A STUDY IN BONE MARROW CHANGES FOUND IN SEVERE ANAEMIA COMMONLY ASSOCIATED WITH ANKYLOSTOMIASIS.

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Preface.

This investigation was undertaken in Tanga Hospital, Tanganyika, East Africa, in 1939, and a condensed version of the results was published in the East African Medical Journal in May, 1944. The lapse of time between the field work and the publication of the results in this thesis was mainly due to the fact that the writer was stationed in out-districts of Tanganyika and had only a very limited personal reference library. However, this lapse of time gave him ample and beneficial opportunity to study the blood diseases of the African native and to convince himself that many of the so-called hypochromic anaemias found in East Africa did not so conveniently fit into the classical picture of iron deficiency anaemia but were more complicated in their etiology than was generally accepted by those practising medicine in East Africa.

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Introduction and Aim of this Investigation.

Anaemia is exceedingly common among the natives of Tanganyika of all ages and occupations, but it is especially prevalent among the lower paid employees and in the coastal belt. It is an everyday occurrence to find varying numbers of natives, men, women and children, waiting patiently before the dispensary window for their dose of iron in the form of "Mist Tonic"; while, in the general wards one is sure to come across a number of cases labelled "Hookworm (or Ankylostomiasis) and Anaemia" and to see on the body of the case sheet the words Hb % = 30% (or 35% or 40%). In these latter cases, as a general rule, the diagnosis is then regarded as complete, and it now remains only for the patient to receive his daily doses of iron and his anthelmintic, and to be discharged in 2-3 weeks when his Hb % has risen to 60 or 70%, with instructions to attend the out-patient department for further iron treatment. He seldom obeys the instructions. More usually he drifts away to his work, far from fit, but not ill enough for admission to or retention in hospital.

This is only one aspect of what constitutes, in East Africa, a major public health and economic problem, viz., the prevalence of so much negative health. Ankylostomiasis represents only one of many causes which include, among others, malaria, bilharzia, malnutrition and nutritional deficiencies, chronic infections, scurvy and so on. When one considers the conditions under which the 5,000,000 native inhabitants of Tanganyika live, it is scarcely surprising that the general level of health is so low. The territory is so vast, the population so dispersed except

in the main towns of Dar-es-Salaam, Tanga, Moshi, Tabora and Mwanza, the native huts so primitive and unhygienic, the diet so deficient in balance and the medical facilities so few that the natives almost inevitably become a prey to so much disease. An onlooker watching a gang of perhaps 100 labourers working on a road outside Tanga would be immediately struck by their thin physique, lack of spirit, lassitude, dull indifference and lack of staying power. The picture painted above is black, but Trowell (1946)[1] paints it even blacker - "It is my considered opinion that few Africans have from childhood received an adequate diet, and that this is reflected in thin, weak adults, incapable of hard physical and mental exertion, poor workers, drawing a poor wage, prone to infections and liable to early death."

Generally speaking, the East African native is accustomed to one large meal per day, a custom which finds ready acceptance by many of the employers of labour since it greatly simplifies any ration issue made by them. On the coastal belt and, in fact, in most parts of East Africa, sound dietetic principles are unknown, and the native contents himself by assuaging his hunger when he is able. This he does by consuming enormous platefuls of cooked maize meal or cassava (the latter the name given to the farinaceous root stalks of two species of euphorbiaceous plants, bitter and sweet cassava, which after preparation by drying to remove hydrocyanic ā and subsequent grinding are cooked in bulk). A native may eat at one sitting as much as can be contained in a 7" - 8" diameter bowl. This extraordinarily unappetising mass of food is occasionally accompanied by a handful of beans cooked in water and

a little salt. Cassava meal can properly be called the poor man's staple in East Africa. Trowell & Muwazi (1945)③ working in Uganda give this analysis -

(a) Poor Labourer's Diet (Daily)

