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Intracellular distribution of vitamin E and vitamin A in chicken liver

Since tocopherol has been found to be an activator or cofactor in the reduction of cytochrome *c* by DPNH[1] and may also be implicated in oxidative phosphorylation, it seemed of interest to examine the intracellular distribution of this vitamin in liver, and, in particular, its concentration in the mitochondria. Investigations of the vitamin K content have already been made in this laboratory[2], and other workers[3] have investigated the distribution of tocopherol in heart muscle preparations. In the present study vitamin A determinations were also carried out.

22 newly hatched chicks were reared on a "normal" ration[4] for 6 days and then divided into two groups. Group 1692, consisting of 12 chicks, received for 39 days a 20% casein, "fat-free" diet (No. 3 in Table I of ref.[5]). Group 1694, consisting of 10 chicks, received a corresponding 15% casein, "fat-free" diet for 42 days. The diets were supplemented with 100 mg *d,l*-α-tocopherol acetate ("Ephynal", Roche) per kg.

TABLE I

AMOUNT OF NITROGEN, α-TOCOPHEROL AND VITAMIN A IN FRACTIONS OF 100 g FRESH CHICKEN LIVER

Group No.	*1692**				*1694***			
% casein in the diet	*20*				*15*			
Liver fraction	*"N"*	*"M"*	*"P + S"*	*Total*	*"N"*	*"M"*	*"P + S"*	*Total*
g N/100 g fresh liver	0.85	0.37	1.15	2.37	0.84	0.23	1.20	2.27
N content as % of total N	36	16	48	100	37	10	53	100
μg α-tocopherol/100 g liver	415	343	792	1550	446	283	1140	1869
α-tocopherol as % of total tocopherol	27	22	51	100	24	15	61	100
μg α-tocopherol/g N	490	930	690		530	1230	950	
μmoles α-tocopherol/g N	1.14	2.16	1.60		1.23	2.86	2.20	
μg vitamin A/100 g liver	480	167	1650	2297				
vitamin A as % of total vitamin A	21	7	72	100				
μg vitamin A/g N	564	450	1440					
μmoles vitamin A/g N	1.97	1.57	5.03					

* 107.2 g liver from 12 chicks were used for the preparation of the fractions.
** 74.5 g liver from 10 chicks were used for the preparation of the fractions.

The animals were killed by decapitation. The livers were removed, weighed immediately and homogenized in 9 vol. 0.25*M* sucrose using a Teflon homogenizer. The fractionation was carried out as described by SCHNEIDER AND HOGEBOOM[6], except that after separation of the mitochondria the remaining supernatant (containing the submicroscopic particles (P) and the soluble fraction (S)) was not fractionated. The three fractions—nuclei (N), mitochondria (M), and supernatant (P + S)—were freeze-dried in a Stokes "Freeze-dryer apparatus type 103-LPM".

The tocopherol content of the fractions was determined in the following way: Weighed amounts of the fractions were saponified by heating under reflux—in an atmosphere of nitrogen

and using pyrogallol as antioxidant with KOH dissolved in aqueous methanol. The unsaponifiable matter was extracted with ether, and after evaporation of the ether, the residue was dissolved in benzene, and an aliquot of the solution passed through a column of activated Filtrol-earth[7]. The benzene was evaporated and the residue dissolved in absolute ethanol and an aliquot chromatographed on paper by a modification of the method of GREEN, MARCINKIEWICS AND WATT[8] using benzene as developing solvent[9]. All the chromatograms showed only one spot, having an R_F value and other properties identical with those of α-tocopherol. In a few cases aliquots of the benzene solutions of the unsaponifiable matter (before the Filtrol-treatment) were evaporated and the residues dissolved in dioxan and refluxed with $SnCl_2$ dissolved in conc. HCl in order to reduce any oxidized tocopherol. After this treatment no significant increase in tocopherol content was detected.

Vitamin A was determined on aliquots of the benzene solutions (before Filtrol-treatment) using the method of HJARDE[10].

Nitrogen was determined on the freeze-dried fractions by the method of Dumas[11].

The results are presented in Table I, which shows the intracellular distribution of vitamin E, in the livers of chicks reared on both levels of casein, *viz.* 20% and 15%. On a nitrogen basis 23% and 20% respectively, of the vitamin E was associated with the nuclear (N) fraction, 44% and 45% with the mitochondria (M), and 33% and 35% with the supernatant (P + S) fraction. Thus, a considerable part of the tocopherol present is associated with the mitochondria, although the other fractions also contain some. The levels of casein in the diet did not seem to influence the intracellular distribution or relative concentrations of vitamin E.

In heart mitochondrial fragments, BOUMAN AND SLATER[3] have found 1.1 μmoles tocopherol /g protein or 6.9 μmoles/g N (assuming 6.25 g protein/g N), a value about three times the amount found by us in chicken liver mitochondria.

The largest amount of vitamin A was found in the supernatant (P + S) fraction. On a nitrogen basis, 23% was in the nuclear fraction (N), 18% in the mitochondria (M), and 59% in the supernatant fraction (P + S). These results for vitamin A are in accordance with those of POWELL AND KRAUSE[12], who showed that vitamin A in the livers from normal rats was mainly associated with fraction "P + S" (*viz.* 64.5%). A similar experiment has been reported by COLLINS[13], who demonstrated that the soluble fraction contained 76% of the vitamin A present in rat liver.

It is of interest to compare the distribution of vitamins A and E in chicken liver with that of another fat-soluble vitamin. GREEN, SØNDERGAARD AND DAM[2] have found a relatively high concentration of vitamin K in the mitochondrial fraction from beef liver (0.38 μmole vitamin K /g N). This observation has been confirmed in chicken experiments by MARTIUS[14].

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