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# Coronary atheroma in the cynomolgus monkey: predictive value of serum and cutaneous lipoprotein measurement

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**Summary.** Hyperlipaemia was induced by a high fat diet in 11 cynomolgus monkeys. Morphological study of coronary arteries was carried out in 5 coronary samples from these 11 monkeys. The degree of arterial involvement was compared with the serum and cutaneous lipoprotein levels. These experimental data confirm that cutaneous apoprotein B measurement is the best marker for evaluation of coronary atheroma.

**Key words:** Coronary atheroma – Monkey – Cutaneous apoprotein B – Serum apoprotein B

## Introduction

Various workers have drawn attention to the value of the measurement of serum and cutaneous apoprotein B (Avogaro et al. 1977; Sniderman et al. 1980; Wayne et al. 1981; De Graeve et al. 1984; Douste-Blazy et al. 1985) in relation to coronary arteriographic findings in human coronary atherosclerosis. We carried out a morphological study of 5 coronary samples from 11 monkeys, in whom hyperlipaemia had been induced by a high-fat diet, and compared the degree of morphological alteration with the serum and cutaneous lipoprotein findings. Results were analyzed by non-parametric tests and discriminant analysis was carried out, confirming the value of cutaneous apoprotein measurement in assessing coronary atheroma.

## Material and methods

*Animals-diet.* Eleven male tattooed cynomolgus monkeys (macaca fascicularis), obtained from Charles Rivers, weighing between 2.7 and 3.75 kg, were kept in individual cages in an air-conditioned room where temperature and humidity were constant. Lighting was artificial (12 h light, 12 h darkness).

During the first month (adaptation period) the 11 monkeys were fed a standard UAR 107 diet. Basic biological tests (blood sampling, skin biopsy) were carried out twice, on the 15th and the 30th day.

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The eleven monkeys were subsequently maintained on Clarkson's[1] (1979) hyperlipidic diet, given as 50 g rations, with water ad libitum. For the first two weeks, all monkeys were given the same quantity, then according to response the diet was adapted on the basis of blood cholesterol levels; standard UAR 107 diet was given as supplement. At six months, the quantity of ration given to each monkey was considered as constant for the rest of the study.

*Protocol.* The monkeys, observed over a 2 year period, were weighed monthly. The quantity of ration ingested was checked, the rest of the food being approximately assessed.

Blood samples were taken monthly (from the tibial vein without anaesthesia) in dry tubes for measurement of total cholesterol (T.ch.) and free cholesterol (F.ch.) (Roschlau et al. 1974), triglycerides (T.G.) (Bucolo and David 1973), phospholipids (P.L.) (Takayama et al. 1977), and apoprotein B (S. Apo B) (Fruchart et al. 1981).

Twelve skin biopsies of 2 $cm^2$ were performed under general anaesthetic (Ketalar[2]) during the experimental study. Skin was taken from the abdominal area and tested for skin cholesterol (Cut.ch.) (Lieberman 1895), and apoprotein (Cut. Apo B) (Wulfert et al. 1984).

At the end of the study, blood was drawn by cardiac puncture after thoracotomy under general anaesthetic (Ketalar[2]). The animals were then killed by exsanguination. The heart and aorta were removed. The left coronary artery was perfused with 10% formalin under 100 mm Hg pressure. The whole heart was fixed in 10% formalin, and the right and left coronary arteries were then dissected. Samples were taken as follows: one from the right coronary and four from the left coronary: common trunk, anterior descending artery (two samples: proximal and distal), circumflex artery. After embedding in paraffin, the samples were cut perpendicularly to the vessel axis and stained with Masson's trichrome blue (for fibrosis), with Verhoeff's iodated ferric haematoxylin (for internal and external elastic lamellae). After freeze-cutting, one sample was stained with Sudan black (for intra- and extracellular lipids).

