From the Neurochemical Institute Copenhagen N (Denmark)

Effect of fat-free diet on enzyme activities of rat testes

By J. CLAUSEN

With 6 tables

(Received February 11, 1969)

Symptoms of deficiency in essential fatty acids (EFA) were originally described in rats (4). Since then the EFA deficiency syndrome has been attributed to nutritional deficiency in linoleic or linolenic acid. Arachidonic acid has also been shown to protect or cure the deficiency syndrome (31).

Since the derivatives of EFA are synthesized through two main metabolic pathways dependent on and influenced by linoleic or linolenic acid (11, 18), and since these derivatives are incorporated into particular structures, the EFA-deficiency syndrome may be associated with functional changes of biomembranes. This seems to be substantiated by the demonstration of decreased oxidative capacity of liver homogenates from rats raised on a diet deficient in EFA (16, 20, 26, 28).

Only a few symptoms of EFA deficiency seem to be specific and, apart from the estimation of water intake in relation to body-weight, the clinical symptoms cannot be used quantitatively. More reliable methods for quantitative estimation of EFA deficiency are based upon estimation of fatty acid composition of different organs. The changes consist of an increase in palmitoleic acid and $C_{20:3}$ and seem clearly demonstrable in testes (23); see also the review (1). Furthermore, as EFA deficiency, affecting the fatty acid composition of biomembranes, is associated with degeneration of the testes and aspermia, the probable enzymic changes induced by the deficiency may be demonstrable in testicular particulate fractions. The present communication intends to elucidate these aspects by demonstration of a reduced content of mitochondrial enzymes of testes and sperm cells. The enzymes studied consist of the succinic acid dehydrogenase (SDH) and the lactate dehydrogenase isoenzymes (LDH) of which one, LDH_X, is characteristic of testes and sperm cells (34, 35, 37). An estimation of these two enzymes may be used for support and quantitative estimation of EFA deficiency.

Experimental

Material

Biological material. Adult male Wister rats, 200 g in weight, were used. They were kept in plastic cages on a metal grating allowing for elimination of faeces. During the experimental period, experimental control animals were killed by decapitation and the testes were excised. The testes were immediately washed in 0.9% (w/v) aqueous NaCl and divided into two parts; one part was used for the determination of the total enzyme activities and the other part for the determination of the LDH isoenzyme pattern. The extract used for measuring the total enzyme activity was prepared by homogenizing the tissue sample with five parts (w/v) of 0.9% w/v NaCl containing 1% Triton-X-100 in a Hostalen centrifuge

tube by means of a rounded glass stirring rod (140 rev/min at 0°). The sample was then centrifuged at 4° for 30 min at 12000 g_{max}. Extracts for electrophoresis were prepared without adding Triton-X-100, as this detergent gave rise to an artificially stained zone in the electrophoresis.

Chemicals. Unless otherwise stated, the chemicals used were those of highest purity obtained from British Drug House Ltd.

Diet

Normal female Wistar rats were mated with the same male. Fourteen days before delivery one group (1) of pregnant females was fed a diet low in EFA and a control group (2) was fed a normal diet (Table 1). The newborn (male) rats were selected for continuous feeding; during a 3 to 8 month period these were fed ad. lib. on one or other of the diets.

Table 1. Composition (g/100 g) of the diets

Normal diet[1])		Diet deficient in polyunsaturated fatty acids	
Barley	20.5	Low-vitamin casein[2])	24.0
Oats	20.0	Sucrose	72.1
Ground wheat bran	20.0	Salt mixture[3])	3.9
Lucerne flour	20.0	Vitamin mixture[4])	
Ground maize	10.0		
Ground sunflower seed	5.0		
$CaCO_3$	1.0		
Fish flour	1.0		
Bone flour	1.0		
Ground nut cakes	1.0		
Salt mixture[3])			
Vitamin mixture[4])			

DL-Methionine, 12 g, Vitamin A (palmitate 0.15 ml), 150000 i. u., Vitamin D_3, 5000 i. u., α-Tocopherol, 1500 mg, Menadione (sodium bisulfite), 100 mg, Thiamine B_1, 200 mg, Riboflavin, 200 mg, Vitamin B_6, 150 mg, Vitamin B_{12}, 0.3 mg, Pantothenic acid (Ca), 500 mg, Niacin, 500 mg, Choline HCl, 10 mg, Folic acid, 100 mg, Biotin, 3 mg, p-aminobenzoic acid, 1 g, Inositol, 1 g, Ascorbic acid, 200 mg.

