The Role of 5-Hydroxytryptamine

in the

Pathogenesis of Endomyocardial Fibrosis

A Thesis

Presented for the degree of P.hD. (Physiology)

in the

University of Ibadan

by

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A C K N O W L E D G E M E N T S

I would like to take this opportunity to thank Professor J. Grayson and Dr. J. R. Parratt for their keen interest, supervision, and encouragement throughout the various stages of this research. My gratitude also goes to Dr. A. C. Ikeme (lately consultant cardiologist U.C.H. Ibadan) for his benevolent attitude to this work as well as his co-operation and help whilst I was in his unit from 1965-1966, to Professor G. H. Edington (Pathology) and Professor W. P. Cookshott (Radiology) for permission to use their valuable photographs, to Dr. A. M. Boyd and his staff for providing facilities at the University Clinic, to all my clinical colleagues and friends for their very helpful suggestions and to all those who volunteered as subjects for this work. Finally, I should like to thank my laboratory assistant and the typist, Mr. Sunday Bozegha.

I N T R O D U C T I O N

Endomyocardial fibrosis (E.M.F) is a common heart disease in certain areas of Africa. Much knowledge has accumulated in recent years about the pathology and clinical features of this disease; but at present, its pathogenesis is still in the realms of conjecture. 5-hydroxytryptamine has been implicated in the pathogenesis of E.M.F. on the basis of the role of this substance in causing cardiac lesions in the carcinoid syndrome. It is believed that E.M.F. and carcinoid heart disease have many features in common. Thus high serum level of 5-HT, as in carcinoid heart disease, is postulated to occur in E.M.F. and to cause cardiac damage in this condition.

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Chapter One

ENDOMYOCARDIAL FIBROSIS

Introduction:

The disease now widely known as Endomyocardial Fibrosis (E.M.F.) was first described by Bedford and Konstam (1946) following examination of East and West African soldiers serving in the Middle East during the Second World War. Soon afterwards Davies (1948) presented a series of cases from Uganda and described the pathology of this disease. Much more detailed accounts of both the clinical features and pathology were later given by Ball et al (1954).

Davies and Ball (1955) claimed that E.M.F. is the commonest form of heart disease in Uganda, and Shaper and Williams (1960) stated that it accounts for about 14% of all cardiovascular diseases seen in Kampala (Uganda). Nwokolo (1955) commented on the high incidence of E.M.F. in Eastern Nigeria whilst Abrahams (1962) asserted that it is rare in all parts of the world except in certain African countries where it is one of the commonest causes of cardiac disability. He went on to suggest that its main distribution is in tropical Africa south of the Sahara. Thus it occurs mostly in East, Central and West Africa (Brink & Weber 1966).

Most of the reported cases have come from Uganda and Nigeria, a few from the Sudan, Kenya and Tanzania, Congo Brazaville and Kinshasa, Southern Rhodesia, Ghana and Gambia. In all these countries, the

disease is noted to affect the indigenous African population predominantly and cases among expatriates are rare. In the United States, the disease occurs mainly in Negroes (Abrahams 1962). Brink and Weber (1966) reporting the first case of E.M.F. in a white patient in South Africa declared that "the fact of this condition occuring in any subject other than an African is unprecedented." E.M.F. is not, however, an absolute rarity in white subjects. The first reported case of E.M.F. in a European was that of Edge (1946), and a few more have been described since then - (Gray 1951, Clark et al 1956, Lynch & Watt 1957, Evans 1957, Coelho and Pimental 1963, Farugue 1963, Andrade and Guimaraes 1964, Brockington et al 1967). A feature common to all these reports is that the white patients were either resident or had lived in the respective areas of Africa for many years. This would suggest that a factor operating locally may be significant in the pathogenesis of this disease.

The name endomyocardial fibrosis was coined by Ball et al (1954). Davies in 1956 remarked that E.M.F. is a clear-cut clinical and pathological entity. Before 1954, many different names had been used to describe lesions of the endocardium which simulated E.M.F. only in belonging to the group of heart diseases labelled as obscure cardiopathy. Most of these cases have subsequently been accepted by one author or another as being examples of E.M.F. Yet in many cases the resemblance is not close. (Abrahams 1962). Parry (1965) therefore objected to the indiscriminate application of the name endomyocardial fibrosis to all forms of heart disease in which patchy endocardial lesions occur, since this

would lead to the erroneous conclusion that the disease has a world-wide distribution. He further declared that though the name endomyocardial fibrosis, is mainly a descriptive and anatomical term, yet it refers exclusively to a specific and peculiar form of heart disease common in Africans. E.M.F. has reasonably consistent epidemiological, clinical and morbid anatomical characteristics and is distinctly different from other forms of obscure cardiopathy. Parry finally stated that E.M.F. has been described often in tropical Africa despite the available meagre medical services; in contrast to the isolated reports from other parts of the world where the more advanced medical services may be expected to reveal its true incidence. He therefore concluded that this is a pointer to the fact that E.M.F. is confined mainly to the hot and wet regions of Africa.

Pathology:

This consists essentially of fibrotic lesions in the endocardium and inner one third of the myocardium, hence the name endomyocardial fibrosis (E.M.F). This fibrotic process may involve one or both sides of the heart, the lesions being generally more severe in the ventricles than in the atria whilst the left ventricle is usually the most severely affected chamber (Davies and Ball 1955, Davies 1960).

Gross inspection at autopsy usually reveals a heart enlarged, mainly through dilatation and hypertrophy of the atria (Davies and Ball 1955). Whilst the right atrium may assume aneurysmal proportions, significant enlargement of the left atrium is exceptional (Abrahams 1959). Another constant feature, especially in severe right ventricular lesions, is the presence of a deep groove on the antero-lateral surface of the heart portraying the obliterated apex of the right ventricle.

The pericardium is almost invariably normal, though occasionally milk spots are seen. An associated effusion is not unusual and may indeed contribute to the cardiomegaly diagnosed clinically. This pericardial collection which may be small or large usually is a clear serous fluid with a low protein content and very few cells (Davies and Ball 1955). Abrahams (1959) noted that effusions were more commonly associated with severe lesions in the right ventricle and in the cases reported subsequently by Abrahams and Parry (1963), these effusions were either straw coloured or blood stained and associated with a high protein content and excess of lymphocytes. Three of the patients studied by the author presented with a yellowish opalescent pericardial fluid containing cholesterol crystals as well as high protein concentration. It is significant in this respect that one case at autopsy revealed ruptured pericardial veins presumably resulting from a very high central venous pressure. Rupture of those veins with bleeding into the pericardium may well account for the differences observed in the character of the pericardial fluid (Abrahams and Parry 1963).

When the heart is cut open, a common finding is the presence of ante-mortem thrombi. These mural thrombi may consist of fibrin alone or they may consist of all the components of a blood clot and are seen in varying stages of organisation. In the ventricles they are usually superimposed on the fibrotic endocardial plaques. Such thrombi are commoner in the left ventricle than in the right but by far the commonest sites, are the atria especially in the right atrial appendage. The thrombi in the atria are mainly the result of gross atrial dilatation

and immobilization (Davies and Ball 1955, Shaper & Wright 1963). Sometimes the entire right atrial cavity may be filled with a large ante-mortem thrombus which may further aggravate the high venous pressure with consequent pericardial venous haemorrhages (Abrahams & Parry 1963).

The ventricular endocardium may be 2 - 3 millimetres (mm.) thick; though thicknesses of up to 12 mm. have been reported (Davies and Ball 1955). This thickened endocardium shows a pearly white rugose surface, its raised rolled edges clearly demarcating it from the surrounding healthy endocardium. The fibrotic process affects the endocardial surfaces of all the walls of the ventricles, the severest lesions occuring at the apex and the posterior wall. The lesion usually starts at or near the apex spreads up the inflow tract and practically spares the outflow portion (Davies and Ball 1955, Edington & Jackson 1963). Even in the rare cases where the outflow tract is affected, the lesions always fall short of the pulmonary valves (Abrahams and Parry 1963). The outflow portion is frequently dilated in constrast to the constricted inflow tract (Andrade and Guimaraes 1964).

The papillary muscles are not spared in the spread of the fibrotic process and their muscle fibres may be completely replaced by fibrous tissue. Involvement of the chordae tendinae causes them to shorten and thicken with consequent loss of function. It is the functional impairment of these valvular 'guy ropes' that is responsible for the atrioventricular valvular incompetence that is the common feature of this disease. Though incompetence is almost invariable, stenosis has occasionally been reported (Nwokolo 1955,

Davies 1956, Shaper and Wright 1963). In severe lesions, the posterior chordae and cusp become completely immobilized in a mass of fibrous tissue, plastering them down to the posterior ventricular wall. Also in some cases, fibrous tissue runs down from the atria to the ventricles with only slight irregularities where the cusps are embedded (Davies and Ball 1955).

In severe right ventricular lesions, its entire chamber may be filled with organising clot and fibrous tissue with obliteration of the right ventricular cavity, a condition known as obliterative endomyocardial fibrosis (Davies and Ball 1955). When this occurs, there is a marked approximation of the apex to the tricuspid valve and a drawing in of the antero-lateral wall of the right ventricle producing the visible external depression at the apex. Also in left ventricular lesion there is an increase in the number and branches of the trabeculae carnae showing a close similarity to tapestry or lacework (Abrahams 1959, Edington and Jackson 1963).

Endocardial lesions in the atria are much less severe than in the ventricles. These may consist of small patches of thickened endocardium often in close relationship to an organising thrombus and it has been suggested that these scattered atrial endocardial lesions may represent complete organisation of small thrombi (Davies and Ball 1955).

The semilunar valves, the great veins entering the atria and pulmonary artery show no abnormality. The major coronary arteries are normal but obliterative changes occur in the small branches.

Histopathology:

The greatly thickened ventricular endocardial plaques show three distinct zones. There is a superficial, acellular often hyalinized layer,

which is occasionally the seat of calcification, calcium being deposited as numerous bulky granular masses sometimes as much as 15 mm. thick. Calcification if present is more often seen in the left ventricle than in the right. The intermediate zone is a layer of loosely textured fibrous tissue, containing an occasional macrophage, lymphocyte or plasma cell but no polymorphonuclear leucocytes or eosinophils. The deepest layer usually referred to as the granulation tissue layer contains numerous small blood vessels and a few chronic inflammatory cells. From this layer tongues of fibrous tissue extend into the myocardium usually not further than the inner one third. These fibrous bands encircle myocardial fibres in varying stages of degeneration. The degenerate myocardial fibres show atrophy, with wavy disintegration of sarcoplasm, hyalin changes as well as interfibrillary or interstitial oedema. These changes are frequently more marked in the subendocardial region, but despite the degenerative myocardial changes, acute necrosis of muscle fibres or evidence of their removal is never seen. Conspicuous also in this layer are many small blood vessels and numerous thin-walled dilated vascular channels, representing the obstructed Thebesian and arterio-luminal vessels consequent on the endocardial changes (Davies and Ball 1955).

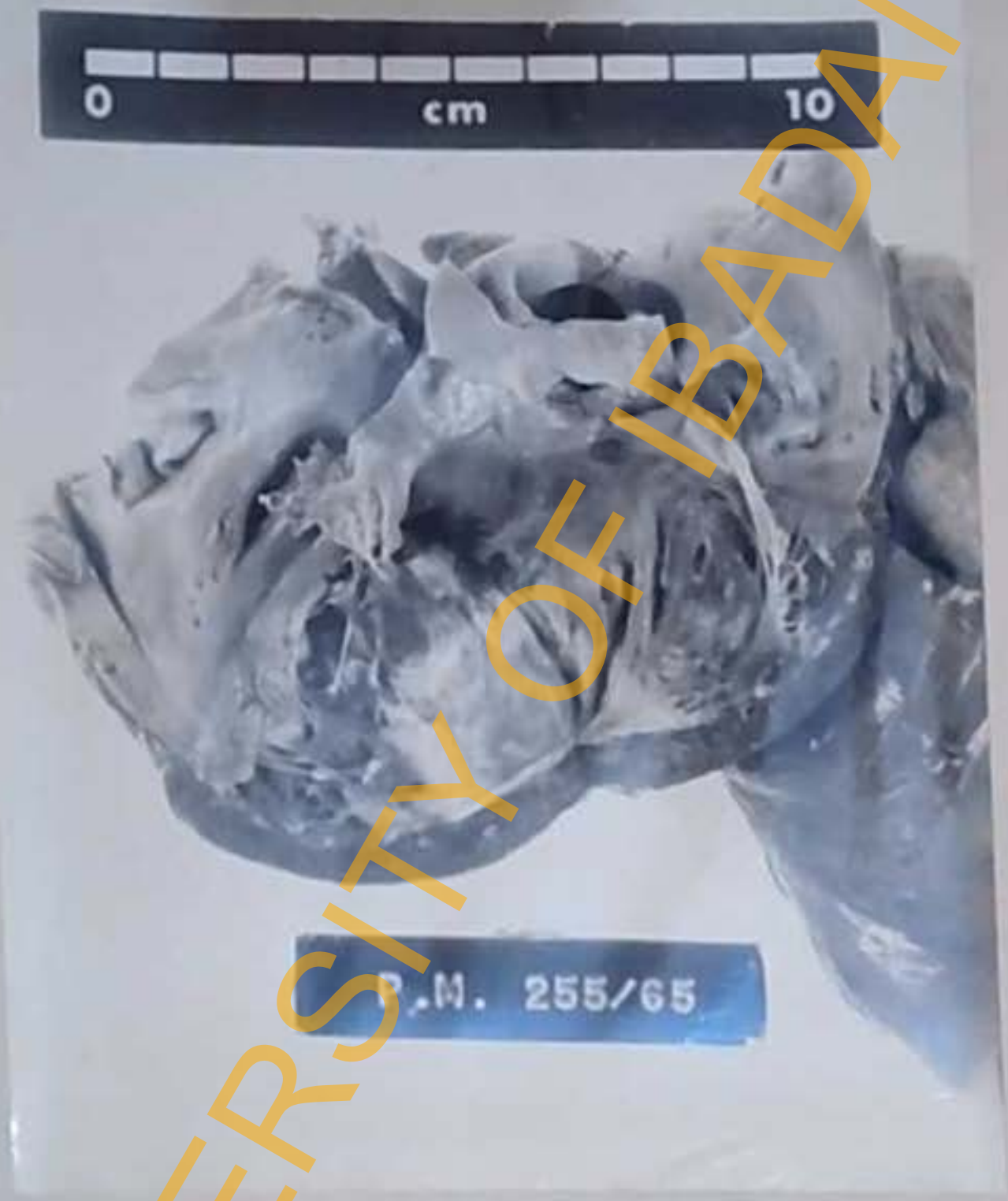
Degenerative myocardial changes occur in all chambers of the heart irrespective of the presence or degree of endocardial fibrosis. Indeed myocardial changes seen in the atria even in the absence of endocardial lesions often involve the entire thickness of their thin muscular walls (Davies and Ball 1955).

Extracardiac Complications:

Significant extracardiac lesions are not seen except for those resulting from cardiac failure or terminal infection. The most consistent pathology outside the heart, especially following right ventricular lesions, is gross hepatomegaly showing histological evidence of cardiac cirrhosis. Often commonly stressed is the relative infrequency of embolic phenomena despite the frequent presence of mural thrombosis (Davies 1956, Shaper and Williams 1960). It is said that embolic phenomena in R.H.F. patients are either associated with bacterial endocarditis or terminal events affecting major blood vessels (Davies 1960). In a recent review of their Uganda cases, Shaper and Wright (1963) noted that 46% of their 123 cases at autopsy had mural thrombi, whilst 15% showed embolic phenomena in the absence of bacterial endocarditis. The usual sites of embolisation are the spleen, and lungs, and less commonly the brain and kidneys.

To summarise, the peculiar pathological features of R.H.F. are the grossness and severity of the endocardial fibrosis, the marked damage to the atrio-ventricular valves and the limitation of the fibrotic process to the apex and inflow tract. The outflow tract if affected at all shows merely the changes due to eddying and turbulence. Damage is confined mainly to the inner third of the myocardium being severest in the subendocardial layer. Thrombus formation is either associated with ventricular endocardial lesions or is the result of atrial dilatation and immobilisation. Embolic complications do not however correlate with the high incidence of ante-mortem thrombosis. No evidence of fibrinoid necrosis, Aschoff nodules, parasitic invasion or bacterial infection is see

Right Ventricular E.M.F. - Gross autopsy appearance of the heart. Note the deep groove on the antero-lateral surface of the heart portraying the obliterated apex of the right ventricle.



Autopsy specimen
Heart of patient with Biventricular E.M.F.
Photograph shows left ventricle only. Portrays gross diffuse apical fibrosis spreading up to involve the posterior and anterior papillary muscles. Chordae tendinae are normal, but anterior mitral leaflet is slightly thickened with a well marked nodular thickening of the free border.

Same patient - Biventricular E.M.F.
Photograph of right side only.
Right atrium shows patchy endocardial fibrosis mainly about the margins of the appendage.
Right Ventricle shows fibrosis of the endocardium, especially at the apex of the 'notch' where it involves the myocardium. Fibrosis extends about half way up the Atrioventricular ring but is more prominent along the pulmonary outflow tract.

CLINICAL FEATURES:

Age Incidence:

Endomyocardial fibrosis occurs more commonly in children and young adults than in the older age groups (Gray 1951). This age incidence serves to differentiate it from another important cardiac condition in this environment at present loosely referred to as heart muscle disease (H.M.D) (Ikeme 1966). Among the forty cases reported by Bedford and Konstam (1946) the majority were of the ages between twenty and thirty and all but two were below forty. In the cases reported from Nigeria, almost all the patients when first seen were below the age of thirty (Abrahams 1959, Cookshott, et al 1967). Mwokolo (1955) reported a case in a young male child of twelve. Fourteen of the thirty patients studied by the author were under twenty and none exceeded the age of forty. Also the average age of a hundred cases reviewed by Parry and Abrahams (1965) was 20 years. Ikeme (1966) further stressed the age incidence by asserting that he would hesitate to diagnose E.M.F. on clinical grounds alone in anyone above the age of forty.

Sex and Social Factors:

Right-sided lesions tend to show an equal sex distribution whilst left sided E.M.F. occurs more frequently in females (Abrahams 1962, Abrahams et al 1963, Cookshott et al 1967). Most of the patients are illiterates. They come from the lower socio-economic strata of the society and are essentially poor indigent farmers (Abrahams 1959, 1962). Reports

from Uganda suggest that it is commoner among the immigrant labourers from Rwanda-Burundi than in the indigenous Baganda people (Davies 1948, Bell et al 1954, Shaper et al 1965).

Clinical Presentation:

In the past, the diagnosis of endomyocardial fibrosis was reached only at necropsy. The situation now is different. Abrahams (1959) discussing the problem of diagnosis especially of right sided lesions stated that it is a form of disease in which the type of patient, symptoms and radiological appearance of the heart, electrocardiographic pattern and above all the physical signs have become so well defined by repetition that they now form a clinical entity which can be accurately recognized at the bedside. Today one has only to encounter a few patients suffering from this disease and the recognition of subsequent cases present no special diagnostic problem.

The clinical presentation depends mainly on the site of the major lesion, thus the brunt of the damage may fall on both ventricles together or on one alone so that at all phases of the disease, the signs and symptoms may vary according to the part mainly affected. E.M.F. is thus a far more dynamic disease than its morbid anatomical title suggests (Parry and Abrahams 1965).

Right Ventricular E.M.F:

Patients with right-sided E.M.F. show a characteristic picture. There is a massive ascites without peripheral oedema, and it is the burden of this grossly protuberant abdomen that brings these patients to hospital. In female patients, a protuberant abdomen coexisting with

amenorrhoea may lead to a mistaken diagnosis of pregnancy. When E.M.F. occurs in the adolescent, growth is usually retarded and in males the secondary sexual characters may not be fully developed. There is gross muscular wasting and the emaciated chest and limbs contrast vividly with the protuberant abdomen. The degree of disability is often amazingly little, dyspnoea is significantly absent but most patients complain of general weakness and a tendency to fatigue easily (Marchesa 1962, Personal observation 1965-66).

All the abnormal findings on clinical examination are consequent upon the haemodynamics of the cardiac lesion. The radial pulse is of small volume and may be paradoxical, whilst atrial fibrillation is not uncommon. The grossly distended jugular veins may show marked pulsation, and this in association with pulsation in the liver and the systolic expansion of the anterior chest wall declare the presence of gross tricuspid incompetence with the concomitant regurgitant flow into an aneurysmally dilated right atrium (Shillingford and Somers 1961, Abrahams 1962). Not infrequently however, these signs of tricuspid incompetence as well as the typical tricuspid systolic murmur are absent even in those where tricuspid incompetence is subsequently shown at necropsy. This is presumably the result of extensive deposits of fibrous tissue in the walls of the right ventricle which prevent it from exerting its full systolic thrust (Bell 1957). The absence of a tricuspid incompetence murmur may also be accounted for in those patients, in which the tricuspid valve cusps are bound down and the right atrium is functionally continuous with the right

entricle. The most consistent ausoultatory finding is a triple rythmn, due to a third heart sound in diastole, best heard at the cardiac apex (Abrahams 1962). This added third sound is a reflection of the gross fibrotic endocardial lesions, placing a marked restriction on ventricular relaxation during the rapid filling phase of the cardiac cycle.

The apex beat is feeble and is rarely felt, the liver is grossly enlarged and may reach the level of the umbilicus. Pericardial and pleural effusions are usually associated, and cyanosis with finger clubbing are not uncommon (Abrahams 1962).

Left Ventricular L.M.F:

Here the clinical picture is dominated by the signs of mitral incompetence and pulmonary hypertension. The patient's main complaint is dyspnoea or chest pain associated with cough and slight haemoptysis, or an irritating non-productive cough. The dyspnoea both nocturnal and on effort, the result of pulmonary oedema and hypertension, may be severe and in the terminal stages incapacitating. The severe mitral incompetence leads to dilatation and compensatory hypertrophy of the left atrium, a high left atrial pressure and marked pulmonary hypertension. The pulmonary hypertension in turn leads to right ventricular hypertrophy as judged by a tapping apical impulse displaced well to the left associated with a vigorous left parasternal heave. A loud apical pan-systolic murmur, often accompanied by a thrill, continuing into the second sound and followed by a third sound confirm the associated gross mitral incompetence. The second sou at the base is often split with marked accentuation of the pulmonary

component. Not infrequently the pulmonary artery pulsation may also be felt. Whilst pleural effusions may be associated, pericardial collections are rare in lone left sided E.M.F. (Abrahams 1959, 1960).

Biventricular E.M.F:

It is claimed that in the early stages of biventricular lesions, the left ventricular damage may appear first because early in the disease the posterior mitral cusps become stuck by fibrous tissue and that if the patient survives this phase the right ventricular impairment becomes dominant (Abrahams 1962). Thus in established biventricular E.M.F., the pulmonary hypertension from the left ventricular lesion may be greatly reduced and signs of mitral incompetence overshadowed by a low right ventricular output. Thus a mitral bruit and significant left atrial enlargement are often absent (Abrahams 1962, Abrahams and Parry 1963). Pericardial effusions may be associated whilst bilateral proptosis is not unusual. Six patients in this group studied by the author had severe proptosis.

CLINICAL INVESTIGATIONS:

Examination of the Blood:

Not much can be gained from the routine examination of the blood. There is usually a moderate degree of anaemia, mainly of the normocytic type, a not unusual finding in most Nigerians of this social status. The plasma albumin-globulin ratio is reduced, a typical finding in Nigerians (Edozien 1958). The anti-streptolysin O-titre may be elevated in the "active cases" (Abrahams 1959, Abrahams and Brigden 1961). Eosinophilia may be associated (Gray 1951, Ive et al 1966, Brockington et al 1967)

19 year old male patient with right ventricular E.M.F. Note the marked ascites, the scrotal oedema, the absence of peripheral oedema and the general retardation in physical and sexual development.

Apparently healthy looking young lady of 17 years with right ventricular E.M.F. Note the slightly protuberant abdomen (X-Rays and angiocardiograms of same patient presented later). (R.U.).

22 year old female patient (M.M.) with biventricular E.M.F. showing the gross ascites, absence of peripheral oedema and a the marked generalised emaciation.

Bilateral proptosis in Biventricular E.M.F. (M.M.)
bilateral ptosis.

whilst the serum transaminase levels are usually within normal range (Campbell and Somers 1960).

There is no specific electrocardiographic pattern in E.M.F. Atrial fibrillation is a common complication. The usual findings consist of abnormalities of the P wave, low QRS complexes associated with flat or inverted T waves. Huge notched P waves are consistent with gross right atrial enlargement, whilst T wave invertion especially in the chest leads may be related to the clockwise rotation of the heart by the enlarged right atrium. Delay in atrio-ventricular conduction probably due to involvement of the Purkinje system in the fibrotic process occasionally gives rise to prolonged PR intervals (Williams et al 1960, Somers et al 1960, Abrahams et al 1963, Parry et al 1965).

RADIOLOGY:

Right Ventricular E.M.F:

A straight X-Ray of the chest shows gross dilatation and enlargement of the heart which may be difficult to distinguish from the globular appearance characteristic of a pericardial effusion. Indeed an associated pericardial effusion is not unusual in E.M.F. The cardiothoracic ratio is invariably greater than 0.7 and values higher than 0.9 are on record. Also the superior mediastinum may be widened in some cases because of the engorged superior vena cava (Cockshott, et al 1967). The most striking radiographic feature is oligaemia of the lung fields seen as a marked reduction in pulmonary vascular markings. Calcification may be seen within

the cardiac shadow, being usually at the right apex or in the fibrotic endocardium in the anterior part of the outflow tract of the right ventricle just below the pulmonary valve (Abrahams 1962, Somers and Williams 1962, Cookshott 1965). The ventricular chamber from the tricuspid valve to the outflow portion exhibits an oblique linear lower border corresponding to the area of calcification. This immobile part of the ventricle is a marked contrast to the vigorous contractions of the distended outflow portion which shows a significant alteration between systole and diastole (Cookshott 1965, Cookshott et al 1967). The movement of the heart are greatly diminished on the screen but sometimes expansile pulsation of the right atrium during ventricular systole can be detected (Abrahams 1962, Cookshott 1965). Chest X-Rays of younger patients with right ventricular endomyocardial fibrosis sometimes show osteoporotic ribs and vertebral column as well as features of hypertrophic bone marrow. As this radiological appearance is also seen in sickle cell anaemia and cyanotic congenital heart disease, it has been suggested that anoxic anoxia associated with the central cyanosis is the factor stimulating the marked proliferation of the bone marrow (Cookshott et al 1967).

Cardiac catheterization of the right ventricle is often impossible since the stretched tricuspid leaflets often obstruct the passage of the catheter tip into the right ventricle which itself has been greatly reduced in capacity. When the right ventricular chamber is catheterised, a dip and plateau form of pressure wave is recorded owing to the restricted

diastolic filling and impairment of systolic ejection (Shillingford and Somers 1961, Abrahams and Parry 1963). The right atrial pressure may be as high as 30 mm. Hg. and arterial oxygen desaturation with values as low as 60% have been recorded. Injections of contrast solution into the right atrium demonstrates its enormous size whilst the presence of pericardial effusion is readily detected by noting the distance separating the opacified atrium and the lateral heart border. Considerable stasis in the right atrium resulting from tricuspid incompetence and obstructive cardiomyopathy is demonstrated by persistence of contrast medium for as long as 12 to 15 seconds after injection (Abrahams 1962, Cookshott 1965, Cookshott et al 1967). Thrombi in the auricular appendage show as persistent filling defects. The aneurysmally dilated right atrium may overlap and obscure the right ventricular apex. As a result of the low right ventricular output, the pulmonary vessels are never well opacified (Cookshott 1965).

The raised central venous pressure results in incompetence of the valves of the large veins of the neck and of the azygos veins. Injections of contrast medium into the right arm veins therefore shows opacification of the dilated azygos veins and some veins of the prevertebral plexus (Abrahams 1962, Cookshott 1965).

Left Ventricular E.M.F:

A straight X-Ray shows an enlarged heart of mitral configuration, a small aorta a prominent pulmonary artery conus with straightening of the left border of the heart. The usual compensatory left ventricular

hypertrophy consequent on mitral incompetence is absent in left ventricular E.M.F. as a result of constrictive endocarditis and apical fibrosis. The enlarged left atrium however is best shown in lateral or anterior oblique positions. Also pericardial collections are rarely seen when only the left side of the heart is involved. Evidence of pulmonary hypertension is portrayed by marked peri-hilar clouding, large main pulmonary vessels and a relative oligaemia of the peripheral lung fields. Pulmonary oedema and septal lines in the costo-phrenic angles are seen very occasionally whilst calcification of the apex of the ventricle is rare (Cookshott 1965, Cookshott et al 1967).

Cardiac catheterization invariably confirms the presence of pulmonary hypertension and systolic pressures as high as 90 mm. Hg. have been recorded in the main pulmonary artery (Abrahams 1959, Shillingford and Somers, 1961). Left ventricular angiocardiography is characteristic and is of much diagnostic significance especially as the clinical presentation of left sided lesions may easily be confused with lesions of rheumatic mitral incompetence and the mitral regurgitation from dilatation of the atrio-ventricular valve ring in heart muscle disease. The ventricular chamber is frequently small with irregularities and apical filling defects. There is often little change in ventricular volume between systole and diastole, the mitral cusps are not seen whilst the enlarged left atrium may show slight systolic expansion (Cookshott 1965).

Another peculiarity of the left ventricular angiocardiogram in left sided E.M.F. is the distortion of the anatomy of the left ventricle and aorta, which have been dislocated by the greatly enlarged right atrium.

Normally the aorta rises from the aortic valves at the left sternal edge, runs upwards backwards and to the left before descending as the thoracic aorta. But when the right atrium is aneurysmal, it rotates the left ventricle laterally and posteriorly so that the aortic valve now lies well to the left of the sternum. From this position the ascending aorta runs straight up and medially until it reaches the arch, it then turns sharply backwards and downwards to attain the left border of the spino whence it descends in the normal position (Abrahams 1962).

