

## VENOUS THROMBO-EMBOLISM IN THE BANTU\*

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Ochsner and DeBaKey<sup>1</sup> found that thrombo-embolism occurred less frequently in Negro patients as compared with White patients. A racial difference has always been suspected in Africa, and venous thrombosis has been considered an uncommon disorder in the Bantu patient.<sup>2</sup> It followed, therefore, that pulmonary embolism was also an infrequent finding in this group, except when it complicated heart failure due to cardiomyopathy. Because of the supposedly rare occurrence of venous thrombosis and pulmonary embolism postoperatively in Bantu and Indian patients in Durban, Franz *et al.*<sup>3</sup> investigated the plasma fibrinolytic activity in Bantu, Indian and White subjects, and concluded that while the plasma fibrinolytic activity was twice as high in healthy Bantu controls as it was in White and Indian controls, the plasma fibrinogen levels were the same in all 3 groups. These authors also commented on the extremely low necropsy incidence of this disorder as reflected in the records of this hospital (King Edward VIII Hospital, Durban) during the 6-year period (1953 - 1958) of their survey. It must be appreciated, however, that the veins of the lower limbs are not examined as a routine and that dissection of the deep veins of the calf is only rarely performed.

In order to assess the occurrence of venous thrombosis in this group it was therefore necessary to investigate the problem on the lines followed by earlier workers.<sup>4-5</sup> Attention was paid to the veins of the lower extremities because of the major role they play in the incidence of this condition in the White races, and also because the clinically silent nature of this disorder is better appreciated today, so that lack of symptoms and physical signs in the lower limbs does not necessarily exclude the presence of venous thrombosis.

### MATERIALS AND METHODS

The material for this study was obtained from consecutive routine necropsies performed on Bantu patients of both sexes, of 10 years of age and over. Similar material was obtained from Indian postmortem examinations for comparative purposes. The majority of patients (314) studied were inpatients of King Edward VIII and Clairwood Hospitals, coming to postmortem during the period February 1964 - June 1965; of these, 49 were medico-legal cases consisting mainly of acute traumatic deaths, and a few other acute deaths following upon possible poisoning.

In addition to routine postmortem examination, the soleus and gastrocnemius muscles were removed from both lower limbs in every case. Removal of these muscles was accomplished by a medial incision extending from the

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Achilles tendon to just above the knee joint. The muscles were then dissected from the posterior aspect of the leg bones and detached high enough to include as much as possible of the popliteal vein. The posterior tibial veins were also dissected down to their small branches. The specimens were labelled and fixed for 3-4 days in trays containing 10% formol saline. The calf muscles were then sliced transversely at intervals of approximately 1 cm. and macroscopic observations recorded in each case. In every instance where a venous thrombus was present, several blocks of tissue were taken for microscopic examination. The superficial femoral vein was examined *in situ* on both sides, and the findings were recorded. Veins of the feet were not examined. On this basis a total of 363 Bantu and Indian patients were investigated for leg-vein thrombosis.

In 343 of these both lungs were also reserved after routine postmortem examination, and were labelled and fixed in 10% formol saline. The pulmonary arteries and their branches were examined for thrombi at the same time as the calf muscles were sectioned. This was achieved by first opening the larger vessels as far as was possible and then making thin sections of less than  $\frac{1}{2}$  cm. for smaller thrombi. It was found easier to feel rather than visualize thrombi in very small vessels. Again, in all instances where thrombi were suspected, blocks were taken for microscopic examination.

All blocks of tissue were sectioned at  $5\mu$  thickness and stained with haematoxylin and eosin, and lung sections were also stained by Weigert's elastic stain. These sections were studied for distinction between antemortem and post-mortem thrombi, size of vessel involved, presence or absence of organization of thrombi, and for the presence or absence of any inflammatory infiltrate in the vessel wall. Those cases where a pulmonary arteritis was found in addition to thrombi (all such cases being associated with infections of the lung) were excluded.

The race and sex distribution of the total number of cases investigated are shown in Table I.

