of them (figure 4). Ovaries of pharate adults which could be characterized by the movements of eclosion, and adults during the 1st 2 h after eclosion, exhibited an intense incorporation of protein precursors. This outstanding synthetic activity does not seem to be related to any visible cytological alteration in the ovaries. Although we have no data on protein synthesis in other tissues, the synthetic activity observed at this stage should not be specifically related to vitellogenesis itself. It could, however, be part of a more generalized metabolic process, at that specific developmental stage of the organism, as discussed by Pan[14]. Considering these observations, yolk deposition in *Rhynchosciara* seems to involve 2 kinds of mechanism: the incorporation of proteins from haemolymph, as suggested by the corresponding electrophoretic patterns detected for fertilized eggs, ovary and haemolymph, and the synthesis by the ovary itself, mainly during the adult stage, as indicated by its intense incorporation of protein precursors.

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Effect of low density lipoprotein on proteoglycan synthesis by aorta cells in culture[1]

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Summary. Increasing the content of human serum low density lipoprotein in the growth medium led to greater incorporation of ^{35}S-sulfate into proteoglycan (mostly into dermatan sulfate) by primary aorta cells but did not affect similar incorporation by fibroblast cells. These results suggest a mechanism which can explain the increased deposition of lipid in aorta due to hyperlipidemia.

Several recent studies suggest that lipid deposition in atherosclerotic lesions may be due, in part, to formation of insoluble complexes of serum lipoproteins with dermatan sulfate proteoglycans secreted by aorta smooth muscle cells[2–4]. It has been shown that of all glycosaminoglycans dermatan sulfate has the greatest affinity for low density lipoprotein (LDL)[5] at physiological pH and ionic strength and is present in high concentration in arterial fatty lesions[6]. Bovine aorta contains a dermatan sulfate-chondroitin sulfate hybrid proteoglycan molecule as the major proteoglycan in the tissue[7,8]. Changes in the proteoglycan composition of aorta tissue can be correlated with the extent of atherosclerotic involvement of the tissue[9,10]. Although aorta smooth muscle cells in tissue and organ culture grow more rapidly in the presence of hyperlipemic serum[11], such increased growth alone cannot explain the lipid accumulation which leads to atherosclerosis. We present evidence that serum lipoproteins can stimulate proteoglycan synthesis by aorta cells in culture and hypothesize that such increased secretion by aorta cells in vivo leads to the increase in lipid deposition observed in the formation of fatty streaks and fibrous lesions in the aorta.

Materials and methods. LDL was prepared fresh for each experiment from pooled normal human serum obtained from fasting people. Separation from other serum components was achieved by the method Ewing et al.[12]. In this method, 2 volumes of serum overlaid with 1 volume of a 1.006 g/ml NaCl solution are centrifuged for 20 h at 40,000 rpm in a Spinco Ti60 rotor at 16–20 °C to remove very low density lipoprotein (VLDL). The bottom layers containing LDL are transferred to a clean centrifuge tube (2 volumes) and overlaid with 1 volume of a 1.182 g/ml NaCl-NaBr solution. After centrifugation at 40,000 rpm in the above rotor for 24 h, the floating LDL is removed. The material from the bottom half of the tube is collected and saved as serum minus VLDL and LDL. The various fractions were dialyzed against 0.15 M NaCl with 0.001 M EDTA, sterile filtered under pressure through a 0.22 μm membrane, and stored for less than 2 weeks in plastic tubes at 8 °C before use.

The human foreskin fibroblast (passage 11) cells were obtained from Dr C. Sanders at the LSU Medical School, New Orleans. Rabbit and monkey aorta cells were obtained by primary explant from aortas of 6 2-week-old rabbits and an adult male rhesus monkey. The aortas

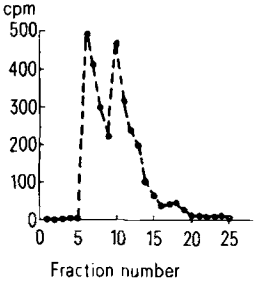

Fig. 1. Bio-Gel P-10 column chromatography of ^{35}S-sulfate-labeled proteoglycans from rabbit aorta cell culture medium (extracellular proteoglycans) after hyaluronidase digestion. The sample was from the experiment in which medium contained 3 times the normal amount of LDL. The sample, 0.2 ml, was applied to a 1.0 × 27 cm column and eluted at a flow rate of 3.3 ml/h.

