voit effectivement dans le culot qui donnera la fraction L, une masse blanchâtre de glycogène qui occupe principalement le fond de ce culot. Il est probable, dans ces conditions que la décantation réalisée après la centrifugation entraîne beaucoup plus facilement les éléments de la fraction L, qui se trouvent tassés au-dessus de ce culot de glycogène, avec comme conséquence leur sédimentation ultérieure dans la fraction microsomale P.

Summary. Significant differences were observed in enzymic composition of L and P fractions between fasting and fed animals. The L fraction contains a smaller percentage and the P fraction a higher percentage of the enzymic activity of the homogenate when the rat is fed, which can be explained by the sedimentation in the L fraction of a high proportion of the glycogen accumulated in the liver of fed animals. The presence of this component changes the organization of the centrifugation pellet so that, during the decantation of the L fraction, subcellular structures sedimenting in this fraction are sucked off and recuperated in the next centrifugation pellet, i.e. in the microsomal P fraction.

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The Effect of UV-Irradiation on Glucobrassicin and Other Glucosinolates (Mustard Oil Glucosides)

The glucoside glucobrassicin (GBR), i.e. 3-indolylmethylglucosinolate, isolated first from Brassica species[1], is the main precursor of the goitrogenic thiocyanate (SCN^-) and of the indole auxins (plant growth-regulators) in these and many other plants[2,3]. These active substances are liberated from GBR by the enzyme myrosinase. The products of this enzymatic cleavage depend on the pH of the medium. At pH 7, GBR splits to yield glucose, sulphate, 3-hydroxymethylindole (3-OH-MI), respectively 3,3'-di-indolylmethane (Di-IM) and SCN^-. At pH 4, 3-indolylacetonitrile (IAN) is the primary indolic cleavage product, along with glucose, bisulphate and sulphur.

Although the aqueous solutions of GBR are relatively stable, they are very sensitive to UV-light. We have investigated this fact in some detail and the essential results are discussed in the present communication.

The GBR used was prepared in our laboratory as the tetramethyl ammonium salt (of 97% purity) according to Gmelin and Virtanen[1]. Aqueous solutions of GBR were irradiated in quartz cells (d = 1 cm) at a distance of 15 cm from the UV source, a 'Chromatolux' tubular lamp of Pleuger Inc. (G8TL, 8 W, emitting principally at 2537 Å without additional filtration).

The time course decomposition of GBR was followed by measurement of the absorbancy of the solution at 230 nm (spectrophotometer CF_4-Optica Milano) (results in Figure 1). The initial steep decrease in absorbancy is supposed to characterize the decomposition of GBR and, after the minimum has been reached (at 3 min), the small rise and slow subsequent change is probably due to the formation of the final decomposition products. The minimum value is supposed to represent the point of intersection of 2 curves. It is important to note that long, continuous irradiation of the sample causes the same changes in absorbancy (curve 1) as a short, only 3 min long exposure (curve 2).

For the identification of the decomposition products, the solutions of GBR (1 mg/ml) were irradiated as already described for 10, 20 and 45 min. At first it may be accentuated that the pH of the solutions fell from pH 7.0 to pH 3.7 after 10 min irradiation and then no further change was observed. The presence of sulphate in the solutions was proved (as Ba-salt). The other products were identified by means of paper chromatography (Figure 2). An unknown product (X on Figure 2; green fluorescence with formaldehyde reagent[4], Rf = 0.56) appeared after 10 min irradiation, but it vanished on further irradiation. The other 4 final products were identified as glucose, SCN^-, 3-OH-MI and Di-IM. From this it is evident that all the products of photolysis are identical with the products of enzymatic cleavage of GBR by myrosinase. Quantitatively, 83.3% of the initial GBR (50 µg/ml) was decomposed after 3 min of UV-irradiation (estimated from the amount of liberated SCN^-). The remaining GBR decomposed probably to IAN, as a result of the fall in the pH of the solution. For comparison, the exposure of the same GBR-solution to sunlight resulted in 55% decomposition of glucoside. Also, after exposing the solution to gamma radiation (100 kR Co^{60}), there followed a 41% decomposition of GBR, but the products formed at UV-irradiation were not found in this case.

The UV-irradiation of some other glucosinolates, sinalbin, sinigrin, glucoiberin and progoitrin, produced a

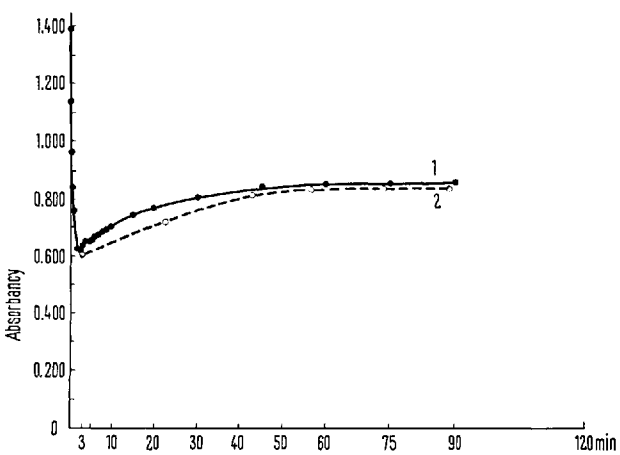

Fig. 1. The changes of absorbancy at 230 nm after UV-irradiation of water solution of glucobrassicin (50 µg/ml). Curve 1, continuously irradiated solution; curve 2, the solution irradiated for 3 min then left without further irradiation.

