is based only on the ζ-potential of these bacteria. Our results also afford experimental evidence of the long-range minimum in the interaction energy curves. S-forms, on the other hand behave in a completely different way from that predicted by the theory. These abnormalities seem to be similar to those observed in the fixation of the H-SHPB (Ca) phage on the S-forms of the same bacteria.

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THE ACID MUCOPOLYSACCHARIDES OF EMBRYONIC SKIN

GERALD LOEWI* AND KARL MEYER

Department of Medicine, Columbia University College of Physicians and Surgeons, and Edward Daniels Faulkner Arthritis Clinic of the Presbyterian Hospital, New York, N.Y. (U.S.A.)

The biological significance of the varied patterns of acid mucopolysaccharides occurring in connective tissue of different organs is still unknown. Various observations, as for example in scorbutic animals and in hypercortisonism, of the failure of collagen fiber formation have been correlated with the failure of the production of sulfated mucopolysaccharides. On the basis of the occurrence of chondroitin sulfate B (ChS-B) in tissues containing coarse mature collagen bundles and its absence in organs containing only thin and immature collagen fibers, it was suggested that ChS-B might be causally connected with the production of the coarse type of collagen[1]. The low concentration of ChS-B in pigskin of embryos at term has been reported[2]. In continuation of such studies, the polysaccharide pattern of skin of various stages of embryonic development has been investigated.

EXPERIMENTAL

Embryo pigs, obtained fresh from the slaughterhouse, were rapidly skinned in the cold. The skins were divided into three groups, according to embryonic age, as estimated by measurement of crown-to-rump length. The skins were processed in a meat grinder.

The analytical procedures have been given elsewhere[3,4]. Digestion with pepsin and trypsin was used throughout this work. The procedures for removal of nitrogenous contaminants and for the separation of the mucopolysaccharide fractions from each other were also similar to those

* Fellow of the Empire Rheumatism Council, London.

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TABLE I

MUCOPOLYSACCHARIDE FRACTIONS FROM SKIN OF PIG EMBRYOS 9–13 cm LONG (83 g DRY WEIGHT)

Fraction	Yield (mg)	% of total polysacch.	N %	Hexosamine		Uronic acid %		SO_4 %	$[\alpha]_D$	Digestion with hyaluronidase	
				%	type	orcinol	Dische			testicular	pneumococcal
I (18% ethanol)	10.0	4.7			galactosamine	27.5	14.1			—	
II (36% ethanol)	165.0	78.0	3.39	41.1	glucosamine	36.4	43.0	< 0.5	—64°		
III (50% ethanol)	37.0	17.4	3.6	29.0	galactosamine + trace glucosamine	26.2	35.2	8.5	—22°	+	±

TABLE II

MUCOPOLYSACCHARIDE FRACTIONS FROM SKIN OF PIG EMBRYOS 15–17 cm LONG (133 g DRY WEIGHT)

Fraction	Yield (mg)	% of total polysacch.	N %	Hexosamine		Uronic acid %		SO_4 %	$[\alpha]_D$	Digestion with hyaluronidase	
				%	type	orcinol	Dische			testicular	pneumococcal
I (18% ethanol)	60.0	12.1	4.03	19.8	galactosamine	32.2	11.3	8.0	—44°	—	
II (36% ethanol)	335.0	67.5	3.36	40.8	glucosamine	42.5	46.3	< 0.5	—84°		
III (50% ethanol)	101.0	20.4	3.9	28.0	galactosamine + trace glucosamine	23.0	29.8	8.1	—20°	+	±

TABLE III

MUCOPOLYSACCHARIDE FRACTIONS FROM SKIN OF PIG EMBRYOS 18–20 cm LONG (208 g DRY WEIGHT)

Fraction	*Yield (mg)*	*% of total polysacch.*	*N %*	*Hexosamine*		*Uronic acid %*		*SO_4 %*	$[\alpha]_D$	*Digestion with hyaluronidase*	
				%	*type*	*orcinol*	*Dische*			*testicular*	*pneumococcal*
I (18% ethanol)	53.7	9.5	2.43	27.6	galactosamine	41.8	13.8	12.0	—63°	—	
II (36% ethanol)	400.0	71.0	3.21	39.8	glucosamine	42.5	51.5	< 0.5	—86°		
III (50% ethanol)	112.3	19.8	3.8	27.0	galactosamine + trace glucosamine	26.0	30.0	8.4	—24°	+	±

TABLE IV

MUCOPOLYSACCHARIDE FRACTIONS FROM SKIN OF ADULT PIGS (4000 g WET WEIGHT)*

Fraction	*Yield (g)*	*% of total polysacch.*	*N %*	*Hexosamine %*	*Uronic acid % Dische*	*SO_4 %*	$[\alpha]_D$	*Digestion with hyaluronidase*	
								testicular	*pneumococcal*
I (20% ethanol)	2.87	64	2.31	23.8	15.1	16.6	—59°	—	—
II (28% ethanol)	1.36	30	3.11	35.5	45.0	2.1	—77°	+	+
III (40% ethanol)	0.22		3.29	30.1	34.0				
IV (55% ethanol)	0.05	0.1		27.1	19.6	hexose 14% (as galactose)			

* taken from MEYER *et al.* 1956[3].

already described[3,5]. Hyaluronate was separated from sulfated fractions by precipitation of the former with $(NH_4)_2SO_4$ and pyridine[6], and subsequent dialysis.

The criteria used in the identification of the polysaccharides have also been given elsewhere[3].

RESULTS AND DISCUSSION

The distribution of acid polysaccharides and their analyses are given in Tables I–IV. Table IV has been added for the purpose of comparison with the values found in adult skin. Among the three groups of embryos, there is a suggestion of less chondroitin sulfate B and more hyaluronic acid in the youngest than the two older groups. However, owing to small yields, the differences between the three groups cannot be regarded as significant. When, however, the values are compared with those in adult pig skin, some striking differences become apparent. Whereas ChS-B constitutes 64% of the total skin acid mucopolysaccharides in the adult, in the embryo this value varies from about 5 to 12%. Conversely, in adult skin, hyaluronic acid constitutes 30%, while in the embryo this value reaches about 78%. Thus, the ratio of ChS-B to hyaluronic acid in embryo skin is 0.14:1, while that in adult skin is 2.1:1. Lastly, in embryonic skin, we found about 20% of the total polysaccharide to be a fraction with the characteristics of ChS-C. This constituted only 0.1% in the case of adult skin.

The differences between embryonic and adult skin are in accord with the correlation of ChS-B with the coarse type of collagen bundles. Histological examination (unpublished observations in collaboration with Dr. M. Moss) of pig embryo skin shows large numbers of fine, randomly orientated, argyrophil fibers, but none showing the morphological or tinctorial characteristics of mature collagen in the light microscope. We suggest, therefore, that the appearance of mature, coarse collagen fibers, as in adult skin, is in time and perhaps also causally connected with the presence of ChS-B. The relatively high percentage of ChS-C in embryonic skin, as in other tissues and in tissue culture, appears to be associated with young fine collagen fibers. The decrease with development may only be an apparent one, since owing to the presence of large amounts of ChS-B, a relatively small amount of C may become overshadowed.

SUMMARY

Acid mucopolysaccharides from embryo skin have been isolated and identified. It was found that embryo skin contained only very small amounts of chondroitin sulfate B, in the presence of relatively large amounts of hyaluronic acid, while adult pig skin contains considerably more chondroitin sulfate B than hyaluronic acid. In addition, embryo skin was found to contain a relatively large amount of chondroitin sulfate C.

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