

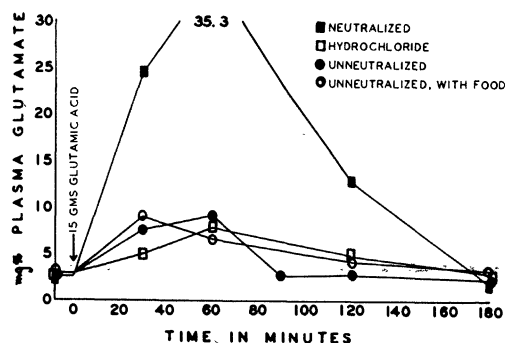


Fig. 1. 1-Glutamic acid tolerance curves on one subject with 1-glutamic acid compounds equivalent 15 g of the acid.

had nausea after taking glutamate experienced none when given the unneutralized acid with or without food.

Two of the same subjects were used for one tolerance test each with glutamic acid hydrochloride. Because of the marked fall in blood pressure and the severe stomach cramps induced by the ingestion of such a large amount of this compound, no additional tests were undertaken. The hydrochloride was given to one subject in 50 ml of water followed by tomato juice and water *ad libitum* and to the other subject in capsules followed by tomato juice, and water *ad libitum*. It is interesting that the subject who received the capsules suffered acute nausea, the first time in six tolerance tests with various glutamic-acid compounds. In the subject who received the hydrochloride in solution, the rise in plasma level was somewhat greater than for the unneutralized acid; in the subject who received capsules, the reverse was true. The tolerance curves for the sodium salt, the unneutralized acid with and without food, and the hydrochloride for one subject are shown in Fig. 1.

It seems possible that the unneutralized glutamic acid is not absorbed to any great extent and that the major part of a given dose passes through the gut without going into solution. The material would not tend to dissolve in the acid pH of the stomach, and, although the duodenal secretions are alkaline, the intestinal contents, with the passage of acid chyme from the stomach, tend to be acid. Even in the absence of food, the passage of a substance as markedly acid as glutamic acid into the duodenum would tend to render that area acid, unless the glutamic acid at the same time stimulated a large flow of pancreatic juice. This latter possibility does not seem likely.

It is also possible that there is an extremely slow absorption of the unneutralized acid and that the blood level is elevated slightly over a comparatively long time. Our data do not suggest such an effect, but blood samples would have to be drawn over a much longer period of time to get a conclusive answer. However, in view of the normal pattern of variation of the plasma glutamate level (6) it does not seem likely that such a small rise would have much physiological effect.

Some of the confusion in the literature regarding the activity of 1-glutamic acid may be due in part to a failure of some investigators to specify which of the three forms of 1-glutamic acid they used. We can assume that 1-glutamic acid given by injection is close to pH 7.0 and, hence, is a salt of 1-glutamic acid, probably monosodium glutamate. Material given orally in solution is probably also a salt, unless otherwise specified, since the hydrochloride in solution has such unpleasant effects; the sludge is probably the unneutralized acid, and the capsules or pills may be any one of the three. The oral ingestion of 1-glutamic acid in a form that does not enter the blood stream readily cannot be expected to have any physiological effects. This situation emphasizes the fact that material placed in the gut is outside the body until it has passed the gastrointestinal mucosa. It is interesting that the majority of favorable reports of the results of administration of glutamic acid to patients or of its physiological effects in animals have been obtained with sodium glutamate or with the hydrochloride.

References and Notes

1. H. Waelsch, *Advances in Protein Chem.* **6**, 299 (1951).
2. D. A. Pond and M. H. Pond, *J. Mental Sci.* **97**, 663 (1951).
3. W. Mayer-Gross and J. W. Walker, *Biochem. J. (London)* **44**, 92 (1949).
4. 1-glutamic acid, 0.7 g/100 g water, 20°C; 1-glutamic acid HCl, 33.3 g/100 ml water, room temperature; monosodium glutamate 73.9 g/100 g water, 25°C.
5. B. A. Prescott and H. Waelsch, *J. Biol. Chem.* **164**, 331 (1946).
6. W. A. Himwich and I. M. Petersen, *J. Appl. Physiol.*, in press.
7. S. P. Bessman *et al.*, *J. Biol. Chem.* **175**, 817 (1948).

5 April 1954.

Pseudo-B₁₂ Activity in the Baby Pig

Jay Firth and B. Connor Johnson

Division of Animal Nutrition,
University of Illinois, Urbana

Pfiffner *et al.* (1) have isolated an organism from rumen contents that produced cobalt containing pigments of the B₁₂ group. One of these compounds, pseudo-vitamin B₁₂, has been isolated by the Parke-Davis group and shown to have B₁₂ activity for *L. leichmannii*, *L. lactis dornier*, and *E. coli* but to be inactive for chick growth. Acid hydrolysis (2) of this pseudo-B₁₂ indicates that it differs from B₁₂ in that it contains adenine instead of 5,6-dimethyl benzimidazole in the nucleotide portion of the molecule. We have previously demonstrated a severe vitamin-B₁₂ deficiency in the pig (3, 4), and the present experiment was made to find whether the adenine-containing form ("pseudo-vitamin B₁₂") would replace the benzimidazole-containing form in the nutrition of the pig.

Baby pigs 2 to 3 days old were used in this experiment. They were housed individually in wire-bottom metal cages and fed *ad libitum* an alpha-protein synthetic milk diet of the following percentage composi-

Table 1. Average weight response to vitamin B₁₂ and pseudo-B₁₂.

