by, *e.g.*, pea extracts (J. TURNER, oral communication). It was shown that the sucrose effect could not be ascribed to an activation of dextrin phosphorylation. That it was based on enzyme action is likely from the fact that it showed an optimum at pH 7 and 35° C. The only way in which a (slight) concentration of activity could be achieved was to keep the press juice in a cold desiccator overnight. Unfortunately, as the autumn season proceeded, the results obtained earlier with untreated juices and concentrates could not be reproduced. Therefore, it is quite possible that the enzyme (if present at all) shows rather strong fluctuations in activity or stability during the course of the year. Experiments to investigate this possibility and to compare various races of potatoes are in progress.

ACKNOWLEDGEMENTS

The authors are indebted to the "Organisatie voor Zuiver Wetenschappelijk Onderzoek" (Z.W.O.) for financial aid, to the Elizabeth Thompson Science Fund for generous help in equipment, to Boots Pure Drug Company for a sample of purified notatin, and to Professor P. E. VERKADE for the preparation of synthetic L-sorbose-1-phosphate.

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Received February 26th, 1952

THE THIAMINE PYROPHOSPHATE CONTENT OF CENTRIFUGALLY-PREPARED FRACTIONS OF RAT LIVERHOMOGENATE. THE INFLUENCE OF A THIAMINE-DEFICIENT DIET*

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By various investigators[1,2,3] it has been fairly well established that the enzymes of the tricarboxylic acid cycle are essentially associated with the mitochondrial units of the cell. It is known that enzymes containing thiamine pyrophosphate are involved in the breakdown of pyruvic- and α-ketoglutaric acids[4], which are both oxidized by means of the tricarboxylic acid cycle.

Furthermore enzymes requiring thiamine pyrophosphate, which catalyze anaerobic reactions, were found to be associated with particles which can be sedimented quantitatively after 30 minutes at 15.000 r.p.m. in the high speed head of the International centrifuge[5].

Therefore it seemed of interest to determine whether thiamine pyrophosphate is bound entirely to the mitochondria. For isolating the mitochondria as well as the other cell components, the procedure of HOGEBOOM, SCHNEIDER, AND PALLADE[6] was followed with the modification that the washing of the microsomes was omitted.

The thiamine pyrophosphate contents of the isolated fractions were determined by the manometric method of WESTENBRINK AND STEYN-PARVÉ. In each experiment 1 g of liver pulp of an adult, male, Wistar rat was fractionated.

Table I shows that the amounts of thiamine pyrophosphate found in the microsomes are insignificant. They may be due to material originating from the soluble fraction because the preparations were not washed.

* This work was supported by a grant from the "Koningin Wilhelmina Fonds" (Queen Wilhelmina Cancer Fund).

TABLE I

THE DISTRIBUTION OF THIAMINE PYROPHOSPHATE IN RAT LIVER FRACTIONS

Rats 1 to 3 normally fed, rats 4-6 after 7 days thiamine poor diet.
Columns I: γ thiamine pyrophosphate derived from 1 g of liver pulp.
Columns II: γ thiamine pyrophosphate per mg N in the fraction concerned.

Rat No.	*Homogenate*		*Nuclear fraction*		*Mitochondria*		*Microsomes*		*Soluble fraction*	
	I	*II*	*I*	*II*	*I*	*II*	*I*	*II*	*I*	*II*
1	11.3	0.38	1.3	0.26	3.5	0.65	0.2	0.02	5.0	0.52
2	12.8	0.46	1.6	0.33	3.7	0.70	0.5	0.06	5.2	0.48
3	12.8	0.41	2.0	0.39	3.8	0.60	0.4	0.04	5.0	0.47
4	4.0	0.11	0.8	0.12	1.4	0.18	0.1	0.01	2.0	0.18
5	3.7	0.10	0.6	0.09	1.0	0.16	0.2	—	2.0	0.16
6	4.2	0.13	0.5	0.08	1.3	0.18	0.2	0.02	1.9	0.22

At least 70% of the cellular thiamine pyrophosphate is recovered from the mitochondrial and the soluble fractions together, the latter containing the greater part. A considerable percentage of the "nuclear" thiamine pyrophosphate must be attributed to intact cells and cytoplasmic contaminations, as could be demonstrated microscopically. Relatively pure preparations of nuclei isolated by the method of DOUNCE[8] contained about 4% of the thiamine pyrophosphate of the whole homogenate. Hence the combined mitochondrial and soluble fractions must contain at least 80% and may even contain more than 90% of the cellular thiamine pyrophosphate if the losses are considered.

Because, contrary to expectation, the largest percentages of thiamine pyrophosphate were found in the soluble fractions, two possibilities of artefact were studied. In the first study no increase of mitochondrial thiamine pyrophosphate was observed when 50 γ thiamine pyrophosphate was added to each ml of homogenate prior to the isolation of the particles. Thus mitochondria do not seem to adsorb thiamine pyrophosphate irreversibly from solution. In the second study no "shearing"[9] of thiamine pyrophosphate from mitochondria was found when a carefully-prepared homogenate was submitted to a rehomogenization by a high-speed mixer, only a proportional breakdown of thiamine pyrophosphate in both the mitochondria and the soluble fractions resulted.

The table shows that, in thiamine deficiency, thiamine pyrophosphate is depleted from both the mitochondria and the soluble fractions. By the method employed no preferential retention of thiamine pyrophosphate could be demonstrated.

This work forms part of investigations on the metabolism and physiological function of thiamine carried out by H. G. K. WESTENBRINK and collaborators. Cytochemical work on enzymes, containing thiamine pyrophosphate, of normal and malignant tissues is in progress.

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Received February 14th, 1952