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SUBSTRATE SUPPLY FOR ENERGY HOMEOSTASIS DURING STARVATION

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KEY WORDS: lipid and carbohydrate substrates; starvation; energy metabolism.

Starvation has been used in clinical practice for a long time as a method of treatment of obesity and various diseases connected with disturbances of lipid metabolism. Nevertheless, the physiological mechanisms of its therapeutic action contain many unsolved problems. Two effects of starvation are not in dispute: increased utilization of endogenous fat for the energetic needs of the tissues and stimulation of glycolysis for the same purpose. However, the use of fatty acids (FA), the basic component of fat, for carbohydrate biosynthesis is denied for mammals and man in most publications.

Sporadic biochemical and ultrastructural data have recently been published on the role of FA as substrates for glucose and glycogen formation [4, 5, 12, 13].

This paper describes a combined ultrastructural and biochemical investigation of the state of the principal lipid and carbohydrate substrates of mammals involved in energy metabolism during starvation.

EXPERIMENTAL METHOD

Experiments were carried out on 235 noninbred male rats weighing initially 300-350 g. Thirty of these rats served as the control, and remained intact, whereas the rest were subjected to total alimentary starvation, with no restriction on drinking. A daily study of fine structural changes in the liver cells was carried out by means of the IEM-7A electron microscope from the beginning of starvation until death of the animals, which usually occurred between the 7th and 14th days. In parallel experiments, uptake of lipids (staining with Sudan) and glycogen (PAS reaction) incorporation in hepatocytes and leukocytes was determined in semithin sections through the liver and in blood films, by cytochemical methods. Each day of starvation data were obtained from five animals. Biochemical investigation of the blood was carried out daily

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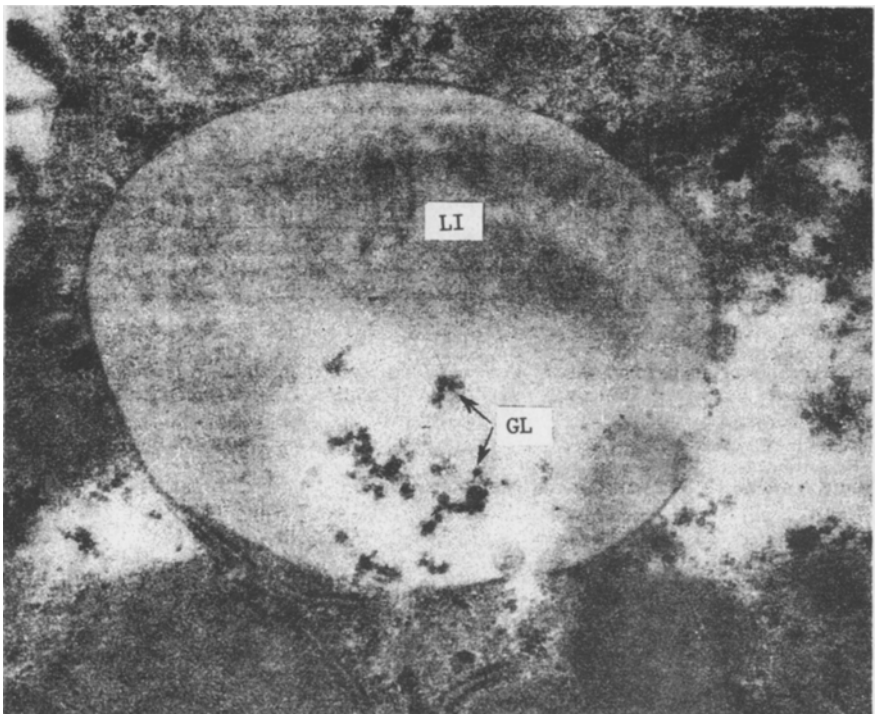

Fig. 1. Formation of glycogen structures (GL) inside resorbing lipid inclusion (LI) of rat hepatocyte on 4th day of starvation. 70,000×.

