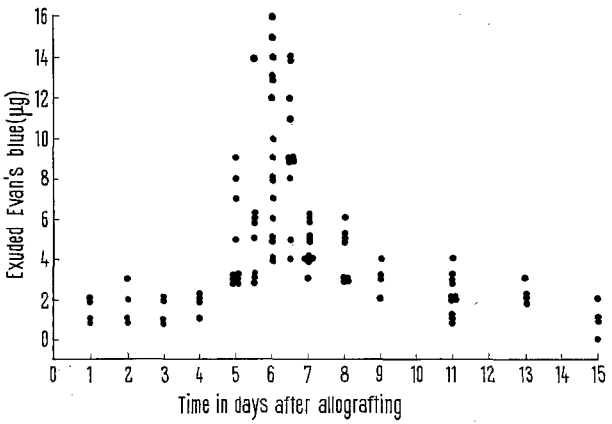
days and subsequently increases in intensity and extent. By day 15 the grafts have the appearance and consistency of leather; thereafter the rate of destruction of the grafts depends on whether the tissues are kept moist or permitted to dry out by removal of the tulle dressing. Drying grafts fragment quickly, whereas those kept moist with dressing persist for up to 21 days before becoming friable scabs.

In the first 2 days after grafting, the tensile strength of attachment of graft to host bed is negligible but then increases until the 15th day. Tensile strength increases rapidly for grafts aged 5–8 days and then more gradually up to the 15th day.

That the strength of attachment should increase so rapidly during the period of the permeability response



The time-course of local increased vascular permeability to circulating Evan's blue evoked by cutaneous allografts in the rat. Each symbol represents the result in individual rats.

was surprising since the onset of the exudative effects suggested impending rejection. This in fact was the case as revealed by histological examination of the grafts. Histological evidence of rejection began to appear in grafts aged 6–9 days, with progressive increase in the density of mononuclear infiltration in and around blood vessels in the junctional zone between graft and host bed, accompanied by endothelial proliferation and later by intraluminal fibrin plugs. Grafts aged 9–11 days are obviously devitalized.

In conclusion, the allograft response provokes inflammation characterized by a prominent permeability response accompanying the cellular infiltration; the increased vascular permeability precedes macroscopic and histological evidence of rejection[4,5].

Résumé. La réaction cutanée de l'immunité de transplantation contre une greffe allogénique est accompagnée d'une forte mais courte augmentation de la perméabilité vasculaire. La réponse vasculaire apparait rapidement et cela avant les effets macroscopique ou histologiques de l'immunité de transplantation.

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Behaviour of Sodium-Potassium-Activated Adenosine Triphosphatase in Rat Kidney Tissue by Folic Acid[1]

At present it is believed that the sodium pump associated with a membrane-bound ATP-hydrolysing enzyme system, i.e. (Na^+K^+) ATPase (SKOU[2] and STONE[3]) is mainly localized within the plasma membrane of the mitchondria-rich region in the tubular cell, i.e. within the basal infoldings. Recently BRADE et al.[4] described temporary functional disorders in the rat kidney after administration of folic acid. Electron microscopic studies[4] revealed alterations, especially within the proximal segments of the tubules, with remarkable loss of basal infoldings.

The purpose of the present study was to investigate the behaviour of (Na^+K^+) ATPase after reduction of basal cell surface in the tubular cells due to folic acid. We used for our experiments a dosage as described by TAYLOR et al.[5,6] to prevent significant cellular damage.

Methods. Male Wistar rats[7] weighing 150–200 g were used. The animals were housed in metabolic cages with free access to water. Urine was collected twice daily in 11 h samples, because an action of folic acid was demonstrated with the production of a water-like urine after an oliguric phase post injection. During the experiment food was given every 11 h for 1 h. Experimental animals were sacrificed 63 and 87 h after i.v. application of folic acid (250 mg/kg body wt.) dissolved in 0.3 *M* sodium carbonate. Control rats remained untreated. Kidneys were removed. The right one was used for light and electron microscopic studies[8] and the left one after determination of wet weight for a homogenate in water (1:11 v/w) prepared in an Ultra Turrax grinder. Aliquots of each homogenate were frozen and lyophilized at −26 °C for 16 h and stored at −31 °C until used. No difference for (Na^+K^+) ATPase activity was noted using

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Ultra Turrax or Potter Elvenhjyem grinders. The frozen-dried homogenates were reconstituted with distilled water for assay. The following enzymes were measured: total ATPase and ouabain insensitive (Mg^{++}) ATPase according to BONTING et al.[9] ((Na^+K^+) ATPase was calculated from total ATPase minus (Mg^{++}) ATPase), alkaline phosphotase (a.Ptase), hexokinase (HK), lactic dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G-6-PDH), malic dehydrogenase (MDH) and isocitric dehydrogenase (ICDH) according to kinetic parameters worked out for rat kidney by DUBACH et al.[10]. Basis for the analysis of dehydrogenases was the fluorimetric measurement of diphosphopyridine nucleotide or reduced triphosphopyridine nucleotide[11] and for ATPase and a.Ptase it was the spectrophotometric measurement of inorganic phosphate[12] and *p*-nitrophenol[13].