4 lbs. cassava (reduced to 3 lbs. when cooked) = 1,750 calories and contains 10 gms. protein; no fat; 0.5 gms. calcium; 4 mgms. iron; no vitamin A & B; no riboflavin or nicotinic acid; 450 mgms. vitamin C (reduced by cooking)

(b) Immigrant Labourer's Diet (i.e., natives entering Uganda mostly from Ruanda and Urundi in the Belgian Congo)

5 lbs. sweet potatoes (3¾ lbs. after preparation) plus ½ oz. meat plus ½ oz. green vegetables = 2,000 calories and contains 25 gms. protein; 1 gm. fat; 0.25 gms. calcium; 8 mgms. iron; 600 units vitamin A; 1.0 mgm. vitamin B; 2.4 mgms. riboflavin; 1 mgm. nicotinic ā; 420 mgms. vitamin C (reduced by cooking).

(c) Suggested Minimum Diet for an African doing Moderate Work

2,600 - 3,000 calories	
60-70 gms. protein	
calcium	0.8 gms.
iron	11 mgms.
vitamin A	5,000 units
vitamin B	1.8 mgms.
riboflavin	2.2 mgms.
nicotinic ā	15 mgms.
vitamin C	75 mgms.

The above analyses (a) and (b) approximately obtain in many parts of Tanganyika and strongly illustrate the poverty of the poor man's diet. Indeed, it is astonishing how he manages to survive far less engage in work. In the writer's opinion, this can be explained by the fact that occasional extras find their way into the diet, such as meat and fish, bananas, tomatoes, rice and native spinach, and by the fact that long years (since childhood) of subminimal feeding have accustomed the body to subsist on less than normal rations. Conditions on large estates

employing native labour appeared on the face of things to be better for here the native was given a daily ration, to be cooked by himself, of 24-28 ozs. maize meal, 4 ozs. beans, a small quantity of salt and red palm oil (a rich source of vitamin A), with a twice weekly issue of meat, approximately ½ lb. However, this issue was usually only made to the worker if he turned up each day for his task and did not take into account any wife and family which he might possess: and, as his wages were normally in the region of 9/- to 12/- per month, from which was deducted a poll tax amounting to 9d. to 1/- per month, it becomes more and more obvious that the native and his family had to struggle to feed themselves adequately. Furthermore, when recruiting for the Forces began in Tanganyika in 1939, it became very soon evident that the physique of many African tribes fell well below standard and in some districts rejection rates amounted to as high as 95 per cent. When a recruit did pass for the army, his physique improved out of all recognition in a large number of cases, following a period of army rations.

This, then, is the background upon which this study of bone marrow changes in the severe anaemia associated with ankylostomiasis is built, and the aim of this investigation is to indicate the type of bone marrow picture which may be found and to suggest that the existence of ankylostomiasis infestation is not, in itself, the main aetiological factor but rather is the "last straw which breaks the camel's back." From the study of 16 cases which form the main basis of this thesis together with the writer's observations over a period of seven years in East Africa his impressions and conclusions have been culled.

Considerably more than 16 sternal punctures have been performed during the past seven years. Some 40 alone have been made on the anaemia associated with ankylostomiasis, but, apart from the 16 presented, the remainder have not been included in the present series for two main reasons, either because they have not been considered as sufficiently illustrative of the main argument of this thesis or because some portion of the investigation has been excluded, such as the omission of differential blood counts or of reticulocyte counts. When it happened that some part of the investigation had been omitted, it was usually because the anaemia was not considered severe enough, as judged by clinical findings and by a Sahli haemoglobinometer reading of over 50% to justify a more detailed examination.

