but the levels were only measurable in samples of kidney homogenate. These results show that after oral administration of a solution of SHAM, its absorption and distribution into the tissues is rapid. The concentrations of SHAM in the kidney, which is responsible for the excretion of unchanged SHAM as well as the glucuronide and sulphate conjugated metabolites of SHAM[5], are noticeably higher than in the other tissues. The levels of SHAM determined in the liver are relatively low (they are of the same order as those found in the brain). Although salicylamide was detected in all sampled tissues, the levels were only measurable in the kidney.

The plasma protein binding of SHAM was assessed by equilibrium dialysis using Visking membrane using human plasma from 1 individual spiked at concentrations over the range between 12 µg/ml and 10 mg/ml. Dialysis was made against pH 7.4 phosphate buffer (0.067 M) at 37 °C. Over this concentration range the overall binding of SHAM was 89.7 (SD 2.2)% for 31 determinations. There was no evidence of saturation of binding at the highest concentrations since the mean binding was 95.7% at 10 mg/ml, 98.6% at 1000 µg/ml and 87.7% at 12 µg/ml. This binding is probably tight and there was no displacement of SHAM at a concentration of 50 µg/ml from its binding by concentrations of salicylate ranging from 5-30 µg/ml. These observations would suggest that the relatively low tissue concentrations of SHAM may reflect its high degree of protein binding. Furthermore, they also indicate that renal elimination may be mediated mainly via tubular secretion rather than by glomerular filtration.

Half-maximal inhibition of oxygen uptake by trypanosomes in vitro requires 15 µM SHAM (approximately 2.3 µg/ml) and 100 µM (approximately 15.3 µg/ml) inhibits over 90% of the trypanosome respiration[2]. Figure a shows that this latter concentration is barely attained in any of the tissues studied, apart from the kidney, and that because of the rapid half-life of SHAM in the tissues, effective levels are not long maintained. These factors presumably explain why even a dose of 500 mg/kg SHAM given to rats infected with *Trypanosoma brucei* does not prolong their survival time[2].

Thus the short half-life (found to be 30 min in the mouse) and the high degree of protein binding may explain the relative lack of in vivo efficacy of SHAM[2,3,6] despite its promising in vitro profile. Although its elimination is probably predominantly renal, interference with this route of excretion is unlikely to prove beneficial in improving the therapeutic ratio.

1 D.A. Evans and R.C. Brown, Trans. R. Soc. trop. Med. Hyg. *67*, 258 (1973).
2 F.R. Opperdoes, P.N. Aarsen, C. Van Der Meer and P. Borst, Expl Parasit. *40*, 198 (1976).
3 C. Van Der Meer and J.A. Versluijs-Broers, Expl Parasit. *48*, 126 (1979).
4 A.J. Barnicoat, W.G. van't Hoff, P.J. Morrison and I.D. Bradbrook, J. Chromat., in press (1981).
5 W.M. McIsaac and R.T. Williams, Biochem. J. *66*, 369 (1957).
6 C. Van Der Meer, J.A.M. Versluijs-Broers, C.T. Vanduin, T.S.G.A.M. Vandennigh, J. Nieuwenhuijs and D. Zwart, Tropenmed. Parasit. *31*, 275 (1980).

Spontaneous rhythmic contractions in isolated human coronary arteries[1]

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Summary. Isolated post mortem human coronary arteries developed rhythmic contractions in physiological saline solution without being exposed to vasoactive agents.

In recent years, coronary vasospasms have been attracting attention as possible trigger mechanisms in the onset of various forms of ischemic heart diseases such as Prinzmetal's variant form of angina pectoris[2,3] or myocardial infarction[4,5]. However, the etiology of such spasms is still unclear. We noticed that isolated post mortem human coronary arteries developed phasic, rhythmic contractions in a period of several min in a nutrient solution without being exposed to vasoactive agents.