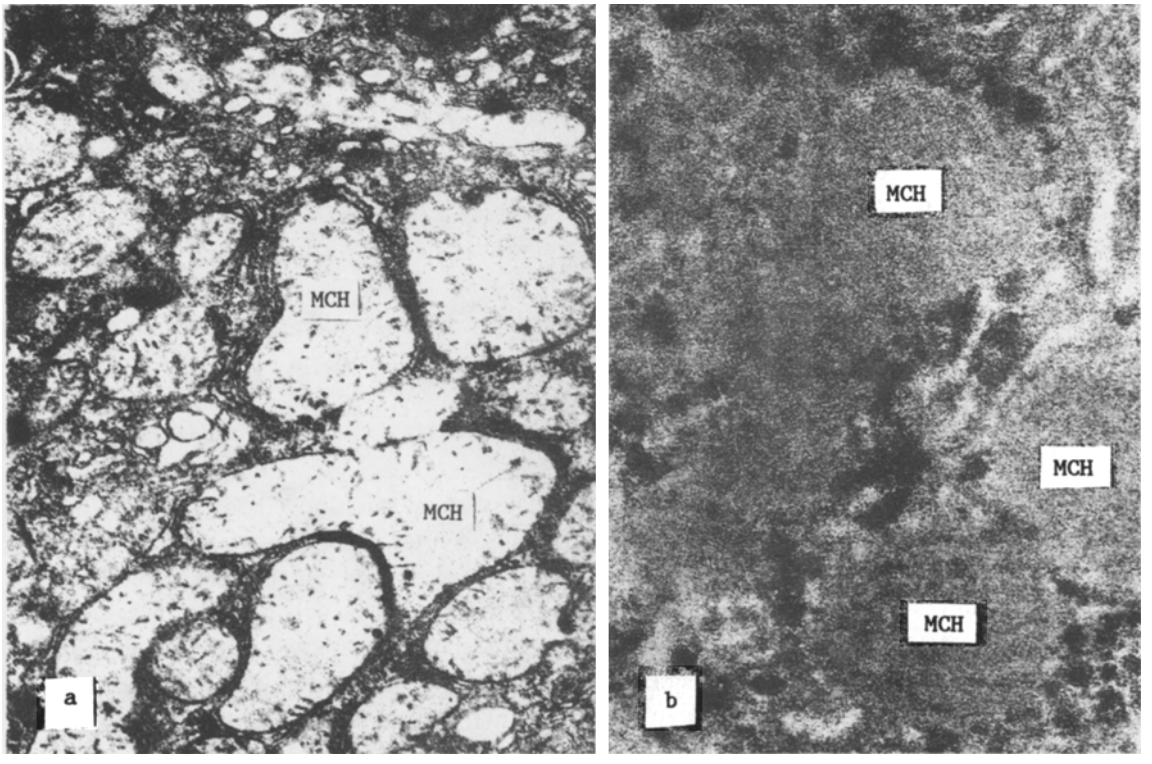

Fig. 2. Destructive changes in mitochondria (MCH) of hepatocytes in starving rats: a) swelling and clearing of matrix (5th day, 35,000×); b) homogenization of contents and disappearance of membranes (40,000×).

