ly no change in fluorescein accumulation compared with the control. In noninbred rats 30 days after the operation the volume of the residual kidney was 65 ± 5% of the volume of both kidneys in animals undergoing the mock operation, compared with 50 ± 5% in Campbell rats. SATOA in the proximal renal tubules of Campbell rats thus does not respond to humoral stimuli associated with unilateral nephrectomy at all times of the investigation, and the residual kidney shows no signs of compensatory hypertrophy 1 month after the operation.

The results thus indicate that SATOA in the proximal renal tubules of Campbell rats is defective both in power and in reactivity. Since SATOA plays an important role in purification of the internal medium of the body from endogenous waste products and foreign substances and since similar systems exist not only in the proximal renal tubules but also in structures of several other organs, including the eye [4, 7, 8, 9], the role of this defect (or defects) in the pathogenesis of retinitis pigmentosa requires further study.

LITERATURE CITED

1. V. M. Bresler and A. N. Kachman, Lab. Delo, No. 2, 109 (1975).
2. V. M. Bresler and A. A. Nikiforov, Tsitologiya, No. 1, 82 (1977).
3. V. M. Bresler and A. A. Nikiforov, Tsitologiya, No. 3, 1005 (1978).
4. V. M. Bresler and A. A. Nikiforov, Transport of Organic Acids through Plasma Membranes [in Russian], Leningrad (1981).
5. G. G. Chusova, I. A. Ostapenko, and M. E. Shabanova, Byull. Éksp. Biol. Med., No. 11, 21 (1982).
6. G. G. Chusova, I. A. Ostapenko, and R. N. Etingof, Byull. Éksp. Biol. Med., No. 9, 305 (1981).
7. E. H. Barany, Acta Physiol. Scand., 82, 12 (1972).
8. B. Becker, Am. J. Ophthalm., 50, 862 (1960).
9. J. G. Cunha-Vaz and D. M. Maurice, J. Physiol. (London), 191, 467 (1967).
10. W. R. Hewitt, P. Wagner, E. Bostwick, et al., J. Pharmacol. Exp. Ther., 202, 711 (1977).
11. I. M. Weiner, in: Handbook of Physiology, Section 8, Renal Physiology, Washington (1973), p. 521.
12. C. M. Yates, A. J. Dewar, H. Wilson, et al., Exp. Eye Res., 18, 119 (1974).

FUNCTIONAL AND STRUCTURAL CHANGES IN THE SMALL INTESTINE IN THE COURSE OF EXPERIMENTAL HYPERCHOLESTEROLEMIA

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The mechanisms of disturbances of cholesterol metabolism, subsequently leading to the development of hypercholesterolemia (HCh) are not yet clearly understood. Under these circumstances the study of the role of the digestive system and, in particular, the intestine and liver, which play a decisive role in cholesterol (Ch) metabolism, in this process assumes great importance [5-7, 9, 11]. It was concluded from the results of experiments on dogs [2, 3, 5, 10] that important factors preventing elevation of the blood Ch level are an intensification of its excretion with the intestinal secretion, the rapid removal of remnants of chylomicrons (CHM) from the circulation, and an increase in excretion of fatty acids.

This paper gives data on functional and structural changes in the digestive organs arising at different stages of experimental HCh in rabbits, which are known to have low resistance to exogenous Ch.

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TABLE 1. Dynamics of Total Ch Level in Blood Serum, Bile, and Chyme of Different Parts of Small Intestine after Single Feeding with Ch

