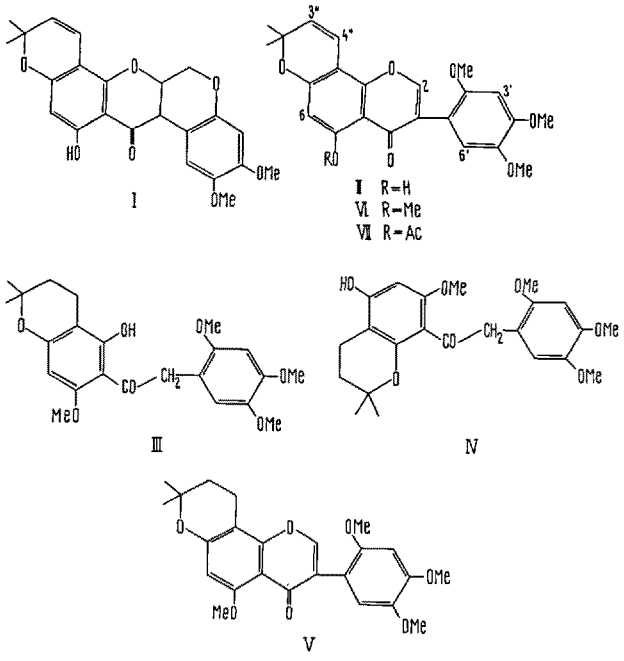

an acetate (VII, mp 212–215°, IR 1777, 1658, 1640 cm^{-1}, NMR 2.39$_s$ (CH_3CO), 7.86$_s$ (C_2-H)) (lit.[1] mp 210°). The spectral properties of the synthetic samples II, VI and VII were superimposable upon those recorded earlier [2] for natural toxicarol isoflavone and its derivatives.

Zusammenfassung. Die Synthese von Toxicarol Isoflavon (2″, 2″-Dimethylpyrano(6″,5″:7,8)-5-hydroxy-2′,4′ 5′-trimethoxyisoflavon) aus 2,2-dimethyl-5-hydroxy-7-methoxychroman wird beschrieben.

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Different Response of Adenine Nucleotide Synthesis de novo in Kidney and Brain During Aerobic Recovery from Anoxia and Ischemia

Anoxia or ischemia causes in various mammalian tissues a substantial loss of nucleotides due to dephosphorylation and further degradative processes[1,2]. In order to clarify whether the different abilities of organs to recover functionally from anoxia or ischemia are related to their capability to restore normal nucleotide levels, studies of the post-anoxic or post-ischemic synthesis de novo of adenine nucleotides were performed on kidney and brain. These two organs are known to exhibit very pronounced differences with respect to their potency of post-anoxic recovery.

Material and methods. In a first series of experiments slices of kidney and brain cortex of rats were incubated anaerobically in Krebs-Ringer-bicarbonate medium containing 0.25 m*M* glycine (preparation of slices, conditions of incubation and composition of the medium see[3]). After 30 min of anaerobic incubation (95% N_2–5% CO_2) the slices were allowed to recover aerobically (95% O_2–5% CO_2) for 60 min. During this period the synthesis de novo of adenine nucleotides (ATP, ADP, AMP) was determined by measuring the incorporation of 2-^{14}C-glycine (0.7 μCi/ml medium) into the adenine ring of the nucleotides (methodical details see[3]). Slices incubated only aerobically but otherwise treated alike served as controls.

A second series of experiments was performed in vivo on kidney and brain of ether anesthetized rats respirated artificially. Unilateral renal ischemia (30 min) was achieved by ligation of the a. renalis, brain ischemia (20 min) by ligation of all arteries originating from the arcus aortae. 30 min after re-establishment of the circulation, a period sufficient for the elution into the blood of protein and nucleotide degradatives accumulating during ischemia, the animals were exposed for 60 min to i.v. injected 1-^{14}C-glycine (25 μCi/100 g body weight), the incorporation of which into adenine nucleotides of kidney and brain was measured. Control values were obtained from sham-operated animals.