TABLE I. RACE AND SEX DISTRIBUTION OF PATIENTS STUDIED

Race	Male	Female	Total
Bantu	189	94	283
Indian	52	28	80
Total	241	122	363

#### Analysis of Cases

This analysis is based on 2 interrelated studies: (i)

venous thrombosis, and (ii) pulmonary thrombo-embolism as found at necropsy. Since primary pulmonary arterial thrombosis is known to occur, and one cannot always be absolutely certain whether a bland antemortem thrombus present in the pulmonary arterial tree was local in origin or was the result of embolization, the term pulmonary thrombo-embolism has been used.

Besides the division into racial groups, the cases studied were further subdivided into 4 main groups, viz. medical, postoperative, traumatic and postpartum. The majority of patients (84.3%) investigated were classified as medical, and included in this group were patients from medical and surgical wards on whom no surgery had been performed. Also included here were 6 medico-legal cases suspected of dying either from poisoning or from causes undetermined. The postoperative group (2.7% of the total) included all patients who had undergone major surgery. Included here were 1 Indian and 2 Bantu patients who had suffered extensive pelvic and abdominal trauma and in whom surgical intervention was necessary. The traumatic group (11.3% of the total) included injured patients on whom no major surgery had been performed. The majority of these were cases of head injuries, stab wounds and multiple limb fractures, who arrived dead or died soon after admission to hospital. The postpartum group, comparatively a very small group (1.7% of the total), included mainly cases of abortion, with one case of rupture of the uterus.

#### RESULTS

##### Venous Thrombosis of the Lower Extremities

Of a total of 363 Bantu and Indian patients in whom deep veins of the thighs and legs were specially examined, thrombosis was observed in 141 (38.8%). The incidence of this disorder in the 4 major divisions of medical service is shown in Table II. Medical cases were separated into cardiac and non-cardiac patients, the former group including cases of congestive heart failure in both races. Venous thrombosis in the lower limbs occurred most frequently in cardiac subjects.

Postoperative Bantu patients followed next, but if one were to exclude the 2 Bantu and 1 Indian patient suffering severe pelvic and abdominal trauma necessitating surgery, the incidence in the Bantu would be 20.0% and in the Indian 0%. The medical non-cardiac group, the largest group studied, showed a significantly high incidence in both the Bantu and the Indian. The lowest incidence occurred in the postpartum group, which was, in comparison, the smallest group investigated. No significant

TABLE II. INCIDENCE OF VENOUS THROMBOSIS AND PULMONARY THROMBO-EMBOLISM IN THE BANTU AND INDIAN AT NECROPSY

	Venous thrombosis						Pulmonary thrombo-embolism					
	Bantu			Indian			Bantu			Indian		
	Total No.	Thrombosis present	%	Total No.	Thrombosis present	%	Total No.	PTE present	%	Total No.	PTE present	%
Medical												
Cardiac	42	27	64.3	10	5	50.0	38	19	50.0	10	4	40.0
Non-cardiac	209	77	36.7	45	18	40.0	193	42	21.2	45	13	28.9
Postoperative	7	3	42.9	3	1	33.3	7	3	42.9	3	2	66.6
Traumatic	22	6	27.3	19	4	21.1	22	7	31.8	19	5	26.3
Postpartum	3	0	0	3	0	0	3	0	0	3	1	33.3
Total	283	113	39.9	80	28	35.0	263	71	27.0	80	25	31.3

racial difference is observed in the over-all incidence of venous thrombosis in the Bantu and Indian.

Table III shows the sex incidence of venous thrombosis in the two racial groups. As is evident from Table III,

TABLE III. SEX INCIDENCE OF VENOUS THROMBOSIS: COMPARISON BETWEEN BANTU (283) AND INDIANS (80)

Race	Males			Females		
	Total No.	Ven. thrombosis No.	%	Total No.	Ven. thrombosis No.	%
Bantu	189	69	36.0	94	44	46.8
Indian	52	15	28.8	28	13	46.4
Total	241	84	34.9	122	57	46.7

there is no appreciable difference between Bantu and Indian males, or Bantu and Indian females, in the incidence of this disorder.

Fig. 1 shows the age incidence of venous thrombosis in the Bantu.

