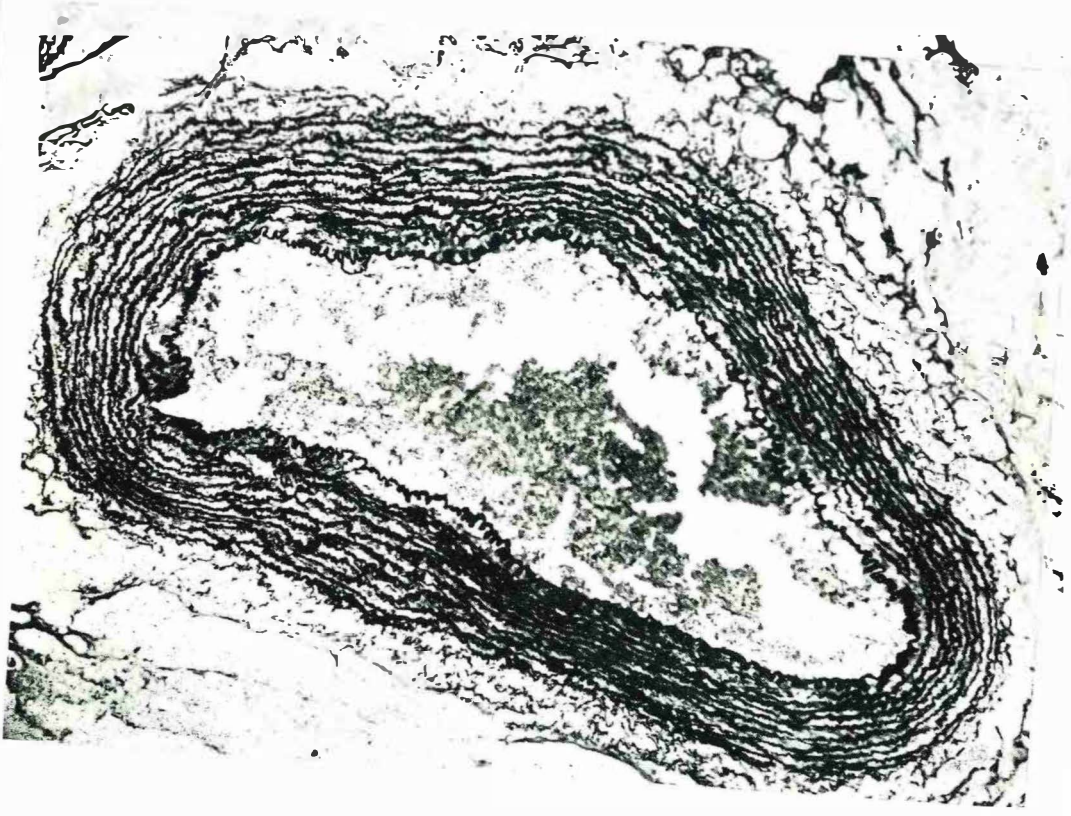
Prevention of vessel wall damage in experimental irradiation



C. Th. Smit Sibinga

PREVENTION OF VESSEL WALL DAMAGE IN EXPERIMENTAL IRRADIATION

STELLINGEN:

1.

Depolymerizatie van mucopolysaccharide grondsubstantie in de arteriële vaatwand moet als het primaire effect van bestraling op de vaatwand worden beschouwd.

2.

Het intreden van 'vet' in de wand van een arterie is niet alleen afhankelijk van beschadiging van de vaatwand, doch tevens van een al dan niet constante verhoging van de vetspiegel in het bloed.

3.

De mitoseactiviteit van de endotheelcel is afhankelijk van op de cel inwerkende beschadigende momenten.

Wright, H. Payling In: The Scientific Basis of Medicine. The Athlone Press, London 1971 p. 320

4.

Alleen al door zijn eenvoud biedt de behandeling van de chronische synovitis haemophilica met intra-articulaire toediening van ^{198}Au grote voordelen boven de langdurige conservatieve tractie therapie en de aggressieve synovectomie.

5.

Ten onrechte wordt in de kliniek de relatie diffuse intravasale stolling en heparine therapie nog als 'a must' beschouwd.

6.

Voor het optreden van de derde harttoon is de aanwezigheid van een mitralisklep-apparaat geen noodzakelijke voorwaarde.

Flemming, J. S. Brit. Heart J. (1969) *31*, 192

7.

Er bestaat onvoldoende aanleiding om van *de* bloed-hersenbarrière te spreken.

8.

Verschijnselen van activiteit van de ziekte van Hodgkin kunnen veroorzaakt worden door infecties; het onderkennen en bestrijden van deze infecties is derhalve noodzakelijk voor een adaequate behandeling van de zieke.

9.

De aanwezigheid van een hoge correlatie tussen het bestaan van een z.g. risicofactor en het voorkomen van een chronische ziekte hoeft niet te betekenen dat algemene bestrijding van de risicofactor een volksgezondheidsbelang is.

10.

Hoewel diagnostische gegevens in onvoldoende mate bekend zijn, is er reden om het advies van Paulus aan Timotheüs in 1 Tim. 5 : 23 ook uit geneeskundig oogpunt verantwoord te achten.

11.

In de naam Erasmus Universiteit voor een Rijksuniversiteit te Rotterdam schuilt voor de Medische Faculteit aldaar een zekere ironie.

12.

De functie van volksvertegenwoordiger moet onverenigbaar geacht worden met het à titre personnel verkondigen van meningen in de volksvertegenwoordiging.

C. Th. Smit Sibinga, *'Prevention of vessel wall damage in experimental irradiation'*

RIJKSUNIVERSITEIT TE GRONINGEN

Prevention of vessel wall damage in experimental irradiation

Proefschrift

ter verkrijging van het doctoraat in de geneeskunde
aan de Rijksuniversiteit te Groningen
op gezag van de Rector Magnificus Dr. A. Wattel
in het openbaar te verdedigen op
woensdag 20 december 1972 des namiddags te 2.45 uur precies
door

Cornelis Theodoor Smit Sibinga
geboren te Makassar (Ind.)

DRUKKERIJ JAN HAAN N.V. GRONINGEN 1972

Promotores: Prof. Dr. H. B. Lamberts
Prof. Dr. H. O. Nieweg

Between the acting of a dreadful thing
And the first motion, all the interim is
Like a phantasma or a hideous dream:
The genius and the mortal instruments
Are then in council, and the state of man,
Like to a little kingdom, suffers then
The nature of an insurrection.

William Shakespeare
„Julius Caesar" act II.

to my wife Lineke
and our children:

Sophie
Antoinette
Hein
Karolien

This thesis was prepared in the Department of Radiopathology (director: Prof. Dr. H. B. Lamberts) and the coagulation laboratory of the Division of Haematology (director: Prof. Dr. H. O. Nieweg), Department of Medicine (director: Prof. Dr. E. Mandema) of the University of Groningen, the Netherlands.

The investigations were supported by the Foundation for Medical Research FUNGO with financial aid from the Netherlands Organization for Advancement of Pure Research ZWO.

The publication of this thesis was made possible by grants from the Netherlands Heart Foundation, Karl Thomae GmbH and Ciba-Geigy N.V.

CONTENTS:

CHAPTER 1

INTRODUCTION

The function of the great elastic arteries like the carotid, iliac and femoral arteries depends on the elastin and collagen fibre network of the vessel wall and the mucopolysaccharide ground substance between the fibres, the muscle cells and the lining endothelium[8]. These vessels have a transport function and for their own metabolism they are dependent on infiltration of substances from the lumen into the intima and part of the media, and on the nutritive vessels in the adventitia[18].

Vessel wall permeability is determined by the mucopolysaccharide ground substance and the rate of contraction and relaxation of the endothelial cells, causing more or less pronounced 'gaps' or 'pores' at the intercellular junction sites[11,19]. The integrity of the basal membrane and the tunica elastica interna are also involved in permeability.

Anatomy and function of the great vessels are essentially different from those of the smaller vessels and capillaries, for instance in the skin and the mucous membranes[18]. These 'microvascular' systems have a nutritive function which is dependent on the permeability of these vessels and the exchange function of the lining endothelial cells[20]. The supporting structures are a single elastin bundle, some smooth muscle fibres and the pericytes of the capillaries[18]. Mucopolysaccharides and elastic fibres are only minor constituents of the walls[22].

These differences in anatomy and function are parallel to the differences in susceptibility to various damaging agents, viz. ionizing radiation, which will be the subject of this thesis.

Damage, reversible or irreversible, produced by radiation stems from molecular events (ionization and/or excitation)[4].

According to the literature two main pathways of damage may occur in the chain of events following irradiation absorption[1,9]:

1. RADIATION CHEMICAL

2. BIOCHEMICAL

ad. 1. *Radiation chemical mechanisms* include all the processes in which molecular changes arise from the absorption of radiation energy[9, 25]. The basic mechanism seems to be the production of free radicals either by water hydrolysis or by direct action on complex target molecules in the tissues[4, 9]. Due to the predominance of water in the soft tissues, the greater part of ionizing events will result in the formation of the free radicals $H^{\cdot}$, $OH^{\cdot}$ and e^{-}_{aq} and the production of H_2O_2 and H_2[9, 15, 25]. Free radicals are highly reactive electrically neutral molecules or atoms, which may attack other molecules in the tissues. This process is called the 'indirect action' of ionizing radiation. In the presence of molecular oxygen, formation of $HO_2^{\cdot}$ radicals takes place, which probably is the base of the well-known oxygen effect in radiation biology[4, 9]. When a more complex molecule or target molecule in the tissues is hit, the free radicals which have been formed, are stabilized by chemical changes, occurring either at the site of the absorption or at a more susceptible point after migration of the charge within the molecule[25]. This process is called the 'direct action' of ionizing radiation.

ad. 2. *Biochemical mechanisms* involve processes like the participation of unimpaired molecules in non-radical reactions with damaged molecules. These processes take much more time than the free radical reactions, which are very fast. Metabolic processes in the cells may then proceed in an aberrant way, because of the interaction of normal undamaged molecules with damaged molecules. Proteins and nucleic acids may play a vital part in these processes[9].

The damaging effect of ionizing radiation on the vessel wall depends mainly on the formation of free radicals from water radiolysis in the mucopolysaccharide ground substance[23] and to smaller extent on the direct action on target molecules in the complex structures of the ground substance, the elastic fibres, the endothelium and the smooth muscle bundles.

As a consequence radiation of the wall of an elastic artery will result in functional and structural disintegration because of depolymerization of the mucopolysaccharide ground substance, destruction with fragmentation, atrophy and thickening of the elastin and collagen supporting fibre network, swelling and disintegration of the smooth muscle cells and the lining endothelium[6, 23]. Changes in permeability and resistance of the vessel wall lead to a further impairment of the normal physiological function of the vessel wall[10-13].

Prevention of the degenerative processes following irradiation may be divided into the following groups according to the outlined damaging mechanisms[4, 17]:

1. RADIATION CHEMICAL MECHANISMS

2. BIOCHEMICAL-PHYSIOLOGICAL MECHANISMS.

Prevention means the ultimate effect of protective mechanisms in ionizing radiation of tissues. Therefore the protective substance or protection inducing agent has to be present at the moment of irradiation[4].

ad. 1. *Radiation chemical mechanisms* act on the immediate processes of damage to target molecules, whether they are the result of 'direct action' or of 'indirect action'[26].

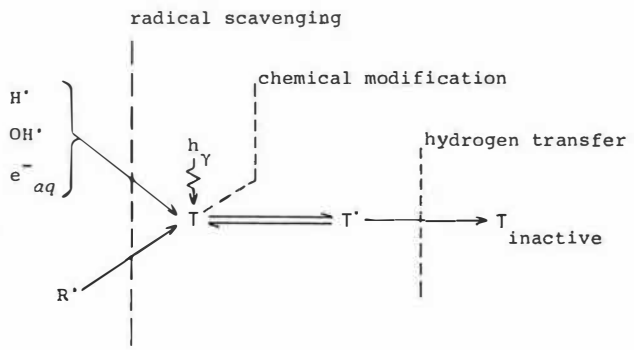


Fig. 1.1 Radiation chemical mechanisms in the prevention of radiation damage to target molecules (T).

The protective agents may act on three levels[4, 17, 26] (see fig. 1.1):

a. the interception with free radicals, known as the radical scavenger mechanism. The complex target molecules in the tissues are protected competitively from the damaging effect of the formed free radicals.

b. when the target molecules in the tissues have been hit either directly or indirectly and hydrogen atoms have been split off from the molecules, instantaneous repair may be obtained by a hydrogen transfer reaction. The initial damage has already occurred on this level, but is in fact reversible.

c. the protective agent may interact with the target molecules in the tissues by altering their intrinsic radiosensitivity. Damage may be prevented on this level.

ad. 2. *Biochemical and physiological mechanisms.*

Biochemical mechanisms may act by intracellular effects on mitochondria and endoplasmatic reticulum, mainly by interaction of the protective agent with SH-groups of enzymes or intracellular membranes[26]. Glucose metabolism, nucleic acid synthesis and protein synthesis are considered to be impaired. Cell division may be delayed or even inhibited[26]. At least three different mechanisms have been suggested on hypothetical and experimental grounds:

a. the release of endogenous protective agents as induced by several protective substances, especially the thiols like cysteamine and others. The released agent is believed to be reduced glutathione (GSH), which scavenges $OH^{\cdot}$ radicals. The release is derived from thiol-disulphide exchange reactions between protein-bound glutathione and the protective substance which has been administered[24]:

$$\text{protein-S-SG} + \text{RSH} \rightarrow \text{protein-S-SR} + \text{GSH}$$

b. the increase of cellular radiosensitivity by synchronization of the generation cycle of the cells. Radiosensitivity of cells seems to be dependent on a.o. the age of the cell at the time of exposure to ionizing radiation[2]. The mechanism is not yet understood, although the reactions are thought to be affected by thiols and disulphides[16].

c. the increase of repair of damaged structures, especially of dividing cells. The delaying effect of sulphhydryl agents on the mitosis and the DNA-synthesis of rapidly dividing tissues seems to be useful in increasing the extent of repair[2, 26].

The physiological effects include the actions on the cardiovascular system, the nervous system and the pituitary-adrenal system.

Hypoxia plays an important part in these protective mechanisms and may be obtained by a general external hypoxia, the blocking of oxygen supply by drugs and the production of local hypoxia in the tissues[28].

The protective mechanism of hypoxia in ionizing radiation may be explained by a reduction in the yield of $HO_2^{\cdot}$ radicals in the absence of oxygen. The consequence of this is a reduced radiation effect. The change in tissue metabolism under hypoxic conditions may lead to a reduced tissue reaction to radiation[28]. The blocking of oxygen supply in the tissues can be achieved by the use of biological amines. These substances have a selective effect on muscles, spleen, bone marrow and lymphatic nodes. According to Semenov[28], radioprotective amines may be classified as follows:

a. substances with sympathetic activity — epinephrine and its derivatives;

b. substances with parasympathetic activity — acetylcholine and its analogues;

c. serotonin and its analogues;

d. histamine.

In addition to the outlined radioprotective mechanisms the effect of platelets, fibrin formation and degradation on the vessel wall in ionizing irradiation may be of theoretical and probably of practical importance, especially when vessels of the elastic transport type are involved in the damaging process.

Changes in the polymerization state of the mucopolysaccharide ground substance of the vessel wall and the intercellular junctions of the lining endothelium, and the destruction with fragmentation of elastin and collagen fibres may result in platelet adherence, the formation of platelet aggregates[21,29] and the ultimate induction of fibrin formation[21]. The release of catalytic enzymes like elastase and collagenase[27], of bioamines like serotonin and histamine from platelets[14], the generation of thrombin and the conversion of plasminogen with the subsequent activation of the kinin system[7], may play a part in the further impairment of the vessel wall permeability and supporting functions.

Thus reduction of the platelet adhesiveness and aggregability (contraction and release phenomena[21]), prevention of the fibrinogen-fibrin conversion on any level and blocking of the plasmin formation and activity may theoretically play an additional part in the prevention of irradiation damage to the vessel wall.

The purpose of the experiments as described in this thesis, is to examine whether prevention of vessel wall damage in an experimental irradiation model can be obtained or at least advanced by the administration of drugs, which have an effect on platelet function, fibrin formation, fibrin degradation or vessel wall permeability.

Chapter 2 describes the experimental model and the methods employed.

Chapter 3 gives a report of the experimental data.

Chapter 4 discusses and criticizes the results.

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CHAPTER 2

MATERIALS AND METHODS

2.1 INTRODUCTION

The carotid arteries of the rabbit are very resistant to atheromatous lesions in hypercholesterolaemia[1, 14]. Even very high serum cholesterol levels do not result in the formation of atheromatous plaques, unless the hypercholesterolaemia has persisted for several months[14].

Only when the vessel wall has been damaged in any way, lipid will infiltrate and be deposited[6]. Formation of atheromatous lesions in the intima and the media then occur.

Thus lipid may serve as a marker substance, measuring the degree of vessel wall damage in experimental irradiation.

2.2 EXPERIMENTAL MODEL

2.2.1 ANIMALS

Rabbits, chinchillas of own breed, of both sexes and 12 to 14 weeks old, were fed on a 0.5 % cholesterol diet one week prior to X-ray irradiation. Each group consisted of 6 rabbits. The irradiation was carried out transcutaneously under general Nembutal® anaesthesia. The diet was continued for another four weeks and then the rabbits were killed for histological examination of the carotid arteries.

Body weights were measured during the whole period of the experiment.

2.2.2 IRRADIATION PROCEDURE

The irradiation was performed with a Philips-Müller MG 300 X-ray apparatus, operating at 200 kVp, 15 mA. A 0.5 mm Cu-filter and a 22 cm

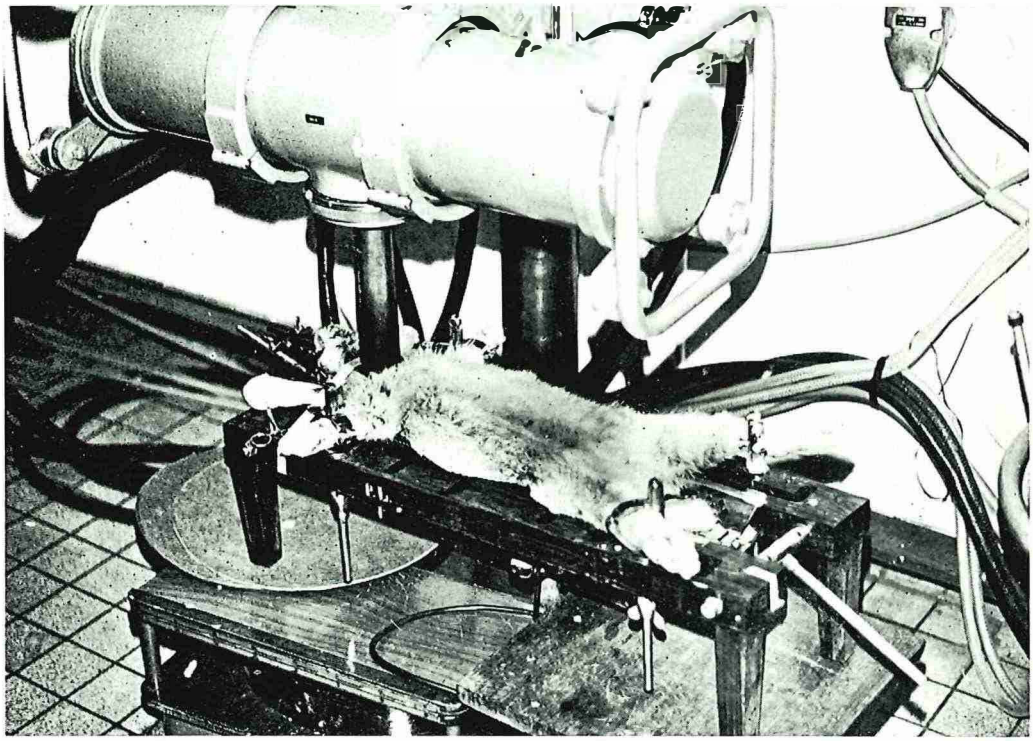

Fig. 2.1 Position of the rabbit during the irradiation procedure.

tube with a diameter of 4 cm were used. Focus distance to the carotid artery was approximately 23.5 cm. Dose rate was 120 R/min. air-exposure as measured with a Philips Universal dosemeter.

A total dose of 2000 R was given in a single session under general Nembutal® anaesthesia of the rabbit. The animals were fixed on a standard animal-experiment operation table (see fig. 2.1).

During the irradiation procedure the neck of each rabbit was shielded from X-rays with a 4 mm lead plate which focussed the X-rays selectively on one carotid artery by a 5 x 0.5 cm groove (see fig. 2.2).

2.2.3 OPERATION TECHNIQUE

Four weeks after the X-ray irradiation, the animals were operated upon under general Nembutal® anaesthesia. Both carotid arteries were carefully prepared from the surrounding tissues and excised; the non-irradiated one being the control to the irradiated one.

The animals were then killed by intracardiac Nembutal® injection.

