

## Discussion

After the Canadian abortion law was liberalized in 1969 the total number and the rate of abortions in residents of Canada increased each year between 1970 and 1978. Similar increases occurred in the United States, where abortion laws were liberalized around the same time. The increases in Canada reflect a response by women to the liberalization of the abortion law, the increase in the number of hospitals providing abortion facilities, the interpretation of the abortion law by hospital therapeutic abortion committees and, perhaps, changes in the attitudes and beliefs of individual doctors about induced abortion. However, the overall abortion rates and the rates for teenagers are lower in Canada than in most of the developed countries for which statistics on legal abortions are available. This may be due to variations in local abortion laws, population structure, social and economic conditions, personal attitudes and beliefs about abortion, and knowledge and practice of methods of birth control.

In Canada more than 25% of the teenagers undergoing an abortion in 1974-77 were more than 12

weeks' pregnant at the time of the abortion, in contrast to only 15% of the women over 19 years of age. This difference explains the higher rate of complications in the teenagers.

The high proportion of abortions performed in teenagers, the slowly rising rate of teenage abortion and the high rate of complications in teenagers underline the need for improved birth control education for Canadian teenagers.

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# Platelet regeneration time and late occlusion of aortocoronary saphenous vein bypass grafts

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The half-time for platelet regeneration was estimated in 16 patients with aortocoronary vein grafts by the use of a non-radioisotopic technique based on the permanent inhibition by acetylsalicylic acid of lipid peroxidation by platelets. Ten patients had patent grafts after 6 years; in the other six at least one graft had become occluded between 2 and 6 years after the operation as shown by serial angiography. The mean half-time ( $\pm$  the standard error) for platelet regeneration was reduced to  $2.5 \pm 0.2$  days ( $P < 0.002$ ) in the group with occluded grafts as compared with  $3.3 \pm 0.1$  days in those with patent grafts and  $3.5 \pm 0.1$  days in 11 healthy volunteers. These results suggest a relation between late graft occlusion and platelet turnover and support the idea that patients with aortocoronary vein grafts could benefit from platelet suppressive therapy. Finally, the method employed appears to be a useful and simple way of evaluating platelet function in vivo.

## On a mesuré le demi-temps de régénération plaquettaire chez

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16 patients avec greffons veineux aortocoronariens selon une méthode non radioisotopique basée sur l'inhibition permanente par l'acide acétylsalicylique de la peroxydation des lipides plaquettaires. Dix patients présentaient des greffons perméables après 6 ans; chez les six autres au moins un greffon s'était obstrué entre 2 et 6 ans après l'opération tel que documenté par des angiographies successives. Le demi-temps moyen ( $\pm$  l'erreur type) de régénération plaquettaire était réduit à  $2.5 \pm 0.2$  jours ( $P < 0.002$ ) dans le groupe avec greffons obstrués en comparaison de  $3.3 \pm 0.1$  jours chez ceux avec greffons perméables et de  $3.5 \pm 0.1$  jours chez 11 volontaires sains. Ces résultats suggèrent une relation entre l'occlusion à évolution tardive des greffons et le cycle de remplacement des plaquettes et appuient l'hypothèse où les patients avec greffons veineux aortocoronariens pourraient bénéficier d'un traitement antiplaquettaire. Enfin, la méthode employée s'est révélée simple et utile pour l'étude de la fonction plaquettaire in vivo.

Measuring the platelet survival time is currently one of the most reliable ways of evaluating platelet involvement in various diseases and assessing the effectiveness of antiplatelet drugs in vivo.

Reduced platelet survival has been reported in several clinical conditions, including angina pectoris, myocardial infarction and thromboembolism.<sup>1</sup> Damage to the blood vessel wall and changes in the platelet surface modify platelet survival<sup>2</sup> and are believed to be responsible for increased platelet consumption. As plate-

let turnover is increased, young platelets, which are more active,<sup>3</sup> enter the blood and may increase the thrombotic tendency.

Until the development by Stuart, Murphy and Oski<sup>4</sup> of a biochemical technique based on the permanent inhibition of lipid peroxidation by platelets exposed to acetylsalicylic acid (ASA), methods for measuring platelet survival implied withdrawal of large amounts of blood, isolation and labelling of the patient's platelets with radioisotopes and reinfusion of those platelets. The new noninvasive method evaluates regeneration rates rather than disappearance of platelets from the blood, and the values obtained correlate well with those obtained in platelet survival studies with chromium 51.<sup>4,5</sup> Patients need only ingest a single dose of ASA and allow blood sampling during the next few days.

