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Encrustation and Atherosclerosis: The Analogy Between Early In Vivo Lesions and Deposits Which Occur in Extracorporeal Circulations

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ABSTRACT

A study was made of the relation between the pattern and topography of thrombus formation in models of various vessel configurations coupled into extracorporeal shunts in swine and the development of atherosclerosis at corresponding sites on swine aortas. The pattern and distribution of deposits formed in the models were strikingly similar to the pattern and distribution of incipient atherosclerosis at comparable sites in the vascular tree. The earliest and only consistent component of the flow chamber deposits was the blood platelet. The platelet deposits would frequently stain with oil red O. The cholesterol level of washed human platelets was found to show a good correlation with that in the plasma. This evidence suggests that deposition of particulate matter (chiefly platelets), largely determined by the hydraulic factors, may be an important factor in the early, as well as later, stages of atherosclerosis.

SEVERAL investigators[1-3] have shown that mural thrombi may form on what appears morphologically to be normal intima. On this evidence, it has been suggested that fibrin encrustations are an important primary mechanism in atherogenesis. In normal vascular systems, however, the formation of fibrin does not ordinarily begin until after platelet thrombi have formed and undergone viscous metamorphosis.[4-6] Several investigators have emphasized that exploration of the encrustation theory necessitates evaluating the role of the blood platelet.[6-8] More and Haust[7] have recently emphasized the importance of this point as follows: "If it can be shown that platelets are the first constituent to be deposited in encrustation giving rise to arteriosclerosis, just as they are the first structures to appear in thrombi, it will be necessary to understand the factors that bring about the local conglutination and deposition of platelets from circulating blood."

In an attempt to explore this problem, we have studied the topography, pattern and histochemistry of atherosclerosis in man and experimental animals and of deposits formed in mechanical models of vessel junctions.

Materials and Methods

Experimental Animals

The experimental animals used were Yorkshire, Landrace or crossed Yorkshire-Landrace pigs of between 40 and 600 lb. Both sexes were used, castrated or uncastrated. They were fed a standard hog-grower ration and fasted for 12 hours prior to the experiments. Over 100 experiments in all have been undertaken. The pigs were anesthetized by the intravenous administration of pentobarbital sodium in doses sufficient to produce moderate anesthesia without significant fall in blood pressure or abolition of deep tendon reflexes.

Operative Procedure

The anesthetized pig was laid on its back in a wooden trough. Catheters were inserted into the

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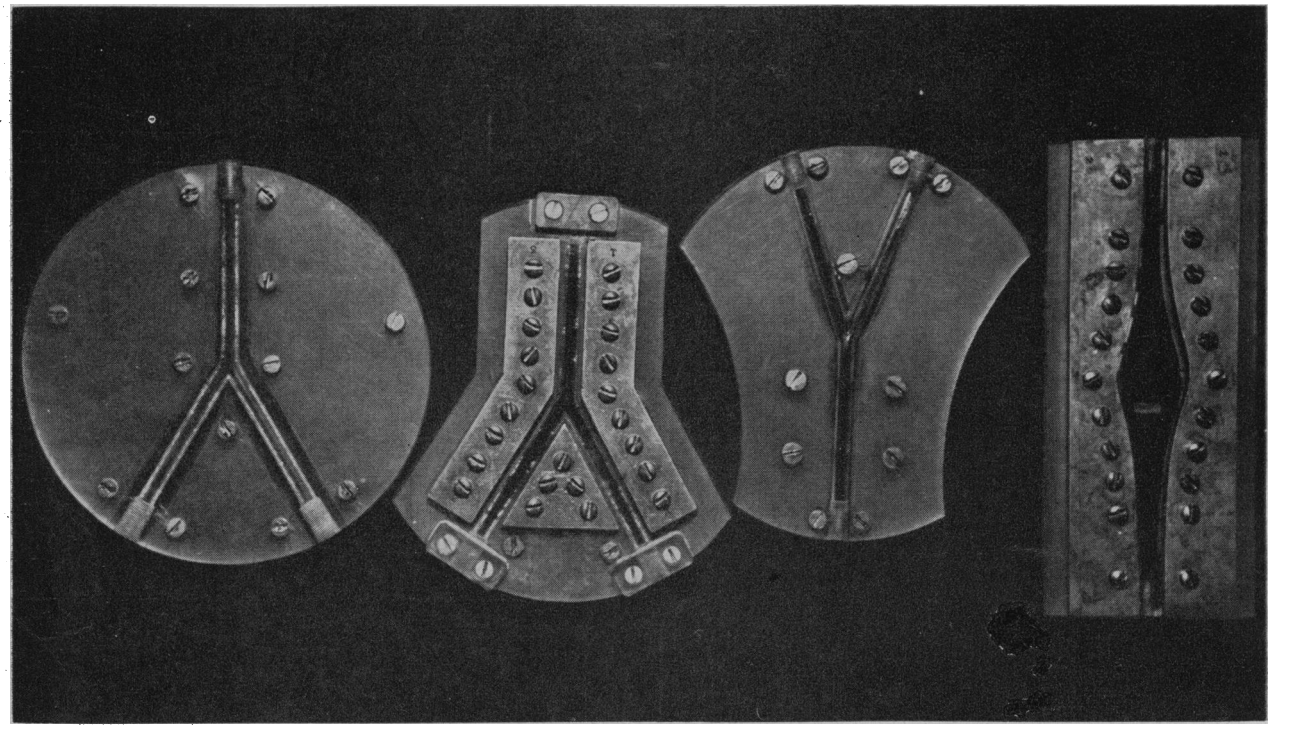

Fig. 1.—Photograph of the plastic models of vessel bifurcations and aneurysm.

left external jugular vein and the right carotid artery after exposure of these vessels through neck incisions. No anticoagulants were administered during these studies.

Flow Chambers

A variety of precision-built plastic models of vascular bifurcations and other vessel configurations were prepared for us by the Hydraulics Division of the Ontario Hydro-Electric Power Commission (Fig. 1). The internal channels were given a high finish by suitable polishing. The flow chambers were attached to polyethylene tubing by means of metal couplers of quarter-inch (0.64 cm.) internal diameter by snugly fitting hard, plastic, exterior sheaths (Fig. 2). The internal diameter of the tubing, the couplers and the adjacent channels of the flow chambers were of equal bore and roughness; and, therefore, turbulence at the junction was reduced to a minimum. The quarter-inch lumen of the plastic connecting tubes was successively reduced by a sequence of tubes so that the catheters inserted into the pig's vessels had an internal diameter of one-eighth of an inch (0.32 cm.). Midway between the flow chamber and the catheter on both the afferent and efferent sides a short strip of red rubber tubing was inserted. This made it possible to take samples of blood through the rubber tubing and to inject bubbles of air into it to estimate rate of flow. Prior to the experiment the entire system was siliconed with G.E. dry-film SC 87 in ether (10 parts silicone to 90 parts ether), rinsed thoroughly with 200 ml. of distilled water (the capacity of the flow chamber system was about 50 to 60 ml.) and charged with 0.85% saline. After each experiment the flow chamber and the couplers were washed very carefully with a detergent and water to remove any traces of deposit. They were then thoroughly rinsed with distilled water and dried at room temperature. The plastic and rubber tubing including the catheters were discarded after each experiment.

The flow chambers were made in two parts, the plane of separation passing symmetrically through the channels. To prevent seepage of blood between the halves, it was necessary to apply firm pressure by through-and-through screws and by external clamps. It was found that petroleum jelly gives positive stains for fats, so that it was not possible to use this substance to secure watertight junctions. After the experiment, the two parts of the

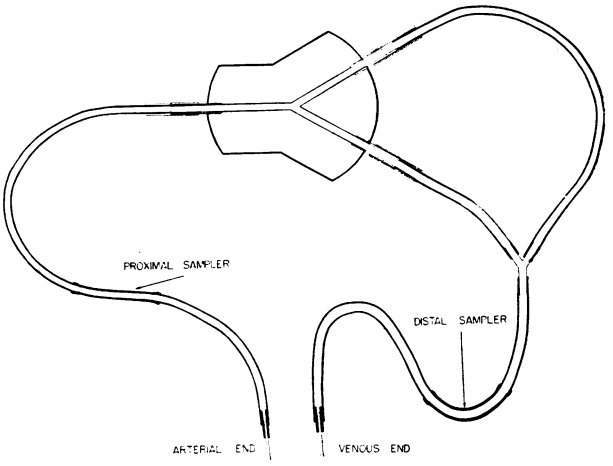

Fig. 2.—Diagram of flow chamber and connections.