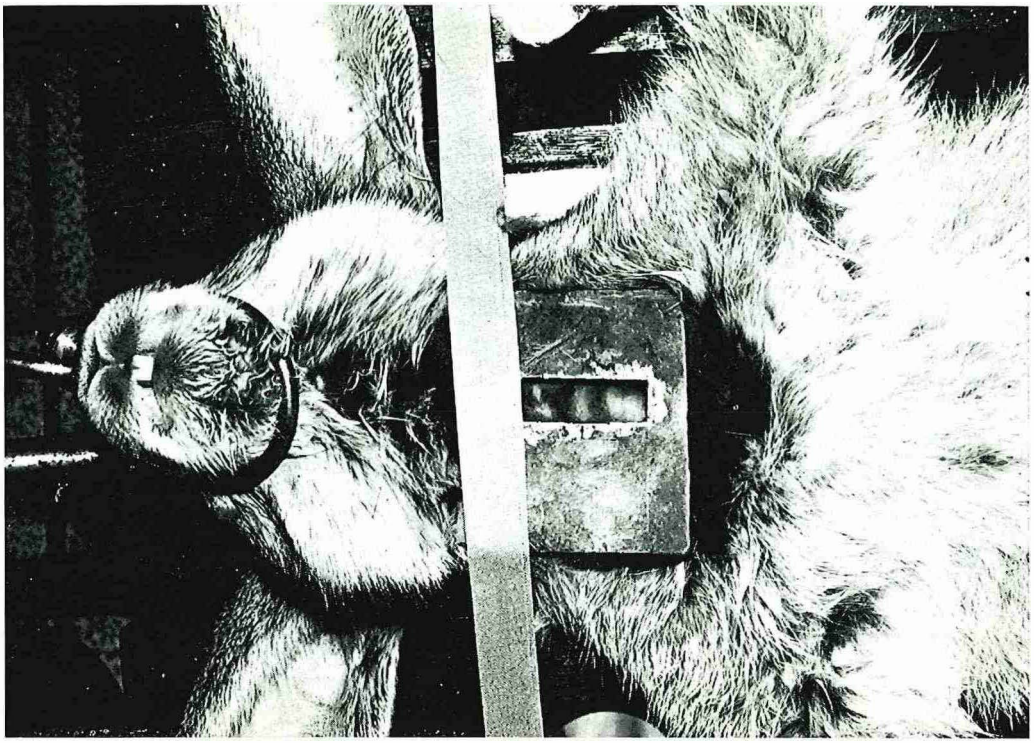

Fig. 2.2 A 0.4 mm lead plate is fixed on the neck of the rabbit during the irradiation procedure. X-rays are selectively focussed on one carotid artery by the 5 x 0.5 cm groove.

2.3 LABORATORY METHODS

Blood was collected by punction of the central ear artery under light Nembutal® anaesthesia at the start (0) of each experiment and immediately before irradiation (1). After the operation procedure at the end of each experiment, blood was collected by cardiac puncture just before the Nembutal® sacrifice (2).

All samples were collected in disposable syringes and siliconized disposable needles were used.

For storage of the samples plastic tubes were used.

2.3.1 COAGULATION STUDIES

Blood for coagulation studies was immediately mixed with 0.1 volume of 3.08 % trisodium citrate dihydrate, and it was kept in plastic tubes covered with Parafilm®.

Platelet-poor plasma (P.P.P.) was prepared in an Optima BHG 600 centrifuge at 2000 **g** (3000 rev/min) for 10 minutes.

Platelet-rich plasma (P.R.P.) was prepared in an Optima BHG 600 centrifuge at 250 **g** (1000 rev/min) for 10 minutes.

All assays were performed immediately.

2.3.1.1 *Fibrin formation*

Kaolin-activated partial thromboplastin time - according to Proctor and Rapaport[17], using cephalin as a phospholipid source.

One-stage prothrombin time - according to Quick[18].

Thrombin time - according to Vermijlen and Verstraete[20].

Thrombotest - according to Owren[16]. A regression curve for rabbit plasma was drawn from serial dilutions of pooled rabbit P.P.P. in the thrombotest system[10]. The regression curve for human plasma as made by the reagent supplier was drawn for comparison, using the same technique and production batch of Thrombotest® (see fig. 2.3).

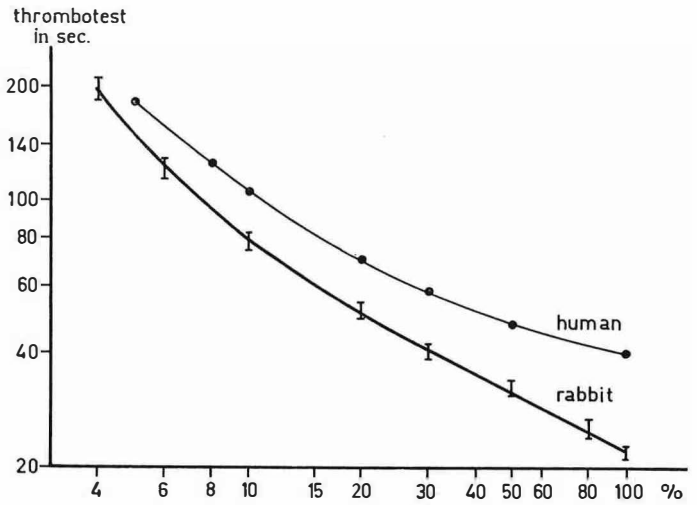

Fig. 2.3 Thrombotest regression curve for rabbit plasma (lower) and human plasma (upper).
Abscissa indicates thrombotest percentages, ordinate indicates the thrombotest in seconds.

Fibrinogen - was measured chemically with a biuret technique according to Leclerc and Khodabandeh[15].

Fibrinolysis - a 24 hours plasma clot lysis system was used as described by Fontein[8].

2.3.1.2 *Platelet function*

Platelet count - according to Feissly and Lüdin[7] with the modified technique of Brentjens[4].

Platelet aggregation - was performed using the Born technique[2] on a Vitatron UC 200 spectrophotometer connected with a Vitatron universal lin/log UR 300 recorder. Aggregation was induced by ADP in a final concentration of 0.75 μgr/ml. The tests were carried out at room temperature.

P.R.P.-clot retraction - as described by Bouma[3]. The percentages of retraction were read after 1, 3 and 24 hours incubation at 25° C.

2.3.2 LIPID

Serum cholesterol - was measured according to the technique of Huang et al.[12].

Serum lipoprotein spectra - were measured electrophoretically on cellulose-acetate strips and stained in an oxydative Schiff technique as described by Kohn [13] and modified by Sluiter[19].

2.3.3 VISCOSITY

The direct effect of X-rays on mucopolysaccharides was studied in a viscosity model as described by Brinkman[5].

Fresh bovine synovia was obtained from the local slaughter-house by puncture of the tibiotarsal joints of freshly slaughtered animals; the synovia was centrifuged in a Christ refrigerated centrifuge at 3250 g (5000 rev/min) for 20 minutes and filtered until a clear solution was obtained.

This synovia had a s.g. = 1.0049.

The synovia was diluted with 0.1 volume of saline in the control, or 0.1 volume of a drug solution.

At a constant temperature of 30° C viscosity was measured in an Oswald viscometer before and 5 minutes after irradiation.

The irradiation procedure was carried out with the same Philips-Müller MG 300 X-ray machine, operating at 200 kVp, 15 mA.A 0.5 mm Cu-filter was used. A plastic cup with 10 ml of diluted synovia was placed directly on the filter. Dose rate 700R, total dose 1400R.

2.3.4 LETHALITY TEST

Mice, males from the C_{57} Black strain, were exposed to a lethal X-ray irradiation dose of 725R.

Immediately before irradiation they were injected intraperitoneally with saline as a control, or a solution of the test drug.

Each group consisted of 20 mice, divided into four cages of 5 mice. Lethality rates were counted over a 30 days period.

2.4 DRUGS

Drugs with an effect on fibrin formation, platelet function, fibrinolysis and vessel wall permeability were tested for their protective effect in the experiment.

During the whole period the drugs were given daily in a dose equivalent to the normal human dose in mg/kg body weight in addition to the diet.

2.4.1 ANTICOAGULANTS

Heparin - calcium heparin was used for subcutaneous injection. A single dose of 3 to 4 mg/kg body weight produced a 24 hours effect on the thrombin time. The dose was varied to achieve a lengthening of at least twice the normal rabbit plasma thrombin time control after 24 hours (see fig. 2.4).

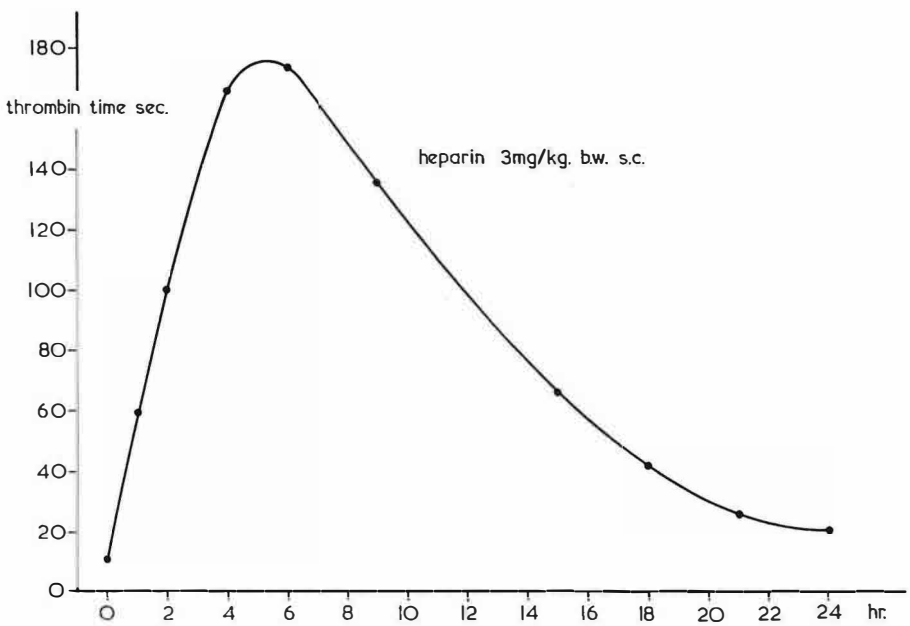

Fig. 2.4 Heparin disappearance curve after administration of 3 mg/kg body weight s.c., registered over a 24 hours period.

Warfarin sodium (3-α-phenyl-β-acethylaethyl-4-hydroxycoumarin) - was given intravenously. An initial dose of 1 mg/kg body weight was given. On the second day 0.5 mg/kg body weight was injected. Dependent on the thrombotest level, a maintenance dose of 0.16 - 0.5 mg/kg body weight was given[11]. Thrombotests were done every other day and the levels were read on the special rabbit plasma reference curve.

2.4.2 ANTI-AGGREGATING AGENTS

Acetylsalicylic acid (A.S.A.) - was mixed through the diet in a daily dose of 20 mg/kg body weight.

Indomethacin - was mixed through the diet in a daily dose of 1 mg/kg body weight.

Phenylbutazon - was mixed through the diet in a daily dose of 10 mg/kg body weight.

VK 744 [2(2-aminoaethyl)-amino-4-morpholinothieno(3,2-d)-pyrimidin dihydrochlorid] - a pyrimido-pyrimidin derivative was given intravenously in a daily dose of 5 mg/kg body weight.

RA 233 [2,6-bis(diaethylolamino)-4-piperidine-pyrimido(5,4-d)-pyrimidin] - a pyrimido-pyrimidin derivative was given intravenously in a daily dose of 5 mg/kg body weight.
Both VK 744 and RA 233 were supplied by Thomae GmbH, Western Germany.

2.4.3 ANTI-FIBRINOLYTIC AGENT

Tranexamic acid (A.M.C.A. or trans aminomethyl cyclohexan carboxilic acid) - was mixed through the diet in a daily dose of 30 mg/kg body weight.

2.4.4 PERMEABILITY AFFECTING AGENTS

Etamsylate - was given intravenously in a daily dose of 25 mg/kg body weight. The drug was supplied by Delalande S.A., France.

Prednisone - was mixed through the diet in a daily dose of 1 mg/kg body weight.

O-(β-hydroxyaethyl)-rutoside (H.R.) - was given intravenously in a daily dose of 30 mg/kg body weight. The drug was supplied by Zyma S.A., Switzerland.

Benzarone (2-aethyl-3-(4-hydroxybenzoyl)-benzofurane) - was mixed through the diet in a daily dose of 15 mg/kg body weight. The drug was supplied by Labaz S.A., Belgium.

2.4.5 CONTROL

Four control groups were included in the experiments:

1. 0.5 % cholesterol diet without any drug; the standard procedure was carried out including irradiation after one week and sacrifice four weeks later.
2. no special diet, normal standard pellet food, no drugs; the rabbits were killed four weeks after the irradiation procedure.
3. 0.5 % cholesterol diet; 15 minutes before X-ray irradiation sodium thiosulphate $5.H_2O$ was injected intravenously in a single dose of 1 G/kg body weight. The experiment was completed in the normal way.
4. clofibrate, added to the 0.5 % cholesterol diet in a daily dose of 30 mg/kg body weight one week prior to X-ray irradiation. The experiment was completed in the normal way.
   Clofibrate had been included because of its effect on fat metabolism, especially the reduction of serum triglycerides, the inhibition of cholesterol synthesis and the increase of neutral sterol excretion[9].

2.5 HISTOLOGICAL TECHNIQUES

Immediately after excision, the carotid arteries were fixed in a 10 % formol solution.

The cryostat sectioned vessels were stained for:

a.	connective tissue	— Azan technique;
b.	elastic fibres	— Verhoeff technique;
c.	smooth muscles and cellular elements	— haematoxylin eosin technique;
d.	lipid	— oil red O, prestained with a light Verhoeff technique;
e.	mucopolysaccharides	— PAS technique.

During the whole experimental period the complete histological procedure was carried out by the same technician.

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CHAPTER 3

RESULTS

3.1 HISTOLOGICAL CRITERIA

In the experimental model described in chapter 2, it was possible to standardize the production of vessel wall damage as measured histologically by the infiltration and deposition of lipid. In the control rabbits the non-irradiated arteries showed no abnormalities, whereas all the irradiated arteries showed marked infiltration and deposition of lipid with destruction of elastic fibres, disturbances of the normal circular arrangement of the smooth muscle bundles and mononuclear infiltrates. There was plaque formation with foam cells in the intima and the media.

Careful histological examination resulted in the following classification of the histopathological phenomena as observed in the model:

Connective tissue

In the Azan stained sections the post-irradiation phenomena were recognized as:

+ widening of the intercellular ground substance spaces of the media, with thickening of the vessel wall;

++ ditto, with disturbances of the normal circular arrangement of the smooth muscle fibres of the media. The muscle cells are ragged;

+++ ditto, with foam cells in the intima;

++++ ditto, with foam cells in the media.

PAS stained sections:

+ normal ground substance distribution;

++ patches of PAS positive material in the media directly underneath the tunica elastica interna;

+++ PAS positive material through the whole media.

Elastic fibres

In the Verhoeff stained sections the destruction and degeneration of elastin and collagen was recognized as:

+ disruption and fragmentation with atrophy and patchy thickening of the subintimal elastic fibres. The tunica elastica interna remains intact;

++ ditto, with destruction of almost all the medial elastic fibres;

+++ ditto, with degeneration and destruction of the tunica elastica interna.

Lipid

Lipid infiltration and deposition as well as fatty degeneration of elastic fibres could be localized and classified in the oil red O and Verhoeff pre-stained oil red O techniques as follows:

+ lipid deposition in the intima, appearing as a lining of fatty material on the tunica elastica interna;

++ patches of lipid, deposited in the subintimal layer of the media, with a fine granular lining of lipid along the elastic fibres;

+++ ditto, with further infiltration and deposition of lipid in the media;

++++ infiltration and deposition of lipid throughout the vessel wall.

Smooth muscle and cellular elements

In the haematoxylin-eosin stained sections, the degenerative processes of the smooth muscle fibres and the infiltration of mononuclear cells were recognized as:

+ vacuolization with nuclear polymorphism and swelling of the smooth muscle cells; disturbances of the normal circular arrangement of the fibres;

++ ditto, with mononuclear cells infiltrating the intima;

+++ ditto, with infiltration of mononuclear cells in the media.

Foam cells or lipophages

The appearance of foam cells or lipophages is a late consequence of vessel wall damage in hypercholesterolaemia. In all the staining techniques the foam cells could easily be identified, localized and classified:

\+ foam cells in the intima;

++ ditto, with foam cells in the media.

Based on the histological criteria outlined above, the following stages of vessel wall damage can be distinguished in the model (table 0):

1. depolymerization of mucopolysaccharide ground substance, and fragmentation, atrophy and destruction of elastic fibres;
2. degeneration of smooth muscle fibres, with vacuolization, nuclear polymorphism, swelling of the cells and disturbances of the normal circular arrangement of the bundles;
3. infiltration and deposition of lipid in the intima and the media;
4. appearance of foam cells and mononuclear infiltrates in the intima and the media.

The overall effect on the irradiated artery is thickening of the vessel wall with sclerosis (decrease in elasticity) and atheromatosis.

3.2 EVALUATION OF THE DIFFERENT GROUPS IN THE EXPERIMENT

A descriptive evaluation of the control groups and the groups which were treated with drugs will be given with presentation of the histological, clotting, lipid and body weight data.

3.2.1 CONTROL GROUPS

Hypercholesterolaemic control (group C)

The eight rabbits of this control group were fed on the standard 0.5 % cholesterol diet during the whole period of five weeks of the experiment. No drugs were administered.

Gross appearance - During the operation it was observed that the irradiated vessels had an irregular, mat and cobbled appearance, with inflammatory reaction of the surrounding tissues. There were yellow-white atheroma-like patches on the vessel walls. The vessels were rigid. The non-

Fig. 3.1 Hypercholesterolaemic control, irradiated carotid artery (rabbit 2267)
PAS stained; 412.5 X
Thickened wall with widening of the spaces between the degenerated elastic fibres, filled with positive material. Foam cells are present in the intima and subintimal layer of the media.

irradiated arteries had a normal appearance, with elastic pulsations and a glossy adventitia. No adhesions were observed.

On external examination of the rabbits no other signs of hyperlipaemia (corneal lipid deposits) were seen.

Histology (table I) - The irradiated vessels showed thickening of the walls with considerable widening of the intercellular spaces in the media, which had been filled with PAS positive material. (fig. 3.1) The medial elastic fibres had been locally damaged. (fig. 3.2) The tunica elastica interna, however, showed no signs of degeneration. The circular arrangement of the smooth muscle bundles had been disturbed. The smooth muscle cells were swollen, with vacuolization and nuclear polymorphism. Lipid had infiltrated into the intima and with a variable depth into the media. The areas of lipid deposition stained deeply red in the oil red 0. Many foam cells were seen, predominantly in the intima, forming plaques

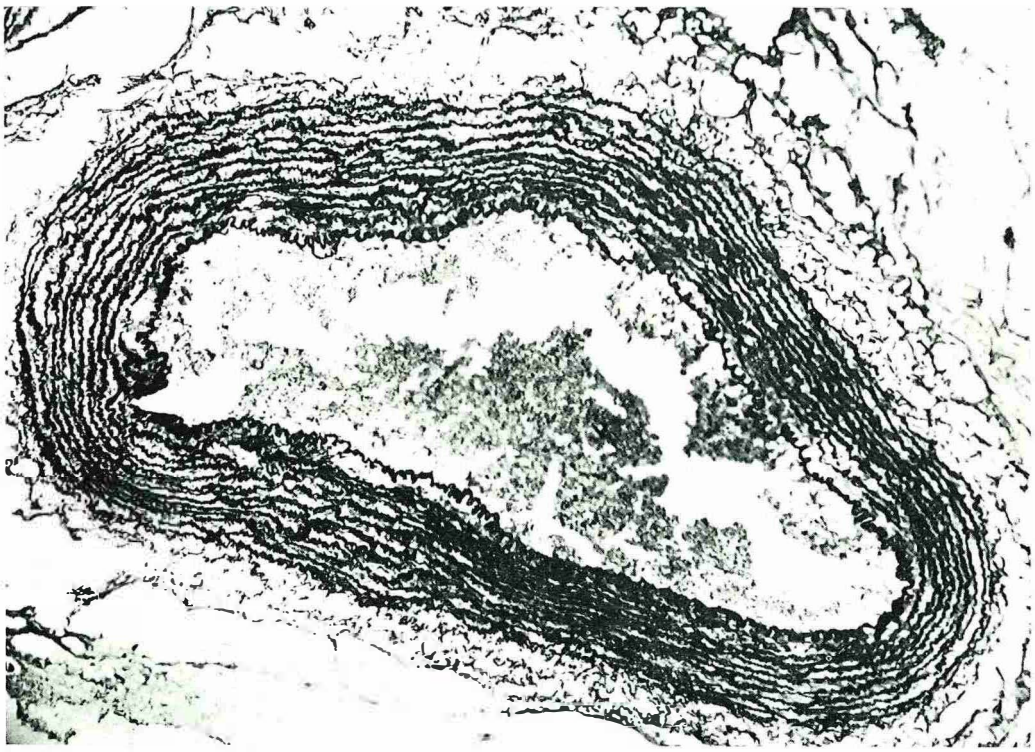

Fig. 3.2 Hypercholesterolaemic control, irradiated carotid artery (rabbit 2267)
Verhoeff stained; 105 X
Thickening of the wall, degeneration with local fragmentation and atrophy of the elastic fibres of the media. Tunica elastica interna remained intact. Foam cells are present both in the media and in the intima, with formation of plaques.

which protruded into the lumen of the arteries. Intimal and medial mononuclear infiltrates were present.

Histology of the control vessels showed a completely normal vessel wall structure. (fig. 3.3)

Blood coagulation studies. - All parameters showed constant and normal values during the whole experimental period. (table A-E, fig. 3.4, 3.5)

Lipid - Serum cholesterol levels increased strikingly from a mean value of 116 mg% at the start, to 417 mg% before the irradiation and 1046 mg% at the sacrifice. (table I, fig. 3.6) Serum lipoprotein spectra changed from normal at the start into a complete loss of pre-beta and alpha bands with very heavy beta bands.

