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A Method of Exteriorizing the Guinea Pig Omentum for Microscopic Examination and its use in the Study of Vascular Collapse in Diphtheria

by

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(With three Plates)

PREVIOUS studies by the author (in preparation) have shown that early toxaemia in rabbits following infection with *Corynebacterium diphtheriae type gravis*, is accompanied by a diminution in the intensity of constrictor responses of the peripheral blood vessels in the skin and splanchnic bed to physiologic, intravenous doses of 1-noradrenaline ; it was also suggested that this failure constituted the vascular factor in diphtheritic shock. The experimental methods used permitted direct visualization of the smaller blood vessels with quantitative measurements of their constrictor responses. The use of similar methods in such studies in the late stages of toxaemia when circulatory failure supervenes, however, entails the error caused by poor transport of the injected drug. It was therefore expected that with onset of failure of the circulation the responses to topical application of the pressor substance on exposed tissue rather than its parenteral injection would provide more reliable data on the pressor drug sensitivity of the vessels. A further advantage of topical use might be the absence of local reflex effects of the generalized cardiovascular response to the parenterally injected drug. This report describes an adaptation of the rat meso-appendix technique of Zweifach (1948) to the omentum of guinea pigs which are very susceptible to experimental diphtheritic infection and its use in the study of the responses of omental arterioles in the later stages of diphtheritic toxaemia, to topically applied l-noradrenaline.

Methods

Animals : Adult guinea pigs of the English and Abyssinian strains were used, comparative observations on normal and infected animals having been made as far as was possible, on pairs of the same strain and sex.

Microscopy : On account of the warmth of the irrigating fluid (37°C-38°C) the use of a high power objective was inconvenient on account of the condensation of moisture on the objective lens. A × 10 objective with a × 17 ocular and an extended tube length provided adequate magnification for quantitative observation aided by a micrometer scale fitted in the ocular. The total magnification used in these experiments was × 240.

Irrigating fluid : The exposed omentum was irrigated by Ringer-Locke solution at pH 7 containing 1% gelatin, maintained at a constant temperature of 37°C-38°C.

Anaesthesia : Light surgical anaesthesia was induced with pentobarbitone sodium 4.0 mg. per 100 g body weight injected intramuscularly. Under this dose the animal was immobilized just sufficiently for continuous observation for at least 1 hour.

Drugs : Pure l-noradrenaline bitartrate powder was dissolved in distilled water to a strength of 1 mg. noradrenaline base per ml. This stock solution was stored in intervals between use at 4°C and was used within 2 weeks of preparation. This solution was diluted for use in 50 ml. normal saline containing 0.2 % N/10 HCl as stabilizer to a strength of 10 μg./ml. From this solution further dilutions were made in normal saline to contain 1.0 μg. and 0.1 μg. respectively ; 3 drops of each solution delivered from a No. 20 needle constituted the dose of the vasoconstrictor. Each dilution was prepared immediately before use.

Cultures : Toxaemia was induced by the subcutaneous injection of 0.2 ml. of a freshly prepared, approximately 750×10^6 organisms per ml. nutrient broth suspension of a 24 hr. Loeffler slope culture of *Corynebacterium diphtheriae type gravis* NCTC No. 7282. Preliminary tests on adult guinea pigs of similar size infected with this dose showed that the survival time infection varied from 22 to 26 hr. In all infected animals observations were therefore begun about 22 hr. after infection.