Enzymatic activities were calculated as moles of substrate converted per kg (dry weight) per h (= MKH units). All enzyme activities are related to dry weight because there is a large increase in the water content of the kidneys after folic acid[5] indicated by the high wet weight of the left kidney (1.47 g versus 0.70 g calculated on the basis of the data shown by [5]) accompanied by only a small increase in dry weight.

Results. After i.v. application of folic acid (250 mg/kg body wt.) the animals suffered from an average loss of 12% from their starting weight up to the time of sacrifice. Histologically and by electron microscope no widespread epithelial necrosis was detected in the kidney tissue[8] but a remarkable diffuse dilatation was noted of proximal and distal tubules in both animal groups (63 and 87 h) associated with a loss of basal infoldings.

A strong decrease of activity is shown by the (Na^+K^+) ATPase representing the transport enzyme (61% below normal for animals D and E and 57% for C and F) followed by a lesser one for (Mg^{++}) ATPase (21% and 20% respectively) (Table I).

Table II demonstrates variable diminution of several dehydrogenases, i.e. ICDH and MDH, and a marked decrease of the activity of a.Ptase. In contrast to the in vitro study of VOGEL et al.[14] we noted a marked increase of G-6-PDH activity by 114%, only a slight diminution of LDH and unchanged HK activity in the kidney homogenate 63 h after application of folic acid.

Discussion. Our results revealed an inhibition of various enzyme systems in the rat kidney homogenate after folic acid. The decreased (Na^+K^+) ATPase activity corresponds with data of BRADE et al.[4] who described reversible reduced reabsorption capacity for sodium ions at 72 h post-injection. Remarkable is the parallelism of the histological findings with a loss of basal infoldings mainly within the proximal tubules[4,8] and the severe loss of (Na^+K^+) ATPase activity. After folic acid administration, proximal and distal tubules are markedly dilated in a manner that is known for human kidney insufficiency[15]. On the basis of our findings, the question may remain unanswered whether the decreased (Na^+K^+) ATPase activity is caused by the diminution of the basal cell surface of the dilated tubuli with lost infoldings or whether the dilatation did result from a specific inhibitory effect by folic acid on several enzyme systems required for active transport function, e.g. (Na^+K^+) ATPase and some dehydrogenases mainly of the Krebs cycle, i.e. ICDH and MDH. Under the experimental conditions of VOGEL et al.[14] folic acid and several of its analogues inhibited completely glutamic acid dehydrogenase, LDH, G-6-PDH and MDH at concentrations as low as $8 \times 10^{-4} M$. Evidence is available for a specific inhibitory effect on enzymes by folic acid: a.Ptase, a membrane-bound enzyme, also showed a severe loss of activity although the brush border remained largely intact.

The increased activity of G-6-PDH corresponds with data from TAYLOR et al.[5] who described stimulated kidney growth and nucleic acid synthesis in the intact animal effected by folic acid. FARQUHAR et al.[16] revealed a coincidence of G-6-PDH activity increase and DNA peaks lasting for 96 h after unilateral nephrectomy in rats. The strong stimulus for initiation of DNA synthesis and cell proliferation after folic acid may explain the lack of an

Table I. Enzymatic activities of rat kidney after folic acid (250 mg/kg body wt.)

Animals	Total ATPase	(Mg^{++}) ATPase	(Na^+K^+) ATPase
D, E (63)[a]	6.691[b] ± 0.251[c] (8)[d]	4.990 ± 0.152 (8)	1.697 ± 0.187 (8)
C, F (87)	6.917 ± 0.219 (8)	5.030 ± 0.306 (8)	1.875 ± 0.201 (8)
Normal (6 animals)	10.624 ± 0.397 (17)	6.305 ± 0.180 (17)	4.331 ± 0.279 (17)

[a] Hours after i.v. application of folic acid. [b] Activity expressed as moles of inorganic phosphate per kg dry weight/h (MKH). [c] S.E.M. [d] Number of samples analyzed.

Table II. Mean enzymatic activities of rat kidney homogenate removed 63 h after folic acid (250 mg/kg body wt.) animal E

Enzyme	Control	Folic acid	Change in %
G-6 PDH	1.42 (3)[a]	3.03 (3)	+114
ICDH	42.07 (3)	18.39 (3)	— 56
MDH	164.48 (3)	86.07 (3)	— 48
LDH	51.95 (3)	43.38 (3)	— 16
A.Ptase	16.98 (3)	5.77 (3)	— 66
HK	1.13 (3)	1.13 (3)	0

[a] Mean activity expressed as moles of substrate converted per kg dry weight/h (MKH). Number of samples analyzed in parenthesis.

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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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