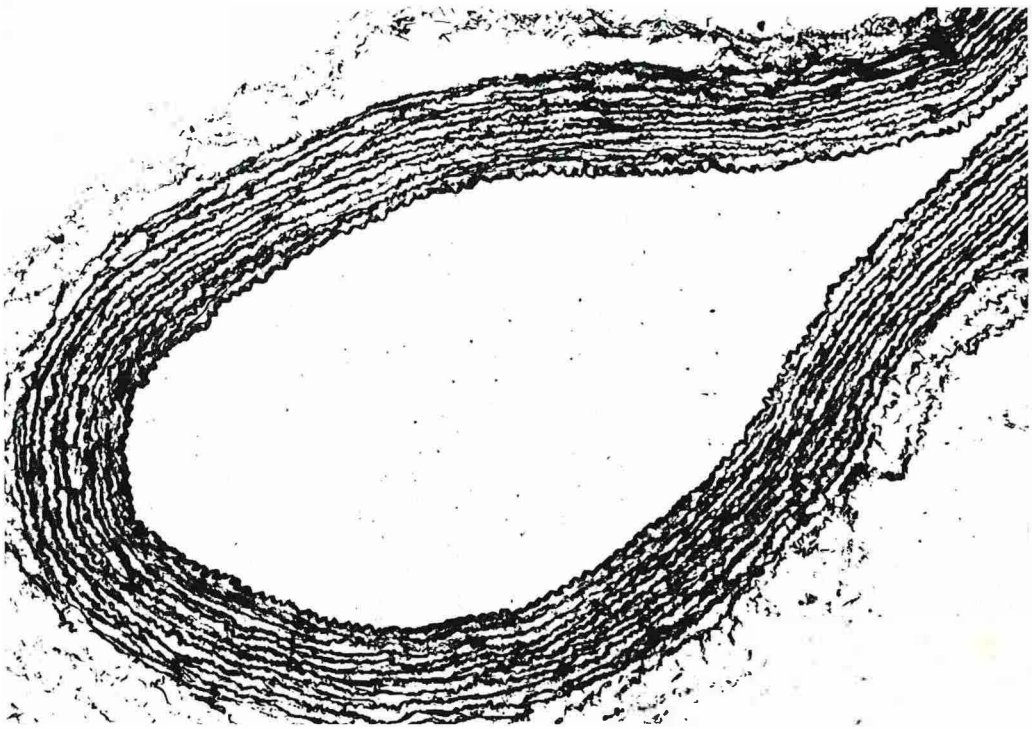

Fig. 3.3 Hypercholesterolaemic control, non-irradiated carotid artery (rabbit 2267) Verhoeff stained; 105 X
Normal regular arrangement of elastic fibres, with regular spaces between the fibre network. No foam cells.

Body weight increase: + 11.4 %

The changes in the irradiated arteries of this hypercholesterolaemic control group were classified as stage 4.

Non-hypercholesterolaemic control

Three rabbits were fed on a standard pellet diet without addition of cholesterol. No drugs were administered.

Gross appearance - The irradiated carotid arteries were easy to prepare, because there were only few adhesions to the surrounding tissues. The vessels were dull and without gloss and were more rigid in comparison with the non-irradiated normal control vessels.

Histology (table II) - The irradiated arteries showed thickening of the walls, due to widening of the intercellular spaces, which had been filled

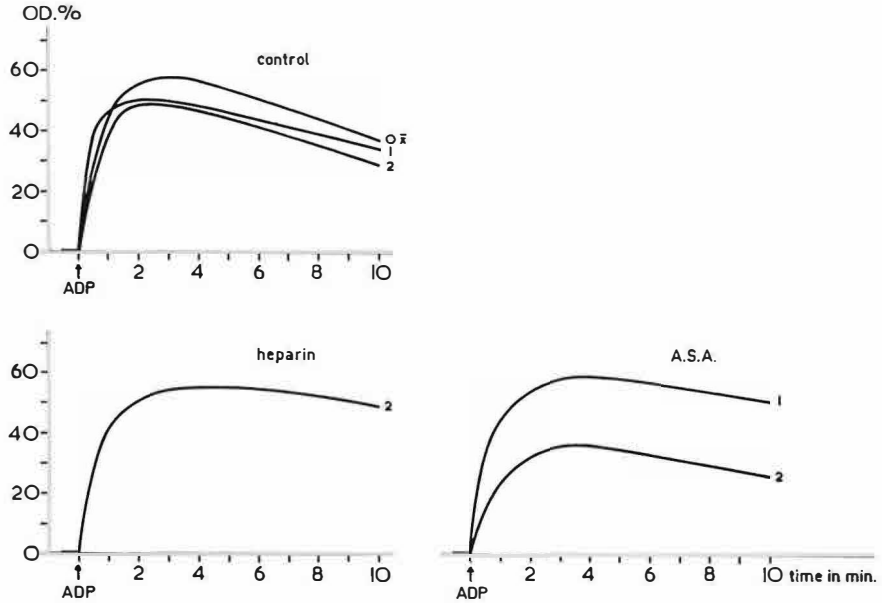

Fig. 3.4 ADP-induced platelet aggregation curves.
Abscissa indicates time in minutes, ordinate indicates percentages optical density loss.
0 = start, 1 = irradiation, 2 = sacrifice.

with PAS positive material. The elastic fibres of the media had been locally damaged. The tunica elastica interna of one carotid artery (2901) showed definite signs of degeneration. The tunica elastica interna of the other two carotid arteries had not been damaged.

Smooth muscle cells were ragged with disturbances of the normal circular arrangement, vacuoles and nuclear polymorphism.

There were no signs of lipid infiltration or deposition. Mononuclear cells were not present.

The non-irradiated control vessels showed no abnormalities.

Blood coagulation studies - These were not performed in this control group.

Lipid - Serum cholesterol levels remained low. (table II)

The serum lipoprotein electrophoretic patterns were normal and did not change.

Body weight increase: + 18.6 %

The changes in the irradiated arteries of this non-hypercholesterolaemic control group were classified as stage 2.

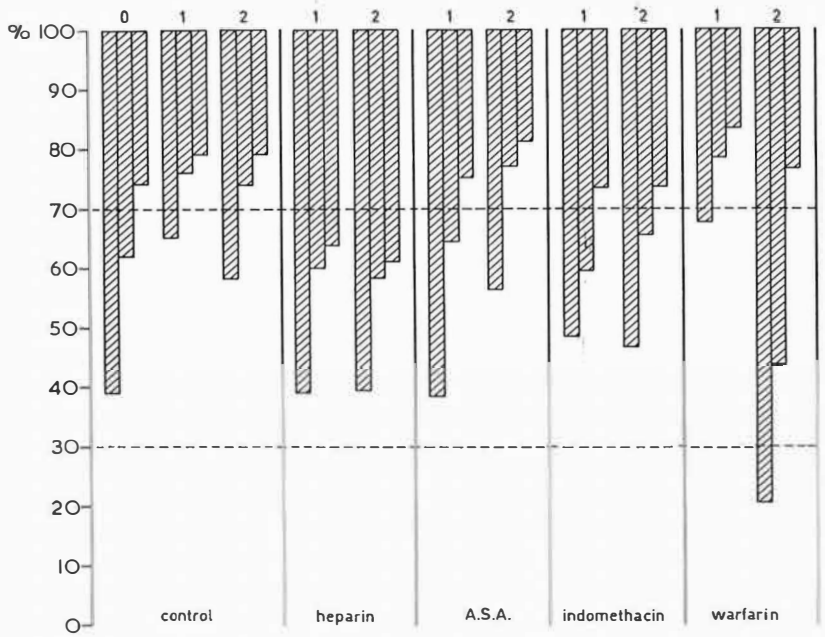

Fig. 3.5 Reverse percentages of clot retraction after 1, 3 and 24 hours incubation at 25° C (left to right).
0 = start, 1 = irradiation, 2 = sacrifice.

Sodium thiosulphate control

Three rabbits were fed on a 0.5 % cholesterol diet, one week prior to X-ray irradiation. Fifteen minutes before irradiation sodium thiosulphate was injected into a marginal ear vein in a dose of 1 G/kg body weight.

The rabbits were kept on the cholesterol diet and four weeks later they were killed for histological examination of the carotid arteries.

Gross appearance - During the operation the irradiated arteries showed a smooth adventitia with very few adhesions and elastic pulsations. The non-irradiated arteries were completely normal.

There were no external signs of hyperlipaemia.

Histology (table III) - The irradiated arteries showed some thickening of the walls, due to widening of the intercellular spaces. However, in the PAS stained sections no increase of positive material was observed. The elastic fibres of the media had degenerated, with fragmentation and atrophy predominantly in the subintimal layer. The tunica elastica interna remained intact.

Smooth muscle fibres showed minor signs of degeneration, with some

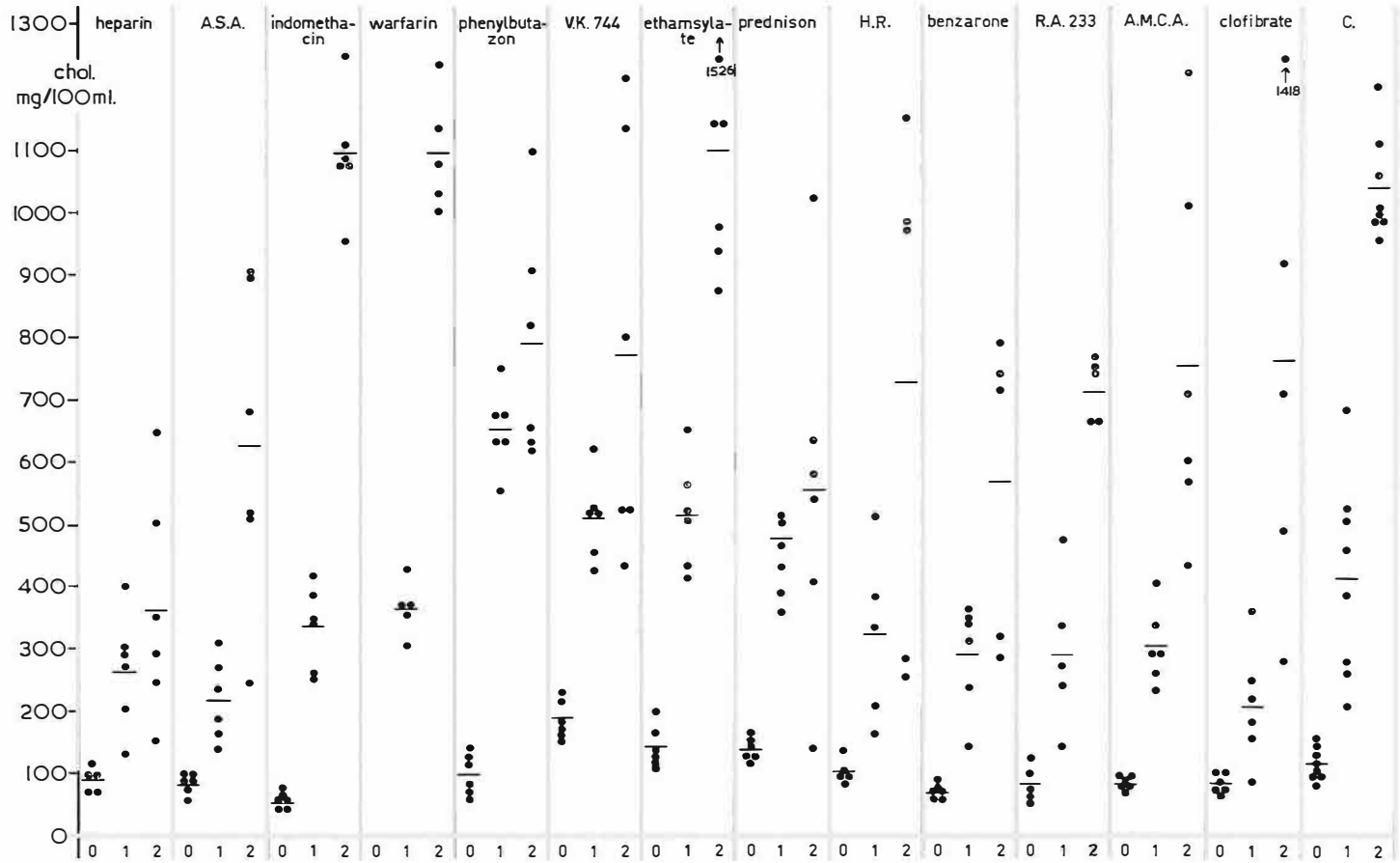

Fig. 3.6 Individual (•) and mean (—) cholesterol levels of the various groups. 0 = start, 1 = irradiation, 2 = sacrifice.

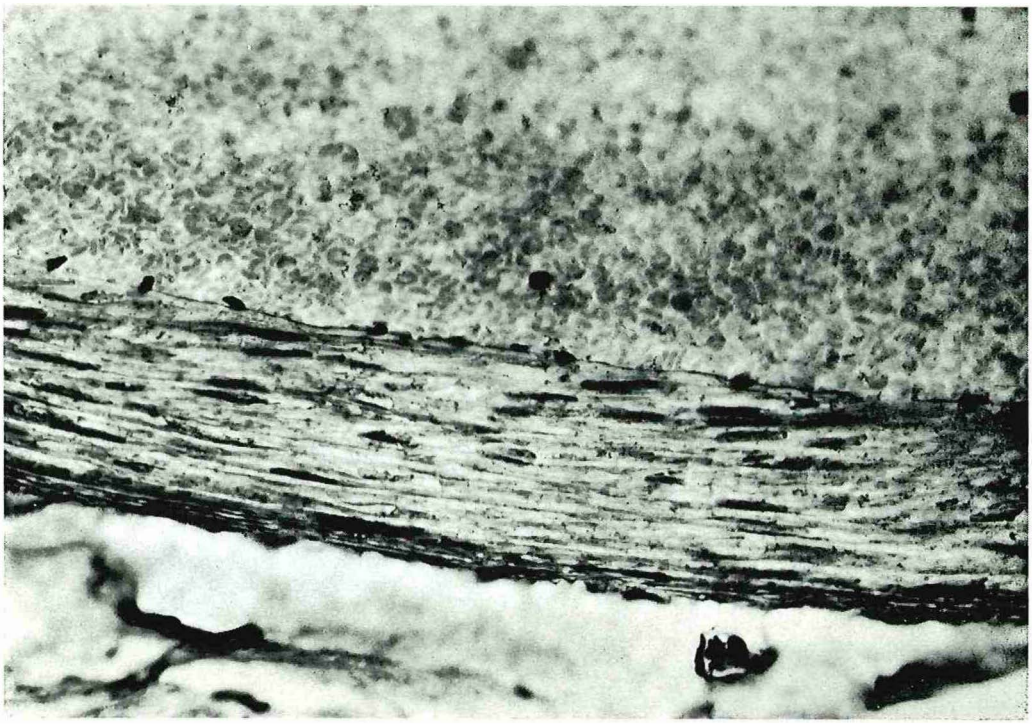

Fig. 3.7 Sodium thiosulphate control group, irradiated carotid artery (rabbit 2906) haematoxylin-eosin stained; 412.5 X
Minor signs of vessel wall damage, with some thickening of the wall and swelling with vacuolization of smooth muscle cells.

vacuolization and swelling. (fig. 3.7) The circular arrangement of the bundles seemed to be intact.

Neither lipid nor mononuclear cells were observed.

The non-irradiated carotid arteries showed completely normal vessel wall structures.

Blood coagulation studies - These were not performed in this control group.

Lipid - Serum cholesterol levels showed a considerable rise, from a mean starting value of 64 mg% to 266 mg% at the irradiation and 912 mg% at the sacrifice. (table III)

Serum lipoprotein spectra changed from normal into heavy beta bands with complete loss of pre-beta and alpha lipoproteins.

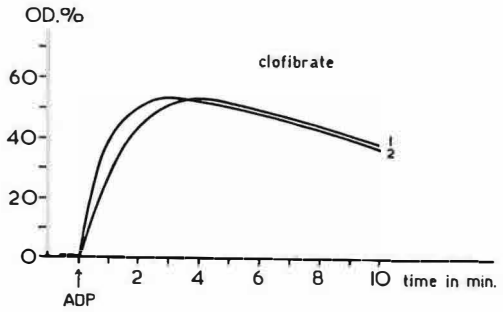

Fig. 3.8 ADP-induced platelet aggregation curve.
Abscissa indicates time in minutes, ordinate indicates percentages optical density loss.
1 = irradiation, 2 = sacrifice.

Body weight increase: + 18.8 %

The changes in the irradiated arteries of this sodium thiosulphate control group were classified as stage 2.

Clofibrate control (group XIII)

This group consisted of 6 rabbits. The standard experimental procedure was carried out, including the 0.5 % cholesterol diet with an addition of clofibrate in a daily dose of 30 mg/kg body weight to the diet.

During the irradiation procedure one rabbit died of a Nembutal® overdosage.

Gross appearance - Examination of the irradiated carotid arteries during the operation revealed mat and rigid vessels with few adhesions to the surrounding tissues. The vessels were regular. No atheroma-like patches were observed.

There were no external signs of hyperlipaemia.

The non-irradiated control vessels were normal with a glossy adventia and elastic pulsations.

Histology (table IV) - The irradiated arteries showed thickening of the walls with widening of the intercellular spaces of the media, which had been filled with PAS positive material. The elastic fibres of the media had been locally damaged while the tunica elastica interna of two rabbit carotid arteries (2870, 2874) showed definite signs of degeneration.

Smooth muscle fibres were ragged with vacuoles and nuclear polymorphism.

The normal circular arrangement had been disturbed.

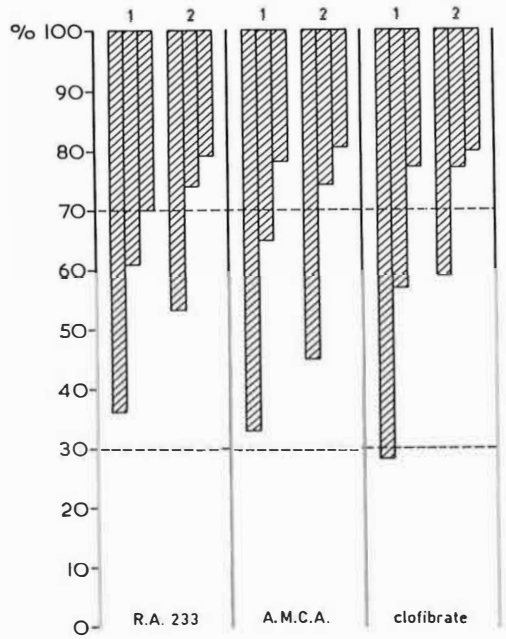

Fig. 3.9 Reverse percentages of clot retraction after 1, 3 and 24 hours incubation at 25° C (left to right).
1 = irradiation, 2 = sacrifice.

Lipid had infiltrated and been deposited into the media to a more or less extent, with foam cells present in the media as well as in the intima.

Two rabbit arteries (2869, 2873) revealed no foam cell reaction, while all the arteries had been infiltrated with mononuclear cells.

The non-irradiated control arteries were completely normal.

Blood coagulation studies - No abnormalities or changes were found in the fibrin formation tests, the platelet function tests or the platelet count. (table A-E, fig. 3.8, 3.9)

Lipid - Mean serum cholesterol levels showed a rise from a starting value of 83 mg% to 207 mg% before the irradiation and 763 mg% at the sacrifice.

There was a very wide range of increase of values of the individual animals during the experimental period, viz. from an increase of 181 mg% to an increase of 1344 mg% of cholesterol. (table IV, fig. 3.6)

Serum lipoprotein spectra changed from normal into heavy beta bands with some faint remaining pre-beta lipoproteins.

Body weight increase: + 16.1 %

The changes in the irradiated arteries of this clofibrate control group were classified as stage 4.

3.2.2 ANTICOAGULANT GROUPS

Heparin (group I)

Heparin therapy was controlled daily with the thrombin time test to ensure adequate levels during the experimental period.

Gross appearance - The irradiated arteries were normal, with only a few adhesions and a mat adventitia. There was a moderate loss of elasticity compared to the normal glossy elastic control arteries.

No external signs of hyperlipaemia were seen. The rabbits were lean.

Histology (table V) - The histopathological phenomena were dominated by widening of the intercellular spaces (fig. 3.10) which had been filled with PAS positive material, thickening of the vessel walls, destruction with fragmentation of the medial elastic fibres and degenerative changes of the smooth muscle fibres - vacuoles and nuclear polymorphism.

The tunica elastica interna were intact.

Neither the intima nor the media showed any infiltration or deposition of lipid. No signs of inflammation were seen.

The non-irradiated control vessels showed completely normal histological structures.

Blood coagulation studies - Apart from the inhibiting effect of heparin on the fibrin formation and the prothrombin conversion no other effects on clotting parameters were found. Platelet function was normal. (table A-E, fig. 3.4, 3,5)

Lipid - During the experimental period serum cholesterol levels rose to a moderate extent from a mean starting value of 86 mg% to 266 mg% before the irradiation and 362 mg% at the sacrifice. The individual increases varied from 58 mg% to 577 mg% (table V, fig. 3.6)

Serum lipoprotein spectra showed a typical pattern: light to moderate beta bands with some pre-beta lipoproteins.

Body weight increase - There was an overall loss of body weight of 23.8 %.

The changes in the irradiated arteries of the heparin group were classified as stage 2.

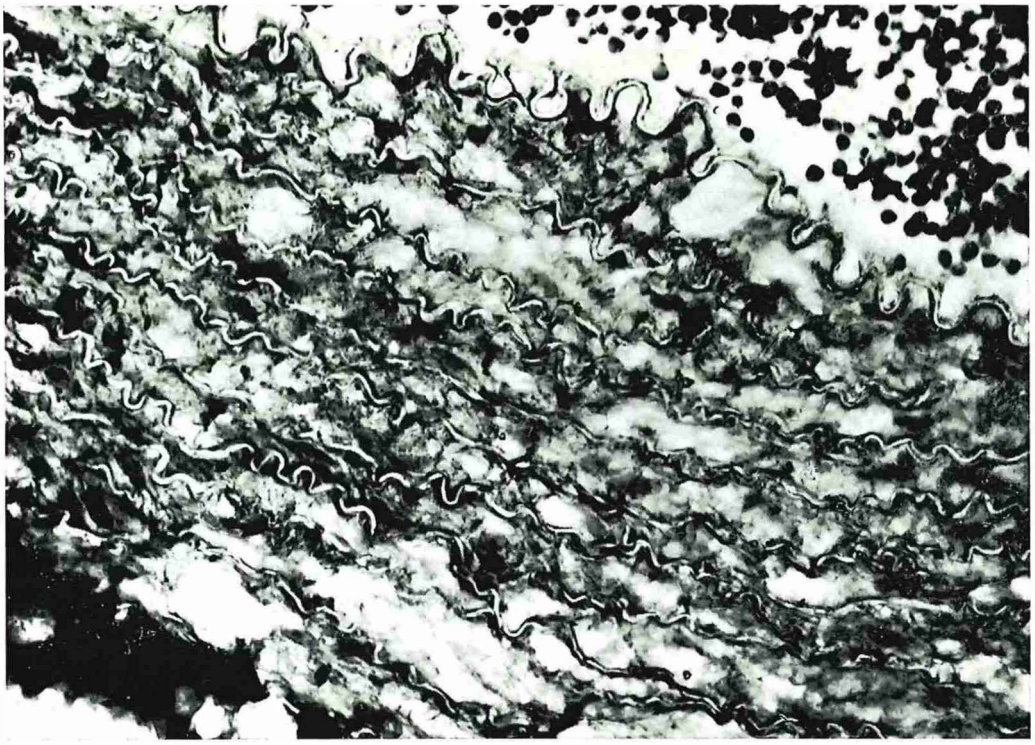

Fig. 3.10 Heparin; irradiated carotid artery (rabbit 2433)
Azan stained; 412.5 X
Thickened wall with widening of the intercellular spaces. No foam cells present.

