inhibitory effect for this substance on hexose monophosphate shunt activity as seen by VOGEL et al.[14] under in vitro conditions. In compensatory hypertrophy following unilateral nephrectomy with stimulated protein synthesis, increased activities of a.Ptase, glutamic acid dehydrogenase and (Na^+K^+) ATPase were shown[17,18]. The latter findings compared with our data indicate an inhibited functional metabolism in favour of an activated proliferative metabolism of the tubular cell after folic acid as described by TAYLOR et al.[5]

Zusammenfassung. Männliche Wistarratten zeigten nach i.v. Gabe von Folsäure einen Aktivitätsabfall der Enzyme, die in den aktiven Ionentransport eingreifen: (Na^+K^+) ATPase (60% gegenüber Kontrollen), ICDH (56%) und MDH (48%) und a.Ptase (66%).

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In vitro Studies of the Stability of Liver Lysosomes after in vivo Treatment of Rats with Sulfapyridine

Sulfapyridine is one of the successfully used drugs in the treatment of the skin disease, dermatitis herpetiformis[1,2]. The histologic picture characteristic of the disease[3] suggest that proteolytic enzymes of the lysosomes[4] are involved in the development of the pathological process. In order to investigate whether the 'stability' of lysosomal membranes is affected by sulfapyridine, the author has examined liver lysosomes from rats treated per os with sulfapyridine. The effect of the drug was examined by studying, under controlled conditions, the release of acid phosphatases from lysosomes in a lysosome-rich subcellular fraction.

Experimental. Male rats of the Sprague-Dawley strain were used. At the beginning of the experiment, the rats were 35–41 days old. In respect of age, 2 equal groups were formed, each containing 6 rats. The control group was given a practical type of diet (mouse pellets, H. Fors and Co. AB, Sweden) and the experimental group was fed the same diet with an addition of sulfapyridine (Pharmacia, Sweden) to a final concentration of 1%. The diet was given for 5 consecutive days in the form of freeze-dried balls made of distilled water and the powdered pellets. When mixed with the powder, sulfapyridine formed a homogeneous material from which selection was impossible. The supply of food was not restricted and the consumption by the rats was measured every day. On the 5th day the rat was killed by a blow and exsanguinated; 3.50 g of the chilled liver were weighed out, and lysosomes were prepared according to WEISSMANN[5]. The following procedure was a combination of WEISSMANN's and DE DUVE's techniques[6]. The lysosome-rich sediment obtained after washing with sucrose was resuspended in 10.0 ml of ice-cold acetate buffer (0.05 *M*, pH 5.0) containing 0.25 mole sucrose per litre of solution. In order to determine the total activity of acid phosphatases per millilitre of suspension, 0.70 ml of the suspension was mixed with 2.30 ml of the above-mentioned sucrose-acetate buffer with added Triton X-100 (final concentration 0.15%). Another sample made up for the determination of the release of acid phosphatases was composed of 2.0 ml of the suspension and 1.0 ml of the sucrose-acetate buffer. Both the samples were incubated at 37 °C for 45 min. After incubation they were cooled in ice-cold water and then centrifuged at 4 °C for 20 min at 15,000 *g*. 1.0 ml of the supernatants was then incubated for 10 min at 37.0 °C with 1.0 ml of acetate buffer with β-glycerophosphate (Sigma, USA; max 0.1% α-isomer) as a substrate. The final concentration of the acetate and of the β-glycerophosphate was 0.05 *M* (pH 5.8). After rapid cooling the reaction was stopped by the addition of 2.0 ml of ice-cold 10% trichloroacetic acid (TCA). Blanks were prepared by adding TCA before the substrate. The supernatant of the initial 15,000 *g* centrifugation[5], hereinafter called 'Supernatant 2', was treated in the same way as the supernatants just mentioned. Part of the acid-phosphatase activity of 'Supernatant 2' corresponds to enzymes which have been discharged into cell sap during life or released during the homogenization. After centrifugation of the incubation mixtures, the amount of inorganic phosphate was determined according to FISKE-SUBBAROW[7]. The mixture with Triton X-100 was diluted in order to avoid disturbances in the phosphate analyses[8]. In the analytical procedure demineralized water and chemicals of analytical grade were used. The suitable final concentration of Triton X-100 was determined in special assays. Triton X-100 was not found to affect the activity of acid phosphatases. Preliminary assays also indicated that under the conditions of the test the activity of the acid phosphatases is proportional to the enzyme concentration.

