plet-, ^2J (C_{5a}–H_{10}): 6 · 1 Hz), and C_{10} (32.6 ppm; tt, ^1J (C_{10}–H_{10}): 127.5 Hz, ^3J (C_{10}–H_4) = ^3J (C_{10}–H_5): 3 · 5 Hz). Therefore, compound (**1**) can be assigned as 6-geranyloxy-3-methyl-1,8-dihydroxyanthrone. The basic skeleton and substitution pattern of the aromatic portion is consistent with the polyketide biosynthetic pathway. Furthermore, chemical shifts of the aromatic protons of compound (**1**) are in agreement with those of aromatic protons in the madagascin anthrone[12] (**2**). Oxidation of compound (**1**) in alkaline dimethylsulfoxide[13] gave compound (**3**) which gave UV absorptions at λ_{max}^{MeOH} (log ε), 455 (3.86), 286 (4.07), 262 (4.10), and 221 (4.35) nm. The chemical shifts for the protons in compound (**3**) are comparable to madagascin[12] (**4**).

Efforts to synthesize compounds (**1**) and (**3**) and to establish the important structural features for antitumor activity in this series are presently underway in our laboratory.

1 To whom correspondence should be addressed. The authors acknowledge the support from USDA for collection of the plant material and the National Cancer Institute for support of this work under contract No. NO1-CM-97296. This is paper 15 in the series 'Potential Antitumor Agents'.

2 J.C. TH. UpHof, in: Dictionary of Economic Plants, 2nd edn., p. 434. Strechert-Hafner Service Agency, Inc., New York 1968.
3 G.H.M. Lawrence, in: Taxonomy of Vascular Plants, p. 603. The MacMillan Company, New York 1964.
4 J.M. Watt and M.G. Breyer-Brandwijk, in: The Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd edn., p. 494. Livingstone Ltd, Edinburgh 1964.
5 R.I. Geran, N.H. Greenberg, M.M. MacDonald, A.A. Schumacher and B.J. Abbott, Cancer Chemother, Rep. *3*, 1 (1972).
6 Cytotoxicity was assayed under the auspices of contract No. NO1-CM-97296. The Division of Cancer Treatment, National Cancer Institute, National Institute of Health, Bethesda, Maryland awarded support for this work.
7 Compound **1** showed borderline but reproducible activity in vivo against P-388 leukemia in mice and cytotoxicity in vitro toward cells derived from human carcinoma of the nasopharynx (KB).
8 P.R. Kinget, Planta med. *15*, 234 (1967).
9 C.-j. Chang, H.G. Floss and W. Steck, J. org. Chem. *42*, 1337 (1977).
10 W. Steck and M. Mazurek, Lloydia *35*, 418 (1972).
11 C.-j. Chang, Lloydia *41*, 17 (1978).
12 B. Ritche and W.C. Taylor, Tetrahedron Lett. *1964*, 1431.
13 D.W. Cameron, J.E. Edmonds and W.D. Raverty, Aust. J. Chem. *19*, 1335 (1976).

Acidic glycosaminoglycans in human coronary arteries, with special reference to the presence of heparin or related glucosaminoglycan[1]

K. Murata and Y. Yokoyama

Department of Medicine and Physical Therapy, University of Tokyo School of Medicine, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113 (Japan), 10 March 1981

Summary. Acidic glycosaminoglycans (AGAG) in 3 branches of the human coronary artery were enzymatically analyzed. The main AGAG were heparan sulfate, chondroitin sulfates and dermatan sulfate. The yield of AGAG decreased in the order of left, right and peripheral branches. Heparin or a related glucosaminoglycan were dominant in the peripheral branch.

Acidic glycosaminoglycans (AGAG) may play a role in the process of atherosclerosis in the coronary arteries as negatively charged macromolecular substances. It is evident that the arterial AGAG possess physiological functions such as antithrombogenic and anti-coagulant properties[2–4]. Numerous studies have reported that the AGAG in the aorta consist of chondroitin sulfates (CS), dermatan sulfate (DS), heparan sulfates (HS) and hyaluronic acid (HA)[3–7]. The presence of a certain amount of heparin in the aorta has been indicated[5,8,9]. In contrast, little is known about the constitution of AGAG in the coronary artery[10,11]. Recently, Likar et al. reported the presence of mast cells in bovine coronary arteries[12]. To the authors' knowledge, no attempt has been made to analyze the AGAG in different branches of the coronary artery.