Exteriorization of the omentum : Having shaved the upper abdomen of the animal, exteriorization of the gastric end of the omentum was made through a $\frac{3}{4}$ cm. median upper abdominal incision with the animal lying on its dorsum. The location of the incision at this relatively high level with the animal on its side, in contrast to the 2.5 cm. incision in the lower right quadrant used in the rat mesoappendix exposure, was considered less likely to cause loss of peritoneal fluid during prolonged examination. The gastric end of the omentum was chosen in preference to the splenic extremity on account of its content of blood vessels of assorted size, and the scantiness of its adipose tissue. A loose swab of absorbent cotton wool borne on a flexible ekel, soaked in warm saline or Ringer-gelatin solution was then introduced through the incision towards the gastric region for a distance of about 2 cm. The swab was then gently withdrawn with the free edge of the omentum lightly adhering to the wool. The exteriorized tissue was never handled except with soft cotton wool swabs moistened with Ringer-gelatin solution. With the omentum thus exteriorized and temporarily covered with moistened cotton wool, the animal was transfered to the stage of the microscope. The animal was kept on its side within a cardboard tube of suitable diameter with its upper abdomen exposed through a wide aperture made in the tube. The tube was strapped onto the stage by means of two rubber bands. With the abdominal incision aligned opposite the irrigation chamber the exposed omentum was very carefully spread by means of mounted cotton wool swabs, over the central observation stage. This chamber fig. 1 consisted of a plate of Perspex with a moulded gutter surrounding a central circular elevated observation stage which was about 1 cm. in diameter. This central stage permitted continuous maintenance of the field under observation in one plane. The irrigating fluid which was directed onto the tissue on this stage was led off through a drain

tube inserted into the gutter. Having selected a suitable area of the omentum for examination the rest of the tissue was covered with thin wisps of absorbent cotton wool which retained the irrigating fluid. The irrigating drip fig. 2 delivering 60 drops per min. (1.7 ml.) at 37°C-38°C was then turned on such that the exteriorized tissue was uniformly bathed.

The criteria described by Zweifach (1948) for assessing the suitability of the rat mesoappendix preparation were used in accepting these omentum preparations for further examination. Preparations deviating from these were abandoned. Judged by these criteria normal tissue showed intermittent capillary circulation fig. 3, with minimal leucocyte sticking in the venules, and absence of stasis of blood and haemorrhages. All observations were made under approximately equal conditions of ventilation of the room and its temperature which was about 30°C.

Type of vessel studied : While general observations of the vessels in the exposed tissue were made to assess the suitability of the preparation, measurements of the constrictor responses were confined to arterioles of approximately 35 μ in diameter on account of their importance in the maintenance of blood pressure.

Measurement of responses

Examination of the preparations was made about 15 min. after installation to allow of the subsidence of any disturbances due to the manipulations. All drug analyses were made within an hour of exteriorization. The test dose of the noradrenaline was applied to the field of tissue at a constant rate with the irrigating drip temporarily deflected from this field. Records were made of the resting diameter of the vessel under observation and of its diameter at the height of contraction following the dose of noradrenaline. The percentage reduction of the vessel diameter was taken as the intensity of the constrictor response. This end point was preferred to that of the threshold concentration of noradrenaline which produces narrowing of the terminal arterioles and precapillaries (Zweifach, 1948) which is just sufficient to cause slowing of the flow through the capillary bed, on account of the subjective decision required of the observer in the latter measurement. The contractions following the application of the solutions containing 1.0 μg. and 0.1 μg. per ml. were uniform and were easily measured. Although the reactions to concentrations of 10.0 μg. and 100.0 μg. per ml. were also observed measurements of their effects were not possible on account of the varicose and irregular contractions which ensued. The irrigation was resumed after the contraction reached a maximum. Tests were made at intervals not less than 3 min. to avoid tachyphylaxis. Three readings were taken, one for each application of a given concentration and the mean response was used in plotting the dose response curve fig. 4. The drug solutions were used in increasing concentrations. Control observations were made of the vessel responses to 3 drops of the diluent alone and to the temporary deflection of the drip for a period equal to that taken by the vessel in reaching a maximum contraction to the lowest concentration of the drug.

Having completed the observations the omentum was reinserted and the incision closed.