The present series of cases, 16 in all, were drawn from various tribes either resident in Tanga, or on adjacent sisal estates, or from neighbouring villages, and were treated in Tanga Hospital in 1939. All the patients were male adults between the ages of 20-60 years, the majority reputedly single or living alone. They belonged to the poorer labouring or peasant class whose economic and social conditions were notoriously bad. Clinically, they presented, in varying degree, the characteristic features associated with severe anaemia, viz., apathy and lassitude, dyspnoea, anorexia, oedema of the legs and/or feet, puffiness of eyelids and face, pallor of the mucosae, haemic murmurs, enlargement of the heart, tachycardia, petechial haemorrhages, albuminuria and others. Diarrhoea was not noted nor was constipation a marked feature, while the stools presented none of the features associated

with sprue. The clinical features suggestive of beri beri arising from vitamin B deficiency (neurological changes) and of frank pellagra resulting from vitamin B complex deficiency (characteristic dermatitis, gastric and intestinal upsets or confusional states) were absent. In all cases the hair presented a dry, soft, lifeless appearance and the skin exhibited a dryness and lack of oiliness which was easily demonstrable by rubbing the forearm gently, thus producing a greyish white powdery layer composed of the superficial cells of the epidermis. The resulting appearance rather resembled a thin layer of white flour on black cloth. The skin over the tibiae in most cases presented a tightly drawn brownish shining look, devoid of subcutaneous fat, curiously unhealthy looking, like the type of skin in which one would readily expect an ulcer to develop easily. The tongue was usually heavily coated and pale, and the gum margins and bases of the teeth heavily coated with a dirty-greyish, porridge like deposit. Obvious fissuring at the angles of mouth and other sites was not noted. In four cases there were definite signs of vitamin A deficiency (dry horny skin with follicular keratitis, especially at the elbow joints - night blindness was not tested). The characteristic eggs of ankylostomiasis were readily demonstrable on straight examinations of faeces and no recourse was necessary to "flotation" methods or other means of concentration. In addition, other pathological conditions noted included pyorrhoea alveolaris and dental caries (eight cases), splenomegaly and hepatomegaly combined (three cases), hepatomegaly without palpable splenomegaly (one case), elephantiasis of legs and/or scrotum

(two cases) and mossy foot (one case). Table No. II summarises the individual cases with reference to their peripheral blood pictures and associated pathological conditions when present. It is interesting to record in passing that all cases came to hospital on foot, even case No. 19 being able to struggle along supported by sticks.

Technique

The following procedure was adopted for each patient. On admission to hospital, and before treatment was begun, each patient was taken to the operating theatre. Peripheral red and white cell counts were made using the Thoma haemocytometer and the usual diluting fluids*. Estimations of haemoglobin percentages were carried out using Sahli's acid haematin method and a new recently tested haemoglobinometer, and allowing the HCl to remain in contact with the freshly drawn blood for approximately 30 minutes before dilution. The final readings were given in gms. of Hb.% according to the equation 14.5 gms. Hb. = 105% Haldane = 85% Sahli. Slides were also prepared for differential W.B.C. counts in addition to supravital brilliant cresyl blue films for reticulocyte counts. The latter films were prepared using 0.3% brilliant cresyl blue in absolute alcohol, a drop of the dye being placed on two coverslips, to one of which a drop of blood was added and

*Diluting Fluid for R.B.Cs.

Sodium chloride	0.6 per cent.
Sodium citrate	1.0 ,, ,,
Formalin	1.0 ,, ,,
Distilled water	97.4 ,, ,,

Diluting Fluid for W.B.Cs.

Glacial acetic a ...	1.5 c.cm.
1% soln. of Gentian Violet in water ...	1.0 c.cm.
Distilled water	98.0 c.cm.

allowed to come into contact with the other prepared coverslip. When the drop of blood had spread between the two coverslips, they were carefully slid apart in opposite directions. The percentage number of reticulocytes to R.B.Cs was then calculated microscopically. The Cooke count (an adaptation by Cooke (1930)[3] of the original Arneth count) was performed on the nuclei of the polymorphonuclear cells in a few of the earlier cases, but was discontinued as it did not give any more information than could be obtained from peripheral and marrow blood examinations (for reference see under Peripheral Blood Findings).