Warfarin sodium (group IV)

Every second day warfarin sodium therapy was controlled with the thrombotest. Thrombotest percentages were read on the rabbit plasma regression curve (fig. 2.3) and remained between 7 % and 15 %. During the irradiation procedure one rabbit died due to Nembutal® overdosage.

Gross appearance - Examination of the irradiated vessels during the operation revealed rigid arteries with a cobble-stone appearance, some atheroma-like patches on the adventitia and many adhesions to the surrounding tissues.

The non-irradiated arteries were completely normal with a glossy appearance and elastic pulsations.

No external signs of hyperlipaemia were found.

Histology (table VI) - There was a moderate destruction of the irradiated vessel walls with widening of the intercellular spaces, PAS positive

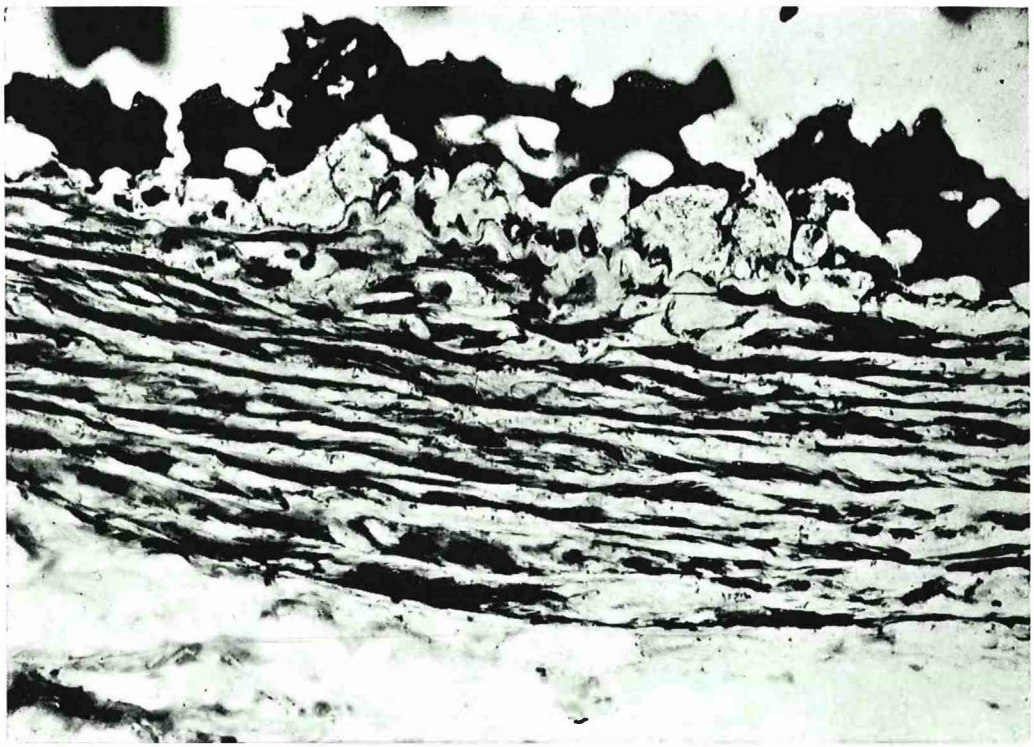

Fig. 3.11 Warfarin sodium; irradiated carotid artery (rabbit 2431)
Azan stained; 412.5 X
Ragged, degenerated smooth muscle fibres, with disturbances of the circular arrangement. Foam cells are present in the intima and the media.

material, thickening of the walls and fragmentation of elastic fibres located in the media. The tunica elastica interna were intact. Smooth muscle fibres had degenerated, with vacuoles, nuclear polymorphism and disturbances of the normal circular arrangement.

Local infiltration and deposition of lipid were seen in the intima and the subintimal parts of the media. Degenerated elastic fibres of the media were lined by fine drops of lipid. Foam cells were present in the media as well as in the intima (fig. 3.11) and mononuclear cells had infiltrated the walls.

The non-irradiated arteries were completely normal without any sign of degeneration or lipid infiltration.

Blood coagulation studies. - Apart from the delaying effect of warfarin sodium on the prothrombin conversion some effect was seen on ADP-induced platelet aggregation: at the end of the experiment ADP-induced platelet aggregation showed a flattening of the initial slope of the aggre-

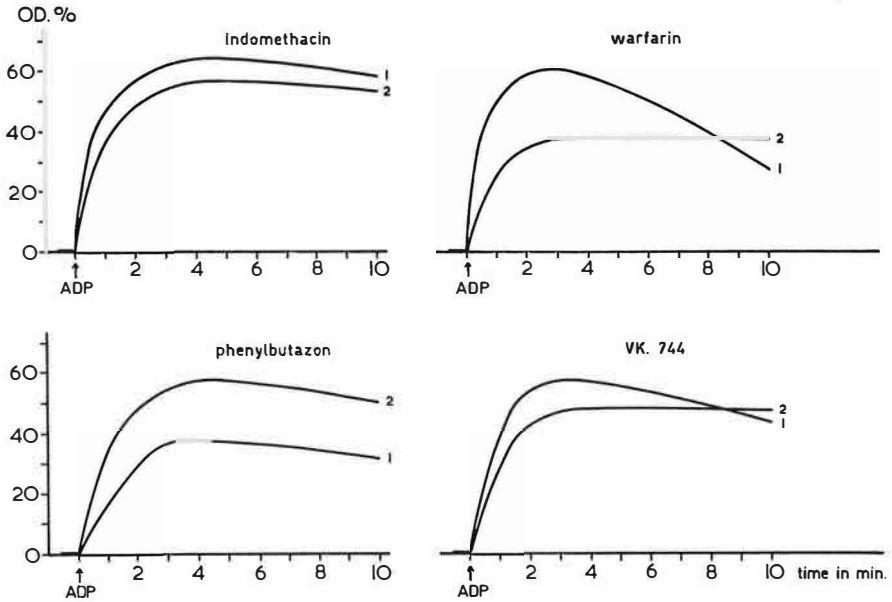

Fig. 3.12 ADP-induced aggregation curves.
Abscissa indicates time in minutes, ordinate indicates percentages optical density loss.
1 = irradiation, 2 = sacrifice.

gation curve, a smaller decrease in maximum optical density loss and a lack of disaggregation. (fig. 3.12)

The other clotting and platelet function tests remained constant and normal. (table A-E, fig. 3.5)

Lipid - Serum cholesterol levels showed a rise similar to the control values (hypercholesterolaemic control group), increasing to 366 mg% before the irradiation and 1099 mg% at the sacrifice. (table VI, fig. 3.6)

Serum lipoprotein electrophoretic patterns changed from normal at the start to spectra with very heavy beta bands and complete loss of pre-beta and alpha lipoproteins.

Body weight increase: + 18.6 %

The changes in the irradiated arteries of the warfarin sodium group were classified as stage 4.

3.2.3 ANTI-AGGREGATING AGENT GROUPS

Acetylsalicylic acid or A.S.A. (group II)

Gross appearance - The irradiated vessels had a smooth surface. The arteries were more rigid than the elastic control arteries.

The adventitia were dull, with only a few adhesions.
No external signs of hyperlipaemia were observed.

Histology (table VII) - The irradiated vessels had thickened, which had been caused by a widening of the intercellular spaces. The smooth muscle fibres were ragged, with vacuoles and polymorphism of the nuclei. The circular arrangement had been disturbed. PAS positive material was found between the ragged muscle fibres. Neither lipid infiltration or deposition, nor mononuclear infiltrates were seen. The elastic fibres of the media had degenerated showing fragmentation, atrophy and thickening. The tunica elastica interna were intact.

The non-irradiated control vessels were completely normal.

Blood coagulation studies - No abnormalities were found in the fibrin formation tests. Fibrinogen levels tended to increase in the course of the first week of the experiment, but had returned to normal by the end of the experiment. ADP-induced platelet aggregation showed a flattening of the initial slope with a decrease in maximum optical density loss. (fig. 3.4) Clot retraction and platelet count had not been influenced by the drug. (table A-E, fig. 3.5)

Lipid - Serum cholesterol levels rose moderately from a mean starting value of 81 mg% to 217 mg% at the irradiation and 626 mg% at the sacrifice. There was a considerable individual variation in the cholesterol level increase, viz. from 190 mg% to 814 mg% during the experimental period. (table VII, fig. 3.6) Serum lipoprotein spectra changed from normal to very heavy beta bands with complete loss of pre-beta and alpha lipoproteins.

Body weight increase - This group showed a small decrease in body weight: —3.7 %.

The changes in the irradiated arteries of the acetylsalicylic acid group were classified as stage 2.

Indomethacin (group III)

Gross appearance - The irradiated arteries showed a marked atheromatosis with little yellow-white cobbles in the adventitia. The vessels were very rigid with barely visible pulsations and dense adhesions. The non-irradiated control vessels were smooth, elastic and glossy, without adhesions. All the animals of the group showed clearly visible lipid depots in the corneae.

Histology (table VIII) - The irradiated vessel walls presented the histological picture of severe damage. The elastic fibres of the media had

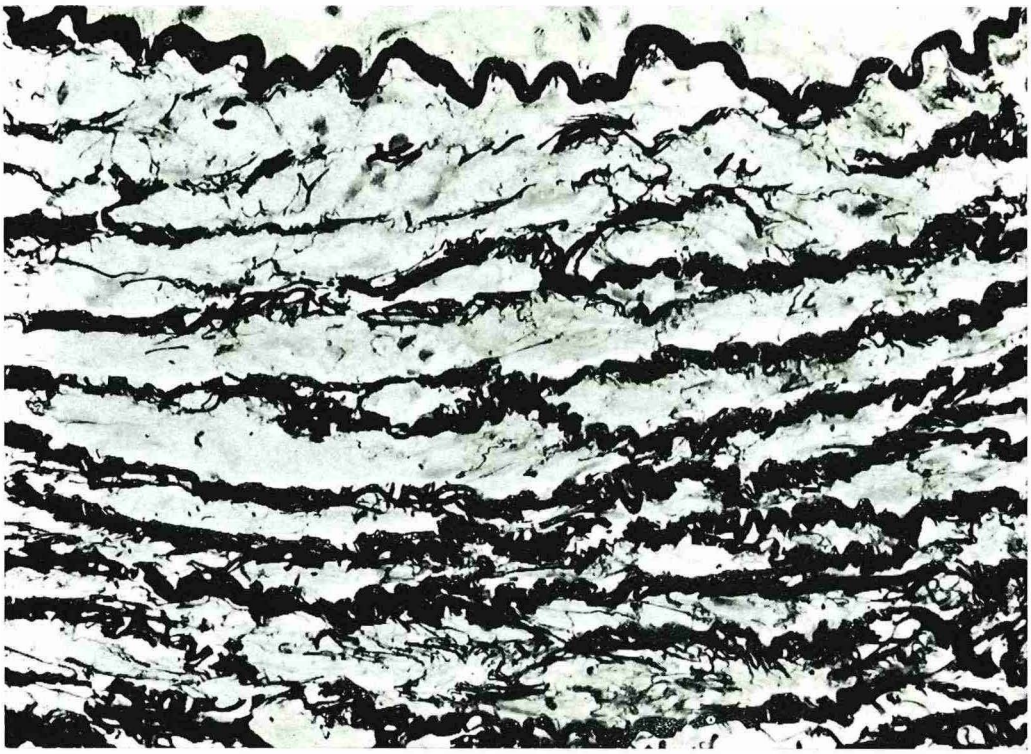

Fig. 3.13 Indomethacin; irradiated carotid artery (rabbit 2519)
Verhoeff stained; 412.5 X
Severe destruction with fragmentation and atrophy of the elastic fibres of the media. Tunica elastica interna intact. Foam cells are present between the degenerated elastic fibres.

degenerated showing fragmentation and atrophy, while the tunica elastica interna remained intact. (fig. 3.13) The intercellular ground substance spaces of the media had widened and had been filled with deposited lipid, foam cells and PAS positive material. (fig. 3.14, 3.15) The walls had thickened. Smooth muscle fibres had been severely damaged with vacuoles, nuclear polymorphism and swelling. The bundles were ragged and the circular arrangement had completely been lost. There was a deep infiltration and deposition of lipid throughout the vessel wall with plaque formation and protrusion of these plaques into the lumina.

Mononuclear infiltrates were part of these extreme pathological changes. Only one rabbit (2523) differed strikingly from the other animals in this group: the irradiated vessel wall showed less severe degenerative changes, although infiltration and deposition of lipid in the intima and the sub-intimal layer of the media were apparent; no foam cells or mononuclear cells were seen.

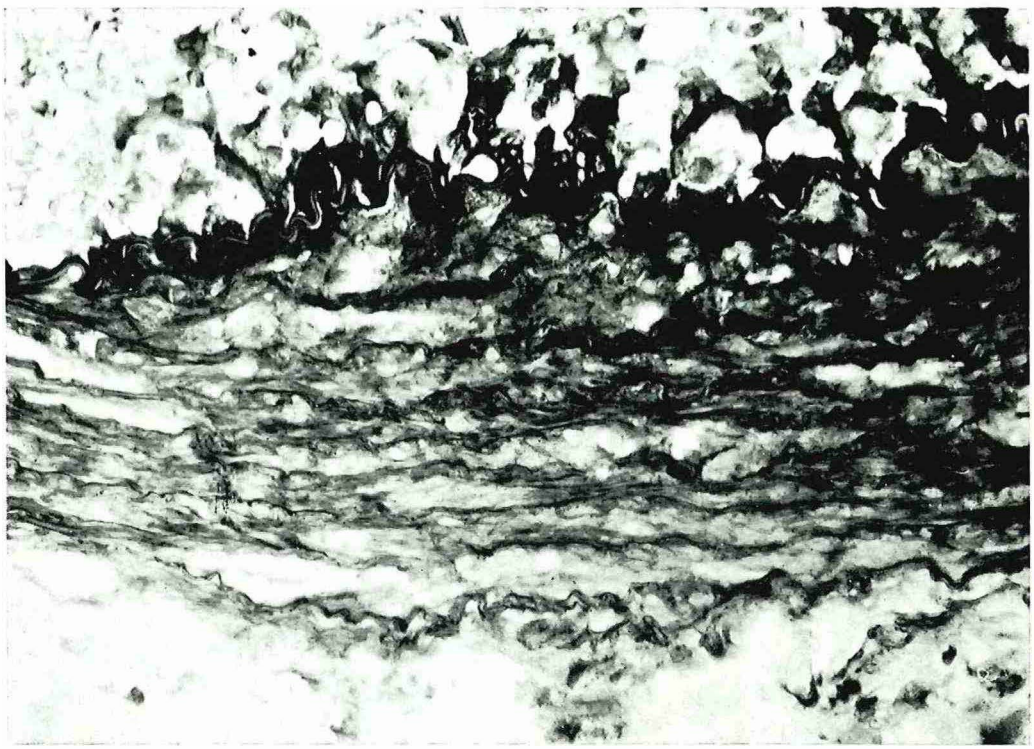

Fig. 3.14 Indomethacin; irradiated carotid artery (rabbit 2522)
PAS stained; 412.5 X
Positively staining material in the intima and the media. Foam cells in the media and the intima, with formation of a plaque.

The non-irradiated control arteries in this group were normal, without any sign of degeneration or lipid infiltration.

Blood coagulation studies - No abnormalities or changes were found in the coagulation tests. The fibrinogen levels tended to rise, but remained within the normal limits.

No effect was seen on ADP-induced aggregation, clot retraction or platelet count. (table A-E, fig. 3.5, 3.12)

Lipid - Serum cholesterol levels increased in the same way as in the hypercholesterolaemic control group, with only slight individual variation. The mean value of 58 mg% at the start rose to 335 mg% at the irradiation and 1097 mg% at the sacrifice. (table VIII, fig. 3.6)

Serum lipoprotein spectra showed heavy beta bands with complete loss of pre-beta and alpha lipoproteins.

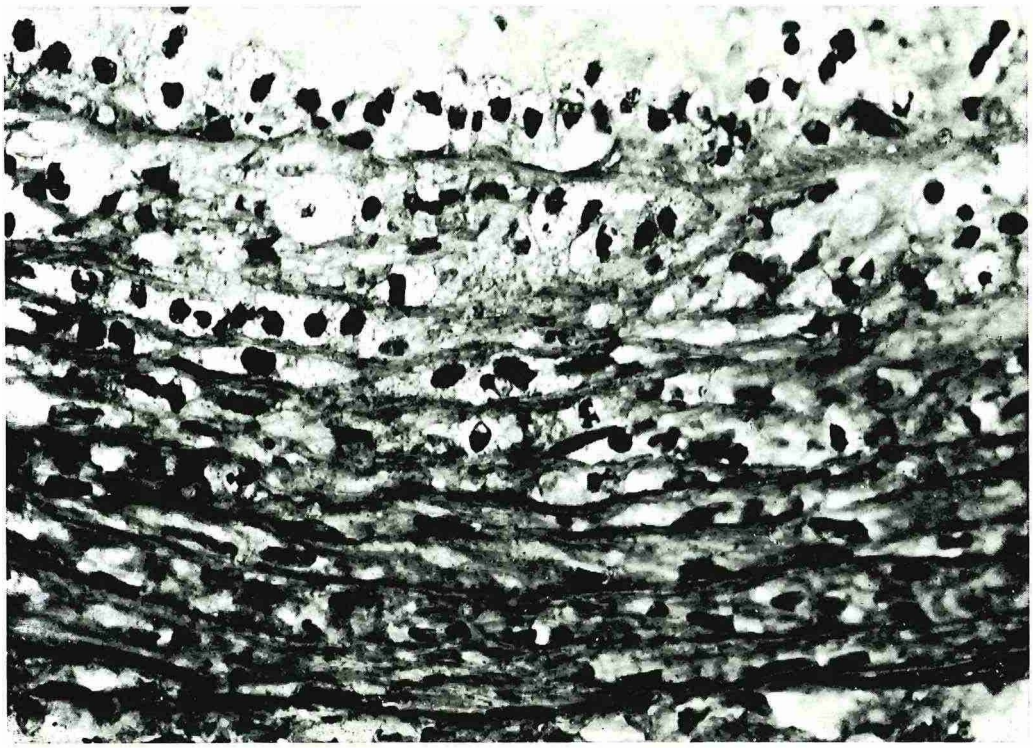

Fig. 3.15 Indomethacin; irradiated carotid artery (rabbit 2524)
haematoxylin eosin stained; 412,5 X
Heavily damaged vessel wall with degenerated elastic fibre network, foam cells in the intima and deeply penetrated into the media. Mononuclear cells.
Vacuolization and nuclear polymorphism of the degenerated smooth muscle cells.

Body weight increase: + 16.3 %

The changes in the irradiated arteries of the indomethacin group were classified as a severe degree of stage 4.

Phenylbutazon (group V)

Gross appearance - The irradiated arteries were rigid with barely visible pulsations. The vessel wall surfaces were dull and cobbled with atheromatous patches in four of the six rabbits (2448, 2449, 2452, 2453). All the irradiated vessels had adhered to the surrounding structures.

Corneal deposition of lipid was seen in all the rabbits of the group.

Fig. 3.16 Phenylbutazon; irradiated carotid artery (rabbit 2448)
Azan stained; 412.5 X
Severely damaged vessel wall with many foam cells in the media and plaque formation in the intima.

The non-irradiated control arteries were smooth and glossy with elastic pulsations.

Histology (table IX) - The irradiated vessel walls of four rabbit (2448, 2449, 2452, 2453) showed severe degenerative changes of the connective tissue, elastic structures and smooth muscle fibres. Lipid had infiltrated and been deposited through the whole media with infiltration of foam cells and mononuclear cells. (fig. 3.16) There was a striking increase in PAS positive material. A typical feature was the degeneration of the tunica elastica interna in two of the irradiated vessels (2448, 2453), with stretching of the laminae and atrophy. (fig. 3.17) The other two rabbits (2450, 2451) showed less severely damaged vessel walls, with lipid deposited in the intima only. The media of these vessels, however, showed widening of the intercellular spaces, with an increase of PAS positive

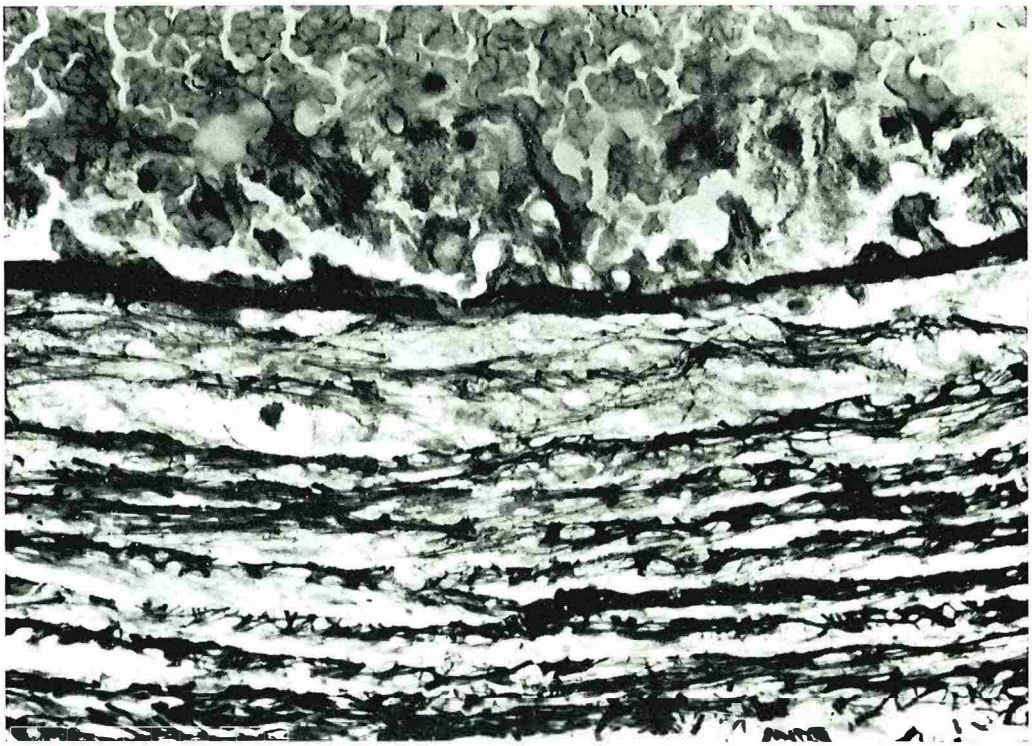

Fig. 3.17 Phenylbutazon; irradiated carotid artery (rabbit 2448)
Verhoeff stained; 412.5 X
Heavily damaged elastic fibre network of the vessel wall. The tunica elastica interna is stretched and degenerated. Foam cells are present in the media and the intima, with a plaque protruding into the lumen.

material and degenerative changes in the smooth muscle fibres. No foam cells were seen in these vessel walls.