Lipid excess and fibrosis were assessed microscopically for each sample, taking into account their surface area and depth of penetration in the vessel wall. These lesions were of course accompanied by varying destruction of the elastic lamina.

An atheroma on the circumference, taking up almost the whole of the media (only one same external zone sometimes remained clear), was graded $++++$; a circumferential atheroma of the intima and the internal media, $+++$; a plaque of atheroma, identical to the preceding lesion but taking up only part of the circumference, was graded, $++$; an isolated plaque of small extent affecting the intima and the internal part of the media, $+$; extracellular and particularly intracellular intimal deposits, $\pm$ (these are only potential atheromas). The overall state of coronary atheroma was estimated by computing the number of plus signs observed: "*A*" indicated very marked atheroma, "*a*" slight atheromatous lesions and "*O*" absence of atheroma.

Comparison of the means was done with the C1 test of Fisher, Yates and Terry. Correlations were computed using the non-parametric test of Spearman. Discriminant analysis was linear between 2 groups: group 1 (5 monkeys) coronary atheroma O or a, group 2: (6 monkeys) coronary atheroma A. Data were calculated using a Vax 780 Vms 3–2 computer from Digital Equipment Corporation.

## Results

The biochemical results of samples taken at the beginning of the study (before diet), and after 4 and 24 months of diet (at autopsy) are given in Table 1.

On diet, overall increase of cholesterolaemia and of apoprotein B were

1 Skimmed milk powder 30%, wheat flour 20%, casein USP 13%, lard 25%, apple juice 7.3%, vitamin mix 2.2%, salt mix USP XIV 27, cholesterol 0.5%

2 Ketalar (ketamine hydrochloride, Parker Davis and Company, Detroit, MI, USA)

**Table 1.** Serum and cutaneous lipid levels in 3 groups *T*0, *T*4 and *T*24

| | Tot. Chol.[a] | | Free Chol.[a] | | Tot. T.G.[a] | | P L[a] | | S. Apo B[b] | | Cut. Chol.[b] | | Cut. Apo B[d] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $T_0$ | 3.29 ±0.253 | | 0.72 ±0.055 | | 0.59 ±0.049 | | 2.27 ±0.155 | | 0.62 ±0.070 | | 0.597 ±0.0219 | | < 5 |
| | *** | | *** | | ** | | ** | | ** | | *** | | ** |
| $T_4$ | 11.16 ±1.122 | ** | 2.18 ±0.220 | ** | 0.32 ±0.036 | NS | 3.30 ±0.197 | ** | 1.28 ±0.164 | * | 1.223 ±0.1725 | NS | 14.7 ±0.99 | *** |
| | NS | | NS | | NS | | NS | | NS | | * | | *** | |
| $T_{24}$ | 14.14 ±2.439 | | 2.21 ±0.334 | | 0.58 ±0.092 | | 3.74 ±0.313 | | 1.13 ±0.168 | | 0.694 ±0.0920 | | 106.1 ±7.94 | |

* $P<0.05$, ** $P<0.01$, *** $P<0.001$

[a] mmol/l, [b] g/l, [c] mmol/100 g fresh skin, [d] μg/g

**Table 2.** Morphological observations on the 5 samples from the right coronary artery (R.C.), left coronary artery: common trunk (T.C.), proximal (A.D.P.) and distal (A.D.D.) anterior descending artery, circumflex artery (C.I.)

| | R.C. | C.T. | A.D.P. | A.D.D. | CI. | Ath. |
|---|---|---|---|---|---|---|
| 1 | ± | ++ | +++ | ± | +++ | A |
| 2 | ± | + | + | o | o | a |
| 3 | ++++ | ++++ | ++++ | ++++ | ++++ | A |
| 4 | o | o | o | o | o | o |
| 5 | o | o | o | o | o | o |
| 6 | ++ | ++ | +++ | + | ++++ | A |
| 7 | o | o | ± | o | ± | o |
| 9 | ++ | ++ | ++ | o | + | A |
| 10 | o | ± | ± | o | ++ | a |
| 11 | ++++ | ++++ | ++++ | ++ | ++++ | A |
| 12 | ++++ | +++ | ++++ | +++ | ++++ | A |

