Operschall, Christine, Loretta Falivene, Jean-Paul Clozel, and Sébastien Roux. A new model of chronic cardiac ischemia in rabbits. J Appl Physiol 88: 1438–1445, 2000.—Chronic cardiac ischemia has mainly been studied in large species such as pigs or dogs. Little research has been performed using small species such as rabbits. In the present study, 1–3 wk after implantation of a novel device (ameroid) on the circumflex coronary artery of New Zealand White rabbits, vessel patency was evaluated by coronary angiography, corrosion cast, and radiolabeled microspheres. Coronary angiograms showed, after 21 days, either total occlusion or severe stenosis in seven of eight arteries, which was confirmed by corrosion casts. The ameroid group had less blood flow in the epicardial (−62%) and endocardial (−54%) layers of the ischemic area compared with sham-operated rabbits (P < 0.05). Blood flow increased in the ischemic area compared with day 0 during acute occlusion, suggesting that progressive coronary occlusion initiated the growth of de novo collateral vessels. Thus we have developed a new model of chronic cardiac ischemia in rabbits with documented progressive coronary stenosis and occlusion that is suitable to test various therapeutic angiogenesis strategies.

A new model of chronic cardiac ischemia in rabbits

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METHODS

The study was subdivided into four parts, all of which were performed in New Zealand White rabbits that were implanted with an ameroid. The first, second, and third parts of the study were designed to document the stenosis or occlusion of the coronary artery by the ameroid with different techniques, namely, angiography of the left coronary artery, corrosion cast of the coronary tree, and coronary blood flow measurements with radiolabeled microspheres. In the fourth part of the study, infarct size was determined.

The experimental procedures and protocols used in this investigation were reviewed and approved by the ethics committee of Basel.

Ameroid constrictor. We have used a newly designed hygroscopic ameroid constrictor specially designed for constriction of coronary arteries of small diameter. It is a two-pored cylinder, stained with a lead dye for X-ray localization and fixed on the epicardial surface of the heart by a silk thread, surrounding the coronary artery of interest (Fig. 1). Thus, by use of this constricting device, the artery gradually narrows and eventually occludes as the space between the ameroid and the myocardium shrinks. After 21 days the average volume of the 13 implanted ameroids increased from 98 to 171 mm3 (75% increase). Measurement of the ameroid size at various time points showed that its final size was reached after ~10 days (Fig. 2).
Fig. 1. Schematic drawing of the novel cylindrical ameroid constrictor tied on the epicardium (5 mm diameter, 5 mm height). Nonabsorbable thread passes beneath intramyocardial coronary branch and is brought to the surface on its other side (left). By progressive swelling, ameroid gradually constricts the coronary artery within ~10 days (right).

Surgical implantation. New Zealand White rabbits (3.5–4.0 kg) were sedated with intravenous 20 mg/kg thiamylat sodium (Surivet, Dr. E. Graeub, Bern, Switzerland), the trachea was intubated (2.5 mm ID; neonatal tube, tracheal end, Cole-Parmer) for mechanical ventilation (Servo Ventilator 900C, Siemens-Elena, Solna, Sweden), and expired air was monitored (Normocap 200, Datex Medical Instruments, Tewksbury, MA). Anesthesia was maintained on 3–4% isoflurane (Forene, Abbott Laboratories, Abbott Park, IL) during sterile surgical procedure. Left thoracotomy was performed at the third intercostal space, and the lungs were gently retracted. After the incision of the pericardium, 1,000 IU heparin (Roche Pharma, Basel, Switzerland) and 150 mg acetylsalicylic acid (Synthelabo, Lausanne, Switzerland) were injected into a marginal ear vein.

The ultrasonic signal of a minor branch of the left coronary artery, either lateral branch of the left circumflex coronary artery or the left circumflex itself, was detected by a Doppler ultrasonic flowmeter (Suction-on Transducer, Iowa Doppler Products, Iowa City, IA). The artery of interest was chosen to provide a sufficiently large myocardial ischemia but without compromising cardiac pump function. Two millimeters proximal to the Doppler probe, a nonabsorbable 5-0 polyester thread (8 mm; Ti-Cron D-1, Davis and Heck, Wayne, N.J.) was driven around the coronary artery branch. This technique does not require dissection of the coronary artery, which is sometimes deeply entrenched in the myocardium. As such, the site of puncture was on the long axis, halfway from the apex of the left ventricle to the atrioventricular groove.