Experimental conditions	Cholesterol concentration, g/liter					Cholic acid in bile, g/liter
	in blood serum	in bile	in chyme			
			from duodenum	from jejunum	from ileum	
Normal (control)	0,23±0,01	0,26±0,02 (n=7)	0,24±0,02 (n=6)	0,31±0,03 (n=6)	0,36±0,02 (n=7)	68±4 (n=6)
After a single feeding with Ch						
1 h	0,42±0,03* (n=6) 183%	0,23±0,01 (n=5) 85%	0,29±0,03 (n=5) 129%	0,36±0,03 (n=5) 118%	0,24±0,01 (n=6) 67%	63±7 (n=5) 92%
3 h	0,56±0,03* (n=5) 243%	0,21±0,01* (n=5) 78%	0,41±0,04* (n=4) 181%	0,43±0,04* (n=5) 139%	0,73±0,08* (n=5) 204%	67±4 (n=4) 98%
6 h	0,37±0,02 (n=3) 160%	0,16±0,01* (n=3) 58%	0,43±0,05* (n=3) 189%	0,54±0,02* (n=3) 174%	0,24±0,03 (n=3) 67%	88±16 (n=3) 131%
15 h	0,99±0,02* (n=7) 430%	0,15±0,01* (n=7) 54%	0,22±0,02 (n=7) 97%	0,38±0,01 (n=5) 124%	0,81±0,12* (n=5) 228%	45±3* (n=5) 67%
24 h	0,57±0,02* (n=8) 248%	0,21±0,02* (n=8) 78%	0,20±0,01 (n=6) 86%	0,21±0,01* (n=7) 67%	0,21±0,02* (n=7) 60%	67±4 (n=5) 99%
48 h	0,35±0,03* (n=8) 152%	0,25±0,03 (n=7) 91%	0,14±0,01* (n=6) 62%	0,21±0,01* (n=7) 68%	0,12±0,02* (n=6) 34%	67±4 (n=5) 99%

Legend. Values under normal conditions taken as 100. Here and in Tables 2-4: *$p < 0.05$ compared with control.

EXPERIMENTAL METHOD

Experiments were carried out on 52 male rabbits weighing 3-3.5 kg. Material was taken from rabbits killed by air embolism 1, 3, 6, 15, 24, and 48 h after they had been fed with a single dose of 0.5 g/kg of Ch together with carrot (100 g). Total Ch was determined in the blood serum, bile, and contents of the duodenum, jejunum, and ileum, by the method of Girard and Assous, and cholic acid in the bile was determined by the method of Reinhold and Wilson. Concentrations of CHM, and of very low-, low-, and high-density lipoproteins (LP) (VLDL, LDL, and HDL, respectively) were determined in blood serum from the auricular vein, abdominal aorta (AA), portal vein (PV), and hepatic vein (HV) [5, 6]. Methods of light and electron microscopy were used to study structural changes in the jejunal mucosa. Intact animals receiving a single dose of carrot only served as the control.

EXPERIMENTAL RESULTS

Only 3 h after Ch feeding its blood level was found to be 243% higher than initially and it continued to rise during the next few hours (Table 1). Its highest value was reached 15 h after feeding (430%). The level of HCh after 24 h was lower, but even after 48 h the blood Ch level remained higher than initially. The total LP concentration 24 h after Ch feeding was higher than initially on account of an increase of 107% in the concentration of the CHM + VLDL + LDL fraction, whereas the HDL level was unchanged (Table 2). The Ch concentration in

TABLE 2. Dynamics of LP Concentration in Blood Serum from a Rabbit Auricular Vein (in g/liter)

Time after Ch feeding, h	Group of animals	LP fraction		
		total LP	CHM + VLDL + LDL	HDL
0	Control	1,48±0,23 (n=4)	0,99±0,27 (n=4)	0,30±0,08 (n=4)
	Experiment	1,39±0,21 (n=7)	1,03±0,07 (n=7)	0,51±0,08 (n=7)
3	Control	1,72±0,22 (n=4)	0,92±0,14 (n=4)	0,98±0,27 (n=3)
	Experiment	1,79±0,15 (n=7)	0,92±0,13 (n=7)	1,11±0,17 (n=6)
6	Control	2,11±0,10 (n=3)	0,79±0,09 (n=3)	1,12±0,14 (n=3)
	Experiment	1,41±0,10 (n=8)	0,91±0,08 (n=7)	0,70±0,11 (n=7)
24	Control	1,81±0,20 (n=3)	1,40±0,09 (n=3)	0,41±0,11 (n=3)
	Experiment	2,80±0,33* (n=3)	2,13±0,19* (n=8)	0,45±0,05 (n=8)

TABLE 3. Concentrations of Individual LP Fractions in Blood Serum 15 h after Feeding with Ch (in g/liter)

