A Maternal Influence on the Incorporation of Methionine into Liver Protein[1]

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It was previously demonstrated (*3*) that two highly inbred strains of rats, differing in body size and growth rate, incorporated labeled S^{35} DL-methionine into surviving liver-slice proteins at significantly different rates. In studying the mode of inheritance of this character, earlier observations have been confirmed and extended to a demonstration of the influence of maternal genotype on the process *in vitro* under foster nursing.

The results were obtained by the technique of Melchior and Tarver (*2*). According to this procedure, the labeled protein, *in vitro*, is freed of adsorbed radioactivity and the cyst(e)ine sulfur separated as the cuprous mercaptide. The remaining (methionine) sulfur is converted to sulfate, precipitated as benzidine sulfate, counted, and titrated. Results are expressed as % replacement, defined as the fraction of the methionine recovered which was replaced by radioactive methionine.

The foster-nursing tests were conducted on four litters of J (Fisher Strain No. 344) and four litters of F (Wistar King Albino) progeny, interchanged between mothers 12–36 hr after birth. The fostered offspring were weaned at 4 weeks and continued on stock diet of Purina chow and greens until they were sacrificed at 100-g body weight. Triplicate tests were performed on individual foster-nursed and control animals, and the results are summarized in Table 1.

TABLE 1

INFLUENCE OF FOSTER NURSING ON PROTEIN SYNTHESIS *in Vitro* IN RAT LIVER SLICES

Litter No.	J ♂ nursed by F		F ♂ nursed by J	
	No. of tests	% Replacement	No. of tests	% Replacement
1	2	0.41	3	0.59
2	3	0.42	2	0.46
3	3	0.44	3	0.41
4	3	0.37	3	0.41
Average	11	0.41*	11	0.46
Standard error		± 0.02		± 0.03
Normal controls	13	0.29	10	0.43
Standard error		± 0.02		± 0.02

* Significantly different from normals at 1% level.

It is apparent that the maternal influence, acting through the milk, has stimulated the liver activity of the J strain to the level of the foster parent. The maternal influence on liver activity is accompanied by a marked increase in the growth of the fostered J progeny. It is also noticeable that the failure of F progeny to respond to J maternal influence on liver activity is reflected in the undisturbed growth of these animals, as shown in Table 2.

TABLE 2

INFLUENCE OF FOSTER NURSING ON GROWTH

	No. of animals	Weight in g at end of weeks		
		4	8	10
J ♂ nursed by F	17	67	200	244
J ♂ " " J	185	55	170	212
F ♂ " " J	15	66	194	230
F ♂ " " F	167	66	184	232

The demonstration of genetic control of the incorporation of an amino acid into the protein of liver tissue under the conditions of this study, and of the strong maternal (milk) influence on its hereditary transmission, raises certain questions concerning the generality of the phenomenon. It is to be wondered whether milk influence on specific metabolic processes in the mammal is part of a normal developmental mechanism. In this regard, the improvement of suckling ability and the elimination of cannibalism by vitamin B_{12} supplementation to the diet of pregnant mothers (*1*), and the correction of developmental deformities by riboflavin supplementation to the diet (*4*), show the gross effect of maternal nutritional deficiency on normal development. The results of this study extend the effect of variable maternal potency to normal metabolic processes and development.

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References

1. LEPKOVSKY, S. Personal communication.
2. MELCHIOR, J., and TARVER, H. *Arch. Biochem.*, 1947, **12**, 301.
3. RUTMAN, R. J., DEMPTSTER, E., and TARVER, H. *J. biol. Chem.*, 1949, **177**, 491.
4. WARKARY, J., and SCHAFFRENBERGER, E. *J. Nutrition*, 1944, **27**, 477.

Phenylhydrazine Oxalate as "Trapping Agent" for the Simultaneous Fixation of Intermediate Products in Lactic Acid Fermentation with Living Cells

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The chemistry of lactic acid fermentation is still based upon that of glycolysis because of the difficulties encountered in separation of the intermediate products which, on the contrary, was rather easy in the case of the cells of yeast and animal tissues.

