nous inoculation caused fatal infection in a horse.

The authors are indebted to Joann Sparks for technical assistance.

1. Campbell, C. C., Saslaw, S., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 469.
2. Schaeffer, J., Saslaw, S., *ibid.*, 1954, v85, 223.
3. Saslaw, S., Schaeffer, J., *ibid.*, 1955, v90, 400.
4. Saslaw, S., *ibid.*, 1956, v91, 412.
5. Farrell, R. L., Cole, C. R., Prior, J. A., Saslaw, S., *ibid.*, 1953, v84, 51.
6. Saslaw, S., Maurice, G. E., Cole, C. R., *J. Lab. and Clin. Med.*, 1955, v46, 948.
7. Saslaw, S., Carlisle, H. N., Sparks, J., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 342.
8. Menges, R. W., Furcolow, M. L., Hinton, A., *Am. J. Hyg.*, 1954, v59, 113.
9. Menges, R. W., *Cornell Vet.*, 1954, v44, 21.
10. Blake, F. G., Cecil, R. L., *J. Exp. Med.*, 1920, v31, 403.
11. Saslaw, S., Campbell, C. C., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 559.
12. Saslaw, S., *J. Lab. and Clin. Med.*, 1948, v33, 811.
13. Howell, A., *Pub. Health Rep.*, 1947, v62, 631.
14. Cole, C. R., Prior, J. A., Saslaw, S., *J. Am. Vet. Med. Assn.*, 1950, v116, 135.
15. Conant, N. F., Smith D. T., Baker, R. D., Callaway, J. L., Martin, D. S., *Manual of Clinical Mycology*, W. B. Saunders Co., Philadelphia, 1954.

Received July 10, 1960. P.S.E.B.M., 1960, v105.

Mucopolysaccharides of Aorta at Various Ages.* (26015)

DAVID KAPLAN† AND KARL MEYER
Dept. of Medicine, Columbia University College of Physicians and Surgeons, and Edward Daniels Faulkner Arthritis Clinic, Presbyterian Hospital, New York

Changes in the patterns of mucopolysaccharides (MPS) of cartilage, nucleus pulposus, skin and aorta, concomitant with ageing, have been described in past years. In general, these studies indicated a decrease of total MPS per unit weight with chronological age coincident with an increase in collagen content. The most profound changes were encountered in human costal cartilage(1). In this tissue, a steady decrease in total MPS was observed. Keratosulfate increased in the first two decades and remained constant thereafter. Chondroitin sulfate steadily decreased with time and chondroitin-4 sulfate (ChS-A) was replaced by chondroitin-6 sulfate (ChS-C). Similar changes have been described in degenerating versus normal nucleus pulposus(2). The pattern of age changes in the MPS of skin has also been described.

While these studies were based on isolation and chemical and enzymatic characterization of the isolated fractions, the reported studies of ageing of aorta have been based on analysis of the whole organ for hexosamine, uronic acid, sulfate, and identification of monosaccharides by paper chromatography after acid hydrolysis, as for example in the work of Dyrbye and Kirk(3,4,5). Those authors found until age 60 no change in ratio of galactosamine to glucosamine, and after 60 a slight increase. Similarly, the sulfate content of the tissues was found not to change till age 60. More recently Kirk has reported a crude MPS fraction of aorta to have the anticoagulant activity of 1% standard heparin(3) while Yü and Blumenthal found approximately 4%(6). Jorpes(7) and others have obtained heparin in small quantities from aorta.

The MPS of bovine and human aortae have been isolated and characterized. In bovine abdominal aorta of 1- to 2-year-old cattle, total isolated MPS was approximately 1% of defatted dry weight. Of this total, ChS-A represented 45%, hyaluronic acid 23%, and ChS-B and heparitin sulfate approximately 16% each(8).

The present study was undertaken to com-

* Supported in part by grant from the U. S. Public Health Service.

† Fellow of Arthritis and Rheumatism Foundation, New York.

pare MPS patterns of isolated fractions in whole human aortae at various ages.