	Basal + B ₁₂	Basal - B ₁₂	Basal + pseudo-B ₁₂
No. of pigs	5	5	5
Avg. individual wt. (kg)	1.93	1.89	1.62
Avg. 4-wk wt. (kg)	10.19	5.13	5.06
Avg. gain (kg)	8.26	3.24	3.44

tion: alpha-protein 29.4; DL-methionine 1.3; cerelose 30.5; lard 30.5; minerals 8.3. A complete vitamin premix was added at the time of feeding with the exception of vitamin B₁₂ or pseudo-vitamin B₁₂, which were given by weekly injection at the rate of 0.8 μ /kg of body weight per day.

Three groups of five pigs each were used. Weight gains for the B₁₂-deficient and pseudo-vitamin B₁₂ groups were essentially equal for the 4-wk period, as is shown in Table 1. Erythrocyte, leucocyte, reticulocyte, and hemoglobin determinations were made every other day and were relatively equal for both the deficient and pseudo-vitamin B₁₂ groups, showing a gradual decrease in red cells and reticulocytes. Injection of B₁₂ produced an immediate appetite response in both groups, followed by a reticulocyte response in 8 to 10 days. Untreated pigs in the deficient and pseudo-vitamin B₁₂ groups began to die of the B₁₂ deficiency during the fifth week.

Pseudo-vitamin B₁₂ is apparently inactive for the baby pig.

References

1. J. J. Piffner et al., *Chemical Abstracts* (Am. Chem. Soc., Columbus 10, O., 1951), p. 22C.
2. H. W. Dion, D. G. Calkins, J. J. Piffner, *J. Am. Chem. Soc.* **74**, 1108 (1952).
3. B. C. Johnson and A. L. Neumann, *J. Biol. Chem.* **178**, 1001 (1949).
4. A. L. Neumann, B. C. Johnson, and J. B. Thiersch, *J. Nutrition* **40**, 403 (1950).

14 April 1954.

Reversible Changes in the Absorption Spectrum of *Chlorella* upon Irradiation

L. N. M. Duysens*

Carnegie Institution of Washington,
Department of Plant Biology, Stanford, California

When a suspension of purple bacteria is irradiated with visible or near infrared radiation, a change in the absorption spectrum occurs within one or a few seconds (1, 2). In the dark, the original absorption spectrum is restored within the same time. When the bacteria are photosynthesizing and the intensity of irradiation does not much exceed the intensity at which saturation of photosynthesis occurs, the difference between the absorption spectra in light and darkness is strikingly similar to the difference between the absorption spectra of oxidized and reduced cytochrome *c* (2).

This finding, combined with what was known about the photosynthesis of purple bacteria, strongly suggests that a cytochrome pigment is an intermediate in the photosynthetic oxidation of the reductant in purple bacteria. The question now poses itself whether in green plants the photosynthetic oxidation of the reductant—water—also is mediated by a cytochrome pigment.

We found that *Chlorella*, too, showed a change in the absorption spectrum upon irradiation, which was reversed upon darkening. This change was measured by a method similar to that used with purple bacteria (1). Red light ($\lambda > 670$ m μ) was used to illuminate the suspension, contained in a square cell, and the change in absorption brought about by this illumination was measured by a second, weak beam of variable wavelength at a right angle to the illuminating one. The optical density of the suspension, corrected for scattering, was about 0.5 at 680 m μ . The difference spectrum determined in this way is plotted in Fig. 1.

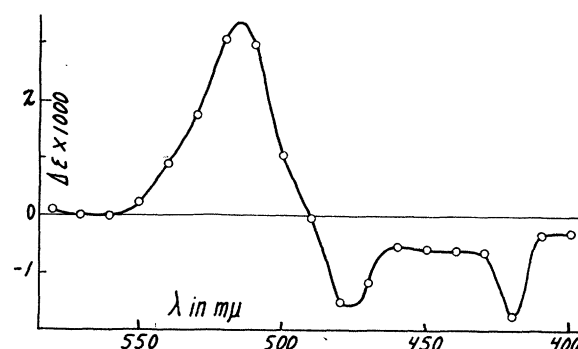


Fig. 1. Change in absorption spectrum of a *Chlorella* suspension upon irradiation with red light; $\Delta\epsilon$ is change in optical density.

It shows a maximum, corresponding to a pronounced increase in absorption during irradiation, at about 515 m μ , and minima, indicating decreased absorption, at about 478 and 420 m μ .

Similar effects around 515 and 480 m μ were found also in a leaf, in the thallus of a sea alga, and in blades of *Vallisneria*. The region around 420 m μ was measured only for *Chlorella*. Thus it seems that the difference spectrum found for *Chlorella* is characteristic of green plants and algae in general.

As a whole, this spectrum of *Chlorella* cannot be identified with the difference between the absorption spectra of oxidized and reduced cytochrome *c* or *f*; it may, however, be the sum of two difference spectra: one belonging to a cytochrome, with the peak at 420 m μ , and one belonging to another pigment, with peaks at 515 and 478 m μ . If this is the correct interpretation, then the absorption drop at 420 m μ can be considered as revealing the oxidation of a cytochrome upon irradiation of *Chlorella* and its reduction in the dark. This would be in accordance with the suggestion of Hill and coworkers that cytochrome *f*, which they found in the chloroplasts of green plants (3) and in