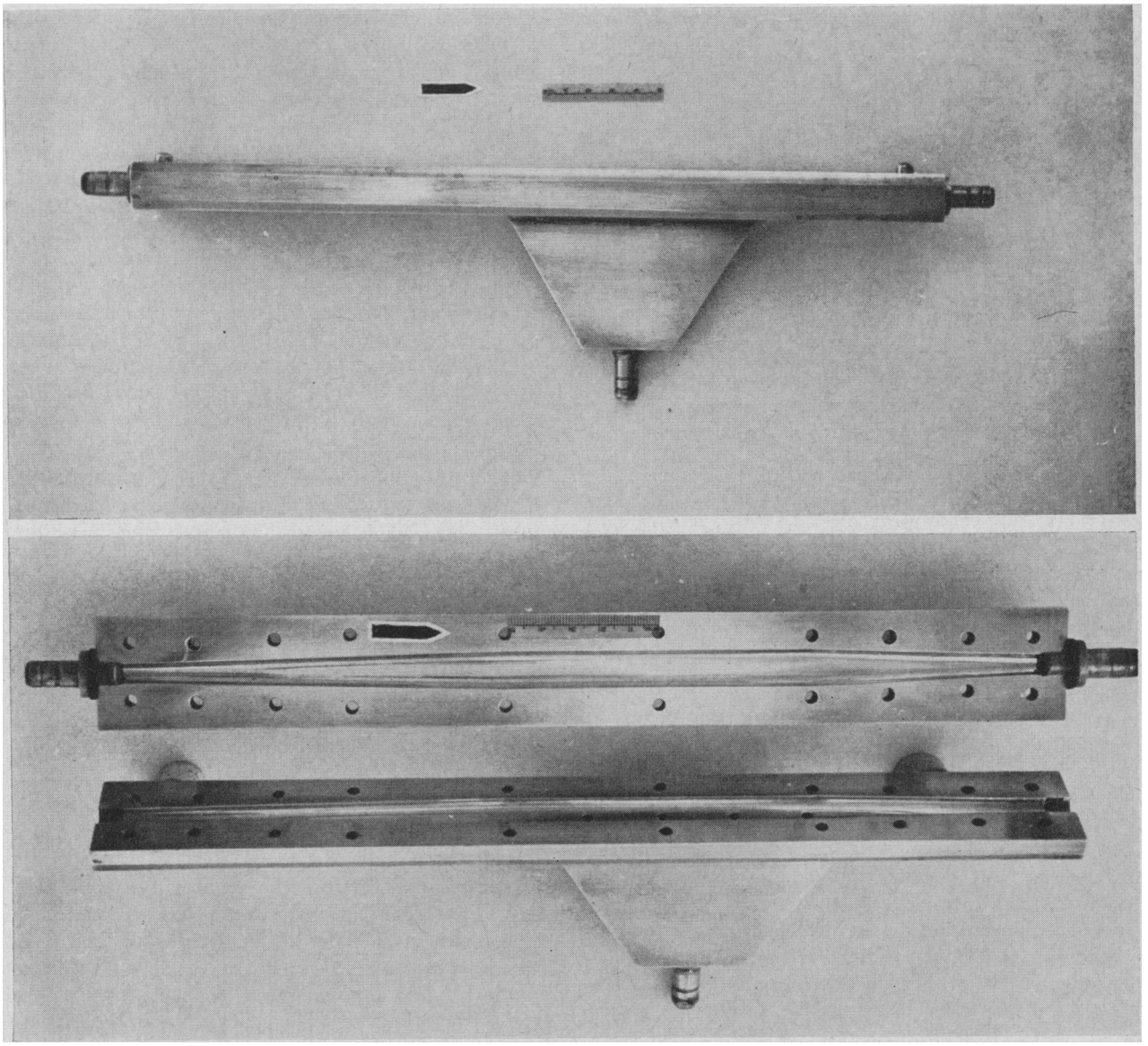

Fig. 3.—The upper photograph shows the assembled brass flow chamber. The keel-shaped structure on the bottom of the chamber is for the channels from four orifices in the main lumen that merge into a single channel shown in the lower photograph. In the lower photograph, the upper half of the chamber has been lifted off the lower half. The upper half of the lumen can be seen between the two surfaces which fit those on the bottom half. The holes in these surfaces are for the screws used to clamp the two surfaces together. The lumen in the bottom half has four openings in a row. These can be seen in the area directly above the keel-like structure. The black arrows in the two photographs indicate the direction of blood flow when the chamber is connected to an extracorporeal circulation.

flow chambers were separated, great care being necessary to prevent the deposits from being dislodged from their sites of formation. It was found that this was best achieved by insinuating a pair of fine scissors between the layers of the chamber and gently cutting any strands bridging the two parts. Even the sharpest of scalpel blades when used to cut these strands tends to dislodge the deposits from their sites of attachment.

A special flow chamber designed as a simulacrum of the intercostal vessel orifices in a pig's aorta was also devised (Fig. 3). This model differed from the others in that it was made of polished brass, and the plane of cleavage between the two halves was perpendicular to the plane of the branching vessels. It was, therefore, not possible to study the deposits in the side vessels in this model. Moreover, to provide a large undisturbed area on either side of the vessel orifices, a gentle taper from a quarter of an inch (0.64 cm.) to half an inch (1.27 cm.) was introduced in the plane of cleavage. The bore remained a quarter of an inch (0.64 cm.) throughout in the lesser axis.

Volume of Flow

The volume of flow was estimated by observing the time taken for the leading edge of a 2-ml. bubble of air which extended across the entire lumen, to traverse a length of the tubing, the volume of which had previously been determined. The bubble was introduced through the proximal rubber sampler at the beginning, half way through, and at the end of each flow period. Estimated volumes of flow varied between 200 and 700 ml.

per minute, varying little in the course of any one experiment.

Examination of Deposits

The usual procedure was to stain one half of the flow chamber with oil red 0, with light green as a counterstain, and the other half with Wright's stain. The distribution of the deposits was recorded by drawings, and by photographs in some instances. Samples of the deposits were removed prior to staining for other examinations such as electron microscopy. The surfaces of the flow chambers were examined by the naked eye, by means of a Zeiss operating microscope and by means of a light microscope. The shape of the flow chamber was designed to make such examination possible.

Electron Microscopy: Material was removed from the flow chambers after fixation *in situ* with Palade's fixative, embedded in methacrylate and cut on a Porter-Blum microtome with glass knives, and examined at 55 KV. on a Phillips EM75 electron microscope.

Fluorescent Antibodies: Antisera against pig platelets were prepared in rabbits, concentrated and conjugated with fluorescein isocyanate by the method of Coons and Kaplan.[9] Controls of specificity of the conjugated antisera were: a failure of the conjugate to stain pig erythrocytes and pig platelets previously exposed to unconjugated platelet antiserum. Fluorescent microscopy was carried out with a Leitz Dialux microscope, with a Phillips mercury high-pressure bulb CS-150 as the light source. Examination for fluorescence was performed according to the following method: (1) by placing conjugated antisera on the channels of the flow chambers and incubating at 37° C. for one hour; and (2) by removing portions of the deposit from selected areas, diluting them in saline as required, spreading the mixture over a glass slide and applying conjugated antibody. The deposit was covered and examined immediately.

Radioactive Isotopes

In some experiments, attempts were made to determine the nature of the deposit by the use of radioactive isotopes. Two isotopes were used; P^{32} in di-isopropyl fluorophosphonate (DFP^{32}),* a drug which forms a linkage, reported to be irreversible, with all esterases including those of circulating platelets;[10] the other isotope was S^{35} given as a sulfate. This is incorporated in certain growing tissues including the megakaryocytes.[11] A donor pig of 40 to 60 lb. was given 50 m.c. of S^{35} intravenously. On the third day when the platelet radioactivity was at its height, the pig was subjected to thrombocytapheresis;* platelet-rich (EDTA) plasma, almost free of red and white blood cells was prepared[12] and then infused in a small volume (less than 40 ml.) into a recipient pig with a compatible blood group. In the recipient pig a flow-chamber experiment was undertaken 40 minutes later, and the radioactivity of the transfused material was checked. Platelet-poor plasma prepared from the same donor was transfused into another recipient pig in which a flow-chamber study was then performed. The red cells, platelets, platelet-free plasma and fibrin prepared by clotting plasma were examined for radioactivity, in the blood of the recipient animals which received the S^{35}-labelled platelets and in those receiving the platelet-poor plasma.

Similar transfusion experiments were carried out with intravenous DFP^{32} as a label, thrombocytapheresis of the donor, however, being performed four to six hours after the injection, since maximum labelling is rapidly achieved.

Radioactivity was measured by means of a Nuclear-Chicago D47 proportional gas-flow detector (with window) and a Model 186 scaler. For fluid preparations, the concentrations of platelets and erythrocytes in the platelet-rich and platelet-poor plasma were determined. One-ml. samples of washed platelet or red corpuscle suspensions and of platelet-free plasma were dried on stainless steel sample pans of one inch (2.54 cm.) diameter. An M5 changer provided constant geometry between sample pans and detector. The total number of platelets infused, and the radioactivity of platelets, red corpuscles and platelet-free plasma, were then calculated. The radioactivities of small segments of aortas and other tissues were measured similarly. For flow-chamber deposits or for large areas of aorta, the detector was mounted in such a manner that the chamber or vessel was approximately 1 cm. from the window. A lead plate, 3 mm. in thickness, in which was located a hole 1.2 cm. in diameter, served as a shield to define a small field for examination.

Aortas

The aortas studied were derived principally from two sources—children below the age of 16 years, and pigs used in various dietary experiments. A total of 40 aortas from children ranging between six months and 14 years of age were examined. One hundred and forty swine aortas were examined, 98 from animals used in dietary experiments, part of which have been reported.[13] Other species seen from time to time in the course of other work provided interesting material for comparison.

*Obtained from Radiochemical Centre, Amersham, England.

*Thrombocytapheresis means removal of the platelets from the body. Blood from the pig is removed in amounts of a litre at a time. The platelets are removed by differential centrifugation and the rest of the blood is returned to the donor. In this way, it is possible to remove a large part of the platelet mass over a short period of time without causing shock.

