crystallization. The reaction tube is then drawn to a fine capillary near its open end (Fig. 1 b). The capillary is cut off at the place marked in Fig. 1 b. A 3 cm long piece of 3 mm tubing is sealed at one end and is heated at one point in a micro flame to produce a small recess. The reaction tube is then placed in this filtering tube, the capillary end inwards; the filtering is achieved by centrifuging the liquid away from the reaction tube (Fig. 1 c). On centrifuging, the crystals usually remain at the closed end of the reaction tube; if they come loose, they are held back by the capillary end, which functions as a filter. If the precipitate is very fine, it is better to use an asbestos filter (Morton, p. 166). If some crystals reach the capillary end of the reaction tube, they can be brought to the bottom by dropping the reaction tube through a long glass tube against some hard object.

For recrystallization of a substance, a little solvent is taken up in the filling capillary. The reaction tube is gently heated, and its capillary end is put into the filling capillary (Fig. 1 d). On cooling, the reaction tube sucks the solvent in. Washing liquids and precipitating agents can be introduced in the same way. After the solvent has been centrifuged to the bottom, the crystals are dissolved by heating the tube in a metal block (not over a naked flame!). It is best to let part of the substance remain undissolved; it then initiates crystallization on subsequent cooling. If a supersaturated solution does not begin to crystallize, a minute amount of liquid is allowed to drain out of the capillary end. On the outer surface of the capillary the solution can be brought to crystallize by rubbing with a capillary glass rod. The crystallization then propagates through the capillary part into the reaction tube.

The crystals are then freed from the liquid by centrifuging as above. The drying of the crystals is best accomplished by heating the reaction tube in an evacuated test tube (Fig. 1 c). The vacuum is released a couple of times during drying. After drying the melting-point of the substance is determined in the same tube. The crystallization can then be repeated if necessary.

For sublimations, the capillary tube is heated in the same way as in melting-point determinations (Kajola's apparatus [2]). The sublimation is greatly accelerated if a minute hole is left at the bottom of the capillary tube. Since the air in the melting-point apparatus is in rapid motion, a slow current of air is produced through the capillary tube; the air current conveys the vapours of the substance to the colder part of the tube, where they are condenced (Fig. 1 f).

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*iso*Thiocyanates IX. The Occurrence of Ethyl *iso*Thiocyanate in Nature

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The naturally occurring *iso*thiocyanate-containing glycosides can, for practical reasons, be classified in two groups according to whether their *iso*thiocyanates are volatile with steam or not. Within the former group the occurrence of allyl, (+)-*sec*-butyl, benzyl and β-phenylethyl *iso*thiocyanate was convincingly established before these studies were initiated two years ago. Since then, we have proved 3-butenyl *iso*thiocyanate to be derivable from a glycoside of, a.o. *Brassica napus* L.[1], and demonstrated by isolation the wide-spread occurrence of *iso*propyl *iso*thiocyanate in the family *Cruciferae* [2]. To these findings we now wish to adduce experimental evidence for the presence in nature of a glycoside containing ethyl *iso*thiocyanate, the occurrence of which has not been suggested previously.

In the course of a paperchromatographic scanning of a large number of seed-samples for their contents of volatile *iso*thiocyanates, the results of which have been partly published [3], a rather unique spot with an R_{Ph}-value [4] of 0.15 was noticed from the seeds of *Lepidium Menziesii* DC. This value is in accord with that of authentic N-ethylthiourea, suggesting the presence of ethyl *iso*thiocyanate in the glycoside of the seed in question. Unfortunately, the small amount of seed available at that time did not allow substantiation of this assumption by isolation. Through the courtesy of

the Botanical Garden of the University of Copenhagen, however, a larger seed sample was provided after the plant had been cultivated in the Garden on a larger scale during the summer of 1953.