The knot was tightly tied while the Doppler probe signal was used to ensure that the coronary blood flow would remain undisturbed. The thoracotomy was closed in layers, and the pneumothorax was evacuated by a chest tube (Uno Mini Vacuum Set, Uno Plast). An analgesic (250 mg thiamylal sodium, Novalgin, Hoechst, Frankfurt am Main, Germany) was injected intravenously.

Angiography day 0, day 7, and day 21. Coronary artery patency was qualitatively assessed by selective coronary angiography on day 0 (before ameroid implantation), day 7, and day 21. The first angiography of the left coronary artery confirmed full patency. The next angiography on day 7 was carried out to confirm proper ameroid position and to detect stenosis or occlusion of the vessel and was repeated on day 21.

Ten rabbits were anesthetized as described above. The right carotid artery was cannulated with a 4F sheath introducer system (Avanti, Cordis, Roden, The Netherlands), and 1,000 IU heparin were injected. 3-Fr radiopaque polyethylene tubing (William Cook Europe) was introduced via the carotid artery to the aortic sinus, and the tip was positioned under fluoroscopy (Angioscop C with a Polydoros 80 generator, a Megalix X-ray tube, and a Sirecon 17/12 high-density image intensifier; Siemens-Albis, Erlangen, Germany). A total of 10–15 ml diluted (1:1) nonionic contrast medium (Iopamiro 370, Bracco, Milano, Italy) was then manually injected for selective opacification of the left coronary artery while serial images of the heart were recorded at a rate of 75 frames/s. With a fixed magnification, the arteriograms of the coronary artery were performed with a tube-to-animal distance of 85 cm. Finally, cine recording was made on a 35-mm GBX-2 Kodak film processed in a Gevamatic R10 developer to a γ-value of 1.5. The angiograms, from various angles (front, lateral right, and right anterior oblique projections), were projected on a Cap-35E (Elmo, Tokyo, Japan) and qualitatively analyzed.

Angiograms on days 7 and 21 were performed according to the same procedure.

Corrosion cast. To confirm the occlusion of the coronary artery, the hearts of the 10 New Zealand White rabbits investigated by angiography were later studied by an exact corrosion specimen.

The rabbits were given 1,000 IU heparin intravenously to prevent clotting and then were euthanized. The heart was removed, including the first proximal centimeter of the thoracic aorta. A polyethylene cannula, dilated by heat, was fixed into the aorta, and the heart was gently flushed with saline while the multicomponent plastic mixture (Batson's no. 17 plastic replica and corrosion kit, Polysciences, Warrington, PA) was prepared. Care was taken to avoid introduction of air into the system (14). When the polymerizing mixture just started to solidify, it was retrogradely injected into the ascending aorta with a constant pressure, over a period of a few minutes. The casts were kept then at room temperature during 1 h to allow solidification (exothermic polymerization reaction). Subsequently, the ameroid was gently detached from the heart, and the tie was fixed with a clamp to later identify its location. Macerating the heart tissue in a concentrated solution of sodium hydroxide for ~5 h at 50°C revealed the cast, and finally the corrosion cast was rinsed with warm running water for at least 10 min.

Microsphere blood flow measurements. Regional blood flow was sequentially determined with radiolabeled micro-
spheres (13). Different time points were chosen to track the time course of myocardial blood flow changes as coronary occlusion progressed: 1) on day 0 at baseline, without any manipulation; 2) on day 0 during a 25-s mechanical occlusion to define the area at risk; 3) on day 7; 4) on day 14; 5) on day 21 to determine the percentage of myocardial blood flow restoration in the area at risk (vs. time point 2; see above); and 6) on day 21 after mechanical occlusion obtained by gently lifting the ameroid to determine the remaining anterograde flow.

Blood flow determinations at time points 3, 4, and 5 were terminal and from different groups of rabbits. As a control, sham-operated animals underwent all of the surgery without ameroid constrictor placement and were evaluated on day 21 after surgery.

