Mechanismus erniedrigt[4,13,15]. Wenn jedoch nach einer Strahlenbelastung der Gehalt dieser Verbindungen absinkt, wie es in der Figur gezeigt ist, dann ist der «feed back» zur Regulation der Acetyl-CoA-Carboxylase teilweise aufgehoben. Damit wären aber im Hinblick auf den Hemmstoff die Voraussetzungen für eine erhöhte Fettsäuresynthese gegeben. Es sei darauf hingewiesen, dass ein enger zeitlicher Zusammenhang besteht zwischen dem Abfall der Fettsäure-CoA-Verbindungen und einem erhöhten Fettgehalt in der Leber bestrahlter, hungernder Mäuse.

Man darf annehmen, dass sich die Konzentrationsänderungen der Fettsäure-CoA-Verbindungen nach der Strahleneinwirkung auch auf die Aktivitäten der beiden anderen eingangs erwähnten Enzyme in ähnlicher Weise auswirken[16].

Summary. The contents of fatty acid CoA derivatives were measured in mouse liver after whole-body X-irradiation with 690 R. While the contents are almost unchanged in fed mice, a decrease occurs in 24 h starved mice after the irradiation. The results are discussed with respect to fatty acid synthesis.

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Studies on Phytohemagglutinins II. Phytohemagglutinins of *Pisum sativum* L. and *Lens esculenta* Moench: Specific Interactions with Carbohydrates

The specific interaction of concanavalin A, the phytohemagglutinin of *Canavalia ensiformis* L., with some polysaccharides has been known for several years[1,2] and studied by some workers in detail[3–6]. Recently, Aspberg et al.[7] reported that the non-specific phytohemagglutinin of *Vicia cracca* exhibited some similar properties. In our work dealing with isolation and characterization of phytohemagglutinins of leguminous plants, we have found that substances with hemagglutinating activity, contained in seeds of the pea (*Pisum sativum* L., var. Pyram) and the lentil (*Lens esculenta* Moench., var. Hrotovická), precipitate some polysaccharides and are strongly adsorbed to the Sephadex matrix. We have also found that monosaccharides and oligosaccharides, which inhibit the hemagglutinating activity of these phytohemagglutinins, likewise influence their rate of flow by electrophoresis on starch gel. As a rule, the inhibitory power of these sugars is directly proportional to the acceleration they impart to phytohemagglutinins when applied with them in solution to the starch gel.

The isolation of the crude phytohemagglutinin was carried out as described by Liener[8] up to the ammonium sulphate fractionation. For further purification adsorption on Sephadex G-200 was used in a simple procedure which will be described in detail later. Desorption was effected with either HCl gradient or glycine-HCl buffer pH 2.0. As shown in Figure 1, the capacity of different Sephadex types decreases in the order G-200 > G-100 > G-50 > G-25. The amount of protein mixture used in this experiment corresponds approximately to the capacity of the Sephadex G-200 column of given size so that part of the active substance appears in the normal saline eluate from G-100 and G-50 columns (Figure 1B, C).

Homogeneity of the isolated phytohemagglutinins was checked by vertical starch gel electrophoresis according to Smithies[9]. In short runs (3 h), agglutinins appeared as 1 homogeneous band, but on prolonged electrophoresis (7–8 h), these bands could be resolved into 2 distinct fractions (Figure 2). When electrophoresis was run on gels prepared in 0.03 *M* acetate buffer pH 5.0 (using 0.1 *M* buffer of the same pH as the bridge solution)[10], agglutinins of both the pea and the lentil invariably showed only 1 band, even on prolonged runs. The main difference in the electrophoretic behaviour of phytohemagglutinins of these 2 species resides in the direction of their migration in borate buffer. While pea agglutinins migrate to the positive pole, lentil agglutinins move to the negative one (Figure 2). Comparative electrophoretic analysis of phytohemagglutinin fractions from different pea and lentil varieties showed quantitative and (in lentil) also qualitative differences in the pattern of active components.

