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Effect of vitamin K deficiency on the adenosine nucleotide content of chicken liver

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A VITAMIN K deficiency produces a hemorrhagic tendency resulting from a depressed synthesis of clotting factors; however, the details concerning the mechanism have not been elucidated. Martius and Nitz-Litzow reported that mitochondria from the livers of vitamin K-deficient animals were uncoupled[1] and suggested that, as a result, the energy supply would be decreased, which in turn would result in a depression of protein synthesis. However, other investigators found the P-O ratio of mitochondria from vitamin K-deficient animals to be no different from that of normal animals.[2, 3] In the present studies, a different approach was used to obtain information concerning this point. Assuming that a depression of the high-energy phosphate content would serve as an index of uncoupling, the adenine nucleotide content of normal and vitamin K-deficient animals was compared. No difference was found between the adenine nucleotide content of control and vitamin K deficient-animals.

MATERIALS AND METHODS

A vitamin K deficiency was produced by placing one-day-old white leghorn chicks (Duckworth Hatcherys, Hanover, Md.) on a vitamin K-deficient diet (General Biochemicals, Chagrin Falls, Ohio). On the basis of preliminary trials, the diet appeared to be deficient in other vitamins as well. The diet was therefore, supplemented with vitamins in the amount and quantities reported by Griminger.[4] The diet of the control animals was supplemented with vitamin K_1 in the form of stabilized beadlets (Hoffman-LaRoche, Inc., Nutley, N.J.), with the equivalent of 2·7 mg vitamin K_1/kg food. The diets and tap water were given *ad libitum* throughout the experimental period. All chicks were reared in electrically heated thermostatically controlled brooders with a screen bottom.

At four weeks of age, blood was collected from the carotid artery of ether-anesthetized chicks and prevented from clotting by the use of sodium citrate. Plasma prothrombin times were determined by the one-stage procedure, with acetone-dehydrated chick brains as a source of thromboplastin.

Immediately after the blood samples were obtained, liver samples of approximately 100 mg were removed rapidly and frozen in liquid nitrogen in order to minimize any changes in nucleotide content that might occur during the period of handling. The tissues were then pulverized in a − 17° room and extracted with $HClO_4$ as described elsewhere.[5]

ATP, ADP, and AMP were assayed by the enzymatic procedure of Kalckar.[6] Adenylic acid deaminase (preparation A) and adenyl pyrophosphatase were prepared as described by Kalckar.[7]

Myokinase was obtained from Boehringer and Soehne. The nucleotide content is expressed as micromoles per gram fresh weight. Glycogen was determined by the procedure of Seifter *et al.*[8] Inorganic phosphate was determined by the method Fiske and Subbarow.[9]

RESULTS AND DISCUSSION

After four weeks on the diet, the chickens weighed about 210 g and all appeared healthy. However, those on the vitamin K-deficient diet had a prolonged prothrombin time, while those on the diet

TABLE 1. THE ADENINE NUCLEOTIDE CONTENT OF VITAMIN K-DEFICIENT CHICKENS

	Prothrombin time (sec)	AMP (μmoles/g)	ADP (μmoles/g)	ATP (μmoles/g)	Total (μmoles/g)	P_I (μmoles/g)
Controls (15)	14·7 ± 0·04	2·39 ± 0·22	2·51 ± 0·19	0·30 ± 0·10	5·21 ± 0·31	5·96 ± 0·19
Expt'l. (15)	27·8 ± 0·27*	2·60 ± 0·21	2·30 = 0·24	0·43 ± 0·15	5·31 ± 0·34	5·50 ± 0·23
P	>0·001	0·5 — 0·6	0·5	0·4 — 0·5	0·8 — 0·9	0·05 — 0·1

Both groups of chicks were started on the same diet which was supplemented with vitamin K_1 for the controls. After four weeks on the diet the blood and livers were analyzed. The values given are means ± standard error.

* Three animals with prothrombic times >10 min are not included in this value.

supplemented with vitamin K_1 had a normal prothrombin time (Table 1). In both cases, the livers were about 2·9% of the body weight. The glycogen content averaged 13·3 ± 3·2 and 13·3 ± 4·9 mg/g wet weight for the controls and experimentals respectively.

A summary of the adenine nucleotide content of the livers is presented in Table 1. There was no difference between control and experimental livers in any of the nucleotides, individually or in total amount. Similarly, the inorganic phosphate contents were about the same. The creatine phosphate content was too low to be measured accurately and therefore was not included in this analysis. The amount of ATP, compared with ADP and AMP, was relatively low in contrast to the proportion found in the livers of mature rats, which contained about 2·0 μmoles ATP/g.[10] Since the methods and assay procedures used were the same in each case, it would appear that the variations in values obtained are reflections of the differences between the species or ages of the animals rather than differences in method of isolation and assay. The similarity in glycogen and inorganic phosphate content of control and experimental animals does not indicate a shift to glycolysis. The possibility that the ATP levels were maintained by accelerated rates of metabolism or greater utilization of other substrates can not be excluded. However, if such an increase or shift had taken place in order to maintain the ATP levels, it would not seem likely that the synthesis of the clotting factors would be affected. Since no difference was found between the ATP levels of control and experimental animals, there is no evidence from these studies to support the view that a vitamin K deficiency produces changes in oxidative phosphorylation which would account for a hemorrhagic condition.

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Evidence for pilocarpine transformation by serum*

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PILOCARPINE is being extensively used for inducing the copious flow of prostatic secretion,[1, 2] although the mechanism of stimulation has barely been examined.[3, 4] Since the pharmacologic stimulation of

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