Ath., Atheroma; A, Very marked overall atheroma; a, Very slight atheromatous lesions; o, no parietal lesion

**Table 3.** Serum, cutaneous lipids and atheroma at $T_{24}$

| | Tot. chol. | Cut. chol. | S. Apo B | Cut. Apo B | Ath. |
|---|---|---|---|---|---|
| 1 | 11.64 | 0.515 | 0.67 | 86 | A |
| 2 | 6.63 | 0.412 | 0.67 | 101 | a |
| 3 | 14.5 | 1.122 | 1.04 | 138 | A |
| 4 | 5.1 | 0.457 | 0.44 | 77 | o |
| 5 | 3.9 | 0.449 | 0.51 | 74 | o |
| 6 | 18.4 | 0.748 | 1.13 | 129 | A |
| 7 | 14.3 | 0.476 | 0.87 | 92 | o |
| 9 | 7.7 | 0.371 | 1.82 | 86 | A |
| 10 | 25.5 | 1.099 | 1.82 | 108 | a |
| 11 | 27.4 | 1.102 | 1.92 | 153 | A |
| 12 | 20.5 | 0.881 | 1.55 | 123 | A |

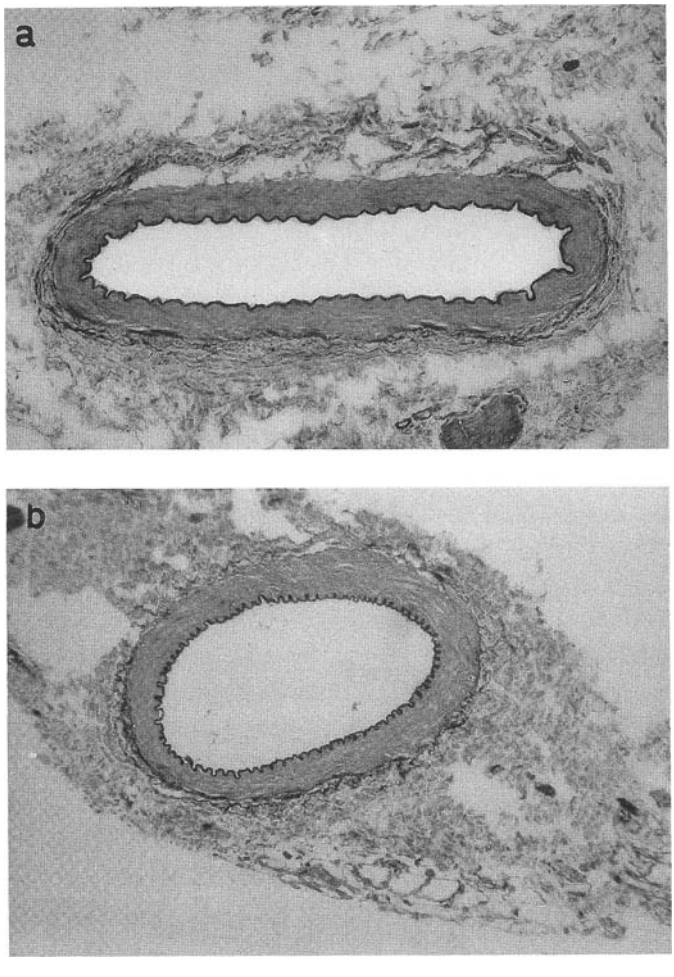


**Fig. 1 a, b.** No vascular lesion (Grade 0). **a** Monkey N° 4. Left proximal anterior descending coronary artery. Normal aspect. Verhoeff × 10. **b** Monkey N° 7. Right coronary artery. Normal aspect. Verhoeff × 10. (In these picture and in the picture 4a the waviness of the internal elastic lamina and the general collapse of the vessels are the result of short pressure perfusion of arteries)

observed. Triglycerides remained practically at the same levels after decreasing at the fourth month; phospholipids increased at the fourth month and the twenty-fourth month. For each animal, variations in the levels of these various serum components were noted at the time of the monthly tests and there was no significant change in monthly serum values for lipids.