Materials and methods. 4 coronary artery segments each were taken from 29 post mortem human hearts (19 males, 10 females, aged from 25 to 80, average 59 years), each segment measuring about 1.5 cm in length. The hearts were extirpated within 18 h after death. The segments were from the proximal portion of the right coronary and left anterior descending arteries and the mid portion and distal portion of the left anterior descending artery (116 coronary artery segments). Immediately after death, the cadavers were placed in a room kept at a constant temperature of 8-9 °C. The time that elapsed from the autopsy to the start of the experiments was no more than 5 h. During this period, the isolated arteries were preserved in a nutrient solution saturated with a mixture of 95% O_2 and 5% CO_2 at a temperature of 4 °C. The arteries were helically cut at an angle of approximately 45° to the longitudinal axis into strips according to the method of Furchgott and Bhadrakom[6]. The helical strips were fixed vertically between hooks in a 50-ml bath containing a nutrient solution. The upper end of the strip was connected to the lever of an isometric tension transducer (TB611T Nihonkoden Kogyo Co., Tokyo, Japan). The resting tension was adjusted to 1.5 g (proximal portion and mid portion) and 0.5 g (distal portion). The bathing solution was bubbled with a mixture of 95% O_2 and 5% CO_2 and was maintained at 37 ± 0.5 °C. The pH of the solution was 7.4. The composition of the bathing medium was as follows (in mmolar concentrations): Na^+, 162.1; K^+, 5.4; Ca^{++}, 2.5; Mg^{++}, 0.76; Cl^-, 157.0; $H_2PO_4^-$, 1.7; HCO_3^-, 14.9; dextrose, 5.6. The sam-

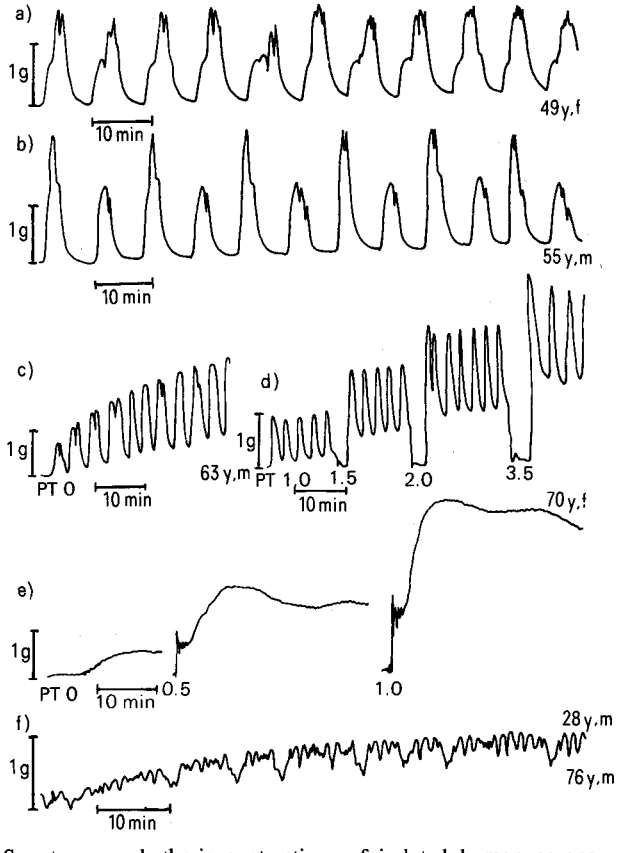
Incidence of rhythmic contractions and developed tension classified by portion of the coronary arteries

	Outer diameter (mm)*	Incidence N	%	Developed tension (g)**
RCA (proximal)	3.95 ± 0.74	14/24	58	1.00 ± 0.49
LAD (proximal)	4.15 ± 0.71	10/24	42	0.66 ± 0.19
LAD (mid)	3.55 ± 0.53	12/24	50	0.77 ± 0.24
LAD (distal)	1.81 ± 0.60	4/24	17	0.88 ± 0.39

N, number of preparations developed rhythmic contractions/number of preparations examined. RCA, right coronary artery. LAD, left anterior descending artery. * Mean ± SD, ** Mean ± SE.

ples were taken from cases diagnosed as having cerebrospinal disease, cancer, peptic ulcer and toxicosis in which no heart diseases had been clinically proved. They were treated with various drugs including catecholamines, digitalis, diuretics and antiarrhythmic agents in the 48 h before death. 3 cases had no medication.