1 Acknowledgments. This work was supported by a Fort Polk-American Heart Association-Louisiana, Inc. Reserch Award.
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Effect of human serum low density lipoprotein on incorporation of ^{35}S-sulfate into proteoglycans of aorta and fibroblast cells in culture

Proteoglycan fraction	Cholesterol concentration (mg/ml)	Serum LDL (% of normal amount in human serum)*	Sulfated proteoglycans (cpm/mg cell protein) Primary rabbit aorta cells	Primary monkey aorta cells	Human foreskin fibroblasts
Cell-associated	0.047	0	ND**	11,661 ± 3,000	50,997 ± 1,025
	0.188	100	53,647 ± 5,195	23,315 ± 3,842	55,322 ± 6,011
	0.376	300	84,922 ± 14,478	71,965 ± 8,003	52,516 ± 11,405
Extracellular	0.047	0	ND	30,152 ± 5,805	224,936 ± 12,264
	0.188	100	145,312 ± 2,797	75,858 ± 9,371	201,602 ± 24,719
	0.376	300	244,929 ± 35,049	210,521 ± 54,449	180,927 ± 9,955

* The low density lipoprotein in the pooled human serum from fasting people, which was used for these experiments, contained 65% of the total serum cholesterol. ** ND, not determined.

were excised and the intima and adventitia partly removed by scraping with a sterile scalpel; each aorta was cut into 1–2 mm^3 fragments with iris scissors and then planted in flasks containing 2 ml Dulbecco's minimal essential medium (DMEM) with 10% fetal calf serum, 20 mmoles HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) pH 7.3, chlortetracycline (100 µg/ml), penicillin (100 units/ml), streptomycin (100 µg/ml), and mycostatin (50 µg/ml). Cells grew out from the explants and reached confluency within 21 days. They were then used for experiments.

Cholesterol assays were performed by the Pearson method[13], and lipoprotein identified by electrophoresis on agarose gel plates[14]. Bio-Gel P-10 columns (1.0 × 27 cm) were equilibrated and eluted with 0.01% sodium dodecyl sulfate (SDS), 0.01 M Tris-HCl pH 7.4. Hyaluronidase (Nutritional Biochemicals) digestions were performed on 0.2 ml aliquots of dialyzed samples using 0.2 ml testicular hyaluronidase (100 units/ml in 0.1 M NaCl), and 0.2 ml citrate-phosphate buffer, pH 5.0. Incubation was at 37 °C for 13 h. For nitrous acid digestion, 50 µl glacial acetic acid and 50 µl 20% sodium nitrite were added to 0.2 ml samples in glass test tubes and the mixture incubated 1.5 h at 27 °C.

Duplicate 25 cm^2 plastic flasks with confluent monolayers were treated as follows. The medium was removed, and either DMEM with 10% human serum minus VLDL and LDL, DMEM with 10% dialyzed human serum, or DMEM with 10% dialyzed human serum plus LDL (0.13 ml of purified LDL, cholesterol content = 2.82 mg/ml) was added. In order to have the same concentration of medium in all samples the incubation medium was adjusted to 2 ml using 0.001 M EDTA, 0.15 M NaCl (the dialysis solution for LDL) as necessary, and ^{35}S-sulfate added to a concentration of 10 µCi/ml. The cell monolayers were incubated at 37 °C for 36 h.

Following incubation and removal of the medium, the cells were washed 4 times with HEPES-buffered saline and then dissolved in 1% SDS. Both medium and cell fractions were dialyzed exhaustively against, first, 0.1 M Na_2SO_4 and then 0.15 M NaCl. Aliquots were assayed for radioactivity and characterized by various techniques, as described above.

Results and discussion. The uptake of ^{35}S-sulfate into proteoglycans was measured in experiments with rabbit and monkey aorta cells and human foreskin fibroblasts. In general, higher levels of LDL in the serum resulted in increased incorporation of label into proteoglycan in the rabbit and monkey aorta cell experiments, but in decreased incorporation in the human foreskin fibroblast study (table). The increase was found in both cell-associated (not lost by washing) and extracellular (secreted) proteoglycans. The increased ^{35}S-sulfate incorporation for rhesus aorta cells was greater than that for rabbit aorta cells, although the rabbit smooth muscle cells had a greater net incorporation on a per mg basis at lower levels of LDL.

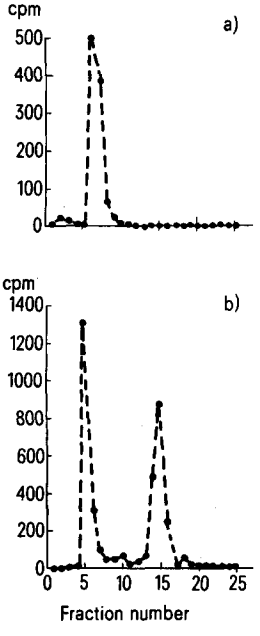

Fig. 2. Bio-Gel P-10 column chromatography of ^{35}S-sulfate-labeled proteoglycans from rabbit aorta cells (cell-associated proteoglycans) grown in medium containing 3 times the LDL content in normal human serum. *a* 0.2 ml of a hyaluronidase digest and *b* 0.2 ml of a nitrous acid-treated sample were applied to the column.