Inhibition of type 0 red blood cell agglutination by sugars[a]

Sugar	Hemagglutinin from pea	lentil
D-Glucose	128	256
D-Galactose	0	0
D-Mannose	512	128
D-Fructose	16	8
D-Arabinose	1	0
Maltose	32	32
Sucrose	32	8

[a] Numbers indicate last degree of 2-fold serial dilution of 2% sugar solution (= 1) which causes perceptible inhibition of hemagglutination by a 0.004% phytohemagglutinin solution[13].

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When eluted separately from the starch gel, the 2 components of phytohemagglutinins from both plant species show a non-specific hemagglutinating activity of about the same titer against the red blood cells of all blood groups. Ultracentrifugal analysis of the 2 hemagglutinating components from pea confirmed their homogeneity. Their sedimentation constants ($s_{20,w}$) are 3.59 and 3.48 *S*, respectively, with mol. wt. between 53,000 and 54,000. Both are glycoproteins containing about 1% neutral sugar.

Both pea and lentil hemagglutinins precipitate some polysaccharides as found for concanavalin A. In our study, the precipitation of yeast mannan and muscle glycogen was followed turbidimetrically[11] (Figure 3), using phytohemagglutinins purified by the Sephadex adsorption procedure.

Hemagglutinating action of pea and lentil hemagglutinins is inhibited to a different extent by a number of monosaccharides and oligosaccharides (Table). As shown in Figures 4–6, sugars causing inhibition, when applied together with phytohemagglutinins to the starch gel, enhance their mobility in electrophoresis. The rate of acceleration is directly proportional to the inhibitory power of the particular sugar and to its concentration.

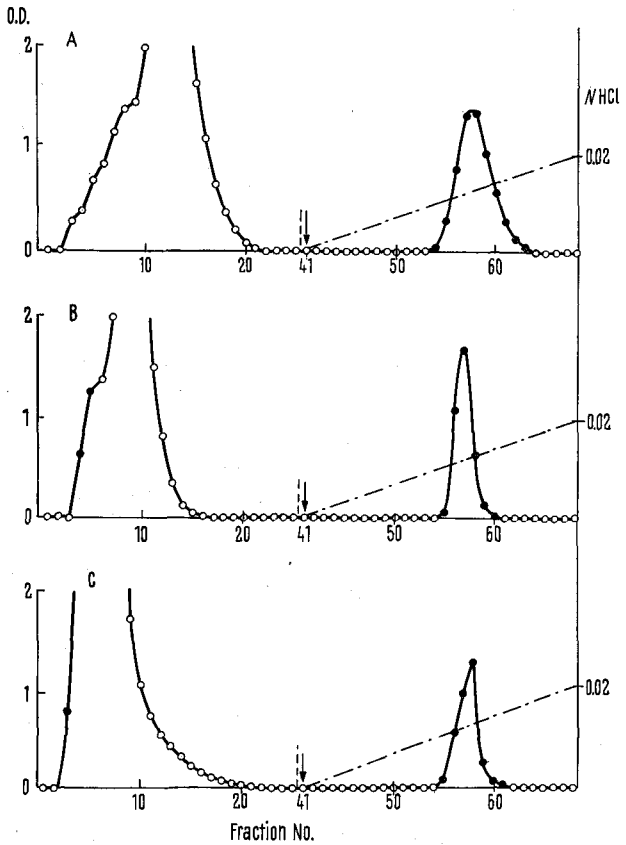

Fig. 1. Chromatography of the crude protein mixture on Sephadex columns (A, G-200; B, G-100; C, G-50). 150 mg of a protein mixture, dissolved in 1 ml of normal saline, was applied to a column (2.5 × 11 cm) and eluted with normal saline. Arrows indicate the start of the HCl gradient. A linear gradient of HCl was achieved by mixing 70 ml of normal saline and 70 ml of 0.02 *N* HCl in normal saline. 5-ml fractions were collected at a flow rate of 15 ml/h. Optical density was measured at 280 nm using an Optica Milano CF 4 NI spectrophotometer. ○—○—○ Fractions with hemagglutinating activity. ●—●—● Fractions without hemagglutinating activity.