Whereas thrombosis of the veins draining the lower limbs was observed in all age-groups (in decades), it was found to increase with advancing age. The drop in incidence in the patients over 70 years may be explained by the smaller number of patients of this age-group included in the present series.

The combinations of veins or groups of veins affected by thrombosis were found to vary considerably and often differed in the two lower limbs of the same patient.

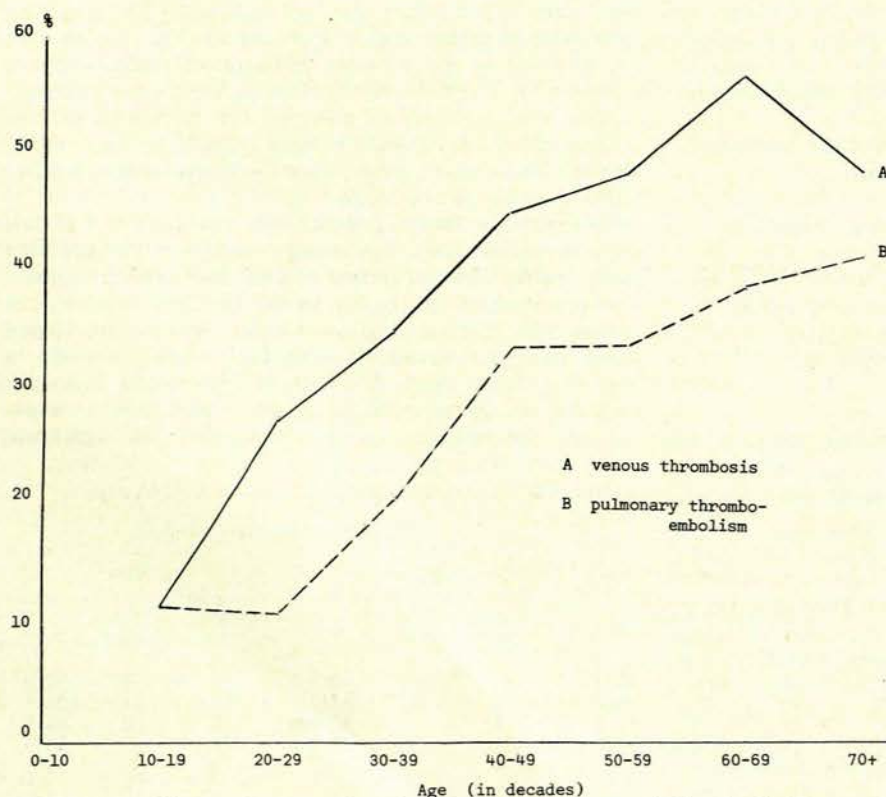


Fig. 1. Incidence of venous thrombosis and pulmonary embolism in the Bantu.

Thrombi, however, were most frequently observed in the deep veins of the calf (Fig. 2) and were often bilateral, and



Fig. 2. Venous thrombo-embolism. Multiple thrombosed veins within the calf muscles.

TABLE IV. THROMBOSIS IN VARIOUS VEINS DRAINING THE LOWER LIMBS IN 113 BANTU AND 28 INDIANS AT NECROPSY

	Thigh veins				Calf veins			
	Femoral		Popliteal		Post. tibial		Soleal and gastrocnemius	
	Bantu	Indian	Bantu	Indian	Bantu	Indian	Bantu	Indian
Limbs: Right	1	0	9	3	17	2	17	5
Left	1	0	14	4	27	5	24	5
Both	4	1	9	4	31	10	56	11
Total	6	1	32	11	75	17	97	21
Percentage	(5.3%)	(3.6%)	(28.3%)	(39.3%)	(66.6%)	(60.7%)	(85.8%)	(75.0%)

the left leg was slightly more often involved. The distribution of thrombi within the veins of the lower extremities in the Bantu and Indian is summarized in Table IV.

