changes of this sort in the cyclic AMP concentration taking place during the first minutes of contact with the microorganisms must have some effect on the host organism as a whole. Cyclic AMP formed in the cells of the intestine may play the role of intermediary (or catalyst) in the development of the diverse secondary responses of the body to microbial contamination, which is known to be followed in some cases by rapidly developing reactions resembling shock in type [8]. The physiological role of the cyclic AMP formed under such conditions in the intestine likewise is unknown, but it may perhaps participate in the early stages of formation of the immune response [5].

The increase discovered in the cyclic AMP content in the macrophages during phagocytosis of bacteria is in agreement with the observations of Park et al. [8, 9], who demonstrated increased synthesis of cyclic AMP during phagocytosis of inert (latex) particles. These processes are evidently autonomous and regulatory in character. Increased liberation of cyclic AMP during phagocytosis may perhaps function as a special type of stop signal, preventing excessive ingestion of microorganisms by phagocytes [6].

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EFFECT OF HYDROCORTISONE ON COMPOSITION OF ACID MUCOPOLYSACCHARIDE FRACTIONS OF THE AORTA

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Five fractions of acid mucopolysaccharides were identified in the rabbit aorta: hyaluronic acid, heparitin sulfate, and chondroitin sulfates, A, C, and B. During prolonged administration of hydrocortisone the concentration of hyaluronic acid rose but that of heparitin sulfate fell. The relative percentages of chondroitin sulfate A, C, and B were lowered 15 days after administration of the hormone ceased.

KEY WORDS: *Mucopolysaccharides; aorta; corticosteroids.*

Previous investigations have shown that adrenal insufficiency developing after cessation of prolonged administration of hydrocortisone changes the state of the mucopolysaccharide filter of the vascular wall, so that its permeability is disturbed and lipids are deposited in it [1, 2]. Information on the composition of the acid mucopolysaccharide (AMPS) of the aorta is limited in amount and contradictory in nature [5-7].

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TABLE 1. Composition of Acid Mucopolysaccharide (AMPS) Fractions of Rabbit Aorta (μg uronic acids/g dried, defatted tissue) (M±m)

Group of animals	Total content of AMPS	Hyaluronic acid	Sulfated AMPS				
			total fraction	heparitin sulfate	chondroitin sulfate		
					A	C	B
1-							
(*n*-8)	3912,93±173,05	1351,2±136,3	2561,73±263,7	1193,35±192,7	186,74±19,9	862,98±133,5	318,69±39,7
2-							
(*n*-8)	4528,9±354.9	2057,64±261,7	2471,2±199,1	819,76±82,5	227,0±72,16	1018,49±180,7	391,6±50,28
P	>0,1	<0,05	>0,5	>0,1	>0,5	=0,05	>0,5
3-							
(*n*-8)	5038,03±188,6	3291,9±163,2	1712,15±164,6	652,9±153,3	171,28±27,6	655,67±89,4	268,71±91,8
P	<0,05	<0,001	<0,001	<0,05		>0,05	

The object of this investigation was to study the composition of AMPS fractions in the rabbit aorta under normal conditions and also during and after prolonged administration of hydrocortisone.

EXPERIMENTAL METHOD

Experiments were carried out on 24 male rabbits weighing 2.5-3 kg. The animals of group 1 were intact and served as the control. The rabbits of group 2 received hydrocortisone (Richter) in a dose of 0.2 mg/kg on alternate days for 3 months; these rabbits were killed on the day after the last injection. The rabbits of group 3 also received the hormone but were killed on the 15th day after the last injection. Tissue of the intima and media, defatted and dried to constant weight, was used for biochemical analysis; AMPS were isolated by papain proteolysis [3]. The mixture of AMPS was separated into its components by fractional elution of cetylpyridine complexes of AMPS from columns of microcrystalline cellulose, mark LK (Chemapol) measuring 0.8×8.0 cm [9]. To calibrate the columns and estimate the yield, a known quantity of standard preparations of hyaluronic acid and chondroitin sulfates (Koch Light Ltd.) and samples with the addition of the standard were chromatographed. Regeneration amounted to 92.5-102%. For quantitative analysis of the fractions the uronic acids were determined by the carbazole reaction with the addition of tetraborate [4], and also by the orcine method, by means of which chondroitin sulfate B could be identified from the carbazole/orcine ratio [8]. The results were expressed in μg uronic acids/g dried, defatted tissue.