Early and late occlusion of aortocoronary saphenous vein bypass grafts is still an important limiting factor in the surgical treatment of coronary artery disease. Shortened platelet survival (determined for platelets labelled with <sup>51</sup>Cr) has been reported by Steele and colleagues<sup>6</sup> in patients with occluded grafts, which indicates that platelets may play an important role in the occlusion. However, in their study Steele and colleagues did not distinguish between early and late occlusion, phenomena that involve different factors or processes.<sup>7-9</sup>

We measured the half-time for platelet regeneration in patients whose grafts had become occluded between 2 and 6 years after the bypass operation. The half-time was reduced in these patients but was normal or near

normal in patients whose grafts had remained patent.

## Methods

The half-time for platelet regeneration was measured in 16 men undergoing angiographic evaluation 6 years (mean  $\pm$  standard error,  $68 \pm 5$  months) after aorto-coronary saphenous vein bypass grafting and 4 years ( $46 \pm 5$  months) after previous angiography.<sup>10</sup> The clinical data are summarized in Table I. Ten patients had patent grafts and six had at least one occluded graft. The control group included nine healthy male volunteers recruited from the hospital staff and two men with no clinical evidence of heart disease and normal coronary arteries as observed during angiography less than 1 year previously.

After a 12-hour fast, including abstinence from drugs, 15 ml of venous blood was collected in plastic syringes containing 2.5 ml of acid-citrate-dextrose anticoagulant. Sampling was performed at the beginning of the study, just before the ingestion of 600 mg of ASA, 2 and 4 days later, and after 7 days when necessary (the half-time for platelet regeneration was more than 4 days in the control group). After the addition of ethylenediamine tetra-acetic acid (EDTA)<sup>11</sup> and platelet counting,<sup>12</sup> platelet-rich plasma was divided into 2-ml aliquots and centrifuged at  $100 \times g$  for 16 minutes. The platelets (range of counts  $0.3$  to  $1.0 \times 10^9/l$ ; mean  $0.6 \times 10^9/l$ ) were resuspended in 2 ml of phosphate buffer containing 1 mmol of *N*-ethylmaleimide and incubated at  $37^\circ C$  for 60 minutes in a shaking bath. The production of malonyl dialdehyde by the platelets was estimated following reaction with 2-thio-barbituric acid.<sup>4,13</sup> We have found that the EDTA allows easier and better suspension of platelets without interfering with the malonyl dialdehyde production.<sup>14</sup>

The half-time for platelet regeneration is defined as the time after ASA ingestion in which samples of a platelet population can produce half the baseline concentration of malonyl dialdehyde. Estimations were made on a group and an individual basis and directly from a graph in which the relative production of malonyl dialdehyde by  $10^9$  platelets was plotted against the time after ASA ingestion.

Student's *t*-test for comparison of means was used for statistical analysis.

## Results

The mean production of malonyl dialdehyde by  $10^9$  platelets before ASA ingestion was  $2.34 \pm 0.2$ ,  $2.31 \pm 0.35$  and  $2.64 \pm 0.22 \mu\text{mol}$  in samples from the control group and patients with occluded and patent grafts respectively. The mean half-time for platelet regeneration was estimated at 3.5 days in the control group,  $3.3 \pm 0.1$  days in the group with patent grafts and  $2.5 \pm 0.2$  days in the group with occluded grafts (Fig. 1); the individual half-times in the three groups ranged between 3.2 and 4.1 (mean  $3.5 \pm 0.1$ ) days, 2.8 and 3.6 (mean  $3.3 \pm 0.1$ ) days and 1.5 and 3.1 (mean  $2.5 \pm 0.2$ ) days respectively (Fig. 2). Even taking into account the fact that multiple comparisons were made, the patients with occluded grafts had significantly shorter half-times for platelet regeneration than the