As is apparent from Table IV, the deep veins of the calf (soleal, gastrocnemius and posterior tibials) were more often affected than any of the other veins or groups of veins examined, and of these the soleal veins were most commonly involved. Of the thigh veins examined, the popliteals contained thrombi more frequently than did the femorals, but the incidence of popliteal vein thrombosis was significantly lower than that of thrombosis in the deep veins of the calf. In only 3 of the 141 positive cases were the thigh veins affected without concomitant involvement of the calf veins.

The majority of thrombi examined were of recent origin (Fig. 3). In 38 cases (29 Bantu and 9 Indians) varying degrees of organization were observed. Thus, while venous thrombosis was present in 38.8% of the total number coming

to necropsy, this was definitely not a terminal event in 10.5% of all cases investigated. Thrombi within femoral veins measured 10-15 mm. in diameter, while those in the popliteal veins ranged from 7 to 10 mm. Thrombi situated within the calf muscles were usually numerous (Fig. 2), and varied in size from 2 to 15 mm. in diameter, the majority being 3-10 mm.

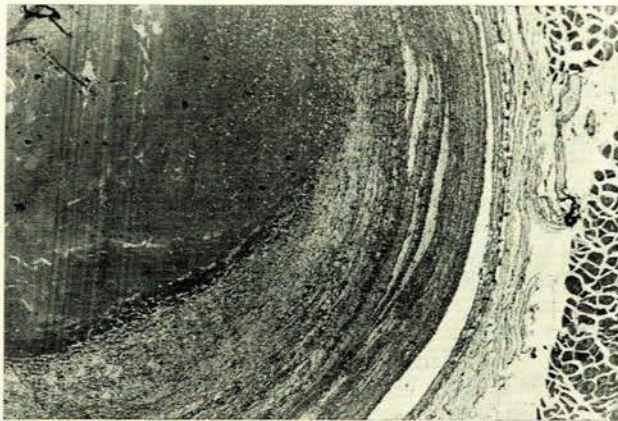


Fig. 3. Venous thrombo-embolism. Photomicrograph showing a fresh antemortem thrombus in a deep calf vein (H & E  $\times$  60).

Venous thrombosis of the lower limbs in this series was almost always classified as phlebothrombosis. Thrombophlebitis was encountered on two occasions only.

#### Pulmonary Thrombo-embolism

The incidence of venous thrombosis and pulmonary thrombo-embolism in Bantu and Indian necropsy subjects is shown in Table II. No significant racial difference was found in the incidence of pulmonary thrombo-embolism. The highest incidence of pulmonary thrombo-embolism in Bantu occurred in patients with heart disease. The frequency of this disorder was found to exceed that of venous thrombosis of the lower limbs in the traumatic group in both races, and in the postoperative group among Indians. In 18 Bantu and 7 Indian patients with pulmonary thrombo-embolism, venous thrombosis of the lower extremities was either absent or insignificant. In these the source was considered to have been sites other than the veins of the lower limbs, e.g. pelvic veins, right heart, inferior vena cava, etc., or pulmonary arterial thrombi may have arisen locally.

Infarction was observed in 9 Bantu and 1 Indian. In the majority (8) infarcts were of recent origin, being older and related to previous embolism in the other 2 cases. Abscess formation was found in 2 of the 10 cases of infarction of the lungs.

Table V shows the sex incidence of pulmonary thrombo-embolism in Bantu and Indian subjects. No significant differences are apparent between the corresponding sexes of the two race groups. As shown in Fig. 1, the incidence of pulmonary thrombo-embolism increases with advancing age and parallels the age incidence of venous thrombosis.

The morphology of pulmonary arterial thrombi recorded in these cases was divided into 3 main groups: (i)

where a thrombus blocked one or both branches of the pulmonary artery; (ii) where a large thrombus was present in one or both main arteries with concomitant involvement of segmental and more peripheral branches; and (iii) where thrombi were present in the segmental and peripheral arteries only.