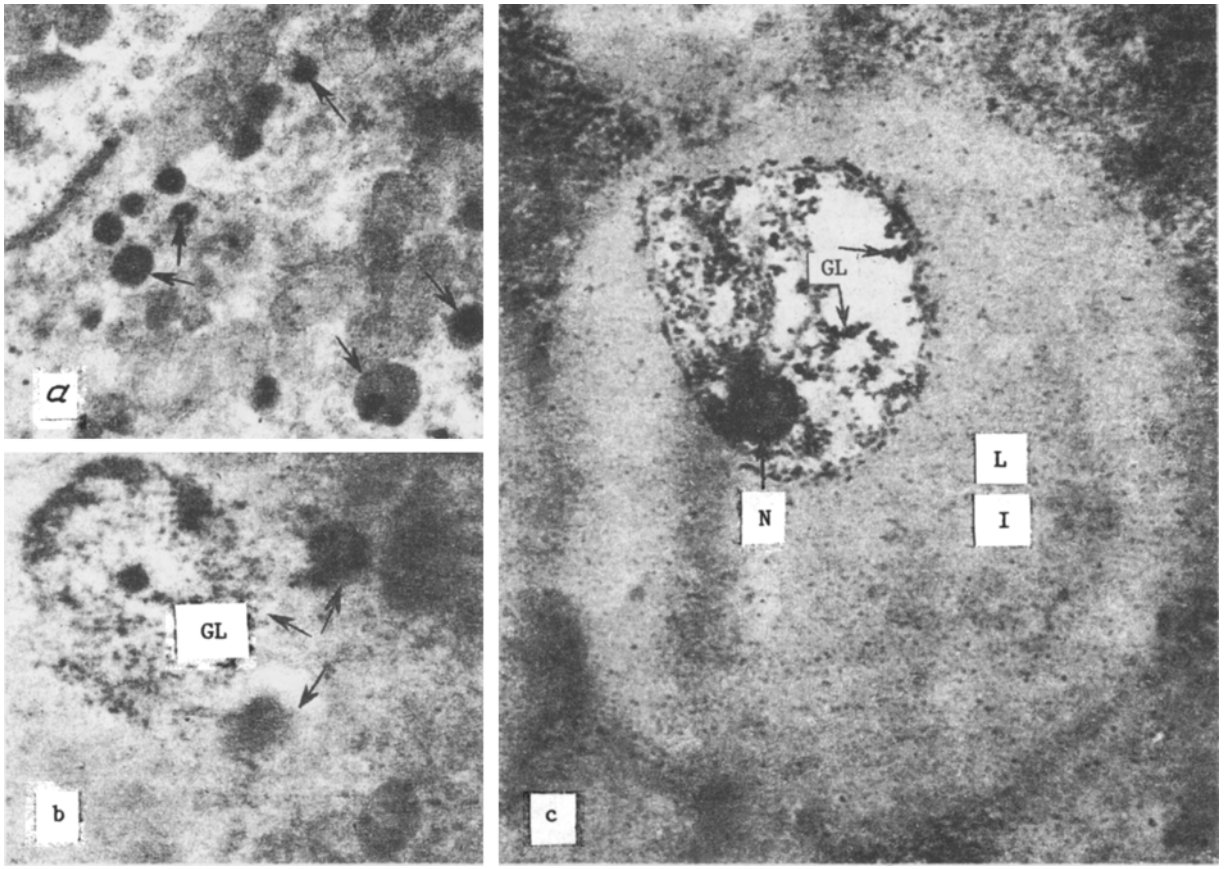

Fig. 3. Involvement of microcorpuscles in glycogen formation in hepatocytes of starving rats: a) concentration of microcorpuscles (arrows) in cytoplasm of hepatocyte (5th day, 10,000×); b) formation of glycogen structures in zone of distribution of microcorpuscles in cytoplasm of hepatocyte (arrows, 6th day, 70,000×); c) formation of glycogen structures in zone of tight junction of microcorpuscle with lipid inclusion in hepatocyte (7th day, 70,000×); N) nucleoid of microcorpuscle.

from the 1st through the 11th days of starvation. Blood was taken from the portal vein of the starving and control rats, anesthetized with pentobarbital, to determine the following parameters: total protein concentration (by the biuret reaction), lactic [3] and pyruvic acids (using kits), activity of enzymes of the pentose phosphate pathway of glucose oxidation (PPP): glucose-6-phosphate dehydrogenase (G6PDH) [11] and transketolase (TK) [10] (activity of PPP enzymes also was tested in liver tissue), the glucose concentration (enzymic method, biological test from Lachema, Czechoslovakia), glycogen, total lipids (kit from Lachema, Czechoslovakia), lipase activity [7], and the cortisol level by radioimmunoassay (sterone-K^{125}-I USSR). The data were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

Analysis of the ultrastructural and cytochemical data showed that the original glycogen reserves (in the liver cells) are completely used up by the end of the 2nd day of starvation. Thereafter, accumulation of lipid inclusions takes place in the cytoplasm of the hepatocytes with newly formed glycogen located near and inside them (Fig. 1). This process can be followed throughout the period of starvation, but most frequently in its middle period (from the 3rd through the 8th days). The lipid content is particularly high in the hepatocytes on the 4th-6th days of starvation, and it frequently leads to fatty degeneration of the hepatocytes. Such hepatocytes contain little glycogen. However, in most hepatocytes and lipophages containing a moderate number of lipid inclusions, the latter undergo distinct transformation into glycogen structures. Similar glycogen formation from fat in cells of the liver and other tissues was described by the writers previously in various extremal and pathological states [4-6]. The marked destructive changes in the hepatocyte mitochondria in the middle period