Cutaneous biochemical results (cholesterol and apoprotein B) are also shown in this Table. Cutaneous cholesterol increased very significantly at the fourth month ($p < 0.001$), but presented no significant difference between the beginning of the study and 24 months. Apoprotein B increased very significantly and constantly. The two tests carried out at an interval of

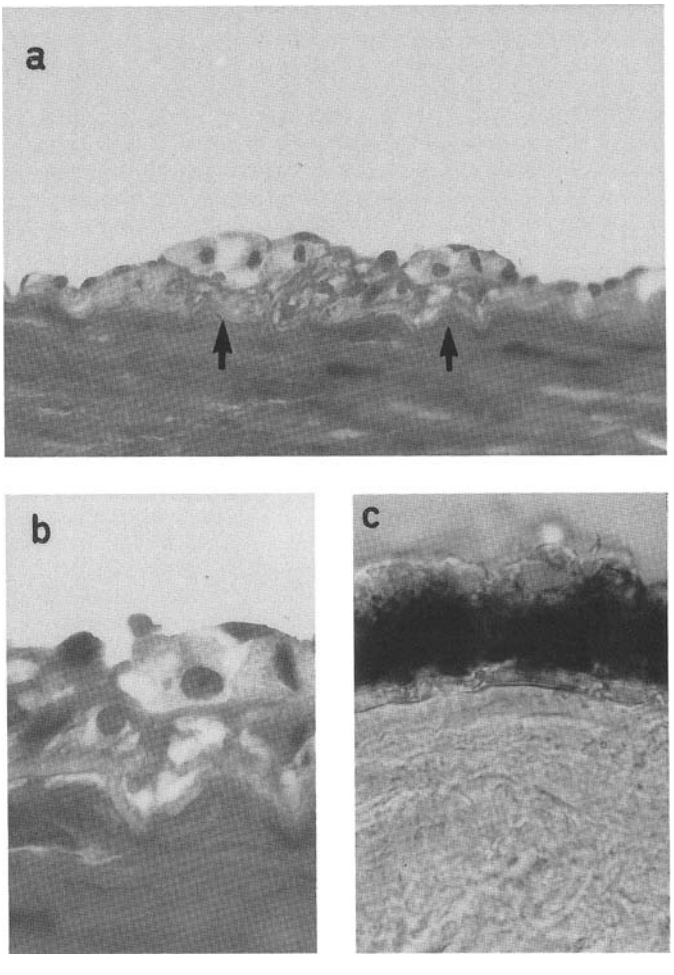


**Fig. 2a–c.** Potential atheroma (Grade ±), Monkey N° 10 common trunk. **a** Lipid intracellular intimal deposits, without lesion of internal elastic lamina (↑). Masson's Trichrome blue. ×40. **b** Part of picture **a**. Endothelial and subendothelial foam cells. Masson's trichrome blue ×100. **c** Aspect of intima only lipid-deposition. Sudan black ×100

two weeks, before diet, showed only trace amounts of apoprotein B. We noted them as being less than 5 μg/g/of fresh skin.

No significant variations in the haematocrit were detectable after repeated blood sampling.

*Morphology*

Table 2 resumes the morphological observations concerning the 5 samples from the coronary arteries of each of the 11 monkeys; 6 of the monkeys had very marked overall atheroma: (A) 2 very slight atheromatous lesions (a); 3 were exempt from any fibrous or lipid vascular lesion (O) (Figs. 1–6).