In both series of experiments, the tissues were quickly frozen in liquid nitrogen. Preparation of $HClO_4$ extracts, quantitative determination of ATP, ADP and AMP and measurements of the total radioactivity of adenine nucleotides were done according to methods already described[1,3]. Glycine was quantitated using a distillation procedure[4]. The radioactivity of glycine was measured either in an aliquot of the distillate (experiments with 2-^{14}C-glycine) or in a portion of the eluate containing the purified glycine after its isolation by column chromatography[5] (experiments with 1-^{14}C-glycine).

The rates of biosynthesis de novo of adenine nucleotides were calculated by relating the total radioactivity of adenine nucleotides, which results only from the incorporation of radioactive glycine during the de novo-formation of the purine ring, to the mean specific activity (MSA) of the tissue glycine precursor pool. MSA values were computed in each experiment from the specific activities of the tissue glycine always determined after 60 min of exposure to labelled glycine, and a correction factor obtained from special kinetic studies on the time dependent changes of the specific activities of the tissue glycine (Figures 1 and 2).

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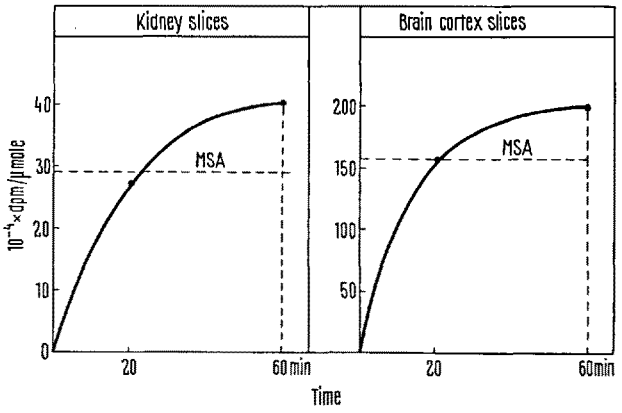

Fig. 1. Time course of the changes of the specific activities of glycine (dpm/µmole) in slices of kidney and brain cortex during 60 min of aerobic incubation. The medium contained 0.25 *M* glycine and 0.7 µCi 2-^{14}C-glycine/ml. Due to the ratio of slices to medium (1:24, w/v) glycine radioactivity in the medium remained fairly constant over the incubation time. Mean specific activities of glycine (MSA) for the period of 60 min (horizontal broken lines) were calculated dividing the planimetrically determined areas below the curves by the incubation time. MSA of glycine thus obtained divided by the specific activity of glycine after 60 min of exposure yields the correction factor, which was used in order to calulate the MSA values of glycine in all the other in vitro-experiments.

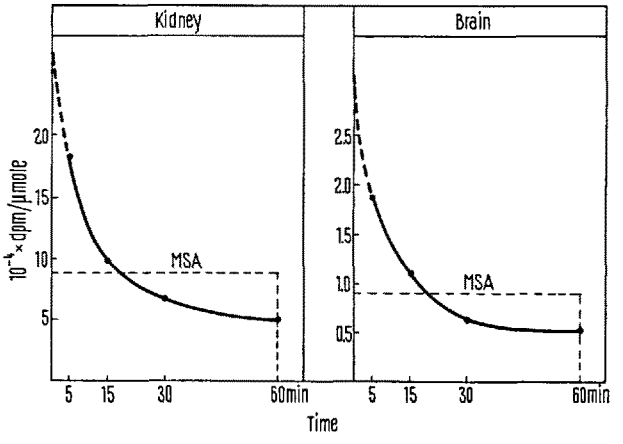

Fig. 2. Time course of the changes of the specific activities of glycine (dpm/µmole) in kidney and brain in vivo under normoxic conditions during 60 min exposure of rats to i.v. injected radioactive glycine (25 µCi 1-^{14}C-glycine/100 g body weight). The exponential decrease of the specific activity of the tissue glycine is mainly due to an intensive metabolic utilization of glycine. For details of calculation of the MSA of glycine and of the correction factor used for the determination of the MSA values of glycine in all the other in vivo-experiments see legend to Figure 1.