Table I—Clinical data for the men in the study\*

Variable	Control group (n = 11)	Patients with vein grafts	
		Patent (n = 10)	Occluded (n = 6)
Age (yr)			
Range	20-66	52-70	49-62
Mean	$31.6 \pm 4$	$57.5 \pm 2^\dagger$	$54.2 \pm 2^\dagger$
Mean serum levels (mg/dl)			
Cholesterol	$193 \pm 8$	$256 \pm 13^\dagger$	$248 \pm 17^\dagger$
Triglycerides	$110 \pm 11$	$171 \pm 13^\S$	$179 \pm 24^\dagger$
Vein grafts			
Total no.	—	19	11
Mean no. per patient	—	1.9	1.8
No. found to be patent by angiography			
Four years earlier	—	19	11
At the time of this study	—	19	4
No. smoking cigarettes	3	3	2
No. using			
Propranolol	—	3	1
Triamterene and hydrochlorothiazide	—	1	1
Isosorbide dinitrate	—	1	3
Diazepam	—	2	2
Dicyclomine and clonidine	—	1	—
Digoxin	—	1	1
Methyldopa	—	1	1
Diphenhydramine and isoxsuprine	—	—	1
Chlorthalidone	—	1	—

\*Each mean is accompanied by the standard error. Level of significance of difference between means in the patient groups compared with the control group:  $^\dagger P < 0.001$ ,  $^\ddagger P < 0.01$  and  $^\S P < 0.02$ .

control group ( $P < 0.001$ ) and the patients with patent grafts ( $P < 0.002$ ). The mean half-time of the patients with patent grafts was not significantly different from that of the control group. One patient in the study had two occluded grafts and the half-time was 2.2 days.

## Discussion

Although roughly 90% of aortocoronary bypass grafts escape occlusion due to thrombosis in the first 2 weeks, none seems to escape, after 1 month, intimal thickening<sup>8,9,15-17</sup> secondary to hyperplasia of smooth muscle cells and fibrosis.<sup>9,17,18</sup> In some patients these lesions progress rapidly and partially obstruct or occlude the grafts. Morphologically these changes are virtually indistinguishable from the fibrous plaques of atherosclerosis,<sup>15,16,18</sup> with the presence of foam cells, dense connective tissue, calcium and lipid.

Atherosclerosis in the grafts is hastened by unusual exposure of the vein to hemodynamic stresses<sup>8,17,19</sup> and by variables such as resistance to flow in the vascular bed distal to the graft,<sup>18</sup> vein anoxia,<sup>17</sup> surgical trauma and high blood lipid levels.<sup>15,18</sup> However, the lesions are not only the response of a vein to pressure but also the result of circumferential formation of nonocclusive intimal thrombi.<sup>16</sup> The release of constituents (mitogenic and permeability factors) from platelets after the cells adhere to an altered endothelial surface and the organization of platelet-fibrin thrombi are believed to be important steps in the initiation and development of atherosclerosis.<sup>20-22</sup>

Platelet survival and regeneration times reflect platelet aging and platelet-vessel wall interaction resulting

in platelet removal and replacement.<sup>1,2</sup> Shortened survival<sup>23,24</sup> and regeneration times<sup>5</sup> have frequently been noted in patients with coronary artery disease. In the present study the half-time for platelet regeneration was 3.1 days or less in all six patients with occluded grafts, and in four of them it was 2.5 days or less (Fig. 2). The mean half-time in this group was  $2.47 \pm 0.2$  days ( $P < 0.002$ ), as compared with  $3.3 \pm 0.1$  days in the group with patent grafts and  $3.5 \pm 0.1$  days in the control group (Fig. 1). These results are similar to those reported by Steele and colleagues<sup>6</sup> for platelets labelled with <sup>51</sup>Cr and suggest a relation between vein graft occlusion and shortened survival and regeneration times. Although additional evidence is needed to establish this relationship as causal, the use of platelet suppressant drugs known to prolong platelet survival or increase it to normal should be tried in an attempt to prevent early and late graft occlusion.

It has been reported that coronary bypass operations may<sup>25</sup> or may not<sup>6</sup> modify platelet survival and that most patients whose grafts become occluded within the first 3 years generally had a shortened platelet survival time before the operation. Therefore, the platelet survival or regeneration time should be determined preoperatively as a possible indicator of later graft occlusion. On the other hand, from the results of our study, even though we did not measure the half-time for platelet regeneration before the operation we doubt whether platelet regeneration was seriously impaired then since 1 to 3 years later all the patients had been shown by angiography to have patent grafts. Occlusion only occurring late may indicate a slow pathologic process in these patients or relatively late