General Observations

Normal animals : Probably as a result of the anaesthetic no spontaneous vasomotion of the arterioles was detected at the magnification used viz. 240, (Clark and Clark, 1932). The application of the diluent alone or the temporary deflection of the drip, caused no alteration of the state of the vessels. The earliest visible reaction to the concentrations of 1.0 μg. and 0.1 μg. per ml. which began about 15-20 seconds after the application of the dose, was a slowing of the arteriolar flow followed by uniform contraction of the vessel. To the larger doses of 10.0 μg. and 100.0 μg. per ml. the diameter of the contracted vessel was highly irregular giving it a varicose appearance. The flow in such states had often ceased. The flow itself and the calibre of the vessel altered independantly of each other for it was often observed that brief stasis occurred in a patent vessel while a contracted one permitted rapid flow. A similar phenomenon of independance of blood flow and spontaneous vasomotion was described by Chambers and Zweifach, (1944). Resumption of the drip which washed off the dose of noradrenaline caused a return of the circulation to its former state.

Infected animals : The corresponding reactions observed in the arterioles of infected animals were different from those of normal animals in the following respects. In the toxaemic animals the onset of retardation of flow and the contraction of the vessel were delayed to about 20-30 seconds after application of the noradrenaline, while the intensity of the contraction especially to the larger doses of 10.0 μg. and 100.0 μg. per ml. was strikingly diminished, with absence of the nodularity and stasis of flow.

Results

The noradrenaline sensitivity of the arterioles of toxaemic animals when compared with that of normal animals, the table and fig. 4, is seen to be diminished. The survival times however do not bear a constant relation to the intensity of the diminution. The absent or diminished flow-retarding effect in the infected animals could indicate a failure of the precapillary sphincter mechanisms.

Discussion

The possibility that a failure of the vasoconstrictor mechanisms of the peripheral vessels occurs in diphtheritic toxaemia has suggested itself to workers on this disease on account of the generalized visceral congestion often seen in fatal cases. However the nature of the lesion responsible for the failure has been in dispute and it has been variously concluded that the smooth muscle of the blood vessels (Brodie, 1899), the peripheral vasoconstrictor nerves (Myers, 1933), the vasomotor centre (Yabe, 1922) or the myoneural junctions in the splanchnic blood vessels (Edmunds and Johnston, 1928) is responsible for this failure. A reduction in pressor amine sensitivity of smooth muscle in tissues injected with diphtheria toxin has been demonstrated by Agalwar and Holt (1959) and by Agalwar and Nasmyth (1959). Since, however diphtheria toxin evokes a severe inflammatory reaction, the inference that

this effect of pressor amine refractoriness is a result of the specific action of diphtheria toxin is open to question on account of the demonstration by Spector and Willoughby (1960) of increased destruction by monoamine oxidase of an adrenaline-like substance in acutely inflamed tissue.

The results reported here indicate that the constrictor responses of the splanchnic arterioles of diphtheritic guinea pigs to l-noradrenaline are of diminished intensity as compared to those of normal animals. A similar failure to endogenous pressor amines may constitute the vascular factor of circulatory collapse later in the disease. Since the precapillary sphincters are even more sensitive to the pressor amines (Chambers and Zweifach, 1944) it is possible that these structures too suffer a similar failure in toxaemic animals as indicated by the feeble or absent flow-retarding effect on the application of noradrenaline. A failure of this mechanism which regulates the circulation in the true capillary bed leads to pooling of blood in this area which is an important shunt in the circulation ; the 'distributory oligaemia' which ensues may also contribute to the shock. In view of the well established supportive role of the glucocorticoids of the Zona Fasciculata of the adrenal cortex in the responses of blood vessels to the physiological pressor amines adrenaline and noradrenaline (Fritz and Levine, 1951 ; Ramey, Goldstein and Levine, 1951) the origin of the loss of responsiveness demonstrated in the present experiments may be attributed to the adrenal cortical lesions seen in guinea pigs dying of diphtheria. Fig. 5 shows the cortex of a guinea pig that died 22 hr. after infection with the same strain of *C.diphtheriae type gravis* as was used in these experiments. The lesions are localized to the Fascicular zone and consist of congestion, cloudy swelling and patches of necrosis. The high incidence of macroscopic lesions seen in the adrenals of guinea pigs dying of experimental diphtheria has been commented on by Barratt (1923, quoted by Wilson and Miles, 1955). It seems more probable that these adrenal lesions are the cause rather than the passive result of splanchnic pooling of blood, through this vasoconstrictor failure which probably ensues from a reduction of the output of the secretion of the fascicular zone resulting from the lesions described above. It has been clearly shown (Bolton, 1905 ; Dudgeon, 1906) that the myocardium is often extensively damaged in diphtheria and as such plays a large part in the shock in this disease. If this myocardial insufficiency does not permit an increase of arterial flow to the adrenal gland to compensate for the heightened activity of the fascicular zone in its response to the shock (Stoner and Green, 1950) the resulting state of relative ischaemia may lead to the degenerative lesions with a fall in the gluco-corticoid output and therefore of the pressor amine sensitivity of the blood vessels. A cycle of events would then be established leading to irreversible shock and death.