Coronary blood flow was determined in 47 female New Zealand White rabbits that were anesthetized and operated as described in Surgical implantation. Catheters were placed in a marginal ear vein (Tricath In, Codan, Esbjerg, Denmark) for drug application and in the right femoral artery (Combitrans, Braun, Melsungen, Germany) for reference blood sampling as well as for continuous monitoring of arterial blood pressure and heart rate (Linearorder WR3300, Hugo Sachs Elektronik, March, Germany). Mean arterial pressure and heart rate were measured before and during adenosine infusion.

Left thoracotomy was performed as described in Surgical implantation, and 1,000 IU heparin as well as 150 mg acetylsalicylic acid were injected into the marginal ear vein to prevent premature coronary occlusion. A Silastic catheter (0.8 mm ID) was placed into the left atrium for administration of radiolabeled microspheres. For maximal coronary vasodilation, which abolished the reactive hyperemic response to a 25-s occlusion, an infusion of adenosine (Krenosine, Sanofi Winthrop, Münchenstein, Switzerland) was given at a rate of 0.15 mg·kg⁻¹·min⁻¹ intravenously 10 min before and during adenosine infusion. In a marginal ear vein (TriCath In, Codan, Esbjerg, Denmark) for drug application and in the right femoral artery (Combitrans, Braun, Melsungen, Germany) for reference blood sampling as well as for continuous monitoring of arterial blood pressure and heart rate (Linearorder WR3300, Hugo Sachs Elektronik, March, Germany). Mean arterial pressure and heart rate were measured before and during adenosine infusion.

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After coronary blood flow measurements (baseline and acute coronary occlusion), the ameroid was implanted, all catheters were removed, and the thoracotomy was closed as described in Surgical implantation. The rabbits were allowed to recover for 7, 14, or 21 days, until the terminal part of the study.

Animals were euthanized with an intravenous overdose of pentobarbital sodium. The heart and kidneys were excised and preserved for 2 days in 4% buffered formaldehyde solution. The left ventricle was isolated and cut into five short-axis slices. The uppermost slice, in most cases situated above the ameroid, was excluded from the analysis. Every slice was cut into eight circumferential wedges: two septal (nonischemic zone) and six from the left ventricle free wall that were further subdivided into epicardial and endocardial halves. Each animal yielded at least 60 pieces, with each of them containing at least 400 microspheres during baseline conditions. All pieces were placed in plastic tubes and counted in a multichannel γ-spectrometer (germanium well-type detector/PCA multiport analyzer). Counts were converted into regional myocardial flows by multiplying by the reference blood withdrawal rate and dividing by the counts in the reference blood sample. Finally, all blood flows were normalized for sample weights. Myocardial blood flow results were expressed as absolute values in milliliters per minute per gram wet weight.

A section of the left ventricle was considered to belong to the area at risk when regional blood flow decreased to <30% of the nonischemic septum during acute occlusion (20, 35).

Histology. Seven rabbits assigned for histological determination of the infarcted area underwent ameroid implantation as described in Surgical implantation but without application of radioactive microspheres. After 21 days the rabbits were euthanized, and their hearts were fixed in 4% buffered formaldehyde solution for 2 days. The left ventricle was cut into six short-axis slices of ~3-mm thickness. Each slice was embedded in paraffin and sectioned by microtome into 4-μm slices. Microscopic sections were cut from the apical surface of each block and stained to differentiate viable myocardium from scar (Goldner trichrome). The slice with the thread and the ameroid were excluded from analysis, because direct contact by the constrictor usually caused artificial epicardial necrotic foci. Similarly, the uppermost slice of every heart, in all cases situated above the ameroid, was excluded from the analysis. Quantification of the left ventricle as well as the infarcted area was obtained with a digitizing tablet (DiaSys, Datalab, Thoerigen, Switzerland). The infarcted area was expressed as a percentage of left ventricle mass, and the average of every heart was determined.

Statistical analysis. Data are expressed as means ± SE. For the statistical analysis, Scheffé’s F procedure for multiple comparisons was used. A P value <0.05 was considered as significant.

RESULTS

The ameroid-implanted animals lost ~7% of their body weight within the first 3 days postoperatively (sham operated lost ~2%), but they regained it within 17 days after surgery.

