The hexane extract was evaporated to dryness under N_2 gas.

The residue was applied on a column of silica-gel, eluted with hexane to remove lipid fraction, and then with hexane-benzene (1:1) to recover the violet quinone band. The quinone fraction was then purified by preparative thin-layer chromatography using Silica-gel G plates. The development with chloroform formed 2 purple bands. The upper band, though it has not yet been obtained in a pure form due to its instability, shows nearly the same UV-absorption as the lower and is assumed to be a precursor of the latter from the fact that the former easily forms the latter by repetition of thin-layer chromatography or other treatment. The lower band was extracted with ether and the residue was further purified by the repetition of preparative thin-layer chromatography using a mixture of hexane-chloroform as the solvent, and finally recrystallization from cold methanol to deep violet crystals of m.p. 66.5–67° (5 mg from 1600 g of the muscle), M^+ 779.618 *m/e* (Calcd. for $C_{53}H_{81}O_3$ N, 779.622), λ_{max}^{EtOH} 285, 515 nm, ν_{KBr} 3470, 3330, 2850–3050, 1643, 1600 cm^{-1}.

Synthetic rhodoquinone, m.p. 38–42.5°, was prepared from ubiquinone-9 by ammonolysis[2,3] followed by the separation by preparative thin-layer chromatography. The natural quinone and the synthetic sample show entirely the same UV- and IR-absorptions, identical fragmentations in mass spectra, and identical Rf values in thin-layer and reversed phase thin-layer chromatography[5].

Discussion. Ubiquinone is the most widely distributed benzoquinone in nature. Its distribution is closely correlated with the aerobic metabolism of a tissue or organism, a pattern which is consistent with the evidence that ubiquinone is a coenzyme in the electron transport system in mitochondria[6]. Although the time course study with labelled *p*-hydroxybenzoate has shown that rhodoquinone is a product of ubiquinone metabolism[7], the occurrence of rhodoquinone has been confined to only few microorganisms. Precise physiological role of rhodoquinone has not yet been determined. While in the microorganisms, *R. rubrum* and *E. gracilis*, both ubiquinone and rhodoquinone are detectable, in *Ascaris* worm rhodoquinone exclusively. Preliminary studies on the intracellular distribution of rhodoquinone-9 in the *Ascaris* muscle provided the evidence that rhodoquinone in the mitochondrial fraction, in which could be observed many mitochondria poor in cristae by the electron microscope, accounted for about 65% of the total in the muscle homogenate. These facts suggest that rhodoquinone may play some physiological role on the mitochondrial function in the *Ascaris* worm. Precise physiological role of this rhodoquinone-9 in the *Ascaris* worm remains to be evaluated.

Zusammenfassung. Es gelang, aus der Epithelmuskelzelle von *Ascaris lumbricoides* var. *suis* Rhodochinon (Methoxylgruppe in Ubichinon mit einer Aminogruppe substituiert) zu extrahieren und kristallin zu gewinnen. Die Spektralmessungen (UV-, IR-, Massen-Spektren) ergeben, dass es sich beim Rhodochinon um Rhodochinon-9 handelt.

H. OZAWA, M. SATO,
S. NATORI and H. OGAWA

Pharmaceutical Institute,
Tohoku University School of Medicine, Sendai, and
National Institute of Hygienic Sciences,
Tokyo (Japan), 25 November 1968.

[5] H. WAGNER, L. HÖRHAMMER and B. DENGLER, J. Chromat. *7*, 211 (1962).
[6] D. E. GREEN and G. P. BRIERLY, in *Biochemistry of Quinones* (Ed. R. A. MORTON, Academic Press, New York 1965), p. 405.
[7] W. W. PARSON and H. RUDNEY, J. biol. Chem. *240*, 1853 (1965).

Selenium Toxicity: Effect of Fluoride

Results from epidemiological studies among children and from animal experiments indicate that consumption of small amounts of selenium during the period of tooth development increases the suceptibility to dental caries[1]. However, the effect of fluoride, which is a well-known agent for prevention of caries, on selenium metabolism has received only scant attention. MOXON and DUBOIS[2] reported that the combined administration of fluoride and selenium to rats increased the toxic action of selenium. The present study was undertaken to provide further data on the effect of fluoride on selenium toxicity.

30 male, weanling rats of the Sprague-Dawley strain were equally divided into 2 groups and housed in individual cages with raised screen bottoms. The first group received drinking water containing 3 ppm of selenium as sodium selenite and 50 ppm of fluoride as sodium fluoride. The second group drank water having only 3 ppm of selenium.

Both groups of animals were fed a diet commonly used in experimental caries research having the following composition (per cent): ground corn, 64; powdered whole milk, 30; alfalfa meal, 3; irradiated yeast, 2; sodium chloride, 1. Food and water were provided ad libitum and the consumption measured accurately by methods described in previous works[3,4]. The intake of water was measured daily but that of food only on 3 consecutive days during each of the 4 weeks of the experimental period.

The results are presented in the Table and indicate that the combined administration of selenium and fluoride to rats did not increase the severity of symptoms characteristic of chronic selenosis compared with the

[1] D. M. HADJIMARKOS, Archs Environ. Health *10*, 893 (1965); Borden's Rev. Nutr. Res. *27*, 3 (1966); in: *Advances in Oral Biology* (Ed. P. H. STAPLE; Academic Press, New York 1968), vol. 3, p. 253; T. G. LUDWIG, B. G. BIBBY and F. E. LOSEE, Caries Res., in press.
[2] A. L. MOXON and K. P. DUBOIS, J. Nutr. *18*, 447 (1939).
[3] D. M. HADJIMARKOS, Experientia *22*, 117 (1966).
[4] D. M. HADJIMARKOS, Archs Environ. Health *14*, 881 (1967).

controls receiving only selenium. All rats survived the experimental period, and there were no statistically significant differences ($P > 0.05$), as measured by the t-test, in weight gain and in the amount of food and water consumed between the 2 groups of animals. It is well known that young rats drinking water containing 2–3 ppm of selenite-selenium develop typical symptoms of chronic selenium intoxication[3,5–7], whereas the addition of 50 ppm of fluoride to the water does not affect their health[8].