Biventricular E.M.F:

In biventricular E.M.F., the cardiothoracic ratio is usually greater than 0.6, and a plain X-Ray closely simulates the cardiac contour of right ventricular E.M.F. (Cockshott et al 1967).

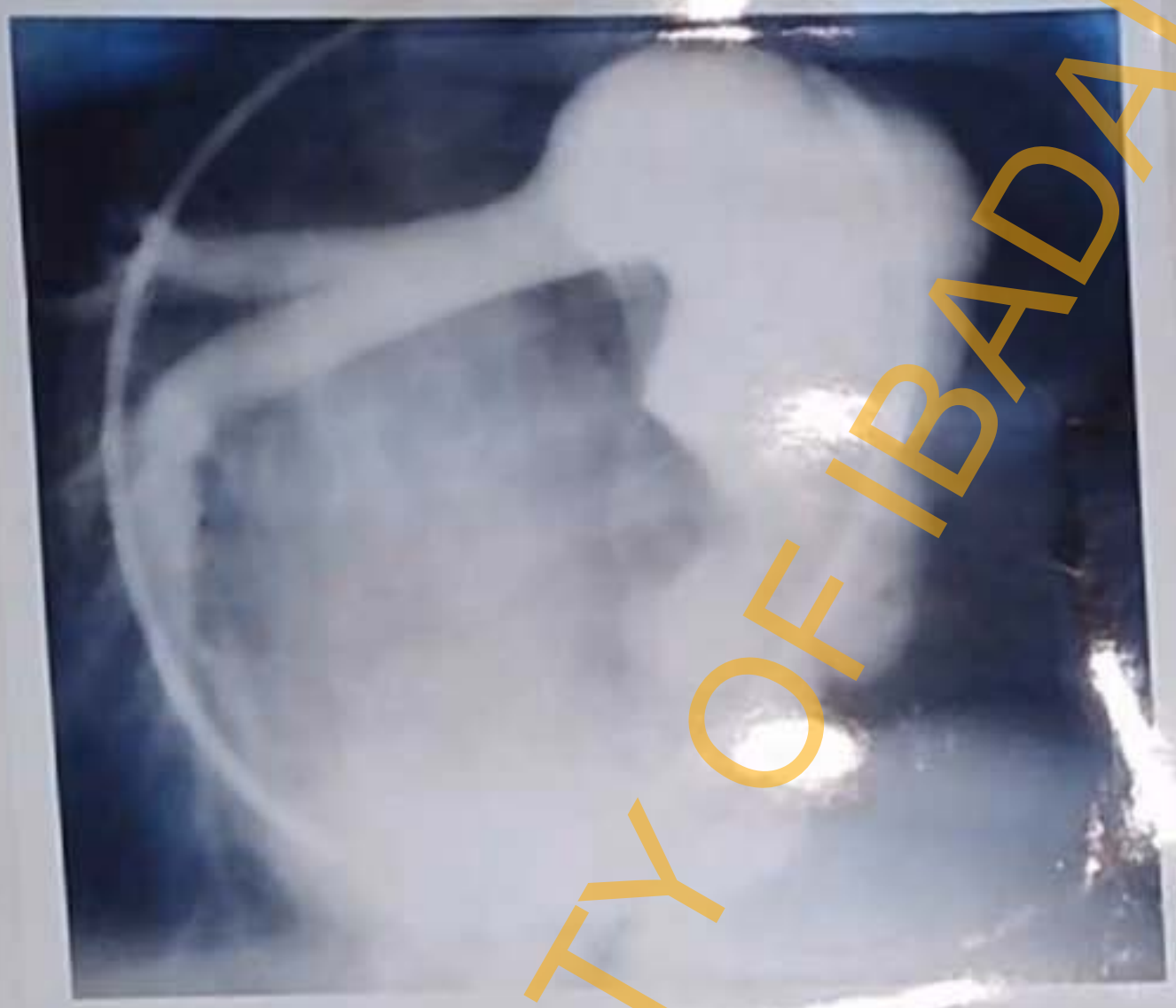
Haemodynamic Considerations:

A regular feature of right sided E.M.F. is a very high central venous pressure. It has been suggested that this may be the factor responsible for the persistent pericardial effusion that is often present (Abrahams 1962). It is significant in this respect that pericardial effusion is rare in long left sided disease, whilst the presence of severe right sided E.M.F. with its gross rise in central venous pressure is almost invariably associated with pericardial effusion (Abrahams and Parry 1963). It is also claimed that the rise in intra-abdominal pressure resulting from the tense ascites plays a role in maintaining the central venous pressure, for removal of ascitic fluid leads to a pronounced fall in central venous pressure which then almost approaches near normal levels

Plain film, Right ventricular E.M.F. (R.U.)
Shows gross dilatation and enlargement of the heart, a globular shaped cardiac contour with a cardiothoracic ratio of about 0.8, opacification of the superior vena cava, and relative oligaemia of peripheral lung fields. There is no visible aortic knuckle, the bulge on the left upper border is characteristic and indicates the dilated ventricular outflow tract.

Angiogram of right ventricular E.M.F. (R.U.)
Opacified right atrium showing its vast size and the absence of pericardial fluid. The dilated outflow portion of the right ventricle is also visible.

Angiogram of severe right sided R.H.F.
Shows the massively aneurysmally dilated right atrium; filling defects in the right atrial appendage, the right ventricular apex is obscured by the huge right atrium; also shows dilatation of the ventricular outflow portion, with [illegible] unfolding of the main pulmonary artery segment indicative of pulmonary hypertension.



Right ventricular angiogram
Selective right ventricular injection with catheter tip below the pulmonary valve. Note the bulge is due to the distended outflow portion of the right ventricle. The apex of the right ventricle is obliterated and there is reflux of contrast medium into a large right atrium.

Plain film of left sided E.M.P.
Shows an enlarged heart of mitral configuration, a small aorta, a prominent pulmonary artery conus, marked peri-hilar clouding, straightening of the left border of the heart due to absence of left ventricular enlargement.

Left ventricular angiogram with gross apical filling defects and reflux of contrast medium into the left atrium.

Angiogram of left sided endomyocardial fibrosis (Lateral projection)(a)
Shows a large left ventricle with minimal filling defects, marked atrio-ventricular incompetence with opacification of a dilated left atrium.
Typifies the difficulty in differentiating between a left sided mitral regurgitation of E.M.F. from that of rheumatic mitral incompetence.

Angiogram of left sided endomyocardial fibrosis
Frontal projection (b)

Plain film of Biventricular E.M.F.
Shows a grossly dilated heart, with a globular contour, indistinguishable from a large pericardial effusion.
Cardiothoracic ratio is 0.85.

Angiogram of left sided R.M.P. in Biventricular disease
Shows small size of ventricle with filling defects and
displaced ascending aortic arch due to a large right atrium.

Angiogram of left sided E.M.F. in Biventricular disease Shows an exceptionally large left atrium. A small left ventricle with obvious filling defects.

associated with a rise in vital capacity (Abrahams 1962, Abrahams and Parry 1963). In biventricular E.M.F., rigidity of the endocardium leads to a rise in ventricular end-diastolic volume with a corresponding increase in left atrial pressure. An associated mitral incompetence and obliteration of the ventricular cavity both serve to raise the atrial pressure further, this pressure is transmitted to the pulmonary veins and is reflected back by a general rise in pressure throughout the lesser circulation (Abrahams and Parry 1963). In severe right sided E.M.F., most of the function of the right heart is taken up by the right atrium and the high venous pressure may help to maintain the flow of blood through the lungs into the left heart (Shillingford and Somers 1961). Indeed a pressure gradient may exist from the right ventricle to the pulmonary artery during a significant part of ventricular diastole. Blood therefore flows through the pulmonary valve during diastole as well as during systole and the cardiac output may be maintained at least in part by this means (Parry and Abrahams 1963).

The dip and plateau pressure tracing in right ventricular E.M.F. though a constant feature of the disease is not specific to this condition, and is only a pointer to the impaired diastolic filling (Abrahams 1962). In right ventricular E.M.F., systolic ejection is impaired and stroke volume considerably reduced due to restriction by the greatly thickened endocardium, and myocardial fibrosis. The endocardial involvement also prevents diastolic relaxation of the ventricles thus greatly impeding diastolic filling. The decrease in cardiac output and impairment of

diastolic filling thus accounts for the pathologic physiology of E.M.F. Therefore any process preventing diastolic relaxation of the ventricles and resulting in marked impairment of systolic ejection thus decreasing cardiac output will produce the same characteristic pressure patterns in the right atrium and ventricles (Clark et al 1956).

The characteristic massive ascites with a complete absence of peripheral oedema in right E.M.F. defies all explanation. The plasma proteins are not particularly low nor can the reduction of albumin globulin ratio account for this. Although histologic evidence of some degree of liver cirrhosis is frequently obtained, the liver function tests do not suggest any severe degree of liver damage. Thus neither hypoalbuminaemia nor liver cirrhosis can adequately explain the presence of ascites. It is also significant that the ascitic fluid invariably contains a high protein content, suggesting that the fluid is an exudate rather than a transudate (Abrahams 1962, Personal observation 1965-66). Ascites without peripheral oedema is a common sign in tricuspid and pulmonary stenosis as well as in constrictive pericarditis due to venous engorgement resulting from diminished cardiac output in the absence of myocardial failure. Thus 'constrictive endocarditis' as evinced by a gross right ventricular lesion may explain the occurence of the ascites without peripheral oedema (Mabayoje 1967). Even then, the high protein content of the ascitic fluid still has to be accounted for.

The characteristic appearance of a patient with right sided E.M.F. simulates the picture of congestive cardiac failure yet these patients are

not in failure. Digoxin fails to lower the right atrial pressure whereas a rapid fall occurs with intravenous digoxin if the elevated central venous pressure is due to myocardial failure (Parry and Abrahams 1963). The right atrial pressure rises and the cardiac output increases on mild leg exercise. It is difficult to see how such hearts can maintain the cardiac output and even increase it on exercise. The right ventricle is useless and the atrium thin and aneurysmally dilated. The stroke volume is fixed, remaining unchanged with exercise so that any increase in cardiac output must be due solely to an increase in heart rate (Parry and Abrahams 1963).

Marked arterial oxygen desaturation with cyanosis and finger clubbing may occur in right sided s.l.f. Abrahams (1962) demonstrated with the conventional right ventricular angiograms retrograde filling of the vena azygos from the superior vena cava as a result of the high central venous pressure. Abrahams therefore concluded that shunting of blood between the connections of the azygos veins and the pulmonary veins may well explain the arterial oxygen desaturation, the cyanosis, as well as the finger clubbing seen in these patients. Cookshott (1965) however believes that the arterial oxygen desaturation is mainly the consequence of lower pulmonary segmental collapse resulting from a marked increase in intra-abdominal pressure due to the tense ascites.

The arterio-venous oxygen difference is small at rest. This is partly due to associated anaemia and partly to the decreased arterial oxygen saturation. Thus the oxygen extraction at rest is obviously greater

than normal and this accounts for the relatively small increase in extraction on effort (Parry et al 1963).

The severe proptosis occasionally seen in biventricular C.H.F., the amenorrhoea not infrequently associated in female patients, the marked diminution of public and axillary hairs, or the retarded body and sexual development that are common findings in right ventricular disease all seem to point to an underlying endocrine disturbance. Thus enlargement of the pituitary fossa reported in one or two patients at Ibadan may be significant.

Differential Diagnosis:

The diagnosis of right ventricular E.M.F. is usually not difficult. Even then, it presents many features similar to other forms of heart disease common in Nigeria. Some of these features can be confused with those of the following conditions:

1. Constrictive Pericarditis:

The clinical picture of right ventricular endomyocardial fibrosis is similar to that of constrictive pericarditis (Clark et al 1956). Indeed the haemodynamics of cardiac constriction corresponds to decreased cardiac filling whether due to pericardial, myocardial or endocardial fibrosis (Turner and Manson-Bahr 1960, Coelho and Pimental 1963). Thus in right E.M.F. as well as in constrictive pericarditis, there is a high central venous pressure, an elevated right atrial pressure associated with ascites and low voltage electrocardiographic pattern. The dip and plateau type of right ventricular pressure tracing is found in both

conditions whilst calcification within the cardiac shadow may also be seen in constrictive pericarditis (Abrahams 1962, Parry et al 1963). However whilst large hearts and gross tricuspid regurgitation are not features of constrictive pericarditis they are the rule in established right ventricular endocyocardial fibrosis (Adi 1963).

2. Tuberculous Pericardial Effusion:

Endocyocardial fibrosis may present clinically as a pericardial effusion (Evans 1957) and here in Nigeria it is a common mode of presentation (Abrahams and Parry 1963). Three of the four cases described from Kenya were initially labelled as cases of tuberculous pericarditis (Turner and Manson-Bahr 1960). Thus with a large pericardial effusion as in E.M.E., the radiograph shows a large globular heart with little pulsation on screening. Auscultatory findings in the absence of tricuspid incompetence murmur may not be helpful whilst the electrocardiographic pattern also shows low voltages with inversion of T waves.

Important factors in the differential diagnosis are the presence of tricuspid incompetence with systolic expansion of the neck veins, a third sound, atrial fibrillation and the presence of cardiac enlargement due to aneurysmal dilatation of the right atrium. Thus atrial fibrillation is uncommon in tuberculous pericarditis and when present is always transient and related to digi- talis therapy. Also pericardial aspiration with air replacement may help, the thinness of the pericardium and the enlarged heart of E.M.E. are in contrast to the thickened pericardium and normal-sized heart underlying tuberculous pericardial effusion. Finally, though not strictly necessary for diagnostic purposes, cardiac catheterization by virtu

of the pressure values and tracings confirm the presence of endomyocardial fibrosis (Abrahams and Perry 1963). It is also said that jugular venous tracings may be diagnostic, E.M.F. showing a combined cv wave as its only positive wave whilst apart from a sharp Y descent in pericardial effusion all the positive a, c, v, waves are distinct (Abrahams 1962).

2. Heart Muscle Disease:

This is one of the commonest forms of cardiopathy in Nigeria (Edington et al 1963, Cookshott et al 1967). The disease usually affects the middle-aged patients and occurs more often in males than in females (Cookshott et al 1967b). Ascites though a constant feature is never as severe as in E.M.F. and is always associated with dependent oedema, even at the very early stages. The characteristic feature is always of biventricular disease unlike E.M.F. which is often restricted to one ventricle alone. The atrioventricular incompetence if present occurs only in the terminal stages from dilatation of the valve rings as a result of myocardial failure. Symptoms of orthopnoea and nocturnal dyspnoea invariably precede right ventricular changes. Though the radiograph also shows a large heart, evidence of pulmonary congestion is always present. Another constant feature is a mild 'decapitated' or diastolic hypertension with blood pressure values of 150/130mm. Hg. This invariably returns to normal when the cardiac failure has been adequately treated with rest, cardiac glycosides and diuretics. At least in the initial stages of this disease the response to digitalis and diuretics is usually more satisfactory than with cases of E.M.F. (Abrahams 1962, Ikeme 1966, Cookshott et al 1967b)

4. Rheumatic Heart Disease:

A few cases of rheumatic heart disease (R.H.D), manifesting with mitral incompetence only, may simulate mitral lesions of left ventricular endomyocardial fibrosis very closely. Indeed it is frequently difficult to differentiate clinically between these two conditions. A previous history of streptococcal sore throat in R.H.D. is rarely often obtained in Nigerians whilst elevated anti-streptolysin O-titre is not a feature of the chronic disease. However whilst a compensatory left ventricular hypertrophy and marked enlargement of the left atrium are often associated with the mitral incompetence of rheumatic origin, these do not occur in left sided E.M.F. The problem can only finally be resolved by the characteristic angiographic appearance of left ventricular E.M.F. (Abrahams 1962).

5. Cirrhosis of the Liver:

The general appearance of a patient with hepatic cirrhosis is not unlike that of one with right sided E.M.F. Thus a protuberant abdomen, engorged jugular veins and the associated hepatosplenomegaly may lead the unwary and inexperienced astray. In fact, some patients originally diagnosed as liver cirrhosis have subsequently proved to be cases of right sided E.M.F. Also cardiac cirrhosis is a known complication of long standing right ventricular endomyocardial lesions (Abrahams 1962, Edington and Jackson 1963, Personal observation 1966). But since heart lesions are not usually complications of hepatic cirrhosis, a careful examination of the heart, in conjunction with chest radiograph, even without

the aid of other special investigations usually settles the problem. Also whilst dependent oedema occurs early in association with ascites in liver cirrhosis, it is a terminal manifestation of right sided H.M.F.

Treatment and Prognosis:

Since the aetiology of this condition has not been discovered nor its pathogenesis conclusively worked out, preventive measures are not available. Also the progress of the pathology in the heart cannot be halted with therapy, the result is that treatment is mainly symptomatic. Patients are admitted into hospital with severe symptoms of cardiac decompensation, get better with the usual regime of cardiac glycosides and diuretics and are discharged for follow up in the cardiac clinic. Some of them default only to reappear perhaps months or even years after, in severer state of cardiac failure. The regular attendants at the cardiac clinic seem to linger between fairly tolerable existence and total incapacitation, and most if not all have not the energy to pursue or cope with the demands of a regular employment.

Parry et al (1965), noted that the prognosis of the established cases is bad when the abdomen is very tense with ascitic fluid, the right atrium is very large and the arterial oxygen saturation is significantly reduced. Until these ominous signs develop, patients with right ventricular H.M.F. have been observed living relatively comfortably, hampered only by a ponderous abdomen whose ascitic fluid is resistant to every drug except spironolactone and hydrochlorothiazides.

Indeed many patients may live for several years, and some of Abrahams' patients first seen in 1958 are still being followed up at the cardiac clinic of the University of Ibadan teaching hospital. Thus, morbidity from the disease is out of proportion to mortality.

CHAPTER TWO

Current Theories on the Pathogenesis of Endomyocardial Fibrosis:

The pathological lesions in E.M.F. are the end result of a process whose cause and development are largely unknown (Williams et al 1954). Its aetiology is unknown and its pathogenesis obscure. The patients are first seen in the terminal stages of their illness so that investigations relating to pathogenesis are hampered by the difficulty of recognising the early cases (Lancet 1962). The early stage of E.M.F. is elusive because it is sub-clinical (Mwakolo 1955) and the insidious onset of the disease probably long proceeds the appearance of clinical signs or symptoms (Gray 1951).

Whatever the initial lesion or the natural history of E.M.F., it is generally accepted that the final picture of this disease is the result of organisation of mural thrombi (Davies et al 1955, Davies 1956). The difficulty is in deciding whether this is the result of a primary endocardial or myocardial lesion (Lynch and Watt 1957). Thrombus is usually deposited on the surface of an abnormal endocardium. Therefore the initial damage must necessarily precede thrombus formation. This might be the result of a direct injury and the relative delicacy of the inflow tract, the region predominantly affected would strongly suggest that some factors operate directly on the inflow tract endocardium that spare the coarser outflow tract (Davies and Ball 1955). However the relative infrequency of embolic phenomena despite the frequent finding of ante-mortem thrombi suggests that thrombus formation is essentially slow, allowing time for the processes of

organization to occur, such that these mural thrombi become relatively adherent and not therefore easily dislodged. Thus it would appear that the initial lesion is primarily myocardial with secondary endocardial damage, thrombus formation and organization (Davies 1956). Against a primarily myocardial lesion is the observation that there is no great loss of muscle tissue as would follow acute necrosis of muscle.

Davies (1956) therefore concluded that the initial site of damage is the sub-endocardial muscle layer and here indeed the severest myocardial changes are located. The sub-endocardial muscle layer is an area where nutrition is precarious, and this area of the inflow tract with its thin delicate endocardial lining may be expected to bear the severest brunt of a blood borne noxious agent. That this may be so is supported by the existence of lesions in the sub-endocardial muscle in chambers and regions that show no endocardial fibrosis as well as in those that do (Davies and Ball 1955). Also in the thin-walled atria, myocardial changes often involve the entire thickness of the muscle walls. Thus the initial lesion may be primarily myocardial; indeed isolated valvular lesions do not occur where the endocardium is not in contact with the heart wall, valvular fibrosis is always in continuity with lesions of the adjacent mural endocardium (Davies and Ball 1955). Unlike the rheumatic process, vegetations are not seen on the valve cusps and valvular distortion in endomyocardial fibrosis is mainly the result of spread of the mural endocardial fibrotic process (Edington and Jackson 1963).

The secondary endocardial damage, with thrombus formation, organisation and subsequent scar formation would be expected to cause obstruction of the Thebesian and arterio-luminal vessels and dilatation of these vessels is an histological feature of E.H.F. With obstruction of these vessels, a vicious cycle is set up, the hypoxia of obstruction adding insult to the injury already received by the myocardium (Davies and Ball 1955).

With only the evidence of advance disease to go on, the search for aetiological agents remains wide open (William et al 1954). The prevalence of the disease in Africa may shed light on its cause (Cruy 1951) and some factor operating locally appears likely (Shaper and Coles 1965). Various authors have expressed their ideas as to the possible aetiological factors involved in the pathogenesis of endomyocardial fibrosis. Whilst most of them appear plausible none in fact is conclusive or universally accepted. These theories will now be briefly discussed.

The Natural History of E.H.F:

Abrahams (1962) gave a tentative enunciation of the natural history of this condition as seen in Ibadan, Nigeria. Later Parry and Abrahams (1965) elaborated on this theory. They stated that endomyocardial fibrosis starts with an initial illness, summarized by them as a carditis with unusual features, and manifesting mainly with malaise, fever and loss of weight. This initial illness lasts for a few weeks. Subsequently it either resolves without any signs of residual damage or terminates in an acute phase leading rapidly to death. Others may however pass into the chronic fibrotic stage of established E.H.F.

Most of their patients who described an initial illness said it began in the rainy season. Parry and Abrahams therefore suggested that an organism or vector of disease prevalent during the rainy season may in some way be responsible for E.M.F. "The early symptoms of E.M.F. are suggestive of a systemic disease and this might be infective" (Parry 1964). Parry and Abrahams (1965) however conceded that the diagnosis of this initial illness can be made only by those who are familiar with or aware of the early phase of the disease. They claim that a definite diagnosis at the early stage may be impossible and that the issue may only be settled if the patient is followed up for months.

Mkokolo (1962) had earlier expressed the view that the initial lesions preceeding E.M.F. are inflammatory oedema, necrosis and cellular infiltration. Parry and Abrahams (1965) declared that the endocardium in the initial stages of E.M.F. is composed of looser fresher fibrous tissue very vascular and rich in chronic inflammatory cells. Changes occur throughout the myocardium, which show pigment laden cells with disappearance of muscle cells. "Obviously," commented Shaper (1966), "the initial lesions of E.M.F. as described by Parry and Abrahams constitute a panearditis analagous to rheumatic infection particularly in its natural history."

Immunological/autoimmune Hypothesis:

The age incidence, the natural history, the occasional finding of Aschoff nodules in the myocardium as well as a raised anti-streptolysin O-titre are all factors that have led to the suggestion that E.M.F. might be an unusual expression of the rheumatic process (Abrahams 1959, Shaper et al 1960, Shaper 1966). That E.M.F. may in fact represent another form

of hypersensitivity response to streptococcal infection (Bonnyns et al 1966, Shaper 1966).

The presence of Aschoff nodules and high anti-streptolysin O-titre has not been confirmed in most cases of E.M.F. It is now believed that such an association as was observed previously may have been merely fortuitous (Parry et al 1965). The auto-immune hypothesis may however be significant, the formation of auto-antibodies could lead to an inflammatory disease ending in fibrosis (Coelho et al 1963).

Using the indirect immuno-fluorescent technique for bound gamma globulins, higher values of circulting heart antibodies have been demonstrated in E.M.F. patients than in other control African patients with or without cardiac disease (Van der Geld et al 1966, Shaper et al 1967). What significance should be attached to this finding in respect of the pathogenesis of endomyocardial fibrosis is not clear, especially as it has not been ascertained whether these antibodies are the cause or the result of endomyocardial fibrosis (Van der Geld et al 1966).

Parasitic Infestation:

The frequency of eosinophilia suggests an unrecognised parasitic infestation or possibly an allergic response of the body (Gray 1951). The parasitic theory may be important in view of the geographical distribution of E.M.F. which occurs in areas where vectors would be most easily found for any possible insect transmitted disease (Nwokolo 1955). Loa-loa, a filarial worm, has been incriminated as an aetiological factor in the pathogenesis of E.M.F. (Gray 1951, Ive et al 1966, Brockington et al 1967). Though eosinophilia is often associated with E.M.F., this is

a non-specific sign in these areas where all types of helminthic infestation are rife. Also an intensive search in some cases revealed neither filarial infestation nor eosinophilia (O'Brien 1954, Coelho et al 1963).

Following up the parasitic theory, and reasoning by analogy with elephantiasis and other conditions due to interference with lymph flow, it has been suggested that chronic impairment of cardiac lymph flow in man may play an important role in the production of endomyocardial fibrosis (Miller et al 1963). It has been shown that chronic interference with cardiac lymph flow in dogs produces ventricular endocardial haemorrhages, which healed with endocardial thickening associated with increased amounts of fibrous and elastic connective tissue (Miller et al 1963). Indeed the cardiac lymphatic system undoubtedly plays an important role in maintaining the integrity of the heart and like lymphatics elsewhere in the body removes excess protein and fluid from the interstitial space and aids in resolving or repairing necrotic foci (Kline et al 1963). The cardiac lesions produced in the dogs bore no resemblance to the established lesions of E.M.F., whilst parasitic invasion of the heart or its lymphatic drainage system are not pathological features of E.M.F. Edington et al (1963) however found "portions of unidentified microfilaria" in the hearts of two of their autopsy cases.

Virus Infection:

The existence of viruses capable of causing myocardial damage is well known (Williams et al 1954, Ball 1957, Arnott 1959, Shaper 1967).

An important experimental factor in inducing cardiac localization of virus is a decrease in the amount of oxygen supplied to the heart, the most susceptible portion of the heart being the inner part of the myocardium and papillary muscles, followed by the endocardium and then the pericardium (Williams et al 1954). Up to date, there is no clinical evidence or experimental proof for or against this hypothesis in the pathogenesis of E.M.F. It is not an unusual practice however to relegate to a viral aetiology, diseases whose pathogenesis are mainly in the realms of conjecture.

Malnutrition Theory:

The predominance of this disease in the lower socio-economic strata of the society may lend support to this theory. The diet of the African is typically low in protein and high in carbohydrate. The relationship of such a diet to fatty degeneration and fibrosis of the liver is known but no cardiac changes have been reported in man or in experimental animals with hepatic fibrosis of dietary cause (Gray 1951). Nwokolo (1955) reported a case of E.M.F. which occured 3 years after treatment for Kwashiokwor in a Nigerian child. There is however at present no evidence suggesting that Kwashiokwor leads to heart disease in later life (Shaper 1967). Also against the malnutrition theory is the fact that E.M.F. has been reported in well nourished Europeans and that E.M.F. has not been correlated with any severe degree of malnutrition in the Africans affected. If malnutrition is an aetiological factor in E.M.F., then the disease should be commoner in other parts of the world such as India and some other parts of Asia and Africa.

Avitaminosis B and other Deficiencies:

Vitamin B deficiency is considered in several accounts of E.M.F. only to be dismissed (Williams et al 1954). Although the African diet is poor in proteins, it contains ample amounts of B complex vitamins. Clinically, beri-beri manifests with a high output failure, a rapid bounding pulse, cutaneous vasodilatation and peripheral oedema, whilst the heart lesions are hypertrophy and dilatation of the ventricles associated with hydropic degeneration and interstitial oedema of the myocardium. Thus E.M.F. is pathologically and clinically different from the cardiac form of beri-beri. Also the pathognomonic therapeutic response to thiamine administration with beri-beri does nor occur in E.M.F. (Gray 1951, Nagaratnam et al 1959).

Fibrotic lesions in the heart have been demonstrated as a result of vitamin E deficiency in animals, but at present there is no evidence that such a specific nutritional deficiency is involved in the evolution of endomyocardial fibrosis (Shaper 1967).

Again myocardial necrosis healing with formation of a thick fibrous scar has been reported in rats treated with steroids and sodium salts. It was claimed that the subendocardial and endocardial scarring so formed bore a close resemblance to the lesions in E.M.F. (Selye 1958). However with prior prophylactic administration of magnesium salts these lesions did not develop. Also cardiac lesions with focal complete destruction of cardiac musculature have been demonstrated in potassium depleted rats (Molnar et al 1962). It is difficult to evaluate the significance of these findings in relation to the pathogenesis of E.M.F. in man.

Nutritional Toxin:

The injurious factor which damages the heart could be nutritional. E.M.F. may be due to some specific poison present either in food or in medicinal concoctions well known in Africa. That chemical factors can cause pathological changes in vascular tissue is now established (Arnott 1959). Edge (1946) attributed arsenical poisoning to the case described by him. Nwokolo (1955) stated that the possible existence of cardiotoxic factors in food acceptable to both Africans and Europeans in areas where E.M.F. occurs may be investigated with profit. Before expatiating further on the theory of cardiotoxic substances in food, it may be pertinent at this juncture to digress for a while and consider briefly the carcinoid syndrome.

In the carcinoid sydrome endogenous mechanisms for the elaboration of large quantities of cardiotoxic drugs have been demonstrated. This syndrome consists of tumours of the argentaffin cells of the gastro-intestinal tract with metastases to the liver and in some cases to other parenchymatous organs. It manifests mainly with right sided fibrotic endocardial lesions resulting in valvular distortion especially of the pulmonary and tricuspid valves. There is episodic flushing of the skin with patchy changing cynosis and telengeotasia. Other symptoms include intestinal hypermotility with frequent watery diarrhoea, paroxysmal dyspnoea with wheezing and respiratory distress and finally oedema and ascites (Thorson et al 1954, Heimark et al 1956, Sjoerdsma et al 1957, Fernow et al 1957).