Phosphorylation was, however, observed by Virtanen (*16, 17*), and Neuberg and Kobel (*6*) obtained a high

yield of methyl glyoxal by the reaction of hexose diphosphate with special enzymatic preparations of *Lactobacillus delbruckii;* the same lactobacillus converts the hexose diphosphate to lactic acid (*14*). As methyl glyoxal was excluded as a key intermediate product in biological processes, it was to be expected that the lactic acid fermentation would follow the pattern of glycolysis, according to the usual scheme of Embden-Meyerhof. However, research in this field is still limited, and no decisive results were obtained in the identification of the intermediate products. It is known, however, that *L. delbruckii* converts phosphoglyceric acid to pyruvic acid (*2–6, 9*), and this to acetyl phosphate (*4*).

Virtanen *et al.* (*18*) were unable to fix pyruvic acid in the presence of bisulfite from lactic acid fermentations, while Simon (*10*) and Neuberg and Kobel (*7, 8*) have questioned the fixation of the same acid and methyl glyoxal in the presence of semicarbazide according to the experiments of Kostytschew and Soldatenkow (*3*). Also, the results obtained with other bacteria are favorable to the applicability of the Meyerhof scheme to bacterial dissimilations. Stone and Werkman (*13*) found phosphoglyceric acid as an intermediate in some bacterial dissimilations. The triose phosphate dehydrogenase of *Escherichia coli* was studied by Still (*12*), and Utter and Werkman (*15*) compared the enzymes aldolase and isomerase of *E. coli* with those in muscle and yeast.

But a direct demonstration of the applicability of the Meyerhof scheme to the activity of lactic acid ferments could be obtained only by isolating the principal intermediates of dissimilation—for instance, the triose phosphates and pyruvic acid. Using phenylhydrazine oxalate (OP) as trapping agent,

$$\begin{matrix} COOH \cdot H_2N \cdot NH \cdot C_6H_5 \\ | \\ COOH \cdot H_2N \cdot NH \cdot C_6H_5 \end{matrix}$$

I was able to fix both products simultaneously in fermentations with living cells, as was possible in alcoholic fermentations (*1*). The simultaneous isolation of triose phosphates and pyruvic acid suggests that the sequence of reactions that leads to the formation of lactic acid is the same as the Embden-Meyerhof scheme for glycolysis, i.e., the reduction of pyruvic acid to lactic acid involves an oxidation-reduction between triose phosphates and pyruvic acid. The complete absence of methyl glyoxal in the medium gives additional evidence that lactic acid cannot arise according to the following reaction:

$$CH_3 \cdot CO \cdot CHO + H_2O \xrightarrow{\text{glyoxalase}} CH_3 \cdot CHOH \cdot COOH$$

Moreover, acetaldehyde was fixed, although the bacilli produced lactic acid from saccharose in a 95% yield. Considering that the bacilli are able to convert pyruvic acid mainly to acetic acid and 2,3-butylene glycol (unpublished results), the conclusion could be drawn that the fixed aldehyde is an intermediate of the degradation of pyruvic acid to acetic acid, via acetyl phosphate (*4*), followed by the conversion of acetic acid to 2,3-butylene glycol via acetaldehyde; a reaction perhaps like that which Slade and Werkman (*11*) observed for *Aerobacter aerogenes.*

The cells of a particular thermophilic strain of *L. delbruckii,* which I isolated recently, were grown on a medium containing beet molasses with up to 5% saccharose. After 20-hr incubation at 50° C with $CaCO_3$, the cells were harvested by centrifugation, washed, recentrifuged, and suspended in the fermentation mixture consisting of: yeast water, 700 ml; distilled water, 300 ml; saccharose, 10 g; Na_2HPO_4, 3 g; salt (OP), 2 g; wet microbial cells, 15–30 g. Salt (OP) was dissolved in boiling yeast water; then the solution was cooled and saccharose was dissolved in it. After 6-hr incubation at 50° C, the medium was centrifuged and the fixed products were identified in the clear supernatant.