TABLE 1. Biochemical Tests of Blood and Liver Tissue of Rats during Starvation (M ± m)

Days of starvation	Blood protein, g/liter	G6PDH activity		TK activity		Blood lactic acid, μmoles/liter	Blood pyruvic acid, mmoles/liter	Blood lipase activity, ml NaOH	Total blood lipids, g/liter	Blood glycogen, mmoles/liter	Blood glucose, mmoles/liter	Cortisol, nmoles/liter
		in blood, μmoles NADPH/g Hb·m²	in tissue, mmoles NADPH/g protein·m²	in blood, μmoles C-7-P/g Hb.h	in tissue, mmoles C-7-P/g protein.h							
1	62±1,3*	3,24±0,5	2,4±0,3	6,9±1,5	27,3±8,9*	541,5±133	0,024±0,009*	1,15±0,15*	6,7±1,8	0,31±0,16*	9,4±1,3	32,1±3,0*
2	65±2,3*	1,42±0,5*	6,9±1,3*	6,6±1,3	6,8±1,6*	480,4±86,6	0,046±0,013	0,71±0,09*	4,5±1,0	0,85±0,3	7,8±0,8	50,5±5,8*
3	51,4±1,6	15,7±2,4*	6,8±1,1*	20,6±1,9*	5±1,8	315±28,8	0,021±0,008*	0,71±0,02*	3,7±1,0	—	9,3±1,0	8,4±1,1*
4	57,9±3,0	7,3±1,5*	4,5±1,0*	10,6±0,7	6,6±1,3*	79±14,1*	0,006±0,003*	0,97±0,34	1,1±0,5*	0,1±0,03*	5,4±3,7	12,5±1,8
5	70±1,4*	2,6±0,5	2,7±0,7	7,3±0,9	2,1±0,5	526,7±159	0	-	1,6±1,1	0,1±0,01*	13,4±6,0	-
6	55,7±1,2*	5,5±0,9*	5,1±1,2*	5,4±0,7*	16,9±2,9*	114,6±52,0*	0,0008±0,0007*	0,5±0,04	1,8±1,0	0,2±0,1*	7,8±1,5	—
7	57,9±4,0	11,6±1,5*	1,7±0,2	8,2±0,6	19,2±3,9*	426,7±53	0,019±0,007*	0,5±0,05	4,9±1,0	0,64±0,25	12,8±2,8	35±1,9*
8	56,4±2,3	13,2±1,5*	1,2±0,3	10,6±1,5	7,9±1,3*	812,5±10,5	0,029±0,008*	0,6±0,06*	6,2±0,9*	0,72±0,25	8,3±1,2	40,7±10,0*
9	56,4±4,8	9,0±0,8*	1,6±0,2	7,1±0,7*	17±2,4	1106±132*	0	0,55±0,05*	0,85±0,4*	1,7±0,5	3,8±0,4*	36,8±4,6*
10	59±3,0	4,8±1,0	1,2±0,2	6,4±1,2*	4,7±0,9*	1643±201*	0	0,55±0,05*	5,4±0,9	0,25±0,1*	9,6±2,0	-
11	62,8±2,8*	1,72±0,3*	1,1±0,1	5,8±1,2*	7,5±1,5*	1485±288*	0	0,42±0,03	4,96±2,0	1,6±0,4	4,2±0,7*	37,9±2,36*
Normal state	52±0,9	3,2±0,3	1,59±0,4	9,6±0,8	1,5±0,36	384±36,5	0,0696±0,0042	0,396±0,06	3,6±0,4	1,51±0,4	10±1,7	13,04±1,6

Legend. *) Significance of differences compared with control.

of observation (Fig. 2) indicate the state of inhibition of FA oxidation in these organelles. Meanwhile the number of lysosomes and, in particular, of microcorpuscles (peroxisomes) in the hepatocytes increases. The microcorpuscles frequently surround the lipid inclusions closely and become centers of formation of new glycogen granules (Fig. 3). In the prelethal and lethal periods of starvation, as a rule no lipid inclusions are present in the hepatocytes. At the same time, they do contain a certain amount of glycogen.