These argentaffin tumours elaborate and secrete large quantities of a vaso-active substance 5-hydroxytryptamine (5-HT) (Lembeck 1953). Chronically high levels of blood 5-hydroxytryptamine (Pernow et al 1954, 1957) and a high urinary excretion of 5-hydroxy indole acetic acid (5-HIAA) a metabolic end product of 5-HT (Page et al 1955, Snow et al 1955, Sjoerdsma et al 1955, 1956, Goble et al 1955, 1956) occur in these patients. The greatly elevated urinary excretion of 5-hydroxyindole acetic acid is pathognomonic of malignant carcinoids and is diagnostic of this disorder (Page et al 1955, Sjoerdsma et al 1956).

It is generally accepted that all the manifestations of this syndrome are explicable on the biological actions of the humoral agent, 5-HT, and are in fact caused by the high blood levels of 5-HT in these patients (Bean et al 1955, Goble et al 1955, 1956, Pernow et al 1954, 1957, Waldenstrom et al 1955, Stacey 1957, Schneeloth et al 1957, 1959, Thorson 1954, 1958). "All these symptoms are due to 5-HT overactivity and can be reproduced in normal subjects if a sufficiently large dose is injected intravenously" (Davies 1959). Also the pathogenesis of the cardiac lesions is attributed directly to 5-HT action. Thus 5-HT is capable of causing endocardial damage with fibrous tissue replacement.

It is therefore very significant that this potent cardiotoxic substance is contained in some normal articles of the diet. Bananas contain high concentrations of 5-HT (Waalkes et al 1958, Foy and Parratt 1960). Also large quantities of 5-HT in association with nor-adrenaline and dopamine have been found in plantains (Waalkes et 1958, Foy et al 1958, 1960, Marshall 1959). Other edible fruits known to contain 5-HT

are tomatoes (West 1958, 1959) red plums, avocado and egg plant (Sjoerdsma 1959). The staple article of the diet of the Baganda people of Uganda is matoke, a banana which is usually eaten when green after steaming for 2 hours (Crawford 1962). In Nigeria, especially in the Southern States, bananas are consumed raw in the ripened stage and plantain ingestion may take one of five forms. It may be soaked in water and extracted as a beverage, may be boiled, fried in oil, roasted, or boiled and pounded into an homogenous paste and taken with stew.

As much as 40-50 micrograms of 5-HT is contained in one Gram of plantain pulp, the amount increasing with ripening (Udenfriend et al 1959, Foy and Parratt 1960). It is claimed that the plantain eating Nigerian consumes on the average per day about 300-500 Gms. of plantain (equivalent to one or two fruits) with a total 5-HT content of 20-40 milligrams (Foy and Parratt 1960, 1962). Also the daily diet of many Ugandans may contain as much as 50-100 mg. of 5-HT (Crawford 1963). The result of a purely accidental finding that ingestion of bananas increased urinary 5-HIAA excretion in rhesus monkeys (Anderson et al 1958) sparked up considerable interest in this urinary amine. Subsequently Crout and Sjoerdsma observed that the consumption of raw sweet bananas increased the 24 hour urinary excretion of 5-HIAA from 5.9 mg. to 54 mg. in man. Similar findings in man were reported by Lewis (1958), Puente-Duany et al (1958). Increased urinary 5-HIAA excretion also occurs in Nigerians after a plantain meal (Foy and Parratt 1962), and in Ugandans after a diet of matoke (Crawford 1962). Thus in the process of cooking plantain in East and West Africa, the contained 5-HT is not destroyed. After oral

administration of 5-HT, it is absorbed very rapidly from the upper gastro-intestinal tract and the probable duration of influence of ingested 5-HT on urinary 5-HIAA excretion is about 8 hours (Lewis 1958), basal levels being attained within 20 hours (Foy and Parratt 1962).

It is claimed that ingestion of bananas and plantain may increase 5-HIAA excretion sufficiently to suggest the diagnosis of malignant carcinoid (Udenfriend et al 1959, Crout et al 1959, Foy et al 1960). Since urinary excretion of 5-HIAA is a pointer to the turn-over rate of 5-HT in the body, then the plantain eating African is subjected to a stress similar to the argentaffin patients (Crawford 1962). Indeed Ball (1957) had earlier drawn attention to the similarity between carcinoid heart lesions and fibrotic endocardial lesions of E.M.F., and suggested that a humoral agent might be responsible in both conditions. Arnott (1959) postulated that a plantain diet may be an important environmental factor in the development of E.M.F. Foy and Parratt (1962) declared that continued ingestion of foods rich in 5-HT might well contribute to lesions similar to those found in endomyocardial fibrosis. The theory that potent cardiotoxic substances in food may be involved in the pathogenesis of E.M.F. was strengthened by the findings of McKinney and Crawford (1963). These workers produced endocardial and myocardial damage in rats on a diet based on the local dietary staple (plantain). They further claimed that the lesions produced in these animals had some similarities to those of E.M.F. in man.

It is extremely pertinent therefore that the geographical distribution of E.M.P. correlates well with the plantain eating habit of the local population. E.M.P. occurs most commonly in areas where plantain is a staple article of the diet (Mckinney and Crawford 1965). E.M.P. is very common in the West Coast of Africa stretching right across from the Cameroons to the Ivory Coast especially in the Southern belts of rain forest where plantain and bananas grow in abundance. E.M.P. is rare in the Northern States of Nigeria, where plantain neither grows nor is regularly consumed. The only proved case of E.M.P. in Northern Nigeria is that of Boot (1954) in comparison to the numerous case reports in the Southern States. E.M.P. is rare in South-Africa (Brink and Weber 1966) Malawi and Southern Rhodesia, (Balachin 1959) Kenya, and South East Africa as well as the West Indian Islands (Nwokolo 1962). Except for the West Indies, plantain is not a dietary staple in any of these countries. The rarity of E.M.P. in Jamaica in spite of a high consumption of bananas and plantain could be related to the method of cooking. The Jamaicans boil the banana in water and this method of cooking elutes and destroys most of the 5-HT (Crawford 1963).

"Though it remains true that E.M.P. and banana diet share a common geographical distribution, there is at present no direct evidence from clinical and experimental studies to incriminate serotonin in the development of E.M.P. The suggestion remains as interesting a hypothesis as when it was first put forward." However, "in a problem as obscure as E.M.P., a firm working hypothesis capable of being proved or disproved is better than no hypothesis at all" (Shaper 1967).

CHAPTER THREE

5-Hydroxytryptamine

Introduction:

The history of 5-HT dates back for almost a century. In 1904, it was shown that the vasoconstrictor property of blood increased when it clotted. This vasoconstrictor principle was isolated and named serotonin by Rapport and his colleagues in 1948. It was later crystallised and identified as 5-HT (Rapport 1949). Meanwhile Erspamer and his co-workers had isolated the specific secretory substance of the entero-chromaffin system and given it the name enteramine. It was subsequently shown that enteramine is serotonin (Erspamer and Asero 1952). Thus 5-HT, enteramine and serotonin are one and the same substance.

5-HT is widely distributed both in the animal and plant kingdoms. It is present in both the invertebrate and vertebrate animals (Erspamer & Boretti 1950; Adams and Weiss 1959). In man it is to be found in the gastro-intestinal tract, the brain, spleen and blood. The biosynthesis of 5-HT occurs only in the brain and gastro-intestinal tract, indeed the gastro-intestinal mucosa is the only source of extra-cerebral 5-HT and thus of all urinary 5-HIAA (Erspamer et al 1959). Blood 5-HT is therefore derived mainly from the gastro-intestinal tract whilst splenic 5-HT is entirely due to its blood content.

The Biogenesis of 5-HT:

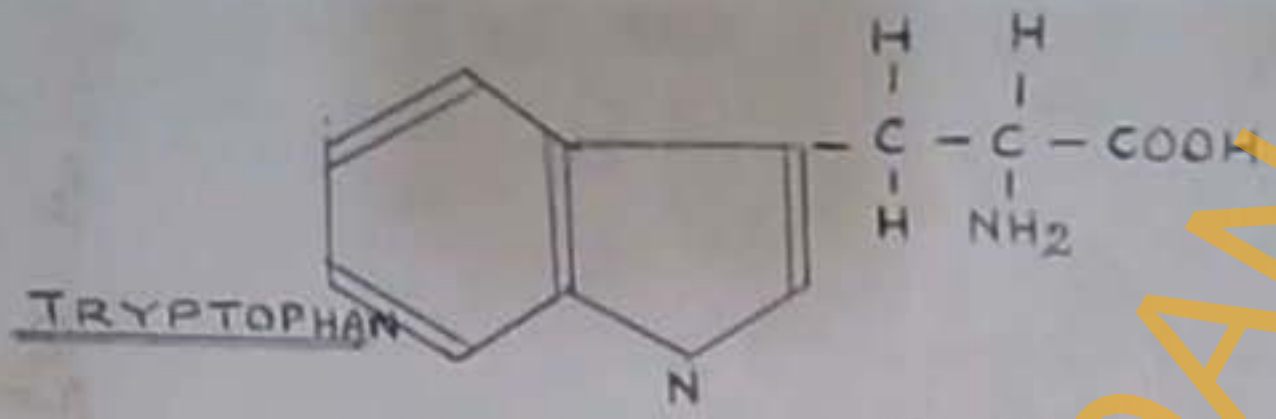
Gastro-intestinal 5-HT is formed in the so called entero-chromaffin system of cells, first described by Heidenhain in 1870. These cells are to be found throughout the gastro-intestinal tract, being most common in the terminal ileum and appendix. They are also found in the gall bladder,

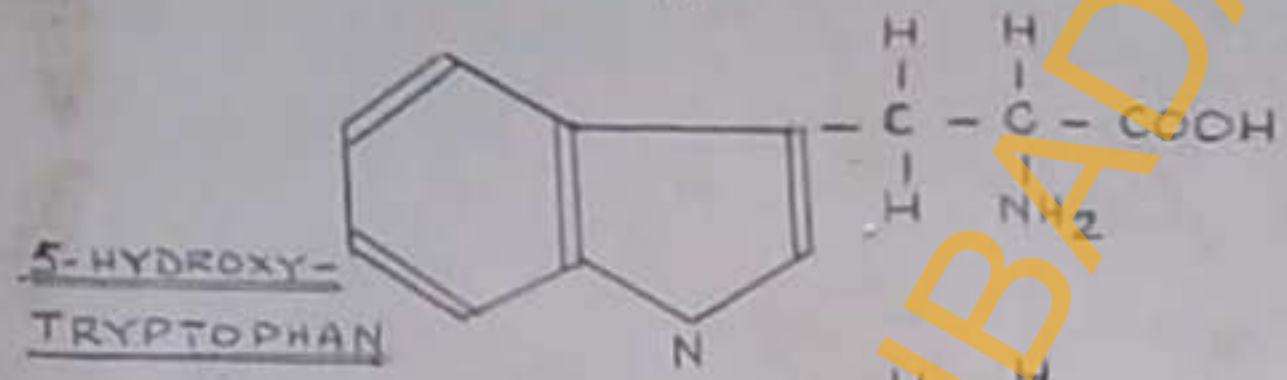
bile ducts and Meckels diverticulum. Kultschitzky (1897) extended on Heidenhain's work and described the granular nature of these cells. The granules do not exist in the unfixed state and are due to a chemical artefact produced by formalin fixative reacting with 5-HT (Davies 1959). The Kultschitzky cells as they were subsequently known, after formol fixation, exhibit granules capable of reducing ammoniacal silver nitrate (argentaffinity) or turning yellow or brown following treatment with bichromate (chromaffinity) and these staining characteristics are essentially due to their 5-HT content (Butler et al 1953, Sheppard et al 1953). Thus the parenteral administration of reserpine to guinea pigs caused a release of chromogenic material of the duodenal entero-chromaffin cells and a parrallel reduction in the amount of extractable 5-HT (Benditt and Wong 1957). The entero-chromaffin or argentaffin system has therefore also been applied to this collection of cells which Erspamer regards as a diffuse endocrine system designed for the elaboration, storage and release of 5-HT (Erspamer and Asero 1952). 5-HT is absent from the intestines of animals lacking in entero-chromaffin cells, and during embryonic development of such animals that do possess this system, 5-HT makes its appearance simultaneously with the first entero-chromaffin cells (Lembeck 1953). Thus a patient whose intestinal tract had been almost entirely removed had neither a detectable amount of 5-HIAA in the urine nor serotonin in the platelets (Weissbach, et al 1959).

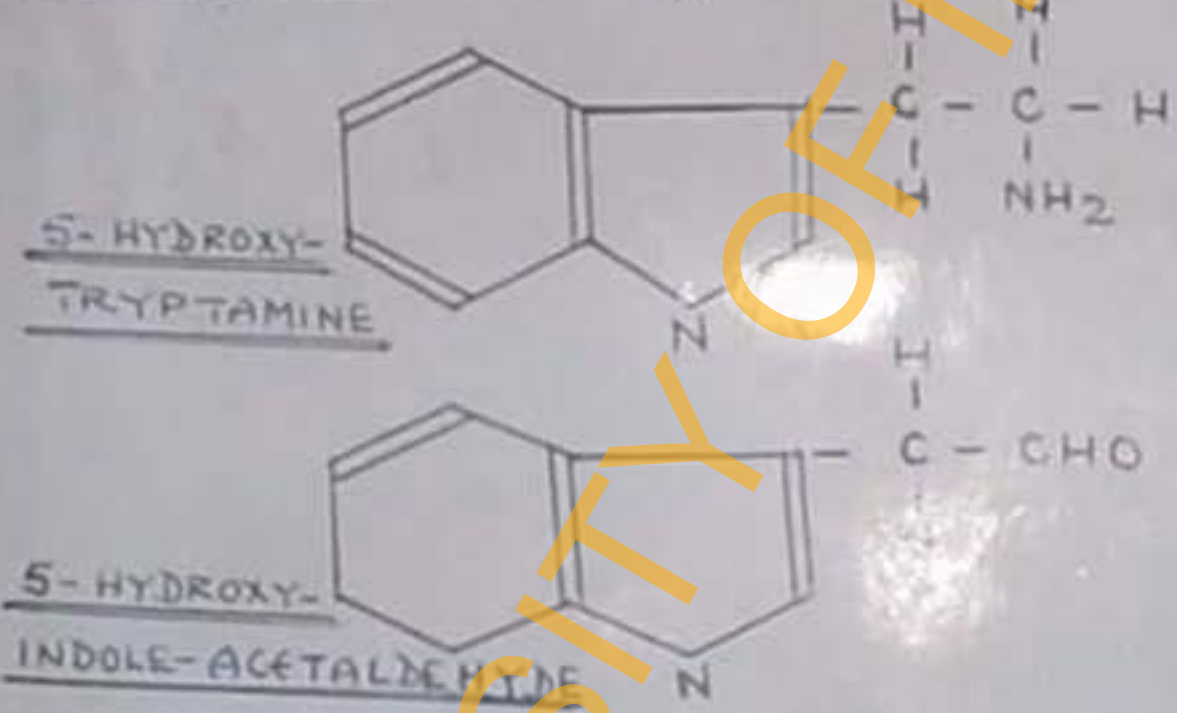
5-HT is formed by the entero-chromaffin system from its dietary precursor, an essential amino-acid tryptophan (Sjoerdsma et al 1956). Administration of tryptophan by mouth produces an increase in the number

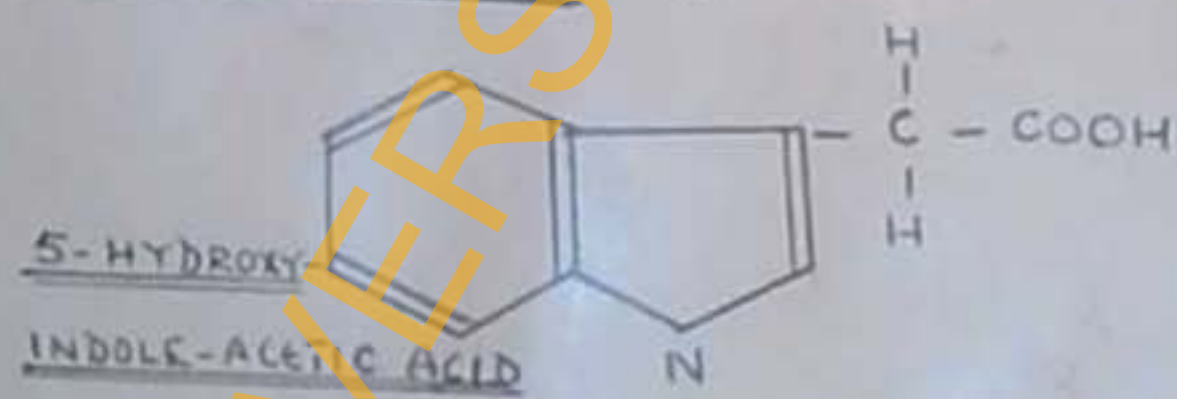
of the entero-chromaffin cells as well as the 5-HT content of the gastro-intestinal mucosa (Hadistry et al 1956). Tryptophan is first hydroxylated to 5-hydroxytryptophan (5-HTP) by the enzyme L-tryptophan 5-hydroxylase and 5-HTP is then decarboxylated to 5-HT by the action of the enzyme 5-Hydroxytryptophan decarboxylase. Thus in a 5-HTP secreting gastric carcinoid tumour, tryptophan hydroxylation increased following oral administration of L-tryptophan as shown by the elevated urinary excretion of 5-HTP (Oates et al 1962). Also when 5-HTP is administered to animals, it is rapidly taken up by the tissues and converted into serotonin. 5-HT brain levels of more than ten times normal have been produced in this manner (Udenfriend et al 1957). The action of 5-HTP-decarboxylase requires the presence of pyridoxine phosphate or Vitamin B6 as its co-enzyme. Buxton and Sinclair (1956) found the activity of renal 5-HTP decarboxylase was low in rats on Vitamin B6 deficient diets, and Pyridoxine phosphate in vitro restored the lost activity. Also 5-HTP decarboxylase enzyme became inactive when its co-enzyme was separated from it. The addition of 5-HTP to the solution passing through the intestinal lumen of guinea pigs or rabbits greatly increased the amount of 5-HT in the effluent whilst the convertion of 5-HTP to 5-HT was greatly enhanced in the presence of pyridoxal phosphate (Bulbring and Lin 1958).

However, only the gastro-intestinal mucosa, the nervous tissue in man, and the mast cells in rats and mice which contain L-tryptophan 5-hydroxylase are capable of carrying out the complete biosynthesis of 5-HT. This restricted distribution of tryptophan hydroxylase contrasts so









TRYPTOPHAN-5-HYDROXYINDOLE METABOLIC PATHWAY

(Udenfriend et al 1956)

sharply with the ubliquitous distribution of 5-HT decarboxylase that it is believed that hydroxylation and not decarboxylation is the step controlling the rate of formation of 5-HT (Udenfriend et al 1957, Sjoerdsma 1959).

Blood and Platelet 5-HT:

5-HT formed in the entero-chromaffin cells and released into the blood stream is quickly taken up by the blood platelets (Udenfriend et al 1954, Humphrey et al 1954, Hadistry et al 1955). Indeed platelets can absorb 5-HT against a concentration gradient (Humphrey et al 1954) and 5-HT uptake is inhibited by metabolic poisons such as fluorides and iodoacetates (Maynert 1967) showing that the uptake of 5-HT is an active process. The platelet 5-HT is concentrated in cytoplasmic granules in which it is associated with adenosine-triphosphate (A.T.P), (Baker et al 1959). Also since a proportional relationship has been demonstrated between A.T.P. in platelet and the platelet 5-HT content, it was suggested that A.T.P. plays a significant role in the binding and release of 5-HT in platelets (Born et al 1958). In support of this view is the fact that blood clotting leads to the disappearance of A.T.P. as well as 5-HT from platelets (Born 1956). On the other hand, reserpine which depletes platelet 5-HT, does not alter the platelet A.T.P. content (Hadistry 1957).

Normal values of 5-HT in whole blood of man range from 0.02 to 0.3 micrograms/millilitre (Erspamer 1954, Snow et al 1955, Davies 1959, Sjoerdsma 1959, Waalkes 1959). Almost all the blood 5-HT is bound to platelets leaving very little free in the plasma (Rand and Reid 1951, Sjoerdsma et al 1956, Armin and Grant 1957). Indeed the appearance of 5-HT

in serum depends on the presence of platelets at the time of clotting (Rand and Reid 1951). Thus platelets constitute packets of very active pharmacological substances which circulate in the blood stream, releasing their contents according to the body's requirement (Humphrey et al 1954).

Not much is known about the physiological factors involved in the regulation of 5-HT release from intact platelets. Udenfriend et al (1954) stated that 5-HT is so firmly bound to platelets that it is released only after platelet disruption. It is also well known that 5-HT is released from platelets during the process of clotting (Zucker et al 1955a). Also thrombin in low concentrations liberates 5-HT from washed platelets suspended in saline, plasma or albumin (Zucker et al 1955b). It has been shown that (nephrosin) contained in the alkaline extracts of kidney and stomach mucosa liberates 5-HT from whole blood or platelet suspensions (Toh 1956). Rat's kidney and brain extracts did also liberate 5-HT from mast cells obtained from a mouse mastocytoma (Giaman and Potter 1958). Reserpine not only releases 5-HT from platelets (Shore et al 1956, Hadistry et al 1956) but depletes the brain and gastro-intestinal tract of their 5-HT content (Pletscher et al 1955, Shore et al 1955). Cardiac glycosides inhibit the absorption of 5-HT by platelets whereas potassium an inorganic phosphates in low concentrations enchance platelet 5-HT accumulat ion (Weissbach et al 1960a and b).

Blood 5-HT therefore consists of two factions, one bound to platelet and the other free in the plasma, the latter being very small as platelets absorb 5-HT with avidity (Toh 1954, 1956) and free 5-HT is rapidly removed

by the tissues (Erspamer 1954). Free plasma 5-HT, as well as that released either from intact platelets or after platelet disruption is rapidly deaminated by the enzyme mono-amine oxidase via 5-hydroxyacetaldehyde to 5-hydroxy indole acetic acid (Bradley et al 1950, Blaschko 1953). Platelet 5-HT is metabolically inert and plasma is indeed the necessary intermediate between platelets which carry 5-HT and have no activating enzymes and the cells of the parenchymatous organs such as liver, kidneys intestines and lungs which are rich in mono-amine oxidase (Erspamer 1955). Thus platelets are circulating reservoirs of 5-HT, drawing upon the richer deposits represented by the entero-chromaffin cells of the gastro-intestinal mucosa and capable of protecting 5-HT from attack by amine oxidase. This ensures a gradual and lasting release of 5-HT into the plasma (Erspamer 1954).

It seems however that platelet 5-HT may be liberated during transit through the lungs, this release has been detected by direct sampling across the organ and takes place even in the absence of platelet breakdown. Although platelet losses occur in passing through the lungs, yet a greater amount of serotonin is liberated than can be accounted for by platelet disruption alone (Crawford 1965). The fact that blood returning from the intestines contain more 5-HT than blood coming from other vascular areas confirms the view that platelet 5-HT must be released with considerable ease and rapidity (Toh 1954, Erspamer et al 1959).

Formation and Excretion of 5-HIAA:

It has been known for many years that a serum vasocontrictor appearing spontaneously in clotted blood disappears when the defibrinated blood is

passed through the lungs (Eichhotts and Verney 1924). Also extracts of sheep's lungs have been shown to destroy serotonin (Rappart et al 1948) and the presence of mono-amine oxidase has been demonstrated in these extracts (Bradley et al 1950). Indeed incubation of 5-HT with homogenates of mammalian intestines, liver and kidney resulted in the formation of 5-HIAA in vitro (Erspamer 1954). The oxidative deamination of 5-HT to 5-HIAA takes place only in these tissues (Blaschko 1952, Erspamer 1954). Titus et al (1955), noted that the ability of liver and kidney homogenates to convert 5-HT to 5-HIAA was blocked by semicarbazide, suggesting that 5-hydroxy-indole acetaldehyde is an intermediate product. This inhibition was accompanied by the accumulation of the substance semicarbazone.

5-HIAA, the breakdown product of 5-HT is a normal constituent of mammalian urine. Normal 24 hour urinary excretion of 5-HIAA in man ranges from 2 mg. to 10 mg. (Titus et al 1954, Sjoerdsma et al 1955, Udenfriend et al 1955). After administration of 5-HT, there is a marked increase in the urinary output of 5-HIAA (Titus et al 1954, Erspamer 1955). Also changes in the release of 5-HT from the body stores as during the administration of 5-HT releasers such as reserpine, or in the production of endogenous 5-HT as in carcinoidosis also result in an increase urinary excretion of 5-HIAA (Erspamer 1954). Carcinoid patients may excrete as much as 60-2,000 mg. of 5-HIAA in 24 hours, whilst corresponding serum 5-HT levels range from 0.2 to 6.5 micrograms/ml. (Udenfriend et al 1955).

It is claimed that less than 50% of administered exogenous 5-HT appears in the urine as 5-HIAA (Erspamer 1954). Melmon et al (1963) however reported a 60-90% recovery of administered exogenous 5-HT as

urinary 5-HIAA. Three factors determine the degree and duration of effect of ingested 5-HT, the absorption of 5-HT from the gastro-intestinal tract of which nothing is known (Page 1958), the rate of convertion of 5-HT and the renal clearance of 5-HIAA, both of which are quite rapid (Udenfriend 1946). The rapidity with which 5-HT is metabolized by mono-amine oxidase suggests that this enzyme may be directly involved in limiting the physiologic action of 5-HT (Sjoerdsma et al 1955).

The renal excretion of 5-HIAA is achieved by glomerular filtration and active tubular excretion, thus the intravenous administration of 2 Gms. of probenecid to patients with the carcinoid syndrome resulted in a decreased urinary excretion of 5-HIAA and its accumulation in the plasma (Despopoulos 1956, Despopoulos et al 1957). This excretion rate is not however significantly affected by changes in urinary pH (Milne et al 1960). There is a diurnal variation in the excretion of 5-HIAA in man, lowest values occuring between midnight and 3 a.m. and peak values in the three hour period on either side of noon (Johsen et al 1958). Very small amounts of 5-HT escape enzymatic degradation and is excreted unchanged in the urine. The daily urinary excretion of 5-HT in healthy subjects range from 20 - 720 micrograms (Twarog et al 1953, Rodnight 1956, Sjoerdsma et al 1956).

Functional significance of 5-HT:

Despite the wide distribution of 5-HT in nature and the presence of endogenous mechanisms for its elaboration in man, its physiological function in the body is still far from clear. Page (1954) declared that it is evident from the enthusiasm of most research workers in this field, that

5-HT is going to have many functions real and fancied before its true role in biological function is told. This comment holds true today as when it was first made. Thus it is said to be a potent stimulus of gastro-intestinal, bronchial and vascular smooth muscle, that it is concerned with the maintenance of normal intestinal motility, the regulation of renal haemodynamics and the general circulation as a whole. It is also believed that 5-HT is an anti-diuretic, that it plays a part in haemostasis and finally that it may be involved in the control of mental processes (Page 1958).

5-HT and the Nervous System:

5-HT is formed locally in the brain, and its presence in the brain was first demonstrated by Twarog and Page (1953). Its distribution in the brain is similar to that of adrenaline (Amin et al 1954). Brain 5-HT is formed from its immediate precursor 5-HTP which diffuses easily across the blood-brain barrier whereas blood 5-HT does not (Sjoerdsma et al 1957). Indeed there is a close correlation between the distribution of brain 5-HT and 5-hydroxy-tryptophan decarboxylase, an enzyme responsible for its final elaboration (Gaddum et al 1956, Bogdanski et al 1957).

It has been postulated that 5-HT participates in cerebral function and in normal mental processes (Wooley and Shaw 1954a and b). Low serum 5-HT and urinary 5-HIAA excretion have been noted in mental defectives, whilst low brain 5-HT levels occur in rats with a similar mental deficiency. This may be due to a defect in the absorption and transport of tryptophan, or to an abnormal hydroxylation of tryptophan to 5-HTP similar to

phenyl-alanine inhibition of L-tryptophan 5-hydroxylase activity, impaired uptake of 5-HTP by the brain or finally to 5-HTP decarboxylase inhibition (Page 1967). Both high and low brain 5-HT values have been associated with psychiatric and psychotic manifestations. Various reports suggest that both higher and lower urinary 5-HIAA excretion are associated with schizophrenic patients than in normal controls (Sjoerdsma 1959). Thus a specific antagonist of 5-HT, lysergic acid diethylamide (L.S.D) administered in small doses to humans produced hallucinations and psychotic like states indistinguishable from schizophrenia (Schenckloth et al 1957). Also the administration of 5-HTP to experimental animals resulted in a considerable increase in brain 5-HT, associated with marked central disturbance similar to that following L.S.D. therapy (Udenfriend et al 1957).

5-HT may also be involved in central synaptic transmission (Marazzi et al 1955, Weight et al 1967) especially in the central levels of the parasympathetic system (Brodie et al 1957, Brodie 1967) as well as in the excitation of certain afferent and efferent autonomic nerves (Douglas et al 1957, Bulle 1959). Reserpine releases bound 5-HT from the brain. Reserpine also potentiates hexobarbital effects on mice by prolonging the barbiturate sleeping time. As this potentiation is inhibited by L.S.D., it has been suggested that reserpine action on the brain is mainly due to 5-HT release, and that its accumulation in small amounts stimulates the parasympathetic centres causing sedation (Shore et al 1955, Pletscher et al 1955, 1956). The action of reserpine on the brain may not be entirely due to 5-HT release alone since it also causes a simultaneous release of

catecholamines such as nor-adrenaline (Muscholl and Vogt 1957). Recently Jouvet (1967) produced data suggesting that 5-HT may contribute to the induction and persistence of sleep. 5-HT and the catecholamines have also been involved in body temperature control and the general reactions to fever (Gisman 1967).