Methods

LDH activity. LDH (EC 1.1.1.27, L-lactate: NAD-oxido-reductase) activity was determined on 100 µl samples of suitable dilution, as previously described [CLAUSEN and ØVLISEN (7)]. All results for total LDH activity are mean values of duplicate determinations.

The LDH activity is expressed as µmoles NADH oxidized per min per 1 ml original samples at pH 7.5 using a reaction mixture containing 1.0 mM-sodium pyruvate and 0.33 mM-NADH in 50 mM-sodium phosphate buffer. The specific LDH activity was similarly expressed in relation to the amount of protein in the sample.

[1]) 26.1% of the total fatty acids is $C_{16:1w7}$ and $C_{18:1w9}$ unsaturated fatty acids.

[2]) Fat free [below 0.02% fat, extracted with chloroform + methanol (2 + 1), polyenoic fatty acids below 0.001% of total diet].

[3]) No. 2, U.S. Pharmacopeia XIII.

[4]) Vitamin mixture (per 10 kg):

SDH activity. (EC 1.3.99.1, succinate: acceptor oxidoreductase) was used as an arbitrary measure of mitochondria content of particulate fractions. The SDH activity was measured using 2:6-dichlorophenol-indophenol (DPIP) as electron acceptor (13). The reaction mixture (2 ml) for routine assay consisted of: 40 μl particulate fraction, 20 mM sodium succinate, 2.0 mM KCN, 0.05 M EDTA (disodium salt of ethylenediaminetetra-acetic acid) and 500 mg bovine albumin (Behringwerke, Germany) per l. The dehydrogenase activity was assayed by reading the change in optical density at intervals of 30 sec for 5 min against a blank containing all components except succinate (temperature 25°).

These assay conditions were fixed upon systematic variation in pH concentration of succinate and DPIP (27). The concentrations used were those giving saturation with succinate and maximum activity with DPIP. Higher concentrations for this component caused inhibition of SDH activity.

The optical density was read at 600 nm. On the basis of the extinction change at 25° and the molar extinction coefficient of DPIP at 600 nm of $1.91\ 10^7$ cm²/mole (2), the number of enzyme units present in 1 ml of the original sample was expressed as μmoles DPIP reduced per min. The specific activity of the samples was expressed as μmoles DPIP reduced/min per mg protein.

Protein content of samples. The protein content of the samples assayed for LDH and SDH activity was determined by a modified Lowry method (21) with tyrosine as standard. The tyrosine values were converted into mg protein on the basis of the tyrosine content of bovine serum albumin. Before the protein determination, 50 μl particulate suspension was solubilized at 37° with 200 μl 2 N-NaOH for 16 h.

Determination of LDH isoenzymes. LDH isoenzyme was determined by microelectrophoresis on agar-gel slides (33) at pH 8.6 in 0.05 M-sodium barbitone buffer containing 1% DIFCO Special Noble Agar (DIFCO Ltd., USA).

The separated isoenzymes were made visible by formazan (12). The relative proportions of the different isoenzyme bands were estimated by scanning the slides at 546 mμ in the Vitatron photometer (Vitatron Ltd., Dieren Holland) equipped with a scanning device, an automatic recorder, and an integrator (7).

Preparation of tissue suspension for ultracentrifugation. Rat testes (6 g) were cut into pieces and rinsed with 0.9% saline to remove blood. One gram of testes was added to 3.0 ml 0.32 M sucrose and homogenized at 250 rev/min for 2 min in an all-glass POTTER-ELVEHJEM homogenizer with a loosely fitting pestle. All fractions were combined. About 1 ml of each tissue suspension was removed for measurement of LDH activity, isoenzyme and protein contents.