Results. The coronary arteries obtained from 24 hearts isolated within 8 h after death developed spontaneous ryhthmic contractions or tonic contractions in a nutrient solution without the addition of vasoactive agents. The coronary arteries obtained from 5 hearts that had been isolated more than 12 h after death showed neither rhythmic nor tonic contractions. When coronary arteries obtained from 3 hearts that had been isolated within 8 h after death were preserved in a nutrient solution at 4 °C, they produced spontaneous rhythmic contractions even up to 20 h later. The spontaneous rhythmic contractions occurred irrespective of the kind of diseases present or drugs used and when no medication was administered. The spontaneous rhythmic contractions developed in 19 out of the 24 hearts (79%) and in 40 out of the 96 coronary artery segments (42%). The incidence classified by portion is shown in the table. The largest number was observed in segments of the proximal portion of the right coronary artery, and the smallest in the distal portion of the left anterior descending artery. The period of cyclic contractions ranged from 42 sec to 10 min, averaging 3 min and 42 sec. The developed tension ranged from 0.1 to 5.7 g, with an average of 0.8 g. The contraction waves were generally consistent (fig., a). However, 5 instances of alternative properties were noted (fig., b). In 4 instances, the spontaneous rhythmic contractions occurred without the application of passive tension (fig., c). A rise in the active tension resulting from a gradual increase of the passive tension was noted. In such instances, however, no changes were observed in the cycles (fig., d). Spontaneous rhythmic contractions did not occur in any of the 5 hearts from patients below 42 years of age; only tonic contractions were noted. The active tension of these coronary artery strips rose gradually as the passive tension was increased (fig., e). In the 19 hearts taken from patients ranging from 49 to 80 years of age, spontaneous rhythmic contractions were noted. In those cases of advanced coronary atherosclerosis, the cycles averaged 1 min and were relatively short and irregular, while the developed tension averged 0.1 g, also relatively small (fig., f).



Spontaneous rhythmic contractions of isolated human coronary arteries: *a* Typical contraction waveform. *b* Case showing alternative properties. *c* Case showing rhythmic contractions occurring when passive tension (PT) is not applied. *d* Changes in contraction reactions caused by a gradual increase in passive tension: *e* Changes in tonic contraction caused by a gradual increase in passive tension. *f* Spontaneous contraction waveform of a case of advanced coronary atherosclerosis.

Discussion. The mechanism and physiological significance of the spontaneous rhythmic contractions in human coronary arteries are as yet unclear. Spontaneous rhythmic contractions have been observed in the small arteries of the dog's paw[7] and in the portal mesenteric veins of the rat[8] and of the mouse[9]. However, it has been considered to be a rare phenomenon in large vessels. D'Hemecourt[9] noted spontaneous rhythmic or sustained contractions in the coronary arteries from the rabbit heart, but in none from its skeletal muscle. Such contractions occurred more often in large than in peripheral small coronary arteries. In our observations, the incidence of spontaneous rhythmic contractions in the small coronary arteries was lower than in the large coronary arteries. Though it cannot be concluded that spontaneous rhythmic contractions of human coronary arteries in vitro represent in vivo physiological contractions and relaxations of the coronary arteries, it is believed that studies on this phenomenon will be of use in the clarification of the trigger mechanisms of ischemic heart diseases that are mainly caused by coronary vasospasms. Thus, we believe our future work is as follows. a) Despite the fact that the cases we dealt with did not include cases of Prinzmetal's variant form of angina pectoris or other ischemic heart diseases, spontaneous contractions occurred. Therefore, similar experiments on coronary arteries affected by these diseases and comparative studies on the reactions produced by non-ischemic heart diseases are necessary. b) Studies on pharmacological differences between normal and ischemic heart diseases should be undertaken.

Addendum. We were unaware of Ross' paper (Cardiovasc. Res. *14*, 613 (1980)) at the time we originally submitted our article. Coronary arteries that were isolated immediately at the time of a cardiac transplantation also produced spontaneous phasic contractions in the same manner as coronary arteries isolated from cadavers.

1 This study was supported by a Scientific Research Fund (No. 56480181) from the Ministry of Education, Japan.

2 J.S. Schroeder, J.F. Silverman and D.C. Harrison, Chest *65*, 573 (1974).
3 A. Maseri, R. Mimmo and S. Chierchia, Chest *68*, 625 (1975).
4 H.R. Hellstrom, Am. Heart J. *90*, 545 (1975).
5 P.B. Oliva and J.C. Breckinridge, Circulation *56*, 366 (1977).
6 R.F. Furchgott and S. Bhadrakom, J. Pharmac. exp. Ther. *108*, 129 (1953).
7 B. Johansson and D.F. Bohr, Am. J. Physiol. *210*, 801 (1966).
8 J. Axelsson, B. Wahlstrom, B. Johansson and O. Jonsson, Circulation Res. *21*, 609 (1967).
9 H. Mislin, Revue suisse Zool. *70*, 319 (1963).
10 A. d'Hemecourt and R. Detar, in: Primary and Secondary Angina Pectoris, p. 177. Ed. A. Maseri. Grune & Stratton, New York 1978.