In the molluscan nervous system, 5-HT is contained in granules of ganglia and peripheral nerves, and on the basis of the high turn-over rate of 5-HT at these sites, (half life 15-20 minutes), it has been suggested that, in molluscs at least, 5-HT might be involved in the transmission of nervous impulses (Welsh 1958, 1967, Gerschenfeld et al 1967). Application of 5-HT to the exposed base of a blister on the human forearm causes pain; and it has been postulated that 5-HT release from platelets contributes together with histamine and bradykinin-like polypeptides to the pain which follows injury to tissues (Keele 1957). 5-HT injected intra-arterially into the skin of rat's leg led to a slow discharge of impulses from fibres of the saphenous nerve that transmit painful and itching sensations (Douglas et al 1959). In this respect, it is pertinent to note that 5-HT is a constituent of itch powder of the mucuna pruriens (Dowden et al 1954). Wasp's venom contains not only histamine but high concentrations of 5-HT and kinins (Holdstock et al 1957). 5-HT has also been detected in venoms of scorpions and toads (Erspamer 1954). That 5-HT may also participate in the development of migrainous headache is supported by the fact that methysergide, a very potent serotonin antagonist has been used with success as a prophylactic drug against attacks (Southwell et al 1964). It is

suggested that arterial dilatation which occurs during a migraine headache may be accompanied by the release of serotonin which lowers the pain-threshold locally. 5-HT certainly possesses vasoconstrictory properties and in low concentrations can give rise to pain (Sicuteri et al 1965). Also mono-amine oxidase inhibitors reduce attacks whilst reserpine may provoke attacks accompanied by an increase urinary excretion of 5-HIAA and V.M.A. (Curzon 1967).

5-HT and Clotting Mechanism:

It has been asserted that 5-HT in platelets plays an important role in the defence mechanism against bleeding. Thus it is said that it enhances clot retraction (Fenichel and Seegers 1955, Ballerni 1955), that it accelerates the convertion of fibrinogen to fibrin in the presence of thrombin (Milne and Cohn 1957, Kato and Gozsy 1958) and finally that it reduces the antithrombin time prolonged by the action of heparin (Keeler 1958). Most of the above resulsts have been obtained with highly unphysiological doses of 5-HT. Thus following complete depletion of platelet 5-HT by reserpine treatment in animals and man, no significant changes were noticed in the clotting process (Haverback et al 1956, 1957; Weiner et al 1957; Hutchison et al 1959; Shore et al 1959). The results of various workers on the capability of 5-HT to reduce bleeding time in patients with haemorrhagic disorders are in the main conflicting. Thus the general concensus of opinion is that 5-HT has no influence whatever on the blood clotting system and that the ability of the substance to shorten the bleeding time is a pure consequence of its vasoconstrictor action (Sucker 1947, Reid 1952, Erspamer 1954).

Vasoconstrictor Properties of 5-HT:

It has long been established that blood after clotting possesses vasoconstrictor properties, Most of this vasoconstrictor action of serum is due to 5-HT (Rand and Reid 1951). Marked vasoconstriction, increased resistance to flow and elevation of pulmonary artery and right ventricular pressures are associated with intravenous injections of 5-HT in cats (Reid 1952) and in dogs (Nahas 1958, Nahas and MacDonald 1959). High doses of 5-HT injected into a dog's pulmonary artery resulted in severe pulmonary oedema, due to increased pulmonary capillary permeability (Kobins et al 1959). 5-HT is not an effective pulmonary vasoconstrictor agent in man and calves, thus intravenous infusion in these species produces neither changes in pulmonary resistance nor pressure (Harris et al 1960, Kuida et al 1961). 5-HT injected into the umbillical artery of perfused human placenta displayed a potent constrictor action (Astron and Samolius 1957) that it has been suggested that 5-HT liberated from blood platelets may participate in the spontaneous closure of the umbillical artery after birth.

The action of 5-HT on the musculo-cutaneous vessels is to constrict the large arteries and veins whilst the small vessels are dilated, thus producing oedema during prolonged infusion due to increased capillary hydrostatic pressure provoked by venous constriction (Hardy et al 1957, Hardy 1960). In man, 5-HT infused into the brachial artery in doses of 1 - 16 ng/minute caused a reduction of blood flow through the forearm and hand, associated with some volume increase in these areas due to oedema formation. A marked flushing of the skin was a usual accompaniment whilst

with continuous infusion cyanosis gradually developed in the skin of the fingers followed by petechiae hours later. These phenomena have been ascribed to constriction of the arterioles mainly responsible for resistance to flow and dilatation of the capillaries responsible for the colour of the skin (Roddie et al 1955, Magalini et al 1956).

5-HT is however a powerful coronary dilator increasing the oxygen supply to the myocardium without increasing ventricular work (Schofield and Walker 1953). The effects of 5-HT on the heart of intact animals are variable depending on animal species and on route and rapidity of administration. In man, both rapid intravenous injection and intravenous infusion generally produce an increase in heart rate which precedes pressure changes suggesting a direct action on the heart. Stroke volume is reduced but cardiac output rises. 5-HT has a marked stimulant action on the heart of molusca increasing the rate but not the strength of contraction, it however slows the rate of intact dog's heart (Erspamer and Ghiretti 1951).

5-HT is neither a pure hypertensive nor a pure hypotensive agent but according to dose, route of administration, neurogenic vascular tone, the general conditions of the cardiovascular apparatus and above all the animal species, 5-HT can elicit hypotensive, hypertensive or mixed responses (Erspamer 1954). As 5-HT can produce both hypotension and hypertension in the same animal, Page and McCubbin (1953) introduced the term amphiberic to describe the blood pressure response. Some of the factors determining its effects on arterial pressure are stimulation of the chemo-and baroreceptors, an action on the blood vessels causing either

vasoconstriction or dilatation as well as inhibition of neurogenic vasoconstrictor tone. The blood pressure response therefore depends largely on the vasomotor tone, thus when this is low as after ganglion blockade or spinal transection, the response is pressor. Whereas when vasomotor tone is high for example after section of the buffer nerves, the response is depressor. Page therefore refers to 5-HT as humoral antagonist of neurogenic vasomotor tone and speaks of it as a chemical buffering system (Page 1954 and 1958). In man single intravenous injection of 5-HT in both normotensive and hypertensive subjects, results in depressor responses with doses less than 0.3 mg, biphasic responses for doses between 0.3 mg. to 1 mg. and pressor responses at 1 mg. or more. These responses are not prevented by prior administration of hexamethonium, rogitine, atropine and antihistamines (Hallander et al 1957).

5-HT and Respiration:

5-HT has a direct action on bronchioles causing constriction. Injections of 5-HT or exposure to 1% aerosol of 5-HT resulted in the development of severe dyspnoea followed by convulsions in guinea-pigs (Herxheimer 1953a). Similar results were obtained in man, and in 3 of 6 asthmatic patients studied, inhalation of 1% aerosol of 5-HT provoked a severe attack of bronchospasm which regressed spontaneously or was abolished with isoprenaline (Herxheimer 1953b). Reflex apnoea or hyperpnoea may occur with intravenous injection of 5-HT in animals according to the species. Douglas and Toh (1953) showed that the hyperpnoeic response to 5-HT was abolished by sinus nerve section. Introduction of

5-HT into a cat's carotid artery resulted in hyperpnoea accompanied by a considerable increase in sinus nerve discharge and a fall in arterial blood pressure. Thus it would seem that 5-HT stimulates both the chemo-and baroreceptors. 5-HT may also have a central stimulating effect on respiration since larger doses of 5-HT after section of the sinus nerve still produced an hyperpnoeic response (Ginsel et al 1954; McCubbin et al 1956). The apnoeic response occasionally observed in cats before hyperpnoea has been attributed to the stimulation of stretch receptors in the lungs. Thus, injection of serotonin stopped respiration in the expiratory phase with an increased number of impulses recorded form these stretch receptors (Schneider and Yonkman 1953). This is in conflict with the opinion of Mott and Paintal (1953) that 5-HT has no effect whatever on pulmonary stretch receptors.

The Anti-diuretic effect of 5-HT:

It is generally accepted that 5-HT has an anti-diuretic action. A marked anti-diuretic effect has been reported after administration of 5-HT in two patients with diabetes insipidus (Amoni et al 1955). It is suggested that the anti-diuretic effect of 5-HT is due to a preferential vasoconstriction of the afferent glomerular arterioles, that "enteramine represents the specific hormone of the enterochromaffin cell system, a product designed to control the tonus of the intra-renal vascular bed" (Erspamer and Asero 1952).

Through simultaneous evaluations of urine flow and renal excretion of test substances (thiosulphate, creatinine, PAS) it was demonstrated that 5-HT antidiuresis in the rat is due primarily and essentially to

reduction in the glomerular filtration rate (Erspamer 1954). 5-HT inhibits not only water diuresis but diuresis produced by osmotic duiretics and xanthine derivatives. It is significant to note however that after 5-HT administration the percentage reduction in urine volume is greater than either that of glomerular filtration rate or renal plasma flow, suggesting some stimulant action of 5-HT on the reabsorption by the tubular epitholium. Erspamer claims however that this may be explained either by the fact that the glomerular filtration being reduced glomerular load is more completely reabsorbed during the slower passage down the nephron or as a result of afferent vasoconstriction there is a partial diversion of the blood from the cortex to the medulla associated with passive reabsorption of water into the blood of the vasa recta through the walls of thin segment of the loop of henle. Abrahams and Pickford (1956) on the other hand believe that the antidiuretic action of 5-HT is consequent on its action on the vascular system in general and not a direct action on the afferent glomerular arterioles. Thus it was found that when 5-HT was injected arterially into the kidneys of dogs, a much larger dose was required to cause antidiuresis than on intravenous injection.

Del Greco et al (1956), confirmed the anti-diuretic effect of 5-HT on rats brought into osmotic diuresis with mannitol. This effect was dependent on decreased glomerular filtration and largely independent of changes in blood pressure. However both the renal and arterial pressure effects of 5-HT were prevented by specific antagonists of 5-HT such as L.S.D. and its analogue. Erspamer (1954) stated that the most effective

way of administering 5-HT is subcutaneously, intravenous injection is several times less effective and when given by mouth is practically inactive. In rats at least where subcutaneous injections cause marked local oedema, part of the anti-diuretic action in this animal may be due to local fluid retention (Abraham and Pickford 1956, Del Greco et al 1956). Also since serotonin is painful when injected subcutaneously, it may be that some part of the inhibition of water diuresis observed in some experiments may have been due to the release of anti-diuretic hormone from the pituitary under the stimulus of pain (Page 1954).

5-HT and Gastro-intestinal Function:

Lembeck (1953) suggested the possibility that the enterochromaffin cells secrete 5-HT to act as neurohormones of Meissner's plexus, thus constituting a physiological stimulus of intestinal motility. Intravenous injection of 5-HT in dogs produced a powerful stimulating action on intestinal motility as well as on the rhythmic automatic activity of the intestinal villi (Haverbach et al 1957). Injected subcutaneously into rats, 5-HT increased faecal excretion, and introduced into the isolated guinea pigs ileum resulted in a lowering of the threshold stimulus required to elicit peristalsis and the contractions so elicited expelled a larger volume of fluid. Also the release of 5-HT from the gastro-intestinal tract was increased by raising the intraluminal pressure, suggesting a correlation between intra-luminal pressure and 5-HT release. It was therefore concluded that 5-HT formed and stored locally in the mucous membrane is released in proportion to the rise in intraluminal pressure

and that it sensitises pressure receptors situated in the mucosa (Bulbring and Lin 1958). Whilst in some cases the gastro-intestinal symptoms of the carcinoid syndrome may be attributable to obstructive lesions in the gut, colicky abdominal pain may occur with no apparent obstructive element. This is due to local release of 5-HT by the tumour causing spasm and hyperactivity of the intestinal muscle (Davies 1959). 5-HT however inhibited the volume and acidity of both spontaneous and histamine induced gastric secretion in man and dogs, whilst it increased the production of mucus (Black et al 1958, Pichel et al 1959).

The intestinal response to 5-HT was potentiated by antihistaminics, inhibited by benzyl-analogue of serotonin and anticholingergic drugs but was unaffected by ganglion blockade with hexamethonium. It was therefore concluded that 5-HT stimulates intestinal motor activity through cholinergic nerves at a site distal to the ganglionic synapse (Hendrix et al 1957). The workers do not however believe that 5-HT has any physiologic function with respect to the intestine but suggested that argentaffin cells and 5-HT may affect intestinal motor activity only under certain conditions.

Most of the above reported effects of 5-HT were recorded with high pharmacological doses of this substance. Also in the normal healthy man, the body's means of removing 5-HT from the circulating plasma are so efficient and rapid, that it is unlikely that 5-HT plays any significant role in normal physiological function. However an insight into the possible pathologic physiology of 5-HT can be gained by a brief review of the carcinoid syndrome.

THE CARCINOID SYNDROME:

The Flush Reaction:

A passing reference has already been made to the carcinoid syndrome (Pages 36 and 37). The periodic episodic flushing attacks characteristic of this syndrome clearly mimic the effects of 5-HT on the musculo-cutaneous vessels; and it is generally accepted that this is a consequence of high blood levels of 5-HT in these patients (Schneckloth et al 1957, Thorson 1958, Sjoerdsma 1959). Small amounts of 5-HT injected into human volunteers produced the typical flush (Reddie et al 1955). Also a distinctly elevated 5-HT level in blood serum and of 5-HIAA in urine has been demonstrated in those carcinoid patients in which flushing was the predominant sympton (Pernow et al 1957). An increase in the free plasma 5-HT in hepatic venous blood taken during flushing attacks was noted in one patient with metastatic carcinoid deposits of the liver (Andrews et al 1960, Peart et al 1961). In another patient, 5-HIAA excretion ranged from 200 - 400 mg. per day and increased during flushing attacks to 600 - 900 mg. (Sjoerdsma et al 1957). Intravenous administration of reserpine, a specific releaser of 5-HT not only increased the frequency and intensity of flushes in carcinoid patients but was also associated with a marked rise in urinary 5-HIAA excretion (Smith 1957; Christian et al 1959).

Though a measurable rise in blood levels of 5-HT is not always obtained during flushing attacks in carcinoid patients, it appears that flushing is accompanied by an increased release of 5-HT from the tumour (Sjoerdsma et al 1956, 1957, Schneckloth et al 1959, Nelson et al 1965).

Flushing attacks were repeatedly induced in some patients by manual massage of testicular metastases (Daugherty et al 1955, Dockerty et al 1956). Also where the tumour arose from an ovarian teratoma, total removal of the argentaffinoma not only put an end to the vasomotor disturbances but the urinary 5-HIAA excretion values also fell to normal (Sauer 1958, Thorson et al 1958). Increased frequency of bowel actions, hyperpnoea with dyspnoea and wheezing, tachycardia, palpitations and hypotension as well as oliguria are well known accompaniments of severe flushing attacks in those carcinoid patients (Snow et al 1955, Heimark et al 1956, Thomson 1956, Sjoerdsma et al 1956, 1964; Schneckloth et al 1959, Peart et al 1961, Melman et al 1965). All these symptoms can be explained on the basis of 5-HT action and there is no doubt that serotonin is capable of producing most of the manifestations of the carcinoid syndrome (Melman et al 1965).

Other flush precipitating factors in this syndrome include small intravenous doses of adrenaline or noradrenaline (Peart et al 1959 and 1961; Andrews et al 1961, Robertson et al 1962) emotional stress or ingestion of alcohol (Zarafonetis et al 1958, Melman et al 1965b) and food intake especially fatty meals (Thorson 1954, Blochen 1955, Goble et al 1956). Elevated levels of plasma and urinary histamine have also been shown to occur in addition to 5-HT and 5-HTP in those patients with argentaffinoma of gastric origin (Pernow et al 1957, Oates and Sjoerdsma 1962). The flushing pattern in these patients is different, being more prolonged and of the more vivid patchy red type. Whilst the role of histamine in these patients with gastric carcinoid is not quite clear, the

fact that alpha-methyl-dopa, a potent inhibitor of 5-HTP decarboxylase activity causes amelioration of flushing in these patients suggests that serotonin too must be involved in these flushing attacks (Oates et al 1962). It is significant that the distribution of 5-HT in the gastric mucosa is very similar to that of histamine, whilst it is also known that 5-HT can release histamine from living cells (Felberg and Smith 1953). Thus either the gastric carcinoid cells produce both 5-HT and histamine or that increase formation of 5-HT leads to a secondary release of histamine (Pernow and Waldenstrom 1957). Thorson (1958) noted that flushing following the ingestion of cheese or alcohol was preceded by severe hyperperistalsis, and hyperperistalsis is known to cause 5-HT release (Bulbring and Lin 1958). Intravenous administration of alcohol is less effective than the oral dose, so that it is likely that alcohol via a gastric mediator may cause 5-HT release (Sandler 1967). Also in some carcinoid patients, flushing induced by intravenous adrenaline or noradrenaline is associated with a rise in the free plasma serotonin in the hepatic vein (Peart et al 1961). Thus adrenaline and noradrenaline can act as 5-HT liberators (Burkes et al 1966).

The Carcinoid Heart Disease:

The heart lesions are late complications of the carcinoid syndrome suggesting that a prolonged action of the tumour is required for their development. Unless carcinoid metastases to the liver are present or there is drainage of the carcinoid tumours by routes other than the portal vein, the endocardial lesions do not occur (Jenkins et al 1955, Thorson 1958). Thus lesions have also been described where the carcinoid tumour

arose from an ovarian teratoma and large quantities of 5-HT were released via anastomotic channels into the pulmonary veins (Torvik 1960, Kephart et al 1960).

The fibrotic endocardial lesions may also be associated with arthritis suggesting the possibility of a more generalised connective tissue disorder than that affecting the heart valves and endocardium (Sjoerdsma et al 1956). In the patient described by Cassidy (1930, 1931) "the fibrotic phenomenon extended to the pelvis filling it with fibrous tissue as if it were filled with plaster of Paris." It was therefore suggested that some substance leaves the abdominal tumour by direct diffusion causing proliferation of peritoneal fibroblasts and by the blood stream influenced the cardiac valves directly (Waldenstrom et al 1955). Indeed gross extra-cardiac fibrous tissue reactions such as retro-peritoneal fibrosis (Fabricius et al 1958), constrictive pericarditis (Dockerty et al 1955) and intestinal obstruction due to fibrous adhesions (Davies 1959) have all been associated with various cases of this syndrome.

The humoral effect of the tumour has been attributed to 5-HT (Goble et al 1955, 1956) contained in large quantities by the tumour (Lembeck 1953). Since platelet 5-HT is inert, it is likely that it is the 5-HT occuring free in the plasma that causes the endocardial lesions (Snow et al 1955). It is suggested that 5-HT may alter the cardiac endothelium by increasing capillary permeability causing oedema (Pickles 1955) and thus allowing platelet deposition on the valve cusps with subsequent fibrosis (Waldenstrom et al 1955, Goble et al 1955, 1956, Thorson et al 1959). It is also believed that 5-HT in concentrated form

produces a sclerosing effect, and when poured directly from the liver metastases into the right side of the heart produces an increase in ground substance of the tricuspid and pulmonary valves with fibrous thickening and eventual sclerosis. Thus the endocardial fibrosis develops by 5-HT influence on connective tissue (Asboe-Hanson et al 1956, Bates and Clark 1961). Later Bates and Clark (1963) postulated several stages in the evolution of endocardial fibrosis. That a direct action of 5-HT on tissue mast cells causes subendocardial oedema and damage to tissue with reactive fibrosis. There is fibrin deposition on the injured endocardial surface followed by tanning of the fibrin as a result of interaction between 5-HT and ceruloplasmin, making the fibrin resistant to fibrinolysis. The deposit of tanned fibrin then enlarges by accession and organises with transformation to dense connective tissue.

In support of the hypothesis that 5-HT can cause fibrous tissue proliferation is the finding that injection of 5-HT into the skin of rabbits gave rise to a marked local fibrotic reaction (McDonald et al 1958). Also Spatz (1965) produced endocardial fibrous lesions in guinea-pigs with intraperitoneal injections of 5-HT associated with the administration of hepato-toxic drugs. The presence of anti-cardiac auto-antibodies was detected in one patient with the carcinoid syndrome and it is very significant that injected 5-HT had the ability to produce similar antibodies in rabbits (Van den Gold et al 1966).

It is argued that the predominance of right sided lesions and the relative scarcity of left sided involvement is due to the inactivation of 5-HT by mono-amine oxidase during its passage through the lungs.

Since hepatic metastases are essential for cardiac lesions to develop (Jenkins et al 1955, Thorson 1958) it follows that the liver which in the normal individual possesses a high mono-amine oxidase activity and thus capable of inactivating 5-HT (Erspamer 1954) is in these carcinoid patients actually secreting enormous quantities of this cardio-toxic drug. Thus blood reaching the right portion of the heart is relatively rich in 5-HT whilst that entering the left portion is depleted of 5-HT as a result of its inactivation in the lungs (Waldenstrom et al 1955, Goble et al 1955, 1956, Sjoerdsma et al 1956, Sjoerdsma 1959). In support of this hypothesis is the reported finding of plasma 5-HT levels of 6.2 micrograms per millilitre in the main pulmonary artery as compared to 2.2 micrograms/ml. in the brachial artery of one carcinoid patient (Goble et al 1955). Left sided lesions were previously described only in association with a right to left shunt due to a patent foramen ovale (McKusick 1956, Fischer et al 1958) or in association with lung metastases (Goble et al 1956, Sjoerdsma et al 1964, Kinloch et al 1965). Indeed in patients with primary bronchial carcinoids, valvular heart lesions were predominantly left sided (Sjoerdsma et al 1964, Melman et al 1965). Recent reports however indicate that mitral valvular involvement may occur with intestinal carcinoids in the absence of a right to left shunt (Jatlow et al 1964, Roberts et al 1964, Fadell et al 1966). This may suggest that in patients whose blood is flooded with enormous quantities of 5-HT, complete inactivation of free plasma 5-HT may not occur in the passage through the lungs probably due to saturation of the inactivating enzyme system. It is known that in these

carcinoid patients about 60% of the trytophane intake is directed to the serotonin path-way (Sjoerdsma et al 1956).

5-HT, Common Denominator in E.M.F. and Carcinoid Heart Disease:

The strong similarity between carcinoid heart lesions and the endocardial lesions in endomyocardial fibrosis led to the suggestion that a common denominator may underlie both diseases. Also the close correlation between plantain ingestion (containing large quantities of 5-HT) and the incidence of E.M.F. in African patients lends support to this theory (Crawford 1963). Indeed endocardial lesions have been produced in rats on a diet similar to that of the plantain eating African. Antia et al (1966) however found no cardiac lesions in dogs after ligating the cardiac lymphatics and feeding them on a plantain diet for nine months.

Mono-amine oxidase, the enzyme responsible for the convertion of 5-HT to 5-HIAA is present not only in the gastro-intestine tract, but also in the liver, lungs and kidneys. Ingested 5-HT is rapidly metabolized in the body and quickly converted into its excretory product, 5-HIAA (Erspamer 1954). It would therefore be expected that most of the exogenous 5-HT, as contained in a plantain rich diet will be destroyed both in the gut and the liver before it reaches the right side of the heart. Thus high concentrations of 5-HT on the right side of the heart as in the carcinoid syndrome cannot be incriminated in the explanation of right sided heart lesions in E.M.F.

Crawford (1963) therefore postulated a defective metabolisin of 5-HT in those Africans that subsequently develop E.M.F. He agreed that healthy

individuals detoxicate 5-HT rapidly but argues that subjects exposed to a long-life diet of serotonin as well as the stress of malnutrition, kwashiokwor and parasitic infections, common in East and West Africa, cannot be considered as biologically normal. He further stressed that the conditions prevailing in these areas, especially when they affect liver function, may indeed give rise to an abnormality in the metabolism of 5-HT. He thus concluded that whilst dietary serotonin might be contributory to the development of fibrotic heart lesions, that impairment of endogenous serotonin metabolism alone might be all that is necessary without the added hazard of a diet of bananas or plantain. Thus since lung metabolism is the reason for the carcinoid heart lesion occuring essentially on the right side, it is clear that in the presence of defective metabolism of serotonin, that endocardial lesions may not be confined to one side of the heart only.

Working on the hypothesis of defective metabolism of 5-HT in those that subsequently develop E.M.F., one would expect that as a result of this defect, 5-HT normally inactivated in the liver and lungs, having this normal metabolic path-way blocked is still available in right and left ventricular blood where it causes the fibrotic lesions. Therefore it is logical to expect 5-HIAA excretion in those prone to the development of E.M.F. as well as in the established cases would be lower than in normal healthy individuals. Also after oral loading with 5-HT as with a plantain rich diet, there will be a chronically high level of serum 5-HT in these patients as opposed to normals, as well as such lower excretion rate of 5-HIAA.

This then was the basis of my investigation, to determine and compare basal excretory levels of 5-HIAA in normals and patients with E. M. P. and to further ascertain the difference if any after a plantain rich diet.

This in fact turned out to be only the first step, and the second phase involved the assessment of renal and liver function tests. Finally it was necessary to estimate serum levels of 5-HT in E.M.P. patients as compared to healthy Nigerians.

CHAPTER IV

Experimental Work and Results

1. Urinary Excretion of 5HIAA:

The urinary excretion of 5HIAA is a pointer to the turnover rate of 5-HT in the body (Erspamer 1954). High urinary excretion of 5HIAA is characteristic of patients with carcinoid tumour, which liberates large quantities of 5-HT into the circulation. Also ingestion of a plantain-rich diet is accompanied by a rise in urinary 5HIAA excretion (For references see page 38). Therefore studies of the basal excretion of urinary 5HIAA in E.M.F. patients and in control healthy subjects should give an insight into the endogenous production and metabolism of 5-HT in these subjects. Also exogenous loading with 5-HT, as contained in a plantain-rich diet, may reveal the capacity of the dominative enzyme system in handling excess 5-HT.

Studies on the urinary excretion of 5-hydroxy-indoleacetic acid were carried out in four different groups of subjects. The first group were 30 healthy Nigerians (consisting of members of the University staff and medical students). The second group were 6 white expatriate staff, the third group consisted of 30 patients with endomyocardial fibrosis whilst the fourth group comprised 10 patients with other cardiac diseases other than E.M.F. This last group unlike the E.M.F. patients were in a severe degree of cardiac decompensation, and were all in-patients at the University Teaching Hospital. Most of the E.M.F. patients on the other hand, were not so severely incapacitated, and were brought into hospital or the University of Ibadan sick-bay mainly for the purpose of this investigation.

Basal Urinary Excretion of 5-HIAA:

All subjects investigated abstained for 24 hours prior to and throughout the period of the study, from articles of food such as bananas, plantain or avocado pears known to contain 5-HT. The period of study extended over the next 24 hours and all urine voided in this period by each subject was collected. Urine collections were at 4 hourly intervals starting from 10 a.m. of one morning to 10 a.m. the following morning (one 8 hour sample was however collected from 10 p.m. to 6 a.m. to avoid the inconvenience of the subjects waking up at a specific time of the night). The bladder was completely emptied before and after each collection period and all bottles into which urine was voided contained glacial acetic acid and toluene; (in the proportion of 20ml glacial acetic acid and 3ml of toluene to 1½ litres of urine) as 5-HIAA is unstable and rapidly disappears from normal urine. All samples collected were kept in the frigedaire until assayed and all assays were performed within 2½ hours of each collection. The method of assay employed was that of Macfarlane et al (1956) based on the colour reaction between 5-hydroxyindoles and 1 nitro-2 napthol reagent.

Brief Description of Assay Technique:

The volume of each sample was accurately measured and recorded. From each sample, 5ml of urine (already at pH of about 3) was transferred into a 70.1 stoppered tube, and to this was added 2Gms of sodium chloride and 25ml of ether. This mixture was thoroughly shaken for 10 minutes to allow the 5-HIAA to separate out into the other layer. The water/ether phases

were later separated by slow centrifugation at 3,000 rev./minute. 20ml of the other layer was transferred to a 100ml quick-fit flask capped under reduced pressure and the content was evaporated to dryness. The residue, anhydrous 5-HIAA left behind, was taken up in 4ml of distilled water. 3ml of this solution was transferred into a test-tube to which were added 1ml of 1% solution of 1-nitroso-2 napthol in 95% alcohol and 1ml of nitrous acid (prepared fresh daily from stock solutions of 2N suphuric acid and 2.5% sodium nitrite reagent). The test-tubes and contents were warmed in a water bath at 55°C for 5 minutes. Finally 10ml of ethyl-acetate was added to the contents of each test-tube, and the mixture thoroughly shaken and then allowed to stand for 30 minutes. The lower layer, purplish in colour (from the reaction between 5-HIAA and nitroso-napthol) was carefully pipetted into a 4ml photo-cell and its optical density read at 540 mμ in a Unicam S.P. 600 spectrophotometer. The reagent blank used for the blank setting of the instrument was prepared by treating 5ml of distilled water in the same manner as the urine. The 5-HIAA content of each sample was calculated using a calibration curve made by carrying known amounts of 5-HIAA dissolved in distilled water through the entire procedure.

Urinary Excretion of 5-HIAA after a Plantain Meal:

The same groups of subjects were investigated. Also 24 hr. prior to investigation, all subjects abstained from articles of food known to contain 5-HT. At 8 a.m. on the morning of the study, the bladder was completely emptied and the urine discarded. From 8 a.m. to 12 noon, all urine passed was collected and this constituted the basal 4 hr. sample.