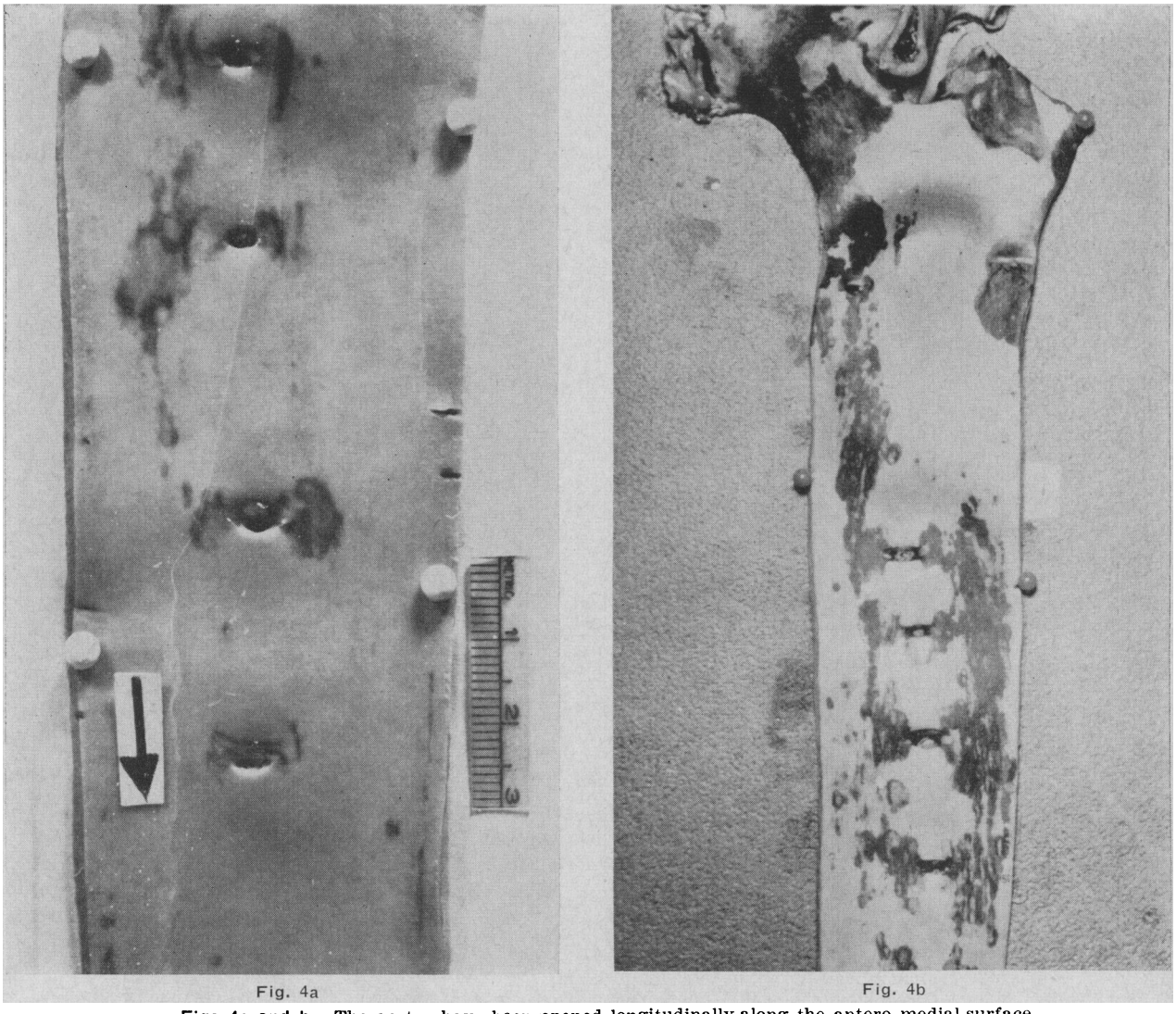

Fig. 4a

Fig. 4b

Figs. 4a and b.—The aortas have been opened longitudinally along the antero-medial surface and stained with Sudan IV. The fatty lesions appear as darker than the surrounding tissue. Fig. 4a shows early changes about intercostal vessels. Fig. 4b shows in addition the involvement along the lesser curvature of the aortic arch and about the aortic valve ring.

The tracing out of the patterns of the lesions was facilitated by staining the aorta with Herxheimer's Sudan IV.

Lipid Analysis

Platelets were prepared from human subjects by techniques which have been described.[12] They were washed once with a large volume of 0.85% saline (0.1 ml. platelet mass to 20 ml. saline) precipitated by centrifugation and re-suspended in a small volume of distilled water which was then transferred to a weighed beaker and dried. The material was then extracted with ethanol-ether (3:1) for two hours at 70° C. The supernatant and the residue were dried in an atmosphere of nitrogen at approximately 45° C. When dry, the products were extracted with petroleum ether, and cholesterol and phospholipid contents of the soluble and insoluble fractions were determined by the techniques of Sperry and Webb,[14] and Zilversmit and Davis,[15] respectively.

Cholesterol and phospholipid values for serum samples collected at the same time were also determined.

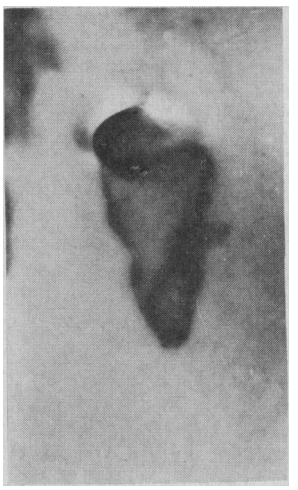

Fig. 5.—Tear-drop lesion extending distally from an intercostal orifice stained with Sudan IV. The dark staining area is the region of involvement.

