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.

E. GERLACH[9], P. MARKO, H.-G. ZIMMER, I. PECHAN[10] and CH. TRENDELENBURG

Abteilung Physiologie, Medizinische Fakultät der Rhein.-Westf. Technischen Hochschule Aachen, D-51 Aachen (Deutschland), 26 Juni 1970.

[7] E. GERLACH, B. DEUTICKE, R. H. DREISBACH, Pflügers Arch. ges. Physiol. *284*, 213 (1965).
[8] Unpublished results of this laboratory.
[9] This work was supported by a grant from the Deutsche Forschungsgemeinschaft No. Ge 129/7.
[10] Fellow of the A.-v.-Humboldt-Stiftung. Permanent address: Biochemical Institute, Comenius University, Bratislava (Czechoslovakia).

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

[1] A. M. SACKLER, A. S. WELTMAN, P. STEINGLASS and S. D. KRAUS, Fedn Proc. *23*, 252 (1964).
[2] A. S. WELTMAN, A. M. SACKLER, R. SCHWARTZ and P. STEINGLASS, Fedn Proc. *24*, 448 (1965).
[3] A. S. WELTMAN and A. M. SACKLER, Proc. Soc. exp. Biol. Med. *123*, 58 (1966).
[4] A. M. SACKLER and A. S. WELTMAN, J. exp. Zool. *164*, 133 (1967).
[5] A. S. WELTMAN, A. M. SACKLER, A. S. LEWIS and L. JOHNSON, Physiol. Behav. *5*, 17 (1970).
[6] H. GRÜNEBERG, *The Genetics of the Mouse* (Martinus Nijhoff, The Hague 1952).
[7] K. G. BARRY, A. W. MCLAURIN and B. L. PARNELL, J. Lab. clin. Med. *55*, 803 (1960).
[8] R. O. BRIERE and J. D. MULL, Am. J. clin. Path. *42*, 547 (1964).

Table I. Hematocrit and plasma protein values in homozygous and heterozygous male whirler mice (age $8^1/_2$ weeks)

Group	n	Body wt. (g)	Hemato-crit (%)	Total plasma protein (g/100 ml)	n	Albumin (g/100 ml)	Globulins (g/100 ml)				
							α-1	α-2	β	γ	Total
Homo. whirler ± S.E.	9	20.2 ± 1.0	48.6 ± 1.3	5.6 ± 0.1	8	3.66 ± 0.09	0.26 ± 0.02	0.50 ± 0.04	0.81 ± 0.07	0.44 ± 0.03	2.01 ± 0.16
Hetero. whirler ± S.E.	8	22.7 ± 0.4	49.7 ± 1.3	6.0 ± 0.1	8	3.28 ± 0.10	0.37 ± 0.01	0.64 ± 0.03	1.09 ± 0.04	0.58 ± 0.04	2.69 ± 0.08
Difference (%)		—11.0	—2.2	—6.7		+11.6	—29.7	—21.9	—25.7	—24.1	—25.0
P value		0.05	0.56	0.02		0.02	<0.01	0.01	<0.01	0.01	<0.001

versus heterozygous whirler males were significantly lower as analyzed by standard *t*-test procedures[9]. No significant differences were noted between the hematocrit observations although the values were moderately lower (—2.2%) in the whirler group. Analyses of the total protein data revealed significant decreases in the plasma protein levels of the whirler mice. Electrophoretic analyses indicated, however, that plasma albumin levels were significantly higher in the whirler mice but the α-1, α-2, β- and γ-globulin values were significantly lower when compared on an individual or total globulin basis. Apparently, the greater decrease in the globulin fractions was sufficient to override the increase observed in plasma albumin.