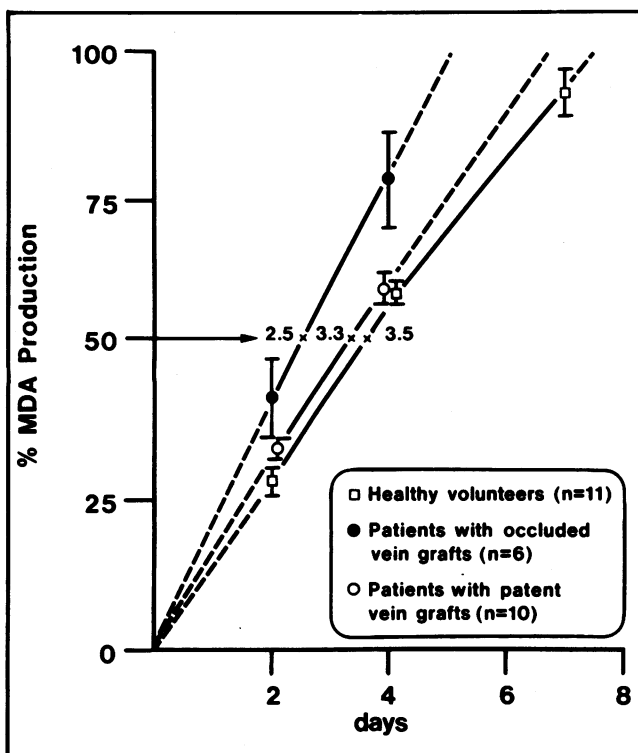


FIG. 1—Percent malonyl dialdehyde (MDA) production and estimations of half-time for platelet regeneration in three groups.

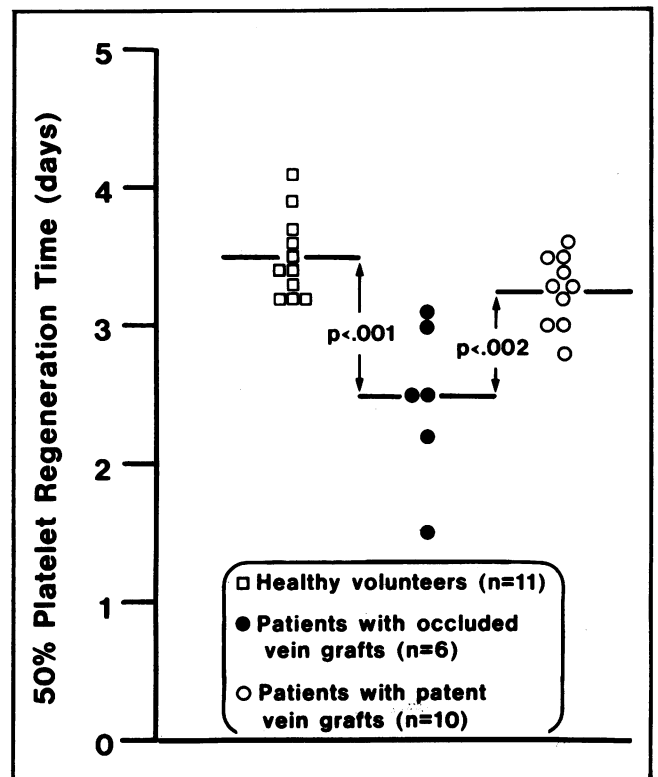


FIG. 2—Individual half-times for platelet regeneration in the three groups.

changes. Therefore, we believe that the platelet survival or regeneration time should be measured not only before but also periodically after the operation. On the other hand, as Grondin and associates<sup>26</sup> reported recently from a larger study, blood lipid levels do not discriminate between patients with patent or late occluded grafts; therefore, late occlusion may be less influenced by blood lipid levels than occlusion occurring in the first months or years after the operation.<sup>27</sup> Nevertheless, blood lipids deserve special attention since hyperlipidemia remains an important influence on graft patency<sup>15</sup> and on platelet survival<sup>28,29</sup> and function.<sup>30</sup>

Platelet survival studies with <sup>51</sup>Cr or other radioisotopes are generally restricted to centres with nuclear medicine facilities and experience in these studies. On the other hand, measuring the half-time for platelet regeneration is simple, can be partially automated<sup>5</sup> and can be done simultaneously for several patients in most hospital laboratories. Therefore, it may prove useful for screening and monitoring platelet function in several clinical conditions.<sup>5</sup> However, the ingestion of ASA and ASA-like drugs must be prohibited for 1 week before and during the test.

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