The non-irradiated control arteries of all the rabbits of the group were completely normal.

Blood coagulation studies - There was a striking increase in fibrinogen levels up to 1425 mg% during the post-irradiation episode of the experiment. The clotting tests remained normal. (table A-D)

ADP-induced aggregation appeared to be influenced by the drug: the curves showed a clear flattening of the initial slope with a decrease of maximum optical density loss during the experimental period. (fig. 3.12)

Clot retraction and platelet count remained normal (fig. 3.18, table E)

Lipid - Serum cholesterol levels showed a moderately severe increase from a mean starting value of 99 mg% to 654 mg% at the irradiation and 790

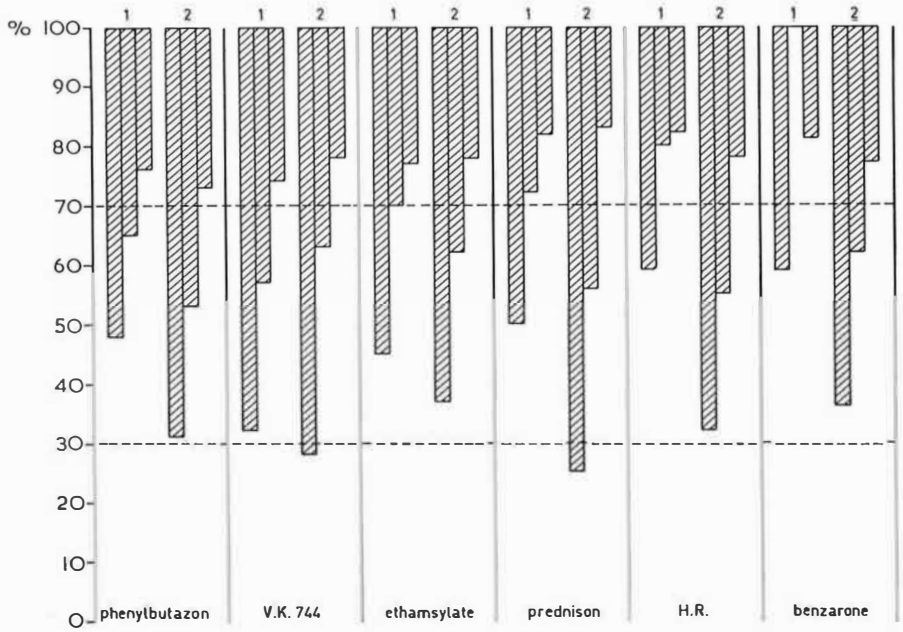

Fig. 3.18 Reverse percentages of clot retraction after 1, 3 and 24 hours incubation at 25° C (left to right).
1 = irradiation, 2 = sacrifice.

mg% at the sacrifice. Individual increase of cholesterol levels varied from 541 mg% to 973 mg% during the period of the experiment (table IX, fig. 3.6) Serum lipoprotein spectra turned from normal at the start to heavily dominating beta bands and a complete loss of pre-beta and alpha lipoproteins.

Body weight increase: + 8.2 %

The changes in the irradiated arteries of the phenylbutazon group were classified as a severe degree of stage 4.

VK 744 (group VI)

VK 744 is an experimental pyrimido-pyrimidin derivative supplied by Thomae, GmbH, Western Germany. The drug has an effect on platelet adhesiveness and aggregability[20].

Gross appearance - Examination of the irradiated arteries during the operation revealed normal smooth vessels, with a dull surface. The vessels

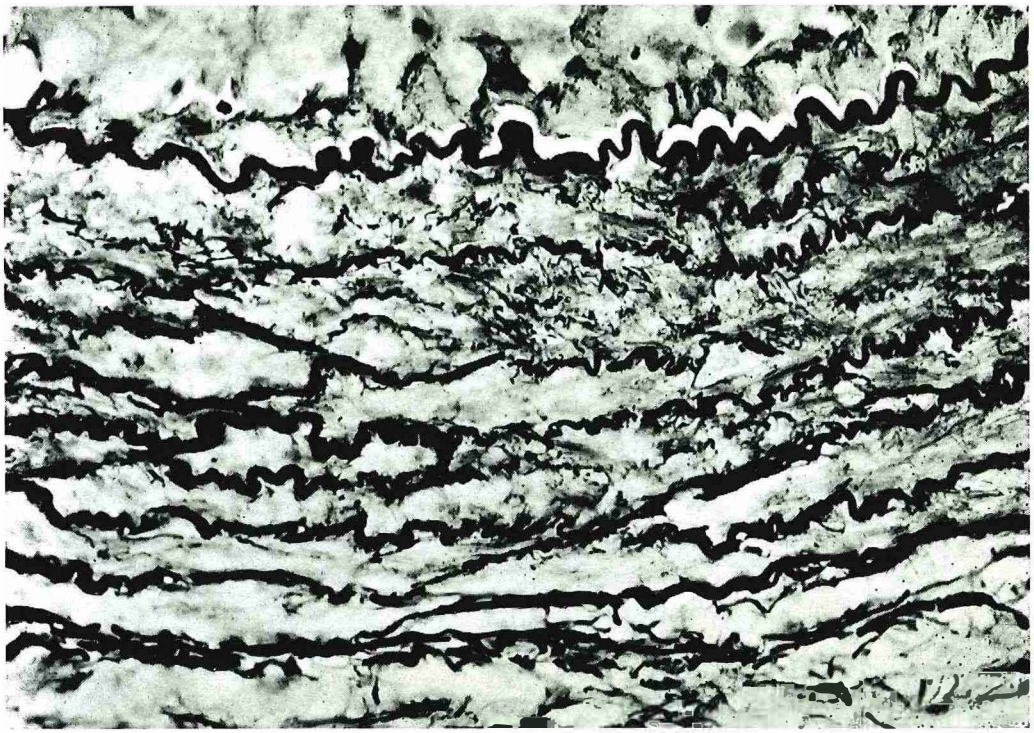

Fig. 3.19 VK 744; irradiated carotid artery (rabbit 2581)
Verhoeff stained; 412.5 X
Destruction of the elastic fibres of the media with local fragmentation and atrophy. The tunica elastica interna remained normal.

were less elastic in comparison with the non-irradiated control arteries. There were good pulsations, and only a few adhesions. No external signs of hyperlipaemia were seen in the rabbits.

Histology (table X) - The irradiated vessel walls showed a distinct thickening of the media with widened intercellular spaces and a considerable increase in PAS positive material.

The elastic fibres of the media had been degenerated. (fig. 3.19) The tunica elastica interna of one of the carotid arteries (rabbit nr. 2579) showed a definite though early degeneration. The smooth muscle fibres were ragged with disturbances of the circular arrangement and vacuolization with nuclear polymorphism. Lipid had infiltrated and been deposited in the intima and the media, with apparent plaque formation. Both foam cells and mononuclear infiltrates were present in the intima and the media.

The non-irradiated control arteries were completely normal.

Blood coagulation studies - No abnormalities or changes were observed in the fibrin formation tests or the fibrinogen levels. ADP-induced platelet aggregation showed no distinct abnormalities. Clot retraction and platelet count remained normal. (table A-E, fig. 3.12, 3.18)

Lipid - Serum cholesterol levels increased with a wide range of individual values. The mean starting value of 189 mg% rose to 511 mg% at the irradiation and 714 mg% at the sacrifice, with individual increases varying between 294 mg% and 1004 mg%. (table X, fig. 3.6) Serum lipoprotein electrophoretic patterns changed from normal into spectra with heavily dominating beta bands. Pre-beta and alpha lipoproteins had disappeared completely.

Body weight increase: + 25.9 %

The changes in the irradiated arteries of the VK 744 group were classified as a moderate to severe degree of stage 4.

RA 233 (group XI)

RA 233 is another experimental pyrimido-pyrimidin derivative, supplied by Thomae GmbH, Western Germany. In vitro the drug is a powerful inhibitor of platelet function, aggregation as well as glass bead adhesion and clot retraction[8, 10 12].

Gross appearance - During the operation the irradiated arteries looked normal, with only a few adhesions and smooth dull surfaces.

The vessels were less elastic in comparison with the glossy, elastic-pulsating, non-irradiated control arteries.

No external signs of hyperlipaemia were seen.

Histology (table XI) - Histological examination showed a marked degree of vessel wall damage in the irradiated arteries. The walls had thickened, caused by widened intercellular spaces, filled with PAS positive material. The degenerated smooth muscle fibres showed vacuoles and nuclear polymorphism. The circular arrangement of the bundles had been disturbed, the fibres were ragged and irregular. Elastic fibres in the media had disintegrated, but the tunica elastica interna had not been damaged.

Lipid had infiltrated and been deposited predominantly in the intima and the subintimal layers of the media, with a fine granular lining of fatty material along the elastic fibre remnants. (fig. 3.20) Foam cells and mononuclear cells were mainly present in the intima. (fig. 3.21)

The non-irradiated arteries showed no abnormalities.

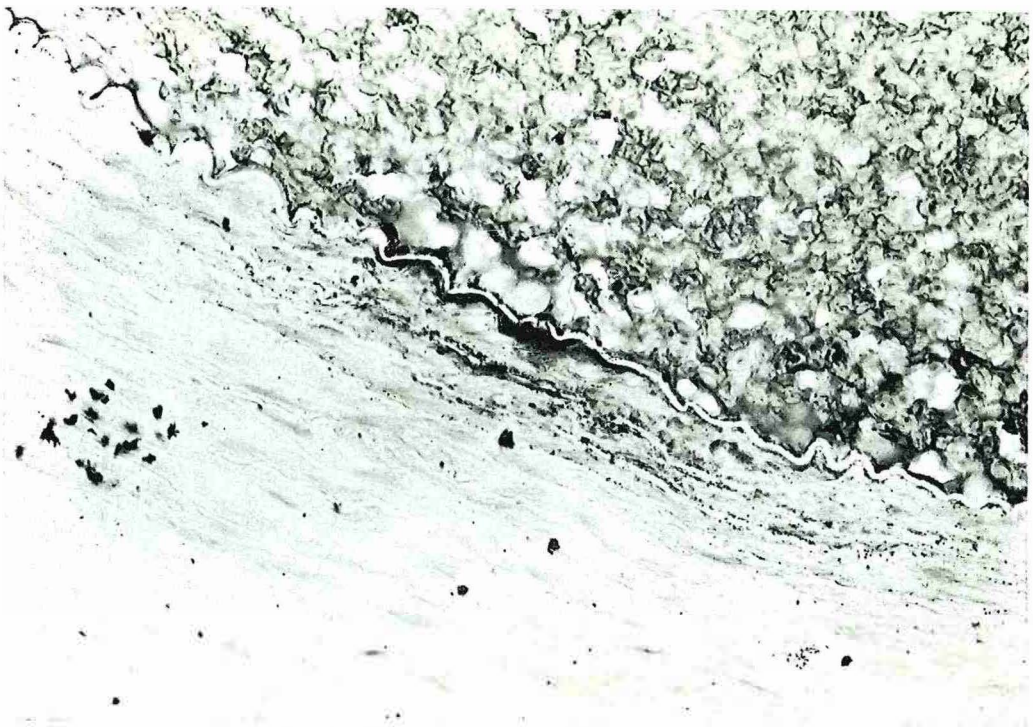

Fig. 3.20 RA 233; irradiated carotid artery (rabbit 2701)
oil red O stained; 412.5 X
Lipid deposition in the intima and the subintimal layer of the media.
There is a fine granular lining of fatty material along the degenerated elastic fibres of the media.

Blood coagulation studies - At the end of the experiment a lengthening of the kaolin-activated partial thromboplastin time was measured. The other tests remained normal. Fibrinogen levels showed an initial increase within the normal variation, and returned to the starting values at the end of the experiment. (table A-D)

ADP-induced platelet aggregation was markedly influenced by the drug: the initial slope was normal and steep, the maximum optical density loss decreased within the normal variation and a remarkable disaggregation was produced. (fig. 3.22) Clot retraction and platelet count had not been influenced. (fig. 3.9, table E)

Lipid - Serum cholesterol levels increased moderately, with very little individual variation. Mean value at the start: 85 mg%, at the irradiation: 305 mg% and at the sacrifice: 760 mg%. (table XI, fig. 3.6)

Serum lipoprotein spectra showed the same changes with heavy beta bands and a complete loss of pre-beta and alpha lipoproteins as observed in the VK 744 group.

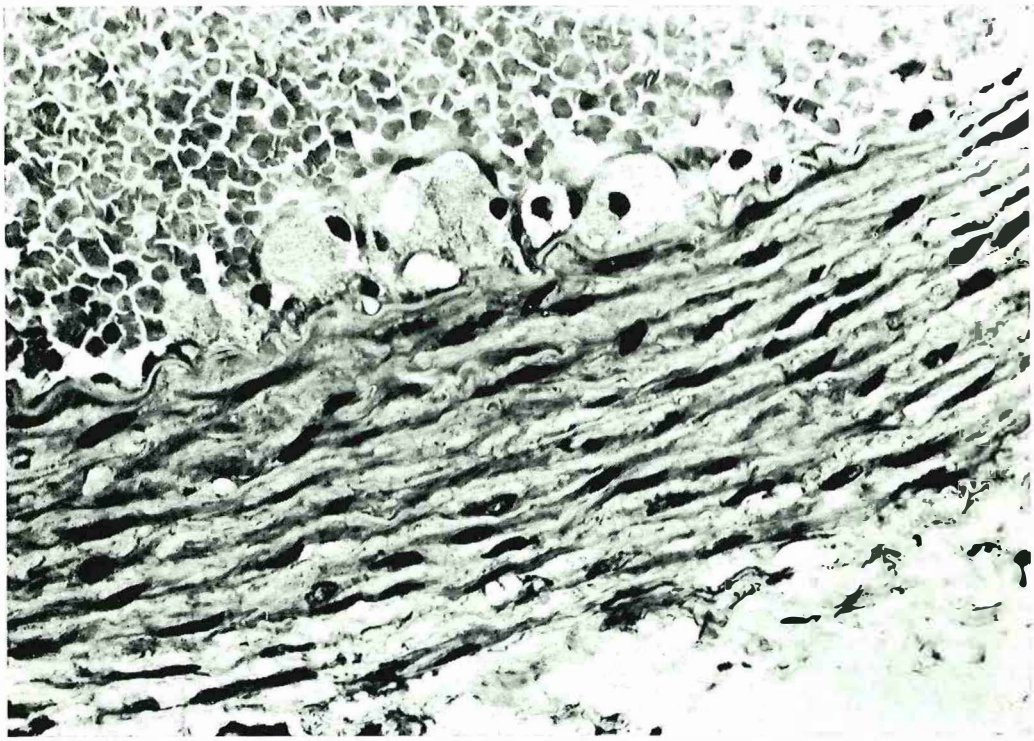

Fig. 3.21 RA 233; irradiated carotid artery (rabbit 2701)
haematoxylin-eosin stained; 412.5 X
Foam cells in the intima, mononuclear cells in the intima and the media.
Nuclear polymorphism and vacuolization of the smooth muscle cells.

Body weight increase: + 14.7 %

The changes in the irradiated arteries of the RA 233 group were classified as stage 4.

3.2.4 ANTI-FIBRINOLYTIC AGENT GROUP

Tranexamic acid or A.M.C.A. (group XII)

Gross appearance - The irradiated arteries revealed quite normal pulsating vessels with some adhesions and smooth dull surfaces. During the operation of rabbit nr. 2787, the wall of the irradiated artery ruptured traumatically.

The non-irradiated arteries were normal.

No external signs of hyperlipaemia were observed.

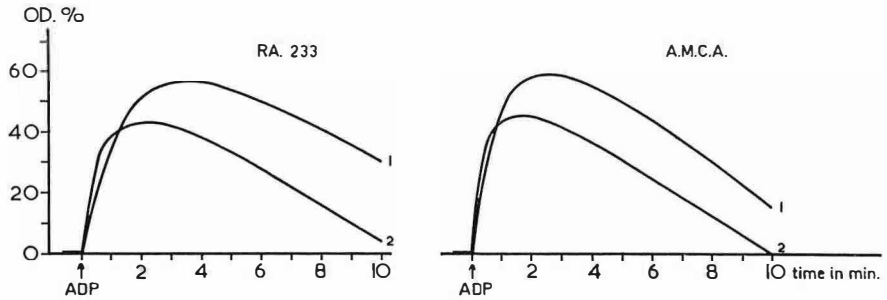

Fig. 3.22 ADP-induced aggregation curves.
Abscissa indicates time in minutes, ordinate indicates percentages optical density loss.
1 = irradiation, 2 = sacrifice.

Histology (table XII) - Microscopical examination of the sections of the irradiated arteries showed thickened walls because of widened intercellular spaces (fig. 3.23), filled with PAS positive material. The PAS positive material had been distributed predominantly in the subintimal layers of the media.

The medial elastic fibres had degenerated, showing local atrophy and fragmentation. (fig. 3.24)

The tunica elastica interna had a normal appearance in all the irradiated arteries.

Smooth muscle fibres were ragged, with disturbed circular arrangement, vacuoles, swelling and nuclear polymorphism.

Lipid had infiltrated into the intima of only one rabbit artery (2788), with foam cells only in the intima; the degenerated elastic fibres of this media were lined by a fine granular deposition of fatty material.

No mononuclear infiltrates were seen.

All the non-irradiated arteries were normal.

Blood coagulation studies - No abnormalities or changes were registered in the clotting tests, clot retraction or platelet count. (table A-E, fig. 3.9)

However, ADP-induced platelet aggregation showed the same surprising effect as was also observed in the RA 233 group: steep initial slope with some decrease of maximum optical density loss at the end of the experiment, and a complete disaggregation. (fig. 3.22)

Lipid - Serum cholesterol levels showed an overall moderate increase from a mean starting value of 87 mg% to 305 mg% at the irradiation and 760 mg% at the sacrifice. Individual cholesterol level increase varied considerably: from 360 mg% to 1132 mg% (rabbit nr. 2788). (table XII, fig. 3.6)

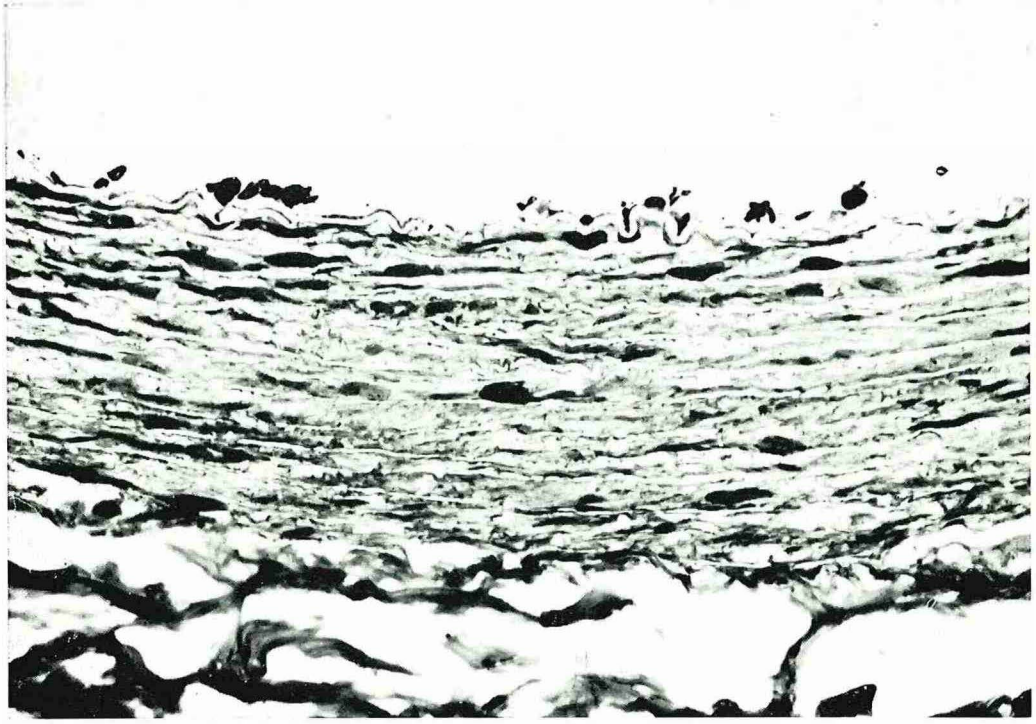

Fig. 3.23 Tranexamic acid or A.M.C.A.; irradiated carotid artery (rabbit 2789)
Azan stained; 412.5 X
Thickening of the wall due to widening of the ground substance spaces. No foam cells present.

Serum lipoprotein electrophoretic patterns changed from normal to spectra with moderate beta bands. Some pre-beta lipoprotein was still present and merged into the beta bands.

Body weight increase: + 5.7 %

The changes in the irradiated arteries of the A.M.C.A. group were classified as stage 2, besides the unexplained exception of rabbit 2788.

3.2.5 PERMEABILITY AFFECTING AGENT GROUPS

The effects of the different drugs used in this group are well known in microcirculation research and phlebology[4, 6, 15-17, 19, 24]. Little, however, is known about the effects on great vessels, especially on arteries of the elastic transport type.