Method. Thirty-three fresh human aortae were obtained at autopsy and segregated into 7 different age groups, containing from 3 to 7 aortae in each group, with average ages of the groups ranging from 21 to 72 years. The tissues showed a general trend of an increasing degree of atherosclerosis with increasing age. The groups of average age 46 and 67 were all female, the 21-year-old group was of mixed sex, and the other 4 groups were male. The aortae in each group were dried in acetone, cleaned of perivascular and adventitial tissue, cut in small pieces, ground in a mill and treated further with acetone. The powders of each aorta were weighed and combined so that each group contained approximately 35 g of dry aorta. This was homogenized (VirTis) in 0.1 N HCl, digested with pepsin, neutralized and digested with trypsin(8), and purified by means of the Sevag procedure, absorption with Lloyd's reagent/kaolin mixture and several alcohol precipitations(8). It was finally fractionated as the calcium salt at 18%, 23%, 28%, 33%, 43%, and 52% alcohol.

The fractions obtained above were analyzed for uronic acid by both the carbazol and orcinol methods(9), for total hexosamine by the method of Elson and Morgan(9), and for glucosamine and galactosamine by Fischer-Dorfel paper chromatography(10). In addition, increase in reducing sugar was measured after enzymatic digestion with combinations of pneumococcal hyaluronidase, testicular hyaluronidase, and A-adapted (this extract digests ChS-B when the organism has been adapted to either ChS-A or B(11)) and heparitin-adapted enzyme. Rarely did any one fraction contain only one polysaccharide, and further separation was required to obtain a quantitative estimation of amount of each MPS.

The 18% and 23% fractions were each found to contain a mixture of ChS-B and heparitin sulfate. They were therefore combined and separated by the method of Cifonelli and Dorfman(12). The 28% fraction was usually small and not susceptible to further separatory procedures. It was therefore necessary to rely on a correlation of the results of the paper chromatogram and enzyme experiments. The 33% fraction contained hyaluronate and estimation of the amount with pneumococcal hyaluronidase correlated well with the actual amount isolated by the method of Scott(13). The residual MPS was identified by infrared spectrophotometry. The 43% fraction usually contained only ChS and was identified again by its infrared absorption. There was no significant precipitation above 43%.

From these data, relative amounts of the different MPS were calculated.

Results. Total yield of MPS showed no correlation with age (nor did total hexosamine done on individual aortas) and varied between 0.85% and 1.2% of the dry weight of aorta. In the following graphs the data are expressed as per cent of each polysaccharide of total MPS against age in years (Fig. 1-4). It can be seen that ChS-B and heparitin sulfate increase with increasing age (and increasing degree of atherosclerosis). Essentially the reverse is the case for hyaluronate and ChS-C.

Of interest in regard to comparison with the data of others cited above is that one can deduce relative amounts of glucosamine and galactosamine of the aortae for each age group—and this does indeed give a quite constant value. For the 7 age groups, in order of increasing age, ratio of galactosamine to glucosamine (ChS-C + ChS-B to HA + heparitin) would be 74/26, 69/31, 74/26, 74/26, 74/26, 70/30, 74/26.

Heparin, keratosulfate, and ChS-A could not be identified in any fraction.

Discussion. Isolation and fractionation of MPS of aorta present greater difficulties than that from any other tissue studied thus far. The reasons for these difficulties are not obvious and may reside, in part, in the protein complexes in which they occur in vascular tissues. By reason of the abnormal behavior towards alcohol fractionation, it is necessary to make the conditions of isolation and fractionation as uniform as possible. Even so, occasionally the 28% alcohol concentration appeared to precipitate all 4 fractions.