Angiography. A total of 10 rabbits was chronically instrumented with an ameroid constrictor in the angiography and corrosion cast group. None of them died prematurely. Two had no coronary angiogram because of technical reasons and were excluded from the analysis.

Representative coronary angiograms recorded on days 0, 7, and 21 are illustrated in Fig. 3. Coronary angiograms on day 0 (before ameroid implantation) were normal in all rabbits. Coronary angiograms on day 7 revealed that the ameroid position was either on the left circumflex artery or on its lateral branch. In two rabbits the artery was already occluded after 7 days, in three the flow was severely impaired as seen in a significant slowdown of the contrast medium in the distal coronary artery, and in three the angiogram was unchanged. On day 21, three animals showed total occlusion of the artery, four animals showed a dramatic (TIMI grade 1) delay in coronary filling, and only one rabbit showed little change in coronary patency. In some animals blood flow in the coronary artery of interest was interrupted during systole, due to compression of the left ventricular muscle.
With the angiographic resolution of 1.6 line pairs/mm, we could not reliably visualize macroscopic collateral vessels.

Corrosion cast. The plastic model of the coronary arteries displays a three-dimensional image of the arterial tree and thus supports the interpretation of the angiograms. Seven of eight casts showed a stump on the vessel of interest, indicating abrupt occlusion, and one artery showed a severe stenosis at the site of the ameroid while the silk thread remained around the intact polymer vessel. One representative cast is shown in Fig. 4.

Interestingly, in one-half of the animals (n = 4) the area that originally was supplied by the occluded vessel was now supplied by retrograde flow from adjacent arteries (e.g., ramus interventricularis anterior or right coronary artery). The area of the occluded artery, the collateral zone, in the other one-half of the animals (n = 3) showed no major arteries.

Thus we could show that this model results in gradual swelling of the ameroid, leading to severe coronary stenosis on day 7 and complete coronary occlusion on day 21.

Myocardial blood flow in microspheres group. A total of 51 rabbits was implanted with an ameroid constrictor in the microspheres group. Eleven (22%) died, probably because of premature thrombotic coronary artery occlusion. Massive infarction as well as a large area at risk (mean 38% of left ventricular mass) accounted for premature death. In the survivors, the mean area at risk represented 22% of the left ventricular mass. Two animals (1 ameroid, 1 sham) with nonreproducible microsphere data were excluded from the coronary blood flow analysis.

Average blood flow in the ischemic area of the 21-day group (ameroid compared with sham) is shown in Fig. 5. Regional myocardial blood flow of the ischemic area is shown in Fig. 6. During acute coronary occlusion, no difference was observed between the ameroid and sham groups in either the endo- or epicardial regions. In both groups, myocardial blood flow decreased almost to zero in the area at risk (−94% blood flow), whereas the blood flow to the nonischemic area decreased slightly compared with preocclusion (−24% blood flow; Table 1). This result suggests that full vasodilation was achieved and that no hyperemia occurred in the adjacent region.

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Fig. 3. Selective angiograms of left coronary artery, lateral view. A: control of vessel patency before surgery. B: 7 days after ameroid implantation [selective angiogram of left circumflex coronary artery (LCX)]. Ameroid (a) is placed on a lateral branch of LCX. No significant stenosis is yet detectable. C: same coronary artery 21 days after ameroid implantation but from a slightly different angle for optimal visualization. Artery of interest is totally occluded by the ameroid.

Fig. 4. Corrosion cast details of left coronary artery performed after angiography (day 21). Branch of LCX was occluded as visualized by stump (arrow). LAD, left anterior descending coronary artery.
After 21 days, blood flow in the ischemic area of ameroid-implanted rabbits was still significantly lower than in the sham-operated rabbits, both in the epicardial as well as in the endocardial region (\(P < 0.05\)). In the ameroid group, endocardial blood flow in the area at risk reached only one-half of baseline value, whereas no significant difference from baseline value was observed in the epicardial area. The endocardium of the ischemic area was less perfused than was the epicardial region in the ameroid group, suggesting that the degree of ischemia was more severe in the endocardial layers.

Mean blood flow during the mechanical occlusion on day 21 was not significantly reduced in either layer (data not shown). This indicated that the artery of interest no longer supplied anterograde blood to the area at risk and that the remaining blood flow originated from the collaterals.