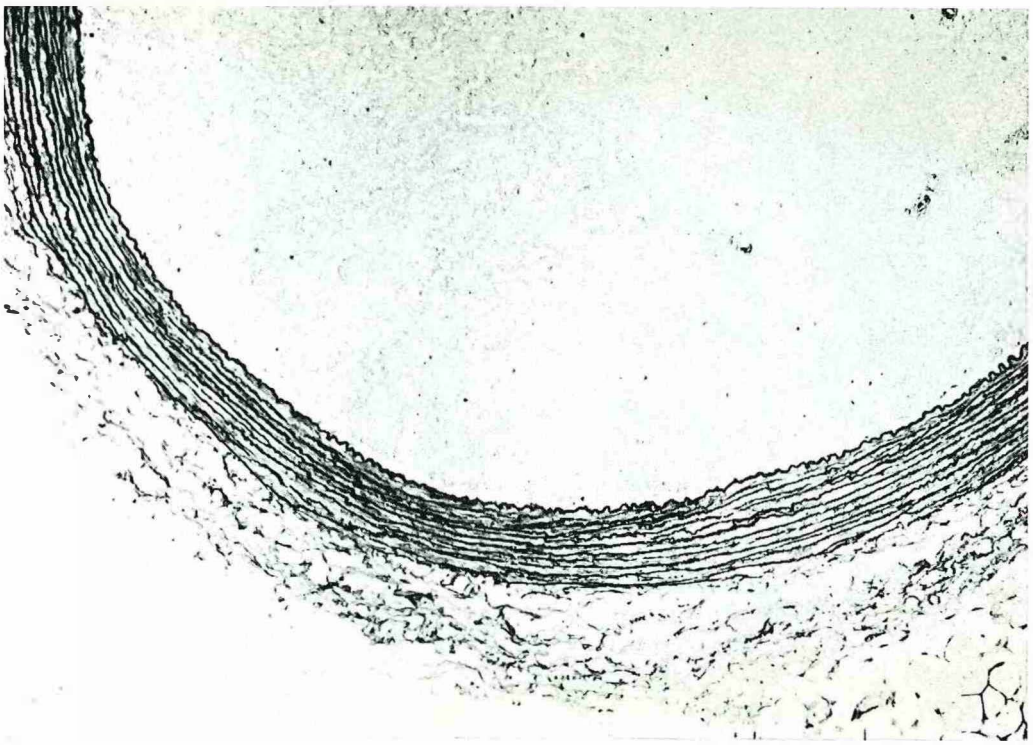

Fig. 3.24 Tranexamic acid or A.M.C.A.; irradiated carotid artery (rabbit 2787) Verhoeff stained; 105 X
Local degeneration of the elastic fibres of the media, with atrophy and fragmentation. Irregularly widened spaces between the degenerated elastic fibres. No foam cells or plaques.

Etamsylate (group VII)

Etamsylate is commonly known as Dicynone®, and was supplied by Delalande S.A., France.

Gross appearance - The irradiated arteries revealed cobbled rigid vessels, with mat surfaces and variably dense adhesions. Some yellow-white atheroma-like patches were present. The non-irradiated arteries were completely normal, showing elastic pulsations and glossy smooth surfaces. No other signs of hyperlipaemia were observed.

Histology (table XIII) - The irradiated arteries showed very heavily damaged vessel walls in almost all the rabbits. (fig. 3.25) There was a considerable widening of the intercellular spaces with accumulation of PAS positive material and thickening of the walls. The smooth muscle fibres were ragged and had degenerated, showing vacuolization and polymorphism of the nuclei.

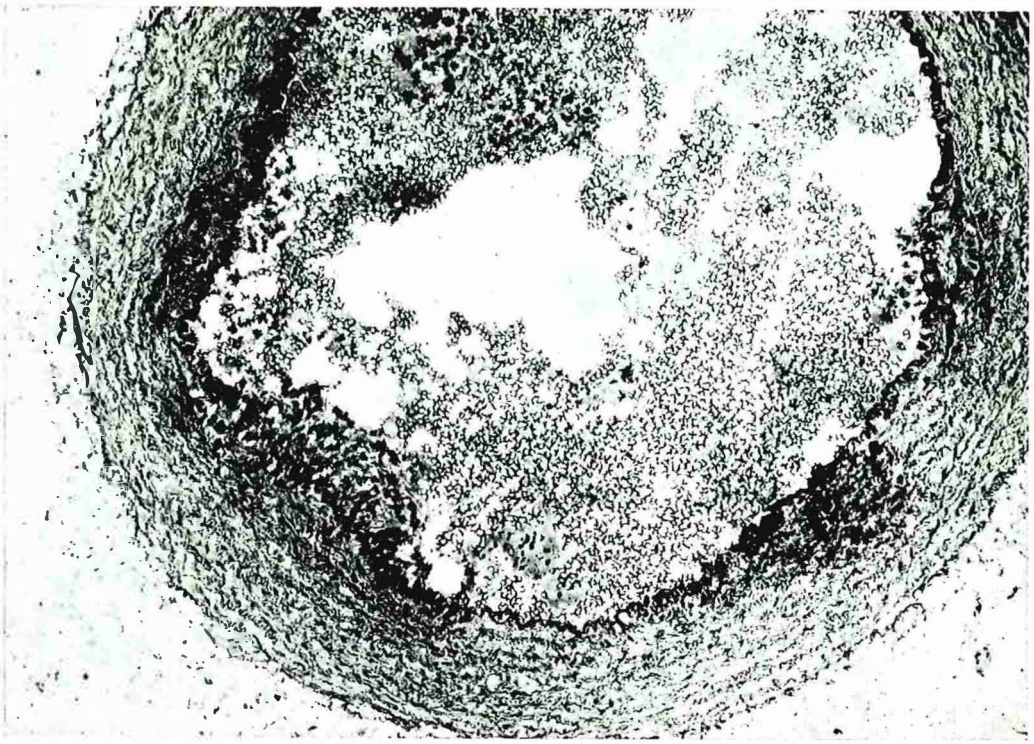

Fig. 3.25 Etamsylate; irradiated carotid artery (rabbit 2596)
Verhoeff prestained oil red 0; 105 X
Thickened vessel wall with extensive deposition of lipid throughout the wall and plaques protruding into the lumen.

The circular arrangement had been lost. The elastic fibres had almost completely been degenerated. Moreover there were initial degenerative changes of the tunica elastica interna. Lipid had infiltrated and been deposited throughout the walls with foam cells present in the media as well as in the intima. Mononuclear infiltrates were seen in the intima and in the media. Only one rabbit (2598) showed less severe damage of the irradiated artery, with an unaffected tunica elastica interna and lipid deposition restricted to the intima.

The non-irradiated arteries showed no signs of damage.

Blood coagulation studies - No abnormalities or changes were measured in the clotting tests, fibrinogen levels, platelet function tests or platelet count. (table A-E, fig. 3.18, 3.26)

Lipid - Serum cholesterol levels increased from a mean starting value of 144 mg% to 517 mg% at the irradiation and 1103 mg% at the sacrifice.

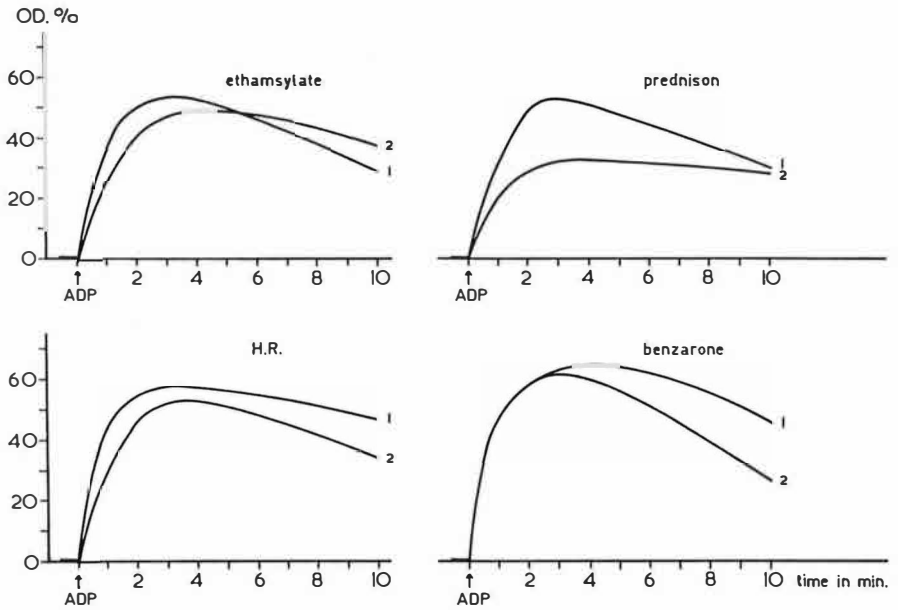

Fig. 3.26 ADP-induced platelet aggregation curves.
Abscissa indicates time in minutes, ordinate indicates percentages optical density loss.
1 = irradiation, 2 = sacrifice.

The individual increase varied between 765 mg% and 1327 mg%. (table XIII, fig. 3.6)

Serum lipoprotein spectra showed heavily dominating beta bands with complete loss of pre-beta and alpha lipoproteins at the end of the experiment.

Body weight increase: + 19.9 %

The changes in the irradiated arteries of the etamsylate group were classified as a severe degree of stage 4.

Prednisone (group VIII)

Gross appearance - The irradiated arteries were quite normal and smooth with few adhesions. The pulsations were less elastic in comparison with the normal non-irradiated control vessels.

During the operation of rabbit nr. 2533 the wall of the irradiated carotid artery ruptured traumatically.

No external signs of hyperlipaemia were seen.

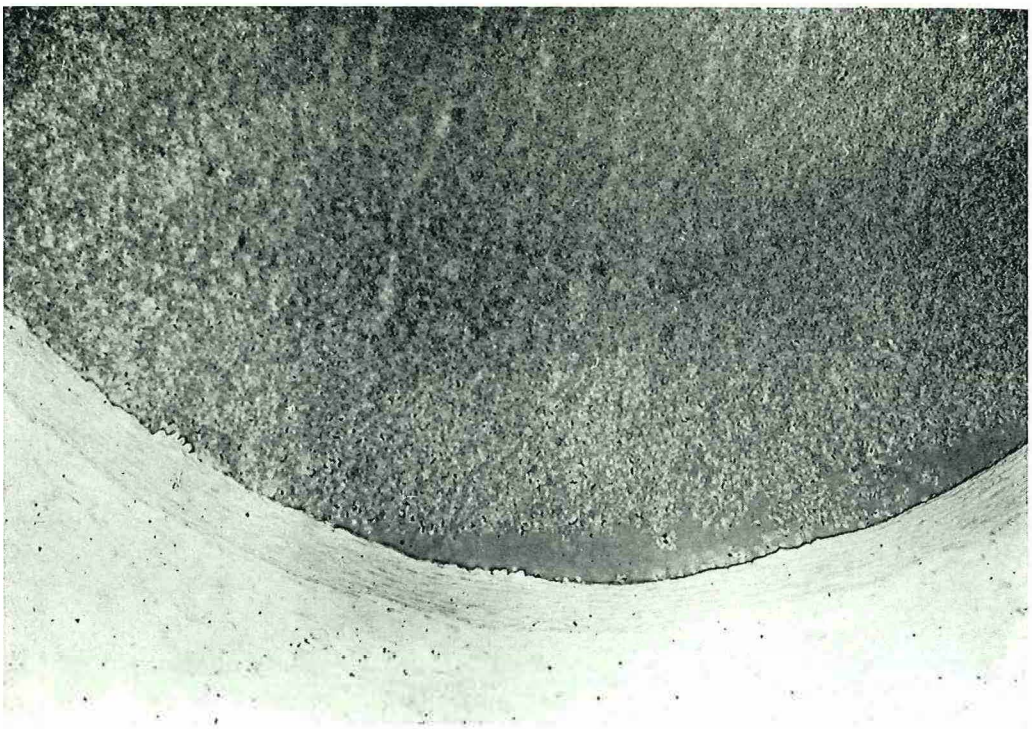

Fig. 3.27 Prednisone; irradiated carotid artery (rabbit 2534)
oil red O stained; 105 X
Characteristic intimal lining of lipid; no deposition of lipid in the media.

Histology (table XIV) - The irradiated vessel walls had thickened because of widened intercellular spaces, in which PAS positive material had accumulated. The smooth muscle fibres, especially of the subintimal part of the media, had degenerated. The bundles were ragged with disturbances of the normal circular arrangement. Vacuoles and nuclear polymorphism were present.

The elastic fibres had disintegrated and atrophied. The tunica elastica interna had not been damaged.

Lipid infiltration and deposition had been restricted to the intima.

A characteristic intimal lining of fatty material was observed in this group. (fig. 3.27) Foam cells and mononuclears were absent.

The non-irradiated arteries were completely normal.

Blood coagulation studies - An increase in fibrinogen levels was measured after the first week of the experiment, returning to the starting value during the rest of the period. (table D)

Clotting tests, clot retraction and platelet count remained normal. (table A-E, fig. 3.18)

ADP-induced platelet aggregation showed a flattening of the initial slope, with a less optimal optical density loss and just a slight tendency to disaggregate. (fig. 3.26)

Lipid - The sera of all the rabbits had a milky appearance at the end of the experiment. Serum cholesterol levels, however, showed a moderate increase from a mean starting value of 139 mg% to 480 mg% at the irradiation and 557 mg% at the sacrifice.

Individual cholesterol changes during the experiment varied from a decrease of 24 mg% (2589) to an increase of 914 mg%. (table XIV, fig. 3.6)

Serum lipoprotein electrophoretic patterns showed characteristic changes, with light to moderate beta bands and merging of the pre-beta and beta bands. The alpha lipoproteins were no longer present.

Body weight increase: + 2.9 %

The changes in the irradiated arteries of the prednisone group were classified as stage 3.

O-(β-hydroxyaethyl)-rutoside or H. R. (group IX)

H.R. is commonly known as Venoruton® and was supplied by Zyma S.A., Switzerland. One rabbit died after the irradiation procedure due to Nembutal® overdosage.

Gross appearance - The irradiated vessels were rigid, with cobbled dull surfaces and dense adhesions to the surrounding tissues. The non-irradiated vessels were normal, with smooth and glossy surfaces and elastic pulsations.

No external signs of hyperlipaemia were observed.

Histology (table XV) - Histological examination of the irradiated arteries revealed heavily damaged, thickened vessel walls. The intercellular spaces had widened and had been filled with PAS positive material and deposited lipid. (fig. 3.28, 3.29) The elastic fibres had degenerated with fragmentation and atrophy, although the tunica elastica interna remained intact. Smooth muscle fibres had almost completely been degenerated in the inner part of the media, and had severely degenerated in the adventitial part of the media, with vacuoles and nuclear polymorphism.

Lipid had infiltrated and been deposited through the whole wall, with foam cells deeply penetrating into the media. (fig. 3.30) Many mononuclear cells were seen and plaques lined the intima.

In only one rabbit (2636) did the vessel wall show less severe damage: lipid had only been deposited in the intima and no foam cells were observed.

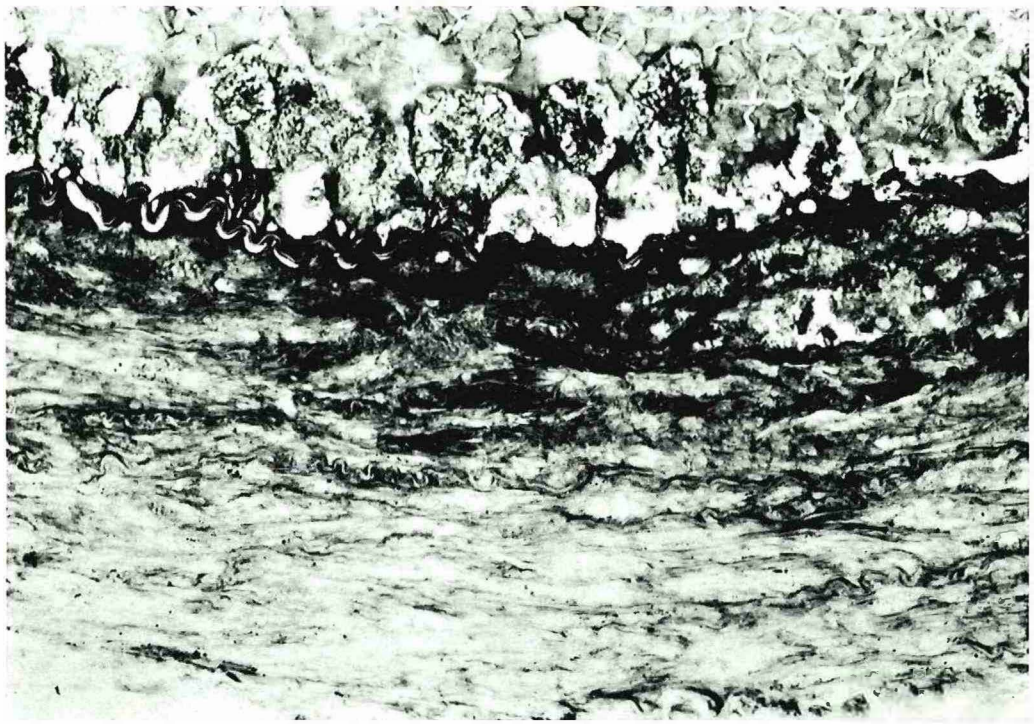

Fig. 3.28 O-(β-hydroxyaethyl)-rutoside or H.R.; irradiated carotid artery (rabbit 2628) PAS stained; 412.5 X
Thickened vessel wall with widened intercellular spaces, filled with positively staining material. Foam cells are present in the media and the intima, with a plaque protruding into the lumen.

The non-irradiated arteries showed normal vessel wall structures, without any sign of degeneration or lipid infiltration.

Blood coagulation studies - All kaolin-activated partial thromboplastin times had been lengthened, though stayed within the normal range. The other clotting tests, fibrinogen levels, clot retraction and platelet count remained normal. (table A-E, fig. 3.18)

ADP-induced platelet aggregation eventually showed slightly more flattening of the initial slope with an increase in disaggregation, but changes remained within the normal variation. (fig. 3.26)

Lipid - Serum cholesterol levels showed a striking variation in the increase during the experimental period.

The mean starting value of 104 mg% rose to 323 mg% at the irradiation and 731 mg% at the sacrifice, with an individual increase varying between 175 mg% and 1052 mg%. (table XV, fig. 3.6)

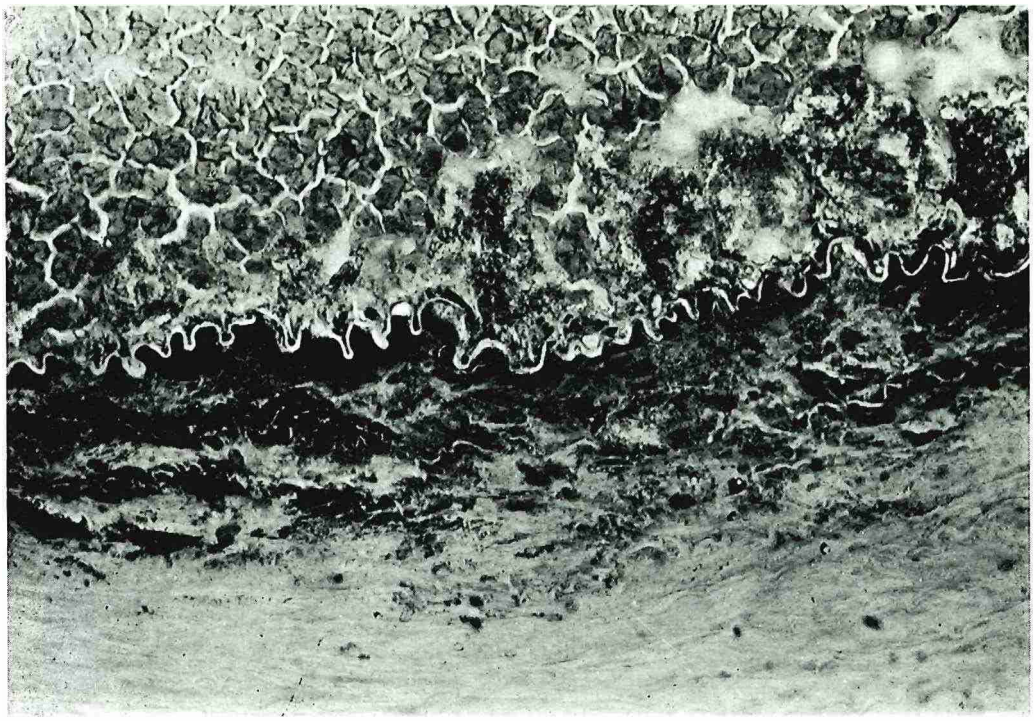

Fig. 3.29 0-(β-hydroxyaethyl)-rutoside or H.R.; irradiated carotid artery (rabbit 2628) oil red 0 stained; 412.5 X
Lipid deposition in the media and the intima. Note the concentration of lipid along the elastic fibre remnants.

Serum lipoprotein electrophoretic patterns showed spectra with heavy beta bands and complete loss of pre-beta and alpha lipoproteins.

Body weight increase: + 18.2 %

The changes in the irradiated arteries of the H.R. group were classified as a severe degree of stage 4.

Benzarone (group X)

Benzarone was supplied by Labaz S.A., Belgium as Fragivix®.

The drug has an effect on microvascular permeability[6]. Animal experiments suggest a plasminogen activating effect[3,6].

Gross appearance - The irradiated arteries showed dense adhesions, a rather smooth and mat adventitia, and rigid pulsating walls.

The non-irradiated control arteries were normal.

No external signs of hyperlipaemia were observed.