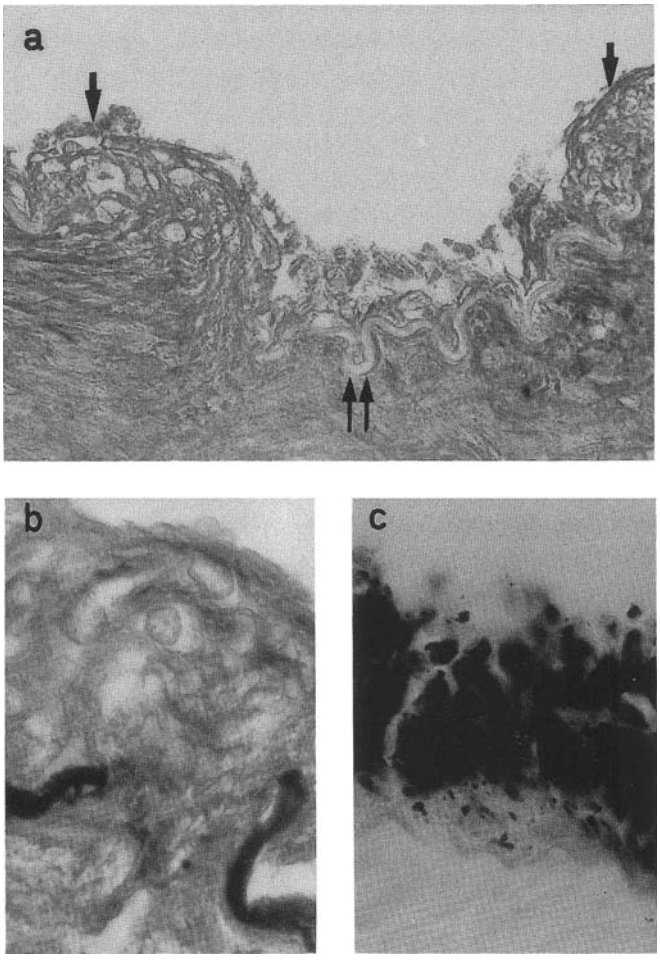


**Fig. 3a–c.** Isolated plaque (Grade+). Monkey N° 2. **a** Common trunk. Two small isolated plaques (↑) separated by portion of abnormal intima with intact internal elastic lamina (↑↑). Masson's trichrome blue ×40. **b** Part of same lesion (**a**). Small plaque affecting the intimal and the internal part of media with rupture of internal elastic lamina. Verhoeff ×100. **c** Left proximal anterior descending coronary artery. Lipids in intima and internal part of media. Sudan black ×100

A comparison between biochemical and morphological results at the 24th month $T_{24}$ is shown in Table 3.

Table 4 presents the correlations between the degree of coronary atheroma observed (C) and the levels of the serum and cutaneous lipid variables (serum apoprotein B, serum cholesterol, free serum cholesterol, phospholipids, triglycérides, cutaneous cholesterol and cutaneous apoprotein B) obtained at the 24th month.

The correlation between cutaneous cholesterol and serum cholesterol is positive ($r$: 0.82, $p < 0.01$); it is also positive between cutaneous apoprotein

