eral anti-histaminic agent. In the anesthetized dog, the major cardiovascular effects of meperidine appear to be mediated through release of histamine, although a combined effect of meperidine and histamine on the peripheral vascular system is not ruled out. Administration of anti-histaminic drugs in large doses has failed to separate these effects and other means are being considered to settle this point.

The differences in configuration of the tracings illustrating the effects of histamine and meperidine (Fig. 2) probably have two causes. First, the initial drop in the histamine tracing is more precipitous because more histamine is perfused per unit of time. These animals received the amine in one or 2 seconds whereas the meperidine group released this amount of endogenous histamine over a one minute period. The return to normal is more rapid in histamine treated animals because there is no prolonged release of the amine. This is evident by the rapid return of the blood values to control levels (Table 1).

Summary. Meperidine is a potent histamine releasing agent in the anesthetized dog. The quantitative and kinetic aspects of this phenomenon strongly suggest that it is responsible for the cardiovascular effects of this drug.

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Employment of Polyethylene Tubing for Production of Intra-Arterial Thrombi in Rabbits and Rats.* (26478)

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Recently we have described(1) a simple method of inducing thrombi of approximately uniform size and weight in both the arteries and veins of rabbits and rats. This technique consisted of insertion into the lumen of a vessel of a small coil of Mg-Al wire previously treated with $ZnCl_2$. The thrombi form within a few hours, apparently triggered by the rapid electrolytic dissociation of the coil.

Other methods were sought for inducing intravascular thrombosis in an intact vessel without interrupting flow of blood, however, because a less fulminant, slower forming thrombus might offer certain advantages in various experimental studies. The present report describes a new method which is simple, relatively reliable and produces thrombi of approximately uniform size, weight and content.

Methods. It was discovered accidentally that small segments of polyethylene tubing† will serve as a nidus for thrombus formation. Further studies indicated that if these segments of tubing are split lengthwise and the resulting gutter-like segments inserted, thrombus formation almost invariably occurs within 72 hours in the trough of the segments.

Accordingly, 30 young male rabbits were anesthetized with pentobarbital and their aortae isolated below the exit of the renal arteries. Each aorta was gently occluded by traction upon ligatures approximately 2 cm apart and a very small incision made. Through this incision was inserted a 0.5 inch segment of polyethylene tubing (i.d. = 0.045″ and

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† Clay-Adams PE-160, Intramedic (Medical Formulation PHF) polyethylene tubing.

o.d. = 0.062″) cut in half lengthwise. Insertion was manipulated so that the cut walls of the semicircular section of the polyethylene segment were usually in close contact with the intimal surface of the aorta and the convex portion of the segment lay free in the lumen. When this is done, the thrombus forming later is directly in contact with the intima. A silk thread carried at one end of the polyethylene segment served as an anchor. The aortic incision was closed by a single, previously placed mattress type of suture.

Essentially the same procedure was followed with 10 rats except that the tubing employed was smaller in diameter (i.d. = 0.023″ and o.d. = 0.038″) and shorter (0.25″).

Rabbits were sacrificed either at 72 hours, 3 weeks or 10 weeks. All rats were sacrificed at 72 hours. The thrombotic processes found in the aorta were examined grossly and microscopically.

Results. The rabbits tolerated insertion of polyethylene segments as well as other rabbits had tolerated insertion of Mg-Al coils (1). Thus 26 of the 30 rabbits survived the process of insertion itself. The remaining 4 died exhibiting paralysis of the hind legs immeditely subsequent to surgery. All of the 10 rats survived the insertion.

Nine rabbits and all 10 rats were sacrificed 72 hours after insertion of tubing. A rather firm thrombus was found at site of operation in 7 of the 9 rabbits and in 8 of the 10 rats. It usually occupied the entire trough of the tubing. The gross appearance of the thrombi in rabbits and rats was identical except for the difference in size. In most cases (Fig. 1), this thrombus was almost pure white, but occasionally small areas obviously rich in red blood cells were scattered here and there in the thrombus. At 72 hours, thrombi of both species were more firmly attached to the wall of the tubing than to the intima of the vessel.

On microscopic examination, the 72 hour thrombi of both species appeared identical. They were composed of an amorphous almost acellular structure interlaced with fibrin strands, (as identified by Mallory's Phosphotungstic Acid Hematoxylin Stain). In some sections, scattered clumps of red and white blood cells were observed. No cellular infiltration from the aortic intima was observed at this time.