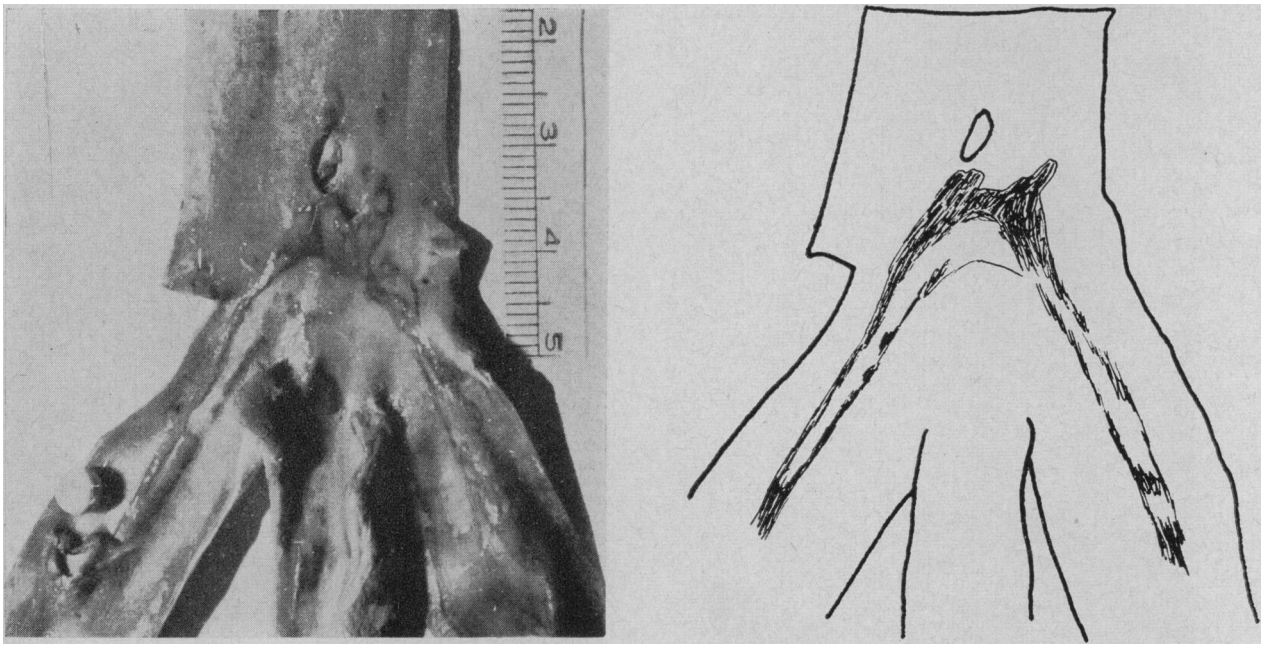

Fig. 6a

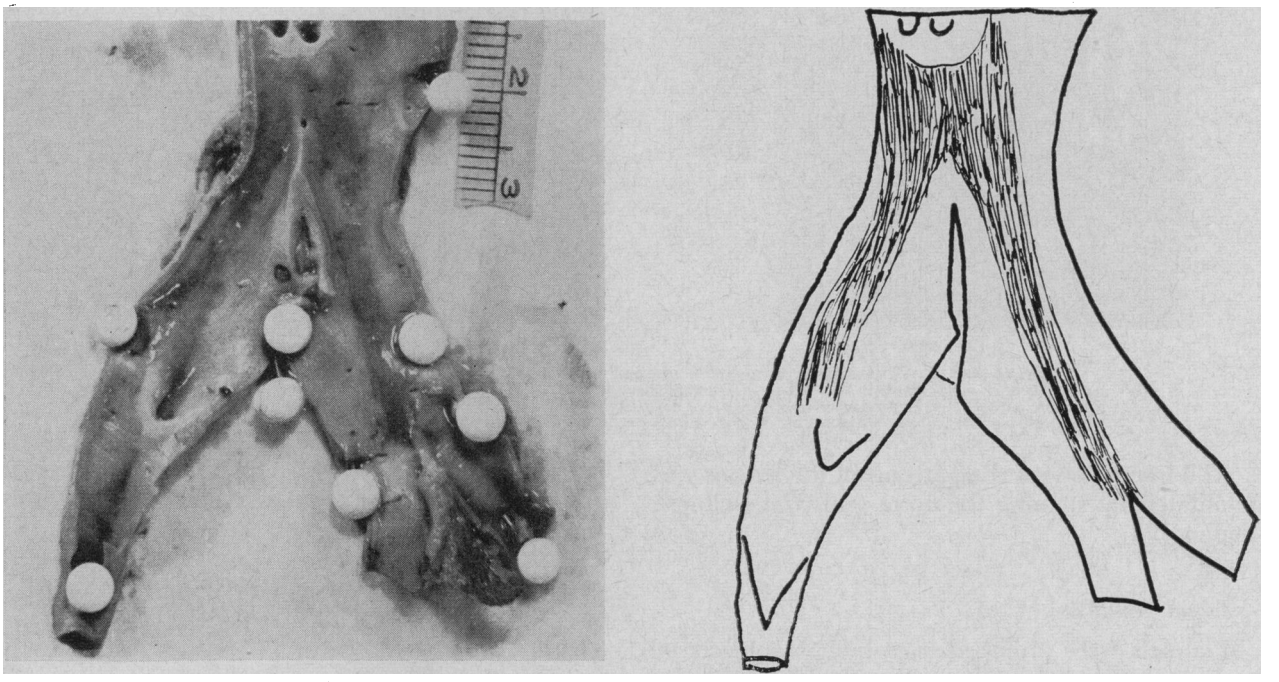

Fig. 6b

Fig. 6a.—The terminal part of a pig's aorta stained with Sudan IV. The central branch, abortive in man, supplies the tail. The common iliac vessels have been split down the medial side. The lesions are indicated by the darker areas, heaviest on the lateral aspects. There is a patch just above the beginning of the trifurcation with limbs extending down both of the common iliac vessels. The hole just above where the lesion is in the aorta is from a punch biopsy used to remove tissue at the time the animal was sacrificed. Fig. 6b.—The bifurcation of the aorta in a child of 6 years of age. The dark staining areas of fatty involvement extend across the hips of the bifurcation and down both iliac vessels.

RESULTS

I. ATHEROSCLEROSIS

The Characteristics of Early Lesions in Swine

Topography: Examination of the early lesions of atherosclerosis in swine showed a remarkable consistency of pattern. Lesions occurred at the commissures of the aortic valve cusps and sinuses of Valsalva (Fig. 4), the lesser curvature of the aortic arch, at the ductus scar, and about vessel orifices. In the thoracic aorta, the lesions had a characteristic relationship to the mouths of the intercostal vessels. When each pair of intercostal vessels arose by a common orifice, as they do in most pigs, the earliest changes generally occurred at the lateral aspect of the orifices, but occasionally started distally (Fig. 5). Almost all swine over one

year of age showed changes at these sites regardless of diet. More advanced lesions extended laterally and flared out into longitudinal patches giving the butterfly-shaped pattern described by Anitschkow[16] (Fig. 4). Butterfly patterns from several adjacent orifices sometimes coalesced, giving a ladder-like appearance (Fig. 4). A detailed description of these changes in relation to diet was presented in a previously published report.[13] There was a parabolic area on the proximal side of the orifice which was relatively spared even in the presence of moderately advanced disease. In the abdominal aorta the pattern was more irregular, but, here again, the proximal lip of orifices tended to escape.

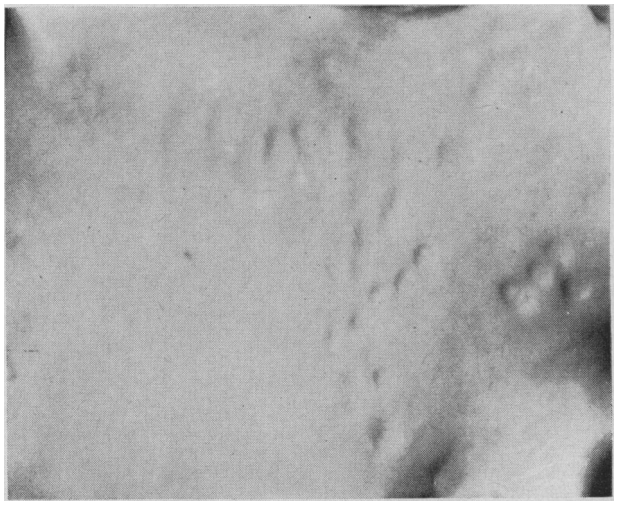

Fig. 7.—Unstained early lesion, showing the floccular pattern in the aorta of a swine.

At vessel bifurcations the medial aspects of distributory vessels were less prone to involvement, and the heaviest concentration was observed laterally (Fig. 6). The beak of the bifurcation was often involved, whereas the distal part of the parent vessel usually escaped. Changes were present at this site in all swine over the age of one year, regardless of diet.

Pattern: The earliest change visible to the naked eye was a peppering of pearly grey floccules (Fig. 7). These were made more evident when a suitable fat stain was applied. These lesions commonly coalesced to form longitudinal streaks, particularly in areas where they were not adjacent to vessel orifices. The streaks sometimes showed transverse ridging (Fig. 8) which resembled the surface of a coralline thrombus or the ripple marks on wave-washed beaches. As the lesions coalesced, they tended to become raised, but they sometimes covered a considerable area without being palpable. More advanced lesions had a raised pearl-like appearance. When such lesions were treated with Sudan IV, visible fat staining was evident, mainly at their periphery.

Histology: Microscopic examination showed that the lesions varied in size from minimal intimal thickening invisible to the naked eye, to discrete

Fig. 8.—Close-up of a Sudan IV stained lesion in a pig's aorta. The overall disposition is longitudinal, but there is clear transverse ridging. Flow is from the top.

nodules. Microscopic evidence of intimal thickening could frequently be found at the classical sites before there was gross evidence of change (Fig. 9). The changes were confined to the intima, and the elastic lamina was usually intact. Section of extensive lesions showed that they were not homogeneous but contained several histologically different layers.[13]

The slightest change consisted simply of a subendothelial fat streak. The nodular lesions showed a diffuse accumulation of fat and a considerable amount of connective tissue. When they were larger still, connective tissue was often the major constituent, with fat present only at the circumference and deep to the fibrous cap. Not all of the lesions were sudanophilic.

Various stains (toluidine blue, Hale's reaction, thionine) showed that early lesions were rich in

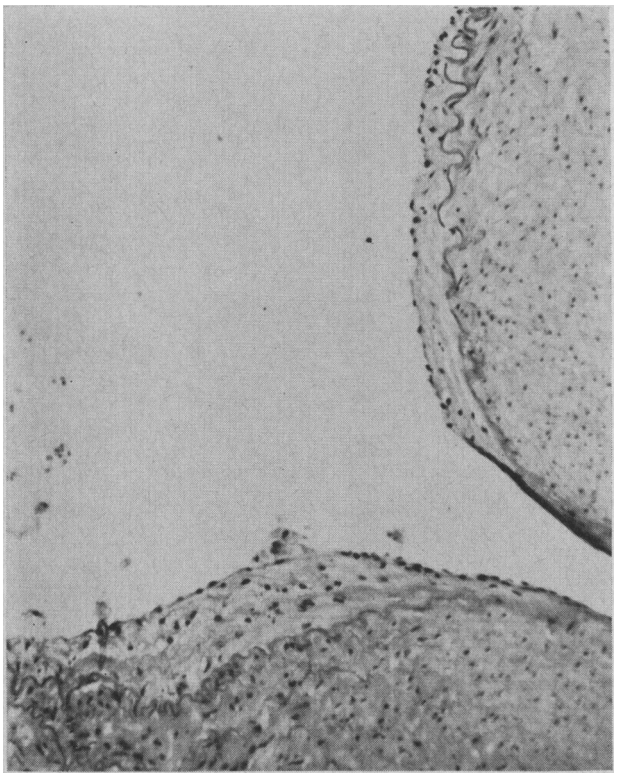

Fig. 9.—Intimal thickening with little fat deposition at the bifurcation of a pig's carotid artery (H & E., x 200).

acid mucopolysaccharides[13] (a common finding where there is proliferating connective tissue). Early changes were often rich in acid mucopolysaccharides but without demonstrable lipid; fat accumulation alone without acid mucopolysaccharides was not encountered. More extensive lesions rich in mature connective tissue, however, frequently showed little evidence of acid mucopolysaccharides, though fat was abundant.

Early Lesions in Man

Examination of the aortas from young human subjects dying from usual childhood causes (leukemia, trauma, etc.) showed features similar to those described for swine. The differences observed could be attributed to anatomical factors. For example, the intercostal vessels in man arise separately from the aorta, while in the pig they usually arise in pairs. In consequence, the characteristic butterfly-shaped lesion described in the pig was modified in man (Fig. 10a), the wings extending on the lateral side with a sparing of the area between the orifices. This is not a species difference, since the lesions in the occasional swine with separate intercostal orifices follow the usual human pattern (Fig. 10b). The similarity of the pattern at the aortic trifurcation in swine and at its bifurcation in man is shown in Fig. 6.

Lesions in Other Animals

Despite species peculiarities, lesions in other animals were analogous. Thus in the rabbit the changes occurred near the intercostal orifices at the usual sites (Fig. 11), as Anitschkow[16] pointed out, but the abdominal aorta and its bifurcation usually escaped.