TABLE V. SEX INCIDENCE OF PULMONARY THROMBO-EMBOLISM: COMPARISON BETWEEN BANTU (263) AND INDIANS (80)

Race	Males			Females		
	Total No.	No. positive PE/T	%	Total No.	No. positive PE/T	%
Bantu	176	44	25.0	87	27	31.0
Indian	52	12	23.1	28	13	46.4
Total	228	56	24.6	115	40	34.8

Thrombi in the pulmonary arteries were commonly multiple, the lower lobes were frequently involved, and usually both lungs were affected. Table VI shows the

TABLE VI. THROMBO-EMBOLISM IN VARIOUS BRANCHES OF THE PULMONARY ARTERIAL TREE IN 71 BANTU AND 25 INDIANS AT NECROPSY

	Main branch of pulmonary artery alone		Main branch plus smaller branches		Smaller branches alone	
	Bantu	Indian	Bantu	Indian	Bantu	Indian
Lungs:						
Right	3	2	0	0	5	4
Left	1	0	1	0	5	1
Both	2	0	8	6	46	12
Total patients	6	2	9	6	56	17
Percentage	(8.4%)	(8%)	(12.7%)	(24%)	(78.9%)	(68%)

frequency with which the various branches of the pulmonary arterial tree were involved according to the above grouping. No significant difference in the distribution of pulmonary thrombo-emboli was observed in the two races. The segmental and peripheral branches alone were most commonly involved. Next in order of frequency was concomitant involvement of one or both main branches and smaller branches, while involvement of the main branches alone was least frequent.

In 15 patients (10 Bantu, 5 Indians) thrombi were found in the segmental and/or more peripheral arteries of the one lung only. Of these, 7 showed evidence of multiple pulmonary emboli in one lung, while the other was apparently unaffected. In the remaining 8 cases involvement was negligible. The great majority of thrombo-emboli present in the pulmonary arteries were of recent origin. Varying degrees of organization were found in 19 cases (13 Bantu, 6 Indians). However, in 15 of these cases fresh occlusions were present in addition to those undergoing organization. The size of thrombo-emboli found in the pulmonary arteries varied from 1 to 15 mm. in diameter. Large thrombi measuring 10-15 mm. in diameter were found in the main branches of the pulmonary artery, while those in the more peripheral branches were between 1 and 6 mm.

In all except one case, occluding thrombi were found to be bland in nature.

## DISCUSSION

Observations at necropsy and their confirmation by histological examination have shown that, contrary to clinical evidence, the occurrence of peripheral venous thrombosis and pulmonary thrombo-embolism is not uncommon in the Bantu. A comparison of their occurrence among Bantu and Indian hospital patients coming to necropsy has shown no appreciable difference. In both races thrombosis was observed most frequently in the calf veins, was more often bilateral, and was not suspected clinically in the great majority. When thrombi were found in the pulmonary arterial tree they were commonly multiple and showed a definite tendency towards involvement of the vessels of the lower lobes. Frequently both lungs were affected, and again in a large percentage of cases clinical evidence of pulmonary embolism was lacking.

Considerable disagreement still exists regarding the sites of origin of pulmonary emboli, although many authors have tried to clarify this point by necropsy studies of various types. It must be borne in mind, however, that these studies have unfortunately differed widely in patient populations as regards age, sex and disease state, and also in the extent of venous dissection, all of which must have a bearing on the experience and interpretations of findings of the different authors.

Important sites for primary thrombus formation are the leg veins, femoral and iliac veins, pelvic veins, inferior vena cava, arm veins and the right side of the heart. Although the reported frequency with which the above sites are involved has varied, it is now believed that pulmonary emboli most often originate in the veins of the lower extremities.<sup>9-20</sup> In the present series venous thrombosis of the lower extremities could have accounted for 74.6% and 72% of all cases of pulmonary embolism in the Bantu and Indian, respectively. These findings, while showing the incidence of venous thrombo-embolism in the Bantu to be similar to that in the Indian, are in agreement with those of earlier authors,<sup>4-7</sup> in that venous thrombosis in the lower extremities most frequently affects the deep veins of the calf. This contrasts with the observations of Ingraham<sup>14</sup> and McLachlin and Patterson<sup>15</sup> that the pelvic and thigh veins are the usual sources of pulmonary emboli. The finding that the soleal veins were the most commonly involved calf veins confirms the observations of Hunter *et al.*<sup>8</sup>

Measurement of the large thrombi within the calf-muscle veins also correlated with the findings of Hunter *et al.* and supported their view that fatal pulmonary emboli can and do arise in these veins. It has been the experience here that the left leg is slightly more frequently involved.