The mechanism postulated here is similar to that described to explain the reduction in noradrenaline sensitivity of vessels in diphtheritic rabbits shown in a previous study, (in preparation). It is again possible that this same mechanism might operate in the shock associated with acute infections in which adrenal lesions of a similar nature are commonly seen (Rich, 1944).

The reversal of this effect by administration of corticoids was not considered conclusive of this suggested mechanism since these hormones may potentiate pressor responses in normotensive persons (Kurland and Freedberg, 1951).

Summary

A method is described for exteriorizing the guinea pig omentum for microscopic examination under irrigation with warm Ringer-gelatin solution. The responses of omental arterioles of normal and diphtheritic guinea pigs infected with *C.diphtheriae type gravis* to topically applied l-noradrenaline have been studied using such preparations. A reduction in intensity of constrictor response to this drug in infected animals has been demonstrated, and it is suggested that a similar failure to endogenous noradrenaline might constitute the vascular factor in diphtheritic circulatory collapse.

Acknowledgement

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LEGENDS TO FIGURES

Figure 1.—Irrigation chamber and animal board in position. × c½.

Figure 2.—Diagram of irrigation apparatus.

Figure 3.—Vessels in guinea pig omentum. V=tortuous venule, A=arteriole, C=capillary with temporary absence of corpuscles. × 240.

Figure 4.—The effect of l—noradrenaline on the diameter of omental arterioles of normal and diphtheritic guinea pigs. Continuous lines refer to mean responses in normal animals, interrupted lines refer to mean responses in infected animals. Numerals and simple letters refer to animals in normal and infected series respectively.

Figure 5.—Adrenal gland of guinea pig 22 hr. after infection with *C. diphtheriae type gravis* showing degeneration and necrosis in Zona Fasciculata (F). G=glomerular zone, R=reticular zone, M=medulla. Haematoxylin and Eosin. × 150.

Table showing relation between vessel diameters in arbitrary units and dose of l—noradrenaline.

Normal Animal No.	*Strain* *	*Sex*	*Weight in g*	*Concentration of l-noradrenaline μg/ml* 0.1 *μg* *a*	0.1 *μg* *b*	1.0 *μg* *a*	1.0 *μg* *b*	*approximate diameter of arteriole μ*	†S *in hr.*
1	E	f	506	0.65	0.55	0.65	0.45	48	..
				0.65	0.55	0.65	0.45		
				0.65	0.55	0.65	0.45		
2	E	m	413	0.5	0.425	0.5	0.3	37	..
				0.5	0.425	0.5	0.3		
				0.5	0.425	0.5	0.3		
3	A	m	416	0.7	0.5	0.7	0.4	52	..
				0.7	0.5	0.7	0.4		
				0.7	0.5	0.7	0.4		
4	E	m	411	0.625	0.55	0.625	0.45	46	..
				0.625	0.55	0.625	0.45		
				0.625	0.55	0.625	0.45		
5	A	f	480	0.5	0.375	0.5	0.3	37	..
				0.5	0.375	0.5	0.3		
				0.5	0.375	0.5	0.3		
6	A	m	395	0.5	0.425	0.5	0.3	37	..
				0.5	0.425	0.5	0.3		
				0.5	0.425	0.5	0.3		
7	A	m	392	0.425	0.275	0.425	0.25	32	..
				0.425	0.275	0.425	0.25		
				0.425	0.275	0.425	0.25		
Infected Animal No.									
a	E	f	425	0.475	0.475	0.55	0.525	35	
				0.475	0.475	0.55	0.525		2
				0.475	0.475	0.55	0.525		
b	E	m	340	0.375	0.375	0.375	0.375	28	
				0.375	0.375	0.375	0.375		¼
				0.375	0.375	0.375	0.375		
c	A	m	319	0.85	0.75	0.85	0.675	63	
				0.85	0.75	0.85	0.675		9
				0.85	0.75	0.85	0.675		
d	E	m	197	0.75	0.725	0.75	0.7	56	
				0.75	0.725	0.75	0.7		10
				0.75	0.725	0.75	0.7		
e	A	f	375	0.375	0.35	0.375	0.275	28	
				0.375	0.35	0.375	0.275		3
				0.375	0.35	0.375	0.275		
f	E	f	..	0.425	0.35	0.425	0.3	32	
				0.425	0.35	0.425	0.3		10
				0.425	0.35	0.425	0.3		
g	A		462	0.6	0.55	0.6	0.45	45	
				0.6	0.55	0.6	0.45		12
				0.6	0.55	0.6	0.45		