Preparation of subcellular fractions of testes. The tissue suspensions were diluted with 30 ml of 0.32 M sucrose. The nuclear fraction (N) was isolated by centrifugation for 10 min (4°) at 900 g in a Sorvall RC-2 centrifuge. The collected supernatant fractions were then centrifuged at 11500 g_{max} for 20 min (4°) in a Spinco (L-2) ultracentrifuge to obtain the crude mitochondrial sediment.

According to the density gradient centrifugation technique (9), the mitochondrial sediment (M) was resuspended in 10 ml 0.32 M-sucrose and layered on a 40 ml gradient consisting of equal volumes of 0.8, 0.1, 1.2 and 1.4 M solutions of sucrose. Centrifugation at 50000 g_{max} for 2 h (4°) produced in each gradient the corresponding mitochondrial subfractions: A, B, C, D and E. The particulate mid-layers were removed separately for re-suspension in 0.15 M-NaCl. After centrifugation at 10000 g_{max} for 10 min (4°), each subfraction was further washed three times in 0.15 M-NaCl. The microsomal sediment was isolated from the 11500 g_{max} supernatant fraction by centrifugation for 2 h (4°) at 100000 g_{max}. Each of the fractions obtained, the nuclear sediment, the crude mitochondrial and microsomal sediments were purified by washing three times with 0.15 M-NaCl.

Extraction of enzymes from particulate fractions. The 1 ml samples of the above-mentioned tissue suspensions and all of the subcellular fractions thus collected were suspended in 0.5 or 1.0 ml 0.15 M-NaCl, partly homogenized at 500 rev/min for 5 min in an all-glass homogenizer, and centrifuged at 18000 g_{max} for 20 min. In some experiments

the mitochondrial and microsomal pellets after homogenization were further extracted with 1.0 ml of a 1.0% (w/v) Triton-X-100 in 0.15 M-NaCl, re-homogenized, and centrifuged as above. The resulting supernatant fractions were tested for total LDH activity, total protein, isoenzyme pattern and SDH activity.

Electron microscopy. Selected samples of the particulate fractions obtained by ultracentrifugation were fixed in a 1% osmium-isotonic veronal buffer of pH 7.4 for 30–60 min, dehydrated in acetone, and embedded in Vestopal (Martin Jaeger Ltd, Switzerland). Sections were cut with glass knives on an LKB ultratome and examined by a Philips 100 B electron microscope. Photographs were taken at magnifications of 1300 and 7500.

Gas chromatography. The fatty acid composition of the testes lipids was evaluated by gas chromatography of the methyl esters. The fatty acids of the lipids were saponified with 2 ml 1 N-KOH in ethanol for 30 min (25). The fatty acids were liberated by addition of 1.5 ml 2 N-HCl + 5 ml H_2O. They were dissolved in 10 ml di-ethyl ether and washed three times with 10 ml 0.1 N-HCl in a separatory funnel. The ether phase was dried over a hydrous $CaCl_2$ for 16 h and concentrated to dryness under N_2. The fatty acids were afterwards methylated with 6 ml superdry methanol saturated with HCl gas; 250 µl dry benzene was added as catalyst. The methylation was performed for 30 min at 80° in sealed bottles. The methyl esters were extracted and washed as described above. Finally, the esters were concentrated by evaporation of the ether below a stream of N_2. Gas chromatography was performed on samples containing 0.2 µl methyl esters in a Perkin-Elmer flame-ionization gas chromatograph F-11 [steel column dimensions 2 m × 1 mm, stationary phase 8% 1.4 butane-diol succinate on chromosorb W (80–100 mesh), carrier gas N_2, temperature 190°]. Peaks on the chromatograms were quantitated by triangulation and the proportions of the individual fatty acids were expressed as percentage of the total. The fatty acids were identified by log plotting and by comparison with standard markers of fatty acid methyl esters obtained from the Hormel Institute, Minnesota, USA.

Statistical evaluation of results. The results obtained were indicated as the arithmetric mean values and evaluated statistically by means of the chi-square test (8). The chi-squares were estimated at the Northern European University Computing Center, using computer Contingency Table Analysis program no. BMD O 2 S (10). From the chi-squares obtained and the number of freedoms ($n = 1$), the level of significance was estimated. Only p-values below the level of 0.05 were indicated in the tables.