![Graph showing average epicardial (epi) and endocardial (endo) blood flow in ischemic area of ameroid-implanted and sham-operated animals measured with radiolabeled-microsphere technique. Blood flow was determined under full vasodilation at day 0 (baseline and acute occlusion (occl) of coronary artery) and day 21 (ischemic blood flow). Acute coronary occlusion elicited a total interruption of blood flow in area at risk and confirmed sparse innate collateral circulation of rabbits. During acute coronary occlusion, no difference was observed between ameroid and sham groups in endo and epi flow. Values are means ± SE; n, no. of animals. *Significant vs. sham, \(P < 0.05\). †Significant vs. occl, ameroid group, \(P < 0.05\). ¶Significant vs. occl, sham group, \(P < 0.05\).

![Graph showing average coronary blood flow in area at risk of ameroid-implanted animals, continuously measured with radiolabeled-microsphere technique beginning on day 0 up to day 21. Blood flow in the ischemic area was reduced 7 days after ameroid implantation, compared with baseline values, and progressively increased on days 14 and 21. It reached one-half the baseline value in endo layer and was almost back to baseline value in epi layer. *Significant vs. day 21, \(P < 0.05\). †Significant vs. occl, \(P < 0.05\).

**Table 1. Myocardial blood flow in the nonischemic zone (septum) of the left ventricle**

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Ameroid (n = 13)</th>
<th>Sham (n = 8)</th>
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<tbody>
<tr>
<td>Baseline</td>
<td>3.92 ± 0.25</td>
<td>4.88 ± 0.40</td>
</tr>
<tr>
<td>Acute occlusion</td>
<td>2.96 ± 0.21</td>
<td>4.46 ± 0.22</td>
</tr>
<tr>
<td>21 Days</td>
<td>6.35 ± 0.26</td>
<td>5.59 ± 0.29</td>
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Values are means ± SE given in ml·min⁻¹·g⁻¹; n, no. of animals. Blood flow was measured with the microsphere technique under full vasodilation at 3 time points: before any manipulation (baseline), during acute occlusion of coronary artery and delineation of later ischemic area (acute occlusion), and 21 days after ameroid implantation (21 days).
The intermediate myocardial blood flow measurements 7 and 14 days after ameroid implantation suggest that the ameroid induced a progressive occlusion of the artery of interest (see Fig. 6). Accordingly, in the 7-day group, epicardial and endocardial blood flow in the area at risk (0.48 ± 0.01 ml·min⁻¹·g epicardium⁻¹; 0.32 ± 0.04 ml·min⁻¹·g endocardium⁻¹) were not significantly higher than during acute occlusion on day 0 but became significant in the epicardial layer only on day 14 (1.53 ± 0.2 ml·min⁻¹·g epicardium⁻¹; P < 0.05). Nevertheless, myocardial blood flow in the area at risk of both layers did not yet reach the 21-day values either on day 7 or on day 14 (P < 0.05).

Histological analysis. Mean infarct size was larger in the ameroid group (9 ± 4% of the left ventricle mass; n = 4) compared with the sham-operated animals (1 ± 1%; n = 3; Fig. 7). The type of the left ventricular infarct was mainly transmural, and the location was generally posterolateral. Thus necrosis could not be avoided despite progressive coronary occlusion. However, variation of the infarct size in the ameroid group was wide, ranging from 3 to 20% of the left ventricle mass.

**DISCUSSION**

This study shows for the first time that endogenous cardiac angiogenesis can be studied in a rabbit model of chronic cardiac ischemia.

The rabbit was our model of choice because of its small size, universal availability, low cost, and the similarity of its coronary circulation to the human vasculature where the number of new coronary collateral vessels usually is insufficient to supply the ischemic myocardium. Similarly, the rabbit heart is poorly collateralized, similar to that of the swine, whereas the dog heart has a highly collateralized myocardium (21, 34).

Acute coronary occlusion elicited a profound decrease in blood flow in the area at risk (later ischemic area). Such a marked decrease in regional flow confirmed the findings of Maxwell et al. (21) that rabbits have a sparse innate collateral circulation, which is remarkably similar to the healthy human heart (27). Therefore, the rabbit heart might provide an appropriate model for studying gradual coronary artery occlusion and therapeutic collateral development.