The present study reports qualitative and quantitative analyses of AGAG in the different branches of the human coronary artery using enzymatic assay[13]. We found a different constitution of the AGAG among the left, right and peripheral coronary arteries. It is of interest to note that heparin or a related glucosaminoglycan are present in human coronary arteries, in the peripheral branch in particular.

Materials and methods. Fresh human coronary arteries were

Composition of acidic glycosaminoglycans in different branches of coronary arteries by enzymatic assay

AGAG	Unsaturated disaccharides	Left (n = 11)[a]	Right (n = 13)	Peripheral (n = 13)
Total AGAG (mg)[b]		5.88 ± 0.43[d]	5.56 ± 0.37	5.12 ± 0.50
Hyaluronic acid (%)[c]	Nonsulfated	5.2 ± 1.2	6.6 ± 0.7	7.1 ± 1.0
Chondroitin 4-sulfate (%)	4-Sulfated	14.6 ± 1.2	11.9 ± 0.7	10.3 ± 0.5
Dermatan sulfate (%)	4-Sulfated	13.2 ± 0.8	15.0 ± 0.8	15.9 ± 1.7
Chondroitin 6-sulfate (%)	6-Sulfated	28.6 ± 1.8	26.4 ± 0.8	26.1 ± 1.9
Oversulfated dermatan sulfate (%)	Di-sulfated	2.8 ± 0.2	3.2 ± 0.6	2.8 ± 0.2
Heparan sulfates (plus heparin) (%)	Undigested with chondroitinase-ABC	35.6 ± 2.2	36.9 ± 2.4	37.8 ± 2.5

[a] Each sample consisted of 3–4 pooled branches based on the closed age in years. [b] Measured as uronic acid and expressed as mg/g defatted dry weight. [c] Measured as uronic acid and expressed as percent to total AGAG. [d] Mean and SE.

dissected from the hearts of 46 male subjects in the 3rd decade. They were separated carefully from perivascular tissue and divided into the left, right and peripheral coronary arteries. The AGAG were prepared by the method reported previously[2–4,9], as follows: Lipid was extracted with methanol-chloroform (1:2/vol). Protein was repeatedly digested by pronase (1,000,000 tyrosine units/g, Kaken Kagaku Co., Tokyo) at a rate of 20 mg/g dry weight at pH 7.8 at 8-h intervals. β-Elimination was performed with 0.5 M NaOH at 5 °C overnight. After cold trichloroacetic acid had been added to a concentration of 10%, the mixture was centrifuged. The supernatant containing the AGAG was dialyzed against running tap water and distilled water for 12 h each and the dialyzate was concentrated in a flash evaporator at 37 °C. Cetylpyridinium chloride (CPC) was then added to precipitate completely the AGAG-CPC complex to which 4 vol. of ethanol was added. The AGAG thus prepared were then passed through Dowex 50W×8 (H^+ form), applied to a Dowex 1-X2 column (Cl^- form) and eluted with 3.0 M NaCl. The eluate was desalted and used for electrophoresis in pyridine formic acid[14], in calcium acetate[15] and barium acetate buffers[16] and for enzymatic assay using chondroitinases and other specific AGAG-lyases[13]. The duplicate experiments showed an average yield of 95%. The AGAG were digested by chondroitinase-ABC and -AC to their constituent unsaturated disaccharides which were then separated in 1-butyric acid – 0.5 M ammonia (5:3/vol) by descending paper chromatography[4,9,13]. The AGAG content was measured by the borate carbazole reaction[17], after the separated disaccharide spots and the origin had been cut out and eluted with water.

