1.9 | 4.2 | 5.0 | 9.2 | 11.8 | 12.0 | 3.1 | 2.8 |
| 2 | 0 | 5.0 | 6.8 | 9.6 | 3.3 | 5.2 | 7.4 | 11.8 | 19.2 | 21.9 | 4.1 | |
| | $3 \times 10^{-4}$ M | 4.4 | 8.2 | 9.8 | 3.0 | 6.1 | 7.6 | 11.6 | 19.6 | — | 4.6 | |
| 3 | 0 | 4.6 | 6.0 | 7.2 | 2.8 | 4.3 | 5.4 | 9.9 | 12.0 | 13.1 | 2.6 | 3.2 |
| | $3 \times 10^{-3}$ M | 3.6 | 6.0 | 6.8 | 2.2 | 4.2 | 5.2 | 9.0 | 11.6 | 12.7 | 3.0 | 3.7 |
| 4 | 0 | 5.0 | 8.6 | 7.3 | 3.3 | 6.4 | 6.0 | 15.0 | 15.2 | 18.0 | 2.7 | 3.0 |
| | $3 \times 10^{-3}$ M | 4.4 | 7.2 | 7.9 | 2.9 | 5.3 | 6.0 | 12.2 | 15.2 | 14.1 | 3.1 | 1.9 |

(a) No glutamic acid added to medium.
(b) Medium contained $3 \times 10^{-3}$ M added glutamic acid.
(c) Medium contained $10^{-2}$ M added glutamic acid.
*The values are calculated from the results with $10^{-2}$ M glutamic acid. The values for brain slices were determined from the 3-hour respiration interval.

reduced the magnitude of the $Q_{O_2}$ increases, but no such reduction was observed. The maximum PGA level which has been reported in the blood of human subjects is 0.8 μg per ml[11] following the intravenous administration of 15 mg of PGA. In the present study levels as high as 1323 μg PGA per ml of medium were without significant effect on glutamic acid metabolism.

In another series of similar experiments, the animals were either made severely deficient in PGA or fed a high level of PGA for a prolonged period. The birds on the deficient diet developed a severe cervical paralysis,[12,13] and the deficient rats showed a mild cytopenia. The animals were sacrificed when the marked signs of the characteristic deficiency developed. Control animals were fed purified diets[4,12] containing adequate levels of PGA (1 mg PGA per kg diet for rats and 2 mg per kg of diet for turkeys). Because of the non-linear oxygen consumption curves obtained with brain tissue the control animals were sacrificed simultaneously with the experimental animals and the tissue slices were prepared from both at the same time. The respiration of the tissue slices was measured as described above, except that no PGA was added to the medium. No difference between the behavior of the tissues from the deficient and control animals was found (Table II).

The effects of feeding large amounts of PGA and of PGA deficiency in the rat on glutamic acid metabolism by brain and kidney tissue *in vitro* were compared (Table II). The "high-PGA" diets contained 100 mg PGA per kg and were fed for several weeks. The tissues from two series of animals showed no significant differences in the tests.

*Discussion.* In studies with various species of animals PGA, like the other B-complex vitamins, has been shown to be a substance of very low toxicity.[14] Prolonged dosage of human subjects with 50 mg of PGA daily has been reported not to produce adverse symptomatology.[15] Recent statements[2] have

[11] Denko, C. W., Abstracts of Papers, American Chemical Society, 112th meeting, New York, 25 C, 1947.

[12] Jukes, T. H., Stokstad, E. L. R., and Belt, M., *J. Nutrition*, 1947, **33**, 1.

[13] Richardson, L. R., Hogan, A. G., and Kempster, H. L., *J. Nutrition*, 1945, **30**, 151.

[14] Harned, B. K., Cunningham, R. D., Smith, H. D., and Clark, M. C., *Ann. N. Y. Acad. Sci.*, 1946, **48**, 289.

[15] Berry, L. J., and Spies, T. D., *Blood*, 1946, **1**, 271.