Vessel	Group of animals	LP fraction		
		CHM	VLDL + LDL	HDL
AA	Control	0,12±0,05 (n=3)	0,37±0,07 (n=3)	0,42±0,09 (n=3)
	Experiment	0,62±0,06* (n=5)	1,21±0,36 (n=3)	0,27±0,06 (n=3)
PV	Control	0,07±0,01 (n=3)	0,47±0,05 (n=3)	0,48±0,14 (n=3)
	Experiment	0,30±0,04* (n=4)	0,96±0,07* (n=4)	0,60±0,20 (n=3)
HV	Control	0,19±0,03 (n=3)	0,40±0,04 (n=3)	0,85±0,27 (n=3)
	Experiment	0,24±0,02 (n=4)	0,82±0,18 (n=4)	0,32±0,14 (n=3)

TABLE 4. Concentrations of Individual LP Fractions in Blood Serum 24 h after Feeding Rabbits with Ch (in g/liter)

Vessel	Group of animals	LP fraction		
		CHM	VLDL + LDL	HDL
AA	Control	0,32±0,13 (n=4)	0,78±0,12 (n=5)	0,16±0,03 (n=5)
	Experiment	0,34±0,07 (n=3)	0,22±0,05* (n=3)	0,27±0,06 (n=3)
PV	Control	0,20±0,09 (n=3)	0,92±0,18 (n=3)	0,36±0,05 (n=5)
	Experiment	0,37±0,03* (n=3)	0,42±0,13 (n=3)	0,83±0,08* (n=3)
HV	Control	0,35±0,04 (n=3)	0,97±0,29 (n=3)	0,30±0,04 (n=4)
	Experiment	0,46±0,05 (n=3)	0,27±0,06 (n=3)	0,54±0,10 (n=3)

the chyme of the small intestine rose during the first 6 h in the proximal portion on account of the entry of exogenous Ch, but after 24 and 48 h its level in this portion was lower than initially. In the distal portion of the small intestine 1 h after feeding the Ch concentration was 67% of its initial value, after 3 h it was increased by 204%, and after 15 h by 228%, also evidently on account of the entry of exogenous Ch. The Ch concentration after 24 and 48 h, just as in the proximal portion, was much lower here than initially. The fall in the Ch concentration below its initial values after 24 h in all parts of the small intestine and the simultaneous rise in its blood level (up to 248%) are evidence that Ch taken in with the food is rapidly absorbed, whereas its excretion by the small intestine is delayed.

The CHM concentration in blood from AA and PV 15 h after feeding of the rabbits was raised to 416 and 328% respectively (Table 3). The difference in the CHM concentration in blood flowing from the intestine (PV) and in blood flowing into it (AA) was 51%, i.e., the CHM were held back by the intestinal wall. In the same period the blood VLDL + LDL level in PV was increased by 103% compared with the control. It can be tentatively suggested that the increased outflow of these LP fractions in blood flowing from the intestine was the result of their increased synthesis in the enterocytes. A certain quantity of these LP also was formed, evidently, from CHM entering the intestine with blood from AA. After 24 h the CHM concentration in blood from PV still remained increased by 86% (Table 4). The VLDL + LDL concentration in blood from AA was reduced by 72%. The increased outflow of HDL into blood from PV will be noted. The difference from the control 24 h after feeding the rabbits with Ch was 131%. The high HDL level in blood from PV and the low degree of their synthesis in the liver at this time indicate that HDL may be formed directly in the intestinal wall during metabolism of CHM and VLDL.

Excretion of Ch with the bile was lower than initially for 48 h after feeding. Excretion of cholic acid with the bile showed a tendency to fall. Both 15 and 24 h after feeding of the rabbits with Ch no appreciable breakdown of CHM was observed in the liver, to judge from the difference in their concentrations in blood flowing into and out of the liver.