Results and discussion. In Table I data are summarized concerning the rates of adenine nucleotide synthesis de novo in kidney and brain tissue. From these figures it becomes evident that during post-anoxic and post-ischemic recovery nucleotide synthesis de novo is considerably enhanced in kidney tissue, but remains almost unchanged in brain tissue. Compared to the corresponding control values, the rates of nucleotide synthesis increase more than 2-fold in kidney slices and almost 10-fold in the kidney in vivo. These results can be considered to reflect the actual status of nucleotide metabolism in these organs, since anoxia or ischemia induced changes of size and/or of radioactivity of the tissue glycine precursor pool, which would lead to erroneous values for the calculated rates of nucleotide synthesis, do not occur (Table II). According to the data in Table II, concentrations and mean specific activities of glycine are much higher in brain slices than in the brain in vivo, indicating an intensified accumulation of glycine in the isolated tissue, in which the accessibility to glycine is not limited by the blood brain barrier.

The greatly increased de novo-formation of adenine nucleotides in kidney during post-anoxic and post-ischemic recovery can be explained by the release of the adenine nucleotide dependent feedback inhibition of purine biosynthesis de novo[6], since the concentrations of ATP, ADP and AMP become considerably diminished during anaerobic incubation or ischemia and remain still on a

Table I. Rates of adenine nucleotide synthesis de novo (nmoles/g/h) in kidney and brain in vitro (slices) and in vivo under normoxic conditions and during post-anoxic or post-ischemic recovery

	In vitro		In vivo	
	Normoxia	Post-anoxic recovery	Normoxia	Post-ischemic recovery
Kidney	226.9 ± 26.1 (*n* 6)	500.4 [a] ± 114.8 (*n* 5)	107.6 ± 16.1 (*n* 6)	953.0 [b] ± 179.8 (*n* 5)
Brain	13.3 ± 1.5 (*n* 8)	12.9 ± 1.2 (*n* 9)	33.3 ± 3.8 (*n* 4)	38.7 ± 9.6 (*n* 4)

Mean values ± SEM; *n*, number of experiments. [a] $P < 0.05$; [b] $P < 0.0025$.

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Table II. Intracellular concentrations and MSA values of glycine in kidney and brain in vitro (slices) and in vivo under normoxic conditions and during post-anoxic and post-ischemic recovery

		In vitro			In vivo		
		n	µmoles/g	MSA (10^{-4}dpm/µmole)	*n*	µmoles/g	MSA (10^{-4} dpm/µmole)
Kidney	Normoxia	6	2.17 ± 0.29	31.55 ± 4.82	6	3.08 ± 0.13	8.40 ± 0.77
	Recovery	5	2.94 ± 0.40	25.90 ± 5.14	5	3.13 ± 0.12	7.98 ± 0.75
Brain	Normoxia	8	9.02 ± 0.61	135.30 ± 7.98	4	2.60 ± 0.25	0.78 ± 0.11
	Recovery	9	7.97 ± 0.60	123.27 ± 6.69	4	2.67 ± 0.28	0.90 ± 0.10

The glycine concentrations were measured 60 min after application of labelled glycine. For calculation of MSA of glycine see methods and legends to Figures 1 and 2. Mean values ± SEM; *n*, number of experiments.

reduced level during the subsequent recovery period of 60 min. A similar mechanism seems to be also responsible for the higher rate of nucleotide formation in aerobically incubated kidney slices as compared to the normoxic kidney in vivo. This conclusion is based on our present and former findings[7], according to which the concentration of adenine nucleotides in kidney slices incubated aerobically amounts only to about 50% of that in the normoxic kidney in vivo.