Acetaldehyde was determined by distillation of the medium with pyruvic acid in a Vigreux column and precipitation in the distillate as 2,4-dinitrophenylhydrazone (2,4-DNP), mp 164° C. Calculated: N%, 25.0. Found: N%, 25.10. Found: amount in g‰, 0.005–0.01. Blank test: Distillation of the OP salt with pyruvic acid gives no acetaldehyde 2,4-DNP.

TABLE 1

METHYL GLYOXAL 2,4-DNP IN 100 ML DISTILLATE

Exp. No.	Without H_2SO_4	With 20% H_2SO_4
1	1 mg	14 mg
2	traces	28 mg

Pyruvic acid can be isolated as phenylhydrazone by ether extraction of the medium concentrated 1: 5, or by precipitation as 2,4-DNP from the medium, concentrated and reacting at high temperature with benzaldehyde. The product is dissolved in *N*/2NaOH and reprecipitated by acidification with HCl and recrystallization from acetic acid, mp 220° C. Calculated: N%, 20.89. Found: N%, 20.96. Found: about 0.15 g‰ as 2,4-DNP.

Trioses have been isolated as methyl glyoxal according to the method of Neuberg *et al.* (*5*), which was conveniently modified as in the preceding work (*1*), i.e., the medium, treated with benzaldehyde and extracted with ether, was distilled after addition of concentrated H_2SO_4, so as to obtain a sulfuric acid concentration of 20%. Methyl glyoxal was precipitated in the distillate as 2,4-DNP, and this purified by dissolving twice in nitrobenzene and precipitating with alcohol, mp 298° C. Analysis: Calculated—C, 41.64; H, 2.77; N, 25.93. Found—C, 41.80; H, 3.02; N, 25.98. Found: 0.15–0.2 g‰ as 2,4-DNP. As the distillate from the fermentation mixture, not acidified with sulfuric acid, contains but traces of methyl glyoxal, it follows, as shown in Table 1, that the intermediate products are really trioses, not methyl glyoxal.

As was previously observed in alcoholic fermentation (*1*), the OP salt fixes mainly the first intermediates of fermentation and only by accelerating the microbial activity is it possible, probably for kinetic reasons, to fix the last intermediates in greater amount.

At the end of every experiment the positive vitality of the cells was tested by separate subcultures. The state of the trioses, i.e., whether they were or were not phos-

phorylated, was not investigated in the course of this research.

References

1. BOLCATO, V. *Nature*, Lond., 1950, **165**, 814.
2. KATAGIRI, H., and MURAKAMI, S. *Biochem. J.*, 1939, **33**, 1257.
3. KOSTYTSCHEW, S., and SOLDATENKOW, S. *Z. physiol. Chem.*, 1927, **168**, 124.
4. LIPMANN, F. *J. biol. Chem.*, 1944, **155**, 55.
5. NEUBERG, C., *et al.* *Biochem. Z.*, 1917, **83**, 263.
6. NEUBERG, C., and KOBEL, M. *Biochem. Z.*, 1929, **207**, 232.
7. *Ibid.*, 1927, **191**, 472.
8. *Ibid.*, 1928, **199**, 230.
9. *Ibid.*, 1933, **260**, 241.
10. SIMON, E. *Biochem. Z.*, 1932, **245**, 488.
11. SLADE, H. D., and WERKMAN, C. H. *Arch. Biochem.*, 1943, **2**, 97.
12. STILL, J. *Biochem. J.*, 1940, **34**, 1374.
13. STONE, R. W., and WERKMAN, C. H. *Biochem. J.*, 1937, **31**, 1516.
14. TYCHOWSKI, A., and KOBEL, M. *Biochem. Z.*, 1929, **209**, 134.
15. UTTER, M. F., and WERKMAN, C. H. *J. Bact.*, 1941, **42**, 665.
16. VIRTANEN, A. I. *Z. physiol. Chem.*, 1924, **138**, 136.
17. *Ibid.*, 1925, **143**, 71.
18. VIRTANEN, A. I., KARSTRÖM, H., and BÄCK, R. *Z. physiol. Chem.*, 1926, **151**, 232.