The results of this investigation show a pattern of ageing totally different from that encountered in costal cartilage. In the latter, chronological ageing is accompanied by

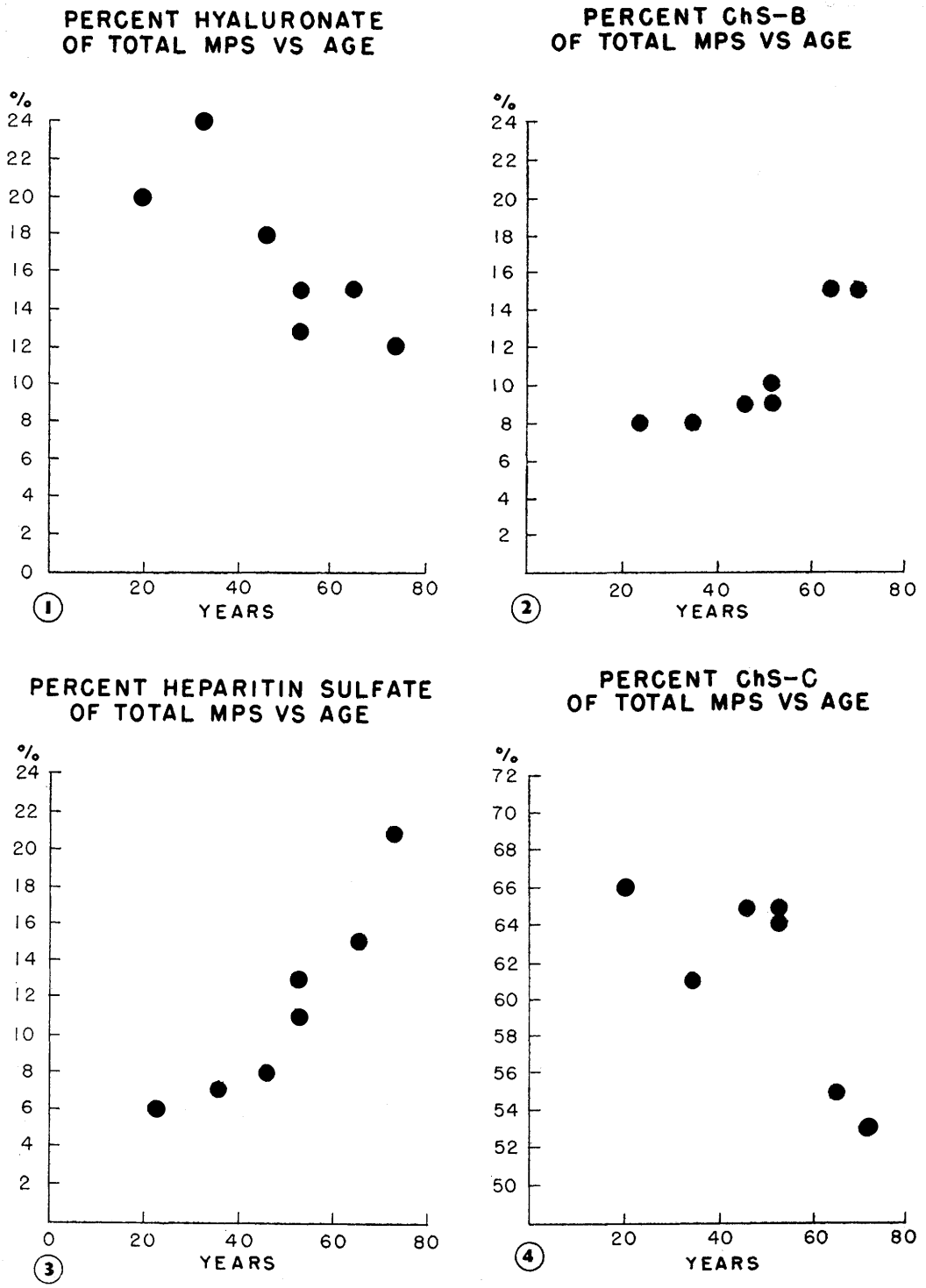
PERCENT HYALURONATE
OF TOTAL MPS VS AGE
%
24
22
20
18
16
14
12
10
8
6
4
2
0
20
40
60
80
YEARS
1
PERCENT ChS-B
OF TOTAL MPS VS AGE
%
24
22
20
18
16
14
12
10
8
6
4
2
20
40
60
80
YEARS
2
PERCENT HEPARITIN SULFATE
OF TOTAL MPS VS AGE
%
24
22
20
18
16
14
12
10
8
6
4
2
0
20
40
60
80
YEARS
3
PERCENT ChS-C
OF TOTAL MPS VS AGE
%
72
70
68
66
64
62
60
58
56
54
52
50
20
40
60
80
YEARS
4