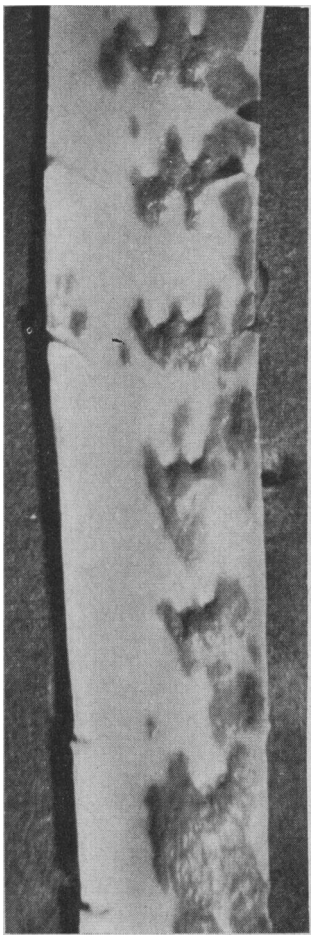

Fig. 11.—Characteristic lesions about intercostal vessel orifices in the aorta of a rabbit (stained with Sudan IV). The rabbit was fed egg yolk. The proximal lips of the intercostal vessel orifices are spared, and the lesions tend to be heaviest lateral and distal to the orifices.

II. DEPOSITS IN EXTRACORPOREAL CIRCULATIONS

Study of the factors which determine the localization of the atherosclerotic lesion is complex, and the amount which can be learned exclusively from the study of postmortem material is limited. It is probable that the form of the vessel contributes hydraulic factors; but it is also evident that various physical stresses may produce pathophysical changes at certain sites of the arterial tree. In an attempt to dissociate these two groups of factors, we have studied the effects of perfusion of blood through models of various vessel configurations. In this way, it has been possible to explore the hypothesis that encrustations, the nature of which will be discussed in subsequent paragraphs, are a factor in the development of early atherosclerosis, and to examine the effects of hydraulic factors on their

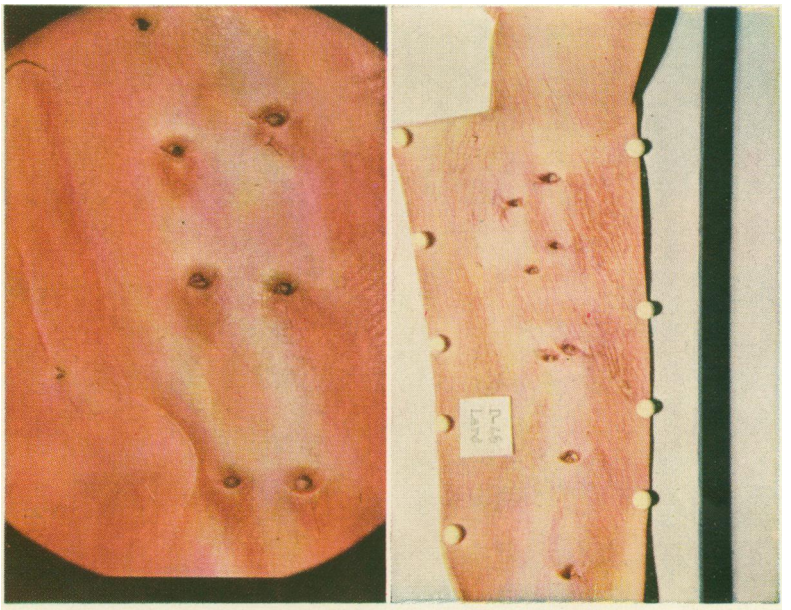

Fig. 10a Fig. 10b

Figs. 10a and b.—The pattern of the lesions about paired intercostal orifices in man (A) and in a pig (B) stained with Sudan IV. Flow is from the top. The proximal lips of the intercostal vessels are spared, and the lesions are greatest lateral to the intercostal vessel orifices.

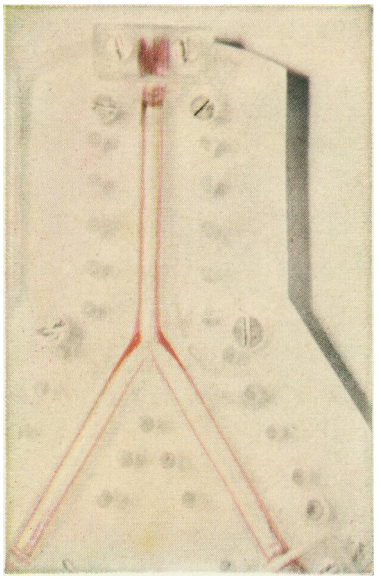

Fig. 13.—An advanced deposit at a 60-degree bifurcation in a plastic flow chamber model. The parent limb is almost completely spared. The deposit can be seen extending across the hips of the bifurcation and down the lateral side of both distributory vessels. Flow is from the top.

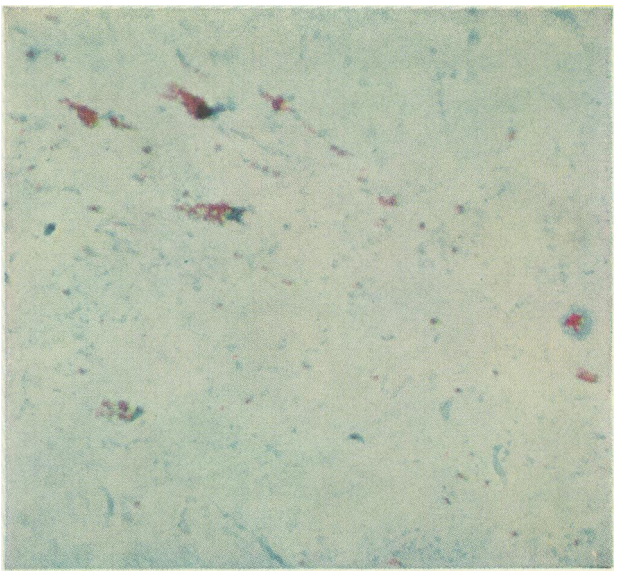

Fig. 21.—Photomicrograph of an early deposit on a flow chamber surface showing the intimate association between the sudanophilic material (red) and the blood platelets. Stained with oil red 0 and counter-stained with light green (x 480).

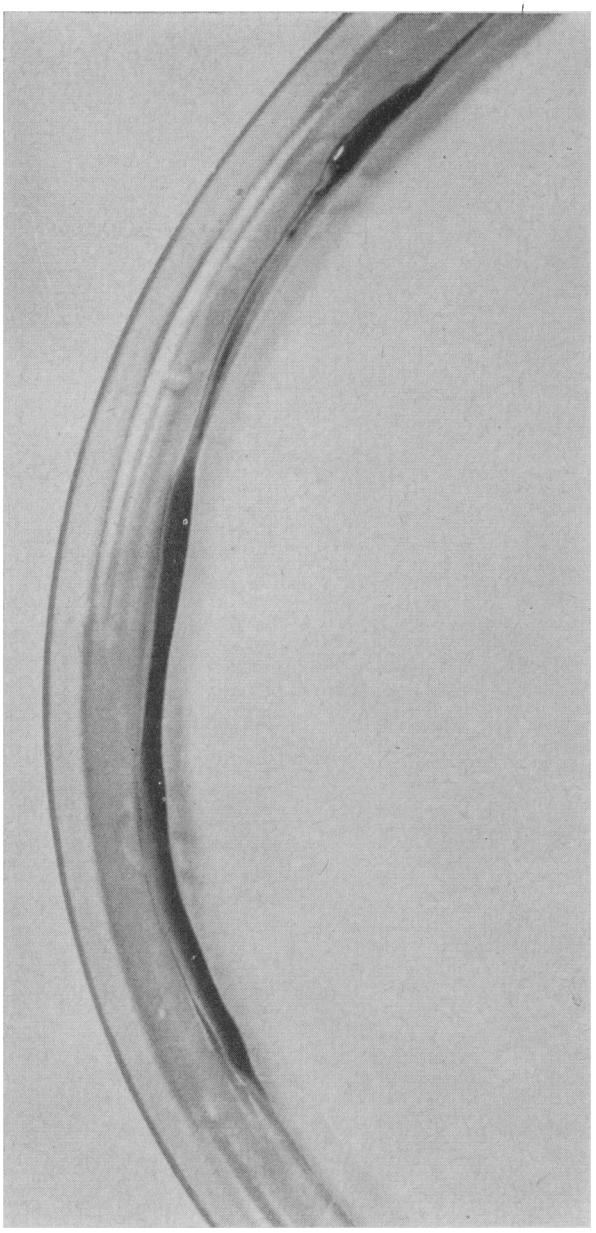

Fig. 12.—This photograph shows the deposit which formed along the lesser curvature. The tubing has been disconnected from the extracorporeal shunt and photographed.

formation. Moreover, the channel surfaces in such models, albeit siliconed, are sufficiently foreign to accentuate and accelerate the process of deposition from circulating blood.

Topography

Many studies were performed on each of the various models of vessel configurations, over 100 experiments in all being carried out. The distribution of the deposits formed in each model was remarkably constant.

Simple Curve (Fig. 12): The maximum area of deposition was on the lesser curvature.