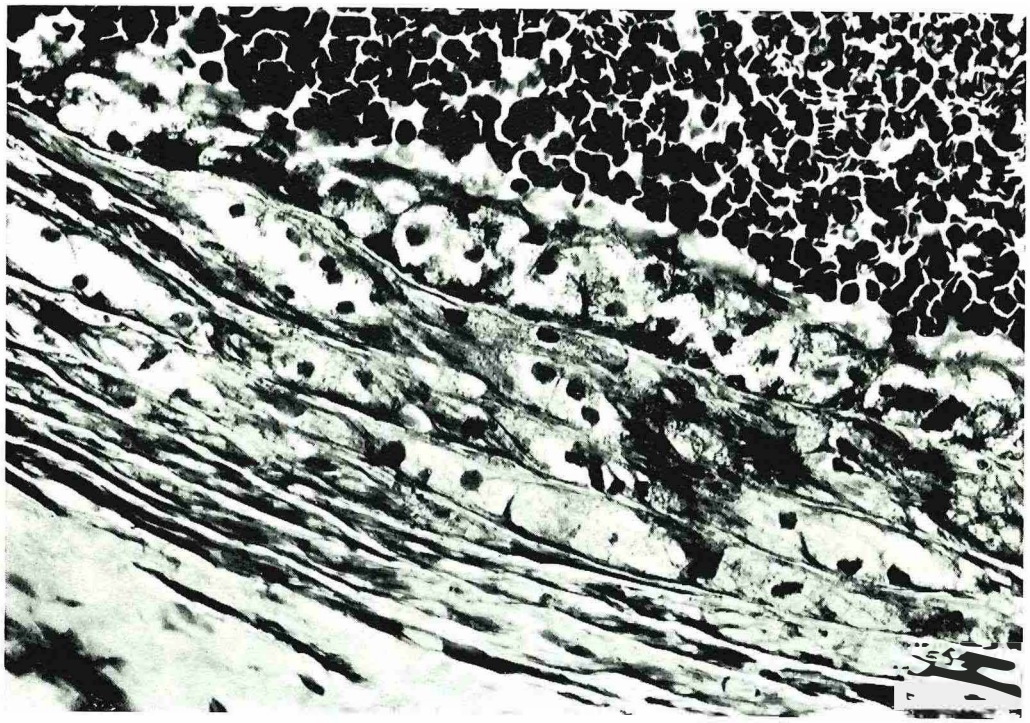

Fig. 3.30 O-(β-hydroxyaethyl)-rutoside or H.R.; irradiated carotid artery (rabbit 2629) Azan stained; 412.5 X
Heavily damaged vessel wall with foam cells deeply penetrated into the media. Mononuclear cells are present. A plaque lines the intima.

Histology (table XVI) - The irradiated vessel walls had thickened because of widening of the intercellular spaces of the media. PAS positive material had predominantly accumulated in the subintimal layers of the media. Subintimal elastic fibres had been damaged with atrophy and fragmentation.

Smooth muscle fibres had a ragged appearance with clear nuclear polymorphism and vacuolization. The circular arrangement had been lost. Lipid had infiltrated and been deposited in streaks, mostly in the inner part of the media. Fine granular fatty material lined the degenerated elastic fibre remnants, predominantly of the middle part of the media. Foam cells were present in the intima, while mononuclear cells had also infiltrated into the media.

The non-irradiated control arteries showed no abnormalities.

Blood coagulation studies - All the clotting tests remained normal. Fibrinogen levels tended to rise during the first week of the experiment, but

returned to the starting values during the rest of the period. Clot retraction and platelet count did not change. (table A-E, fig. 3.18)

ADP-induced platelet aggregation showed a definite disaggregation at the end of the experimental period, with a steep initial slope and an optimal optical density loss. (fig. 3.26)

Lipid - Serum cholesterol levels increased moderately from a mean starting value of 72 mg% to 293 mg% at the irradiation and 572 mg% at the sacrifice. Individual increase varied between 241 mg% and 701 mg%. (table XVI, fig. 3.6)

Serum lipoprotein spectra showed heavily dominating beta bands with complete loss of pre-beta and alpha lipoproteins during the experimental period.

Body weight increase: + 20.1 %

The changes in the irradiated arteries of the benzarone group were classified as stage 4.

3.3 EVALUATION OF THE SUPPLEMENTARY RADIOPATHOLOGICAL EXPERIMENTS

Very little is known about radioprotective effects of the various drugs used in the experiment[2, 4, 7, 9, 11, 13-17, 20-23, 25, 27].

Especially the radiochemical or biochemical-physiological effects of these drugs in the prevention of degenerative vessel wall processes following irradiation are still obscure or have not been investigated[2].

In an attempt to unravel the effects observed in the model in a more radiopathological way two supplementary experiments were carried out:

1. the protective effect of the drugs on X-ray induced depolymerization of mucopolysaccharides was examined in the viscosity model of Brinkman[5];

2. the protective effect of the drugs on post-irradiation lethality of mice was studied.
   Only those drugs, having a favourable protective effect in the rabbit model were investigated.

3.3.1 VISCOSITY EXPERIMENTS

Mucopolysaccharides are highly sensitive to X-rays, which cause an immediate depolymerization and a consequent drop in relative viscosity[1, 2, 5, 15, 18].

Drug solutions were mixed with the bovine synovia in concentrations

of mg/gramme synovia equivalent to the concentrations used in the rabbit experiments. As a control, saline 0.9% was used. Sodium thiosulphate, which is a definitely effective radioprotector[1,2,5], was used for comparison.

The viscosity of each sample was measured five times in an Oswald viscometer, before and five minutes after irradiation. The difference in mean recorded time is expressed as a percentage relative viscosity and relative viscosity drop, and compared to the control values. (table XVII)

For statistical analysis the Student t-test was used.

Heparin showed a slightly significant favourable effect, but prednisolone was even more active.

Tranexamic acid had a slightly significant unfavourable effect. In order of increasing activity acetylsalicylic acid, benzarone, salicylic acid, indomethacin and RA 233 had a highly significant unfavourable effect.

VK 744 showed a definite unfavourable effect in the viscosity model.

Warfarin sodium, phenylbutazon, etamsylate and H.R. did not differ statistically from the saline control.

3.3.2 LETHALITY TEST

The effect of heparin, acetylsalicylic acid, prednisone and tranexamic acid on post-irradiation lethality rates of C_{57} Black male mice was examined in comparison with the saline control.
No differences were observed in the groups. All the mice died between the 10th and 20th day after lethal irradiation.

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it is not madness
That I have utter'd: bring me to the test,
And I the matter will re-word, which madness
Would gambol from.

William Shakespeare

'Hamlet' act III.

CHAPTER 4

DISCUSSION AND CONCLUSION

4.1 INTRODUCTION

Ionizing radiation produces immediate chemical alterations in irradiated tissues[3, 4, 13, 37]. These initial chemical changes result in metabolic derangements which in the course of days or weeks may lead to manifest cellular damage and eventually to functional disintegration of the tissues or organs[37]. In the elastic arteries, the mucopolysaccharide ground substance and elastic fibres play a major part in the normal functional state[10], and are considered to be the primary target structures in irradiation[5, 20, 25, 27, 37].

Mucopolysaccharides are highly sensitive to irradiation[9]. An immediate depolymerization of complex polymers[1, 9, 23] as well as special components like hyaluronic acid[11, 26] has been described.

Collagen and elastin, the main structural proteins of the extracellular spaces of the elastic vessel wall[30], aid in forming a structural continuum of the arterial wall, binding groups of smooth muscle cells together[30].

Of these fibrous proteins particularly elastin seems to be susceptible to irradiation, which causes disintegration of the complex molecular structures[5, 20, 32].

Of the cellular elements the endothelial cells are moderately sensitive to irradiation due to their relatively slow and irregular mitotic activity[37, 39] and moderate degree of differentiation[37].

Smooth muscle cells are largely long-living, fixed, post-mitotic cells and are highly resistant to the direct destructive action of radiation[37].

The immediate damaging effects of X-rays on all these major vascular constituents result in an increase in permeability of the vessel wall and in local inflammatory reactions[5, 8, 27, 37].

The inflammatory reaction is thought to be mediated by released endogenous 'mediators' like histamine, serotonin, plasmin and proteolytic

products like bradykinin, kallikrein and other kinins[34], and prostaglandins[33].

The effect of these mediators on the contraction of endothelial cells with a subsequent opening of the intercellular junctions[16], may be the direct cause of the 'gaps' or 'pores' which occur at these intercellular junction sites[31]. Infiltration and accumulation of fluids, proteins and other colloids like lipids and lipoproteins in the tissues of the vessel wall will then occur[16, 31, 34].

The infiltration and deposition of lipid as observed in the model, may be explained by an increase in vessel wall permeability induced by X-rays. This phenomenon is a late result of irradiation damage, effecting irreversible changes in structure and function of the arteries[5, 24, 37]. The accumulation and deposition of lipid as unanimously reported in the literature[15] seem to be dependent on at least slightly elevated serum cholesterol levels. Even intermittent elevations of serum lipid levels may promote atherogenesis in injured arterial walls[15].

From the data reported in chapter 3 (fig. 3.6, table I to XVI) it can be seen that the mean and individual serum cholesterol levels distinctly increased in all the experimental groups treated with drugs. As the damaging agent, the X-ray irradiation, had been standardized, the response of the rabbit arteries had to be dependent on the effect of the administered drug, whether protective, neutral or adverse.

4.2 EVALUATION OF THE HISTOLOGICAL DATA

Apparent differences were seen in the histological picture of the various groups in comparison with the hypercholesterolaemic control. The irradiated carotid arteries of this control group showed considerable thickening of the vessel wall with fragmentation of the elastic fibres of the media and accumulation of PAS positive material. Lipid had infiltrated and been deposited in the media as well as in the intima. Foam cells and an inflammatory reaction were present. Plaques lined the intima. (table I)

The irradiated carotid arteries of the rabbits treated with heparin and acetylsalicylic acid showed no signs of infiltration or deposition of lipid, not even in the intima, although the arterial walls definitely revealed the damaging effect of irradiation on the mucopolysaccharide ground substance and the elastic fibres, with secondary changes of smooth muscle bundles. No inflammatory cells were seen. (table V, VII)

The irradiated carotid arteries of the rabbits treated with tranexamic acid showed even less clear cut signs of destruction of the connective tissue constituents of the vessel wall in four of the six irradiated arteries, with only minor accumulations of PAS positive material. In one of the irradiated arteries, however, there was a definite infiltration and deposition of lipid in the media with foam cells in the intima. Fine granulae

and droplets of lipid lined the degenerated elastic fibres of this media. No inflammatory cells were seen in this group. (table XII)

The irradiated carotid arteries of the rabbits treated with prednisone gave a limited infiltration and deposition of lipid with a characteristic lining of fatty material deposited in the intima. PAS positive material was present in all the vessel walls as a substrate of depolymerized mucopolysaccharide ground substance. No lipid diffused into the media. Inflammatory cells were not seen; prednisone has been shown to have an inhibiting effect on exudation with leucodiapedesis[17, 23]. (table XIV)

The histological data of the groups treated with heparin, acetylsalicylic acid, tranexamic acid and to a lesser extent prednisone revealed a definitely favourable effect on post-irradiation changes in vessel wall permeability and on post-irradiation inflammatory reaction.

The irradiated carotid arteries of the rabbits treated with indomethacin, phenylbutazon, VK 744, etamsylate and H.R. showed even more severe vessel wall damage after the irradiation in comparison with the hypercholesterolaemic control. There was severe destruction of the media with deep infiltration and extensive deposition of lipid throughout the walls. A striking foam cell and inflammatory reaction had occurred in these groups, in spite of the potent antiphlogistic effects of indomethacin and phenylbutazon[17], and the reported protective effect of etamsylate[28] and H.R.[6, 23] on vessel wall permeability. (table VIII-X, XIII, XV)

The irradiated carotid arteries of the rabbits treated with warfarin sodium, RA 233 and benzarone did not differ from the hypercholesterolaemic control. (table VI, XI, XVI)

The non-hypercholesterolaemic control group showed radiation damage of the media without infiltration and deposition of lipid (table II)

Only minor signs of degeneration were observed in the cholesterol-fed group, protected by sodium thiosulphate during the irradiation, (table III) while the clofibrate group did not differ from the hypercholesterolaemic control. (table IV)

4.3 EVALUATION OF THE BLOOD COAGULATION DATA

Fibrin formation was predictably influenced by heparin (thrombin time control) and warfarin sodium (thrombotest control).

Of the other drugs which were used, only RA 233 induced a lengthening of the kaolin-activated partial thromboplastin time at the end of the experiment. In comparison with the other groups tranexamic acid increased the kaolin-activated partial thromboplastin times slightly, although the values remained within the normal limits. (table A)

The final fibrinogen levels of the phenylbutazon group showed a surprising and unexplained elevation. (table D)

One-stage prothrombin times, thrombin times and platelet count were not influenced. (table B, C, E)

Some effect on ADP-induced platelet aggregation was observed in the rabbits treated with warfarin sodium, prednisone, phenylbutazon and benzarone (fig. 3.12, 3,26):

— flattening of the initial slope (adhesion phenomenon): warfarin sodium and phenylbutazon.

— decrease in maximum optical density loss (adhesion and contraction phenomenon): warfarin sodium, phenylbutazon and prednisone.

— decrease in disaggregation (contraction and release phenomenon): warfarin and prednisone.

A distinct effect on ADP-induced platelet aggregation was observed in the rabbits treated with acetylsalicylic acid, RA 233 and tranexamic acid (fig. 3.4, 3.22):

— acetylsalicylic acid: flattening of the initial slope and a decrease in maximum optical density loss, reflecting a decreased adhesiveness.

— RA 233 and tranexamic acid: steep initial slopes with some decrease in maximum optical density loss and a definite and complete disaggregation (contraction and release phenomenon).

Platelet rich plasma-clot retractions were not influenced. (fig. 3.5, 3.9, 3.18)

4.4 EVALUATION OF THE LIPID DATA

In all the groups treated with drugs, serum cholesterol levels showed a definite increase, with marked individual variations. Some groups showed less increase in serum cholesterol levels: heparin, acetylsalicylic acid, prednisone, benzarone and RA 233. The other groups did not differ from the hypercholesterolaemic control. (fig. 3.6)

A striking feature is a lack of correlation between the absolute cholesterol levels, the increase and the variation of the levels of the individual rabbits, and the degree of histological damage, especially of the infiltration and deposition of lipid, as can be seen in the tables I-XVI. From the data of the non-hypercholesterolaemic control group further evidence can be obtained for the statement that at least some elevation of serum lipid levels must be present to produce some degree of lipid deposition in a damaged vessel wall[15].

Serum fat spectra showed characteristic changes: heavy beta bands with complete loss of pre-beta and alpha lipoproteins, resembling the Fred-

rickson type II human hyperlipaemia electrophoretic pattern.

The rabbits treated with heparin, prednisone and tranexamic acid, however, showed different electrophoretic patterns: light to moderate beta bands with some pre-beta lipoproteins left, mostly merging into the beta bands.

4.6 EVALUATION OF THE BODY WEIGHT DATA

The rabbits in the heparin treated group showed a definite decrease in body weight, probably induced by the post-heparin lipolytic activity of lipoprotein lipase or 'clearing factor'[17].

The animals treated with acetylsalicylic acid and prednisone showed no differences in body weight during the experimental period; acetylsalicylic acid is said to have a stimulating effect on thyroid function[29] and cell metabolism[17,38], prednisone has a well-known catabolic effect[17]. Some increase in body weight was observed in the groups treated with tranexamic acid and phenylbutazon. All the other groups showed definite increases in body weight, which did not parallel the increases in their serum cholesterol levels in comparison with the hypercholesterolaemic control. (table XVIII, fig. 3.6)

4.7 GENERAL CONCLUSION

The late effects of X-ray irradiation on changes in vessel wall permeability of the carotid artery in cholesterol-fed rabbits treated with drugs were evaluated. Whether these effects reflect a radioprotective or radiosensitizing action of the drugs administered, remains uncertain. The data of the supplementary experiments on mucopolysaccharide depolymerization suggest that prednisone provides a slight protective effect, whereas in the presence of acetylsalicylic acid and its metabolite salicylic acid (S.A.), benzarone, indomethacin, RA 233 and VK 744 the depolymerizing effect of the X-rays is increased.

Warfarin sodium, H.R., phenylbutazon, etamsylate, tranexamic acid and heparin did not have clearly unfavourable or favourable effects on X-ray induced mucopolysaccharide depolymerization.

Post-irradiation lethality rates of mice were not influenced by heparin, acetylsalicylic acid, prednisone or tranexamic acid in comparison with the saline control.

Therefore only prednisone seems to have some tendency to a radioprotective effect, although the ultimate histopathological changes in the irradiated arteries were not so favourable as they were in the groups treated with heparin, acetylsalicylic acid and tranexamic acid, inspite of

increases in cholesterol levels (58 mg% to 923 mg%) and in vitro decreased post-irradiation synovial viscosity.

Van Caneghem has shown an in vitro protective effect of heparin on permeability increase after irradiation of corium membranes of the rat[3].

Heparin efficiently blocks the fibrinogen conversion and prevents the formation of the prothrombin converting principle[19, 22].

Heparin has an inhibiting effect on in vitro thrombin induced platelet aggregation, because of its direct thrombin neutralising effect[19]. However, ADP-induced platelet aggregation is not influenced[19].

Heparin promotes the release of lipoprotein lipase or 'clearing factor', an effect which in usually called the post-heparin lipolytic activity or p.h.l.a.[17].

The interaction of these effects probably promoted the favourable result of heparin on the prevention of post-irradiation increase in vessel wall permeability.

Acetylsalicylic acid, everyman's medicine, is a substance with a variety of effects. Among others it has an inhibiting effect on platelet release induction[35], on platelet adhesiveness[7, 12], on prothrombin synthesis in the liver[17]. It has an effect on connective tissue metabolism and inflammation[17, 38], probably by reducing or blocking the synthesis of prostaglandins[14, 33, 36]. It has an effect on lipid metabolism, a.o. lowering serum cholesterol, phospholipid and free fatty acid levels, probably by an inhibition of acetyl co-enzyme A carboxylase[17, 38], and it has an effect on thyroid gland metabolism, increasing the T_3 and T_4 levels[17, 29, 38]. These activities may all play a part in the special effect of acetylsalicylic acid on the prevention of post-irradiation increase of permeability of the vessel wall in this model.

Tranexamic acid has a blocking effect on the plasminogen conversion[2], with a subsequent effect on the production of kallikrein, which is a well-known precursor of mediators of inflammation like bradykinin and other plasma kinins[21, 34]. These substances also have an effect on the contraction of the endothelial cell[16, 17, 31, 39]. In animal experiments tranexamic acid promotes the fibrinogen conversion[18]. Special effects on fat metabolism are not known. The inhibition of the kallikrein and bradykinin production may be responsible for the ultimate favourable effect of tranexamic acid on post-irradiation changes in vessel wall permeability[21].

Prednisone, like acetylsalicylic acid, is a substance with a variety of effects. It influences inflammatory processes[17], especially exudation with leucodiapedesis[23]. Kärcher has shown that prednisone restores the polymerized state of mucopolysaccharides after irradiation[23]. Prednisone has a catabolic action and an effect on the mobilization and deposition of lipid in the tissues[17], and may induce diabetes[17].

The restorative effect of prednisone on depolymerized mucopolysaccharides, in combination with its anti-inflammatory activities pro-

bably determined the moderately favourable result on the prevention of post-irradiation changes in vessel wall permeability.

It is clear that no uniform explanation can be given for the definitely favourable effects of heparin, acetylsalicylic acid, tranexamic acid and prednisone: reduction of post-irradiation increase in vessel wall permeability, as measured on the infiltration and deposition of lipid. (table XIX)

However, inhibitory effects on platelet adhesiveness (acetylsalicylic acid), and aggregability (tranexamic acid and prednisone), on fibrinogen conversion (heparin), on fibrin degradation and kinin production (tranexamic acid) and on vessel wall permeability (heparin, acetylsalicylic acid, tranexamic acid and prednisone) seem to play an additional part in the prevention of structural and functional vessel wall damage in experimental irradiation.

Although it is not yet permissible to extrapolate these experimental data to the clinical situation, the definitely unfavourable effects of indomethacin, phenylbutazon, etamsylate and H.R. on vessel wall damage after X-ray irradiation justify a more reserved and critical approach to the clinical application of these drugs in radiotherapy.

In the clinical situation X-ray irradiation as used in patients subjected to radiotherapy may induce or at least accelerate the process of atherosclerosis in the larger elastic arteries as well as the coronary arteries[5, 15, 32, 37]. These atherosclerotic lesions closely resemble those described in previous animal experiments[5, 15, 24, 27], and in this thesis.

Because it is still too early to extrapolate from these experimental data data to human pathology, further experiments in this field will be necessary for a more precise evaluation and understanding of atherosclerosis produced by irradiation and its relationship to other forms of atherosclerosis.

However, the definitely favourable effects of heparin, acetylsalicylic acid and tranexamic acid as obtained in this experimental model, suggest a potentially rewarding approach to at least some aspects of the pathogenesis and consequentially of the prevention of atherosclerosis.

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TABLES

C — hypercholesterolaemic control

I — heparin

II — acetylsalicylic acid or a.s.a.

III — indomethacin

IV — warfarin sodium

V — phenylbutazon

VI — VK 744

VII — etamsylate

VIII — prednisone

IX — 0-(β-hydroxyaethyl)-rutoside or h.r.

X — benzarone

XI — RA 233

XII — tranexamic acid or a.m.c.a.