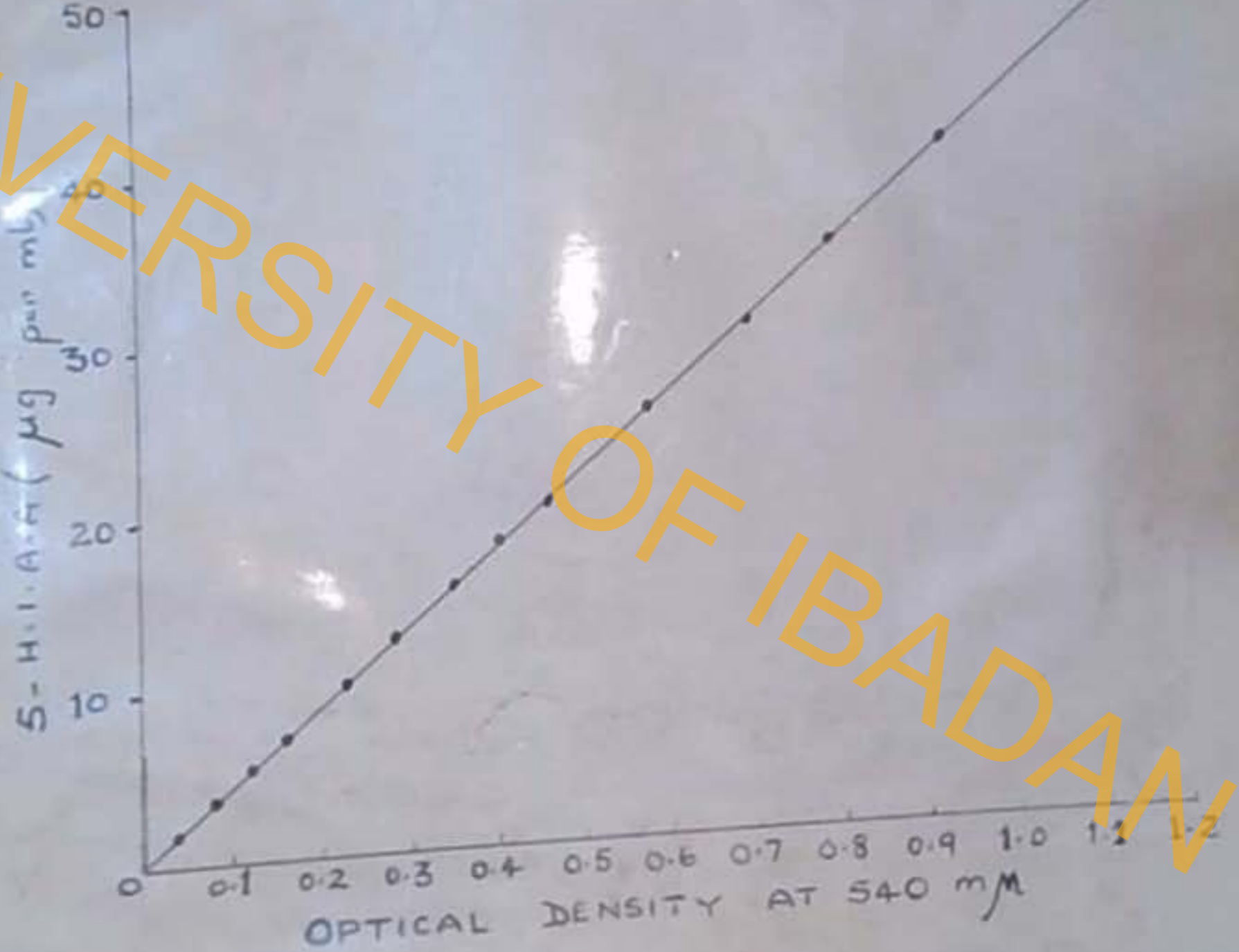
At 12 noon, each subject was given a weighed quantity of fried plantain. Urine was collected thereafter at 2 hourly intervals for the next six hours and subsequently at 6 hourly and 8 hourly intervals respectively until 20 hours after the plantain meal. Each urine sample was treated as before and assayed in the same way for the 5-HIAA content.

The quantity of 5-HT ingested was calculated from the weight of plantain eaten working on the reported findings of Foy and Parratt (1962) that 1Gm of plantain pulp contains 50 micrograms of 5-HT. 5-HIAA excreted due to plantain meal was obtained by subtracting total 5-HIAA excretion for each two hour period from the predetermined corresponding basal values.

The results of urinary 5-HIAA excretions, both basal and after plantain ingestion are tabulated on pages 73 - 93.

Estimation of urinary 5-HIAA
On the left, is shown the distilling apparatus for evaporating off ether to leave behind a residue of 5-HIAA; on the right, incubation of 5-HIAA in water bath at 55°C, after addition of 1 nitroso-2 napthol, and nitrous acid reagents. Reagents and samples of urine in the background.

Spectrophotometric assay of urinary 5-HIAA
Final solutions after incubation and addition of ethylacetate.
Unicam SP 600 spectrophotometer with photocells and holder;
optical density of solution read 540 uv.



URINE VOLUMES AND BASAL 5HIAA EXCRETION
NORMAL - NIGERIANS

TABLE 1

INITIALS	10am - 2pm		2pm - 6pm		6pm - 10pm		10pm - 6am		6am - 10pm		Total 24 hr UV in ml.	Total 24 hr URINARY 5HIAA Exc. in µg
	UV in ml.	5HIAA Exc. in µg	UV in ml.	5HIAA Exc. in µg	UV in ml.	5HIAA Exc. in µg	UV in ml.	5HIAA Exc. in µg	UV in ml.	5HIAA Exc. in µg		
A.B.	205	582	316	467	378	630	408	1,408	254	603	1,561	3,690
A.C.	162	772	418	696	288	834	327	1,328	296	738	1,485	4,368
A.O.	320	619	195	635	208	688	164	1,582	273	598	1,160	4,122
A.S.	146	660	302	648	153	704	169	1,380	403	670	1,173	4,062
B.M.	427	752	395	566	253	669	210	1,087	388	795	1,673	3,869
B.P.	115	968	268	890	103	864	403	1,526	251	758	1,230	5,006
D.N.	96	583	151	721	260	870	132	745	214	695	853	3,614
D.T.	226	651	163	495	289	609	195	862	314	528	1,187	3,145
F.A.	271	1,172	403	1,024	256	1,259	301	1,927	183	846	1,414	6,228
G.T.	109	873	162	719	394	598	226	968	345	805	1,236	3,963
K.O.	135	810	200	1,660	150	450	210	1,554	190	304	895	4,868
L.H.	210	672	450	630	460	980	475	380	570	342	2,175	3,024
M.O.	490	490	910	728	140	700	520	1,148	262	383	2,322	3,904
O.T.	85	646	315	945	290	1,160	520	728	175	1,050	1,385	4,529
O.O.	230	506	248	644	145	507	420	672	155	1,414	1,198	3,743

URINE VOLUMES AND BASAL 5HIAA EXCRETION

NORMAL - NIGERIANS (Continued)

TABLE 1

INI-TIALS	10am - 2pm		2pm - 6pm		6pm - 10pm		10pm - 6am		6am - 10pm		Total 24 hr UV in ml.	Total 24 hr URINARY 5HIAA Exc. in µg
	UV in ml.	5HIAA Exc. in µg	UV in ml	5HIAA Exc. in µg	UV in ml.	5HIAA Exc. in µg	UV in ml.	5HIAA Exc. in µg	UV in ml.	5HIAA Exc. in µg		
O.H.	520	750	290	928	200	920	385	1,831	390	1,032	1,785	5,461
O.B.	85	401	100	440	545	1,526	100	415	180	532	1,030	3,314
	100	905	125	939	420	1,475	335	603	106	784	1,086	4,306
O.A.	155	1,054	210	924	95	622	266	1,330	155	1,054	881	4,984
S.R.	135	480	328	502	240	700	152	1,205	196	725	1,105	3,612
S.V.	137	656	168	714	249	707	186	1,186	254	674	995	3,937
T.B.	160	504	96	382	152	486	218	872	193	475	819	2,719
T.K.	105	606	113	827	128	655	289	1,963	320	692	1,005	4,743
T.E.	291	648	234	590	207	613	386	1,134	217	605	1,335	3,590
U.A.	132	832	167	789	152	788	205	1,412	261	872	917	4,653
U.J.	402	779	368	721	300	701	248	1,076	234	668	1,552	3,845
W.O.	387	380	469	413	321	516	189	895	264	435	1,630	2,639
W.A.	225	627	230	654	290	507	314	986	271	503	1,330	3,357
W.P.	192	586	217	611	168	601	125	926	394	618	1,096	3,342
Y.K.	154	643	182	687	165	621	189	998	123	584	813	3,533
Y.A.												

URINE VOLUMES AND BASAL 5HIAA EXCRETION
NORMAL EUROPEANS

TABLE 2

INITIALS	10am - 2pm		2pm - 6pm		6pm - 10pm		10pm - 6am		6am - 10pm		Total 24Hr	
	URINE VOL. IN ml.	5HIAA µG	URINE VOL. IN ml.	5HIAA µG	URINE VOL. IN ml.	5HIAA µG	URINE VOL. IN ml.	5HIAA µG	URINE VOL. IN ml.	5HIAA µG	URINE VOL. IN ml.	5HIAA µG
J.G.	1,010	874	362	764	1,014	960	500	827	135	732	3,391	4,357
C.C.	240	648	508	609	360	586	510	1,406	460	904	2,578	4.155
M.I.	344	463	490	652	160	535	255	912	336	681	1,585	3,243
J.P.	158	501	400	472	345	667	435	837	245	601	1,583	3,078
M.C.	175	394	264	496	95	501	190	932	170	488	894	2,811
T.V.	334	846	501	730	473	784	401	1,236	695	724	2,404	4,320

URINE VOLUMES AND BASAL 5HIAA EXCRETION
E. M. F. PATIENTS

TABLE 3

INI-TIALS	10am - 2pm		2pm - 6pm		6pm - 10pm		10pm - 6am		6am - 10am		Total 24 hr UV in ml.	Total 24 hr 5HIAA Exc. in µg
	UV in ml.	5HIAA Exc. in µg	UV in ml.	5HIAA Exc. in µg	UV in ml.	5HIAA Exc. in µg	UV in ml.	5HIAA Exc. in µg	UV in ml.	5HIAA Exc. in µg		
A.O.	215	586	301	495	287	532	185	905	266	614	1,254	3,135
A.R.	187	495	201	521	155	418	108	822	263	466	914	2,722
A.D.	498	348	235	501	311	416	215	735	193	392	1,452	2,392
A.M.	415	296	440	308	302	326	263	587	274	336	1,694	1,392
A.S.	370	462	216	438	288	386	184	852	306	434	1,484	2,572
A.S.	218	654	304	506	260	574	346	960	196	532	1,324	3,306	
B.J.	98	482	154	426	118	540	214	856	135	468	819	2,772
C.T.	166	396	135	428	408	406	301	785	266	452	1,276	2,467
E.F.	138	412	307	384	263	405	224	692	294	436	1,226	2,329
E.E.	370	376	284	392	302	354	187	582	214	334	1,357	2,038
F.F.	325	764	285	716	303	689	207	1,245	187	668	1,307	4,082
H.G.	517	684	415	582	304	488	387	1,218	246	493	1,869	3,465
I.O.	312	268	350	215	397	245	125	487	288	340	1,452	1,655
J.S.	227	463	194	415	95	392	167	894	225	432	908	2,196
J.G.	107	316	250	361	169	300	218	669	280	410	1,024	2,076

URINE VOLUMES AND BASAL 5HIAA EXCRETION
E. H. P. PATIENTS (Continued)

TABLE 3

INI-TIALS	10am - 2pm		2pm - 6pm		6pm - 10pm		10pm - 6am		6am - 10am		Total 24 hr UV in ml.	Total 24 hr 5HIAA Exc. in µg
	UV in ml.	5HIAA Exc. in µg	UV in ml.	5HIAA Exc. in µg	UV in ml.	5HIAA Exc. in µg	UV in ml.	5HIAA Exc. in µg	UV in ml.	5HIAA Exc. in µg		
K.A.	271	546	307	603	228	584	294	887	345	595	1,446	3,615
M.T.	475	516	570	625	307	447	267	942	187	512	1,806	3,042
M.M.	346	619	258	663	374	602	246	873	245	894	1,469	3,651
O.M.	95	281	105	347	95	247	165	644	121	381	571	1,900
O.I.	704	656	582	467	387	561	493	1,004	365	567	2,531	3,255
O.J.	187	346	196	385	174	289	301	674	216	345	1,074	2,039
O.A.	265	461	249	507	312	486	269	855	312	421	1,407	2,730
R.A.	321	268	281	179	314	301	217	468	304	266	1,437	1,482
S.M.	220	448	306	469	345	452	268	715	245	301	1,384	2,385
T.K.	417	613	400	587	502	643	415	946	387	493	2,121	3,282
T.N.	82	214	93	265	78	210	113	397	137	251	503	1,337
U.J.	261	368	205	347	254	562	320	816	115	420	1,155	2,513
U.R.	376	431	445	478	326	440	247	667	219	381	1,613	2,397
Y.A.	432	581	368	495	337	480	356	821	246	430	1,739	2,807
Y.S.	341	426	336	410	389	456	298	671	320	472	1,684	2,435

URINE VOLUMES AND BASAL 5HIAA EXCRETION
PATIENTS WITH OTHER HEART DISEASES NOT
E.H.F.

TABLE 4

INITIALS	10am - 2pm		2pm - 6pm		6pm - 10pm		10pm - 6am		6am - 10am		Total 24 hr UV in ml.	Total 24 hr 5HIAA exc. in µg
	UV in ml.	5HIAA µg	UV in ml.	5HIAA µg	UV in ml.	5HIAA µg	UV in ml.	5HIAA µg	UV in ml.	5HIAA µg		
A.A.	163	608	204	516	317	584	462	938	180	570	1,326	3,216
A.S.	187	365	102	418	132	347	218	643	172	401	816	2,174
F.T.	142	317	156	343	158	395	364	587	166	361	1,022	2,003
G.O.	84	128	108	155	93	172	145	314	107	139	537	908
K.A.	187	480	203	412	196	468	301	826	170	422	1,057	2,608
L.O.	172	283	145	252	207	228	168	503	194	260	776	1,526
L.S.	326	531	175	495	207	478	236	897	192	516	1,136	2,917
C.F.	270	211	156	148	163	165	294	358	204	187	1,087	1,069
I.T.	255	478	186	495	273	520	308	763	235	526	1,257	2,782
E.C.	311	586	209	472	157	597	258	958	233	513	1,168	3,126

RATE OF URINARY EXCRETION OF 5HIAA AFTER A PLANTAIN MEAL NORMAL (NIGERIANS)

TABLE 5

INITIALS	Total 5HIAA Exc. in ug	Basal 5HIAA Exc. in ug	5HIAA due to Plantain meal in µg	Rate of Urinary 5HIAA Excretion due to Plantain meal									
				0 - 2HRS		2 - 4HRS		4 - 6HRS		6 - 12HRS		12 - 20HRS	
				5HIAA in µg	%	5HIAA in µg	%	5HIAA in µg	%	5HIAA in µg	%	5HIAA in µg	%
A.B.	14,217	3,690	10,527	1,158	11.0	3,432	32.6	2,884	27.4	2,980	28.3	73	0.7
A.G.	12,931	4,368	8,563	1,824	21.3	3,296	38.5	2,064	24.1	1,002	11.7	377	4.4
A.O.	16,727	4,122	12,605	1,298	10.3	3,000	23.8	4,676	37.1	2,836	22.5	795	6.3
A.S.	14,912	4,062	10.850	2,778	25.6	4,350	40.1	2,420	22.3	1,237	11.4	65	0 6
B.M.	18,807	3,869	14,218	2,474	17.4	5,474	38.5	3,853	27.1	1,607	11.3	810	5.7
B.P.	14,632	5,006	9,626	2,243	23.3	2,945	30.6	2,715	28.2	1,020	10.6	703	7.3
D.N.	18,722	3,614	15,108	2,931	19.4	6,466	42.8	3,097	20.5	2,221	14.7	393	2.6
D.T.	14,727	3,145	11,582	1,656	14.3	4,065	35.1	3,092	26.7	2,479	21.4	290	2.5
F.A.	14,073	6,228	7,845	1,436	18.3	3,695	47.1	1,608	20.5	1,105	14.1	-23	-
G.T.	17,230	3,963	13,267	3,317	25.0	5,094	38.4	2,826	21.3	1,552	11.7	470	3.6
K.O.	12,800	4,868	7,932	1,932	24.3	3,615	45.6	1,305	16.3	952	12.0	128	1.6
L.H.	9,685	3,024	6,661	1,386	20.5	2,964	44.6	1,001	15.03	899	13.0	311	4.7
M.C.	11,302	3,904	7,398	834	11.3	3,516	47.5	2,147	29.03	625	8.4	276	3.7
O.T.	15,158	4,529	10,629	2,168	20.4	5,206	48.9	1,863	17.6	1,102	10.4	290	2.7
O.O.	8,451	3,743	4,708	1,261	26.8	1,842	39.1	643	13.7	829	17.6	133	3.5

RATE OF URINARY EXCRETION OF 5HIAA AFTER A PLANTAIN MEAL
NORMAL - (NIGERIANS) (Continued)

TABLE 5

INITIALS	TOTAL 5HIAA Exc. in µg	BASAL 5HIAA Exc. in µg	5HIAA due to Plantain Meal	Rate of Urinary 5HIAA Excretion due to Plantain Meal									
				0 - 2HRS		2 - 4HRS		4 - 6HRS		6 - 12HRS		12 - 20HRS	
				5HIAA in µg	%	5HIAA in µg	%	5HIAA in µg	%	5HIAA in µg	%	5HIAA in µg	%
O.M.	12,107	5,461	6,646	1,540	23.2	2,017	30.3	1,416	21.3	627	9.4	46	0.7
O.B.	10,586	3,314	7,272	932	12.8	3,064	42.1	2,118	29.1	1,108	15.2	50	0.7
O.A.	16,902	4,306	12,596	2,301	18.3	5,276	41.9	3,218	25.6	1,537	12.2	274	2.2
S.R.	13,284	4,984	8,300	1,164	14.0	2,802	36.2	3,468	41.8	954	11.5	-88	-1.1
S.V.	8,922	3,612	5,310	877	16.5	1,965	37.0	1,062	20.0	890	17.2	516	9.7
T.B.	11,238	3,937	7,301	1,618	22.2	2,665	36.5	1,908	26.1	926	12.7	670	9.2
T.K.	10,506	2,719	7,787	2,187	28.1	3,628	46.6	1,089	14.0	843	10.8	40	0.5
T.E.	17,842	4,743	13,099	2,665	20.3	4,904	37.4	4,811	36.7	1,061	8.1	-342	-2.6
V.A.	8,527	3,590	4,937	609	12.3	2,148	43.5	1,826	37.0	274	5.6	80	1.6
V.J.	21,174	4,693	16,481	2,861	17.4	7,201	43.7	2,654	16.1	1,261	7.7	304	1.9
W.C.	9,334	3,845	5,489	1,251	22.8	1,964	35.8	1,038	18.9	872	15.9	364	6.6
W.A.	10,628	2,639	7,589	1,853	24.4	3,071	40.5	2,113	27.8	818	10.8	-266	-3.5
W.P.	14,365	3,357	11,008	3,168	28.8	4,963	45.1	1,803	16.4	1,005	9.1	69	0.5
Y.K.	9,941	3,942	6,599	1,726	26.2	1,841	27.9	2,074	31.4	1,021	15.5	63	0.96
Y.A.	15,622	3,533	12,089	3,168	26.2	4,346	36.0	3,012	24.9	1,386	11.5	277	2.3

TABLE 6

RATE OF URINARY EXCRETION OF 5HIAA AFTER A PLANTAIN MEAL (NORMAL EUROPEANS)

INITIALS	BASAL 24 HR 5HIAA Exc.	TOTAL 24 HR 5HIAA Exc.	5HIAA due to Plan-tain	Rate of Urinary 5HIAA Excretion due to Plantain Meal									
				0 - 2Hrs		2 - 4Hrs		4 - 6Hrs		6 - 12 Hrs		12 - 20Hrs	
				5HIAA in µg	% Total Exc.	5HIAA in µg	% Total Exc.	5HIAA in µg	% Total Exc.	5HIAA in µg	% Total Exc.	5HIAA in µg	% Total Exc.
J.G.	4,357	10,211	6,854	1,54	22.5	2,692	39.3	1,326	19.3	950	13.9	255	3.7
C.C.	4,155	12,782	8,527	1,968	23.0	3,501	41.1	2,577	29.6	780	9.1	249	-
M.I.	3,243	3,576	5,333	1,062	19.9	1,875	35.2	1,528	28.7	698	13.1	170	3.2
J.R.	3,078	9,285	6,207	1,317	21.2	2,960	47.7	991	16.0	876	14.1	263	4.2
M.C.	2,811	8,651	5,840	1,263	21.6	2,360	40.5	1,089	18.6	837	14.3	293	5.0
T.V.	4,320	21,407	16,087	13,752	23.3	7,401	46.0	3,123	19.4	1,894	11.8	97	0.6

RATE OF URINARY EXCRETION OF 5HIAA
AFTER A PLANTAIN MEAL E.H.P. PATIENTS

TABLE 7

INITIALS	Total 24 hr 5HIAA Exc. in µg	Basal 24 hr 5HIAA Exc. in µg	5HIAA due to Plantain Meal in µg	Rate of Urinary 5HIAA Excretion due to Plantain Meal									
				0 - 2HRS		2 - 4HRS		4 - 6HRS		6 - 12HRS		12 - 20HRS	
				5HIAA in µg	%	5HIAA in µg	%	5HIAA in µg	%	5HIAA in µg	%	5HIAA in µg	%
A.O.	9,565	3,135	6,430	978	15.2	1,511	23.5	2,386	37.1	1,246	19.4	309	4.8
A.R.	7,802	2,722	5,080	508	10	940	18.5	1,270	25	1,614	31.8	748	14.7
A.D.	7,218	2,392	4,826	328	6.8	690	14.3	1,356	28.1	1,650	34.2	806	16.7
A.H.	6,855	1,323	5,532	383	7.2	958	17.4	1,632	29.5	2,102	38	438	7.9
A.S.	12,076	2,572	9,504	1,178	12.4	1,920	20.2	2,233	23.5	2,766	29.1	1,407	14.8
A.S.	9,644	3,306	6,338	1,287	20.3	1,629	25.7	1,990	31.4	1,772	18.5	260	4.1
B.J.	6,624	2,772	3,852	547	14.2	690	17.9	859	22.3	1,378	35.8	377	9.2
C.T.	7,510	2,467	5,043	217	4.3	681	13.5	1,059	21	2,456	48.7	630	12.5
E.P.	7,224	2,329	4,895	538	11	1,131	23.1	1,332	27.2	1,537	31.4	357	7.3
E.E.	8,962	2,038	6,924	1,281	18.5	2,015	29.1	1,648	23.8	1,530	22.1	450	6.5
F.F.	11,624	4,082	7,542	1,282	17.0	1,885	25.0	2,262	30	1,492	20	603	8.0
H.G.	8,868	3,465	5,403	477	8.8	1,918	35.5	2,043	37.9	957	17.7	8	0.1
I.O.	5,432	1,655	3,877	347	9.0	647	16.7	1,211	31.2	1,162	30	508	13.1
J.S.	12,625	2,196	10,429	410	3.9	4,090	39.2	3,006	28.8	2,663	25.5	591	5.6
J.G.	9,074	2,076	6,990	2,060	29.4	1,792	25.6	778	11.1	1,873	26.6	501	7.2

RATE OF URINARY EXCRETION OF 5HIAA AFTER A PLANTAIN MEAL E.M.F. PATIENTS (Continued)

TABLE 7

INITIALS	Total 24 hr 5HIAA Exc. in µg	Basal 24 hr 5HIAA Exc. in µg	5HIAA due to Plantain Meal in µg	Rate of Urinary 5HIAA Excretion due to Plantain Meal									
				0 - 2HRS		2 - 4HRS		4 - 6HRS		6 - 12HRS		12 - 20HRS	
				5HIAA in µg	%	5HIAA in µg	%	5HIAA in µg	%	5HIAA in µg	%	5HIAA in µg	%
K.A	9,060	3,615	5,445	287	5.3	2,224	40.9	251	4.6	2,215	40.7	468	8.6
M.T.	9,773	3,042	6,731	269	4.0	1,368	20.3	2,980	44.3	1,246	14.7	868	12.9
M.M.	11,406	3,651	7,755	1,500	19.4	1,604	20.7	2,860	36.9	1,252	16.2	539	6.9
O.M.	9,419	1,900	7,519	2,121	28.2	1,104	14.7	3,109	41.4	843	11.2	344	4.8
C.I.	8,736	3,255	5,481	735	13.4	1,618	29.5	1,689	30.8	564	10.3	25	0.4
O.J.	5,801	2,039	3,762	630	16.7	1,294	34.6	700	18.6	670	17.8	468	12.4
O.A.	6,612	2,730	3,882	342	8.9	684	16.6	1,212	31.1	1,164	29.8	510	13.1
R.A.	4,690	1,482	3,208	508	15.8	510	15.9	482	15	822	25.5	906	28.2
S.M.	9,385	2,385	7,000	778	11	1,792	25.7	1,868	26.6	2,060	30	501	7.1
T.K.	13,802	3,282	10,520	290	2.7	3,006	28.7	4,090	38.8	2,665	25.4	349	3.3
T.N.	7,288	1,337	4,951	933	18.8	1,286	28	706	14.2	1,479	29.8	347	7.0
U.J.	8,842	2,513	6,329	1,066	16.8	1,252	19.7	2,862	45.2	1,500	23.7	539	8.5
U.R.	9,808	2,397	7,411	269	3.6	1,368	18.4	1,146	15.7	3,760	50.8	868	11.7
Y.A.	18,530	2,807	15,723	2,113	13.7	4,780	30.4	6,738	42.8	2,015	12.8	97	0.6
Y.S.	7,961	2,435	5,526	894	16.2	962	17.5	1,316	23.8	1,658	30.1	696	12.4

RATE OF URINARY EXCRETION OF 5HIAA AFTER A PLANTAIN MEAL
WITH OTHER HEART DISEASES NOT R.H.F.

TABLE 8

INITIALS	BASAL 24 HR 5HIAA Exc. in µg	TOTAL 24 HR 5HIAA Exc. in µg	5HIAA due to Plantain	Rate of Urinary 5HIAA Excretion Due to Plantain Meal									
				0 - 2Hrs		2 - 4Hrs		4 - 6Hrs		6 - 12Hrs		12 - 20Hrs	
				5HIAA in µg	% Total Exc.	5HIAA in µg	% Total Exc.	5HIAA in µg	% Total Exc.	5HIAA in µg	% Total Exc.	5HIAA in µg	% Total Exc.
A.A.	3,216	7,263	4,047	847	20.9	928	22.9	1,096	27.0	1,105	27.4	61	1.5
A.S.	2,174	9,745	7,521	1,028	13.7	1,673	22.2	2,034	27.0	1,145	15.2	641	8.5
F.T.	2,003	6,993	4,990	913	18.3	852	17.1	1,536	30.9	963	19.3	726	14.5
G.O.	908	3,107	2,199	306	13.9	463	20.6	527	23.9	745	33.9	168	7.6
K.A.	2,608	5,002	3,3394	468	13.8	725	21.4	813	23.9	617	18.2	781	23.0
L.O.	1,526	3,966	2,440	397	16.2	516	21.2	409	16.7	585	28.9	335	13.9
O.F.	1,069	4,591	3,522	207	5.8	992	28.2	916	26.0	844	24.0	563	16.0
R.T.	2,782	7,419	4,637	528	11.4	1,901	41.0	1,835	39.6	419	9.0	36	0.7
U.C.	3,126	8,520	5,394	803	14.9	985	18.3	1,132	21.0	1,561	29.0	911	16.9
M.S.	2,917	5,308	2,391	451	18.0	601	25.1	528	22.1	496	20.4	335	14.0

RELATIONSHIP BETWEEN 5HT INGESTED AS PLANTAIN AND 5HIAA EXCRETED - (NORMAL NIGERIANS)

TABLE 9

INITIALS	Weight of Plantain Ingested in Gm.	Calculated Weight of 5HT in µg	5HIAA Exc. due to Plantain meal	% 5HT Ingested Excreted as 5HIAA
A.B.	446.8	22,350	10,572	47.3
A.G.	533.5	26,675	8,563	32.1
A.O.	618.0	30.900	12,605	40.8
A.S.	502.3	25,115	10,850	43.2
B.M.	493.7	24,685	14,218	57.6
B.P.	389.0	19,450	9,626	49.5
D.N.	500.0	25,000	15,108	60.4
D.T.	502.5	25,125	11,582	46.1
F.A.	403.6	20,170	7,846	38.9
G.F.	637.8	31,890	13,267	41.6
K.O.	415	20.750	7,932	38.2
L.M.	392	19,600	6,661	33.9
M.O.	320	16,000	7,398	46.2
O.T.	305	15,250	4,708	31.0
O.O.	400	20,000	10,629	53.1

RELATIONSHIP BETWEEN 5HT INGESTED AS PLANTAIN AND 5HIAA EXCRETED - (NORMAL NIGERIANS) (Contd.)