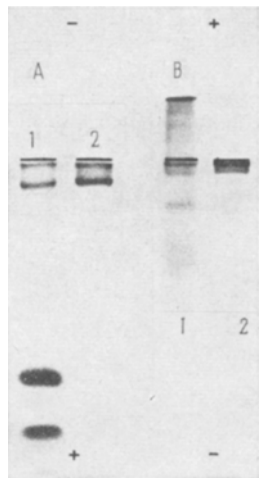

Fig. 2. Vertical starch gel electrophoresis of active protein fractions from seeds and of phytohemagglutinins obtained by chromatography on Sephadex. Gels were prepared from Connaught starch in borate buffer pH 8.6 and were run at 250 V/35 mA for 6 h (A) and 4.5 h (B). A 1, 3% solution of the protein fraction obtained from a pea seed extract by 35–55% saturation with ammonium sulphate; A 2, 2% solution of the pea hemagglutinin as eluted from Sephadex G-200; B 1, 3% solution of the protein fraction obtained from a lentil seed extract by 35–55% saturation with ammonium sulphate; B 2, 2% solution of lentil hemagglutinin as eluted from Sephadex G-200.

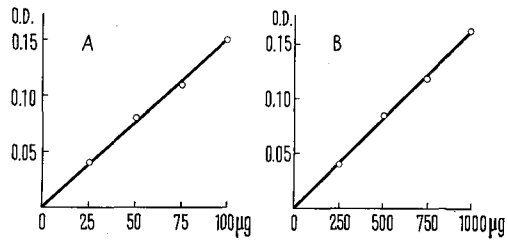

Fig. 3. Interactions of pea hemagglutinins with polysaccharides. Turbidimetric estimation of the precipitate formed by increasing concentration of polysaccharides. O.D. values (420 nm) have been corrected by substracting the O.D. values found after dissolving the precipitate by addition of 0.1 ml of 2% D-mannose solution. A, precipitation of yeast mannan (from *Saccharomyces cerevisiae*). 25–100 µg of the polysaccharide and 2 mg of phytohemagglutinin in 2 ml final volume of 0.05 *M* phosphate buffer pH 7.0. B, precipitation of muscle glycogen. 250–1000 µg of the polysaccharide and 4 mg of phytohemagglutinin in 2 ml final vol. of 0.05 *M* phosphate buffer pH 7.0.

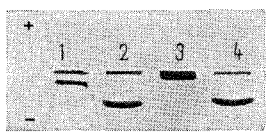

Fig. 4. Effect of sucrose on the electrophoretic mobility of pea and lentil hemagglutinins. Starch gel electrophoresis of 2% solutions of phytohemagglutinins eluted from Sephadex G-200 run at 170 V/40 mA for 5 h in 0.03 *M* acetate buffer pH 5.0 (using 0.1 *M* buffer of the same pH for bridge solution). 1, lentil hemagglutinin in acetate buffer; 2, same with 20% sucrose; 3, pea hemagglutinin in acetate buffer; 4, same with 20% sucrose.

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When the electrophoresis is carried out in borate buffer, pH 8.6, the mobility of phytohemagglutinins is influenced to a much smaller degree.

The similarity of interactions with carbohydrates of the non-specific hemagglutinins from *P. sativum*, *L. esculenta*, *V. cracca* and *C. ensiformis* suggests that all of these substances belong to the same class of phytohemagglutinins with very similar or identical active sites. In addition to the practical usefulness of these reactions for isolation of phytohemagglutinins and quantitative determination of specific carbohydrates[3,11,12], they can no doubt contribute to a better understanding of the mechanism of hemagglutination.

Our work in this direction is being continued.