When the above procedure was completed, 1-2 c.c. of 2% novocaine was infiltrated into the skin, subcutaneous tissue and periosteum over the sternomanubrial junction opposite the upper border of the second costal cartilage at its junction with the sternum. A stout, dry lumbar puncture needle sterilised by boiling was now used in preference to the more solidly built and more popular sternal puncture needle which Messrs. Allen & Hanbury have modified from that devised by Salah (1934)[4]. The lumber puncture needle was introduced into the site at an angle of approximately 60° and, when it was felt to have entered the sternomanubrial junction, it was depressed to an angle of some 15° and pushed home into the sternal bone marrow. Very little discomfort was felt by the patients, and the only difficulty, in my experience, in a large number of cases, has been in old patients with some degree of calcification of the manubrial cartilage. This complication has resulted twice only in some 50 cases resulting in failure to reach the marrow with the lumbar puncture needle. What little discomfort

was experienced by patients did occur when the marrow fluid was being withdrawn through the needle into a dry sterile 1-2 c.c. syringe. The amount withdrawn was, usually, approximately 0.25 c.c., and the fluid was then immediately spread on specially prepared fat-free slides either dry or containing supravital Janus green and neutral red. For the latter, freshly prepared saturated solutions of Janus green and neutral red chloride (both Grübler's stains) in absolute ethyl alcohol were used in the proportions Janus green 13-15 drops to neutral red 0.4 c.cm. in 10 c.cm. absolute ethyl alcohol, following the method of Sabin.[6] The correct proportions of Janus green and neutral red were difficult to obtain and were only reached by trial and error. The slides were covered with coverslips, ringed with vaseline to exclude air, and then placed in an incubator at 37°C. for 20-30 minutes and thereafter examined. Numerous failures were encountered. Sometimes the mitochondria showed too faintly, at other times the cells died rapidly, and, at others, aggregates of cells spoiled even staining. Eventually, a small number of reasonably satisfactory films were obtained and these served to confirm the findings of the ordinary straight films stained by Leishman's stain.

From the marrow fluid placed on the dry unstained slides, as thin films as possible were prepared in the same way as ordinary R.B.C. thin films are prepared. In each case, not less than six marrow films were made and stained with Leishman's stain. Usually little difficulty was experienced in spreading the films directly from the marrow, although on a few occasions some films

had to be rejected because of uneven distribution, which gave an appearance to the naked eye of numerous lacunae in an otherwise homogeneous setting of the dried marrow fluid.

In all cases, between 400 and 800 cells were counted on each Leishman's stained slide and classified differentially, using the terminology outlined below. To ensure greater accuracy, further counts were made from different marrow films in each individual case and a final average cell count taken. The cells of the myeloid and erythroid series were taken together to total 100%. This procedure excluded smear cells, degenerated cells of uncertain origin and isolated nuclei.

Following the above technique, the patient was returned to the wards and placed on a diet consisting of maize meal, ground nuts, red palm oil, milk, meat, and ferri et ammon. cit gr. xxx t.i.d. In no cases was blood transfusion performed. When the Hb. % had risen to 50-60% the ankylostomiasis infestation was treated with carbon tetrachloride and oil of chenopodium.