TABLE 9

INITIALS	Weight of Plantain Ingested in Gm.	Calculated Weight of 5HT in µg	5HIAA Exc. mg to Plantain meal	% 5HT Ingested Excreted as 5HIAA
O.M.	360	18,000	6,646	37.0
O.B.	350	17,500	7,272	41.5
O.A.	502	25,600	12,596	49.2
S.R.	355	17,750	8,000	45.2
S.V.	300	15,000	5,310	35.4
T.B.	375	18,750	7,301	38.9
T.K.	390	19,500	7,787	39.9
T.E.	550	27,500	13,099	47.6
U.A.	296	14,800	4,937	33.4
U.J.	625	32,250	16,481	52.8
W.O.	300	15,000	5,489	36.6
W.A.	365	18,250	7,589	41.6
W.P.	438	21,900	11,008	50.2
Y.K.	340	17,000	6,599	38.8
Y.A.	572	28,600	12,089	42.2

RELATIONSHIP BETWEEN INGESTED 5HT AS PLANTAIN AND THE URINARY RECOVERY OF 5HIAA, NORMAL EUROPEANS

TABLE 10

INITIALS	Wt. of Plantain Ingested in Gms	Calculated 5HT Value in µg	Amount of 5HIAA Ex. in µg	% 5HT Ingested excreted as 5HIAA
J.G.	320 Gms	16,000	6,854	42.8
C.C.	350 "	17,500	8,527	48.7
M.I.	288 "	12,400	5,333	43.0
J.P.	327 "	16,350	6,207	37.9
M.C.	375 Gm	18,750	5,840	31.1
T.V.	620 Gms	31,000	16,087	51.9

RELATIONSHIP BETWEEN INGESTED 5HT AS PLANTAIN AND THE URINARY RECOVERY OF 5HIAA - E.K.P. (PATIENTS)

TABLE 11

INITIALS	Weight of Plantain Ingested in Gms.	Calculated 5HT Value in µg	Amount of 5HIAA Excreted in µg	% 5HT Ingested Excreted as 5HIAA
A.O.	336.6	16,830	6,430	38.2
A.R.	214.8	10,740	5,080	47.3
A.D.	316.4	15,820	4,826	30.5
A.M.	247.3	12,375	5,532	44.7
A.S.	354.6	17,730	9,504	53.6
A.S.	379.5	18,975	6,338	33.4
B.J.	153.8	7,690	3,852	50.1
C.T.	231.2	11,590	5,043	43.5
E.P.	348.4	17.420	4,895	28.1
E.E.	441	22,050	6,924	31.4
F.F.	325	16,250	7,542	46.4
H.G.	300	15,000	5,403	36.0
I.O.	300	15,000	3,877	25.8
J.S.	450	22,500	10,429	46.2
J.G.	315	15,750	6,998	44.4

RELATIONSHIP BETWEEN INGESTED 5HT AS PLANTAIN AND THE URINARY RECOVERY OF 5HIAA - E.M.D. (PATIENTS) (Contd.)

TABLE 11

INITIALS	Weight of Plantain Ingested in Gms.	Calculated 5HT Value in µg	Amount of 5HIAA Excreted in µg	% 5HT Ingested Excreted as 5HIAA
K.A.	300	15,000	5,445	36.2
M.F.	340	17,000	6,731	39.6
M.M.	305	15,250	7,755	51.0
O.M.	388	19,400	7,519	39.3
O.I.	295	14,750	5,481	27.2
O.J.	250	12,500	3,762	30.1
O.A.	239.6	11,980	3,882	32.4
R.A.	298	14,900	3,208	21.5
S.M.	365	18,250	7,000	38.3
T.K.	403	20,150	10,520	52.2
T.N.	300	15,000	4,951	33.0
U.J.	355	17,750	6,329	35.6
U.R.	368	18,400	7,411	40.3
Y.A.	517	25,850	15,723	60.7
Y.S.	300	15,000	5,526	36.8

RELATIONSHIP BETWEEN INGESTED 5HT AS PLANTAIN AND URINARY RECOVERY OF 5HIAA - OTHER CARDIAC PATIENTS

TABLE 12

INITIALS	Wt. of Plantain Ingested in Gms	Cal. Wt. of 5HT in µg	5HIAA Excretion in µg	% 5HT excreted as 5HIAA
A.A.	320 Gms.	16,000	4,047	25.3 %
A.S.	482 Gms	24,600	7,521	30.6 %
F.T.	250 "	12,500	2,199	17.6 %
G.O.	335 "	16,750	4,990	29.8 %
K.A.	250 "	12,500	3,394	27.2 %
L.O.	208 Gms	10,400	2,440	23.5 %
M.S.	300 Gms	15,000	2,391	15.9 %
O.F.	335 Gms	16,750	4,657	27.7 %
R.T.	295 Gms	14,750	3,522	23.9 %
U.C.	318 Gms	15,900	5,394	33.9 %

TABLE 13

EFFECT OF A PLANTAIN MEAL ON URINARY EXCRETION OF 5HIAA

	NORMAL		E.N.F. (30)	Other Cardiopathies (10)
	NIGERIANS (30)	EUROPEANS (6)		
Basal Excretion of 5HIAA (mg per 24hr)	4.0±0.75	3.7±0.7	2.58±0.68 *	2.2±0.8
Amount of Plantain eaten (Gms)	432±102	380±12	324±71	321±74
Estimated amount of 5HT ingested(mg)	21.6±50	19.0±6.2	16.2±3.6	16.05±4.0
5HIAA, above basal levels, excreted within 20hr after plantain meal (mg)	9.46±2.9	8.1±4.1	6.34±3.1	4.1±1.6
Percentage of 5HT excreted as 5HIAA	43±7.2 +	42.7±7.9	39.5±9.0	25.5±5.6
Urine Volume (litres over 24hrs)	1.28±0.37	2.07±0.89	1.38±0.3	1.04±0.22

Values are means + standard deviations significantly different from normal individuals at probability level of P = 0.001 (*)

TABLE 14

RATE OF URINARY EXCRETION OF 5HIAA (mg), IN EXCESS OF BASAL LEVELS, FOLLOWING INGESTION OF PLANTAIN MEAL

HOURS AFTER PLANTAIN MEAL	NORMAL NIGERIANS (30)		E.M.F. PATIENTS (30)		OTHER CARDIOPATHIES (10)	
	5HIAA in mg due to meal	%	5HIAA in mg due to meal	%	5HIAA IN MG due to meal	%
0 - 2	1.894±0.76 (0.6-3.2)	20.1±5.4 (10.3-28.8)	0.82±0.56 (0.22-2.12)	12.75±6.7 (3.6-29.4)	0.6±0.3 (0.2-1.0)	14.7±3.96 (5.8-20.9)
2 - 4	3.7±0.43 (1.84-7.2)	39.12±5.4 (23.8-48.9)	1.91±0.62 (0.51-4.78)	20.56±7.3 (13.5-40.9)	1.0±0.4 (0.5-1.9)	23.8±6.5 (17.1-41.0)
4 - 6	2.33±0.34 (0.64-4.8)	24.46±7.25 (14.0-37.1)	1.9±0.4 (0.25-6.74)	27.9±9.93 (4.6-45.2)	1.1±0.5 (0.4-2.0)	25.8±5.9 (16.7-39.6)
6 - 12	1.23±0.66 (0.27-2.98)	13.1±4.7 (5.6-28.3)	1.67±0.69 (0.56-3.76)	26.6±9.91 (10.3-50.8)	0.8±0.3 (0.4-1.6)	22.5±7.0 (9.0-29.0)
12 - 20	0.3±0.73 (0.04-0.81)	3.3±2.6 (00-9.7)	0.52±0.88 (0.008-1.41)	9.01±5.6 (0.1-28.2)	0.5±0.3 (0.1-0.9)	11.7±5.7 (0.7-23.0)

Values are means ± standard deviation with range in parentheses
% of total urinary 5HIAA due to Plantain recovered in first 4 hours after meal

= Normal Nigerians = 60.2 ± 10.8
E.M.F. Patients = 41.3 ± 14.0
Other Cardiac Patients = 38.5 ± 10.5

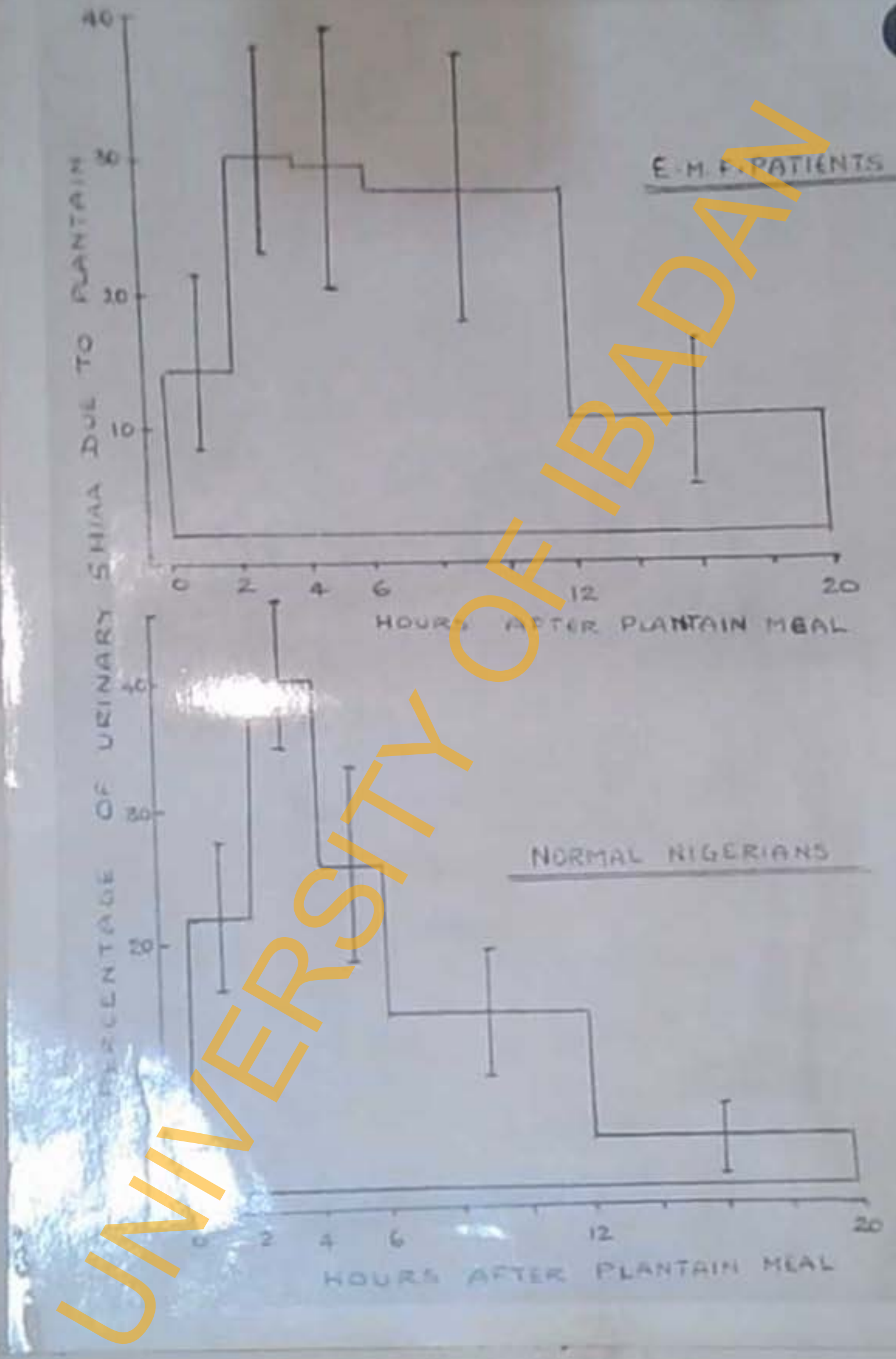


Fig. II Comparision of the rate of excretion of 5-HIAA in normal Nigerians and Nigerians with endomyocardial fibrosis after a plantain meal.

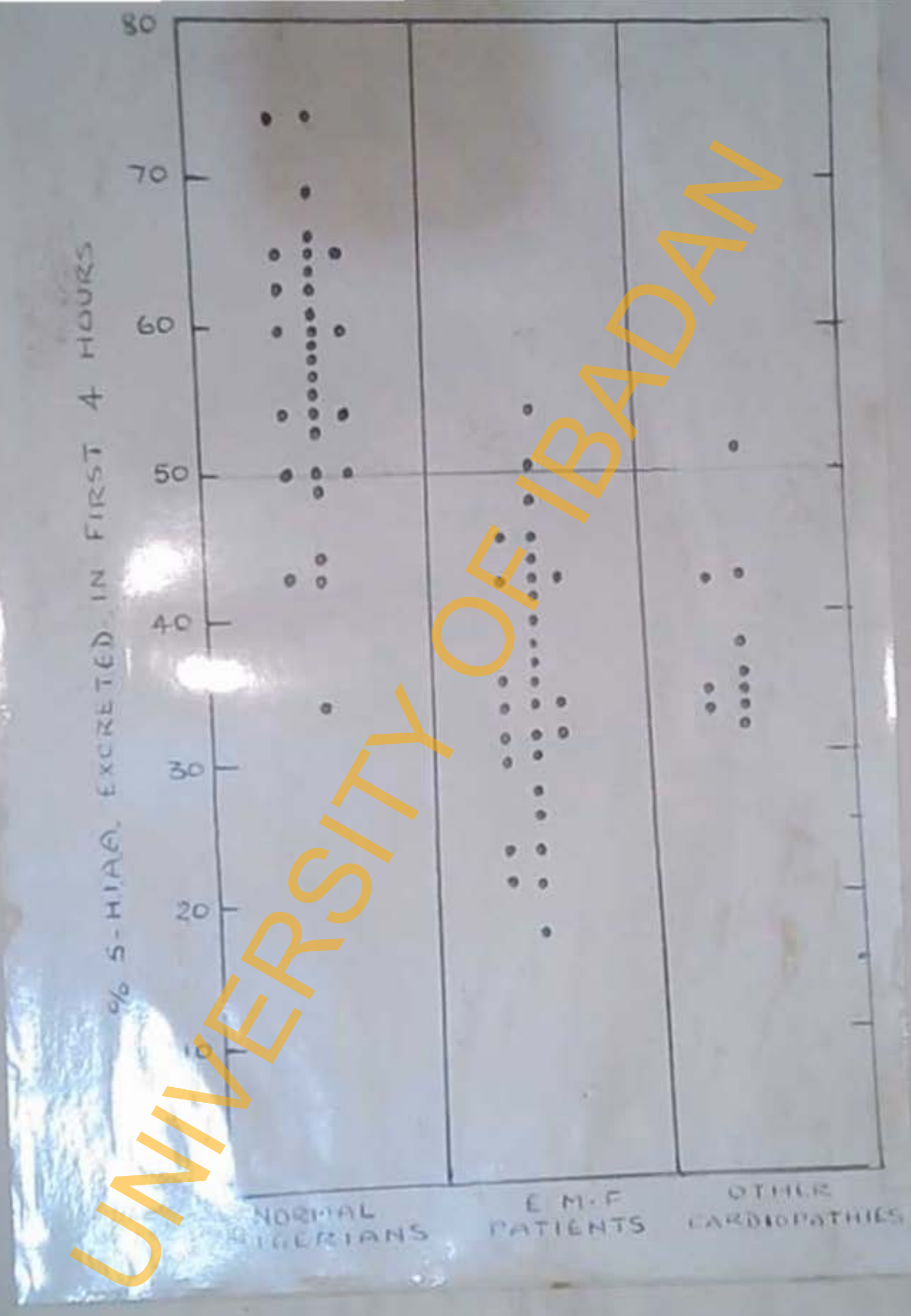


Fig. III Comparision of the percentage excretion of 5-HIAA in first 4 hours after a plantain meal in normal Nigerians and in Nigerians with cardiopathies.

Analysis and Brief Discussion of Results:

As shown in table 13, patients with E.M.F. excreted significantly less 5-HIAA than either the control group of Nigerians or Europeans. There was however no significant difference in the basal urinary excretion of 5-HIAA between normal healthy Nigerians and Europeans. This is in agreement with the finding of Foy and Parratt (1962). The rate of excretion of 5-HIAA, derived from the 5-HT absorbed from a plantain meal, was considerably slower in the subjects with E.M.F. than in the control groups (table 14 and Fig. 2), although the amount of plantain eaten and the total amount of 5-HIAA excreted in the 20-hour period after the plantain meal in the two groups were similar (table 13). Thus in normal subjects more than 60% of the 5-HT load excreted as 5-HIAA appeared in the urine within the first 4 hour period after the plantain meal. The corresponding figures for the subjects with E.M.F. and other cardiopathies were 1.1.3 and 38.5% respectively. Figure 3 is a scattergram of these data.

The results demonstrate clearly that patients with E.M.F. excrete significantly less 5-HIAA than do normal individuals. Further, the ability to excrete 5-HIAA formed as a result of metabolism of ingested 5-HT is significantly retarded in these patients (fig. 2). Since there is no evidence to suggest that the absorption of 5-HT is any slower in these patients, it seems likely that in this condition there is either a delayed breakdown of the amine or a delayed excretion of its main metabolite. This would indicate that blood levels of 5-HT and / or 5-HIAA after the ingestion of plantain would be higher for a longer time in such patients than in normal invidivuals.

Although the results could indicate an impaired metabolism of endogenous and exogenous 5-HT in E.M.P., they do not determine whether such an impaired metabolism precedes the cardiac lesions or merely results from them. The fact that patients with miscellaneous cardiopathies also excreted much less 5-HIAA than did normal individuals, and also metabolised exogenous 5-HT more slowly and incompletely, might suggest that impaired metabolism was, in some indirect way, the result rather than the cause of the cardiac lesions. Thus a reduced ability to detoxicate ingested 5-HT could theoretically result from damage to the liver which is a major site of breakdown of 5-HT in man. Indeed cardiac cirrhosis is a complication of long standing right ventricular E.M.P. This in conjunction with a probable reduction in renal blood flow, consequent on the low cardiac output, may in fact be all that is responsible for the low urinary excretions of 5-HIAA in E.M.P. patients. It was therefore considered, that an assessment of hepatic and renal function tests may shed more light on the interpretation of these results.

Hepatic and Renal Function Tests in Patients with Endomyocardial Fibrosis:

These tests were carried out by the author in collaboration with the Chemical Pathology Department of the University College Hospital. Detailed accounts of methods and techniques will not therefore be given.

Liver Function Tests:

10ml of blood was withdrawn from each subject by vene-puncture for the estimation of serum proteins, cholesterol, the serum transaminases, alkaline phosphatse and thymol reactions.

For the bromsulphalein retention test, the patients were fasted overnight. At 8 a.m. on the following morning, 10ml of venous blood was withdrawn for the blank sample. A quantity of 5% solution of bromsulphalein dye in normal saline (equivalent to 5 mg. of dye per kilogram body weight) was injected slowly intravenously. Blood samples were taken at 5 and 45 minutes after injection and these were analysed for their dye content. This test depends mainly on the ability of the liver to remove bromsulphalein and excrete it into the bile. In normal individuals the bromsulphalein concentration of 45 minutes sample is usually between 0 and 5%.

Renal Function Tests:

The tests performed included estimation of serum urea, and the renal clearances of urea and inulin. It is claimed that the urea clearance test is probably one of the most reliable general tests of renal function (Dawson & Goldie 1958).

Urea Clearance Test:

At 9 a.m. on the morning of study, after the normal usual breakfast, each subject completely emptied his bladder and the specimen was discarded. The subject was then given about a pint of water to drink. At 10 a.m. the bladder was again completely emptied and its volume noted. A 2ml sample of blood was also withdrawn. At 11 a.m. the bladder was again emptied and another sample of blood withdrawn. The urea clearances estimated from these samples were expressed as percentages of average normal maximum urea clearance (when Av. normal max. urea clearance is taken as 75ml/minute).

Inulin Clearance Test:

No special preparation was needed for this test. At the beginning of the study, each patient was given 2 pints of water to drink. The bladder was catheterised and urine allowed to drain continuously. 5ml of blood and 10ml of urine were collected for use as blanks. A priming dose of a mixture of 30ml 10% inulin plus 4ml 20% p-amino hippurate was injected slowly intravenously over a period of 2-3 minutes. This was followed by an intravenous infusion of inulin (containing 70ml of 10% inulin and 20ml of 20% p-amino hippurate in a pint of normal saline) at a rate of 4ml per minute. About half an hour was allowed for stabilisation to occur. Then over the next one hour, urine samples were collected at 20 minute intervals and blood samples also collected at the beginning and the end of each urine collection period. All the samples were sent to the laboratory for the estimation of Inulin Clearance. Results are expressed as percentages of average normal clearance (taking normal inulin clearance as 125ml/minute).

TABLE 15

LIVER FUNCTION TESTS IN L.M.F. PATIENTS

INI-TIALS	Bilirubin in mg/100 ml Blood		Alkaline Phosph in K.A. Units	Thymol Turbi-dity	Thymol Floccu-lation	S.G.O.T. in Cabmid Units	S.G.P.T. in Cabmid Units	45 mins B.S.P.
	Total	Direct						
A.O.	0.6	0.3	5.2	11.2	++++	27	36	16%
A.R.	0.8	0.3	9.4	9	+++	30	34	12%
A.D.	0.9	0.1	3.8	2.1	NEG	13	29	4%
A.H.	1.0	NIL	14	1.7	NEG	17	8	5%
A.S.	1.3	NIL	4	5	++	11	30	2%
A.S.	0.7	NIL	8	2	NEG	24	20	6%
D.J.	0.8	NIL	9	1	NEG	7	12	NIL
C.T.	0.5	NIL	16	5	++	20	16	8%
E.P.	0.5	NIL	7	2	NEG	17	8	3%
E.E.	0.7	0.1	12	2	NEG	15	23	7%
F.F.	0.4	0.6	13	1	NEG	16	12	17%
H.G.	1.1	NIL	12	4	+	34	22	20%
I.O.	1.4	0.6	28	11.4	++	21	38	15%
J.S.	1.4	0.2	9	2	+	14	23	6%
J.G.	0.8	NIL	11	2	NEG	15	2	3%
K.A.	0.7	NIL	9	2	NEG	7	12	4%
M.T.	0.7	NIL	9	2	NEG	15	2	9%
M.M.	0.6	NIL	7.5	31	++	25	46	5%
O.M.	0.4	NIL	13	3	++	12	38	3%
O.I.	1.6	1.0	17	4	++	24	14	19%
O.J.	0.6	NIL	9	3	+	12	7	4%
O.A.	1.0	NIL	13	5	++	27	23	11%
R.A.	0.7	0.1	13	4	NEG	14	19	3%
S.M.	8	NIL	11	3	NEG	30	12	4%
T.K.	0.4	NIL	9	2	NEG	7	8	7%
T.N.	6	NIL	13	2	NEG	25	14	3%
U.J.	1.2	0.3	16	8.3	+++	38	42	16%
U.R.	0.5	NIL	12	2	+	16	23	6%
Y.A.	0.9	0.2	13	3	++	11	6	4%
Y.B.	1.4	0.2	11	1	NEG	6	13	7%

TABLE 16

LIVER FUNCTION TESTS U.M.P. PATIENTS

INI-TIALS	PLASMA PROTEINS IN Gms/ 100ml blood							Serum Cholesterol in mg/100 ml. blood
	Total	Albu-min	GLOBULINS					
			Alpha	Alpha 2	Beta	Gamma	Total	
A.O.	8.8	2.6	0.6	0.0	1.4	3.4	6.2	123
A.R.	6.2	1.8	0.4	0.5	0.9	2.6	4.4	113
A.D.	7.9	2.3	0.8	1.2	1.3	3.3	5.6	152
A.M.	9.0	2.8	0.7	0.8	1.3	3.4	6.2	149
A.S.	10.3	3.9	0.8	1.2	1.3	3.5	6.2	119
A.S.	5.1	2.1	0.4	0.6	0.8	1.2	3.0	162
B.J.	7.3	3.8	0.2	0.6	1.2	2.5	3.5	178
C.T.	4.9	1.3	0.3	0.8	0.8	1.7	3.6	116
E.P.	6.9	2.8	0.4	0.5	0.7	2.5	4.1	97
E.E.	8.3	3.8	0.5	0.45	0.05	2.0	4.5	104
F.F.	8.1	4.2	0.2	0.3	0.7	2.7	3.9	226
H.G.	6.0	2.4	0.2	0.4	0.9	2.1	3.6	168
I.O.	8.2	3.5	0.6	0.7	1.4	2.0	4.7	115
J.S.	5.9	1.72	0.48	0.46	0.8	2.44	4.18	139
J.G.	7.4	3.1	0.3	0.3	0.7	3.0	4.3	165
K.A.	6.5	3.1	0.2	0.3	0.7	2.2	3.4	101
M.T.	8.3	3.8	0.2	0.6	1.2	2.5	4.5	146
M.M.	4.6	1.5	0.3	0.3	0.7	1.8	3.1	137
O.M.	5.2	2.3	0.2	0.3	0.5	1.8	2.9	217
O.I.	9.3	3.1	0.4	0.6	1.3	2.3	4.6	156
O.J.	6.1	2.4	0.3	0.5	0.9	2.0	3.7	109
O.A.	5.4	2.1	0.3	0.4	0.7	0.9	3.3	96
R.A.	4.6	1.7	0.2	0.2	0.5	2.0	2.9	128
S.M.	8.1	3.8	0.3	0.4	0.9	2.8	4.3	203
T.K.	7.7	2.9	0.3	0.4	1.2	2.9	4.8	177
T.N.	5.8	2.4	0.2	0.5	1.0	1.7	3.4	152
U.J.	6.3	2.5	0.2	0.3	0.7	2.6	3.8	136
U.R.	4.9	2.3	0.1	0.15	0.15	1.85	2.5	108
Y.A.	7.0	2.8	0.3	0.4	0.9	2.6	4.2	231
Y.B.	6.1	2.4	0.2	0.3	0.8	2.2	3.7	187

TABLE 17

RENAL FUNCTION TESTS
E.N.F. PATIENTS

INITIALS	Serum Urea in mg/100ml of blood	% of Average Normal, Max. Urea clearance	% of Average Normal Inulin clearance
A.O.	21	68	76
A.R.	26	57	74
A.D.	23	58	69
A.M.	48-126	39	55
A.S.	25	73	84
A.S.	18	66	72
B.J.	30	50	61
C.T.	24	67	69
E.P.	22	75	82
E.E.	20-25	70	84
F.F.	17	86	90
H.G.	26	72	84
I.O.	18-55	58	63
J.S.	41	45	57
J.G.	22	80	86
K.A.	19	77	79
M.T.	28	65	80
M.M.	41	59	77
O.M.	33	60	68
O.I.	29	67	70
O.J.	32	75	82
O.A.	26	70	73
P.A.	28	68	75
S.M.	40	63	69
T.K.	22	84	85
T.M.	35	72	78
U.J.	30	61	65
U.R.	46	55	67
Y.A.	23	68	70
Y.S.	25	78	80

Results of Liver Function:

The results of liver function tests portray some degree of liver impairment probably due to the long standing central venous congestion associated with endomyocardial fibrosis. Thus B.S.P. excretion tests were abnormal in about half of the number of patients investigated but this test is far too sensitive and may be abnormal even with the mildest degree of liver damage. The serum enzymes were all within normal limits, the alkaline phosphatase values do not confirm that excretion function of the liver is impaired. The fact that the transaminases, especially the S.G.P.T. which are released from damaged liver cells were normal shows that there was no extensive damage to the liver cells. However elevated serum transaminases are usually associated with acute lesions. The thymol reactions were abnormal in about 12 of the 30 patients investigated but these may be due to the abnormaly high globulin fraction as well as the reduced albumin globulin ratio, a common finding in most Nigerians. The total serum proteins was low only in 3 of the patients and well within normal limits in the rest. It would appear therefore that liver function is not severely deranged in E.M.F. patients. It is however known that nature endows the individual with excess of liver tissue and that normal function may still be carried out with less than half of the normal liver tissue. Thus in confirmation of Abrahams' findings (1962) the liver function tests did not correlate with any severe degree of liver damage.

Results of Renal Function:

Serum urea values were within normal range in all except 4 of the 30 E.M.F. patients studied. Also the urea clearance tests portrayed no severe

degree of renal impairment. It is said that urea clearance values above 70% of average normal, indicate normal renal function, 40 - 70% mild renal impairment, 20 - 40% moderate renal insufficiency and that values below 20% suggest a severe degree of renal failure (Baron 1957). 12 of the 30 E.M.F. patients studied therefore had normal urea clearance values, one patient portrayed moderate renal impairment whilst the remainder were in the range of mild renal insufficiency. Thus it is logical to presume that products of metabolism in these patients were normally cleared by the kidney and excreted in the urine, which in this instance should also apply to the end product of 5-HT metabolism. Indeed inulin clearance values suggest that glomerular filtration was not severely impaired in spite of the probable reduction in cardiac output.

Discussion of Results:

Even though the E.M.F. patients had a certain degree of liver damage, there is no evidence that gross liver damage results in abnormal 5-HIAA excretion. Normal urinary values have for example, been reported in severe liver cirrhosis (Donaldson et al 1959). Also the percentage recovery of exogenous 5-HT (administered either as plantain or as 5-HT in capsules) as urinary 5-HIAA in patients with liver cirrhosis was identical with that of normal healthy control subjects (Nelson and Sjoerdsma 1963). Furthermore, normal urinary 5-HIAA levels have been reported in arterio-sclerotic and rheumatic heart disease with kidney involvement (Haverback et al 1956) and in some patients with chronic pyelonephritis (Borges and Bessman 1956).

In conclusion, the results of both hepatic and renal function tests do not suggest that the diminished urinary excretion of 5-HIAA in E.M.F. patients is the result rather than the cause of the cardiac lesion. It would seem from the data so far available, that Crawford's theory of impaired metabolism of 5-HT in E.M.F. patients may be true. Therefore endogenously produced 5-HT in these patients, having its metabolic pathway blocked, will accumulate in the serum, and blood 5-HT levels will be further elevated after plantain ingestion. The next stage in this investigation was therefore to determine blood 5-HT levels in E.M.F. patients and in normal healthy Nigerians.

Assay of Blood 5-HT:

Various methods have been described for the assay of 5-HT. These fall mainly into two categories, the chemical and bioassay techniques. Three chemical methods are available, the first is by measurement of its ultraviolet absorption, the second by colorimetric estimation of the chromophore produced by reaction with 1-nitroso-2 napthol and the third is by measurement of its fluorescence in solution. In humans, the concentration of 5-HT in whole blood is approximately 0.1 to 0.3 µg per ml, and such small quantities cannot be detected either by colorimetric or spectrophotometric procedures unless large amounts of blood (30-50ml) are used. The development of a spectrophotofluorimeter capable of activating compounds, revealed that 5-HT fluoresces in the ultra-violet. The intensity of the emitted fluorescence is sufficient to permit measurement of 5-HT on 5 - 10ml samples of blood (Udenfriend et al 1955). Unfortunately such sophisticated instruments are very expensive and not very easy to come by.