It would appear that the significantly heightened locomotor activity and metabolism rates[4,5] in the whirler mice pose greater nutritive demands on the homozygous mutant. The significant increase in plasma albumin may be in accord with significantly higher food consumption observed in whirler mice[3]. Albumin is affected more readily than globulins by nutritional factors[10]. Since albumin synthesis appears to be limited to the liver[11], the significant increase in plasma albumin of the whirler mice may be in accord with previously noted increases in relative liver weights[2,4,5].

The significant decreases in the globulins, especially the γ-globulins or antibody fraction, could likewise have been anticipated from earlier findings. Approximately 20% of the total globulins are synthesized in extrahepatic tissues (lymphoid organs and tissues and the reticuloendothelial system)[11]. Significant decreases have been noted in the relative weights of lymphoid organs such as the thymus in homozygous vs. heterozygous female and male whirler mice[1,2,4]. Total white blood cell and eosinophil counts have also been found to be significantly lower in the whirler males[2]. Undoubtedly, the alterations in lymphoid organ, white blood cell counts and gamma globulin content are in turn related to the significant increases in adrenocortical activity of the homozygous whirler mice[4,5]. The decrease in γ-globulin may explain the decreased viability of the whirler mice. The simultaneous presence of an increase in the albumin level and decreases in the globulin fractions, or vice versa, agree with changes found in various metabolic disorders and diseases[12].

Experiment II involved 13-week-old male mice sacrificed by rapid decapitation for plasma free fatty acid determinations[13]. Table II again indicates significant decreases in the body weights of the whirler vs. heterozygous male mice. The 8.7% increase in the plasma free fatty acid levels of the whirler group was not statistically significant. Possibly, the increases may be indicative of somewhat higher mobilization of the lipids but further studies are required. It should be noted that whirler animals appear lean compared to the plumpness of the 'normal', less active littermates. Internal examination revealed a lack of adipose tissue deposits in the abdominal cavity[5] of the whirler mice. In conclusion, while no definitive differences could be noted in the free fatty acid levels, the plasma protein studies revealed significant differences in the protein metabolism and fractions of the whirler mice. These alterations, together with differences in carbohydrate metabolism, reflect the changes due to behavioral characteristics, nutritive requirements, and endocrine function of the whirlers.

Résumé. Des analyses électrophorétiques d'échantillons de plasma sanguin de souris tourneuses mâles, homozygotes et hétérozygotes ont montré des taux de protéines plasmatiques totales nettement inférieurs chez les homozygotes. Bien que diverses fractions globuliniques soient nettement diminuées, les taux d'albumine furent sensiblement plus élévés chez le mutant homozygote.

A. S. Weltman, A. M. Sackler and G. O'Connor[14]

Laboratories for Therapeutic Research, Brooklyn College of Pharmacy, L.I.U., Brooklyn. N.Y. 11216 (USA), and University of Vermont, College of Medicine, Burlington (Vermont, 05401 USA), 24 February 1971.

Table II. Plasma free fatty acids in homozygous and heterozygous male whirler mice (age 13 weeks)

Group	n	Body wt. (g)	n	Plasma F.F.A. (μg/l)
Homo. whirler ± S.E.	10	22.1 ± 0.8	10	935.91 ± 78.57
Hetero. whirler ± S.E.	11	25.8 ± 0.4	10	860.92 ± 96.30
Difference (%)		—14.3		+8.7
P value		<0.001		0.55

[9] G. W. Snedecor, *Statistical Methods* (Iowa State College Press, Ames 1949).
[10] A. Cantarow and B. Schepartz, *Biochemistry* (W. B. Saunders, Philadelphia 1967).
[11] A. White, P. Handler and E. L. Smith, *Principles of Biochemistry* (McGraw-Hill, New York 1968).
[12] E. S. West, W. R. Todd, H. S. Mason and J. T. Van Bruggen, *Textbook of Biochemistry* (Macmillan and Co., New York 1966).
[13] V. P. Dole, J. clin. Invest. *35*, 150 (1956).
[14] University of Vermont, College of Medicine, Burlington (Vermont 05401, USA).