The Experimental Feeding of Parathion to Dairy Cows[1]

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The increasing use of parathion (O,O-diethyl, O,*p*-nitrophenyl thiophosphate) as an insecticide on forage crops has resulted in speculation as to its excretion in the milk of dairy cows fed residual amounts of the chemical. Consequently an experiment was designed to determine the presence or absence of parathion in the milk of dairy cows fed parathion in capsules.

Ten dairy cows in heavy lactation, and representing the Ayrshire, Jersey, Guernsey, and Holstein breeds, were divided into two groups and fed commercially available parathion in the form of a 25% wettable powder (analysis: 23.75% parathion) continuously for 81 days. Cows in one group were fed parathion in capsules at the level of 5 ppm of an estimated roughage dry matter intake of 2.25 lbs/100 lbs of body weight daily. Cows in the other group were fed parathion in capsules at a level of 1 ppm of the estimated roughage dry matter intake. The two feeding levels were equivalent to 0.11 mg of parathion per kg of body weight for the 5-ppm group and 0.02 mg of parathion per kg of body weight for the 1-ppm group. These feeding levels represent an intake of parathion greater than that which would be ingested as residues (less than 1 ppm) on forage crops treated with amounts of parathion necessary for good insect control (*3*, *5*). In order to study the effect of feeding parathion to cows in late lactation, an Ayrshire cow in late lactation was added to each of the above groups. These cows were fed parathion for only 2 weeks, at which time they were turned dry.

At the end of 81 days, all but 2 cows in the 5-ppm group were dropped from the experiment. At this time, the 2 remaining cows were administered parathion in amounts that were doubled each successive week until a parathion intake equivalent to 40 ppm of the roughage dry matter had been fed. At this final level they were receiving 0.88 mg of parathion per kg of body weight daily.

Samples of carefully mixed milk were taken on alternate days for 6 days prior to the beginning of the experiment and then on alternate days for 6 days subsequent to the beginning of parathion feeding, after which samples were taken at semiweekly intervals for 3 weeks. Thereafter, samples were taken once a week for the duration of the experiment.

Application of the sensitive colorimetric method of Averell and Norris (*1*) for the estimation of small amounts of parathion was tried on 100-g samples of milk, to which known amounts of parathion were added, and extraction attempts were made using the methods developed by Schechter *et al.* (*4*) and Carter (*2*). The presence of interfering substances and a very low recovery of the parathion added to the milk did not permit the use of either of the extraction methods. The difficulty seemed to lie with the development of a selective extraction procedure that could be used to separate the parathion from the milk. Upon the suggestion of the American Cyanamid Company, a procedure involving the use of a liquid-liquid extraction apparatus was tried; essentially this method involved a prolonged percolation of petroleum ether through a column of milk and ethyl alcohol. The mixture in the extraction chamber was stirred at ½-hr intervals with a wire stirrer inserted through the reflux condenser. Standard curves were prepared from data obtained from analyses of milk to which were added known amounts of parathion. Amounts ranging from 20 to 120 μg were added to 100-g samples of milk. The liquid-liquid extraction procedure was carried out for an optimum period of 6 hr, followed by the concentration of the petroleum ether and analysis by the Averell and Norris method. The percentage transmittance values were obtained using a wavelength setting of 555 mμ with a Coleman Model 14 spectrophotometer. It was found that milk blanks varied from 95% to above 100% transmittance when compared with the reagent blanks; therefore, in the actual analyses, no reading above 90% was considered significant. Technical petroleum ether (Skelly-solve "B") was used in all the analyses, since it was found that the use of purified petroleum ether did not improve the results. The use of 95% ethyl alcohol and distilled water to dissolve the residue remaining after the evaporation of the petroleum ether extract prevented the for-

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