Glycogen can be seen in blood leukocytes throughout the period of starvation. According to the biochemical data, G6PDH activity is increased from the 2nd day in liver tissue and from the 3rd day in the blood (Table 1). After the 6th day activity of this enzyme in the liver returns to normal, but it remains high in the blood with a considerable rise on the 7th-8th days and a fall toward the 11th day of starvation. TK activity in the liver was high at all times of observation, but in the blood it was raised only on the 3rd day. The serum glucose concentration was significantly lowered only on the 9th and 11th days, glycogen on the 1st, 4th-6th, and 10th days (at other times the glucose and glycogen levels fluctuated around normal values). The lactic acid fell on the 4th-6th days, but from the 8th day until the end of observation it increased significantly; the pyruvic acid level was low after the 1st day, and after the 8th day it could not be detected at all The serum total lipid concentration rose during the first 2 days and on the 7th-8th days of the experiment, and it was low on the 4th-6th and 9th days; serum lipase activity was raised at all times of observation. A high blood cortisol concentration was noted on the 1st-2nd and 7th-9th days of starvation. The blood protein level was raised throughout the period of starvation.

Analysis of the complex ultrastructural, cytochemical, and biochemical data showed that an adequate glucose and glycogen level can be detected in the blood of the experimental animals at all times of starvation, accompanied by high activity of the PPP of glucose oxidation. evidence of the supply of substrates for glycolysis. Since glycogen disappears completely from the liver as early as after 2 days of starvation, the subsequent supply of glucose and glycogen for the needs of the body can take place only through gluconeogenesis from intermediate products of protein and lipid metabolism.

We know that in the 1st and 2nd periods of starvation proteins are utilized mainly in supplying plastic material for vitally important organs [8] and that the initial substrates for gluconeogenesis are limited to those amino acids whose breakdown is accompanied by the formation of pyruvic acid, either directly or indirectly [1]. In our experiments, from the first days of starvation the pyruvic acid concentration fell, and subsequently it could not be detected; it must therefore be supposed that amino acids may be the main source of gluconeogenesis, there are no bases, and even the ketone bodies, whose level rises during starvation, preventing mobilization of muscle alanine (the key amino acid of gluconeogenesis) inhibit gluconeogenesis from amino acids [1]. However, in the prelethal and lethal periods of starvation, the gluco- and glyconeogenic role of amino acids is probably enhanced in the tissues, and in particular, in the liver, due to the sharp increase in the rate of breakdown of cellular structures, which may perhaps be responsible for the presence of glycogen in the hepatocytes at this time, which was noted above.

During starvation, just as in a stress situation, mobilization of lipids takes place, lipolysis is increased in adipose tissue, but under the influence of glucocorticoids superposed on the high intensity of lipolysis, release of glycerol is not observed [2]. In our experiments, as was noted above, the blood level of glucocorticoids was high, and for that reason glucose formation from glycerol could not be significant.

A biochemical path of carbohydrate formation from FA through ω-oxidation of the latter has recently been elucidated [14]. On this path, hydroxylation of FA is catalyzed by the P-450-monoamine oxidase system of the microsomes [13]. Evidence of activation of microsomal processes in our present experiments was given by the raised blood cortisol level and the high PPP activity in the liver.

An increase in the number of peroxisomes, ultrastructural signs of their involvement in glycogen formation, and the presence of glycogen synthetase in them [5] suggest functioning of a glyoxalate cycle also in the liver cells. Recent investigations have demonstrated the existence of this pathway of FA conversion into carbohydrates in mammals [12]. It can be tentatively suggested that activation of these particular pathways of FA conversion into glucose helps to maintain a sufficiently high level of activity of anaerobic glycolysis during starvation, and under conditions of a sharp decline in oxidative phosphorylation, this is the main source of energy, especially for the liver, for the liver does not utilize ketone bodies as an energy-yielding substrate [2].

Data also have been obtained to show that carbohydrates can be formed from acetone, most of which is a product of FA catabolism [9].

Thus in the initial period of starvation energy homeostasis is maintained chiefly by the liver glycogen reserve, but later, for the longest period, by carbohydrates formed from endogenous FA; only in the prelethal period, however, is the gluco- and glyconeogenic role of amino acids in the liver and, probably, in other organs increased in connection with a sharp increase in the rate of breakdown of cellular structures.

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