Bifurcation (Fig. 13): The proximal or parent limb usually remained free of material up to its termination. While deposits occasionally formed on the beak of the bifurcation, they were most profuse on the lateral and inferior aspects of the distributory vessels. This evidently represents an area of "deadwater"; it is at just this site, for example, that bubbles accumulate when the model is perfused with water at low pressure. Stehbens,[17] in studies on fetuses and infants, found intimal proliferation at these sites, at bifurcations in cerebral

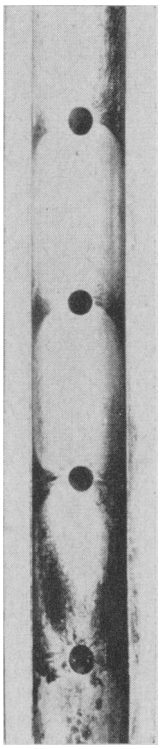

Fig. 14.—Deposits about the multiple orifices in the brass flow chamber. The top half of the chamber has been unscrewed and removed. The direction of flow is from above. About each of the orifices can be seen the dark areas of the deposits. The proximal lips of the orifices are spared, with the heaviest deposit laterally.

arteries. He attributed them tentatively to the occurrence of turbulence and stagnation at these locations. Distribution of flow in the models was often unequal between the two limbs—this we know to be true of simple mechanical systems where the cause is usually not evident—and the deposit was the heavier in the limb in which flow was the less. For an inch or so downstream from the actual bifurcation the vessel often remained clear of deposit, but distal to this, linear accretions were common.

Multiple Right-Angled Branches: Little deposit was formed in the proximal part of the chamber. At the first orifice the deposit extended laterally, fanning out in a smooth curve proximally and distally (Fig. 14). The area immediately proximal to the orifice remained relatively clean. The pattern of the sediment frequently indicated what was evidently the lines of flow (Fig. 15). At the second orifice which lay one inch downstream, a similar pattern was observed, but at the third and fourth

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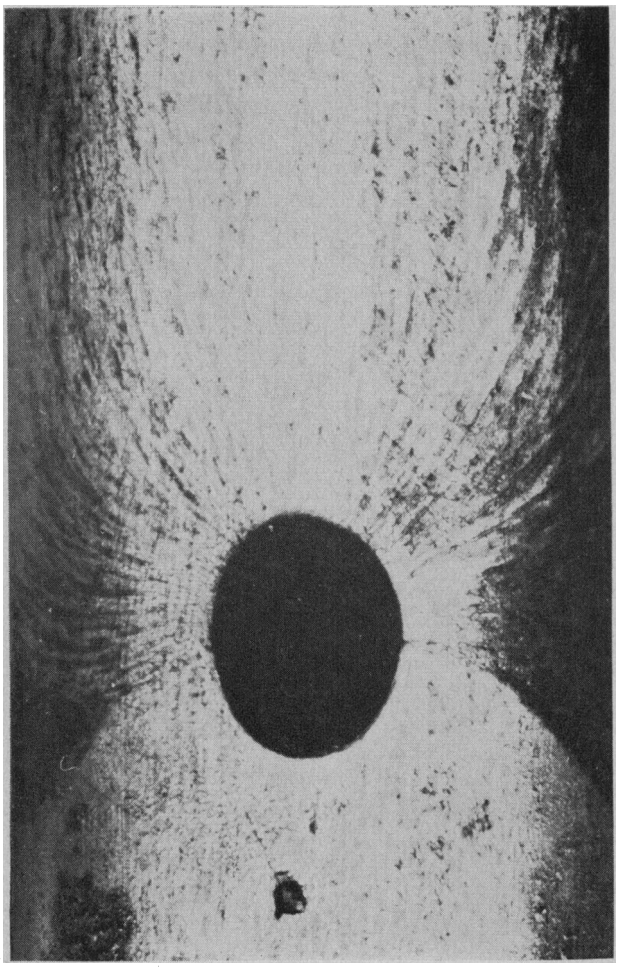

Fig. 15.—A close-up of the deposit about most proximal of the orifices in the brass flow chamber. The flow is from above. The curvilinear characteristic of the deposit, with the heavy lateral formation, can be seen.

orifices, which were similarly spaced, the pattern was less constant. In these experiments, the main and subsidiary channels were kept in the horizontal plane: it was repeatedly noted that the deposit was heavier on the dependent side.

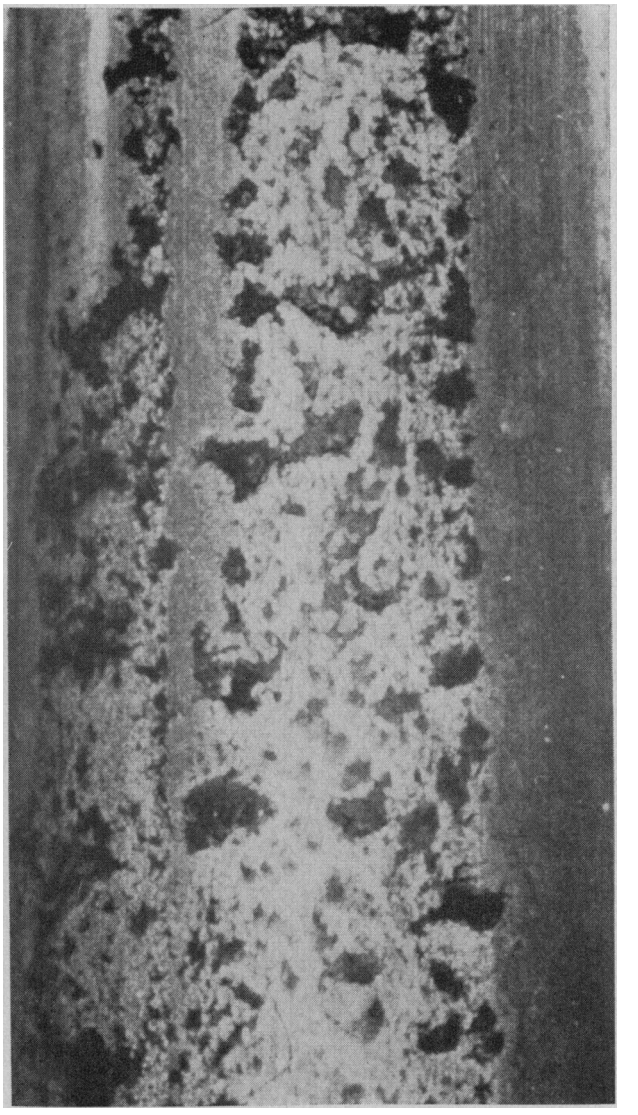

Fig. 17.—Heavy floccular deposit in a flow chamber, showing a tendency to the formation of transverse bars. Stained with Wright's stain. Flow is from the top to the bottom.

Aneurysm: A central channel which was in line with the two unexpanded parts of the flow

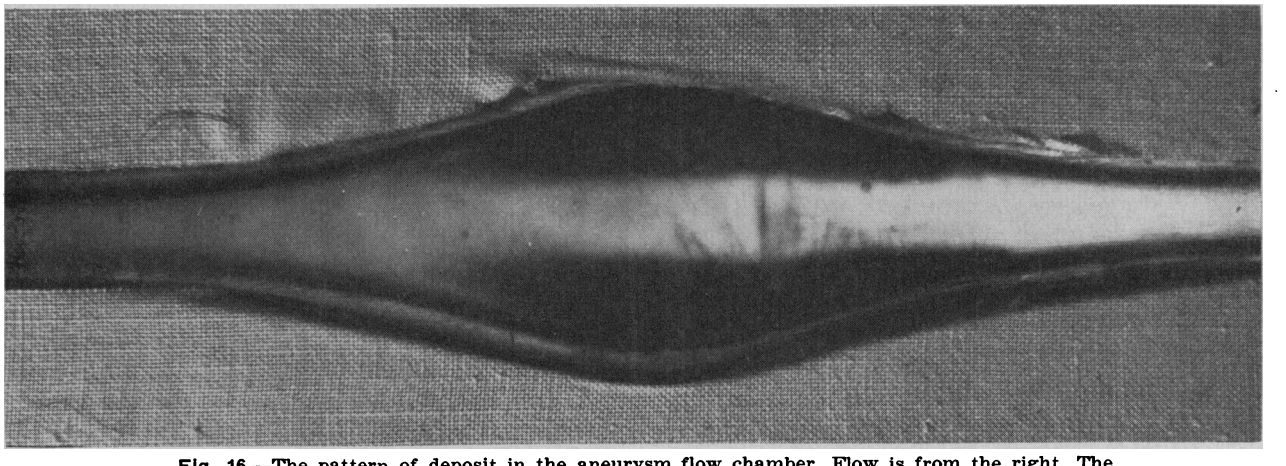

Fig. 16.—The pattern of deposit in the aneurysm flow chamber. Flow is from the right. The central channel is relatively free of deposit, while there is a very heavy deposit at the sides of the dilatation.