The present findings are contrary to those of the only earlier study conducted by MOXON and DUBOIS[2]. They reported that the addition of 5 ppm of fluoride to the drinking water of young rats eating a diet containing 11 ppm of selenium, as seleniferous wheat, increased mortality and caused considerable depression in weight gain and in food and water intake compared with controls receiving only selenium. These symptoms seem to suggest an increased retention of selenium in the body of the animals probably through the combined action of selenium and fluoride. However, it should be pointed out that although in the present experiment rats drank water containing 10 times more fluoride than in the earlier study[2], nevertheless, the symptoms of selenium toxicity were not intensified. The effects of trace elements on chronic selenosis have remained largely unexplored, with the possible exception of arsenic which was found to alleviate the toxic action of selenium in experimental animals[9,10]. Recent studies with rats indicated that the combined administration of zinc or uranium and selenium increased the toxicity of selenium[11].

In view of the findings of this study, it is suggested that in high seleniferous areas where people may be exposed to increased consumption of dietary selenium through locally produced and consumed foodstuffs, intake of fluoride for prevention of caries, either from a water supply or as medication, is not likely to increase the retention of ingested selenium. It was also shown recently[4], that the presence of selenium in the diet does not decrease the amount of fluoride deposited in bones and teeth.

Zusammenfassung. Wenn junge Ratten gleichzeitig Selen und Fluor erhalten, sind die toxischen Eigenschaften von Selen nicht grösser, als in den Kontrollen, die nur Selenium erhielten. Dieses Resultat steht im Widerspruch zu früheren Feststellungen.

D. M. HADJIMARKOS

Department of Preventive Dentistry, University of Oregon Dental School, Portland (Oregon 97201, USA), 31 December 1968.

Effect of fluoride on selenium toxicity in rats

Variables	Treatment	
	Se + F	Se
No. of rats	15 (15)[a]	15 (15)
Initial weight (g)	50.4 ± 1.8	50.1 ± 1.7
Weight gain (g)	82.1 ± 5.7	73.7 ± 4.4
Food intake (g)	9.7 ± 0.3	9.1 ± 0.3
Water intake (ml)	10.0 ± 0.5	9.2 ± 0.4

Values are given as means ± S.E. [a] No. of survivors in parenthesis.

[5] W. BUTTNER, J. Dent. Res. *42*, 453 (1963).
[6] D. M. HADJIMARKOS, Experientia *23*, 930 (1967).
[7] H. A. SCHROEDER, J. Nutr. *92*, 334 (1967).
[8] H. G. HODGE and F. A. SMITH, in *Fluorine Chemistry* (Ed. J. H. SIMONS; Academic Press, New York 1965), vol. IV, p. 112.
[9] I. ROSENFELD and O. A. BEATH, *Selenium*, 2nd edn (Academic Press, New York 1964), p. 182.
[10] O. A. LEVANDER and C. A. BAUMANN, Toxic appl. Pharmac. *9*, 98 (1966).
[11] I. ROSENFELD, Agric. Exp. Station Bull. 414 (University Wyoming 1964), p. 27.
[12] The study was supported by the General Research Support Grant from the National Institute of Dental Research, USPHS.

Effect of Continuous Illumination on Mitochondria of the Rat Pineal Body

The influence of constant light upon the pineal body has been extensively studied by several investigators. A marked increase in pineal 5-HTPD activity[1,2] and a decrease of the serotonin content by one-half in rats under diurnal lighting conditions[3], are some of the effects already described. They indicate that the metabolism of serotonin in the pineal body is highly stimulated by continuous illumination. In a previous paper[4] we described changes in the 2 hypothalamic neurohumors of the rat, norepinephrine and serotonin, following 30 days of continuous illumination as well as 30 days of total darkness. The participation of mitochondria was also mentioned at that time.

This paper deals with mitochondrial alterations in the pinealocytes as revealed by the electron microscope. For a period of 30 days, male Sprague-Dawley rats were exposed to light of 70 lux, as measured at the bottom of the cage. One group of control animals was kept under total darkness (< 0.001 lux) and another group had 14 h of light (70 lux) to 10 h of darkness (< 0.001) for the same period of time.

The Figure shows part of a pinealocyte. The striking feature of the electron micrograph are the enlarged and swollen mitochondria scattered over the cytoplasm. There is almost a complete absence of cristae mitochondriales. The matrix is pale and contains sparse flocculent material. Mitochondria are elongated with club-like deformities. The outer, double-membrane is intact.

[1] S. H. SNYDER and J. AXELROD, Biochem. Pharmac. *13*, 803 (1964).
[2] S. H. SNYDER, J. AXELROD, R. J. WURTMAN and J. E. FISCHER, J. Pharmac. exp. Ther. *147*, 371 (1965).
[3] W. B. QUAY and A. HALEVY, Physiol. Zool. *35*, 1 (1962).
[4] E. RÜTHER, A. HALARIS and N. MATUSSEK, Med. Pharmac. exp. *17*, 139 (1967).