*E — English strain.
*A — Abyssinian strain.
a — diameter before application of noradrenaline.
b — diameter after application of noradrenaline.
†S — survival time after experimentation.

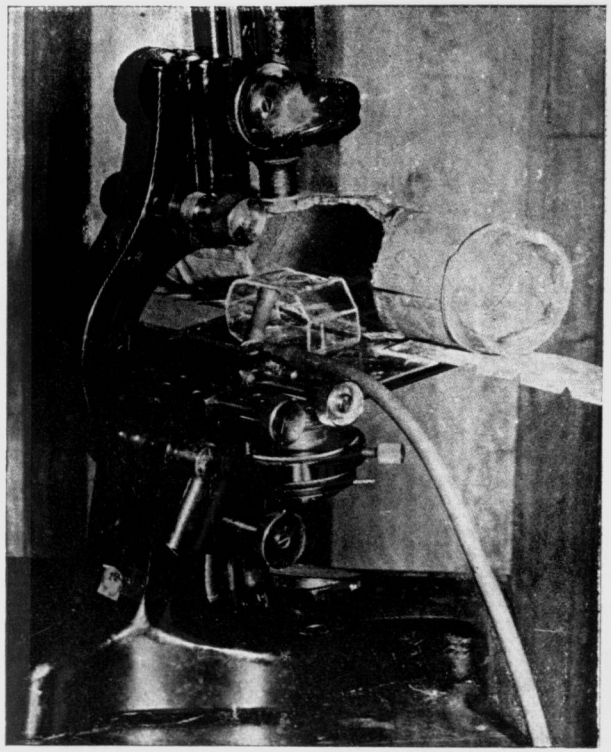

Figure 1

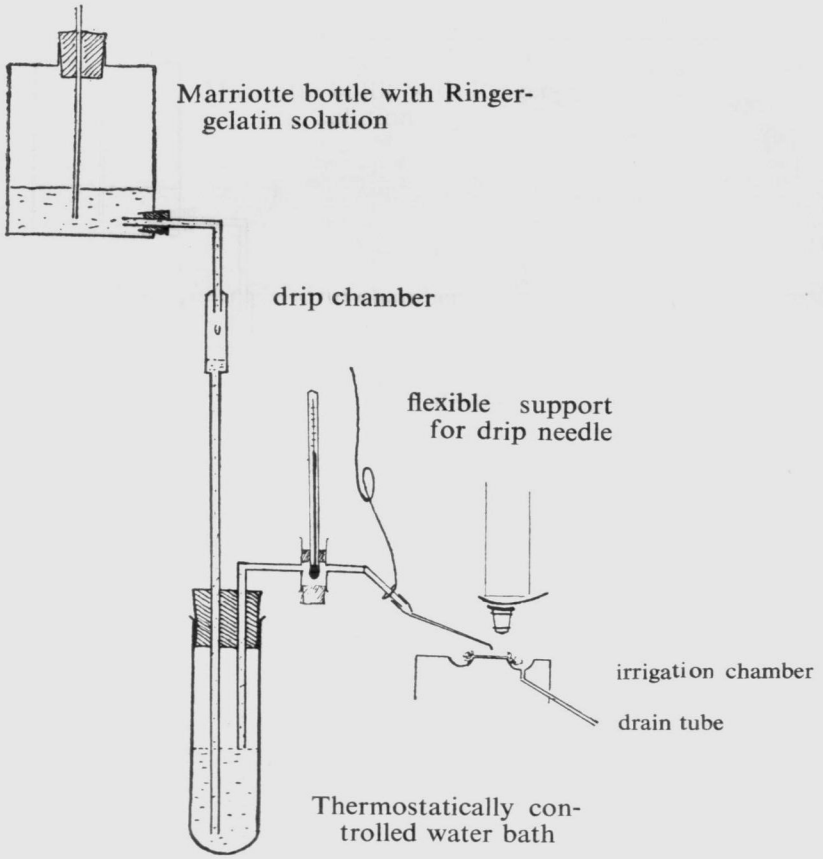

Figure 2