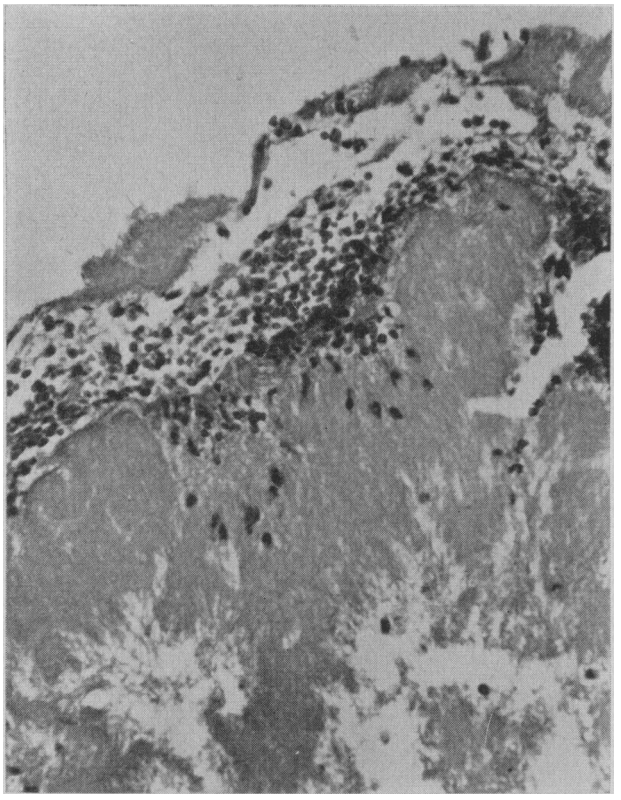

Fig. 18.—Photomicrophotograph of a mixed thrombus formed in the flow chamber. It consists of an amorphous mass composed of fused platelets and some fibrin, with layers of red and white cells sandwiched in between (H & E., x 480).

chamber remained clear (Fig. 16). The expanded parts were obliterated by massive thrombus. Eddying of the blood was readily visible in the areas where the heavy deposits subsequently formed.

Pattern

The earliest deposits were fine, floccular and translucent. This floccular pattern does not occur in a static system. We have occasionally seen experiments in which the seams between the couplers and the sockets have not been tight and a little blood has seeped through and stagnated. A uniform sheet of thrombus formed, which abruptly changed to the floccular pattern where there was flow.

As the duration of perfusion was increased, the floccules became coarser, and coalesced. They were usually elliptical in shape in the neighbourhood of branch orifices: downstream from centres of turbulence they were more linear with transverse ridging (Fig. 17).

Composition

Advanced deposits were gelatinous with many trapped red cells. Histochemical examinations showed that they were composed of a fibrin network with red cells, white cells and blood platelets (Fig. 18). In some deposits, these were arranged in alternating bands of fibrin and amorphous material consisting probably of fused platelets.

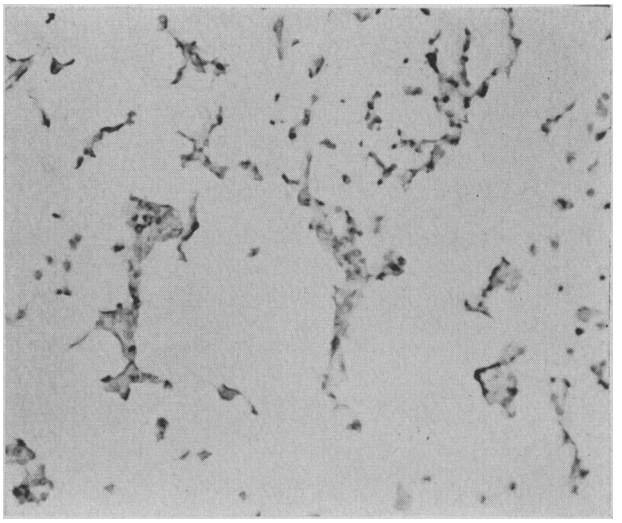

Fig. 19.—Photomicrograph of the components of an early deposit on the surface of a flow chamber. This shows the appearance of platelets which have undergone viscous metamorphosis. Wright's stain (x 480).

Microscopically, this gave the appearance of a coralline thrombus. The experiments were usually stopped short of this advanced state. In the early stages, demonstrable fibrin was absent and the scant encrustation, stained with Wright's stain, had the morphological appearance of platelets (Fig. 19). However, the structure of platelets is so extensively altered once they are deposited on surfaces that we are not satisfied that morphological identification alone is enough. To identify the deposit with greater specificity, three further techniques were applied.

1. Antibodies specific for big platelets were prepared as described (*q.v.*), and conjugated with fluorescein isocyanate. When they were added to the deposits they gave a greenish fluorescence. The fluorescence was more obvious when the deposit contained little fibrin. It was more easily demonstrable when the material was transferred to a glass slide, which adsorbs less ultraviolet light than the plastic of the flow chamber.

2. In isotope studies (*vide supra*), radioactivity was demonstrated in the deposits, but not in fibrin precipitated out of the recipient's plasma or in the red blood cells (Table I). This is taken as good

TABLE I.—RADIOACTIVITY VALUES FOR FLOW CHAMBER DEPOSITS

	C.P.M. (500 counts)*		
	Total	*Background*	*Above background*
Platelet-rich plasma recipient			
Deposit	153	53	100
Platelets†	380	28	352
R.B.C.†	26	26	0
Fibrin†	26	26	0
Plasma†	28	26	2
Platelet-poor plasma-recipient			
Deposit	58	54	4

*Per mg. of deposit, platelets, r.b.c. and fibrin and per 10 ml. of plasma.

†Prepared from recipient animal's blood at termination of experiment.

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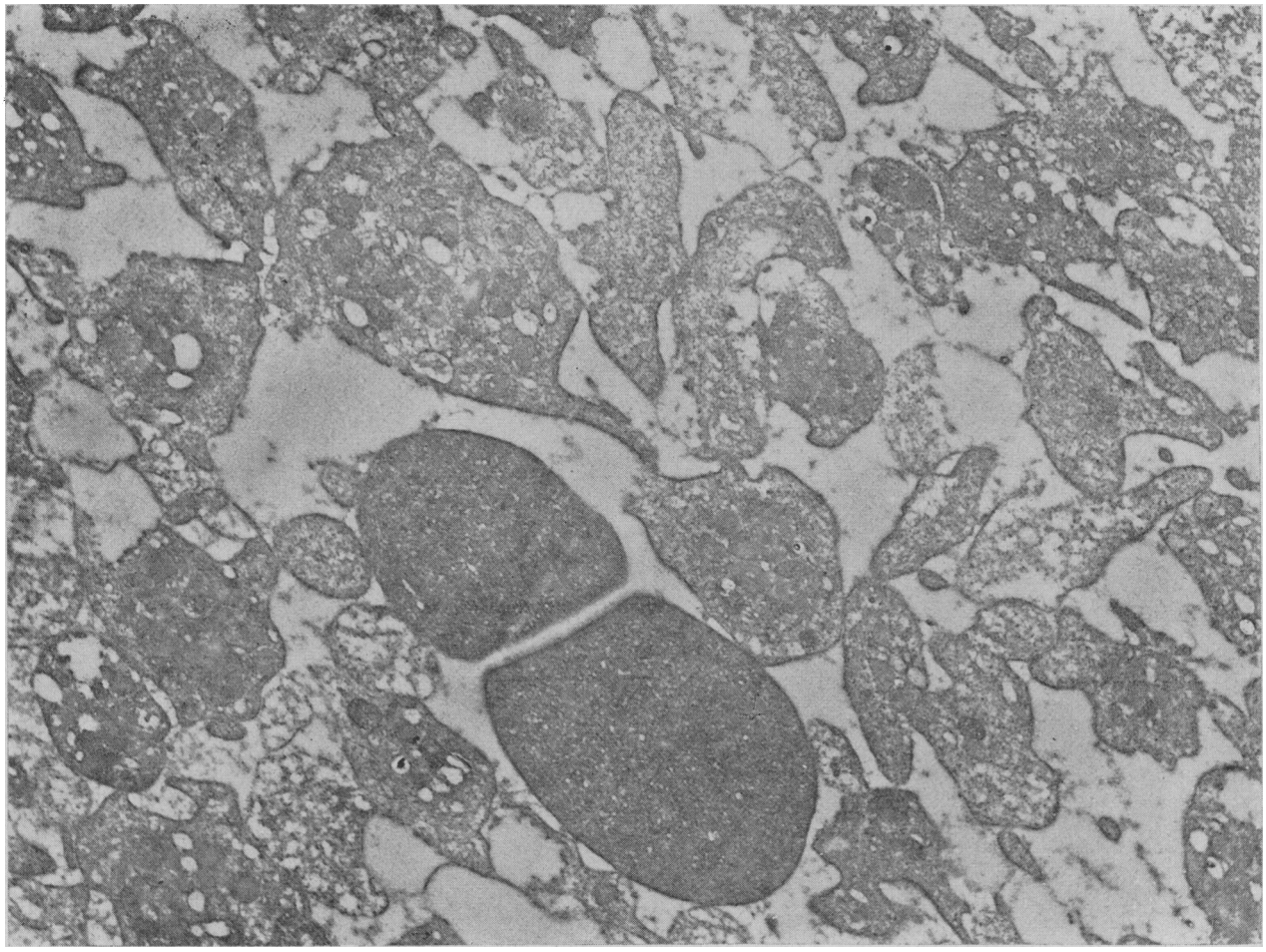

Fig. 20.—Electron-microphotograph of an early gelatinous deposit. In bottom centre is an erythrocyte. The rest of the material consists mainly of clumped and fused platelets (x 16,500).

evidence that early deposits consisted mainly of blood platelets. The survival of the radioactive platelets in the recipient pig was found to be within the range for normal pigs, which suggests that their function was not grossly impaired by the processing prior to transfusion. The experiment was performed five times. The deposit from the pig receiving platelet-poor plasma prepared from the same donor was not radioactive (Table I).