When one considers the occurrence of thrombo-embolism in the various age-groups (Fig. 1) it is evident that while thrombosis and embolism occur in all groups, there is a definite tendency towards a higher incidence as age advances. In the present series almost 50% of Bantu patients, 40 years and over in age, showed evidence of venous thrombosis at necropsy. With increasing age many diseases favouring the production of venous thrombo-embolism are encountered, but diseases of the heart, particularly when decompensation is present, appear to be

the most important single predisposing factor in this study.

The frequency with which leg-vein thrombosis has been recognized clinically, varies from 4 to 20% in different series. Of the 141 patients with venous thrombosis shown here, the condition was suspected clinically in 2 instances and was diagnosed with certainty in another 3. Pulmonary embolism or infarction was also infrequently diagnosed clinically, being suspected on 5 occasions and diagnosed with certainty in another 6 cases.

While the over-all incidence of pulmonary thrombo-embolism was found to be 28% in 343 patients investigated, the patterns of distribution of occluding thrombi and the degree of arterial involvement varied widely. However, it was usual for multiple smaller pulmonary arteries to be involved. The incidence of 28% is high, but it should be appreciated that this includes even solitary emboli in the central and peripheral portions of the lungs in addition to those involving major branches. Twenty-three patients (15 Bantu, 8 Indians) were found to have thrombus obstructing, either completely or partially, one or both main branches of the pulmonary artery. In 16 (10 Bantu, 6 Indians) both main arteries were blocked. In no case was involvement of the main stem of the pulmonary artery observed. Of the group with smaller thrombi, 7 patients (6 Bantu, 1 Indian) were observed to have a significant number of segmental and/or more peripheral branches of one lung occluded by thrombi, while the contralateral lung appeared unaffected. The only explanation for this is that a large, friable thrombus must have lodged temporarily in the one main branch of the pulmonary artery, then broken up and passed into smaller branches centrally and peripherally, Gorham,<sup>16</sup> in his extensive study of pulmonary embolism, mentioned this phenomenon and added that its occurrence was very rare and that in such circumstances recovery was likely. It is possible that of the two cases reported recently by Sautter *et al.*<sup>17</sup> as examples of complete resolution of massive pulmonary embolus, one may fall into this category.

In 20 patients (17 Bantu, 3 Indians) significant numbers of thrombi were observed in segmental and smaller branches of both lungs. Without the aid of angiography, however, it is impossible to comment with any degree of accuracy on the true extent of vascular obstruction. Of the remaining 46 cases, a solitary thrombus involving a segmental or smaller artery was found in 8 patients, while the rest (38) showed evidence of multiple but less significant affection of both lungs. Whereas the latter degree of arterial obstruction may not be fatal, it is reasonable to assume that it could place an added burden on an already embarrassed heart.

While both observations on patients and experimental evidence in animals<sup>18,19</sup> support the mechanical theory and agree that obstructive embolus in the main pulmonary stem or in one or both major branches must be present to be fatal, there is no unanimous opinion regarding the effects of multiple, smaller emboli. Reflex vasospasm may be an important factor here, but will not be considered in this paper.

Measurements of venous thrombi in calf veins (2-15 mm. in diameter) and those in the pulmonary arterial

tree (1 - 15 mm. in diameter) do suggest that the majority of the latter could have arisen in the deep veins of the legs, and are therefore examples of pulmonary embolism as opposed to primary arterial thrombosis.

The incidence of venous thrombo-embolism was significant in the postoperative group, but negligible in the postpartum group. It must be appreciated, however, that these two groups contain comparatively fewer patients, and the findings may therefore not be truly representative. Its frequency in the traumatic group is significantly high. The incidence of pulmonary involvement here exceeded that of venous thrombosis (Table III), thereby suggesting sources other than leg veins, or possible local thrombus formation.

