It must be emphasized that these results are *not* based upon any kinetic aspects of the actin transformations. The rates of polymerization and depolymerization *in vivo* are not known (compare PARRISH AND MOMMAERTS[2]) and would in any case not be a suitable base for calculation due to the discontinuous nature of the events.

As has been pointed out repeatedly by the author (*e.g.*, references[7,11]; compare also BRAVERMAN AND MORGULIS[12]; PERRY[13]), the enzymic activity of myosin-ATPase cannot account for the rate of breakdown of ATP in physiological activity. The present considerations suggest that physiological dephosphorylation is not due to straight enzymic hydrolysis, but is linked with the repeated molecular transformations of actin.

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A STUDY OF AMINO ACID INTERRELATIONSHIP USING SIMULTANEOUS ADAPTATION

by

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The adaptation of a micro-organism for the utilisation of a given substrate involves the simultaneous adaptation for the intermediate metabolic substances derived from this substrate. This logical implication has been used by STANIER[1], KARLSON AND BARKER[2], AJL[3] in the study of oxidation by bacteria.

The same method can be usefully employed in the investigation of amino acid metabolism. In this case one can conveniently measure the growth of the bacteria as the response to the utilisation of a given amino acid. The lag phase can be interpreted, at least partially, as the time necessary for the synthesis of the enzyme involved in the utilisation of a given substrate[4]. In the course of a study on the metabolism of glutamic acid in *B. subtilis* (strain M_1), it has been observed that this bacterium requires one of the following amino acids: glutamic acid, aspartic acid, arginine, ornithine or proline. The relationship between these amino acids is well known[5,6,7,8,9]. One may ask, whether the bacteria use the glutamic acid in replacement of arginine or proline or if these two amino acids are converted into glutamic acid, which is secondarily used as the normal source of nitrogen.

In the experiments reported here, the bacteria are grown in aerated media containing the ordinary mineral salts, glycerol *M*/20, and one of the above cited amino acids at *M*/200. When bacteria, which have been grown on glutamic acid are washed and used as large inoculum in culture

media containing respectively, glutamic acid, ornithine or arginine, one gets the growth curves reported in Fig. 1. If the bacteria have previously been grown on arginine one gets the growth curves of Fig. 2. The same cross experiment can be made with glutamic acid and proline. The results are reported in Fig. 3 and 4.

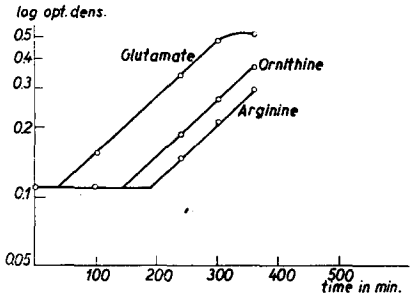

Fig. 1. Growth of bacteria previously grown on glutamic acid

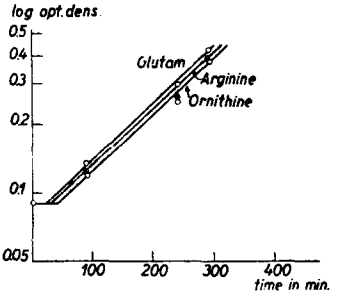

Fig. 2. Growth of bacteria previously grown on arginine

The results show that there is a prolonged lag phase with arginine, ornithine and proline after a previous growth with glutamic acid. In this case the bacteria have to be adapted to these amino acids before a visible growth starts. The arginine, ornithine and proline are not on the main metabolic pathway of utilisation of glutamic acid. On the contrary, after growth on arginine, or proline, the bacteria are ready to grow on glutamic acid with the same lag phase as with arginine or proline.