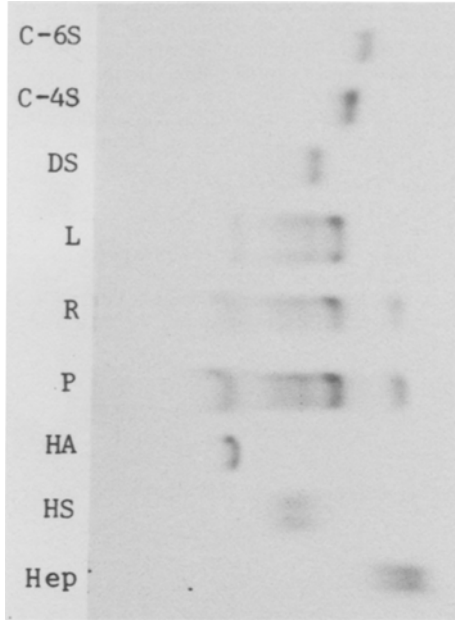

Figure 1. Electrophoretic characterization of AGAG in three branches of the coronary arteries in 0.1 M pyridine formic acid at 0.5 mA/cm for 180 min. Note the fastest band corresponding to standard heparin (Hep) stained denser in the peripheral (P) branch than in the main branches (R = right, L = left). The band remained undigested with chondroitinase-ABC and -AC (not shown in the figure).

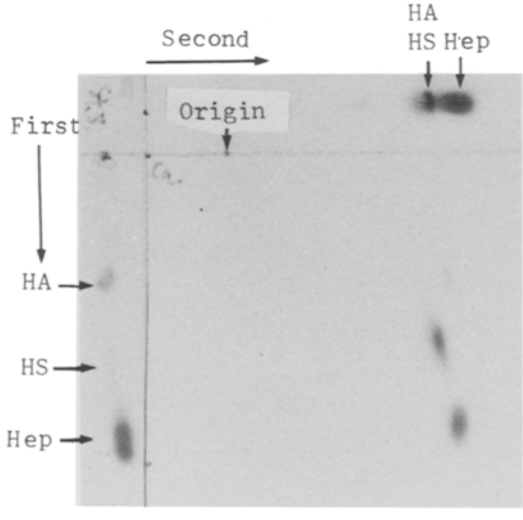

Figure 2. Separation of heparin from the AGAG in the peripheral branch of the coronary artery by two-dimensional electrophoresis. The AGAG had been digested with chondroitinase-ABC and the undigested AGAG were separated by 0.1 M pyridine-formic acid, pH 3.0, at 0.5 mA/cm for 50 min (= 1st run) and by 0.1 M calcium acetate, at 0.5 mA/cm for 150 min (= 2nd run). Three spots were detected which corresponded to standard heparin (Hep), heparan sulfate (HS) and hyaluronic acid (HA).

Results and discussion. Electrophoretic patterns of the AGAG indicated that human coronary arteries contain mainly HS, CS, DS and HA. In pyridine formic acid buffer, the faster band corresponded to standard chondroitin-4-sulfate plus -6-sulfate (C-4S plus C-6S); the intermediate diffuse band to standard DS and HS; and the slow band to standard HA (fig. 1). The reddish, fastest moving spot corresponding to standard heparin was detected in the peripheral as well as in the right branches. The characterization of AGAG was confirmed by electrophoresis in the other two buffer systems. The enzymatic analysis of AGAG in human coronary arteries is summarized in the table. The AGAG content in the coronary artery decreased going from the left to the right and then the peripheral branches. The main AGAG was found to be HS (possibly plus some heparin or related glucosaminoglycan) followed by C-6S, DS, C-4S and HA. The proportion of HS, DS and HA to the total AGAG increased in order of the left, right and peripheral branches, whereas that of C-6S and C-4S decreased in this order.