The bioassay methods are as specific and sentive for 5-HT estimation as the chemical methods. They are also suitable for estimating small quantities of 5-HT in animal tissues. The bioassay method is based on the smooth muscle stimulant effect of 5-HT, and these assays have been performed on various organs, such as the isolated oestrus rat uterus, the rat's colon, the crop of a dry old chick and the fundus of rat stomach.

The bioassay method employed in these estimations was described by Vane (1957) and subsequently modified by Lin and Yeoh (1965). This utilises the response of the longitudinal muscle strip of the fundus of rat stomach

suspended in 15ml bath perfused with magnesium free Kreb's solution at 39.5°C, and aerated with a gas mixture of 95% oxygen and 5% carbon-dioxide.

Collection of Blood Samples and Extraction of Blood 5-HT.

24 hours prior to each estimation, the subjects abstained from articles of food known to contain 5-HT. Since the greater part of 5-HT in blood is contained in platelets, adequate steps were taken against platelet loss. Thus all syringes and glassware used were siliconized. Blood samples were collected by vene-puncture from each subject into siliconized syringes. The first few ml of shed blood were discarded and 10ml samples received into siliconized centrifuge tubes containing 1ml of 1% E.D.T.A. (ethylene diamine tetra acetic acid) made up in normal saline. These tubes were centrifuged at 3,000 rev/minute for 30 minutes. The supernatant platelet rich plasma was carefully removed using siliconized pasteur pipettes, and this was transferred into a non-siliconized glass stoppered flask containing 250ml of acetone. The mixture was thoroughly shaken for ten minutes and then kept in the refrigerator overnight. By morning complete lysis of platelets had taken place. The filtrate was later evaporated to dryness and the residue of 5-HT left behind was taken up in 10ml of distilled water (modified from Hadistry and Stacey 1955).

Preparation of Assay tissue and Method of Assay:

Rats of either sex weighing about 250 gms. were killed with a blow on the head, followed by slitting of the throat. The stomach was dissected free from the abdomen and dropped in a petri-dish containing magnesium free Kreb's solution. The fundus was separated from the pylorus and cut open

along the lesser curvature. The fan-shapped tissue so produced was later cut (as illustrated in fig. 4) along its longitudinal muscle to form a single thin strip 6-10 cms. long.

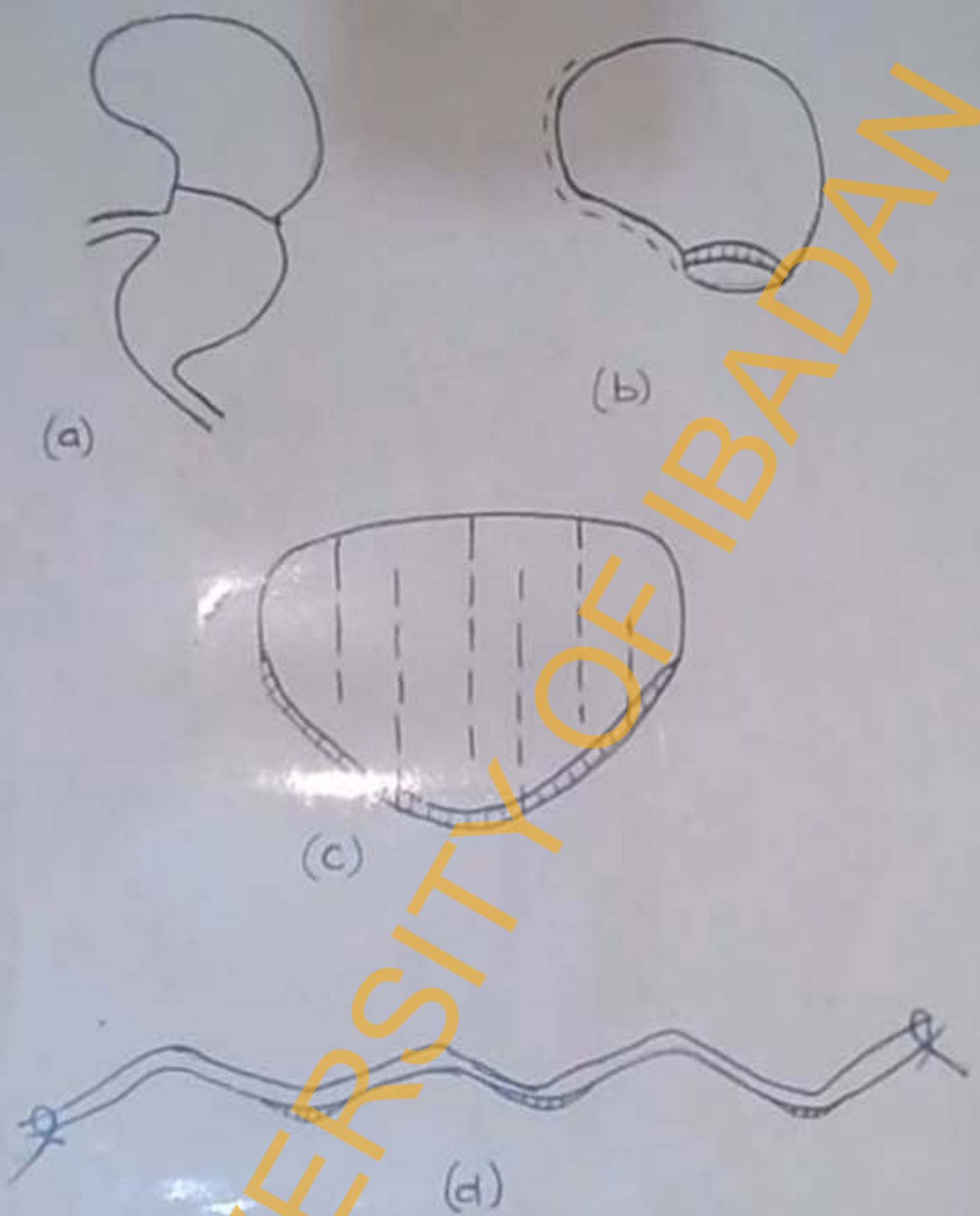
After cotton threads had been tied to the ends, the strip was gently stretched, one end of the strip was tied to the tissue holder and lowered into the organ bath. The cotton tied to the other end of the strip was attached to the auxotonic frontal writing pendulum lever. The bath was perfused with Kreb's solution (containing atropine sulphate in concentration of 10^{7}/litre) and aerated with a mixture of 95% oxygen and 5% carbon-dioxide. The tissue was left for 2 hours for stabilization to occur, before commencement of assay.

At the same time as blood 5-HT samples were extracted, suitable dilutions of freshly prepared 5-HT were made from 5-HT creatinine sulphate (Mann Research Laboratory). The sensitivity of the fundal strip to 5-HT was initially assessed by its response to graded doses of 5-HT (fig. 6). If a satisfactory dose response relationship was obtained, the extracts of 5-HT from blood samples were applied. One ml of each solution of 5-HT was introduced into the perfusing solution. The tissue responded within 10-15 seconds of adding 5-HT to the bath and reached maximum contraction in 45 seconds. Relaxation to baseline occured 1 minute after 5-HT was washed out, and another dose of 5-HT could be added after 3 minutes. The concentration of 5-HT/ml of blood was determined by the method of bracketing between known doses, until a suitable dilution of 5-HT solution gave approximately the same response as the blood sample. Diberyline in a

concentration of 10^{-7} ng/ml completely abolished the response to 10^{-9} ng/ml of 5-HT. This inhibition by dibenyline, on the fundal strip was used as a criterion of the identity of the active substance in the blood extract with 5-HT.

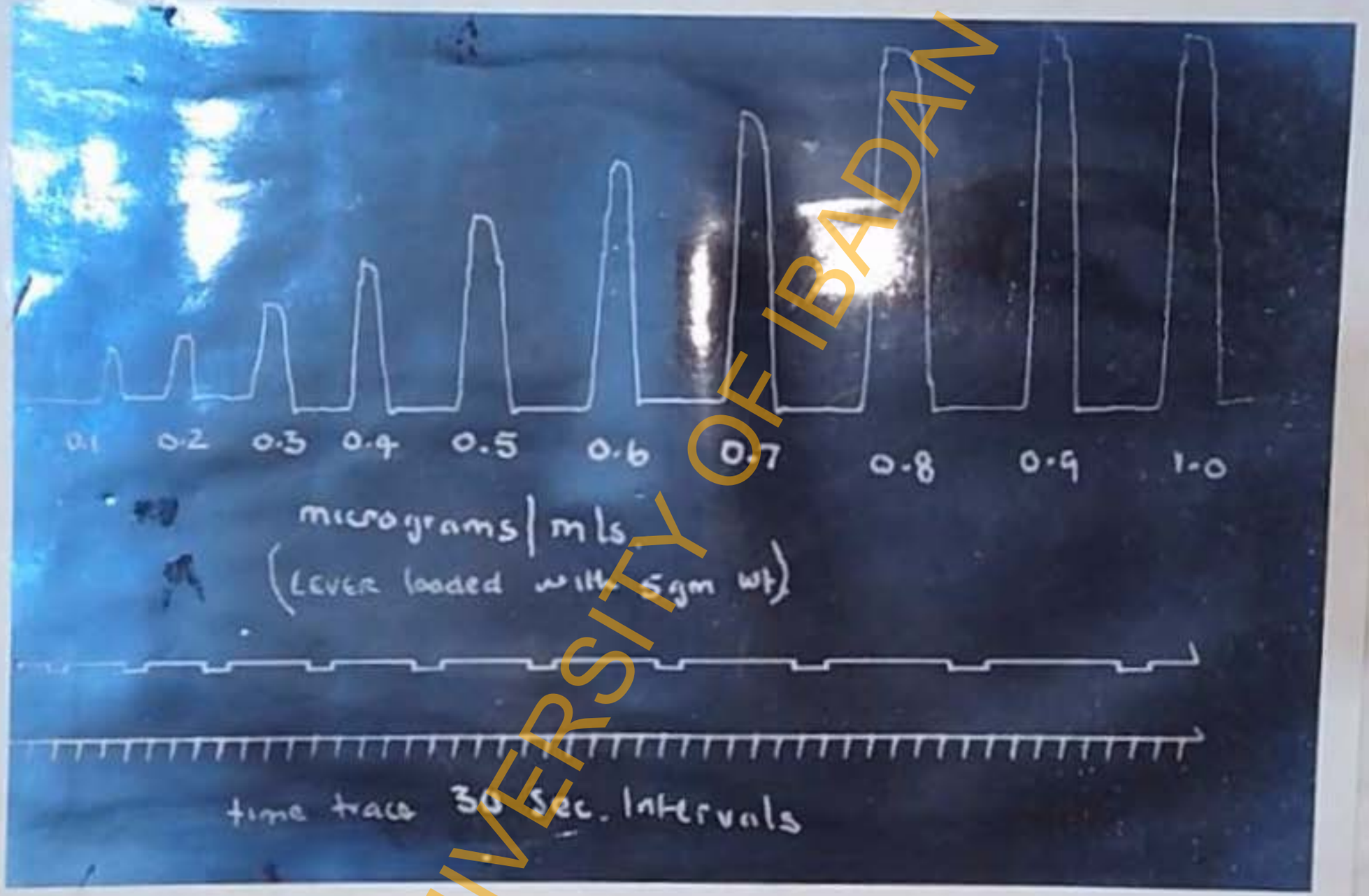
The basal levels of blood 5-HT were determined in 30 normal healthy Nigerians and in 30 patients with endomyocardial fibrosis. Three separate determinations were made on each subject with an interval of one month between each estimation. Subsequently the subjects were fed on known weights of fried plantain. Blood samples were taken with the usual precautions at one and 3 hour intervals after plantain ingestion. These samples were assayed for 5-HT to determine the effect of plantain ingestion on serum 5-HT levels.

The results are tabulated on pages 108 - 112.

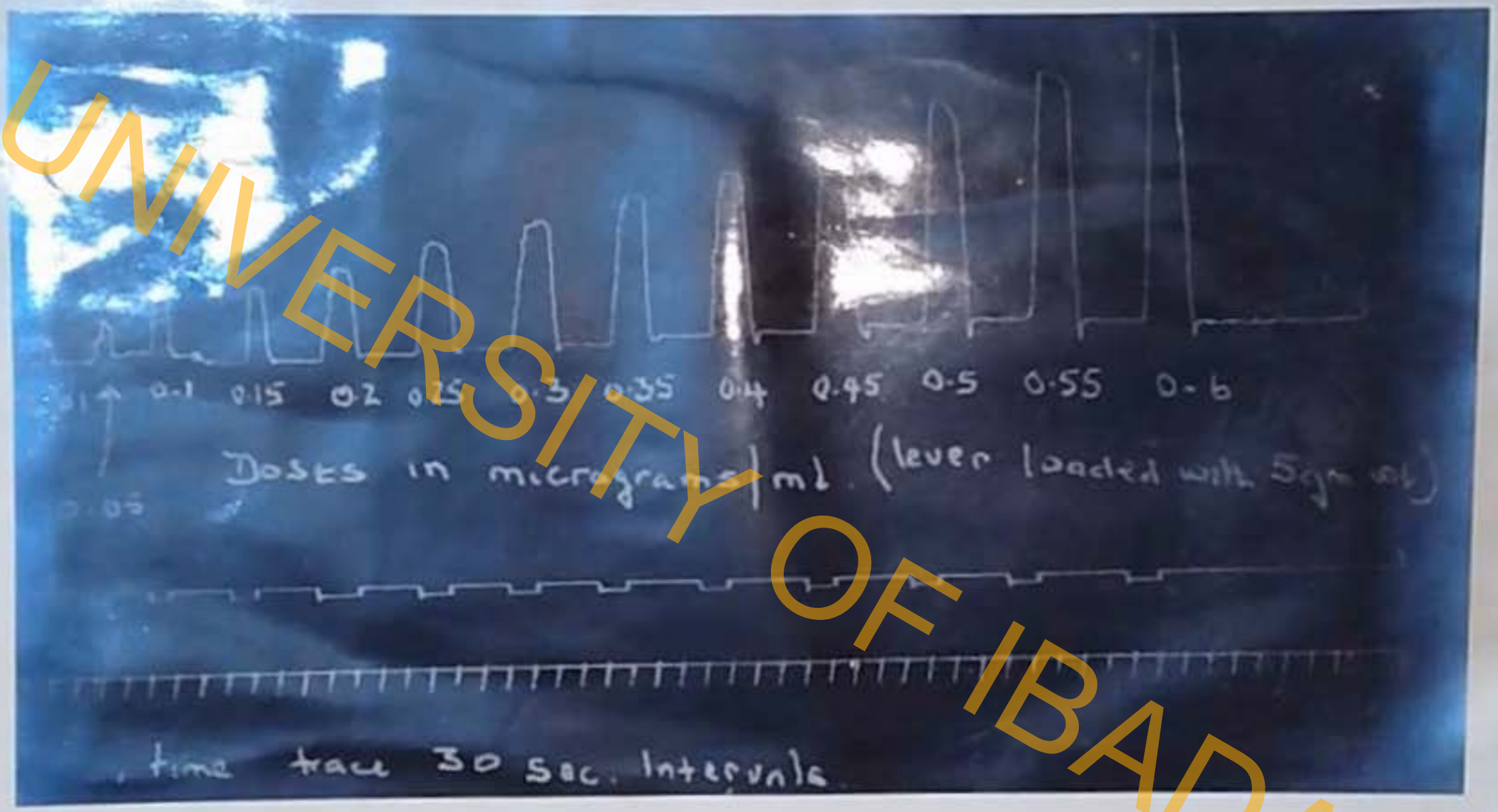


Preparation of fundal strip of rat stomach
(a) The whole rat stomach. (b) The fundus of stomach only, cutting along the lesser curvature - dotted lines, produces (c) a fan shaped specimen; and incomplete cuts are made along the dotted lines as illustrated, gives(d) a thin strip of longitudinal muscle 6-10 cms. long.

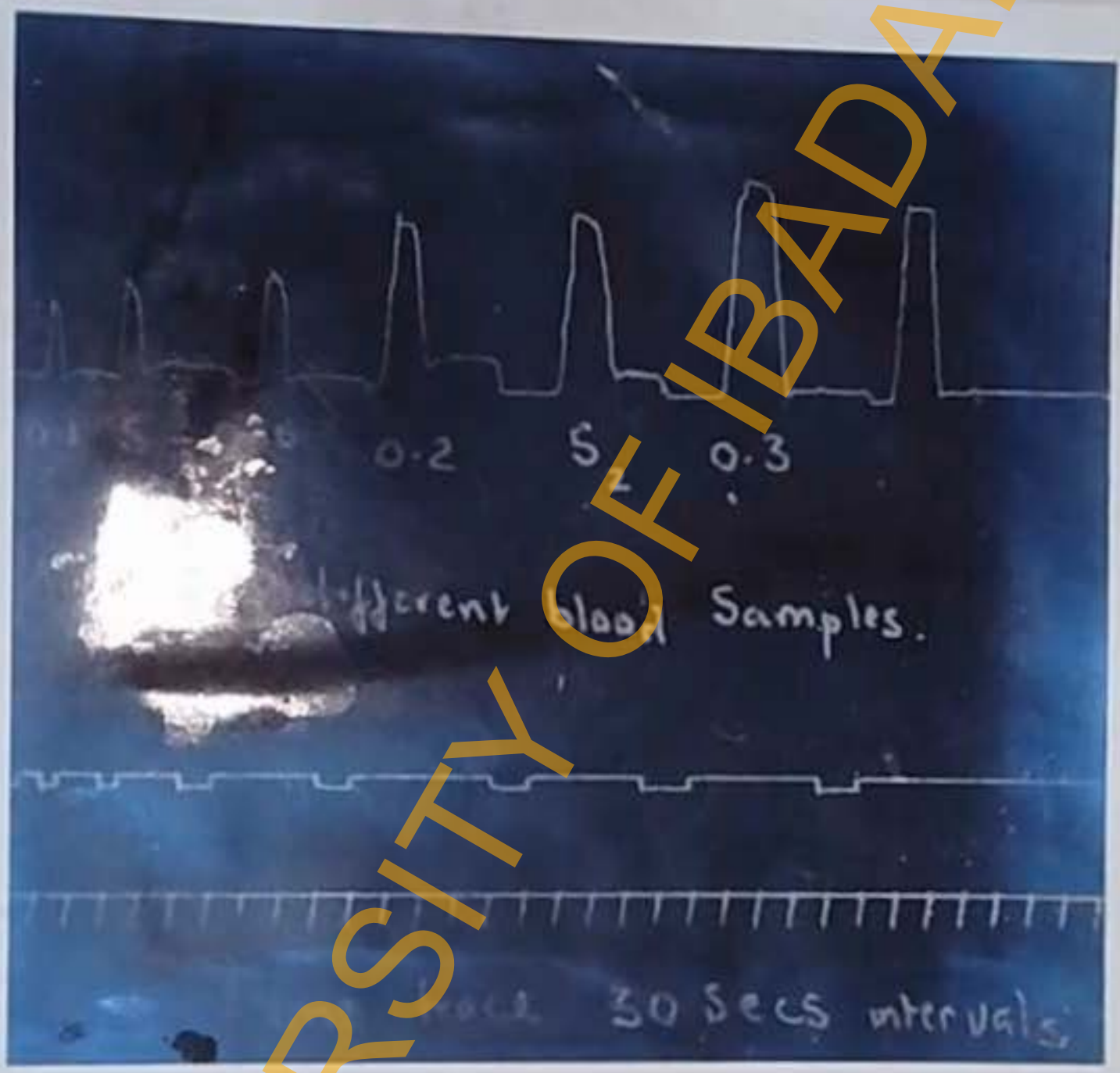
Apparatus for the bioassay of 5-HT shows magnesium free Kreb's solution in perfusion bottle. Tank containing a mixture of 95% O_2 and 5% CO_2. Fundal strip of rat's stomach in organ bath is attached to the auxotonic frontal lever; and contractions are recorded on a smoked drum with a time marker attachment, 5-HT solutions in centrifuge tubes.



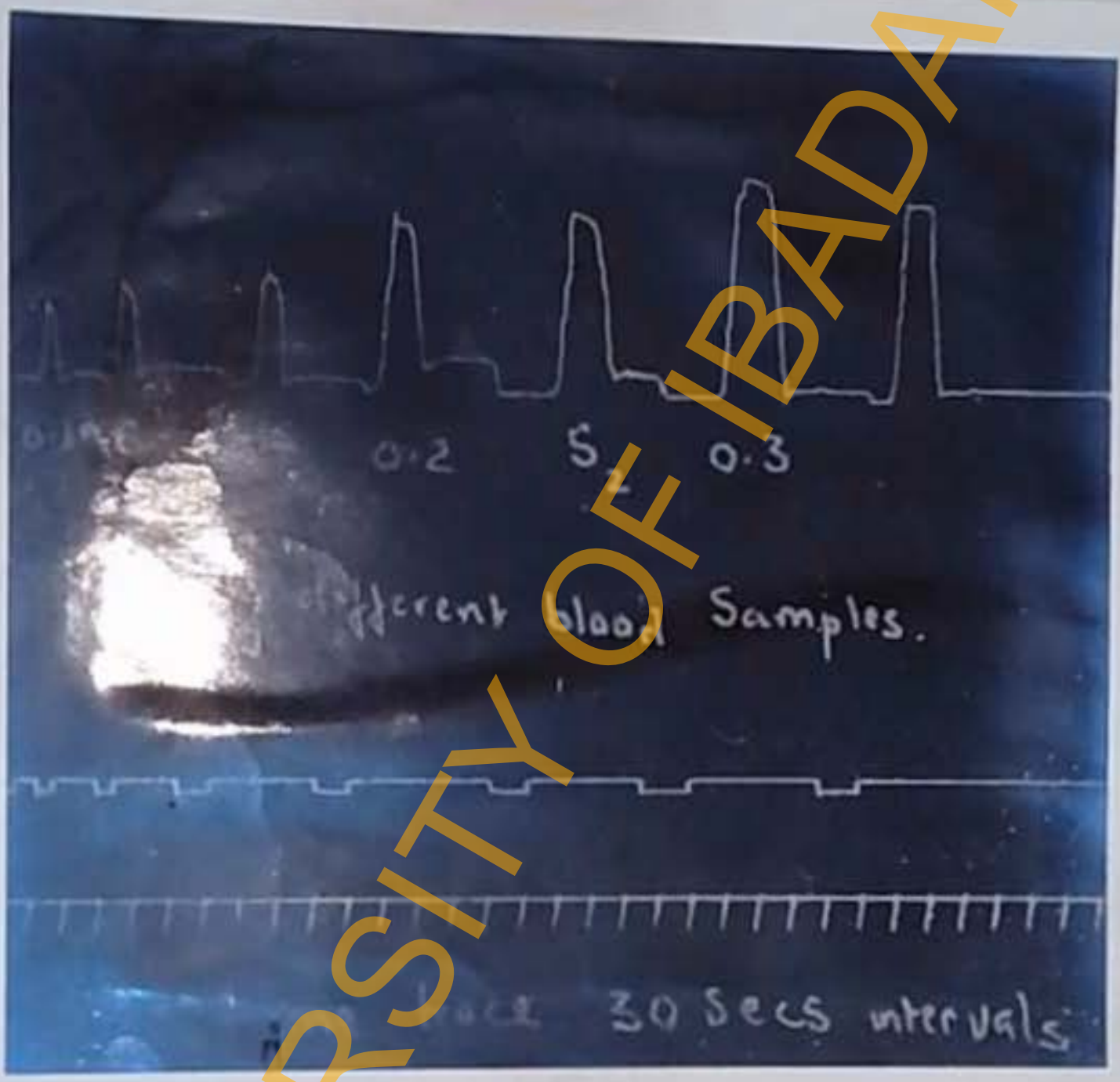
Bioassay of 5-HT on fundal strip of rat's stomach
Showing the dose-response relationship.
Note tachyphylaxis at 1.0 n u/ml.
Liver loaded with a 5 g. weight.



Bioassay of 5-HT on fundal strip of rat's stomach
Dose-response relationship
Low dose range - Lever loaded with 5gm weight.



Bioassay of 5-HT on fundal strip of rat's stomach Showing the system of bracketing of 5-HT in blood sample between known doses of 5-HT.



Bioassay of 5-HT on fundal strip of rat's stomach Showing the system of bracketing of 5-HT in blood sample between known doses of 5-HT.

TABLE 18

BASAL WHOLE BLOOD 5HT VALUES IN µg/ml
NORMAL NIGERIANS
Examinations at intervals of one month

INITIALS	1st Examination	2nd Examination	3rd Examination	Mean Value
A.B.	0.35	0.3	0.32	0.32
A.G.	0.18	0.16	0.18	0.17
A.O.	0.2	0.2	0.23	0.21
A.S.	0.08	0.06	0.05	0.06
B.M.	0.25	0.2	0.24	0.23
B.P.	0.6	0.52	0.56	0.56
D.N.	0.14	0.15	0.12	0.14
D.T.	0.42	0.45	0.40	0.42
F.A.	0.06	0.06	0.06	0.06
G.T.	0.28	0.28	0.3	0.28
K.O.	0.10	0.09	0.09	0.09
L.H.	0.36	0.38	0.3	0.35
M.C.	0.15	0.08	0.16	0.16
O.T.	0.02	0.02	0.03	0.02
O.O.	0.6	0.65	0.62	0.62
O.M.	0.48	0.45	0.5	0.48
O.D.	0.36	0.36	0.3	0.34
O.A.	0.14	0.12	0.2	0.15
S.R.	0.05	0.04	0.04	0.04
S.V.	0.26	0.25	0.22	0.24
T.B.	0.5	0.52	0.5	0.50
T.K.	0.07	0.03	0.09	0.08
T.B.	0.2	0.3	0.18	0.23
U.A.	0.46	0.38	0.44	0.43
U.J.	0.15	0.16	0.15	0.15
W.O.	0.24	0.2	0.36	0.27
W.A.	0.04	0.05	0.08	0.06
[illegible].P.	0.35	0.30	0.36	0.34
Y.K.	0.22	0.28	0.25	0.25
Y.A.	0.09	0.10	0.15	0.11

TABLE 19

BASAL WHOLE BLOOD 5HT VALUES IN µg/ml (E.M.F. PATIENTS)
Estimations at intervals of one month

INITIALS	1st Estimation	2nd Estimation	3rd Estimation	Mean Value
A.O.	0.25	0.22	0.28	0.25
A.R.	0.09	0.10	0.10	0.10
A.D.	0.03	0.07	0.02	0.04
A.M.	0.34	0.33	0.35	0.34
A.S.	0.12	0.11	0.09	0.11
A.S.	0.16	0.15	0.16	0.16
B.J.	0.23	0.25	0.20	0.23
C.T.	0.18	0.20	0.20	0.19
E.P.	0.02	0.01	0.06	0.03
E.E.	0.08	0.13	0.12	0.11
F.F.	0.26	0.28	0.25	0.26
H.G.	0.45	0.44	0.49	0.46
I.O.	0.36	0.12	0.25	0.26
J.S.	0.04	0.04	0.03	0.04
J.G.	0.13	0.18	0.12	0.14
K.A.	0.07	0.05	0.08	0.07
M.T.	0.15	0.12	0.14	0.14
M.M.	0.03	0.03	0.02	0.03
O.M.	0.01	0.01	0.01	0.01
O.I.	0.3[illegible]	0.25	0.30	0.29
O.J.	0.14	0.11	0.12	0.12
O.A.	0.28	0.27	0.32	0.29
R.A.	0.05	0.05	0.03	0.04
S.M.	0.16	0.18	0.12	0.15
T.K.	0.07	0.05	0.06	0.06
T.M.	0.38	0.32	0.34	0.35
U.J.	0.07	0.09	0.09	0.09
U.R.	0.12	0.10	0.13	0.12
Y.A.	0.02	0.07	0.03	0.04
Y.S.	0.15	0.26	0.18	0.20

TABLE 20

WHOLE BLOOD 5HT VALUES IN µg/ml
AFTER PLANTAIN MEAL - (NORMAL NIGERIANS)

INITIALS	Calculated wt. of 5HT ingested in µg	Mean Basal 5HT Levels	5HT Levels 1st hr after Meal	5HT Levels 3 hrs after Meal	Mean 5HT Value 3hr Period after Meal
A.B.	11,500	0.32	0.3	0.35	0.325
A.G.	16,000	0.17	0.22	0.2	0.21
A.O.	14,550	0.21	0.2	0.26	0.23
A.S.	10,200	0.06	0.04	0.07	0.055
B.H.	15,500	0.23	0.21	0.3	0.25
B.P.	12,000	0.56	0.6	0.6	0.6
D.H.	17,800	0.4	0.12	0.28	0.2
D.T.	16,050	0.42	0.5	0.4	0.45
F.A.	18,750	0.06	0.4	0.03	0.035
G.T.	11,150	0.28	0.3	0.25	0.27
K.O.	14,000	0.09	0.15	0.16	0.15
L.H.	16,250	0.35	0.3	0.28	0.29
M.C.	13,110	0.16	0.12	0.10	0.11
O.T.	10,750	0.02	0.04	0.01	0.025
O.O.	15,800	0.62	0.55	0.51	0.53
O.M.	21,625	0.48	0.5	0.56	0.53
O.B.	15,600	0.34	0,25	0.38	0.31
O.A.	14,550	0.15	0.2	0.08	0.14
S.R.	19,725	0.05	0.08	0.1	0.09
S.V.	12,650	0.26	0.36	0.12	0.29
T.B.	13,500	0.5	0.45	0.4	0.425
T.K.	17,220	0.08	0.10	0.08	0.09
T.E.	15,300	0.23	0.2	0.28	0.24
U.A.	17,230	0.43	0.5	0.46	0.48
U.J.	22,175	0.15	0.18	0.24	0.2
W.O.	8,500	0.27	0.23	0.22	0.22
W.A.	14,625	0.06	0.07	0.13	0.10
W.P.	14,150	0.34	0.3	0.25	0.28
Y.K.	16,625	0.25	0.3	0.3	0.3
Y.A.	21,110	0.09	0.16	0.1	0.13

TABLE 21

WHOLE BLOOD 5HT VALUES IN µg/ml
AFTER PLANTAIN MEAL - E.M.P. PATIENTS

INITIALS	Calculated wt. of 5HT ingested in µg	Mean Basal 5HT Levels	5HT Levels 1st hr after Meal	5HT Levels 3hrs after Meal	Mean 5HT Value 3hr Period after Meal
A.O.	18,675	0.25	0.3	0.24	0.27
A.R.	8,600	0.10	0.05	0.09	0.07
A.D.	13,125	0.04	0.04	0.06	0.05
A.M.	10,500	0.34	0.4	0.28	0.34
A.S.	13,220	0.11	0.15	0 17	0.16
A.S.	15,775	0.16	0.10	0.14	0.12
B.J.	10,430	0.23	0.35	0.2	0.27
C.T.	11,650	0.19	0.16	0.15	0.15
E.P.	14,870	0.03	0.2	0.22	0.21
E.E.	13,370	0.11	0.14	0.09	0.12
F.F.	19,520	0.26	0.22	0.24	0.23
H.G.	9,285	0.46	0.5	0.45	0.47
I.O.	11,680	0.25	0.25	0.25	0.25
J.S.	18,560	0.04	0.06	0.09	0.08
J.G.	13,350	0.14	0.1	0.12	0.11
K.A.	10,470	0.07	0.03	0.06	0.05
N.T.	16,300	0.14	0.08	0.12	0.1
N.N.	11,500	0.03	0.05	0.08	0.07
O.N.	14,220	0.01	0.04	0.07	0.06
O.I.	12,300	0.29	0.26	0.18	0.22
O.J.	13,950	0.12	0.17	0.2	0.18
O.A.	9,610	0.29	0.36	0.3	0.33
R.A.	12,315	0.04	0.08	0.1	0.09
S.H.	15,265	0.15	0.13	0.12	0.12
T.K.	14,160	0.06	0.08	0.06	0.07
T.N.	9,650	0.35	0.34	0.31	0.325
U.J.	16,175	0.09	0.06	0.08	0.07
U.R.	11,330	0.12	0.09	0.11	0.1
Y.A.	18,620	0.04	0.07	0.08	0.075
Y.S.	14,760	0.20	0.15	0.22	0.18

TABLE 22

BLOOD 5HT LEVELS IN µg/ml
BASAL AND AFTER PLANTAIN MEAL

	Calculated wt. of 5HT ingested in mg	Mean Basal 5HT Levels in ug/ml	Mean 5HT Level in ug/ml 5hr Period after Plantain Meal
Normal Nigerians (30)	15.27±3.28 (10.2-22.18)	0.245±0.154 (0.02-0.62)	0.251±0.15 (0.025-0.6)
E.H.F. Patients (30)	13.04±2.97 (8.6-18.68)	0.157±0.11 (0.03-0.46)	0.165±0.03 (0.05-0.47)

Values are means ± standard deviations with range in parentheses.