The seeds (*ca.* 60 g) were defatted and cleaved enzymatically in the usual way [3], whereupon a strong smell of *iso*thiocyanate was noticed. The ethereal oil was removed by steam distillation, collected in aqueous ammonia and the resulting crude thiourea isolated upon evaporation. After unsuccessful attempts to provide a homogeneous specimen by numerous recrystallizations and sublimations, the tenaciously adhering impurities were eventually removed by partition chromatography (*cf.* experimental part). Repeated recrystallizations resulted in an analytically pure sample (31 mg) of N-ethylthiourea, the identity of which was further established by mixed melting point, paper chromatography and infrared spectroscopy.

It appears very remarkable that the seed of *Lepidium Menziesii* DC. is the only specimen among approximately one hundred investigated so far, which contains ethyl *iso*thiocyanate. Other species belonging to the genus *Lepidium* have consistently been rich in benzyl *iso*thiocyanate. From the initial routine investigation it seemed as if *Lepidium Menziesii* DC. contains solely ethyl *iso*thiocyanate. However, paper chromatography of the mother liquors from the above crystallizations disclosed the presence also of very small amounts of *iso*propyl, *sec*-butyl and benzyl *iso*thiocyanate which had been sufficiently enriched for detection by the use of a larger seed-sample.

Experimental. A seed-sample (56.8 g) of *Lepidium Menziesii* DC. (source: *Hortus Botanicus Hauniensis*), which by our spectrophotometrical assay [3] was estimated to contain *ca.* 135 mg of ethyl *iso*thiocyanate, was pulverized and thoroughly defatted by repeated hot extractions with a mixture of ligroin (230 ml) and ethanol (115 ml). The fat contents were about 29 %. The finely divided material was then submitted to enzymatic cleavage by suspension in 400 ml of water and addition of 5 ml of a cell-free myrosinase preparation. After having been kept for 18 hours at room temperature in a tightly stoppered flask the liberated volatile *iso*thiocyanate was removed in a stream of steam and the distillate collected in 200 ml of concentrated ammonia. After standing for 24 hours at ordinary temperature the solution was evaporated to dryness *in vacuo* at a bath temperature not exceeding 50°. The residue consisted of crystalline material embedded in an oil, which was removed by careful treatment with cold chloroform. There remained 199 mg of a crystalline product which, however, melted over a very wide range. The crystals were treated with ethanol (4 ml) and the insoluble residue (23 mg) was discarded. Extraction with ethyl acetate removed another 7 mg of insoluble material. Two recrystallizations from ethyl acetate and one from ethylene chloride afforded a sample with a considerably sharper melting point (103—109°). After repeated sublimations *in vacuo* had failed to remove the final impurities, recourse was taken to partition chromatography.

A column of kieselguhr (Hyflo-Supercel), 250 × 18 mm, made up from 40 g of kieselguhr with water as the stationary phase and ethyl acetate as the moving phase, was loaded with 108 mg of the above thiourea. The chromatography was performed in a constant temperature room (23.4°) and 50 fractions of each 3.0 ml were taken by means of an automatic fraction collector. The contents of the tubes Nos. 13—29 gave a positive Grote-reaction (*cf.* Ref.[4]) and were pooled and evaporated to dryness *in vacuo*, leaving 95 mg of a crystalline, slightly yellow material. This product was carefully recrystallized three times from ethyl acetate and an analytical specimen (31 mg) was thus obtained as beautiful, rhombic plates, m.p. 110—111°, alone, or in admixture with an authentic sample of N-ethylthiourea (m.p. 110—111°). Calc. for $C_3H_8N_2S$ (104.2): C 34.58; H 7.74; N 26.89; S 30.77. Found: C 34.68; H 7.76; N 26.96; S 30.83. Paper chromatography of the sample in chloroform-water, ethyl acetate-water and 2-butanone-water gave spots which were undiscernible from those given by authentic ethylthiourea. Furthermore, the infrared absorption spectra determined in Nujol mulls of the two samples to be compared coincided completely. When the mother liquors from the ethyl acetate recrystallizations above were pooled and submitted to paper chromatography in chloroform-water, four spots appeared with R_{PK}-values of 0.15, 0.40, 0.73 and 0.90 (*cf.* Ref.[4]). From previous experience these can be assigned with reasonable safety to ethyl, *iso*propyl, *sec*-butyl and benzyl *iso*thiocyanate, respectively.