XIII — clofibrate control

TABLE A

	C	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
0	26.5	29.6	26.1	31.9	26.0	24.7	25.3	22.0	27.1	27.5	26.0	31.6	38.6	33.7
1	—	—	28.0	35.6	—	29.1	30.2	22.5	21.1	31.7	27.6	25.9	37.9	31.2
2	25.7	—	29.9	30.6	—	27.6	29.8	24.0	28.4	38.6	35.6	46.2	36.4	29.8

kaolin-activated partial thromboplastin times in sec. 0 = start, 1 = irradiation, 2 = sacrifice
$\bar{x}$ = 28.7 SD = 5.8

TABLE B

	C	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
0	6.7	6.7	6.1	7.4	6.3	6.2	6.9	6.3	6.3	7.0	6.1	6.3	7.6	7.3
1	6.1	—	6.1	6.3	—	6.3	5.8	6.4	5.1	7.7	5.7	6.5	8.1	8.7
2	6.7	—	5.9	6.5	—	7.1	5.7	6.4	5.9	7.1	6.7	8.5	8.3	7.7

one-stage prothrombin times in sec. 0 = start, 1 = irradiation, 2 = sacrifice
$\bar{x}$ = 6.6 SD = 0.7

TABLE C

	C	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
0	8.3	9.0	7.4	7.0	6.8	8.0	9.7	7.6	8.5	10.1	8.7	8.2	8.8	8.0
1	8.3	—	7.6	7.9	7.6	7.6	8.1	8.0	7.9	8.7	7.7	7.0	8.6	9.2
2	9.0	—	6.6	7.9	8.0	9.8	8.3	8.4	8.1	8.6	8.3	8.7	9.1	8.7

thrombin times in sec. 0 = start, 1 = irradiation, 2 = sacrifice
$\bar{x}$ = 8.3 SD = 1.0

TABLE D

	C	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
0	314	369	281	262	356	268	269	308	259	318	223	295	299	313
1	246	—	439	332	382	326	260	383	403	292	423	453	251	300
2	233	—	278	410	240	1425	239	309	277	225	258	281	290	227

fibrinogen levels in mg/100 ml. 0 = start, 1 = irradiation, 2 = sacrifice
$\bar{x}$ = 298 SD = 84

TABLE E

	C	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
0	2425	—	—	2322	3332	2988	—	3011	1783	3932	5736	3088	3982	3166
1	3086	—	3799	3190	—	4128	3748	3652	4299	1987	3997	2671	2905	4716
2	2358	—	2577	3992	3339	4957	3098	2501	2186	2612	3399	3498	3295	1840

thrombocyte counts x 100 / mm^3. 0 = start, 1 = irradiation, 2 = sacrifice.
$\bar{x}$ = 3126 SD = 1082

TABLE F

	C	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
0	2641	2238	2382	2142	2672	2860	2165	2495	2693	2275	1650	2130	2612	2360
1	2855	2179	2282	2382	2670	3000	2308	2680	2910	2475	1815	2092	2619	2401
2	2945	1704	2293	2492	3170	3096	2723	2992	2773	2692	2218	2516	2751	2740

body weights in g. 0 = start, 1 = irradiation, 2 = sacrifice

TABLE 0 stages of vessel wall damage in relation to the histological criteria

	Azan	o.r.O	Verhoeff	h.e.	PAS	foam cells
stage 1	+	–	+ to +++	–	++ to +++	–
stage 2	++	–	+ to +++	+	++ to +++	–
stage 3	++	+ to ++++	+ to +++	+	++ to +++	–
stage 4	+++ to ++++	+ to ++++	+ to +++	++ to +++	++ to +++	+ to ++

TABLE I hypercholesterolaemic control group (C)

rabbit nr.	Azan	o.r.O	Verhoeff	h.e.	PAS	foam cells	0	1	2
2263	++++	+++	++	+++	+++	++	127	390	1003
2264	+++	+++	++	++	+++	+	160	283	997
2265	+++	+++	++	+++	+++	+	95	265	960
2267	++++	+++	++	+++	+++	++	83	210	1120
2268	++++	+++	++	+++	+++	++	146	688	1207
2270	+++	+++	++	+++	+++	+	107	530	1067
2271	++++	+++	++	+++	+++	++	98	463	998
2273	++++	+++	++	+++	+++	++	114	505	1046

Histological data of the irradiated carotid arteries in the various staining techniques, in comparison with the cholesterol levels in mg/100 ml.
0 = start, 1 = irradiation, 2 = sacrifice.

TABLE II non-hypercholesterolaemic control group

rabbit nr.	Azan	o.r.O	Verhoeff	h.e.	PAS	foam cells	1	2
2896	++	–	++	+	++	–	96	101
2901	++	–	+++	+	+++	–	80	76
2902	++	–	++	+	+++	–	63	84

Histological data of the irradiated carotid arteries in the various staining techniques, in comparison with the cholesterol levels in mg/100 ml.
1 = irradiation, 2 = sacrifice.

TABLE III sodium thiosulphate control group

rabbit nr.	Azan	o.r.O	Verhoeff	h.e.	PAS	foam cells	0	1	2
2904	++	–	+	+	+	–	73	257	988
2905	++	–	+	+	+	–	60	288	902
2906	++	–	+	+	+	–	59	252	846

Histological data of the irradiated carotid arteries in the various staining techniques, in comparison with the cholesterol levels in mg/100 ml.
0 = start, 1 = irradiation, 2 = sacrifice.

TABLE IV clofibrate control group (XIII)

rabbit nr.	Azan	o.r.O	Verhoeff	h.e.	PAS	foam cells	0	1	2
2869	++	++	++	+++	++	–	74	181	489
2870	++++	++++	+++	+++	+++	++	74	362	1418
2873	++	++	++	+++	+++	–	100	244	921
2874	++++	+++	+++	+++	+++	++	71	159	709
2876	+++	++	++	+++	+++	+	98	218	279

Histological data of the irradiated carotid arteries in the various staining techniques, in comparison with the cholesterol levels in mg/100 ml.
0 = start, 1 = irradiation, 2 = sacrifice.

TABLE V heparin group (I)

rabbit nr.	Azan	o.r.O	Verhoeff	h.e.	PAS	foam cells	0	1	2
2432	++	–	++	+	+++	–	92	302	150
2433	++	–	++	+	+++	–	88	202	500
2434	++	–	++	+	+++	–	114	402	290
2435	++	–	++	+	+++	–	68	128	349
2460	++	–	++	+	+++	–	—	270	240
2461	++	–	++	+	+++	–	68	293	645

Histological data of the irradiated carotid arteries in the various staining techniques, in comparison with the cholesterol levels in mg/100 ml.
0 = start, 1 = irradiation, 2 = sacrifice.

TABLE VI warfarin sodium group (IV)

rabbit nr.	Azan	o.r.O	Verhoeff	h.e.	PAS	foam cells	0	1	2
2431	+++	++	++	++	+++	++	—	371	1005
2458	++++	+++	++	+++	+++	++	—	355	1135
2459	++++	+++	++	+++	+++	++	—	304	1032
2527	+++	++	++	++	+++	+	—	430	1241
2529	++++	+++	++	+++	+++	++	—	370	1081

Histological data of the irradiated carotid arteries in the various staining techniques, in comparison with the cholesterol levels in mg/100 ml.
0 = start, 1 = irradiation, 2 = sacrifice.

TABLE VII acetylsalicylic acid (a.s.a.) group (II)

rabbit nr.	Azan	o.r.O	Verhoeff	h.e.	PAS	foam cells	0	1	2
2424	++	–	++	+	+++	–	83	270	510
2425	++	–	++	+	+++	–	94	139	680
2426	++	–	+	+	+++	–	94	309	902
2427	++	–	++	+	+++	–	85	235	899
2428	++	±	++	+	+++	–	56	163	246
2429	++	–	++	+	+++	–	73	187	516

Histological data of the irradiated carotid arteries in the various staining techniques, in comparison with the cholesterol levels in mg/100 ml.
0 = start, 1 = irradiation, 2 = sacrifice.

TABLE VIII indomethacin group (III)

rabbit nr.	Azan	o.r.O	Verhoeff	h.e.	PAS	foam cells	0	1	2
2519	++++	++++	++	+++	+++	++	46	260	1078
2520	++++	+++	++	++	+++	+	63	419	1254
2521	++++	++++	++	+++	+++	++	56	338	1110
2522	++++	++++	++	+++	+++	++	48	265	1089
2523	++	++	++	+	+++	–	76	387	1075
2524	++++	++++	++	+++	+++	++	59	343	954

Histological data of the irradiated carotid arteries in the various staining techniques, in comparison with the cholesterol levels in mg/100 ml.
0 = start, 1 = irradiation, 2 = sacrifice.

TABLE IX phenylbutazon group (V)

rabbit nr.	Azan	o.r.O	Verhoeff	h.e.	PAS	foam cells	0	1	2
2448	++++	++++	+++	+++	+++	++	141	751	908
2449	++++	+++	++	+++	+++	++	127	552	1100
2450	++	+	++	+++	+++	–	115	676	656
2451	++	+	++	+++	+++	–	70	632	632
2452	+++	+++	++	+++	+++	+	59	678	622
2453	++++	+++	+++	+++	+++	++	80	634	822

Histological data of the irradiated carotid arteries in the various staining techniques, in comparison with the cholesterol levels in mg/100 ml.
0 = start, 1 = irradiation, 2 = sacrifice.

TABLE X VK 744 group (VI)

rabbit nr.	Azan	o.r.O	Verhoeff	h.e.	PAS	foam cells	0	1	2
2579	++++	+++	+++	+++	+++	++	214	622	1218
2581	++++	+++	++	+++	+++	++	185	518	1136
2582	+++	+	++	++	+++	+	171	457	807
2583	+++	+++	++	+++	+++	+	230	523	524
2584	+++	+++	++	++	+++	+	153	428	524
2585	+++	+	++	+	++	+	181	511	714

Histological data of the irradiated carotid arteries in the various staining techniques, in comparison with the cholesterol levels in mg/100 ml.
0 = start, 1 = irradiation, 2 = sacrifice.

TABLE XI RA 233 group (XI)

rabbit nr.	Azan	o.r.O	Verhoeff	h.e.	PAS	foam cells	0	1	2
2700	++++	+++	++	++	+++	++	100	338	755
2701	+++	++	++	++	+++	+	125	475	688
2702	+++	++	++	++	+++	+	67	241	750
2703	++++	++	++	++	+++	++	72	274	767
2710	+++	+++	++	++	+++	+	59	144	672

Histological data of the irradiated carotid arteries in the various staining techniques, in comparison with the cholesterol levels in mg/100 ml.
0 = start, 1 = irradiation, 2 = sacrifice.

TABLE XII tranexamic acid (a.m.c.a.) group (XII)

rabbit nr.	Azan	o.r.O	Verhoeff	h.e.	PAS	foam cells	0	1	2
2786	++	–	++	+	++	–	95	260	710
2787	++	–	++	+	+++	–	83	296	602
2788	+++	+++	++	+	++	+	94	407	1226
2789	++	–	++	+	+++	–	81	340	569
2790	++	–	++	+	++	–	77	236	437
2791	++	–	++	+	++	–	90	392	1013

Histological data of the irradiated carotid arteries in the various staining techniques, in comparison with the cholesterol levels in mg/100 ml.
0 = start, 1 = irradiation, 2 = sacrifice.

TABLE XIII etamsylate group (VII)

rabbit nr.	Azan	o.r.O	Verhoeff	h.e.	PAS	foam cells	0	1	2
2596	++++	++++	+++	+++	+++	++	120	510	1149
2597	++++	+++	++	++	+++	++	127	435	980
2598	+++	+	++	++	+++	+	112	654	877
2599	++++	+++	++	+++	+++	++	167	416	940
2600	++++	++++	+++	+++	+++	++	139	565	1145
2601	++++	++++	+++	+++	+++	++	199	522	1526

Histological data of the irradiated carotid arteries in the various staining techniques, in comparison with the cholesterol levels in mg/100 ml.
0 = start, 1 = irradiation, 2 = sacrifice.

TABLE XIV prednisone group (VIII)

rabbit nr.	Azan	o.r.O	Verhoeff	h.e.	PAS	foam cells	0	1	2
2533	++	+	++	+	+++	–	115	467	1029
2534	++	+	++	+	+++	–	129	515	638
2586	++	+	++	+	+++	–	143	391	410
2587	++	+	++	+	+++	–	129	433	581
2588	++	+	++	+	+++	–	152	361	542
2589	++	+	++	+	+++	–	164	510	140

Histological data of the irradiated carotid arteries in the various staining techniques, in comparison with the cholesterol levels in mg/100 ml.
0 = start, 1 = irradiation, 2 = sacrifice.

TABLE XV 0-(β-hydroxyaethyl)-rutoside (h.r.) group (IX)

rabbit nr.	Azan	o.r.O	Verhoeff	h.e.	PAS	foam cells	0	1	2
2628	++++	++++	++	+++	+++	++	103	336	1155
2629	++++	+++	++	+++	+++	++	139	387	986
2630	++++	+++	++	+++	+++	++	95	517	976
2635	++++	+++	+++	+++	+++	++	82	164	257
2636	++	+	++	++	+++	–	96	209	282

Histological data of the irradiated carotid arteries in the various staining techniques, in comparison with the cholesterol levels in mg/100 ml.
0 = start, 1 = irradiation, 2 = sacrifice.

TABLE XVI benzarone group (X)

rabbit nr.	Azan	o.r.O	Verhoeff	h.e.	PAS	foam cells	0	1	2
2704	++++	++++	++	+++	+++	++	65	349	286
2705	+++	+++	++	+++	+++	+	87	366	793
2706	++++	++++	++	+++	+++	++	71	317	744
2707	++++	++++	++	+++	+++	++	74	342	719
2708	+++	++	++	+++	+++	+	62	143	319
2709	+++	++	++	+++	+++	+	71	239	—

Histological data of the irradiated carotid arteries in the various staining techniques, in comparison with the cholesterol levels in mg/100 ml.
0 = start, 1 = irradiation, 2 = sacrifice.

TABLE XVII	rel.visc. %	rel.visc.drop %	difference to control	paired t value	p value
control	76.1	23.9	0.0 %	—	—
sodium thiosulphate	82.8	17.2	+6.7 %	38.8958	<0.0005
heparin	76.9	23.1	+0.8 %	4.1666	⩾0.0025
a.s.a.	73.7	26.3	—2.4 %	7.2916	<0.0005
s.a.	73.0	26.9	—3.1 %	16.1458	<0.0005
indomethacin	72.0	27.9	—4.0 %	21.3541	<0.0005
warfarin sodium	75.8	24.2	—0.3 %	1.5625	⩾0.05
phenylbutazon	75.6	24.4	—0.5 %	2.6041	⩾0.0125
VK 744	70.1	29.9	—6.0 %	33.8541	<0.0005
etamsylate	75.6	24.4	—0.5 %	2.6041	⩾0.0125
prednisolone	77.9	22.1	+1.8 %	6.7708	<0.0005
h.r.	75.7	24.3	—0.4 %	2.0833	⩾0.05
benzarone	73.4	26.6	—2.7 %	16.6666	<0.0005
RA 233	71.7	28.3	—4.4 %	25.5208	<0.0005
a.m.c.a.	75.3	24.7	—0.8 %	4.1666	⩾0.0025

TABLE XVIII increase in body weight

hypercholesterolaemic control (C)	+11.4 %
non-hypercholesterolaemic control	+18.6 %
sodium thiosulphate control	+18.8 %
clofibrate control (XIII)	+16.2 %
heparin (I)	—23.8 %
warfarin sodium (IV)	+18.6 %
acetylsalicylic acid (II)	— 3.7 %
indomethacin (III)	+16.3 %
phenylbutazon (V)	+ 8.2 %
VK 744 (VI)	+25.9 %
RA 233 (XI)	+14.7 %
tranexamic acid (XII)	+ 5.7 %
etamsylate (VII)	+19.9 %
prednisone (VIII)	+ 2.9 %
H.R. (IX)	+18.2 %
benzarone (X)	+20.1 %

TABLE XIX stages of vessel wall damage

	1	2	3	4
hypercholesterolaemic control (C)				+
non-hypercholesterolaemic control		+		
sodium thiosulphate control		+		
clofibrate control (XIII)				+
heparin (I)		+		
warfarin sodium (IV)				+
acetylsalicylic acid (II)		+		
indomethacin (III)				+
phenylbutazon (V)				+
VK 744 (VI)				+
RA 233 (XI)				+
tranexamic acid (XII)		+		
etamsylate (VII)				+
prednisone (VIII)			+	
H.R. (IX)				+
benzarone (X)				+

SUMMARY

Damage of the arterial wall by ionizing radiation depends mainly on the rate of depolymerization of mucopolysaccharides and the destruction of elastic fibres. This results in changes in permeability and resistance, leading to a further disintegration of the normal structure and physiological function of the vessel wall.

These degenerative processes following irradiation with X-rays may be influenced by dose modifying factors, viz. radioprotectors or radiosensitizers. Changes in the polymerization state of the mucopolysaccharide ground substance of the vessel wall and the intercellular junctions of the lining endothelium, and destruction with fragmentation of elastin and collagen fibres may secondarily result in platelet adherence, the formation of platelet aggregates and the induction of fibrin formation. This leads to the release of catalytic enzymes like elastase and collagenase and of bioanimes like histamine and serotonin from platelets, the generation of thrombin and the activation of the fibrinolytic and kinin systems.

Thus reduction of platelet adhesiveness and aggregability, prevention of the fibrinogen - fibrin conversion and blocking of the plasmin formation and activity may in theory play an additional part in the prevention of irradiation damage of the vessel wall.

Vessel wall damage had been produced in an experimental irradiation model: rabbits were fed on a 0.5 % cholesterol diet, irradiated one week later on one carotid artery and killed four weeks after the irradiation procedure. The carotid arteries (the non-irradiated one being the control to the irradiated one) were excised for histological examination. Twelve groups of 6 rabbits each were treated with a drug in addition to the diet:
anticoagulants - heparin and warfarin sodium,
anti-aggregating agents - acetylsalicylic, indomethacin, phenylbutazon, and the dipyridamol derivatives VK 744 and RA 233,
anti-fibrinolytic agent - tranexamic acid or A.M.C.A.,
permeability affecting agents - 0-(β-hydroxyaethyl)-rutoside or H.R., etamsylate, prednisone and benzarone.

Four control groups were included in the experiments:

1. 0.5 % cholesterol diet without drugs,
2. normal standard pellet food, no drugs or cholesterol diet,
3. 0.5 % cholesterol diet; 15 minutes before irradiation sodium thiosulphate was given intravenously,
4. clofibrate added to the 0.5 % cholesterol diet.

In all these control groups the standard experimental procedure was carried out, including irradiation one week after the start, and sacrifice four weeks later.

Fibrin formation, platelet count and platelet function (ADP aggregation and clot retraction) were measured.

Serum cholesterol and serum fat spectra were determined.

The carotid arteries of the rabbit are very resistant to atheromatous lesions in hypercholesterolaemia. Only when the vessel wall has been damaged in some way, lipid will infiltrate and be deposited. Formation of atheromatous lesions in the intima and the media then occurs. Thus lipid may serve as a marker substance measuring the degree of vessel wall damage in experimental irradiation. On histological grounds the following stages can be distinguished in this experimental model:

1. depolymerization of mucopolysaccharides, and fragmentation, atrophy and destruction of elastic fibres;
2. degeneration of smooth muscle fibres, with vacuolization, nuclear polymorphism and disturbances of the normal circular arrangement;
3. infiltration and deposition of lipid in the intima and the media;
4. appearance of foam cells and mononuclear infiltrates in the intima and the media.

The overall effect is thickening of the vessel wall, with sclerosis and atheromatosis.

The results suggest a preventative effect of heparin, acetylsalicylic acid, tranexamic acid and prednisone, mainly on the changes in permeability of the vessel wall, as measured by the infiltration and deposition of lipid.

Indomethacin, phenylbutazon, VK 744, etamsylate and H.R. caused even more severe vessel wall damage after the irradiation in comparison with the hypercholesterolaemic control. Warfarin sodium, RA 233 and benzarone did not differ from the control.

However, the administration of heparin, acetylsalicylic acid, tranexamic acid and prednisone could not prevent depolymerization of mucopolysaccharides, destruction of elastic fibres and degeneration of smooth muscle fibres, although the rate of damage of the particular structures was much less pronounced in these groups.

Apart from some effect of heparin (p.h.l.a.), prednisone and tranexamic acid on fat spectra, no correlation was found with the absolute cholesterol levels, the increase in the levels and the variance of the levels in the individual animals in all the groups.

Apart from the effect of heparin and warfarin sodium on fibrin for-

mation, no effects on fibrin formation tests, platelet count or plasma clot retraction were observed in all the groups.

ADP-induced platelet aggregation was influenced by acetylsalicylic acid, phenylbutazon, prednisone, RA 233 and tranexamic acid.

Two supplementary radiopathological experiments were carried out:

1. viscometry of bovine synovia before and after irradiation in the presence of the drugs; a test on mucopolysaccharide depolymerization.
2. survival rates of mice after lethal irradiation in the presence of the drugs.

Prednisone showed a favourable effect on the drop in viscosity, all the other drugs did not differ from the control, or even had a more or less unfavourable effect on post-irradiation viscosity drop.

No effect of the drugs was observed in the lethality test.

On the whole the results of the experiments stress the multiconditional aspect of X-ray damage and the resulting processes, and point out the possibility of reducing long-term effects particularly on changes in vessel wall permeability by some clinically well-know drugs.

The definitely unfavourable effect of indomethacin, phenylbutazon, etamsylate and H.R. do justify more reserve and criticism in the clinical application of these drugs in patients subjected to radiotherapy.

ACKNOWLEDGEMENT

This thesis moulds a new variation on an old theme into a concrete form, and has its origin in clinical problems in radiotherapy.

Prof. Dr. H. O. Nieweg initiated my interest in this subject.

Prof. Dr. H. B. Lamberts and his staff hospitably provided the facilities for the performance of the experiments. Their critical discussions assisted in deepening my understanding in radiopathology.

I am greatly indebted to them for their cordial friendship.

Without the competent and accurate technical assistance of Mrs. E. de Noord-Arendsen the experiments would never have been completed.

Mr. J. Brouwer, Mr. F. P. W. Hoffs and Mr. S. Pasma made the illustrations.

With patience, skill and interest Mr. R. A. Wieringa took care of the microphotographic documentation.

Miss M. H. M. Börger assisted in the typewriting of the manuscript.

Many grammatical and style problems were discussed with and solved by Mrs. G. A. de Jong-Hadderingh and Dr. J. H. P. Wilson M.B., CH.B.

Last but not least I wish to express my gratitude for the skilful and pleasant cooperation with Mr. J. J. Bolman and his excellent staff at Jan Haan N.V. printing-office, and to all those who have not been mentioned for their contributions and support.

ERRATA

p. 24 fig. 3.6

p. 38 fig. 3.18

p. 47 fig. 3.26

read: ethamsylate = etamsylate

prednison = prednisone

p. 26 line 10 from the bottom:
adventia = adventitia

p. 59 line 11 from the top:
warfarin = warfarin sodium

p. 61 line 8 from the top:
neutralising = neutralizing

p. 62 line 6 - 7 of the last paragraph:
data data = data