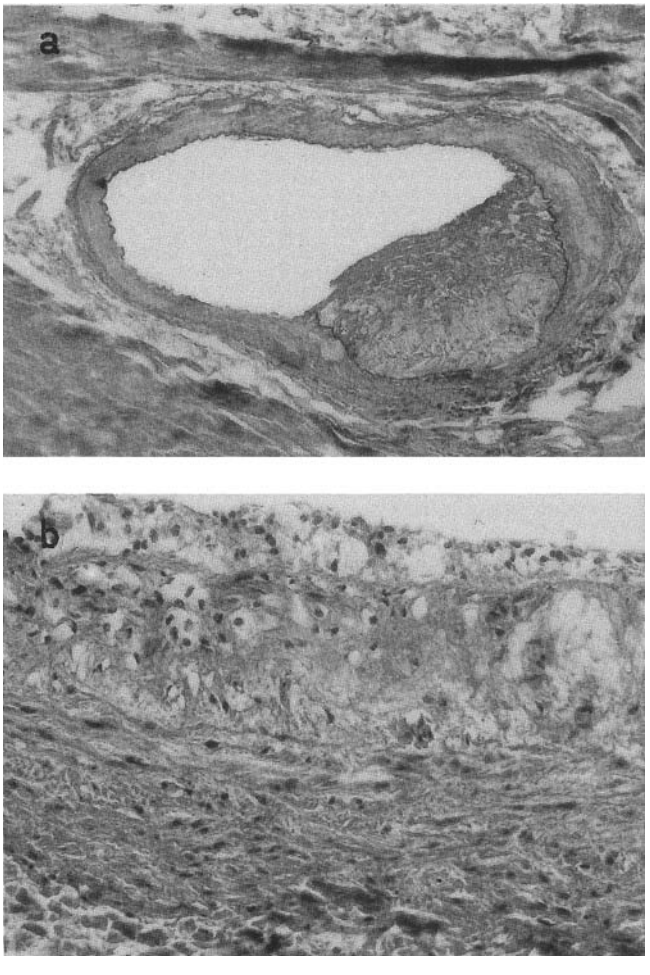


**Fig. 4a, b.** Atheroma grade ++. **a** Monkey N° 11 left distal anterior descending coronary artery. Plaque of atheroma of the intima and internal media on one part only arterial circumference. Verhoeff ×10. **b** Monkey N° 1 common trunk. Abundant foam cells near the endothelial surface. Thickened intima. Thinned media. The original internal elastic lamina is missing. Masson's trichrome blue ×25

**Table 4.** Correlations between coronary atheroma (C) and serum lipid levels (serum apoprotein B: S. APO B; total cholesterol: T. ch.; free cholesterol: F. ch.; phospholipids: P.L.; triglycerides: T.G.) and cutaneous lipids (cutaneous cholesterol: CUT ch.; cutaneous APO B: CUT. APO B)

| | *r* | *p* | | *r* | *p* |
|---|---|---|---|---|---|
| C/CUT. APO B | 0.81 | 0.01 | C/CUT. ch. | 0.71 | 0.05 |
| C/S. APO B | 0.62 | 0.05 | C/S. ch. | 0.71 | 0.05 |
| C/F. Ch. | 0.71 | 0.05 | C/P.L. | 0.53 | NS |
| C/T.G. | 0.03 | NS | | | |

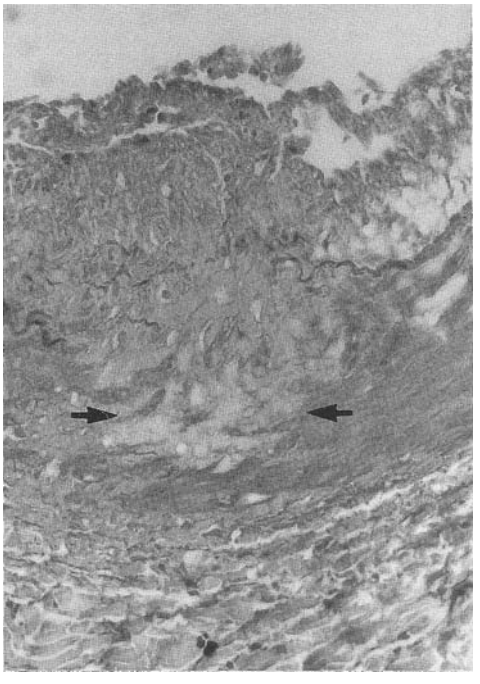

**Fig. 5.** Atheroma grade + + +. Monkey N° 1. Left proximal anterior descending coronary artery. Part of circumferential atheroma. Much of the original internal elastic membrane is missing. Destruction of a part of media. Lipids deposits in the intima and in the part of destroyed media (→). Col. Verhoeff Gr ×25

B and cutaneous cholesterol ($r$: 0.79, $p<0.01$), and between cutaneous apoprotein B and serum apoprotein B ($r$: 0.68, $p<0.05$).