Results

Evaluation of EFA deficiency. During a period of 3 months in which the group 1 rats were raised and fed on a diet deficient in EFA, symptoms chracteristic of EFA deficiency developed, including increased water intake, retarded growth, and skin eruptions. As further evidence of EFA deficiency, gas chromatography of testes tissue and fatty acids carried out on these animals at the time of killing revealed a significant decrease in the amounts of linoleic acid and linolenic acids associated with a relatively significant increase in the saturated fatty acids C_{16}, C_{18} and $C_{18:1}$ (Table 2). Thus, the amount of linoleic acid and linolenic acid was decreased to 2.4 and 0.0% respectively. Furthermore, the animals showed lack of sperm cells in the epididymic tracts as judged by microscopic examination.

Topographic studies of the occurrence of LDH and SDH in particulate fractions of rat testes homogenates. Table 3 shows the highest specific SDH activity to be in the mitochondrial fraction D obtained by sucrose gradient ultracentrifugation of homogenate of whole rat testes. This finding corresponds well with the results of the electron microscopical investigations revealing the mitochondrial fraction D to be rich in morphologic structures characteristic of mitochondria, e. g. of ellipsoid structures internally equipped with cristae.

Table 2. The fatty acid composition (%) of rat testes from two groups of rats – one group fed an optimal diet and one fed a diet deficient in polyunsaturated fatty acids (PUFA) for a twelve week period. Only the significant changes in fatty acids are expressed here. The gaschromatographic analysis is based on assays of fatty acids possessing a chain length of C_{10} to $C_{22:6}$. The values indicated are arithmetic means of 10 independent determinations in each group

Fatty acid	Normal diet	Diet lacking PUFA	Chi-squares	Level of significance
C_{16}	10.7	43.9	12.8000	$P < 0.001$
C_{18}	2.5	10.0	5.4945	$P < 0.02$
$C_{18:1}$	16.3	26.2	3.3333	$P < 0.052$
$C_{18:2}$	17.9	2.4	12.3148	$P < 0.001$
$C_{18:3}$	8.5	0.0	6.4286	$P < 0.01$

Tables 3 and 4 demonstrate that the highest specific LDH activity was also localized in the mitochondrial subfraction D. However, the nuclear fraction also contained a high specific LDH activity. After extraction of the particulate fractions with 0.9% aqueous NaCl solution, the precipitates (washed three times) obtained by centrifugation at 20,000 g-max for 0.5 h were re-extracted with 1.0% Triton-X-100 dissolved in 0.9% aqueous NaCl. As shown in Table 3, the re-extraction with Triton-X-100 gave rise to a further liberation of LDH activity. This liberation was especially pronounced in the mitochondrial subfraction D.

Agar-gel microelectrophoresis combined with the Formazan staining technique for LDH activity revealed an uneven distribution of LDH isoenzymes (Table 4) in the particulate fractions. Saline extracts of the particulate fraction gave rise mainly to liberation of LDH isoenzymes containing H and M subunits. Thus the H_4 isoenzyme predominated in the nuclear and the mitochondrial fractions A and B. However, the mitochondrial fractions C, D and E and the microsomal fraction, as well, seem to exhibit a larger content of isoenzymes containing the M-subunit-containing isoenzymes. The LDH_x was found only in saline extracts of the nuclear fraction and the mitochondrial fraction E.

Enzymic changes induced by EFA deficiency. Table 5 demonstrates the differences in total LDH and SDH activities in a group of rats raised on a normal diet and a group of rats raised on a diet deficient in EFA. In the first group, fourteen animals, and in the second group, sixteen animals were studied. The means of the enzyme activities are indicated. From Table 5 it is obvious that a high statistically significant decrease in SDH activity ($P < 0.001$) occurred in the EFA deficient group compared with SDH activity in the normal group. The total LDH activity seemed to be significantly increased in the group deficient in essential fatty acids compared with that of rats on the normal diet.

The changes in the LDH isoenzyme pattern induced by deficiency in EFA are shown in Table 6. During a 3 month period of deficiency the LDH_x activity decreased more than four fold. This decrease was statistically highly significant ($P < 0.001$). The decrease in LDH_x activity appeared to be associated with slight changes in the relative quantities of the other LDH isoenzymes. However, these changes were insignificant.