Although in brain tissue too, the concentrations of adenine nucleotides decrease remarkably due to anoxia or ischemia, the rate of nucleotide synthesis does not increase in the subsequent periods of post-anoxic or post-ischemic recovery. It seems therefore reasonable to assume that brain tissue – as a consequence of different properties of the first regulating enzyme of purine biosynthesis de novo (glutamine phosphoribosylpyrophosphate amidotransferase) – does not respond to a release of feedback inhibition.

Our results reveal a parallelism between the ability of the kidney to increase the de novo-formation of nucleotides and its well-known capacity to recover functionally after lack of oxygen. In recent investigations, a similar parallelism could be demonstrated also in the heart[8]. On the other hand, the inability of brain to recover functionally after longer periods of anoxia is paralleled by its inability to increase nucleotide synthesis de novo. It therefore seems very likely that organs capable of a complete post-anoxic metabolic and functional restoration are characterized by their ability to enhance nucleotide synthesis de novo subsequent to severe lack of oxygen.

Zusammenfassung. Unter Verwendung von ^{14}C-markiertem Glycin wurden durch Bestimmung der mittleren spezifischen Aktivitäten von Glycin im Gewebe und durch Messung der Glycin-Inkorporation in die Adenin-Nucleotide die de novo-Syntheseraten dieser Verbindungen in Niere und Gehirn in vitro und in vivo unter normoxischen Bedingungen sowie nach vorausgegangener Anoxie bzw. Ischämie bestimmt. Zwischen der Fähigkeit eines Organs zur postanoxischen Nukleotidsynthese-Steigerung und seiner funktionellen Erholungsfähigkeit nach Sauerstoffmangel scheinen enge Beziehungen zu bestehen.

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Plasma Protein and Free Fatty Acid Levels in Male Whirler Mice

Previous investigations of body weights, metabolic and endocrine differences between homozygous male and female whirler mice (wi wi) and their phenotypically 'normal' heterozygous whirler littermates (wi+) have indicated significantly decreased body weights and growth rates, increased adrenocortical function and heightened metabolism rates in the whirler mice[1–4]. Plasma glucose and liver glycogen studies of male mice revealed significant decreases in these biochemical parameters[5] indicative of significant alterations in the carbohydrate utilization and metabolic processes of the whirler animals. The homozygous whirler mice, one of a group of waltzing recessive mutations, are extremely nervous, restless and excitable, displaying rapid clockwise and/or counterclockwise circling activity as well as head-shaking and deafness[6]. Neurological and labyrinthine abnormalities have been associated with the waltzing syndrome[6]. The present study sought to determine possible variations and differences in protein and lipid metabolism by analyses of plasma protein fractions and free fatty acid levels in the homozygous whirler vs. phenotypically 'normal' heterozygous whirler littermates.

The breeding stock of homozygous and heterozygous whirler mice were obtained from Jackson Laboratories, Bar Harbor, Maine. Male homozygous and heterozygous littermates were selected for experimentation from the matings of phenotypically 'normal' heterozygous females to whirler males. The mice were raised in air-conditioned quarters maintained at 73–75 °F. and were housed 2 per cage.

All homozygous and heterozygous male littermates were weaned at 4 weeks, and body weights were determined at weekly intervals. Experiment I, entailing the plasma protein investigations, used homozygous and heterozygous male whirler mice averaging 9 weeks of age; 4 tail blood samples per animal were collected in heparinized, precalibrated, micro-hematocrit tubes. The hematocrit values were obtained with an Adam Readacrit Micro-Hematocrit centrifuge. Total plasma protein[7] levels were measured by a Goldberg Refractometer (American Optical Co.).

The plasma specimens were subjected to Gelman rapid electrophoretic procedures[8] to separate the albumin, α-1, α-2, β- and γ-globulin fractions on cellulose polyacetate strips. A Beckman-Spinco analytical instrument equipped with Scan-A-Tron was used to quantitatively analyze the findings.

Table I presents the body weights and blood values for the 2 groups of mice. The body weights of the homozygous

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