Discriminant analysis was performed with 4 variables (cutaneous cholesterol, cutaneous apoprotein B, total serum cholesterol and serum apoprotein B) between 2 groups: Group 1: 5 monkeys with normal coronary arteries or with slight, isolated atheromatous lesions (O or a); Group 2: 6 monkeys with high degree of coronary atheroma (A).

With the 4 discriminant variables, the percentage of those correctly classified was 81.8%. Cutaneous apoprotein B is the variable which plays a statistically significant role ($p<0.05$).

Classification obtained by analysis was as follows: of the 5 subjects in Group 1, 80% were in fact Group 1, and 20% Group 2; of the 6 subjects in Group 2, 83% were in fact Group 2, and 17% Group 1.

At the end of the experimental study, the eleven monkeys were in excellent condition: constant weight increase during the first year, becoming stable during the second; the body weight at TO was $4.79 \pm 0.75$ esm kg at $T_{24}$: $5.58 \pm 0.87$ esm; activity and liveliness constant, steady appetite, stools rarely discoloured, coat thick and sleek. The behaviour of monkeys 3, 6, 11 and 12 was not modified in spite of the degree of their coronary atheroma, and the various myocardial samples taken on autopsy showed no morphological sign of myocardial infarction. Moreover, all the organs examined were seen to be normal, particularly the liver (except in monkey 3, where hepatic micro and macrosteatosis were observed).

Repetition of the blood sampling had no significant effect on the values of the haematocrit.