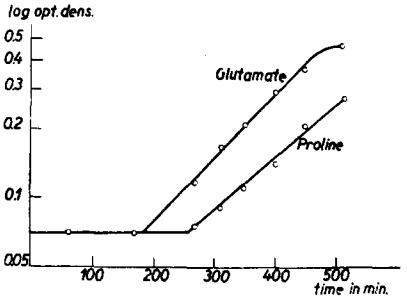

Fig. 3. Growth of bacteria previously grown on glutamic acid

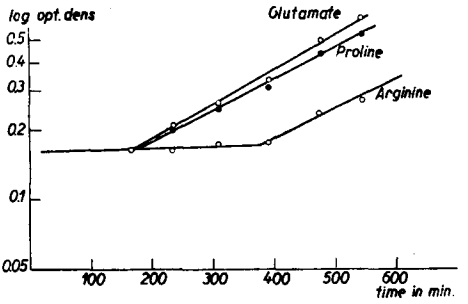

Fig. 4. Growth of bacteria previously grown on proline

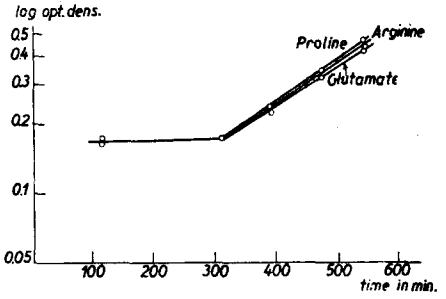

Fig. 5. Growth of bacteria previously grown on arginine

The glutamic acid is on the normal pathway of the utilisation of proline, ornithine or arginine.

It has already been shown that ornithine is reversibly convertible into proline[10]. Proline could be the normal intermediary between arginine and glutamic acid. This is proved to be the case: after growth on arginine, the lag phase on arginine, glutamic acid and proline are identical (Fig. 5), although after growth on proline, the bacteria needs further lag for growth on arginine (Fig. 4).

The method of simultaneous adaptation, as here applied to the growth of bacteria, appears to be a very simple and very reliable test for the study of metabolic pathways *in vivo*. This method could be specially useful in conjunction with nutritional variants of micro-organisms.

A detailed report, included in a study of the central role of glutamic acid as a donor of nitrogen in the metabolism of this bacteria, will be published later.

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SYNTHESIS OF FAT FROM CARBOHYDRATE IN THIAMINE DEFICIENCY

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A number of papers published by MC HENRY and his collaborators[1,2,3] have led to a wide-spread acceptance of the view that the synthesis of fat from carbohydrate is impaired in thiamine deficiency. The evidence put forward by these authors to substantiate this claim is, however, inconclusive. In most of their experiments the groups of rats and pigeons compared had consumed different amounts of food and only one experiment is published in which paired feeding of a fat-free diet was applied to rats (Ref. 1, p. 294). As only the mean results of this experiment are stated it is impossible to decide whether or not the small difference observed between the means is statistically significant.

BOXER AND STETTEN[4], using the incorporation of deuterium into the body fatty acids of rats as a measure of fat synthesis, arrived at the conclusion that the failure of synthesis and deposition of fatty acids in thiamine deficient rats "is attributable chiefly to the diminished food intake rather than to any specific action of thiamine". Whether any specific action of thiamine exists at all cannot be decided as the amount of food consumed was the limiting factor in these experiments.

Therefore we have carried out experiments on pigeons with forced feeding of large amounts of carbohydrate. In a first experiment 30 pigeons received 2 g thiamine-free casein and 18 g sucrose daily, supplemented with a salt and vitamin mixture including thiamine. After 18 days ten animals were sacrificed and their fat contents determined (Group I); half of the remaining number continued on this food (Group II), while the others received the same food, but with omission of thiamine (Group III). When, after 14 days, symptoms of thiamine deficiency appeared in the animals of Group III, all animals of Groups II and III were sacrificed. The mean figures for the fat contents found are 36.9 g (9.3% of body weight) for Group I, 44.6 g (10.3%) for Group II, and 43.6 g (11.5%) for the thiamine deficient Group III. The differences between these figures are not statistically significant. Presuming that the carbohydrate ingested is at least partly metabolized via fat[5,6], one should conclude that the synthesis of fat from carbohydrate is not impaired in thiamine deficiency.

In a second experiment with 24 pigeons, which had been subjected to semi-starvation (in order to exhaust their fat deposits) and to partial depletion of the thiamine pyrophosphate stores of their tissues, a direct proof of unimpaired fat synthesis with a net increase of fat content in thiamine-deficient pigeons could be given. The pigeons were divided in three groups of eight pigeons each, numbered IV, V and VI. Group IV was sacrificed after the starvation and depletion period; Group V received 3 g casein and 24 g sucrose daily with salts and vitamins, including thiamine in abundance. Group VI received the same food without thiamine. When, after six days, the animals of Group VI showed grave symptoms of thiamine deficiency all animals were sacrificed. Thiamine pyrophosphate determinations in the hearts showed that during this period Group VI had a moderate to grave