Results and discussion. At the end of the experiment the weight of the animals in the control group had increased, on the average, by 27% and that of the animals in the experimental group decreased by 1.5%. For weights and intake of food, see Table I. From the consumption of food it appears that the daily intake of sulfapyridine amounts to 0.6–0.8 g/kg body weight. It is of interest here to note that in the treatment of patients with dermatitis herpetiformis, up to 7.5 g of sulfapyridine are given per day[1], i.e. about 0.1 g/kg body weight. On the other hand, the half-life of sulfapyridine is about 10 times as great in man as in the rat[9].

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For analytical results, see Table II. Taking into consideration the number of analyses, a non-parametric statistical method, the Mann-Whitney U Test, was used in the treatment of the data[10]. It is evident that there is no significant difference between the total activities of acid phosphatases in the sediment of the control group and the experimental group. This would seem to mean that the phosphatases are not inhibited by the sulfonamide or its metabolites. However, it must be stressed here that this conclusion is valid only if the production of lysosomes or lysosomal enzymes is not stimulated by the drug. Another prerequisite is that the composition of the liver is not changed. Against the assumption of an elevated enzyme production, it may be stated that sulfapyridine inhibits the production of thyroxine[11,12] and thereby probably also indirectly the synthesis of proteins, regulated by the hormone[13]. Concerning the composition of the liver and, indirectly, the choice of the wet weight of the liver as a reference, it is interesting to compare the composition of the livers of normal rats and starved rats. To judge from the analyses of rat livers made by FENN[14], it is evident that the reduced intake of food by the experimental group is of very little importance, if the wet weight is used as a reference.

Table I. Body weight and intake of food

		Body weight (g) mean values		Intake of food per day (g) mean values				
Group	No. of animals	Initial weight	Final weight	Day 1	Day 2	Day 3	Day 4	Day 5
Control	6	138	175	19.9	19.3	20.1	21.1	18.3[a]
Experimental	6	130	128	7.9	8.9	10.4	10.7	8.5[a]

[a] This consumption corresponds to an intake for 16 h only.

Table II. Activity of acid phosphatases in control and in experimental group

Group	No. of animals	Total activity of phosphatases/ml of resuspended sediment µmol P/ml/ 10 min	Released activity of phosphatases in % of total sedimental activity	Activity of phosphatases/ml 'Supernatant 2' µmol P/ml/ 10 min
Control	6	9.9 ± 0.32[a]	29 ± 4.2	1.3 ± 0.12
Experimental	6	9.3 ± 0.99	19 ± 1.5	1.8 ± 0.05
p[b]		> 0.39	0.002	0.002

[a] Mean $\pm$ S.D. [b] The p values were obtained in the non-parametric statistical analyses (Mann-Whitney U Test) of the differences between the 2 groups.

The acid-phosphatase activity of the 'Supernatant 2' of the treated animals shows a significantly higher value than that of the control group. An analysis of the individual values of the experimental group reveals that the activity of 'Supernatant 2' is fairly constant, irrespective of variations in the sedimental activity. Furthermore, calculations based upon the activities and the volumes of resuspended sediment and of 'Supernatant 2' (11 ml and 25 ml, respectively) show that the total activity of these cell fractions of the experimental group is 5% greater than that of the control group. It seems likely that the increase in activity of 'Supernatant 2' is due mainly to an extraneous source, probably erythrocytes, since sulfonamides are known to be hemolytic[15].

After incubation one finds that the released phosphatase activity of the sediment expressed as a percentage of the total sedimental activity is lower in the sulfapyridine-treated group of animals than in the control group. The decrease is 34%. This fact may mean that the decreased release of enzymes from the lysosomes is due to a direct stabilizing effect of sulfapyridine or its metabolites. This interpretation must, however, be advanced with caution, because sulfapyridine may exert its effect indirectly. The results obtained suggest further similar experiments with other sulfonamides and also in vitro experiments with the metabolites of the drugs, in order to get a clear relationship between drug and effect.

Zusammenfassung. Sulfapyridin, das unter anderem bei der Behandlung von Dermatitis herpetiformis benutzt wird, wurde Ratten per os gegeben. Eine 34%ige Senkung der Freisetzung von sauren Phosphatasen der Leberlysosomen wurde in vitro bei einem Vergleich mit einer Kontrollgruppe erhalten.

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Susceptibility to Strychnine Convulsions in Maturing Rats

One of the several studies of ontogenesis in young rodents of seizure responses to various convulsant drugs has demonstrated a progressive increase in median convulsive dose (CD_{50}) for intraperitoneal strychnine in rats after the eighth day of life[1]. This change was attributed to a diminishing permeability of the central nervous system to the drug, although some other researches[2-4] question whether significant postnatal maturation of blood-brain barrier occurs in the rat. KATO et al.[5,6] later

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