The fastest spot, corresponding to standard heparin, in pyridine-formic acid was not detected electrophoretically after digestion with purified heparinase[18]. The observation that this substance contained glucosamine, using thin-layer chromatography[19], supported the view that it is heparin or a related glucosaminoglycan. Two-dimensional electrophoresis[20] confirmed the presence of heparin in AGAG undigested with chondroitinase ABC (fig. 2). The relative amount of heparin was estimated as less than 5% by electrophoretic analysis. The occurrence of heparin in the coronary artery would be possible, since the presence of mast cells was reported in the arterial tissue by Jorpes[21] and by Pollak[22], and in bovine coronary arteries by Likar et al.[12]. Recently some heparin was found to be present in aortic tissue[8,9]. The AGAG containing HS, DS and HA were dominant in the peripheral branch, whereas the AGAG with the CS isomer were enriched in the main branch. This indicates that the AGAG component in the peripheral branch is rather close to that of human cerebral artery[23], aortic outer layer[3,9] and venous tissue[2].

It would not be surprising to find heparin and related glucosaminoglycans in the coronary arteries. The significance of the presence of heparin in the peripheral branch is not clear. Such sulfated AGAG may play a role in part in reducing thrombogenic lesions by producing anti-coagulant

activity in the coronary arteries, in the peripheral branch in particular. It may be associated with the fact that atherosclerotic lesions are more frequent in the main branches than in the peripheral branch of the coronary artery.

1 The study was supported, in part, by grants-in-aid for scientific research from the Ministry of Education, Science and Culture of Japan, and the Adult Disease Clinic Memorial Foundation, Tokyo. Thanks are due to Dr M. Okudaira and Dr R. Kono for collecting specimens.

2 K. Izuka and K. Murata, Atherosclerosis *16*, 217 (1972).
3 K. Murata, K. Nakazawa and A. Hamai, Atherosclerosis *21*, 93 (1975).
4 K. Murata, K. Izuka and K. Nakazawa, Atherosclerosis *29*, 95 (1978).
5 E. Buddecke, Z. physiol. Chem. *318*, 33 (1960).
6 V. Kumar, G.S. Berenson, H. Ruiz, E.R. Dalferes, Jr, and J.P. Strong, J. Atheroscler. Res. *7*, 573 (1967).
7 R.L. Stevens, M. Colombo, J.J. Gonzales, W. Hollander and K. Schmid, J. clin. Invest. *58*, 470 (1976).
8 K. Izuka and K. Murata, Experientia *29*, 655 (1973).
9 K. Murata, Connect. Tissue Res. *6*, 131 (1978).
10 C. Velican and D. Velican, Atherosclerosis *29*, 141 (1978).
11 M. Tammi, P.O. Seppälä, A. Lehtonen and M. Möttönen, Atherosclerosis *29*, 191 (1978).
12 I.N. Likar, R.W. Robinson and L.J. Likar, Atherosclerosis *38*, 231 (1981).
13 K. Murata, in: Methods in Carbohydrate Chemistry, vol. 8, p. 81. Ed. R.L. Whistler and J.N. BeMiller. Academic Press, New York 1980.
14 K. Murata, T. Ogura and T. Okuyama, Connect. Tissue Res. *2*, 101 (1974).
15 N. Seno, K. Anno, K. Kondo, S. Nagase and S. Sato, Analyt. Biochem. *33*, 197 (1970).
16 E. Wessler, Analyt. Biochem. *26*, 439 (1968).
17 T. Bitter and H. Muir, Analyt. Biochem. *4*, 330 (1962).
18 Z. Yoshizawa, Biochim. biophys. Acta *141*, 600 (1967). The heparinase (EC.4.2.2.7) was prepared from *Flavobacterium heparinum* and purified through ion-exchange chromatography. The enzyme digests specifically heparin from HS.
19 E. Moczar, M. Moczar, G. Schillinger and L. Robert, J. Chromat. *31*, 561 (1967).
20 R. Hata and Y. Nagai, Analyt. Biochem. *45*, 462 (1972).
21 J.E. Jorpes, H.J. Holgren and O. Wilander, Z. mikrosk.-anat. Forsch. *42*, 279 (1937).
22 O.J. Pollak, Circulation *12*, 490 (1955).
23 K. Murata and K. Nakazawa, Atherosclerosis *25*, 31 (1976).