The medical group was the largest of all groups investigated, and the occurrence of this disorder in these patients was common. On separation into cardiac and non-cardiac patients, the former showed the highest incidence of both venous thrombosis and pulmonary embolism in Bantu patients (Table II). Whereas a difference of opinion exists regarding the source of emboli in cardiac patients, it should be appreciated that in many instances incomplete search in the veins of the lower limbs was carried out, or that thrombi were found both in the right heart and in the leg veins. In the present series, 64.3% of Bantu and 50% of Indian patients with congestive heart failure were found to have venous thrombosis of the lower extremities, and the incidence of pulmonary embolism/thrombosis in this group is also high, being 50% in Bantu and 40% in Indian patients. Infarction was noted in 10 of 96 patients with pulmonary arterial obstruction caused by thrombus. Congestive heart failure was present in 8 of the 10 patients showing pulmonary infarction. Abscess formation was noted in 2 instances and was believed in both to result from secondary infection of a bland infarct.

While it is agreed that other sources for embolization, particularly the right side of the heart, may also have been present in these patients, it is nevertheless obvious that the incidence of venous thrombosis of the lower extremities in patients with cardiac decompensation is extremely high.

The fact that the majority of cases were classified as phlebothrombosis indicates that at necropsy thrombo-

phlebitis is the rarer of the two conditions in the Bantu, as in the Indian. Because of the very few cases of thrombophlebitis encountered here it is impossible to draw any conclusions regarding the likelihood or otherwise of embolization in such patients.

#### SUMMARY

Necropsy evidence has shown venous thrombosis in the African to be very much more common than is presently believed. While the over-all incidence of this disorder in Bantu subjects of 10 years and over in age was found to be in the order of 40%, the highest incidence occurred in patients with congestive heart failure.

Venous thrombosis of the lower limbs involved particularly the deep veins of the calf, and such thrombi were sometimes large enough to give rise to fatal pulmonary embolism. Pulmonary emboli were usually multiple and more frequently involved the smaller intrapulmonary branches of the pulmonary artery.

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#### REFERENCES

- Ochsner, A. and DeBakey, M. (1951): *Surgery*, **29**, 24.
- Gelfand, M. (1957): *The Sick African*, 3rd ed., p. 452. Cape Town: Juta.
- Franz, R. C., Kark, A. E. and Hathorn, M. (1961): *Lancet*, **1**, 195.
- Rossle, R. (1937): *Virchows Arch. path. Anat.*, **300**, 180.
- Neumann (1938): *Op. cit.*<sup>8</sup>
- Putzer (1939): Quoted by Hunter, W. C., Krygier, J. J. and Kennedy, J. C. (1945): *Surgery*, **17**, 178.
- Frykholm, R. (1940): *Surg. Gynec. Obstet.*, **71**, 306.
- Hunter, W. C., Sneed, V. D., Robertson, T. D. and Snyder, G. A. C. (1941): *Arch. Intern. Med.*, **68**, 1.
- Hampton, A. V. and Castleman, B. (1940): *Amer. J. Roentgenol.*, **43**, 305.
- Cohn, R. and Walsh, J. (1946): *Stanf. Med. Bull.*, **4**, 97.
- Ravdin, I. S. and Kirby, C. K. (1951): *Surgery*, **29**, 334.
- Byrne, J. J. and O'Neil, E. E. (1952): *Amer. J. Surg.*, **83**, 47.
- Short, D. S. (1952): *Brit. Med. J.*, **1**, 790.
- Ingraham, E. S. (1942): *Canad. Med. Assoc. J.*, **47**, 553.
- McLachlin, J. and Patterson, J. C. (1951): *Surg. Gynec. Obstet.*, **93**, 1.
- Gorham, L. W. (1961): *Arch. Intern. Med.*, **108**, 76.
- Sautter, R. D., Fletcher, F. W., Emanuel, D. A., Lawton, B. R. and Olsen, T. G. (1964): *J. Amer. Med. Assoc.*, **189**, 948.
- Knisely, W. H., Wallace, J. M., Mahaley, M. S. jr and Satterwhite, W. M. (1957): *Amer. Heart J.*, **54**, 483.
- Williams, M. H. (1956): *Circulat. Res.*, **4**, 67.