was observed, as judged by the distribution of both protein concentration and specific enzymic activity (Fig. 2). The electrophoretic mobilities of ribonuclease I and II as a function of pH were investigated in borate buffers of 0.1 ionic strength, and the isoelectric points were found to be located at pH 7.8 and 7.1 respectively. The value for ribonuclease I is identical with that given by ROTHEN[8] for the crystalline ribonuclease. A detailed account of the present investigation as well as results of the application of zone electrophoresis to other biologically active proteins will be reported at a later date.

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RESTORATION OF ACETOIN AND SUCCINIC SEMIALDEHYDE FORMATION IN PIGEON MUSCLE HOMOGENATES IMPAIRED BY THIAMINE DEFICIENCY

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As reported in previous notes in this journal[1] the formation of acetoin and succinic semialdehyde (SSA) from pyruvate and α-ketoglutarate added to homogenates of various muscles of pigeons, which had been on a diet without thiamine and rich in carbohydrate[2] for 12 days, is considerably decreased as compared to the formation of these compounds in the corresponding muscle homogenates from normal pigeons. Even after 4 days of deficiency a decrease could be already observed. In view of work since then carried out in this laboratory by FRANKEN AND STAPERT[3], it now appears desirable to complete these notes by reporting the results obtained upon adding thiamine pyrophosphate (TPP) to the homogenates.

The effect of adding TPP was studied in simultaneous experiments, employing the same muscle preparations as used in the experiments which have been reported previously in another connexion[1,4].

The results concerning acetoin formation are assembled in Table I, those concerning succinic semialdehyde formation in Table II (for experimental details see MONFOORT[4]). In these tables *B* denotes breast muscle, *H* the muscle of the left ventricle of the heart, *L* a mixture of leg muscles and *n* the number of pigeons examined. The standard deviations mentioned are standard deviations of the means.

The small effect of addition of TPP on the enzymic activity of the homogenates from normal muscles may be explained either by the presence of small amounts of apo-pyruvic decarboxylase and apo-α-ketoglutaric decarboxylase in the muscles *in situ* or by the dissociation of part of the holoenzymes during the preparation of the homogenates.

The point to be stressed in particular is that the production of acetoin from added pyruvate, as well as that of SSA from added α-ketoglutarate, in the homogenates of the deficient muscles, is raised to the same level as reached upon addition of TPP to the normal homogenates. Hence the

TABLE I

INFLUENCE OF TPP ON ACETOIN FORMATION IN HOMOGENATES OF PIGEON MUSCLES

	6 γ TPP	Normal		4 d def.		12 d def.	
		n	*μM acetoin*	*n*	*μM acetoin*	*n*	*μM acetoin*
B	—	8	4.3 ± 0.2	8	3.5 ± 0.3	15	1.9 ± 0.2
	+	8	5.2 ± 0.2	8	4.8 ± 0.4	10	5.0 ± 0.4
H	—	8	2.3 ± 0.1	8	1.8 ± 0.2	10	0.3 ± 0.02
	+	8	3.6 ± 0.1	8	3.8 ± 0.1	10	3.8 ± 0.1
L	—	13	0.6 ± 0.04	13	0.4 ± 0.06	11	0.1 ± 0.02
	+	8	1.0 ± 0.05	10	0.9 ± 0.03	6	0.8 ± 0.1

TABLE II

INFLUENCE OF TPP ON SUCCINIC SEMIALDEHYDE (SSA) FORMATION IN HOMOGENATES OF PIGEON MUSCLES

	6 γ TPP	Normal		4 d def.		12 d def.	
		n	*μM SSA*	*n*	*μM SSA*	*n*	*μM SSA*
B	—	7	7.3 ± 0.5	9	4.6 ± 0.4	9	4.8 ± 0.4
	+	7	9.0 ± 0.4	9	9.2 ± 0.3	9	8.8 ± 0.4
H	—	7	5.8 ± 0.4	8	6.0 ± 0.5	9	4.5 ± 0.1
	+	7	6.3 ± 0.3	8	7.0 ± 0.3	9	5.8 ± 0.3
L	—	7	1.1 ± 0.2	8	0.8 ± 0.05	9	0.7 ± 0.1
	+	7	2.5 ± 0.1	8	1.9 ± 0.1	9	2.0 ± 0.2

protein component of the enzymes appears to be maintained during a period of avitaminosis approximating the length of time after which the pigeons die on the diet employed (see GRUBER[5]). These protein components are believed to be identical with the protein components of the pyruvic dehydrogenase and the α-ketoglutaric dehydrogenase. It is quite probable that the coenzyme of these dehydrogenases is lipothiamide pyrophosphate[6]. My results are not in disagreement with the view of REED[6] according to which TPP, and not lipothiamide pyrophosphate, would be the coenzyme responsible for the anaerobic decarboxylation of both α-ketoacids.

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

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