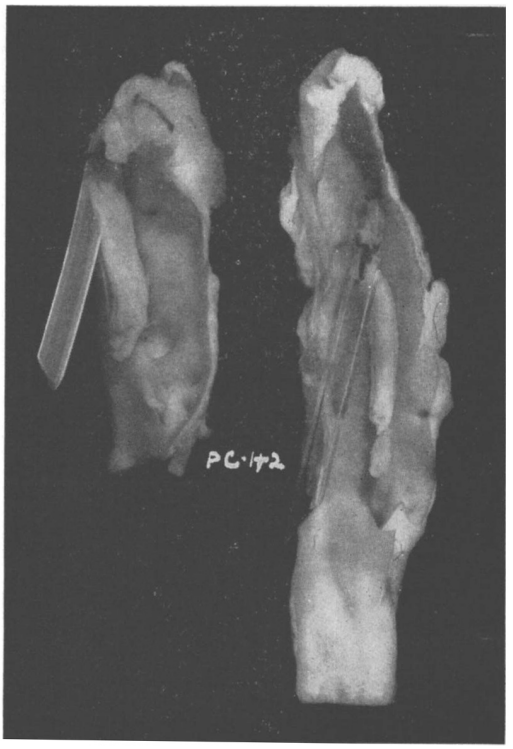

FIG. 1. Two rat aortas, 72 hr after insertion of polyethylene tubing. The thrombus in each aorta has been freed from the trough of the tubing. Note the white rather solid appearance of both thrombi and lack of attachment to the aorta. Silk thread anchoring the segment of tubing can be seen in aorta on right.

Eight of the 10 rabbits sacrificed at 21 days exhibited thrombus formation in the space between the concave surface of the polyethylene tubing and wall of the aorta. The thrombi were adherent to the vessel walls and gross intimal reactions were easily discerned. Also, clumps of cells were beginning to extend over the entire length of polyethylene tubing (Fig. 2). On microscopic examination (Fig. 3), the interior of the thrombus was found to resemble closely the 72 hour thrombus. In the 21 day thrombi, however, proliferating intimal cells could be discerned extending from the intimal tissue directly beneath the thrombus. New small blood vessels also were observed accompany-

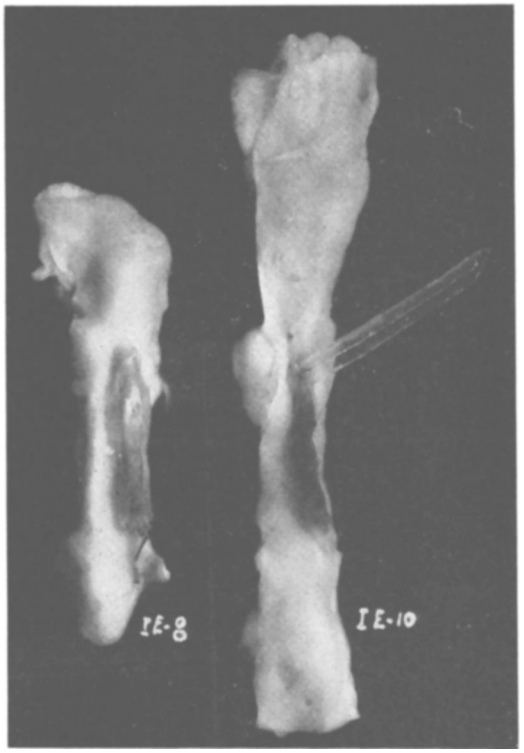

FIG. 2. Two rabbit aortas, 21 days after insertion of tubing. Tubing has been removed from aorta on the left and lifted from aorta on the right. A superficial post mortem film of fresh clot rich in red blood cells is attached to the primary thrombus in aorta IE-10. In aorta IE-G, the new intimal growth had begun to ascend the external convex sides of the tubing. Both thrombi are now firmly attached to wall of aorta.

ing the proliferating intimal tissue. That part of the media directly underlying the thrombus also usually showed partial fragmentation and disorganization. In none of the thrombi was there an evidence of past or present inflammatory processes.

In the 9 rabbits sacrificed at 10 weeks, the polyethylene segment was found to be totally covered by newly formed tissue. This covering tissue was incised and in 7 of the 9 rabbits the troughs were filled by rather firm tissue of pinkish white color (Fig. 4).) In the remaining 2 rabbits, the tubing troughs had faced the luminal flow of blood. Nevertheless, the tissue in these latter 2 was identical with that observed in the others.

Microscopic examination of these latter 2 plaques (Fig. 5) revealed that the original thrombus had been totally replaced by living tissue newly derived from the intima of the aorta. No histological differences were observed between the plaques regardless of the orientation of the tubing within the aortic lumen.

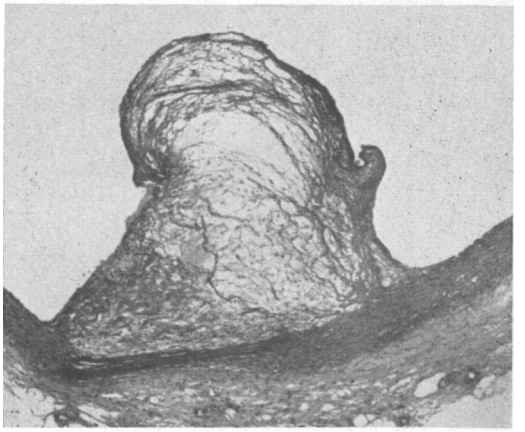

FIG. 3. Low power view of thrombotic process in the rabbit aorta (× 22) 21 days after insertion of tubing (PTAH stain). The rather dense structure of the thrombus with few cells and a few interlacing strands of fibrin is shown. The newly formed intimal tissue beginning to ascend the lateral walls of the thrombus also can be seen. Note thinning and partial disorganization of that portion of the media immediately subjacent to thrombus.