Starting from 3 and 6 h and, in particular, 15 h after administration of Ch to the animals with their diet, dilatation of the postcapillaries and venules (Fig. 1a), aggregation of erythrocytes, and capillary stasis were observed in microvessels of the mucous membrane of the small intestine. Changes occurred in the shape of the circulating erythrocytes with the appearance of multiple regularly alternating projections on their surface (Fig. 1d). Signs of increased functional activity were observed in the endotheliocytes of the microvessels, and the intercellular junctions were widened and filled with amorphous material with increased electron density. The basement membrane was loosely textured in some places and its electron density reduced. Perivascular concentrations of macrophages, lymphocytes, and plasma cells

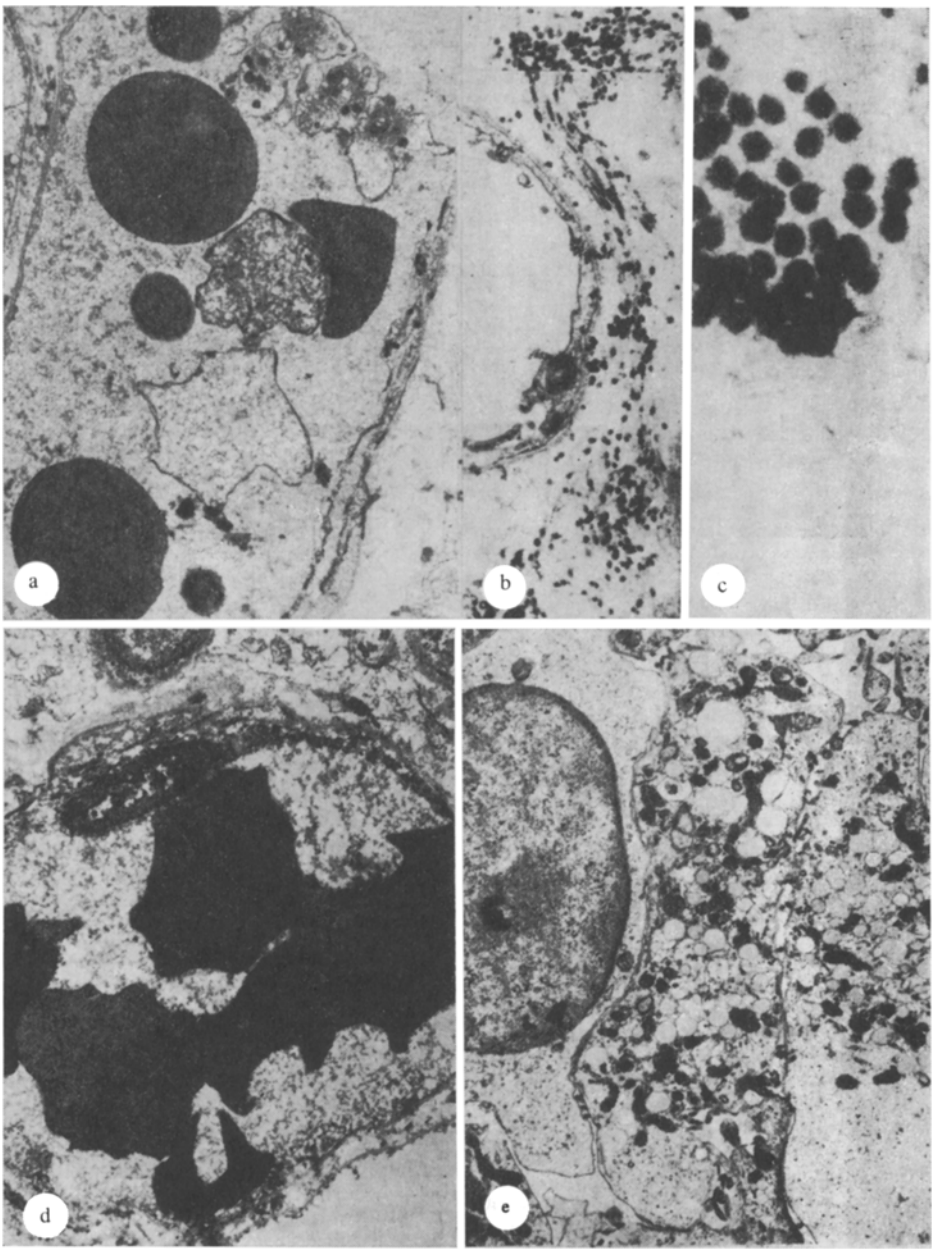

Fig. 1. Structural changes in microvessels and epitheliocytes of mucous membrane of small intestine in rabbits with HCh: a) 15 h after feeding rabbits with Ch, dilated venule with reduced thickness of endothelium, 7500 ×; b, c) 48 h after feeding: accumulation of electron-dense particles in perivascular tissue. Magnification 10,000 and 82,000 ×, respectively; d) 4th day of diet. Aggregation of erythrocytes with altered surface in lumen of microvessels, 7500 ×; e) 3 months of diet. Accumulation of lipid droplets of different sizes and of lysosomes in epitheliocytes, 7200 ×.