The progressive occlusion was achieved with a newly designed ameroid constrictor that required no dissection of the coronary artery with its intramyocardial situation. We investigated changes in myocardial blood flow and the time course of the coronary artery patency at baseline and after 7, 14, and 21 days after ameroid placement. The results from the microsphere data demonstrated that, 21 days after implantation, myocardial blood flow in the area at risk was significantly reduced compared with that in sham-operated rabbit hearts. The origin of this was a complete occlusion or severe narrowing of the artery as assessed by serial angiograms that showed a dramatic slowdown of the contrast medium.

We could demonstrate a progressive build up of collateral flow as denoted by various blood flow measurements on days 7, 14, and 21. Similarly, blood flow in the ischemic area decreased on day 7 and progressively increased on days 14 and 21. Indeed, partial restoration of blood flow after 21 days is in line with the concept that chronic ischemia is a strong stimulus for endogenous coronary collateral development (28).

In some hearts, we could macroscopically show on corrosion casts that blood supply to the ischemic area stemmed from other branches, such as the right coronary artery or the ramus interventricularis anterior, a branch of the left coronary artery. White et al. (35) stressed that the extracardiac collateral vessels in swine sprout into the epicardial region and that the intercoronary collaterals occur primarily in the midmyocardial and endocardial regions. Whether the origin of the increased coronary blood flow in this ameroid model was from radial growth of preexisting capillaries or, alternatively, was the result of vascular sprouting was not addressed in this study.

Progressive increase of myocardial blood flow in the area at risk for up to 21 days contrasts with results obtained from iterative and short occlusions in a rabbit model that did not induce improvement of coronary blood flow (8). This discrepancy suggests that in the rabbits a sustained coronary occlusion is needed to trigger the cascade of events leading to the development of coronary collateral circulation.

Coronary blood flow in the ameroid group reached one-half of the baseline value in the endocardial layer and was, surprisingly, almost back to the baseline value in the epicardial layer despite total coronary occlusion. This difference can be explained by the fact that subendocardial layers are more vulnerable to ischemia than are subepicardial layers (3, 20) and that, similar to humans, major collaterals grow from the epicardium in rabbits. This observation is reminiscent of the situation in the dog in which collateral development mainly occurs at the epicardial level (3, 36) and contrasts with that in the pig in which collateral development is rapid but predominantly within the endo- and midmyocar-

Fig. 7. Short-axis section of a representative heart with viable myocardium (reddish) and scar (blue green) (Goldner trichrome). Infarct size of this section was 10% of left ventricle mass. S, septum; P, papillary muscle.
dium (25, 35). Thus although coronary blood flow (in the area at risk of ameroid-implanted rabbits) did not reach the level of the nonocluded arteries in the sham group, collateral vessel development was adequate to prevent extensive myocardial infarction and allowed normal resting myocardial flow, at least in the epicardial layers.

Ameroid-induced coronary occlusion could not prevent some degree of myocardial infarction. Moreover, autopsy of rabbits that died before the study was completed revealed massive infarct because of early occlusion of a coronary branch supplying a large myocardial area. Consequently, the extent of infarction must be a function of the size of the area at risk and of the amount of collaterals that were either preexisting or newly developed. Thus without preventing infarction, collaterals may limit the damage and may result in smaller infarcts than would have been predicted by the region at risk (30). In contrast, it is well established that acute occlusion in rabbits in the branch of the circumflex coronary artery can result in a large infarct that can commonly involve up to 30% of the left ventricle area (34). In species such as dogs that have extensive native collaterals, progressive occlusion of a coronary artery with an ameroid constrictor results in smaller infarcts, between 1 and 5% of the left ventricle area (2, 32). Sudden and premature cardiac death was frequent (22%) and corresponded to large infarct as 

 Fatal arrhythmias as cause of early death could not be excluded on top of an acute thrombotic coronary occlusion.

In conclusion, we have developed for the first time a small-animal model of progressive coronary constriction associated with coronary angiogenesis. This model should provide a powerful tool to assess the in vivo effect of new angiogenic drugs that represent today a new animal model of controlled coronary artery occlusion in conscious rabbits. Cardiovasc Res 28: 61–65, 1994.