Pseudocholinesterase in obesity: Hypercaloric diet induced changes in experimental obese mice[1]

K.M. Kutty, S.N. Huang and K.T. Kean

Department of Laboratory Medicine, The Dr Charles A. Janeway Child Health Centre and Discipline of Pathology, Memorial University of Newfoundland, St. John's (Newfoundland, Canada A1A 1R8), 2 March 1981

Summary. Pseudocholinesterase activity is significantly higher in liver and serum, but lower in adipose tissue of genetically obese, diabetic and gold thioglucose treated mice. Similar enzyme changes were also observed in lean mice on a high carbohydrate diet. A marked reduction (40%) in PChE activity occurred in the liver of genetically diabetic mice when starved for 24 h. These observations suggest that pseudocholinesterase induction in the liver and repression in the adipose tissue is affected by excessive calorie intake in obesity. This provides a model to study the biological function of PChE in health and disease.

Since its discovery in human plasma 50 years ago, pseudocholinesterase (PChE, acylcholine acyl hydrolase, E.C. 3.1.1.8) has been found in many tissues and animals[2,3]. There have been, however, very few studies conducted to understand the biological function of this enzyme. Several studies suggested that PChE may have a function in the metabolism of lipids[4,5] and low density lipoprotein[6–8]. We and others have observed a significant increase in serum PChE activity in obese and hyperlipemic patients[9,10].

We now report the results of studies conducted in experimental models of obesity in order to obtain a better understanding of the relationship between PChE and obesity.

Male obese mice (ob/ob) and its lean controls (ob/+) of the strain C57BL/6J of age groups 10 and 5 months, diabetic mice (db/db) and its nondiabetic controls (db/+) of the strain C57BL/KsJ of 10 weeks old were used in this study.

The animals were all kept in individual cages under ideal conditions and fed ad libitum with regular mouse chow (Purina, protein 17.5%, fat 11%, carbohydrate 53.9% and gross energy 4.3 kcal/g) and free access to water. The mice in group II, subgroups c and d, were treated differently, as indicated in the table. They were given a high carbohydrate diet (ICN-Pharmaceuticals, protein 18%, fat 8%, carbohydrate 68% and gross energy 4.2 kcal/g) with free access to water containing 10% sucrose for a period of 3 months. The animals in subgroup d were also injected with gold thioglucose (0.5 mg/g b.wt in 2 equal doses, one at the beginning and another 30 days later) i.p. to induce obesity[11]. 4 (db/db) mice in group III were fasted for 24 h prior to sacrifice. The mean daily calorie intake was calculated on the basis of daily food intake over a period of 15 days. The group I was a preliminary study, and no measurement was made in this respect.

Before collecting the tissue and blood samples, animals were immobilized with the anesthetic Ethrane (2-chloro-1,1,2-trifluoroethyl difluoromethyl ether). Blood was collected by heart puncture and livers were first perfused with normal saline before isolation. Epididymal fat pads were dissected out very carefully avoiding major blood vessels. Sera and tissues were stored at −20 °C until ready for use. For analysis, livers were homogenized with 1% triton X-100 in 0.25 molar potassium chloride in a ratio of 1:4 and supernatant obtained by centrifuging at 3000 rpm for 15 min in the cold. Adipose tissues were homogenized with cold distilled water in ratio of 1:2 and spun at 1500 rpm for 10 min. The clear infranatant aqueous phase was used for enzyme studies.

PChE was measured[12] using the substrate propionylthiocholine iodide and the color forming reagent 5,5′ dithiobis-(2-nitrobenzoic acid) (DTNB) and recording the absorbance change at 410 nm of the thiocholine-DTNB colored complex formed. The enzyme activity was expressed in terms of the μmoles of thiocholine formed in 1 min by 1 ml of serum or g wet wt of tissue. Serum glucose was measured by the glucose oxidase method using the autoanalyzer Gilford 3500[13].

I.p. injection of tetramonoisopropylpyrophosphor tetramide (Iso-OMPA, 2.3×10^{-8} mole/g b.wt), a specific PChE inhibitor[14] almost completely abolished the enzyme activity in the liver, serum and adipose tissue.