Terminology

A. Erythropoiesis (a) Normal

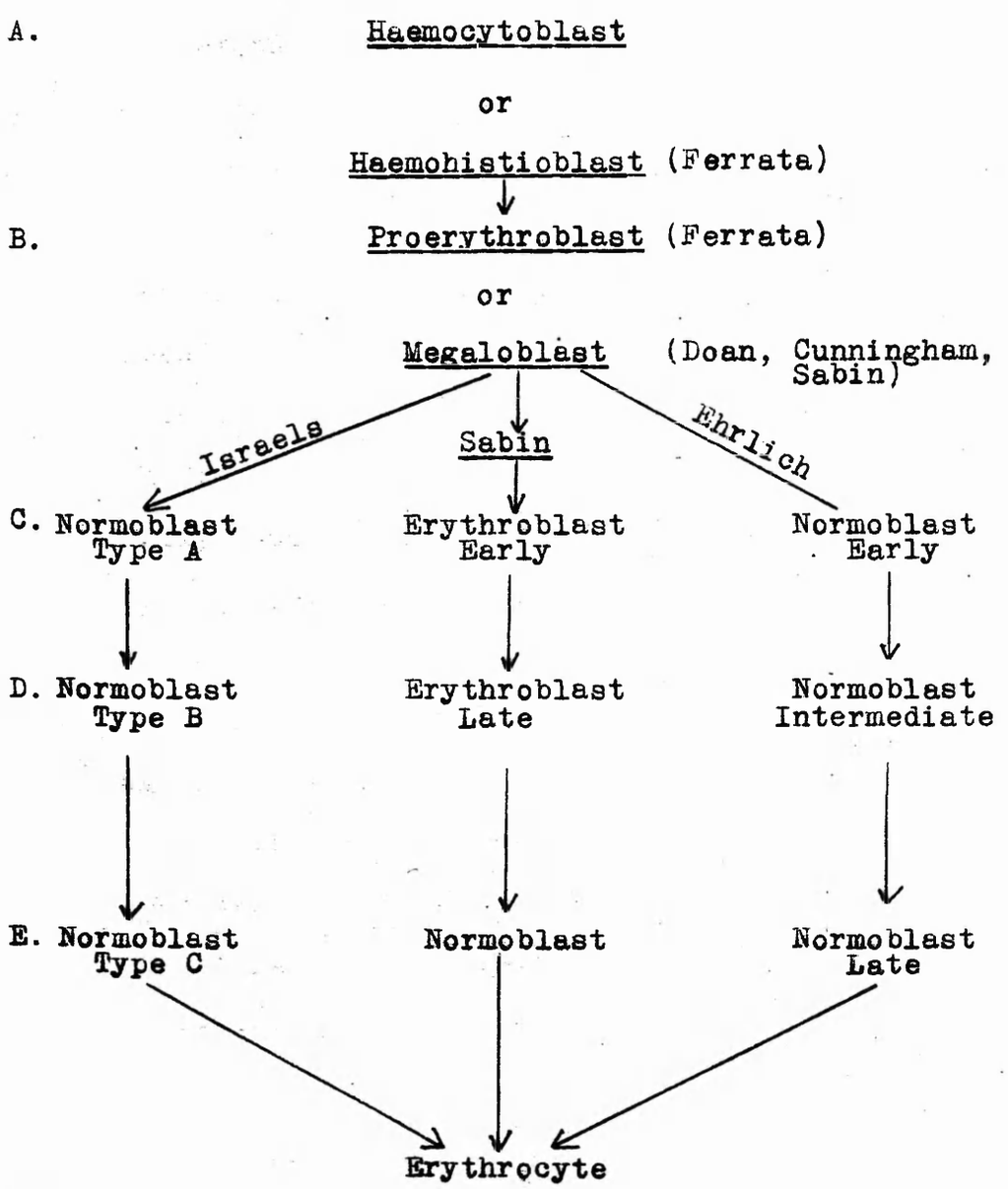
Considerable confusion exists with regard to nomenclature, a confusion largely contributed to by the inclusion in the terminology of both abnormal and embryonic cells which are not found in the normal process of blood development. The term "megaloblast", to quote one example, was originally used by Ehrlich to describe the large, primitive, embryonic, nucleated red cell found in megaloblastic erythropoiesis (pernicious anaemia, sprue, etc.). Its original definition has, unfortunately, become obscured by

its extension to include the earliest differentiated nucleated red cell found in the normal marrow (Doan, Cunningham, Sabin, 1925)[6]. To obviate this conflict of definitions, the term pro-erythroblast was suggested by Ferrata for the normal early precursor of the erythrocyte to distinguish it from the megaloblast, the use of which term to be confined to the early abnormal pathological cell found in megaloblastic erythropoiesis. Unanimity also is lacking in the terms used to describe the later stages in the normally developing red cell in the bone marrow. In an endeavour to simplify the interpretation of the findings of the blood examinations of this thesis, the following table is designed to give a description of the various stages of normal blood erythropoiesis together with the equivalent terms used by various authors. This table has been adopted principally from Whitby & Britton (1946)[7] and the terminology used by the writer follows the haemocytoblast ⟶ proerythroblast ⟶ normoblast, early, intermediate and late ⟶ erythrocyte nomenclature.