with greatly dilated cisterns of the rough endoplasmic reticulum could be seen surrounding the blood vessels, evidence of the more intensive synthesis of immunoglobulins. The presence of unusual electron-dense circular or oval formations outside the basement membrane of the microvessels, and in particularly large numbers near the venules, was noteworthy (Fig. 1, b and c). Measurements of the mean diameter of these particles on the Zeitz ASM semiautomatic image analysis system and their distribution by classes enabled their minimal and maximal diameters (from 32 to 69 nm) to be determined, by which they were accordingly classed as VLDL. Changes noted above in the microvessels of the mucous membrane still persisted 24 and 48 h after a

single dose of Ch. In the epitheliocytes of the villi edema was present with dilatation of cisterns of the smooth endoplasmic reticulum, changes in the mitochondria, and an increase in the number of primary lysosomes. At the same time accumulation of CHM and lipid droplets of different sizes (Fig. 1e) and widening of the spaces between neighboring cells could be observed.

The early stages of experimental HCh are thus characterized by injury to and structural changes in the microcirculatory system of the small intestine, increased permeability of the microvessels, changes in the rheological properties of the blood, the development of dystrophic changes in the mucous membrane, and marked immunologic disturbances.

The high peripheral blood Ch level 15 h after feeding of the rabbits with Ch was combined with increased entry of CHM, VLDL, and LDL from the intestine into the circulation. However, movement of CHM in the reverse direction into the intestinal wall also increased. Accumulation of Ch in the blood evidently promotes a decrease in its excretion by the small intestine, which was found at this time in the proximal portion. HCh 24 h after feeding with Ch was superposed on a decrease in Ch excretion along the whole length of the small intestine. By this time new mechanisms aimed at removal of the excess Ch from the intestine were appearing: Liberation of HDL into blood flowing from the intestine was increased and the retention of CHM by the intestinal wall was no longer present.

Against the background of HCh, destruction of CHM in the liver was limited for the first few days. The low Ch concentration in the bile under the experimental conditions used can be interpreted as an adaptive reaction in response to excessive intake, for we know that the Ch concentration in the bile is an indicator of its synthesis in the liver [5]. However, the absence of any significant changes in the excretion of cholic acid with the bile indicates that in rabbits the ability of the liver to convert its excess into bile acids is not increased in rabbits in response to a single dose of Ch.

In the early stages of HCh development in rabbits substantial changes thus take place in the functions of the small intestine and liver, connected with the reorganization of LP metabolism and with disturbances of the microcirculation.

LITERATURE CITED

1. A. N. Klimov, T. N. Lovyagina, and É. B. Ban'kovskaya, Lab. Delo, No. 5, 276 (1966).
2. M. S. Martsevich, Byull. Éksp. Biol. Med., No. 10, 43 (1973).
3. M. S. Martsevich, S. I. Filippovich, et al., Vestn. Akad. Med. Nauk SSSR, No. 12, 11 (1968).
4. O. N. Nikol'skaya and V. P. Tikhonov, Lab. Delo, No. 10, 579 (1968).
5. L. K. Finagin, Cholesterol Metabolism and its Control [in Russian], Kiev (1980), pp. 28-48.
6. L. A. Shekun, M. S. Martsevich, and E. D. Klimenko, in: Physiology and Pathology of the Cardiovascular System [in Russian], Moscow (1965), pp. 111-112.
7. J. M. Andersen and J. M. Dietschy, J. Biol. Chem., 252, 3646 (1977).
8. W. T. Beher and G. D. Baker, Am. J. Physiol., 197, 1339 (1959).
9. F. Chevallier and T. Magot, Experientia, 31, 627 (1975).
10. G. W. Melchior, R. W. Mahley, and D. K. Buckhold, J. Lipid Res., 22, 598 (1981).
11. C. C. Schwartz, L. G. Halloran, and Z. R. Vlahcevic, Science, 200, 62 (1978).