3. Electron photomicrographs showed that the early deposits consisted of a mass of platelets. The platelets were in a solid fused state but contained interstices with trapped plasma (Fig. 20).

Histochemistry: The platelet deposits exhibited intense metachromasia when stained for acid mucopolysaccharides. They invariably took up fat stains, although sometimes this was evident on microscopic examination only. Gross fat-staining could be readily demonstrated in platelet deposits (Fig. 21). The intensity was tremendously enhanced if an intravenous emulsion of fat was given before the experiment. This suggested that it might be worth while to explore the relationship between the blood lipids and the lipid composition of platelets. This is the subject of the following paragraph.

Lipids in the Serum and the Platelets

Blood was taken from 30 fasting human male subjects (ages 30 to 60 years). The cholesterol and phospholipid levels of the platelets were compared with those in the serum. It is known that the distribution of serum cholesterol is approximately lognormal. The correlation coefficient between the logarithm of the serum total cholesterol and that of the once-washed platelets was good ($r = +0.64$, $p < 0.001$). A similar correlation was found between the serum and platelet phospholipid values which are also lognormally distributed ($r = +0.48$, $p < 0.01$). These observations suggest that the lipid level of the circulating platelet may in part reflect the serum lipid level.

DISCUSSION

The topography and pattern of early atherosclerosis are distinctive. Duguid[18] suggested that the pattern is determined by mechanical stresses to which the aorta is subjected at points where it is anchored by distributory vessels. This does not seem to explain the details of the distribution of atheromatous lesions (e.g. on the lateral wall of a bifurcation). Duff and MacMillan,[19] Willis,[20] Texon, Imparato and Lord[21] and Rodbard[22] recog-

nized the importance of mechanical factors, but stressed the response of the arterial wall to mechanical stimuli. While we agree that mechanical factors are important, we would suggest that they can operate primarily by determining the sites at which sedimentation occurs, without invoking the vital property of the artery. The flow chamber surfaces can be considered similar to a diffusely injured endothelium. The deposits, however, are not uniform but occur mainly at the points where there is a change in the flow pattern. Young[23] has recently published some quantitative data concerning the pattern of deposits in rabbits. It is possible that this process in the intact animal is enhanced by microtrauma to the vascular wall produced by mechanical stresses (e.g. jet lesions).[24] In our mechanical models, there is no vital structure and the channels are not expansile, and yet there is an impressive similarity between the sites of the deposits and the sites of nascent atherosclerosis.

Furthermore, the pattern of the deposits in the brass flow chamber shows that if the earliest phenomenon be deposition, the pattern produced would be just that of the characteristic "butterfly" lesion observed about unpaired intercostal orifices in early atherosclerosis. It is possible that the pressure theory could be adapted to explain the "butterfly" pattern *in vivo*, but it is difficult to see how this explains such formations in mechanical models. Even if deposition occurs only on damaged surfaces, it still seems probable that its extent will be modified by flow factors.

The finely rippled pattern encountered *in vivo* is also explainable as a sedimentation phenomenon: it is encountered not only in the mechanical models but elsewhere in systems where the rate of flow is fluctuating, as on the sand of a wave-washed seashore.

These experiments show that the topography and pattern of early atherosclerotic lesions can be mimicked by the deposits formed by the passage of blood through mechanical models. We cannot evaluate the part played by the vital properties of the arterial wall in atherogenesis: we merely wish to point out that it is not necessary to suppose their participation, since the flow chamber is not a vital structure. It has been shown by Duguid,[2] and by Movat, Haust and More,[3] that in atheromatous subjects, fibrin and platelet-fibrin thrombi may form on areas of endothelium which appear morphologically to be normal.

The pathological findings referred to above suggest that microthrombi could be an early component of atherosclerosis. It seems probable then that if atherosclerotic lesions can originate from minute thrombi, the platelet, as emphasized by French[8] and by More and Haust,[7] must participate both as a source of thromboplastin for the formation of thrombin and as an adhesive. Furthermore, the eddies at the sites where deposition occurs exert a centrifugal force which tend to throw particulate matter against the surface. Platelets and red and white cells are particulate. Fibrin, however, is not known to exist in normal blood. Its precursor, fibrinogen, is present in a colloidal state and is not readily precipitated by centrifugal force. It is converted into fibrin only as a result of complex processes. In a static system with optimum tissue thromboplastin, this takes at least 10 seconds. It is, therefore, more likely that the adhesive particulate matter already circulating in the blood will be important in the early deposits and that fibrin can then form on the surface of these deposits. There is a considerable body of evidence which supports the concept that thrombi, in particular arterial thrombi, begin by the clumping and fusion of platelets,[4-6, 25-28] though it is possible that trace amounts of fibrin form on the surface of the platelets very early in the reaction. It should be pointed out that there are investigators who consider fibrin deposition on the endothelium to be a normal process.[29, 30]

Our observations on the formation and composition of deposits in the flow chambers have shown that three independent techniques—microscopy, fluorescent antibodies and radioactive tagging of platelets—can be used to reveal that the blood platelet is *always* present in these deposits. With the smallest deposits, altered platelets were frequently the only demonstrable constituent. These findings confirmed by a different technique the observations of Shinoya[31] and of Best, Cowan and MacLean.[27] Poole,[32] using an *in vitro* flowing system (as distinct from clotting of static blood in a test tube), showed that early thrombi consist almost exclusively of platelets and a few white blood cells.

In all the flow chamber deposits, sudanophilia was demonstrable. This constituted a point of similarity to the fat streaking seen in aortas stained with Herxheimer's Sudan IV. Various factors in plasma are known to adhere to, or to be adsorbed by, the platelet.[33] The question arose whether lipid was one such constituent. Correlation between the cholesterol content of the serum and of the blood platelets in human subjects was good. This evidence suggests that the lipid content of encrustations and, therefore, possibly of atheromatous lesions may be determined by the lipid content in the blood. Furthermore, it is apparent from the electron microphotographs that platelet thrombi are not solid but contain interstices with trapped plasma. Thus the trapped plasma will also contribute its lipid content to the deposit.

Although this evidence suggests that hydraulic factors and the particulate matter of the blood could go a long way towards explaining some of the features of early atherosclerosis, certain points must be remembered. Although it has been suggested[34] that platelets react with normal endothelium, complete proof of this is still lacking. When evaluating the possible role of encrustations in the early stages of atherogenesis, this question will have to be answered.

SUMMARY

In extracorporeal circulations, the pattern and distribution of deposits formed in flow chamber models of various vessel configurations are strikingly similar to the pattern and distribution of lesions believed to represent incipient atherosclerosis at comparable sites in the vascular tree.

The earliest and only consistent components of flow chamber deposits are the blood platelets. This has been demonstrated by microscopy, by radioactive tagging of platelets and by the use of fluorescent antibodies.

The cholesterol and phospholipid content of blood platelets, washed once, shows a good correlation with that in the plasma.

It is suggested that a fundamental mechanism of atherogenesis may prove to be the deposition of platelets, largely determined by hydraulic factors and that plasma may be of importance in determining the lipid content and extent of the deposit.

The models used in these experiments were designed with the assistance of the Hydraulics Division of the Ontario Hydro Electric Power Commission; the models were made at their hydraulic models section. We would like to express our gratitude to the Chairman of the Commission and to Dr. D. K. Grant for their co-operation, and to the members of the Hydraulics Division, Mr. O. E. Johnston, Mr. J. B. Bryce, Mr. D. G. Harkness, Mr. D. M. Foulds, Mr. W. G. Crane and Mr. R. Griffiths, whose assistance made this work possible.

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Mr. I. Gryner of the Department of Pathology and Bacteriology, Ontario Veterinary College, prepared and examined the sections with the electron microscope. Drs. D. Secord and H. D. Geissinger aided in the preparation of many of the animals.

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PAGES OUT OF THE PAST: FROM THE JOURNAL OF FIFTY YEARS AGO

The amendment to the "Ontario Medical Act," which permits of the registration of osteopaths, is not such a monstrous thing as it seemed at first. I take the view that a man is justified in practising any pathy he wishes, provided he has obtained a sufficient knowledge of the anatomy of the human body, its physiology, and the disease processes to which it is liable. It will be obvious to every sane man that such knowledge is absolutely essential; for how can any one attempt to treat a disease without understanding the nature of the disease in question or normal conditions?

At the present time the public is at the mercy of a large number of uneducated charlatans, whose work is not only of no value in any real disease, but is often of a highly dangerous character. We have all met with cases in which this lack of knowledge has resulted disastrously to the unfortunate patient.

If, as is proposed in the Bill, those wishing to practise osteopathy must pass an entrance examination equal to that of any practitioner of medicine, and in addition pass a primary and final examination, which would include all the essential subjects, substituting their pathy for medicine, we should have no objection to their being licensed by the Ontario Medical Council. In this way the public would be protected by requiring of osteopaths a sufficient knowledge of these fundamental subjects, which is absolutely essential before attempting to treat the sick. If, after they have passed these examinations, they still think there is any value in their particular pathy, we have no objection to their practising. I would take a similar attitude towards any other pathy.

When the Ontario Medical Council was organized the homoeopaths and eclectics were taken in and the same examinations precribed for them as for regular practitioners. What has been the result? The eclectics have practically ceased to exist. Very few homoeopaths have been taking the examinations, as is shown by the fact that at the present time only forty-eight are practising in the province of Ontario.—H. A. Bruce: Presidential Address to the Ontario Medical Association: *Canad. Med. Ass. J.*, 2: 580, 1912.