Discussion. The desirability of inducing thrombi in the arterial system has been intensified ever since Duguid(2) revived Rokitansky's concepts(3) concerning the possible origin of atherosclerotic plaques. Most of the various methods that have been employed in inducing thrombi (4,5,6,7,8,9,10, 11) have consisted either of injecting already formed thrombi into the venous system or of inducing massive thrombosis in a totally occluded arterial segment. Such methods, however, are not suitable for experiments concerned with the possible relationship between thrombus formation and atherosclerosis(2) because the thrombi induced by most methods either form in the venous system or in an artery whose flow has been totally obstructed by the thrombus itself. Stone and Lord(12) observed that Mg-Al wire induced thrombi in the rapidly flowing blood of arteries. This method as modified by us(1) appeared to offer a very satisfactory technique for study of arterial thrombus formation and subsequent plaque placement. The

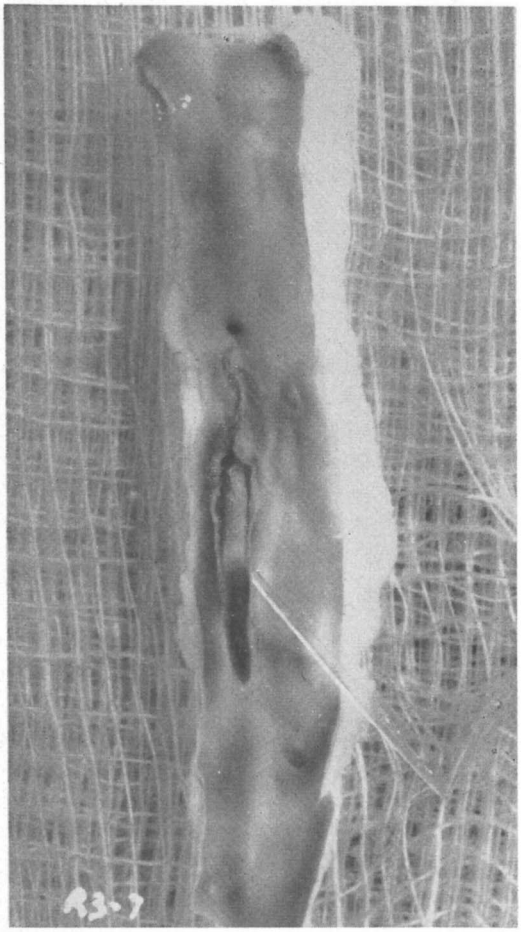

FIG. 4. Aorta of rabbit 10 wk after insertion of tubing. Tissue covering the tubing was incised and tubing removed for photography. Although the central mass exhibits the same external form as thrombi in Fig. 1 and 2, it is no longer a fresh thrombus but has been organized and replaced by fibrous tissue. A small and superficial postmortem film of fresh clot is attached to lower half of plaque. Note lateral prolongations of intimal tissue that had covered external surface of the tubing.

presently described method offers a second technique by which arterial thrombi may be regularly induced and their transformation into plaques observed at all stages. The aortic plaques resulting both from introduction of polyethylene tubing and insertion of Mg-Al coil bear a very close resemblance to the pearly white plaques observed in the human aorta.

Summary. Arterial thrombi were produced in the aortae of rabbits and rats by insertion of specially fashioned polyethylene tube segments. These thrombi differ from clots formed in static blood in that they are relatively acellular precipitates containing fibrin and probably platelets. These thrombi eventually are organized and lead to plaques in which all elements of the initial thrombus are replaced by tissue from the intima. The resemblance of these plaques to the pearly white plaque frequently seen in the human aorta is striking.

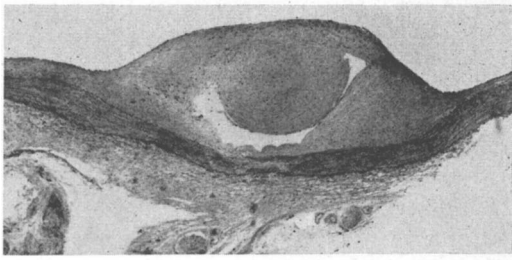

FIG. 5. Low power view of thrombotic process in rabbit aorta (× 16) 10 wk after insertion of tubing (H and E stain). In this animal, the trough of the tubing had faced the lumen rather than wall of aorta. The thrombus within the trough has been completely organized by intimal tissue that had ascended the walls of tubing, then had descended into its trough. The empty space separating the central mass from walls of plaque was produced by the tubing removed prior to section and staining. Note thinning and partial disorganization of media beneath site of tubing.

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