TABLE II. Effect of Glutamic Acid on Respiration Rate of Surviving Brain and Kidney Slices from Animals Which Had Received Diets Containing Varying Levels of PGA.

| Exp. | Animal | Wt, g | PGA dietary supplement, mg/kg | Time on diet, days | $Qo_2$ Brain Slices 1st hr (a) | 1st hr (b) | 1st hr (c) | 3-hr interval (a) | 3-hr interval (b) | 3-hr interval (c) | $Qo_2$ Kidney slices (a) | Kidney slices (b) | Kidney slices (c) | Increase in $Qo_2$ with addition of glutamic acid* Brain | Kidney |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Turkeys | 190 | 0 | 21 | 6.5 | 8.6 | 9.8 | 4.9 | 7.0 | 7.9 | | | | 3.0 | |
| | | 165 | 2 | 21 | 6.5 | 9.1 | 9.8 | 4.7 | 7.6 | 7.9 | | | | 3.2 | |
| 2 | " | 210 | 0 | 24 | 7.9 | 10.4 | 10.8 | 5.7 | 7.7 | 9.0 | | | | 3.3 | |
| | | 220 | 2 | 24 | 8.9 | 9.8 | 9.8 | 6.2 | 7.8 | 8.3 | | | | 2.1 | |
| 3 | Rat | 110 | 0 | 42 | 3.5 | 7.7 | 8.5 | 2.3 | 5.8 | 6.3 | 14.0 | 17.2 | 17.6 | 4.0 | 3.6 |
| | | 130 | 1 | 42 | 4.6 | 9.9 | 10.0 | 3.0 | 7.0 | 7.3 | 14.0 | 16.8 | 21.2 | 4.3 | 7.2 |
| 4 | " | 120 | 0 | 44 | 6.4 | 8.4 | 9.2 | 4.0 | 6.4 | 6.9 | 17.4 | 18.0 | 22.0 | 2.9 | 4.6 |
| | | 182 | 1 | 44 | 6.6 | 8.3 | 9.7 | 4.3 | 6.3 | 7.3 | 13.6 | 16.8 | 21.6 | 3.0 | 8.0 |
| 5 | " | 230 | 100 | 23 | 3.7 | 5.7 | 8.6 | 2.3 | 5.1 | 6.9 | 10.0 | 15.7 | 17.8 | 4.6 | 7.8 |
| | | 270 | 1 | 23 | 4.6 | 6.6 | 8.2 | 2.8 | 4.7 | 6.6 | 11.5 | 15.2 | 18.2 | 3.8 | 6.7 |
| 6 | " | 270 | 100 | 27 | 5.3 | 6.9 | 6.7 | 3.5 | 5.4 | 5.7 | 10.3 | 14.2 | 18.6 | 2.2 | 8.3 |
| | | 210 | 1 | 27 | 4.1 | 6.4 | 7.2 | 2.7 | 4.8 | 5.5 | 10.3 | 16.7 | 18.6 | 2.8 | 8.3 |

(a) No glutamic acid added to medium. (b) Medium contained $3 \times 10^{-3}$ M added glutamic acid. (c) Medium contained $10^{-2}$ M added glutamic acid. * See footnote in Table I.

implied that glutamic acid is important for the functioning of the choline acetylase system in the brain and that PGA might competitively interfere with glutamic acid in nerve metabolism. Although earlier studies[16] related glutamic acid to the choline acetylase system, reactivation of this system by glutamic acid has been since shown to be a non-specific effect, also exerted by various other amino and organic acids, especially citric acid.[17] In fact, recent purification of the system has led to the conclusion that acetate rather than citrate[18] is responsible for the effect. That PGA plays a positive role in the functioning of the central nervous system has been shown by the development in young turkeys of generalized paralysis on PGA-deficient diets[12,13] and that the paralysis disappears within a few hours after the injection of PGA.[13] The present investigation indicates that the respiration *in vitro* of brain tissue slices in turkeys with this paralysis, was not grossly different from that of brain tissue slices from control birds.