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Conversion of Orotic Acid to Uridine Phosphates by Soluble Enzymes of Liver

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In rat liver *in vivo* radioactive orotic acid is converted to a group of uridine nucleotides before the label is incorporated into the pyrimidines of the ribonucleic acid [1]. These nucleotides, which are uridine-5-phosphate (UMP), uridine diphosphate (UDP), uridine triphosphate (UTP) and three compounds consisting of UDP linked to a carbohydrate, have recently been found in rat liver as well as in other sources (*cf.* Ref.[1,2]). Since it has furthermore been established [3,4] that orotic acid can be formed *in vitro* in rat liver from the essential metabolites aspartic acid, CO_2, and NH_3, it is apparent that the compound is an ideal precursor for the study of some of the enzymatic mechanisms in the normal biological synthesis of nucleic acids. The objective of the present project is to examine *in vitro* the nature of the ribosidation stage in the conversion of orotic acid to the uridine-5-phosphates.

The soluble enzymes of rat and pigeon liver are found to be capable of converting orotic acid-2-C^{14} to the uridine nucleotides

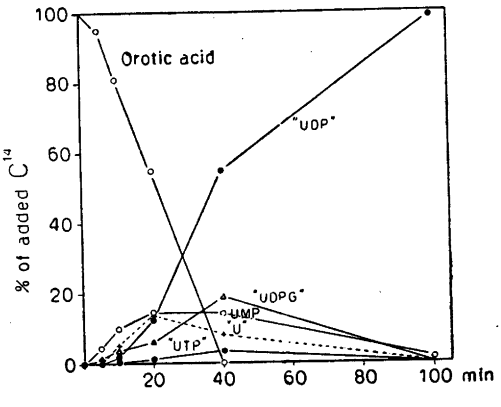

Fig. 1. The dialyzed supernatant fraction [5] from 400 mg of liver is incubated aerobically at 37° with 0.5 µmoles of orotic acid-2-C^{14} (500 000 c/min), 20 µmoles of hexose diphosphate, 3 µmoles of DPN and 120 µmoles of nicotinamide in a volume of 5.0 ml. The reaction is stopped by chilling the flask and adding perchloric acid to 0.4 N. The proteinfree extract is neutralized with KOH and chromatographed directly. ⊙ = *orotic acid;* + = *"U";* ○ = *UMP;* ● = *"UDP";* △ = *"UDPG";* ◐ = *"UTP".*

in vitro. The enzyme preparation is the dialyzed supernatant fraction of the liver after homogenization and high speed centrifugation (*cf.* Ref.[5]). The conversion of the orotic acid is obtained when fructose-1,6-diphosphate or ribose-5-phosphate with the cofactors DPN, ATP and Mg ion are added. A number of assisting and competing enzymes appear to be involved and the relative effectiveness of various combinations of these added components is different in preparations of pigeon liver as compared with rat liver.

The reaction products are separated by chromatography on 6 × 1 cm Dowex-2 (formate) columns by gradient elution with formic acid and formic acid-ammonium formate (*cf.* Ref.[6]). In addition to the unchanged orotic acid, five other radioactive peaks are found. One of these has been identified as UMP and three of them contain UMP which can be liberated by acid hydrolysis. They correspond in chromatographic behaviour to the components identified in rat liver *in vivo* [6] and have been designated here as "UDPG", "UDP" and "UTP" pending complete identification. The other radioactive fraction, "U", is found in the effluent from

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