

oxidase and less malic dehydrogenase. Differences in acid phosphatase activity in the two sample types (Table I) were not great enough to be judged significant.

If one considers that *change* in peroxidase activity may be a general indicator of differentiation in plant root material regardless of whether the direction is toward or away from further differentiation, then our results mesh with those of Avers and Grimm(5) who found that peroxidase activity of roots increased as differentiation into root hairs began. Reduced malic dehydrogenase activity in our cultured material is in keeping with the idea that the cultured material may be likened to tumors and that aerobic metabolism is reduced in both plant and animal tumor tissue(6,7). Our findings with malic dehydrogenase are the first in which reduction of activity of a Krebs cycle component has been measured directly. Whether activity of other enzymes in the cycle is similarly reduced remains to be shown.

Summary. Activity of 7 enzymes in carrot taproot secondary phloem and tissue in culture derived from secondary phloem was measured. Peroxidase, acid phosphatase, and

malic dehydrogenase were present in both preparations but the cultured material contained more peroxidase and less malic dehydrogenase. Possible interpretations are discussed. Alkaline phosphatase, glyceraldehyde-3-phosphate dehydrogenase, glucose-6-phosphate dehydrogenase, and aldolase were not found in either the taproots or cultured material.

1. Steward, F. C., Mapes, M. O., Mears, K., *Am. J. Botany*, 1958, v45, 705.
2. Kato, H., Takeuchi, M., *Plant Cell Physiol.*, 1963, v4, 243.
3. White, P. R., *The Cultivation of Animal and Plant Cells*, 2nd ed., The Ronald Press, New York, 1963.
4. Anonymous, *Worthington Enzymes*, Worthington Biochemical Corp., Freehold, N. J., 1963.
5. Avers, C. J., Grimm, R. B., *Am. J. Botany*, 1959, v46, 190; Avers, C. J., Grimm, R. B., *J. Exp. Botany*, 1959, v10, 341.
6. Warburg, O. H., *Weiterentwicklung der zell-physiologischen Methoden*, Interscience, New York, 1962.
7. Steward, F. C., Mapes, M. O., Kent, A. E., Holsten, R. D., *Science*, 1964, v143, 20.

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Paper Electrophoresis of Serum in Rats Fed Various Carbohydrate Diets.*† (30820)

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The levels of free plasma amino acids, following protein meals, have been studied in swine(1) and rats(2) and found to correspond approximately to the amino acid composition of the ingested protein. The concentration of serum albumin is also related to the quality and quantity of dietary protein and has been found to decrease markedly in cases of protein malnutrition(3). Altera-

tions in the electrophoretic pattern of specific serum protein fractions in the rat, due to type of protein in the diet, have been reported (4).

In addition to protein composition, Guggenheim *et al*(5) have shown that the nature of the carbohydrate fed exerts an influence upon the levels of free plasma amino acids. Previous studies(6) have demonstrated that the type of carbohydrate also influences protein utilization. Most methods which have been used to evaluate protein utilization have been based on standard procedures of nitrogen balance, growth, protein digestibility, and

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TABLE I. Effect of Dietary Treatment on the Serum Composition of Rats.*

Diet	Protein fraction (g %)					
	Total protein	Albumin	Alpha-1	Alpha-2	Beta	Gamma
Series I (no lysine)						
Potato starch (raw)	5.65 ± .25	2.87 ± .30	.76 ± .06	.48 ± .21	1.11 ± .26	.50 ± .07
" " (cooked)	5.42 ± .30	2.96 ± .19	.63 ± .02	.53 ± .98	.98 ± .02	.30 ± .03
Cornstarch	6.68 ± .29	3.99 ± .24	.88 ± .03	.51 ± .01	.87 ± .06	.40 ± .05
Sucrose	5.66 ± 1.15	3.16 ± .15	.77 ± .07	.49 ± .01	.90 ± .09	.31 ± .09
Glucose	6.47 ± .10	4.56 ± .07	.52 ± .03	.37 ± .04	.69 ± .03	.30 ± .02
Dextrin	6.79 ± .10	3.88 ± .15	.80 ± .04	.52 ± .05	1.22 ± .09	.30 ± .07
Series II (0.7% L-lysine added)						
Potato starch (raw)	7.39 ± .14	4.65 ± .11	.84 ± .08	.40 ± .04	1.10 ± .08	.38 ± .03
" " (cooked)	7.49 ± .20	4.48 ± .17	.99 ± .15	.57 ± .04	1.09 ± .06	.35 ± .03
Cornstarch	8.05 ± .16	4.92 ± .38	1.33 ± .12	.53 ± .03	.87 ± .16	.37 ± .05
Sucrose	8.37 ± .14	4.94 ± .36	1.39 ± .17	.56 ± .03	1.19 ± .07	.27 ± .06
Glucose	8.35 ± .30	5.28 ± .09	.91 ± .19	.51 ± .04	.78 ± .11	.37 ± .04
Dextrin	7.88 ± .31	4.84 ± .11	1.03 ± .13	.58 ± .05	1.38 ± .08	.49 ± .05

* Average values of 8 rats per group and standard error of mean.

availability of amino acids. The present study was initiated in order to determine whether the influence of various carbohydrates on utilization of dietary protein as previously determined(6) was accompanied by an alteration in serum protein patterns.

Methods. Ninety-six weanling male rats of the Sprague-Dawley strain, weighing 70-80 g, were divided into 2 series of 6 groups with 8 rats in each group. The rats were fed the experimental diets *ad libitum* for a period of 12 weeks.