*Summary.* The utilization of glutamic acid by *Lactobacillus casei* and *Streptococcus faecalis* R was not affected by increasing the pteroylglutamic acid (PGA) content of the culture medium a thousandfold. The respiration of rat brain slices was not inhibited by adding PGA to the medium at a level of $3 \times 10^{-4}$M, more than 100 times the highest concentration observed in the blood of human subjects following the intravenous injection of 15 mg of PGA. The increased respiration of rat brain slices produced by adding glutamic acid was not reduced by adding PGA to the medium at concentrations of $3 \times 10^{-3}$M and $3 \times 10^{-4}$M. The respiration rate of brain slices of PGA-deficient turkeys, with or without added glutamic acid, was not consistently different from that of brain slices from control birds which had received an adequate

[16] Nachmansohn, D., and John, H. M., *J. Biol. Chem.*, 1945, **158**, 157.

[17] Feldberg, W., and Mann, T., *J. Physiol.*, 1946, **104**, 411; Lipton, M. A., and Barron, E. S. G., *J. Biol. Chem.*, 1946, **166**, 367.

[18] Kaplan, N. O., and Lipmann, F., *Fed. Proc.*, 1947, **6**, 266.

dietary supplement of PGA. Brain and kidney tissue slices from rats receiving a high dietary level of PGA respired at the same rate as corresponding tissues from control rats, with or without added glutamic acid.

ADDENDUM: After this manuscript was submitted, an article appeared (Grossowicz, N., *J. Biol. Chem.*, 1948, **173**, 729) which reported that glutamine inhibited the growth-promoting effect of glutamic acid for *Staphylococcus aureus*, and and that this inhibitory effect of glutamine could be overcome by pteroylglutamic acid, by glutathione, or by extra glutamic acid. These results apparently indicate that under certain circumstances pteroylglutamic acid and glutamic acid may function interchangeably in the nutrition of micro-organisms.

## 16362 P

## Effect of Tween 80 on Certain Strains of *C. diphtheriae.*

MARTHA K. WARD.* (Introduced by M. Frobisher, Jr.)
*From the Department of Bacteriology, Johns Hopkins School of Hygiene and Public Health, Baltimore, Md.*

In attempts to prepare a fluid synthetic medium for general use in diphtheriology, difficulty was encountered in that recently discovered minimus strains[1,2,3] and a few, closely related, minute-colony strains, failed to grow in a medium suitable for most other strains. The synthetic medium used consists of amino acids, inorganic salts, carbohydrates and growth factors in as pure form as could be obtained. It has no peptone, casein or other constituents of variable or unknown composition. Details of the medium will be published elsewhere.

The minimus strains have also uniformly failed to grow well in infusion broth. The granular nature of growth of the minimus strains in infusion broth suggested the possibility that a surface tension reducent might facilitate growth, following the line of thought suggested by Dubos *et al.*[4,5,6] in their studies of the tubercle bacillus. Accordingly, Tween 80 was added aseptically from a freshly prepared, sterile, aqueous solution, in a concentration of about 0.05%, to the synthetic medium just before inoculation. Immediate and striking stimulation of growth of the minimus-type strains was observed. Concentrations of Tween up to about 0.5% have since been used with no apparent inhibition of the minimus type. No optimum or inhibitory range has yet been determined.

With other types of diphtheria bacilli, the results of adding Tween 80 to the medium are somewhat variable. Some strains, particularly of the mitis type, appear slightly inhibited; others show little or no difference in growth response to Tween. Gravis and gravis-like strains which normally grow with pellicle formation, lose their pellicle and grow diffusely throughout the medium. No types other than minimus are stimulated to any great degree, with the exception of a few minute-colony forms sent by Dr. McLeod from Leeds sometime ago. These strains were designated by McLeod as the intermedius type. It is of interest that the 4 strains of this group which Frobisher found to be identical with minimus type[3] were more markedly stimulated by addition of Tween than were the other 6.

Similar stimulation of growth of the mini-

* National Institute of Health Pre-doctorate Research Fellow.

1 Eller, C. H., and Frobisher, M., Jr., *Am. J. Hygiene*, 1945, **42**, 179.

2 Frobisher, M., Jr., *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 330.

3 Frobisher, M., Jr., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 304.

4 Dubos, R. J., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 361.

5 Dubos, R. J., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 56.

6 Dubos, R. J., and Davis, B. D., *J. Exp. Med.*, 1946, **83**, 409.