FIG. 1-4.

profound qualitative changes in the types, besides a decrease in total MPS. Ageing of pig skin appears to be characterized by a decrease in hyaluronic acid content and an increase in chondroitin sulfate B. In aorta, while the histologically demonstrated changes in elastic and collagenous components are very striking, the changes in the MPS are more subtle and complex. The complexity can be assumed to be a result of ageing *per se,* of degenerative and reparative processes occurring simultaneously. The decrease in hyaluronate and ChS-C is similar to that found in other ageing tissues and perhaps would be still more marked if ageing without inflammatory and reparative processes could be studied. In other publications, the association of hyaluronate with elastic fibers was indicated(14). In fact, the known fragmentation of elastic fibers with ageing may well be a consequence of changes in this component. A study of aorta in species not subject to atherosclerosis probably would allow a clearer picture of the process in man.‡

Summary. Mucopolysaccharides were isolated from whole human aortae and characterized by analysis, enzymatic digestion, optical rotation and I.R. spectroscopy. Average age of the groups ranged from 21 to 72 years. Total MPS and galactosamine/glucosamine ratios showed no significant change with age. Of the 4 MPS fractions identified, hyaluronate and ChS-C decreased with age while ChS-B and heparitin sulfate increased. The data are discussed in relation to age changes in other tissues of mesodermal origin. It is assumed that the complex pattern of human aorta is the resultant of age changes *per se* superimposed on degenerative and reparative processes.

We wish to thank the Dept. of Pathology for their cooperation in obtaining aorta.

‡ After completion of this manuscript, a study of MPS of human aorta with and without arteriosclerosis was published by Buddecke(15). The author obtains a mucoid fraction (on phenol extraction) similar to serum mucoid. His findings regarding the acid MPS disagree substantially with our own. Thus, he finds no heparitin sulfate fraction, which has been isolated from bovine and all human aorta studied, but a substantial fraction which he designates as keratosulfate. Methods of isolation and characteristics of the fractions are not sufficiently documented to permit their identification.

1. Kaplan, D., Meyer, K., *Nature,* 1959, v183, 1267.
2. Davidson, E. A., Woodhall, B., *J. Biol. Chem.,* 1959, v234, 2951.
3. Kirk, J. E., *Nature,* 1959, v184, 309.
4. Dyrbye, M., Kirk, J. E., *J. Geront.,* 1957, v12, 20.
5. ———, *ibid.,* 1957, v12, 23.
6. Yü, S. Y., Blumenthal, H. T., *ibid.,* 1958, v13, 366.
7. Jorpes, E., Holmgren, H., Wilander, O., *Z. mikr.-anat. Forsch.,* 1937, v42, 279.
8. Meyer, K., Davidson, E. A., Linker, A., Hoffman, P., *Biochim. Biophys. Acta,* 1956, v21, 506.
9. Rapport, M. M., Meyer, K., Linker, A., *J. Am. Chem. Soc.,* 1951, v73, 2416.
10. Fischer, F. G., Nobel, H., *Z. physiol. Chem.,* 1955, v302, 10.
11. Hoffman, P., Linker, A., Sampson, P., Meyer, K., Korn, E. D., *Biochim. Biophys. Acta,* 1957, v26, 658.
12. Cifonelli, J. A., Ludowieg, J., Dorfman, A., *J. Biol. Chem.,* 1958, v233, 541.
13. Scott, J. F., *Biochim. Biophys. Acta,* 1955, v18, 428.
14. Meyer, K., *Molecular Biology,* Academic Press, New York, 1960.
15. Buddecke, E., *Z. physiol. Chem.,* 1960, v318, 33.

Received July 13, 1960. P.S.E.B.M., 1960, v105.