* Significantly different from normal individuals at a probability level of 0.01

Results of Blood 5-HT Determinations:

The basal blood 5-HT levels seem fairly constant for each subject investigated over the three month period. Ashcroft et al (1965) had observed that in contrast to the large variation in blood 5-HT concentration between individuals, the concentration of blood 5-HT proved to be remarkably constant in a given individual. Mean basal 5-HT level in normal Nigerians was 0.245 ± 0.145 micrograms/ml, the same value for E.M.F. patients was 0.157 ± 0.11 micrograms/ml. (table 22). The 5-HT levels in E.M.F. patients was significantly lower than normal Nigerians at a probability level of more than 0.01. Also after loading with 5-HT as contained in a plantain meal, there was no significant elevation of blood 5-HT in both normal Nigerians and E.M.F. patients. This can only mean that 5-HT was rapidly deaminated to 5-HIAA in both groups of subjects. Thus significantly elevated serum levels of 5-HT did not occur after plantain ingestion, either in normal Nigerians or in patients with endomyocardial fibrosis. Therefore it would seem that E.M.F. patients metabolise 5-HT similarly and as rapidly as normal control healthy Nigerians.

The lower basal 5-HT blood levels in E.M.F. patients is very revealing, especially as this was most unexpected. It is however in consonance with the diminished basal urinary excretion of 5-HIAA in these patients as compared to normal Nigerians. If serum 5-HT levels are low, it follows that the urinary metabolite of 5-HT must be low. The slower recovery rate of exogenous 5-HT as urinary 5-HIAA is probably related to the already demonstrated combined though mild derangement of liver and renal

function in E.M.F. patients. That the degree of this impairment cannot be severe and that deamination of 5-HT to 5-HIAA is not affected, is supported by the similarity in the total percentage recovery of exogenous 5-HT as 5-HIAA in both E.M.F. patients and normal Nigerians, over the 20 hour period of urine collection after plantain meal.

CHAPTER V
DISCUSSION AND CONCLUSION

The prime purpose of this thesis is to examine the proposition that 5HT is a factor in the development of endomyocardial fibrosis. This is a proposition that rests mainly on two counts. First, there is the evidence that 5HT can produce marked cardiac pathology. Part of this evidence is experimental involving animals but a large factor in conditioning thinking along these lines is the occurence of marked cardiac lesions as a result of carcinoid tumours.

From our present point of view the most important manifestation of these tumours is the production of 5HT in large quantity; and that high blood 5HT associated with this syndrome has been clearly implicated in the production of cardiac pathology. It is reasoned that since in this one situation, 5HT appears to produce cardiac pathology, which at first glance has much in common with endomyocardial fibrosis, then, perhaps, in somewhat different situations it might produce the rather different manifestations of E.M.F.

The second major factor in this line of thinking is the apparent geographical relationship between E.M.F. and 5HT which occurs in the diet. The validity of the assumption that carcinoid heart lesions and E.M.F. are similar will be considered first.

Pathology of Carcinoid Heart Disease:

The morphologic appearance of endocardial lesions in the carcinoid syndrome suggest that this may be the result of deposition of a material from the blood. A strong evidence in favour of the theory that this deposition

may come from the blood and not from the underlying endocardium or myocardium is the observation that the internal elastic lamina remains intact. 5-HT, produced in large quantities by the tumour has been implicated to play a major role in the pathogenesis of these lesions. Myocardial lesions have not been described, and the endocardial changes which are specific to this syndrome are present where the primary tumour or metastases have a direct venous inflow into the heart, i.e., right sided lesions when there are hepatic metastases and left sided involvement commonly associated with a right to left shunt, primarily bronchial carcinoids or pulmonary secondaries.

The peculiar carcinoid fibrous plaques are smooth glistening whitish-grey elevations usually focally distributed and very rarely diffuse. Cardiac involvement in this syndrome is predominantly right sided, and the changes are always localised to the endocardium around the valves, the chordae tendinae and may to a lesser extent involve the atrium. Carcinoid fibrous plaques have not been reported in the right atrium or right ventricle unless the tricuspid or pulmonary valves are involved, whilst lesions in the right ventricle are always less extensive than in the right atrium.

The pulmonary valvular plaque is deposited almost entirely on the arterial aspect of the pulmonic valves, sometimes contracting the intima of the pulmonary trunk, thereby causing infolding of the distal portion of the valvular cusp towards the sinus of the valve. On the tricuspid valve, the lesions are severest on the posterior and septal leaflets where the fibrous tissue is located predominantly on the ventricular aspects of the leaflets. These fibrous deposits cause thickening, inter-adherence and eventual

constriction of the pulmonic and tricuspid valves terminating in fixed valvular orifices. The pulmonary valve becomes predominantly stenotic whilst the tricuspid valve, despite the narrowing of its annulus, is usually fixed in a wide open position and its main functional derangement is incompetence with regurgitation. The reason for the different functional results of the same pathological process in these two valves is mainly determined by the size of the natural orifice. Thus in the pulmonary valve the natural orifice of which is initially small, the contracting process leads to stenosis whilst with the initially large tricuspid valve ring, although it becomes slightly contracted, stenosis is not the predominant lesion produced. Carcinoid plaques when present in the pulmonary artery are limited to the portion lining the sinus of the pulmonary valve or immediately above it.

The carcinoid plaques appear to be preferentially located in areas of excessive turbulence of blood or in the paths of regurgitant streams. Thus apart from the valvular lesions, the plaques are seen most commonly on the right atrial septum immediately above the junction of the septal and atrial valvular leaflets and in the atrial appendage. Right ventricular involvement is usually confined to the apices of the papillary muscles - "white capping" of the papillary muscles, due to extension down the chordae tendinae from the tricuspid valve. Rarely an associated right ventricular mural plaque may be present. It is consistent with the humoral theory that pronounced carcinoid changes occur where pressure and turbulence act for this would increase the influence of a given humoral factor as the greater turbulence makes for a closer contact between contents and surface. Also the fact that

the changes are mainly localized to the valves suggests that the humoral effects develop best on fibrous, poorly vascularized tissue. This poor vascularization of the affected fibrous tissue is further accentuated by the arteriolar constriction during flushing attacks, causing a prolonged route of diffusion and thus a protracted effect of a humoral agent.

Involvement of the left side though much less common than right sided lesions, probably occurs more commonly than is generally realised. Biventricular carcinoid heart lesions have been described with primary intestinal carcinoids in the absence of a right to left shunt (For references see page 65). In these cases, the mitral valve most commonly, followed by the left ventricle and finally the aorta are the seat of carcinoid plaques. However vascular changes in the coronaries, especially in predominantly right sided lesions, have been described only with simultaneous occurence of a patent foramen ovale and consists of hyaline thickening of its arteriolar walls.

Extension of the fibrous plaques to the systemic veins is not unusual and these plaques occur commonly on the intima of the superior and inferior vena-cavae, the innominate veins as well as the coronary sinus. Carcinoid fibrous plaques in these sites are attributed to contact with inferior vena-caval blood which has regurgitated through the incompetent tricuspid valve during ventricular systole.

Histopathology:

Carcinoid fibrous plaques are not intra-valvular, thickening of the valve cusps is due to the presence of a peculiar type of fibrous tissue devoid

of elastic fibres superimposed upon relatively intact endothelium. Both the valvular cusps and the mural endocardium remain normal and each is clearly separated from the fibrosing process by the internal elastic membrane. Indeed in formalin fixed hearts, the carcinoid plaques overlying atrial or ventricular endocardium can sometimes be neatly displaced or plucked from the underlying endocardium.

Histological examination shows valvular fibrosis generally without active inflammation, and after elastic staining, the increase in stroma is seen localized chiefly beneath the membrana elastica and only slightly superficial to this is the overlying intact endothelial lining. Within the superimposed atypical fibrous tissue, there is a metachromatic ground substance, a few spindle shaped cells (fibroblasts) blood vessels, oval or round spaces which frequently contain small hyperchromatic nuclei, some lymphocytes, plasmocytes and mast cells but no fat or fibrin. These oval spaces give the fibrous tissue a cartilage-like appearance (MacDonald et al 1957, Fischer et al 1958, Roberts et al 1964).

Contrasting Features of Carcinoid Heart Disease and E.M.F:

The pathological features of carcinoid heart disease have been described at length so that they may be compared with those of E.M.F. It is clear that the two processes are different. Even the endocardial lesions are different (Thomson, 1958; Roberts et al , 1964). In carcinoid heart disease the changes begin, as it were, on the endocardial surface; in E.M.F. the endocardial changes probably follow lesions which are sub-endocardial, beginning, perhaps, in the myocardium itself.

In E.M.F. myocardial lesions occur in the inner one third of its walls irrespective of the degree and localisation of the endocardial fibrosis, whereas myocardial lesions are not features of the carcinoid heart disease.

In the carcinoid heart disease, a peculiar type of fibrous tissue is superimposed upon relatively intact endocardium whilst the endocardial lesion of E.M.F. is essentially a destructive lesion, in which the normal endocardium is swept away and replaced by a dense fibrous scar. Whilst it is generally accepted the final picture of endomyocardial fibrosis is the result of organization of mural thrombi, the concensus of opinion is that the carcinoid fibrous plaques is caused by deposition of material on intact endothelium.

In E.M.F. the severest lesions are in the mural endocardium especially in the apices of the ventricles, valvular functional defects occur only as a result of spread from the mural endocardial plaques and are not seen where these valves are not in contact with the mural endocardium. On the contrary mural carcinoid plaques do not occur either in the atria or ventricles unless the valves are involved, and here indeed the severest lesions are seen. Thus mural carcinoid plaques occur only in paths of regurgitant and turbulent flow of blood consequent on the valvular lesions.

Whilst the delicate veiling of the endocardial inflow tract is the target in E.M.F., it is the coarse outflow tract where predominant lesions are seen in carcinoid heart disease. Lesions in the systemic veins, or coronaries as well as the great arteries are not seen in E.M.F. but are frequently associated with carcinoid heart disease, and these lesions as well as the endocardial plaques are to be found only where the carcinoid tumours

and metastases have a direct venous inflow. Finally Antemortem thrombosis, and embolic phenomena seen in endomyocardial fibrosis have not been described in carcinoid heart disease.

It is extremely unlikely that the grossly different pathological cardiac lesions in E.M.F. and carcinoid syndrome can be the result of a common aetiological factor. What is more, none of the diverse biological effects of 5-HT as seen in the carcinoid syndrome have been encountered in E.M.F. Constipation rather than diarrhoea is the after effect of a heavy plantain meal in Nigerians. Flushing and other vaso-motor features of carcinoidosis do not occur in E.M.F. Oliguria and bronchospasm if present in E.M.F., are seen only in the terminal stages and are the consequence of the cardiac lesion. This is in accordance with the observation that whilst 20mg. of serotonin may be taken orally without any effects whatsoever, as little as 1mg. injected intravenously to a 70 kilogram man produces marked systemic disturbances in normal individuals (Weekles et al 1958). In carcinoid syndrome, the 5-HT as it were is injected in large quantities directly into the blood stream from the carcinoid tumour, whilst in E.M.F. there are many obstacles to the ingress of 5-HT to the general circulation due to the rapid deaminative process in the tissues.

Humoral inter-relationships in the mediation of cardiac pathology:

The association between E.M.F. and 5-HT has been conditioned in part by the superficial similarities between E.M.F. and the carcinoid heart, and arising from this, by the established role of humoral agencies in the production of the lesions of the carcinoid heart. We have, however, just

seen that the pathological similarities between the two conditions are, in fact, spurious. Nevertheless this does not, in itself, completely discount the role of humoral factors. The theory that 5-HT may be an aetiological factor in the pathogenesis of E.M.F. has, of course, been further strengthened by the alleged correlation between the incidence of E.M.F. and the plantain eating habits of certain African groups. However, before considering the validity of this correlation, it is probably desirable to assess further the possible way in which 5-HT could act. The main situation in which it has been invoked is carcinoid heart disease. Even here its role as the sole mediator has been challenged and it is now obvious that the peripheral vascular effects manifested as flushing, at least may not entirely be due to hyperserotonaemia.

Since the early sixties, it has become increasingly obvious that that serotonin cannot be the only mediator in the carcinoid syndrome. Thus the levels of blood serotonin did not always correlate with the severity of clinical symptoms, and with a few exceptions, higher levels of serotonin or its metabolites in plasma or urine have not been demonstrated during flushing attacks than at control levels in carcinoid patients (Sjoerdsma et al 1956, 1964, Schneckloth et al 1959, Roberts et al 1962, Levine et al 1963, Nelson et al 1965). Thus severe flushing reactions have been observed in some carcinoid patients in the absence of elevated urinary 5-HIAA, the metabolic end product of 5-HT (Levine et al 1963, Sjoerdsma et al 1964). Also intravenous injections of

serotonin in normal subjects as well as in carcinoid patients did not consistently induce typical flushes and reactions associated with spontaneous flushing in the carcinoid patients (Roberts et al 1962, Levine et al 1963). Whilst chlorpromazine, (Sjoerdsma 1959) as well as Deserit in combination with No. 5-1025, a potent mona-amine oxidase inhibitor (Dubach et al 1963) have occasionally been useful in the symptomatic treatment of diarrhoea and flushing in some patients, most of the anti-serotonin drugs currently available although potent antagonists of 5-HT in vitro are completely ineffective in the treatment of patients (Sjoerdsma 1959, Schneckloth 1959, Dubach et al 1963). Even the potent 5-HTP decarboxylase inhibitors capable of reducing serotonin synthesis have not been useful in controlling flushing attacks (Oates et al 1964, Sjoerdsma et al 1964).

Bradykinin and the Carcinoid Syndrome:

The greatest challenge to 5-HT as the main mediator in the carcinoid syndrome came with the appearance of the kinins. Single intravenous injections of synthetic bradykinin (0.4 microgram per kilogram body weight) produced flushing reactions similar to the spontaneous flushes in carcinoid patients (Peart et al 1961). Also infusion of bradykinin into healthy individuals reproduced the vaso-motor symptoms seen in the carcinoid syndrome (Fox et al 1961, Bishop et al 1965). Oates et al (1964) demonstrated the appearance of kinin-like polypeptides in the hepatic venous effluent of carcinoid patients. And it has been shown that the kinin levels in the hepatic venous blood increased during flushing attacks (Oates et al 1964, Zeithn et al 1966). It is also claimed that a clear relationship was noted between the release of

kinins into the circulation and the onset and severity of flushes in some patients (Robertson et al 1962, Peart et al 1963).

A rise in bradykinin level of hepatic venous effluent is also associated with adrenaline and nor-adrenaline induced flushes in carcinoid patients (Oates et al 1964). Also during these adrenaline and nor-adrenaline induced flushing, arterial levels of tissue kallikrein, a kinin forming enzyme, rose from 0.03 to 0.2 frey units instead of the normal range of 0.03 to 0.04 (Melmon et al 1965). Adrenaline and nor-adrenaline have for a long time been shown to release kinin-forming enzymes in the submandibular salivary glands of cats. (Hilton et al 1956).

It is significant that whilst no kallikrein activity was detectable in liver tissue of patients without carcinoid tumours, high kallikrein values were obtained in the hepatic metastases of six carcinoid patients in whom flushing was the predominant symptom whilst the lowest levels occured in those carcinoid patients that did not manifest with symptoms of flushing. Also during a spontaneous flush, bradykinin was found to rise strikingly in the arterial plasma of some carcinoid patients but there was no alteration in the kinin level and no appearance of the peptide forming enzymes in normal subjects (Mason et al 1966).

Bradykinin is present in normal human plasma only in minute quantities but its precursor, kallinogen, an alpha$_2$ globulin formed in the liver is present in plasma in high concentrations. Lysyl-bradykinin is split off from kallinogen in plasma by the proteolytic enzyme kallikrein (Holdstock et al 1957), and lysyl-bradykinin or kallidin is then acted upon by the

amino-peptidases also present in plasma (Webster et al 1963) to convert it to bradykinin. The kallikreins from carcinoid tumours, like other tissue kallikreins were shown in vitro to liberate lysyl-bradykinin from its plasma substrate (Melmon et al 1965). Also incubation of fresh human plasma with lysyl-bradykinin resulted in the rapid formation of bradykinin (Oates et 1966). Indeed the kinins released by carcinoid tumours have been shown to be identical all in all respects with bradykinin (Oates et al 1966).

Thus carcinoid tumours possess multiple endocrine properties, secreting not only 5-HT, but bradykinin and occasionally histamine. Plasma values of bradykinin as high as 25 micrograms per 100 ml. have been obtained in hepatic venous effluent of some carcinoid patients during flushes, and it is claimed that those values are in the range of those required to produce peripheral vasodilation in man (Oates et al 1966). It is therefore suggested that the vaso-motor changes in the carcinoid syndrome are mediated by the release of vaso-active kinins into the circulation (Oates et al 1964, 1966, Melmon et al 1965). Indeed bradykinin like 5-HT can account for most of the manifestations of the carcinoid syndrome. Thus bradykinin produces vasodilation, bronchiolar constriction and is a known stimulant of gastro-intestinal smooth muscle (Bjoerdsma et al 1964, Mason et al 1965). It is thus possible that this substance may be important in the pathogenesis of asthma and diarrhoea both of which are clinical features of the carcinoid syndrome (Mason et al 1966). This vaso-dilatory action of bradykinin is consistent with hypotension accompanying flushing attacks in some patients as well as with the high output failure characteristic of the syndrome, probably consequent on the chronic reduction of peripheral vascular resistance.

Holdstock et al (1957) had earlier predicted the inflammation producing potentialities of bradykinin. Holdstock and his co-workers thus remarked "The ability of these polypeptides to evoke pain, to enhance capillary permeability and to cause vasodilation would make them extremely effective mediators of inflamatory reactions though definite evidence of their contribution to any type of injury reaction has not yet been presented." Here the matter rested for almost fifteen years before bradykinin could be linked with any particular inflammatory response. It is therefore not unexpected or surprising that with the finding that carcinoid tumours also produce large quantities of these highly vaso-active polypeptides, the endocardial lesions in the carcinoid syndrome have also been attributed to bradykinin action. It is postulated that bradykinin alters the endothelial permeability with initiation of inflammatory processes to which a proliferative overgrowth of fibrous tissue occurs. Also in consonance with the predominantly right sided carcinoid endocardial plaques is the finding that there is more then a ten-fold difference in levels of bradykinin in right heart blood as compared to femoral arterial blood levels (Fadell et al 1966). Also it had been observed that the evidence of valvular involvement in the syndrome appeared to correlate with the kinin forming ability of the carcinoid tumours (Oates et al 1964).

5-HT/Bradykinin Synergistic Action:

Some carcinoid patients have however been described who did not liberate any appreciable amount of bradykinin into the hepatic venous blood during hypotensive flushing attacks. Indeed it would seem that in those

cases where bradykinin release is not demonstrable, 5-HT may be the sole mediator (Oates et al 1966). However where both substances are released, the flush reaction may be the result of the combined action of 5-HT and bradykinin at their various receptor sites (Vane 1960). Though 5-HT may not be the sole mediator of the paroxysms of vasodilatation seen in the carcinoid flush, neither is bradykinin the universal dilator in this syndrome (Oates et al 1966). Whilst bradykinin release may in fact be responsible for the actual timing of the flush, it is pertinent to stress that bradykinin release takes place in an organism already flooded with 5-HT. Thus it is likely that the carcinoid flush is due to synergism between bradykinin and 5-HT. Indeed such synergism has been evoked in the pathogenesis of migraineous headaches and has also been shown to mediate venular constrictor action on rats mesentery (Sandler 1967).

It is also known that in the carcinoid syndrome, serotonin production is so great that it results in a profound disturbance of tryptophan metabolism. In these carcinoid patients, the serotonin pathway may take as much as 60% of the total tryptophan intake as compared to only 1% in normal individuals. It is believed therefore that pellagra-like skin lesions occasionally seen in this syndrome may be accounted for by the loss of nutrients from the intestinal tract due to diarrhoea, in combination with diminution in niacin production resulting from the abnormality of tryptophan metabolism (Sjoerdsma et al 1956, 1957).

Thus most of the manifestations of the carcinoid syndrome can no longer be explained only on the elevated blood 5-HT levels found in

these patients. 5-HT, bradykinin, as well as abnormal tryptophan metabolism with associated niacin deficiency are involved in the pathogenesis of this peculiar and apparently complex syndrome.

Metabolism of 5-HT in E.M.F. Patients:

If the role of 5-HT in the pathogenesis of carcinoid heart lesions is inconclusive, then its part as an aetiological factor in the production of endomyocardial fibrosis is even more doubtful. The daily basal urinary 5-HIAA excretion in E.M.F. patients is less than that of healthy control Nigerians and Europeans. This has been shown to result from the correspondingly lower serum 5-HT levels in these patients as compared to normal Nigerians. Even though there is a lower excretion rate of 5-HIAA after plantain ingestion, the total percentage recovery of exogenous 5-HT as 5-HIAA is similar in both normal Nigerians and E.M.F. patients.

If there were impairment of 5-HT deamination especially associated with increased ingestion, it might be expected that serum 5-HT levels would be raised in E.M.F. patients. However serum 5-HT determinations showed that serum levels were significantly lower in E.M.F. patients than in healthy Nigerian control subjects. There were only negligible increases after plantain ingestion. This failure to detect any significant demonstrable rise in serum 5-HT levels despite elevated 5-HIAA excretions after plantain meals would suggest that in E.M.F. patients as in normal individuals, deamination of 5-HT to 5-HIAA is fairly rapid. It would therefore seem that the low basal serum 5-HT levels in E.M.F. patients may be due to diminished endogenous production of 5-HT. It is significant in this respect that

vitamin B6 or pyridoxine phosphate deficiency has been established in some Nigerians with tropical neuropathic ataxic syndrome (Oshuntokun and Monikoso 1967). Even though this clinical condition has not been an associated feature of E.M.F. patients, and a-vitaminosis B does not appear to play any role in these patients (Ball et al 1954). Pyridoxine phosphate deficiency, a coenzyme of 5-hydroxytryptophan decarboxylase will adequately explain the diminished endogenous production of 5-HT. This may be further aggravated by a probable dietary tryptophan deficiency since protein malnutrition is a common feature in endemic areas.

E.M.F. and Plantain as Dietary Staple:

It is clear that though high serum 5-HT values are implicated in the causation of endocardial carcinoid heart disease, this is not so in the pathogenesis of endomyocardial fibrosis. 5-HT is not a common denominator in both diseases. Moreover, detailed questionaires presented to these patients did not confirm the idea that they habitually consumed bananas and plantain. Indeed, the practice of plantain ingestion is commoner only amongst the more highly placed Nigerians, for in the urban areas at least, this article of food is relatively expensive. Whereas it is not unusual for "better" class Nigerians to have plantain as a full meal, illiterates and low income earners would rather resort to the cheaper, more easily available and bulkier cassava diet (Garri, Eba or Lafun). Reports from Uganda also confirm that the wealthier Baganda people amongst whom E.M.F. is less common in fact eat more plantain than the immigrant labourers from Rwanda-Burundi in whom E.M.F. has been more frequently described (Shaper and Coles 1965).

Williams (1967) declared that it is not absolutely true that E.M.F. and the plantain diet share a common geographical distribution. He went further to assert that the incidence of E.M.F. in the plantain eating areas does not correlate with the plantain eating habits of the people. Even in carcinoid heart disease, a prolonged exposure to 5-HT is required before endocardial lesions occur, therefore the reported cases of E.M.F. in young children around the ages of five (Mokolo 1955, Parry and Abrahams 1965) does not seem in consonance with the theory that plantain ingestion is an aetiological factor in E.M.F.

Indeed, plantain growing and its ingestion are not the only operative factors in the tropical rain forests of the endemic areas of Africa. Parry and Abrahams (1965) stressed the importance of the soil on which this heart disease seems to thrive. In these areas, anaemia is usual, intestinal parasitism rampant, bacterial and protozoal infections are widespread whilst protein malnutrition is rife especially in infants and young children. These workers therefore concluded that in the presence of persistent chronic ill health of this magnitude, it is unlikely that the myocardium could remain unaffected and healthy. It has also been stated that whatever the initiating factor in E.M.F., a state of increased susceptibility is associated with extremely poor socio-economic conditions and that a degree of protection is conferred by a dietary and social background not far different from "Western" standards (Shaper and Colas 1965). The observation made by Mackie (1962) apparently contradictory to the above is indeed extremely pertinent. He emphasized that the selective geographical

distribution of E.M.P. in West, Central and East Africa and its apparent rarity in South Africa, South East Africa, Kenya, and the Caribbean, (and here the author adds to his list, India, Burma and other Asian countries) parts of the world which share with Nigeria the characteristic stigmata of under-development, protein malnutrition, parasitism, and chronic anaemia is extremely significant, and may be important in the ultimate discovery of an aetiological agent.

Fatal Atypical Carditis in Young Nigerians:

A few unexplained cases of acute pancarditis have been seen in young children and adolescents at Ibadan. These lesions bore no resemblance to acute rheumatic carditis but simulated the initial lesions, earlier described by Nwokolo (1962) and subsequently by Parry and A brahams (1965). Parry and Abrahams (1965) stated that endomyocardial fibrosis starts with an initial illness, summarized by them as "a carditis with unusual symptoms." "The early symptoms of E.M.P. are suggestive of a systemic disease and this might be infective" (Parry 1964). If infection is the cause of endomyocardial fibrosis, the infecting organism has so far alluded detection. Perhaps with improvement of medical services especially at village level, many more of these cases of acute carditis will be encountered. A more intensive clinical investigation of such cases, coupled with a detailed study of their distribution as well as the environmental and other factors that may be operative in these patients, may yield the answer to the pathogenesis of this condition.

Conclusion:

Van Der Geld (1966) observed that the hypothesis that serotonin is

instrumental in the pathogenesis of endomyocardial fibrosis rested on highly tenuous grounds. This association has stemed mainly from the following false premises. Firstly the controversial role of 5-HT in causing endocardial damage in carcinoid heart disease. Secondly, the spurious similarity between heart lesions in E.M.F. and carcinoid syndrome. Finally that chronically high levels of serum 5-HT occur in the plantain eating inhabitants of the 'endemic areas.' It is now quite clear, that carcinoid heart disease and E.M.F. are different clinico-pathological entities. High serum 5-HT levels do not occur in E.M.F. patients or in normal individuals after a meal of plantain. If there is impairment of serotonin metabolism in E.M.F. patients, as postulated by Crawford (1963), it is rather that endogenous production of 5-HT is low, than that the enzyme systems cannot cope with deamination of normal production of 5-HT. The theory that 5-HT may be an aetiological factor in the pathogenesis of endomyocardial fibrosis emanated from scientific conjecture and mere speculation and should now be discarded in the face of overwhelming experimental and clinical data.

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