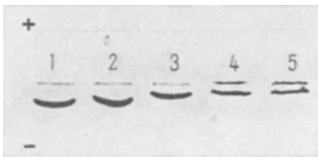

Fig. 5. Effect of D-glucose concentration on the electrophoretic mobility of pea hemagglutinin. 2% solution of hemagglutinin with different amounts of D-glucose in 0.03 *M* acetate buffer pH 5.0. Other conditions of electrophoresis identical with those given in the legend to Figure 4. D-glucose concentrations: 1, 4.0%; 2, 2.0%; 3, 1.0%; 4, 0.5%; 5, 0.25%.

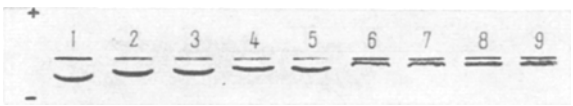

Fig. 6. Effect of various monosaccharides and disaccharides on the electrophoretic mobility of pea hemagglutinin. Conditions of electrophoresis were identical with those given in the legend to Figure 4. 2% solution of phytohemagglutinin in the buffer containing 2% of the following sugars. 1, D-mannose; 2, D-glucose; 3, maltose; 4, D-fructose; 5, sucrose; 6, D-galactose; 7, D-arabinose; 8, D-xylose; 9, cellobiose.

Zusammenfassung. Die in den Samen der Erbse (*Pisum sativum* L.) und der Linse (*Lens esculenta* Moench) enthaltenen Phythämagglutinine reagieren spezifisch auf einige Kohlehydratsubstanzen. Mono- und Oligosaccharide, welche die Agglutinationswirkung dieser Phythämagglutinine hemmen, bewirken auch eine Erhöhung ihrer elektrophoretischen Wanderungsgeschwindigkeit auf Stärkegel. Diese Beschleunigung ist der Hemmwirkung direkt proportionell entgegengesetzt.

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Ethionine and Methionine Biosynthesis in Rat Liver

It is known that the administration of ethionine significantly reduces the transmethylation from methionine for the synthesis of choline[1] and decreases the activity of some enzyme systems involved in choline metabolism (choline oxidase, E.C. 1.1.99.1; sarcosine oxidase, E.C. 1.5.3.1)[2]. Furthermore, ethionine decreases the synthesis of methionine interferring with the metabolic pathway of 1 carbon unit[3].

On the other hand Steckol et al. have shown that ethionine increases the catabolism of methionine to CO_2, whilst methionine increases the catabolism of ethionine[4].

In order to further clarify the action of ethionine on the methyl transfer in vivo, in this paper the influence of ethionine on the betaine-homocysteine-transmethylase enzymatic system (E.C. 2.1.1.5) has been studied.

Furthermore, since Steckol[5] has supposed that ethionine could be de-ethylated into homocysteine, comparatively the action of this compound on the same enzyme has been checked.

Experimental procedure. 4 groups of 6 male Wistar rats (250–300 g) were given i.m. L-ethionine. Each of the 4 groups received respectively, 5, 10, 20 and 40 mg of L-ethionine in 2% acqueous solution for 3 days. Another series of rats were given i.m. equimolecular doses of DL-homocysteine.

On the fourth day the animals were bled by decapitation and the betaine-homocysteine-transmethylase activity was determined on the liver by the method of Dubnoff and Borsook[6] and modified by Williams[7] and Ericson[8].

The L-ethionine was supplied by the Sigma Chemical Co., the homocysteine by the Nutritional Biochemicals Corp. and the betaine by C. Erba.

Results. From the results reported in Table I it is evident that the transmethylase activity is significantly increased ($p < 0.01$) upon the supply of daily doses of 5, 10 and 20 mg of ethionine. The maximum effect corresponds to the dose of 10 mg; with higher doses the transmethylase activity decreases again toward normal levels.

A similar result has been observed following the administration of homocysteine (Table II). The regression of the enzymatic activity, however, is not so marked as in the case of ethionine.

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