The diets were the same as those previously described(6) and were divided into 2 series of 6 groups each. Series I contained the following respective carbohydrates: raw-potato starch, cooked-potato starch, sucrose, cornstarch, dextrin, and glucose. Series II contained the same sources of carbohydrates as in Series I except that 0.7% of L-lysine HCl was added to each group. The diets were composed of 24% wheat gluten, 8% hydrogenated vegetable oil, 2% cod liver oil, 4% salt mixture (USP 14), vitamins in sucrose 1%, and carbohydrates 61%. The addition of L-lysine to the diet in Series II was compensated for by subtracting the same percentage of carbohydrate. Vitamins included were as described previously(6).

At the end of the 12-week period, the rats were anesthetized by injection of sodium amytal and blood samples were collected by heart puncture. After standing for a few hours, the blood samples were centrifuged and

the resulting serum drawn off for subsequent analysis. Serum protein fractions were separated by paper electrophoresis in a Durrum cell in sodium veronal buffer (pH = 8.6, $\mu = 0.75$) for 16 hours and dyed with bromphenol blue.

The paper strips were scanned on a Spinco integrating analytrol (Beckman Instrument Co.) and the percentage distribution of each protein fraction calculated. Total serum protein was determined by the micro-Kjeldahl method(7) and the gram per cent of each protein fraction determined by multiplying total serum protein by the relative percentage of the individual fraction.

Results and discussion. Five serum protein fractions designated as albumin and alpha-1, alpha-2, beta, and gamma globulin were separated by paper electrophoresis. The total protein and the specific protein fractions, in grams per 100 ml of serum, are shown in Table I. Without L-lysine supplementation, rats fed either raw or cooked potato starch exhibited significantly lower ($P < 0.05$) total serum protein levels than those which had received cornstarch, glucose, or dextrin. No differences in serum protein levels were noted in rats fed cornstarch, sucrose, glucose, or dextrin. With the addition of 0.7% L-lysine to the diets, total serum protein in rats fed raw potato starch was lower ($P < 0.05$) than that of rats fed cornstarch, glucose, or sucrose. Lysine supplementation increased the total serum protein in the rats regardless of the

type of carbohydrate in the diet.

Of the 5 serum protein fractions, the type of dietary carbohydrate affected the albumin level most prominently. Without L-lysine supplementation, serum albumin was decreased ($P < 0.05$) by the feeding of both raw and cooked potato starch or sucrose whereas glucose caused an increase ($P < 0.05$) in the serum albumin level. Lysine supplementation increased the level of serum albumin in all of the 6 groups and appeared to negate any effect of dietary carbohydrate.

Significant differences were also noted with respect to the levels of the globular proteins in the serum from rats fed the various carbohydrates. In Series I, the levels of alpha-1, alpha-2, and beta globulin in the serum of the glucose fed rats were lowered ($P < 0.05$) in comparison with rats receiving other carbohydrate sources while in Series II, supplemented with dietary L-lysine, an increase in the alpha-1 protein fraction was noted in the case of the rats receiving cornstarch, sucrose, and glucose. Alpha-1 protein was increased by addition of dietary L-lysine ($P < 0.05$) in all cases except in those animals which consumed either form of potato starch or dextrin. No differences due to dietary treatment were noted in levels of serum gamma globulin.

In previous studies(6), it was noted that the nature of the carbohydrate in the diet affected protein utilization by the rat and that, in this respect, protein availability was markedly affected by either raw or cooked potato starch as compared to cornstarch, sucrose, glucose, and dextrin. The lowering of total protein and albumin levels in the serum of rats fed raw or cooked potato starch in the present study appears also to be correlated with lowered protein utilization by the rats in these groups. It has been suggested(8,9) that the levels of free plasma

amino acids may be useful as an index for evaluation of the nutritive adequacy of protein. Longnecker and Hause(10) reported that the free amino acid levels in the plasma of dogs reflected the protein quality of the diet. This study indicates that serum protein and albumin levels may also be additional factors which can be used in conjunction with free plasma amino acid levels for evaluation of protein utilization.

Summary. The effect of various carbohydrates on the serum protein and serum protein fractions in rats have been investigated. Without L-lysine supplementation, rats fed raw or cooked potato starch showed the lowest serum protein and serum albumin concentration; whereas rats fed glucose showed the highest serum albumin concentration. Lysine supplementation increased serum protein and serum albumin concentration in rats regardless of the sources of carbohydrates. The type of carbohydrate in the diet influences the serum protein pattern in rats.

1. Puchal, F., Hays, V. W., Spear, V. C., Jones, J. D., Catron, D. V., *J. Nutrition*, 1962, v76, 11.
2. Wheeler, P., Morgan, A. F., *ibid.*, 1958, v64, 137.
3. Flodin, N. W., *J. Agr. & Feed Chem.*, 1953, v1, 222.
4. Erwin, E. S., *Proc. Soc. Exp. Biol. and Med.*, 1960, v103, 396.
5. Guggenheim, K., Halevy, S., Friedmann, N., *Arch. Biochem. and Biophys.*, 1960, v91, 6.
6. Chang, Yet-Oy, *J. Nutrition*, 1962, v78, 21.
7. Association of Official Agricultural Chemists, *Official Methods of Analysis*, 1960, 9th ed., Washington, D. C.
8. Smith, R. E., Scott, H. M., *J. Nutrition*, 1965, v86, 37.
9. ———, *ibid.*, 1965, v86, 45.
10. Longnecker, J. B., Hause, N. L., *Arch. Biochem. and Biophys.*, 1959, v84, 46.

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