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These results are in agreement with those of several workers who have found an increased incorporation of ^{35}S-sulfate into glycosaminoglycans in aorta tissue incubated under atherosclerotic conditions[15–18]. Mammalian cells are known to incorporate sulfate only into proteoglycan, and the amount of sulfate incorporation is considered to be a good estimate of actual amounts of proteoglycans synthesized by the cells[19]. Therefore, it seems likely that elevated serum lipoprotein levels cause heightened production of proteoglycans in aorta cells. Phase microscopy studies of morphology indicate that the cell populations consist mainly of smooth muscle cells. The facts that the cells synthesize predominantly dermatan sulfate proteoglycan(s) (see below) supports this conclusion.

Radiolabeled proteoglycans from rabbit aorta cultures incubated with 3 times the normal human serum LDL were chromatographed on P-10 columns before and after hyaluronidase or nitrous acid treatment. After incubation with hyaluronidase, a retarded peak (mol. wt $< 20{,}000$) was found in all medium samples (figure 1), but not in any cell samples (figure 2, a). After nitrous acid degradation, the cell fraction also showed a retarded peak (figure 2, b). The untreated samples from cell and medium fractions showed only a single radioactively labeled peak migrating with the column void volume. Similar results were obtained from analyses of the monkey aorta proteoglycan fractions. With normal and reduced content of LDL in the serum, no change in the P-10 profile was found (data not shown). The secreted proteoglycan material also shows no significant differences in composition with different amounts of serum LDL in the cell growth medium.

We conclude from these results that the composition of cell-associated and extracellular proteoglycans does not vary significantly with the lipoprotein content of the medium. The cell-associated proteoglycans are not degraded by hyaluronidase (figure 2, a), although nitrous acid treatment degrades $\sim 45\%$ of this material (figure 2, b). The cellular proteoglycan material, therefore, consists of 55% dermatan sulfate proteoglycan (hyaluronidase, nitrous acid-resistant) and 45% heparan sulfate (nitrous acid-sensitive). The extracellular proteoglycans contain a portion that is degradable by hyaluronidase ($\sim 55\%$) and a portion resistant to enzyme degradation. We have found (results not shown) that this hyaluronidase-resistant material is not degraded by nitrous acid but is extensively degraded by chondroitinase ABC. These results indicate that the peak in the P-10 chromatograph eluting in the column void volume probably is composed of dermatan sulfate proteoglycan.

Compared to other glycosaminoglycans, dermatan sulfate, identified by its resistance to hyaluronidase and nitrous acid and susceptibility to chondroitinase ABC, has the greatest affinity for LDL under physiological conditions and has been found in high concentrations in arterial lesions[10]. Our results suggest a mechanism to explain the increased incidence of fatty lesions in aortas of animals whose serum lipid content is elevated. The increased serum LDL content in the aorta stimulates an increase in growth of those aorta smooth muscle cells exposed to blood flow due to prior injury of the arterial wall[3, 11]. The growth increase is accompanied by an increased synthesis and secretion of dermatan sulfate proteoglycan which complexes with the serum LDL to form insoluble products. The buildup of insoluble lipids causes loss of cell viability. Eventually, calcification may occur to render the proteoglycan-LDL complex even more resistant to removal from the lesions by enzymatic degradation. This hypothesis is consistent with the increase in dermatan sulfate found in arteries that develop lesions after extensive lipid infiltration[10].

Selective inactivation of catalase during protoporphyrin induced photohemolysis of human red blood cells

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Summary. The level of some enzymatic activities in red blood cells before and after photohemolysis induced by protoporphyrin IX was studied. A 30% decrease in catalase activity was found both in normal erythrocytes and those from patients affected by favism. Other proteins though present in larger amounts inside the erythrocytes are not affected by the photohemolytic process.

The photodynamic effect of protoporphyrin on red blood cells has recently been studied, in view of its significance as a model system for porphyrias[1]. A further interesting aspect is to identify the early damaged component of the membrane[2–6]. While the previous papers were concerned with the membrane damage in general, in the present communication we report about the inactivation of intraerythrocytic catalase during photohemolysis.

Materials and methods. Human red blood cells were obtained by venipuncture from healthy people and from patients affected by favism, using oxalate as anticoagulant. The red cells were collected by centrifugation and washed 3 times with isotonic saline. Then the packed cells were suspended in 100 vol. isotonic saline and incubated in the presence of 1 μM protoporphyrin IX (Sigma, St. Louis, Mo, USA). Samples were irradiated within a thermostated bath with 2 Osram projector 150 W lamps situated at 30 cm distance.

After 5–30 min incubation under light, the samples were stored in the dark for 2 h, then centrifuged and the extent of hemolysis was evaluated by measuring the absorbance of supernatant at 550 nm. Control samples were obtained either by incubation in the dark or by hypotonic hemolysis.

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