EXPERIMENTAL RESULTS

The AMPS of the aorta of the intact animals were separated into five fractions (Table 1): hyaluronic acid (34.5% of the total AMPS content), heparitin sulfate (30.5%), and chondroitin sulfates A, C, and B (4.7, 22, and 8.2% respectively). These figures agree completely with results obtained by the use of other methods of analysis [5-7].

In the animals of group 2 a somewhat higher content of total AMPS was observed, chiefly on account of an increase in the amount of hyaluronic acid, which was 10% greater than in the control animals. The content of total sulfated AMPS was unchanged, but heparitin sulfate amounted to only 17.8%, and not 30.5% as in group 1; meanwhile the content of chondroitin sulfate C, calculated per microgram, increased so that its relative percentage remained at the control level (22%).

In the animals of group 3 the total AMPS content in the aorta was increased on account of hyaluronic acid, which now accounted for 65.3%. The total sulfated AMPS was reduced and the ratio between their fractions was changed: a concentration of heparitin sulfate was down to 12.9% and fraction C to 13%. The hyaluronic acid/sulfated AMPS ratio in the aorta of these rabbits also was appreciably altered.

The writers showed previously that prolonged administration of hydrocortisone increases the total AMPS in the aorta, reduces β-glucuronidase activity and, despite the hyperlipemia, is not accompanied by the deposition of lipids in the vessel wall; however, two weeks after withholding hydrocortisone a completely different picture developed: activity of β-glucuronidase was restored and the permeability of the aorta was increased; these changes were accompanied by deposition of lipids in its wall [1, 2]. Considering the results of the present investigation, it can be postulated that the leading role in these processes is played by heparitin sulfate and chondroitin sulfate C. The decrease in the absolute and relative con-

tent of heparitin sulfate, while the relative percentage of fraction C remained unchanged, under the influence of the hormone evidently somehow prevented changes in the hydrodynamic properties of the gel filter. On withholding the hormone, against the background of an even greater fall in the heparitin sulfate level, the relative percentages of the other sulfated fractions also decreased, possibly in connection with a change in permeability due to the deposition of lipids in the aortic wall.

The results thus indicate that changes in permeability and deposition of lipids in the wall of the aorta, coupled with relative adrenal insufficiency caused by withholding hydrocortisone after its prolonged administration, are based on changes in the relative content of the various fractions of AMPS.

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SPONTANEOUS FLUCTUATIONS IN NAD-KINASE ACTIVITY OF RABBIT SKELETAL MUSCLES

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Spontaneous fluctuations in the time of activity of a 280-300-times purified preparation of NAD-kinase from rabbit skeletal muscles are described after its dilution. No fluctuations of activity were found in an unfrozen but undiluted preparation. After preincubation of the diluted enzyme with substrates (NAD and ATP) its activity did not fluctuate.

KEY WORDS: *NAD-kinase – dilution; fluctuations in activity; skeletal muscle.*

Previous investigations showed that NAD-kinase isolated from rabbit skeletal muscles has a complex quaternary structure and exists in solution as a system of dissociated oligomers, differing in their catalytic activity [1-3]. Interconversion of the oligomers takes place under the influence of various factors including the concentration of the reaction substrates and the protein concentration. The special features of the quaternary structure of NAD-kinase are also reflected in the complex kinetic behavior of the enzyme.

The results of an investigation of spontaneous fluctuations in enzyme activity of NAD-kinase in time are described in this paper.

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