nate the field of observation. There remained scattered irregular dark patches, but many areas were completely devoid of any dark pigment. The effect was more pronounced on smaller animals but was noticeable on all crayfish that received ACE. Control animals exhibited normal chromatophore distribution, black chromatophores being prominent as well as those of reddish hue.

In order that the crayfish-reddening effect of ACE might be described more precisely, uropods were removed from normal crayfish and placed in water in spotplate depressions. The uropods from each animal were separated, one exopodite and one endopodite serving as the experimental material and their opposite members as the control. Each of the former received 0.05 ml of ACE in the water, while the controls received additional water or van Harreveld's balanced-ion solution (*2*). Both groups were examined at intervals with the aid of a compound microscope at 100× magnification. The entire experiment was repeated more than a dozen times. During a 12-hr observation period, no changes were observed in the colored chromatophores on ACE-treated uropods. The black chromatophores, however, became generally gray in appearance, and irregular patches of black granules appeared. The dark chromatophores appeared to have been bleached or denuded of pigment, without noticeable change in degree of pigment dispersion. No observable changes occurred in the chromatophores of control uropods. The crayfish-reddening effect apparently results from destruction of dark pigment, leaving unaltered red chromatophores to create the dominant visual impression.

Although the effect of ACE on arthropod chromatophores has not been reported previously, there is considerable published evidence that adrenal cortical substances may influence pigmentation in vertebrates. Desoxycorticosterone suppresses the formation of melanin, inhibits melanophore differentiation, and causes melanophores to degenerate in explants of chick-embryo skin (*3*). Black or brown rats turned gray as a result of dietary vitamin-B deficiency and resumed deposition of melanin upon adrenalectomy, but the effect is suppressed by exogenous ACE or desoxycorticosterone (*4, 5*). It has been observed for many years that human patients with Addison's disease show varying degrees of darkening or pigmentation, presumably as a result of adrenal cortical insufficiency (*6*). It seems reasonable to hypothesize that the adrenal cortical mechanisms that suppress or destroy pigment in vertebrates and in the crayfish are basically similar.

References and Notes

1. Tap water at the university is not chlorinated and is not toxic to aquatic organisms.
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Absorption of l-Glutamic Acid

Williamina A. Himwich

Thudichum Psychiatric Research Laboratory, Galesburg State Research Hospital, Galesburg, Illinois

The possibility that the salts of l-glutamic acid have a different physiological effect than the acid has been raised by Waelsch (*1*). Pond and Pond (*2*) reported that a given dose of the acid raised the plasma level of glutamic acid less markedly but over a longer period of time than did an equivalent dose of either the sodium or potassium salt. Mayer-Gross and Walker (*3*) found that the oral administration of as much as 100 g of l-glutamic acid hydrochloride was not efficacious in arousing patients from insulin hypoglycemia, whereas the administration intravenously of 20 g of glutamic acid as sodium glutamate was.

A survey of the literature on l-glutamic acid administered either to animals or to man shows that three forms of the compound have been used: the salt of sodium or potassium, the unneutralized acid, and the hydrochloride. These three compounds differ markedly in solubility (*4*) and, thus, might be expected to be absorbed at different rates. In order to test this hypothesis, equivalent amounts of the three compounds were given to human subjects in a fasting condition. Blood samples were drawn routinely before the ingestion of the test substance and at 30, 60, 90, 120, and 180 min afterward; in four cases samples were also taken at approximately 4 and 5 hr. Plasma glutamic acid levels were determined by the method of Prescott and Waelsch (*5*). Since 16 such tolerance tests had been completed previously with 15 g of the monosodium salt of glutamic acid in tomato juice and water (*6*), the same dosage level and mode of administration were used for the other compounds.

The unneutralized glutamic acid was given twice to each of four subjects who had previously shown typical responses to the administration of sodium glutamate. In the first test of the unneutralized acid, in each of the four subjects, it was given as a sludge with tomato juice followed by water *ad libitum*. It was felt, however, that the ingestion of the acid with or immediately after food would more nearly approach the condition existing in the treatment of mentally defective children and that the presence of food might aid in the solution and absorption of the acid. Therefore, in the second test the acid was given with a liberal breakfast of hot cereal, eggs, milk, tomato juice, and toast, with water being allowed *ad libitum*. In three cases, the administration of food with the acid raised the absorption slightly but not at all to a level comparable to that of the salt. In the fourth individual no rise in blood level occurred after ingestion of the acid with or without food. In only one case was there any indication of a delayed rise, even though in two cases blood was drawn as late as 5 hr after the ingestion of the acid. The rises in these cases with unneutralized acid are similar to those reported by Bessman *et al.* (*7*) with a dose of 1 g/10 kg of body weight. One subject who

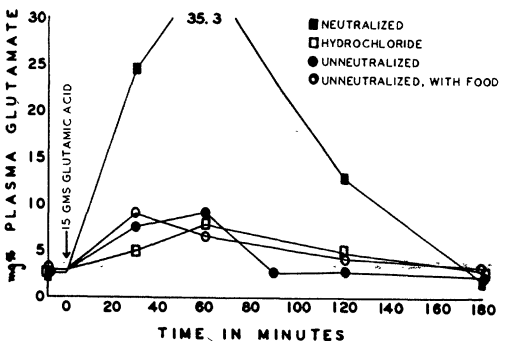

Fig. 1. 1-Glutamic acid tolerance curves on one subject with 1-glutamic acid compounds equivalent 15 g of the acid.

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 tolerence 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 tolerence 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 *p*H 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 *p*H 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

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Pseudo-B_{12} 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 B_{12} group. One of these compounds, pseudo-vitamin B_{12}, has been isolated by the Parke-Davis group and shown to have B_{12} activity for *L. leichmannii, L. lactis dorner,* and *E. coli* but to be inactive for chick growth. Acid hydrolysis (*2*) of this pseudo-B_{12} indicates that it differs from B_{12} 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-B_{12} deficiency in the pig (*3, 4*), and the present experiment was made to find whether the adenine-containing form ("pseudo-vitamin B_{12}") 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-