Table 3. Total lactate dehydrogenase and succinic acid dehydrogenase activities of testes of rats reared and fed on a diet deficient in essential fatty acids. The particulate fractions have been separated by gradient ultracentrifugation in a sucrose gradient (see text). In order to clearly evaluate the possible enzymic differences among the individual particulate fractions, only the central portions of each fraction were isolated by suction from the centrifuge tube. Therefore the yields of total LDH units in the fractions could only be evaluated roughly. The lowest yield encountered was about 1% of total LDH units of crude homogenate in the mitochondrial subfraction A. The highest yield – 10.4% – was found in the mitochondrial fraction D

Fraction		Specific LDH activity (μmoles/min mg protein)	Specific LDH activity (μmoles/min mg protein)
	Expt. 1	Expt. 2	Expt. 3
Nuclear:			
0.9% NaCl extract	0.75	0.32	0.00
Triton-X-100 extract	0.06		
Mitochondrial:			
A			
0.9% NaCl extract	0.16	0.11	0.032
Triton-X-100	0.01		
B			
0.9% NaCl extract	0.92	0.20	0.0091
Triton-X-100	0.06		
C			
0.9% NaCl extract	1.79	0.60	0.087
Triton-X-100 extract	0.06		
D			
0.9% NaCl extract	4.19	1.07	1.83
Triton-X-100 extract	1.13		
E			
0.9% NaCl extract	2.52	0.54	0.64
Triton-X-100 extract	0.30		
Microsomes:			
0.9% NaCl extract	0.27	0.43	0.034
Triton-X-100 extract	0.46		

Subcellular distribution of lactate dehydrogenase isoenzyme during essential fatty acid deficiency. Testes of EFA deficient rats revealed, after differential ultracentrifugation, the presence of the LDH_x isoenzyme only in the nuclei and the mitochondrial subfraction E similar to that found in testes of normal rats.

Table 4. Activity of lactate dehydrogenase and LDH isoenzymes in particulate fractions of testes from normal rats

			Distribution of activity in 0.9% NaCl extract											
	Total activity		% (relative)						μmoles/min mg protein					
Fraction	in 0.9% NaCl extract	In Triton-x-100 extract	H_4	H_3M	H_2M_2	HM_3	LDH_X	M_4	H_4	H_3M	H_2M_2	HM_3	LDH_X	M_4
Nuclear	0.322	0.705	41.9	34.6	3.6	9.1	5.4	5.4	0.135	0.111	0.0116	0.0293	0,0174	0.0174
Mitochondrial: A	0.112	0.414	100.0	0.00	0.00	0.00	0.00	0.00	0.00	0.112	0.00	0.00	0.00	0.00
B	0.196	0.409	78.6	21.4	0.00	0.00	0.00	0.00	0.00	0.154	0.0419	0.00	0.00	0.00
C	0.596	0.244	46.6	53.4	0.00	0.00	0.00	0.00	0.00	0.278	0.318	0.00	0.00	0.00
D	1.070	0.330	36.0	40.0	8.0	16.0	0.00	0.00	0.385	0.428	0.0835	0.171	0.00	0.00
E	0.535	0.406	16.7	10.4	6.3	29.2	18.7	18.7	0.0894	0.0556	0.0337	0.156	0.100	0.100
Microsomal	0.430	0.437	41.6	58.4	0.00	0.00	0.00	0.00	0.149	0.251	0.00	0.00	0.00	0.00
Supernatant of microsomal	0.393	0.830	34.6	46.2	11.5	7.7	0.00	0.00	0.136	0.183	0.0398	0.0302	0.00	0.00

Table 5. The total lactate dehydrogenase (LDH) and succinate dehydrogenase (SDH) activities of normal and EFA deficient rat testes (3month EFA-deficiency; see the text). A statistically significant decrease in LDH and SDH activities occurs in the case of EFA deficiency

	Total LDH μmoles/min/mg prot.	Total SDH μmoles/min/mg prot.
Normal Diet 14 rats	0.479	0.104
EFA deficiency 16 rats	0.541	0.0697
Chi-squares	8.6227	4.6930
P-level	$P < 0.005$	$P < 0.025$