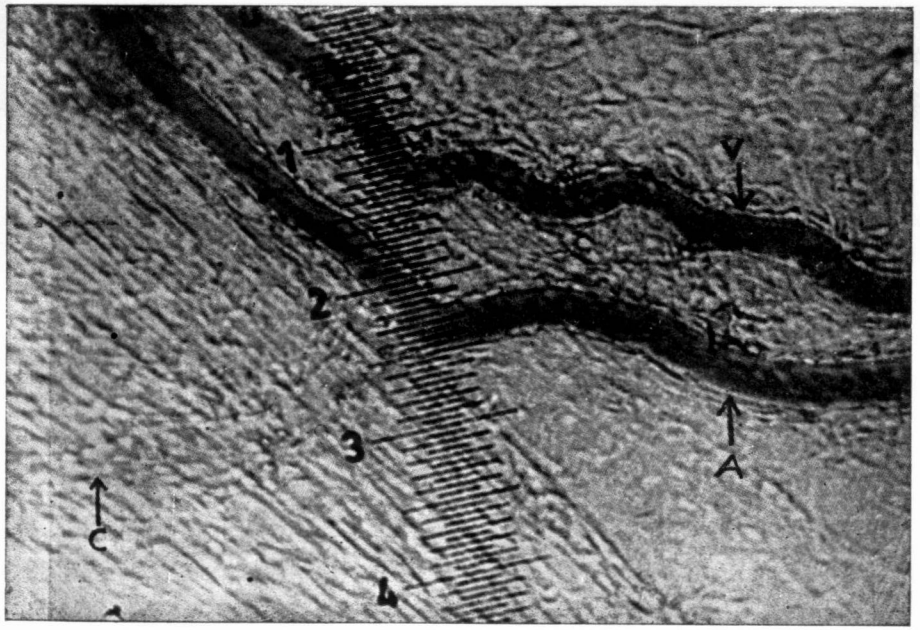

Figure 3

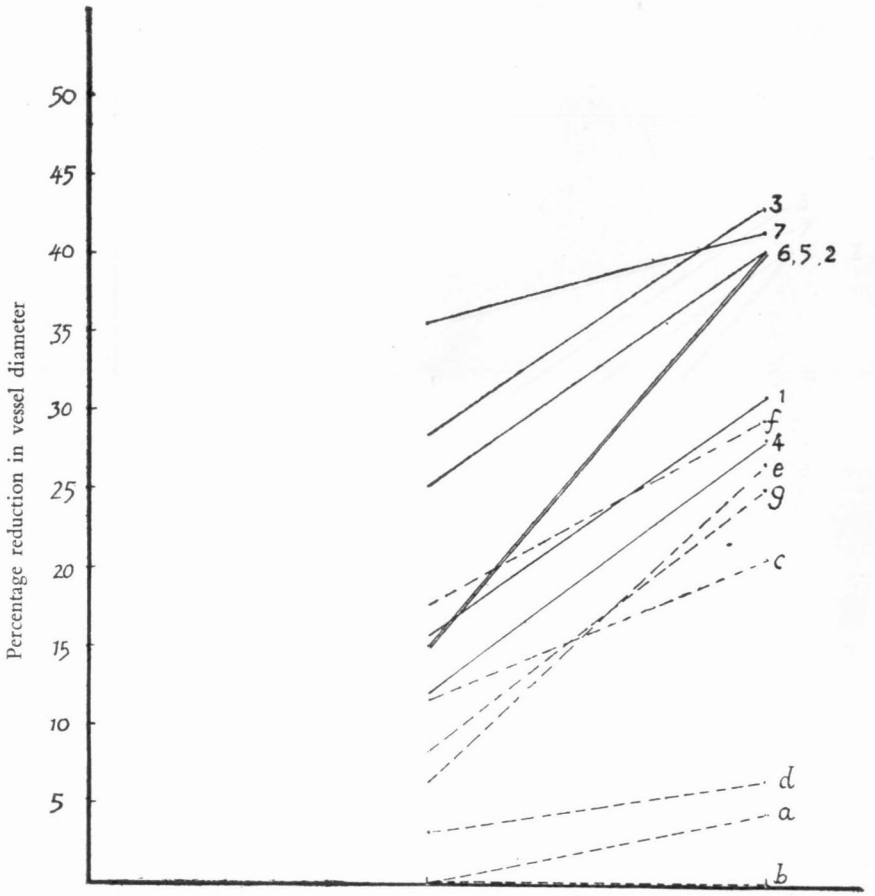

Figure 4

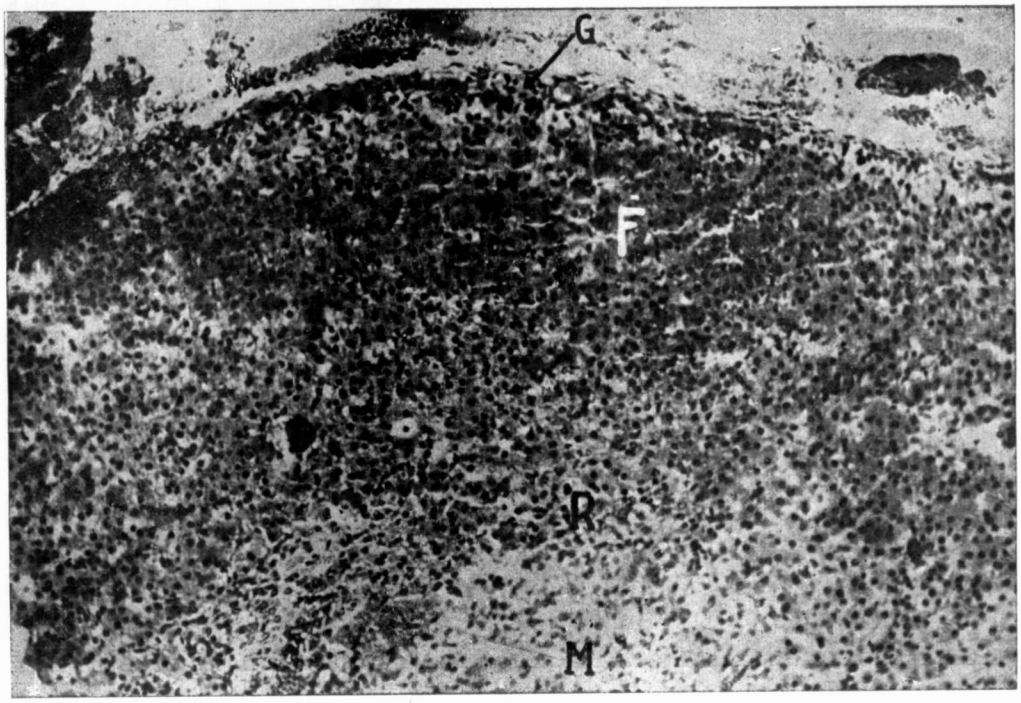

Figure 5