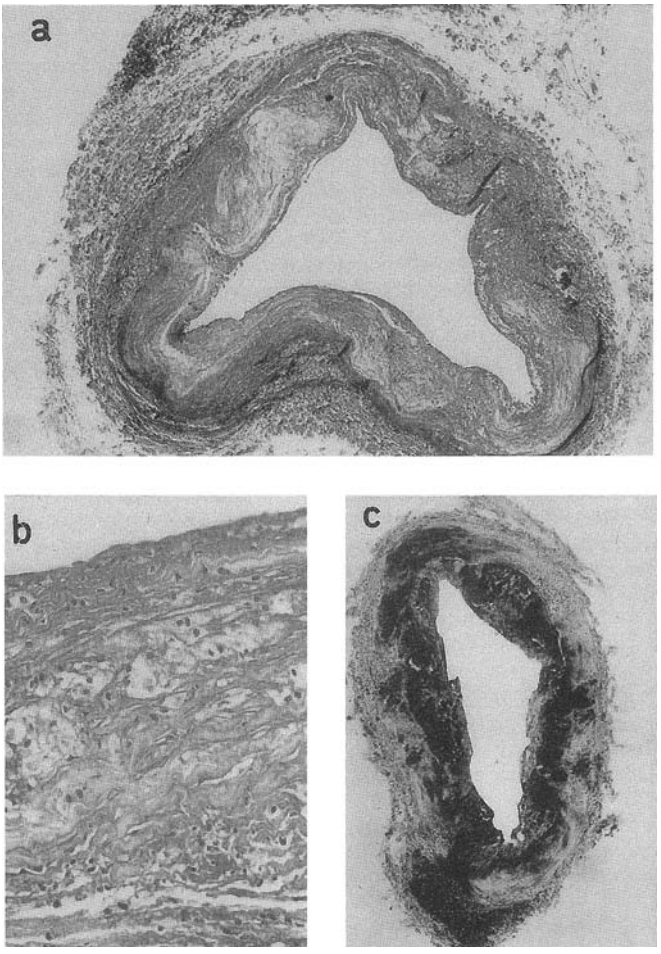


**Fig. 6.** Atheroma grade $+++$. **a.** Monkey N° 3. Right coronary artery. Atheroma on the circumference taking up almost the whole of the media. Col. Masson's trichrome blue Gr $\times 4$. **b.** Monkey N° 3. Left circumflex artery. Destruction of the media. The internal elastic lamina is missing. Penetrating numerous foam cells in the arterial wall. Col. Masson's Trichrome blue Gr $\times 25$. **c.** Monkey N° 11. Left circumflex artery. Extensive lipidic deposits in intima and media. Col. Sudan black Gr $\times 4$

## Discussion

### *Coronary morphology*

Cynomolgus monkeys (macaca fascicularis) are animals which, when maintained on a high-fat diet, easily develop coronary atheroma comparable to that of man. Many authors, particularly Armstrong (1976) and Malinow et al. (1978); Stary and Malinow (1982) have used these animals. We found the same fibrous and fibro-fatty lesions, with no ulceration or mural thrombosis. As is shown in all research on atherosclerosis in non-human primates, there were study considerable variations in the degree and severity of atherosclerosis in our experimental. Involvement varied greatly both between indi-

vidual monkeys and between the coronary lesions of any one monkey. In addidtion to the coronary lesions, we noted, though to a lesser degree and in a smaller number of animals, atheroma of the aorta, the common carotid and iliac arteries. The femoral artery was rarely involved, and the cerebral arteries never (the latter are known to be involved in only about 20% of cynomolgus monkeys (Bond et al. 1980)). In accordance with the findings of Vesselinovitch (1979), in macacus fascicularis atheromatous lesions are predominant in the coronary arteries.

The importance of coronary and aortic atheromas observed may be compared with that observed in man. The same heterogeneity is found: atheromatous lesions predominating on the aorta or on the coronary artery, or identical in both localizations. Moreover, in our series there was a correlation between coronary and aortic atheroma ($r=0.73$, $p<0.05$). We observed no infarction, unlike Taylor et al. (1963) and Bond et al. (1980) in other species of non-human primates.

On the high-fat diet, the level of cutaneous cholesterol rose and then remained at high but widely fluctuating levels. It is known that in man the level increases with age, the atheromatous status of the aorta, and in coronary atherosclerosis (Bouissou et al. 1982). In the latter case, the level of cutaneous cholesterol is extremely high, whatever the age at which myocardial infarction occurs (Bouissou et al. 1983).

Apoprotein B, which is not present in the skin of monkeys on normal diet, reached 100 μg/g of skin from the first month of hyperlipidaemic diet. The level then remained high but was also subject to extreme fluctuation; on the whole, levels rose and then showed a tendency to decrease.

The cutaneous measurements carried out during autopsy and compared with the morphological involvement of the coronary arteries enable us to affirm that cutaneous apoprotein B is reliable marker of coronary atherosclerosis in the monkey, since serum cholesterol and serum apoprotein B are not correlated. This result is identical to that found in man, where cutaneous apoprotein B proves to be the best indicator of coronary status (De Graeve et al. 1984).

In conclusion our study of 11 monkeys maintained on a high-fat diet enables us to say that the diet, which was perfectly tolerated, modifies most serum lipids, increases cutaneous apoprotein B and induced coronary atheromatous lesions in 9 monkeys. The coronary atheroma observed is comparable to that of man and as in man, there is a relationship between coronary status and cutaneous cholesterol level ($p<0.05$) also as in man, a clear relationship appears between the level of cutaneous apo B and coronary atheromatous status ($p<0.01$).

Using morphological analysis of atheromatous involvement of the coronary arteries, this experimental study confirms an hypothesis which in man had only been affirmed indirectly by coronarography: cutaneous apoprotein B measurement is the best marker of the severity of coronary atheroma.

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