Table 6. The relative distribution (in percent) of lactate dehydrogenase isoenzymes in normal and in EFA-deficient rat testes (3 month EFA-deficiency; see the text). The values indicated are arithmetric means of 16 independent determinations in each group

	H_4	H_3M	H_2M_2	HM_3	LDH_X	M_4
Normal diet 16 rats	35.6	31.0	11.8	12.0	7.4	3.9
EFA-deficient 16 rats	35.0	37.5	11.1	10.4	1.6	4.1
Chi-squares	0.5357	1.4341	0.0168	0.0024	11.8080	0.5357
P-level					$P < 0.001$	

Discussion

Studies on the topographic distribution of the cytochrome system in the sperm cell have revealed that this enzyme system is located in the middle piece (and tail) rather than in the sperm head [Zittle and Zitin, (36); Hrudka, (19); Mann, (22)]. These results, together with the electron microscopic finding of a spiral-like mitochondrial apparatus localized to the middle piece (see the survey by Mann, 1964), seem to indicate a topographical distribution of organelles (and enzymes) unique for sperm cells. Although Clausen, (6) found the highest LDH activity to be in the supernatant of particulate fractions from sperm cells, the middle piece contained LDH_X exclusively. More than 40% of the total LDH_X activity of total sperm cell homogenate was found in the middle piece fraction. The middle piece was also demonstrated to contain the highest SDH activity [Clausen, (6)]. Re-extraction of particulate

fractions with Triton-X-100 revealed that a specific activity as high as that already extracted with 0.9% aqueous NaCl from head and tail could be liberated [CLAUSEN, (6)] from the middle piece, exclusively of the LDH_x type. These findings support the idea that LDH_x and SDH are particularly bound to the middle piece.

The compartmentalization of SDH and LDH_x was also found in the work described here; however, in testes tissue these two enzymes do not occur in the same subfractions as they do in sperm cells. Thus, during fractionation of rat testes the LDH_x was not preferentially localized to mitochondrial subfraction D of rat testes obtained by centrifugation on a sucrose gradient, although this fraction exhibited greater SDH activity than subfraction E containing LDH_x. The results of our studies of the distribution of the isoenzymes in particulate fractions of the rat testes are similar to those of studies of the calf kidney [GÜTTLER and CLAUSEN, (15)] which indicated an affinity of kidney mitochondria for LDH-H_4. However, unlike the kidney nuclei, rat testes nuclei fractions exhibited no specific affinity for LDH-M_4.

As the two enzymes mentioned above thus seem to be more or less firmly attached to particulate fractions of the sperm cell and testes, the activity of these enzymes may be related to membrane structure, e. g. fatty acid composition. This relationship may be caused either by loose binding of the enzyme (LDH_x) to the membrane or probably by a direct (catalytic) involvement of the membrane in the enzymic function (SDH). This is supported by the results of the present experiments, indicating that EFA deficiency is associated with a highly significant decrease in both enzymic activities, and may explain why dietary EFA-deficiency is associated with morphological signs of swelling of the mitochondria (29). This is also supported by the fact that polar lipids are involved in the function of several mitochondrial enzymes, e. g. β-hydroxybutyric acid dehydrogenase and enzymes belonging to the electron transfer chain (14). CERLETTI et al. (5) have reported a decrease in SDH activity during solubilization of SDH. This decrease could be compensated by adding mitochondrial lipids, and as the micellar state of lipids is determined by their content of polyunsaturated fatty acids (32), the present finding of a decrease in SDH activity in EFA deficiency may be accounted for by a change in the micellar state of the lipids necessary for SDH activity.

The present experiments indicate that the determination of the activities of SDH and LDH isoenzyme (LDH_x) in the rat testes is useful in qualitative and probably quantitative evaluation of dietary deficiency in EFA. Both the SDH and the LDH_x fraction of testes and sperm cells seem to be bound to subcellular particles, therefore since the EFA are enriched, especially in ethanolamine phosphatides and phosphatidylinositols, and since these polar lipids are abundant in biomembranes (24), it is proper that EFA of the mitochondrial membrane of testes tissue as well as of the middle piece of the sperm cell should be of importance for the optimal function of enzymes attached to these structures.

