In a number of cases the activity of pyruvic decarboxylase in breast muscle was also measured, in the same manner as described by MONFOORT[2]. The effect of adding TPP was studied simultaneously. The results are given in Table II. As may be seen, the activity of pyruvic decarboxylase is markedly reduced after feeding pyrithiamine. It can almost completely be restored by adding TPP to the homogenates.

TABLE II

ANAEROBIC FORMATION OF CO_2 IN PIGEON BREAST MUSCLE HOMOGENATES

Each Warburg flask contained 200 mg breast muscle, homogenized in 0.1 *M* phosphate buffer pH 6.2, containing 0.001 *M* magnesiumchloride and made up to a volume of 2.0 ml. 20 μMol sodium pyruvate were added after temp. equilibrium. Temperature: 37° C. Gas phase: nitrogen.

Group	*Number of animals*	*PT*	*Average surv. time*	*μMol CO_2 in two hours*	
				— TPP	*+ 10γ TPP*
A	4	—	19 d.	5.1 ± 0.6	6.1 ± 0.7
B	4	+	19 d.	2.6 ± 0.2	5.5 ± 0.2

These experiments show that the disappearance of TPP from the tissues and the behaviour of the pyruvic decarboxylase after feeding pyrithiamine run a course, closely parallel to that observed in a thiamine deficiency induced by omitting the vitamin from the diet[2,10]. As yet there seems to be no occasion for presuming that the deficiencies brought about in these two manners are indeed different from one another.

This work forms part of investigations on the metabolism and physiological function of thiamine by H. G. K. WESTENBRINK and collaborators.

J. C. KOEDAM
E. P. STEYN-PARVÉ
D. L. VAN RHEENEN

Laboratory for Physiological Chemistry, The University, Utrecht (The Netherlands)

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Intracellular distribution of vitamin K in beef liver

An analysis of the intracellular distribution of vitamin K seemed of interest especially in view of the recent evidence that the vitamin is involved in oxidative phosphorylation (MARTIUS AND NITZ-LITZOW[1]). Data on the distribution of the vitamin in liver are presented in this note.

90 g of beef liver were divided into portions and homogenized in a Waring blendor for 15–30 sec. The blended liver was centrifuged by the method of SCHNEIDER AND HOOGEBOOM[2] except that after separation of the mitochondria the remaining supernatant (containing the submicroscopic particles (*P*) and the soluble (*S*) fraction) was not further separated. Janus green staining indicated few mitochondria in the supernatant. The three fractions—nuclei (*N*), mitochondria (*M*), and supernatant (*P-S*)—were lyophilized. Nitrogen was determined by the method of DUMAS[3]. Attempts to identify the vitamin in the lyophilized nuclear fraction by paper chromatography (GREEN AND DAM[4]) of a petroleum ether-ethanol extract and by its reaction (GREEN AND DAM[5]) with aluminium oxide were not successful. The fractions were therefore examined

biologically for their vitamin K activity on vitamin K-deficient chicks (DAM, KRUSE AND SØNDERGAARD[6]).

The beef liver mitochondria and supernatant (Table I) contained nearly the same amount of nitrogen as corresponding rabbit fractions (LE PAGE AND SCHNEIDER[7]), but the nitrogen content of the nuclear fraction was about twice that of rabbit. This may represent either a species difference or the presence of a large number of unbroken cells in our nuclear fraction due to the short period of homogenizing (POTTER, RECKNAGEL AND HURLBERT[8]). It is thus possible that the total analysis of vitamin K found in the nuclear fraction is too high.

TABLE I

AMOUNTS OF NITROGEN AND VITAMIN K ACTIVITY EXPRESSED AS MENADIONE IN FRACTIONS OF 100 g FRESH BEEF LIVER

	N per 100 g of fresh liver	*N % of total N*	*γ menadione in fraction of 100 g liver*	*menadione in % of total menadione*	*γ menadione per g N of fraction*	*μM vitamin K per g N of fraction*
"N"	1.21	43	32	45	27	0.15
"M"	0.28	10	18	25	64	0.38
"P-S"	1.33	47	21	30	16	0.09
Total	2.82	100	71	100		

The vitamin K values shown in Table I are averages of two determinations. The vitamin K activity of 100 g fresh beef liver was equivalent to that of 71 γ menadione. On a nitrogen basis 24 % of vitamin K was associated with the nuclear (*N*) fraction, 61 % with the mitochondria (*M*), and 15 % with the supernatant (*P-S*) fraction. The low concentration of vitamin K in the *P-S* fraction is in all likelihood a maximum value, as the Waring blendor is known to disintegrate nuclei and mitochondria and disperse their fragments and contents into the supernatant (POTTER, RECKNAGEL AND HURLBERT[8], SCHNEIDER AND HOOGEBOOM[9]).

Thus, in liver vitamin K appears to be associated primarily with mitochondria. Its association with both mitochondria and chloroplasts (DAM, GLAVIND AND NIELSEN[10]) (analogues of mitochondria) seems to be more than coincidental.

It is of interest to compare the distribution of vitamin K with that of some respiratory catalysts. Thus, in rat liver homogenate riboflavin (PRICE, MILLER AND MILLER[11]) is concentrated in the mitochondria and in the supernatant fractions, and cytochrome *c* is concentrated in the mitochondrial fraction (SCHNEIDER AND HOOGEBOOM[2]). On a nitrogen basis the molar concentration of vitamin K found in our experiments in beef liver mitochondria is about one-third that of cytochrome *c* and one-fifth that of riboflavin (PRICE, MILLER AND MILLER[11]) found in rat liver mitochondria by other investigators.

J. P. GREEN*
E. SØNDERGAARD
H. DAM

Department of Biochemistry and Nutrition, Polytechnic Institute, Copenhagen (Denmark)

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* Fellow of the Life Insurance Medical Research Fund. Present address, Yale University, School of Medicine, New Haven, Conn., U.S.A.