of freshly prepared material was 105–106.5°C with decomposition. (Found: C 11.30; H 3.99; N 26.03. Calc. for $CH_4N_2S_2$: C 11.10; H 3.73; N 25.90).

Diselenocarbazic acid (II). In the preparation of the selenium-containing compounds it proved important to use oxygen and peroxide-free solvents and to flush all apparatus with nitrogen before and during use. The moist compounds were extremely susceptible to oxidation as readily observed by the red colour of the selenium formed by the oxidation.

A solution of carbon diselenide (1.7 g) in dry ether (75 ml) was added, over a period of 2 h, to a stirred solution of anhydrous hydrazine (0.64 g) in dry ether (250 ml) cooled in an ice-bath. The sticky, red-black hydrazinium diselenocarbazate was precipitated at the walls of the reaction flask during the reaction and was finally separated from the supernatant by decantation. By addition of a small amount of absolute ethanol and scratching with a spatula the compound could be brought to crystallization. It was then filtered off, washed with ether, and rapidly dried in vacuum.

On attempting dissolution of the salt in water, varying amounts of an unidentified, insoluble material could be isolated. The filtered aqueous solution, saturated with hydrazinium diselenocarbazate, was cooled in an ice-bath. The precipitated salt disappeared when the calculated amount of 1 N hydrochloric acid was added over a period of 20 min to the stirred suspension. After some time the yellowish diselenocarbazic acid began to precipitate, and when the addition of hydrochloric acid was completed the acid was filtered off, washed with *small* amounts of cold water, and dried immediately *in vacuo*. The dry compound had a characteristic yellow-green colour. It decomposes on heating, but in a closed tube the m.p. could be determined to *ca.* 76°C with decomposition. When stored at −40°C it is more stable than dithiocarbazic acid. (Found: C 5.77; H 1.97; N 13.57; Se 78.00. Calc. for $CH_4N_2Se_2$: C 5.95; H 2.00; N 13.87; Se 78.19).

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In vivo Conversion of Vitamin A_1 to Vitamin A_2

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It is well known that vitamin A_2 (3,4-dehydroretinol) predominates over vitamin A_1 in fresh water fish intestines and liver.[1] β-Carotene is well established as provitamin A_1 and Morton and Creed [2] found as early as 1939 that intake of β-carotene in perch and dace resulted in increased amounts of both vitamins A_1 and A_2 in the intestines and livers. This indicated a common provitamin for both forms of vitamin A. Besides β-carotene, the keto-carotenoids have been suggested as provitamins A, and particularly the widespread astaxanthine, 3,3′-dihydroxy-4-4′-diketo-β-carotene.[3–6] A particular provitamin A_2 has been implied, but has not been found in natural material.[6] Grangaud *et al.*[7] and more recently, Gross and Budowsky [6] found, however, evidence of a possible conversion of keto-carotenoids to β-carotene in fishes. Morcos and Salah [8] fed vitamin A_1 to Nile fish (*Clarias lazera* and *Tilapia nilotica*), and they concluded that vitamin A_1 cannot be converted by these species into vitamin A_2 to any appreciable extent, if at all. Regarding the occurrence of vitamin A_2, the present authors showed in a survey of vitamin A in fishes [9] that vitamin A_2 is present in comparable quantities in all fishes, regardless of marine or fresh-water environment, while higher concentrations of vitamin A_1 mask the presence of vitamin A_2 in most marine fishes.

We were able to confirm the conversion of vitamin A_1 to vitamin A_2 in a recent study on growth and uptake of nutrients in young rainbow trout. Investigating

Table 1. Vitamin A values in rainbow trout kept on feed containing 2000 I.U./g (microgram per fish).

I: First experiment, mean water temperature 12°C.
II: Second experiment, mean water temperature 7°C.

Weeks:		1/2	1	2	4	6
Liver I	A_1	8.42	16.6	19.6	93.1	242
	A_2	*7.22*	*10.0*	*14.3*	*38.6*	*93.5*
Liver II	A_1	2.87	8.71	23.9	56.6	70.1
	A_2	*6.10*	*6.28*	*14.1*	*19.4*	*22.3*
Pyloric c. I	A_1	37.2	94.7	100	392	965
	A_2	*9.56*	*14.7*	*24.4*	*42.0*	*79.8*
Pyloric c. II	A_1	21.0	40.7	87.3	232	287
	A_2	*18.6*	*13.1*	*18.6*	*28.8*	*33.6*

the liver storage of vitamin A_1, groups of young rainbow trout were fed different levels of synthetic vitamin A_1-palmitate mixed in a commercial fish feed originally containing less than 50 I.U. synthetic vitamin A_1 per gram and no carotenoids. Fishes were removed for analysis after ½, 1, 2, 4 and 6 weeks on these diets, and the pyloric caeca and livers were analysed for vitamin A_1 and A_2. Chromatography of the unsaponifiable matter of the samples on alumina sufficed to give vitamin A-fractions which were spectrophotometrically pure. The spectra were calculated to give vitamin A_1 and vitamin A_2 as microgram per fish in the two tissues.[9] Synthetic vitamin A_1-palmitate and pure vitamin A_2 isolated from perch were used to calculate conversion factors.[9,10]

Table 1 gives A_1 and A_2 values for two groups given 2000 I.U. vitamin A_1 per gram of feed. The second of the two experiments was done on trout in a 5°C lower water temperature than the first one, resulting in a lowered uptake of vitamin A.

The vitamin A_1-values show substantial increases, particularly in the pyloric caeca. There is a further smaller, but steady increase in the vitamin A_2 values. Vitamin A_1 was therefore converted into vitamin A_2 in the pyloric caeca of this fish, and probably also in the liver. The rate of conversion was smaller than the rate of vitamin A_1 uptake in these experiments resulting in a decrease of the vitamin A_2 percentage in both organs. The high rate of vitamin A_1 uptake tends to mask analytically the lower but significant conversion of vitamin A_1 to A_2. Analytical methods comprising proper identification of both forms are necessary.

Detailed results from the study on vitamin A uptake and storage in rainbow trout will be published elsewhere.

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