Acknowledgements

Gratitude is expressed to Mr. JARL JENSEN, M. Sc., Northern European University Computing Center, for his kind assistance and advice during the statistical evaluation phase of the experimental data obtained.

Zusammenfassung

1. Die bei Ultrazentrifugierung gewonnenen Membranfraktionen der Rattenhoden wurden auf ihre Succinatdehydrogenase-Aktivität (SDH) sowie für ihre Laktatdehydrogenase-isoenzym-Aktivität (LDH_X) analysiert.
2. Die klinischen Symptome an EFA (essentielle Fettsäuren) der Ratten, die auf Futter von geringen EFA-Gehalt erzogen waren, manifestierten sich als Hypo- oder Aspermie. Die Anzeichen waren mit signifikanten Erhöhungen der C_{16}, C_{18}, $C_{18:1}$. Fettsäuren und mit einer Ermäßigung der $C_{18:2}$ and $C_{18:3}$ Fettsäuren verbunden.
3. Die Ermäßigungen der SDH und LDH_X, die durch Mangel an EFA verursacht wurden, waren von hoher statistischer Signifikanz. Die totale LDH-Aktivität (alle 6 LDH-Isoenzyme der Hoden erfassend) zeigte sich dagegen signifikant erhöht.
4. Die hier beschriebenen enzymatischen Veränderungen stimmen mit der Theorie überein, daß die EFA der Membranfraktionen eine Rolle in den biologischen Funktionen dieser Fraktionen spielen.
5. Es wird postuliert, daß die Resultate der Bestimmungen von SDH und LDH_X der Hoden als ein Indikator von EFA-Mangel verwendet werden können.

Summary

1. Particulate fractions of rat testes obtained by ultracentrifugation were studied for their succinic acid dehydrogenase (SDH) and lactate dehydrogenase (LDH) (isoenzymic) activities. The maximal succinic acid dehydrogenase activity was found localized to mitochondrial subfraction D by ultracentrifugation, but the LDH_X isoenzyme was preferentially localized to testicular mitochondrial subfraction E. These findings support the view of a particulate binding or localization of the above-mentioned two enzymes in testes.
2. In rats fed a diet deficient in essential fatty acids (EFA) the clinical symptoms of this deficiency, manifest as hypospermia or aspermia, were associated with a statistically significant increase in C_{16}, C_{18} and $C_{18:1}$ fatty acids and a decrease in $C_{18:2}$ and $C_{18:3}$ fatty acids.
3. A statistically significant decrease in SDH and LDH_X activities was shown to be induced by the deficiency. However, the total LDH activity (including that of all six isoenzymes of LDH in testes) was significantly increased.
4. The enzymic changes described are consistent with the idea that EFA, present in particulate fractions, may play a part in the biological (catalytic) function of these fractions.
5. It is suggested that estimation of SDH and LDH_X activities of testes may be used as an index of the degree of deficiency in EFA.

References

1. Aaes-Jørgensen, E., Physiol. Rev. **41**, 1 (1961). — 2. Basford, R. E. and F. M. Huennekens, J. Amer. chem. Soc. **77**, 387 (1955). — 3. Blanco, A. and W. H. Zinkham, Science **139**, 601 (1963). — 4. Burr, G. O. and M. Burr, J. biol. Chem. **82**, 345 (1929). — 5. Cerletti, P., R. Strom and M. G. Giordano, Biochem. Biophys. Res. Commun. **18**, 259 (1965). — 6. Clausen, J., Biochem. J. **111**, 207 (1969). — 7. Clausen, J. and B. Øvlisen, Biochem. J. **97**, 513 (1965). — 8. Croxton, F. E., Elementary Statistics with Applications in Medicine and the Biological Sciences, pp. 267 and 328 (New York 1959). — 9. DeRobertis, E., A. Pellegrino de Iraldi, G. Rodriguez de Lores Arnaiz, L. Salganicoff, J. Neurochem. **9**, 23 (1962). — 10. Dixon, W. J. (ed), BMD Biomedical Computer Programs, p. 341 (Bekeley and Los Angeles 1967). — 11. Garcia, P. T. and R. T. Holman, J. Amer. Oil Chem. Soc. **42**, 1137 (1965). — 12. Gerhardt, W., J. Clausen, E. Christen-