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similar decomposition, but evidently not as rapid as that of GBR. The formation of glucose and sulphate and in case of sinalbin also of SCN^- (in accordance with its enzymatic cleavage[5]), as well as the decrease of the pH value of these samples was the same as in the GBR.

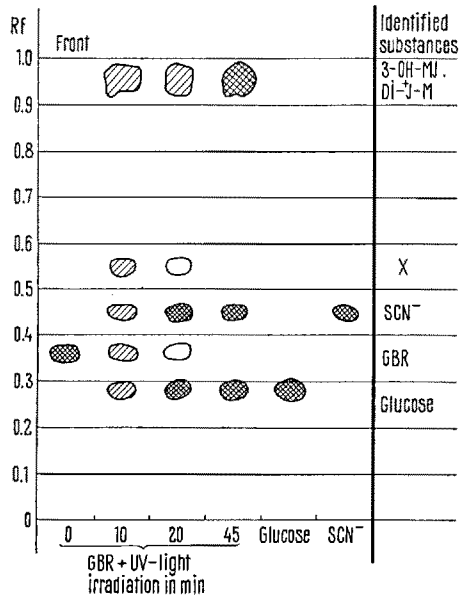

Fig. 2. Identification of products of glucobrassicin decomposition by means of paper chromatography. After applying aliquote parts of the investigated solutions on the paper Whatman 1, the developing was effected in the system butanol-acetic acid-water (4:1:3). Detection: indoles by the EHRLICH[1] and PROCHÁZKA (formaldehyde) reagent[4]; glucose and GBR: $AgNO_3 + NH_4OH$[1]; SCN^-, GBR: $Fe(NO_3)_3 + HNO_3$[1].

However, the isothiocyanates expected from other glucosinolates were not detected even after attempted conversion to thiourea derivatives. This indicates a certain difference between the photolysis and the enzymatic hydrolysis of these glucosides.

The identity of the products of UV-photolysis of the examined glucosinolates, especially of GBR, and of the products of their enzymatic hydrolysis suggests aspects on the function of these substances in plant physiology, previously not considered[6-8].

Zusammenfassung. Durch die Wirkung von UV-Licht auf wässrige Lösungen von Glucobrassicin und Sinalbin entstehen dieselben Zersetzungsprodukte wie bei der Spaltung durch das Enzym Myrosinase (HSO_4^- und SCN^-, Glucose und diesbezügliche Hydroxyverbindungen). Bei anderen Glucosinolaten führte der UV-Einfluss zwar auch zur Abspaltung von HSO_4^- und Glucose, eine gleichzeitig erwartete Bildung von Isothiocyanaten wurde hier jedoch nicht gefunden.

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Pigment Formation from Tyrosine Derivatives by UV-Irradiation in Thin-Layer Chromatography

Thyroxine is known to be sensitive to light, undergoing oxidation with the formation of a visible yellow pigment[1]. Brown pigments were described as appearing from tyrosine and dopa, following oxidation by UV-light[2]. Autooxidation of tyrosine[3-5], dopa[6-7] and dopamine[8] leading to melanin formation has been widely reported. Since all the compounds mentioned are derivatives of tyrosine, the conditions for pigment appearance on thin-layer chromatography plates, particularly from thyroid hormones, have been investigated.

Ascending chromatography was carried out in ethyl acetate:methanol:2*N* ammonia (100:40:60 V/V)[9] for 1 h on glass plates coated with a 250 μ thick layer of Kieselgel G.

Three plates, applied with 25 μg of 3-monoiodo-L-tyrosine (MIT), 3,5-diiodo-L-tyrosine (DIT), 3,5-diiodo-L-thyronine (T_2), 3,5,3'-triiodo-L-thyronine (T_3), L-thyroxine (T_4), DL-thyronine (T), L-tyrosine (Tyr.) and with a mixture consisting of MIT, DIT, T_2, T_3, T_4, 5 μg of each dissolved in 3*N* ammonia, were chromatographed. One plate was sprayed with a 0.2% ninhydrin solution in acetone and heated at 100 °C for 5 min, the second was UV-irradiated and the third was left uncovered in the laboratory for 5 days. Two h of UV-irradiation or exposure to ordinary light for 2–5 days revealed dark spots at the sites corresponding to all examined compounds, as located in the ninhydrin stained plate. The presence of iodine in the molecule does not seem to be essential, since tyrosine and thyronine also yielded pigmented spots, although lighter than those of the iodinated compounds.

Similarly chromatographed plates, when UV-irradiated through an ordinary glass cover or when kept in the dark for nearly 2 months, did not reveal any spots. However, upon exposure to irradiation (the former one after re-

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