SEN, and J. RIISHEDE, Acta Neurol. Scand. **39**, 85 (1963). — 13. GREEN, D. E., S. MII, and P. M. KOHOUT, J. biol. Chem. **217**, 551 (1955). — 14. GREEN, D. E. and S. FLEISCHER, in Metabolism Physiological Significance of Lipids. Ed.: DAWSON, R. M. C. and D. N. RHODES, pp. 581–618 (London 1964). — 15. GÜTTLER, F. and J. CLAUSEN, Enzymologia biol. clin. **8**, 456 (1967). — 16. HAYASHIDA, T. and O. W. PORTMAN, J. Nutr. **81**, 103 (1963). —17. HOLMAN, R. T., Proc. Fedn. Amer. Socs. exp. Biol. **23**, 1062 (1964). — 18. HOLMAN, R. T. and H. MOHRHAUER, Acta chem. Scand. **17**, 84 (1963). — 19. HRUDKA, F., Acta histochem. **19**, 346 (1964). — 20. ITO, T. and R. M. JOHNSON, J. biol. Chem. **239**, 3201 (1964). — 21. LOUS, P., C. M. PLUM, and M. SCHOU, Nord. Med. **55**, 693 (1956). — 22. MANN, T., Biochemistry of Semen and of the Male Reproductive Tract, p. 34 (London 1964). — 23. MEAD, J. F., J. biol. Chem. **227**, 1025 (1957). — 24. NEUDOERFFER, T. S. and C. H. LEA, Br. J. Nutr. **21**, 691 (1967). — 25. RATHBONE, I., Biochem. J. **97**, 620 (1965). — 26. SHARMA, S. K., R. T. TALWALKAR, N. K. GARG, and C. R. K. MURTI, Indian J. Biochem. **3**, 122 (1966). — 27. SINGER, T. P. and E. B. KEARNEY, Methods of Biochemical Analysis. D. GLICK, ed. Vol. 4, p. 307 (London-New York 1965). — 28. SMITH, J. and H. F. DELUCA, J. Nutr. **79**, 416 (1963). — 29. SNIPES, R. L. and J. E. SMITHSON, J. Cell. Biol. **31**, 162 A (1966). — 30. THOMASSON, H. J., Int. Z. Vitaminforschg. **25**, 62 (1953). — 31. TURPEINEN, O. J., J. Nutr. **15**, 351 (1938). — 32. VAN DEENEN, L. L. and G. H. DE HAAS, Ann. Rev. Biochem. **35**, 157 (1966). — 33. WIEME, R. J., Agar-gel Electrophoresis (Brussels 1959). — 34. WILKINSON, J. H. and W. A. WITHYCOMBE, Biochem. J. **97**, 663 (1965). — 35. ZINKHAM, W. H., A. BLANCO and L. KUPCHYK, Science **142**, 1303 (1963). — 36. ZITTLE, C. A. and B. ZITIN, J. biol. Chem. **144**, 105 (1942).

Author's address:

Dr. JØRGEN CLAUSEN
The Neurochemical Institute, DK 1350 Copenhagen (Denmark)

Aus dem Pharmakologischen Institut der Universität Hamburg

Die akute und chronische Toxizität der Ameisensäure und ihrer Formiate*)

Von G. MALORNY

Mit 1 Abbildung und 6 Tabellen

(Eingegangen am 19. Februar 1969)

Ameisensäure und Formiate wurden verschiedentlich schon als Konservierungsstoffe für bestimmte Lebensmittel verwendet. Über ihre akute und chronische Toxizität sowie ihr Verhalten im Stoffwechsel war aber bis jetzt nur wenig Sicheres bekannt.

Im "Eight Report of the Joint FAO/WHO Expert Committee on Food Additives, Genf 1965" (1) finden sich über die Wirkungen der Ameisensäure nur spärliche Angaben. Es heißt dort: "Exact LD_{50} values are not available." Und an einer anderen Stelle: "Since long-term toxicity studies are lacking, it is not possible to give guidance on an unconditional acceptable daily intake level in man."

*) Bericht an die Fremdstoffkommission der Deutschen Forschungsgemeinschaft.