Table I

Description of the Various Stages in Normal Erythropoiesis with Terminology in Use by Various Authors

Nomenclature of Developing Red Cell used by Various Authors



Description of Developing Red Cell at Various Stages

A. This large primitive cell 18-23μ is believed to be the reticulo-endothelial precursor of erythrocytes and leucocytes. The nucleus occupies most of the cell, has a distinct cell membrane, a fine reticular chromatin network with well marked nucleoli. The cytoplasm, deeply basophilic, is reduced to a thin rim.

B. Derived from the haemocytoblast. Measures 14-19μ with a deeply basophilic non-granular cytoplasm. The nucleus occupies the greater part of the cell and nucleoli are usually distinct. The reticulum of fine threads of chromatin presents a delicate mosaic appearance, and the nucleus as a whole stains palely eosinophilic when contrasted with later normoblasts. The cell is difficult to distinguish from earlier megaloblasts and stains rather more pronouncedly than does the myeloblast.

C. Measures 11-17μ. Cytoplasm still densely basophilic. Nucleus still occupies greater part of the cell but the chromatin tends to be less reticulate and to clump into a coarser pattern. Nucleoli are lost. This cell multiplies by karyo kinetic division.

D. Measures 10-14μ. Nucleus is smaller. The chromatin material is more condensed and the cytoplasm less densely basophilic than its predecessor. The nucleus may present a cartwheel appearance as a result of chromatin condensation. It is at this stage in the normal development of the erythrocyte that the cytoplasm begins to acquire haemoglobin. No further cell division occurs, the next stage resulting from further maturation.

E. Measures 7-10μ. Product of maturation of previous stage. Cytoplasm eosinophilic of moderate to full intensity. Nucleus becomes steadily denser and smaller until it stains uniformly dark - is frequently eccentric and can often be seen in the process of extrusion from the cell or, less frequently, in a state of fragmentation prior to extension. The cell outline as a whole is frequently irregular.

F. No description is required.

A. Erythropoiesis (b) Abnormal.

For the purpose of this thesis and the interpretation of the blood findings contained in it, two aspects of abnormal erythropoiesis may be conveniently and shortly indicated -

(i) Changes observed in bone marrow and peripheral blood resulting from iron deficiency in the body;

(ii) Changes observed in bone marrow and peripheral blood resulting from a deficiency of the haemopoietic principle in the body.

(i) As in any other system of the body, any simple increased demand on the blood-forming mechanism (e.g., in haemorrhage) gives rise to a simple hyperplasia. In the bone marrow, as a result, there is a marked increase of haemopoietic tissue, varying in degree in accordance with the demands for new blood. The process of blood formation and the production of red cells becomes speeded up, the number of normoblasts, early, intermediate and late becomes increased, and the process of haemoglobinisation shifts backwards towards an earlier stage of the developing red cell, usually the intermediate normoblast. Under conditions of iron deficiency, to the simple hyperplasia is added the extra factor of lack of iron necessary for the formation of haemoglobin. Therefore, in addition to the increased production of red cells as in normal hyperplasia, there is evidence of the struggle going on within the blood-forming mechanism to utilise the depleted haematinic reserves of the body. Variations in the size and shape of the red cells become apparent; the normoblasts are greatly increased and frequently distorted, and their degree of haemoglobinisation variable, micro- and macro-normoblasts being relatively common.

(ii) When the haemopoietic principle is deficient in the body, erythropoiesis begins to become abnormal at the stage between pro-erythroblast and early normoblast, giving rise to megaloblasts, early, intermediate and late, and ultimately to macrocytes. The stages of megaloblastic development correspond with the stages of the normoblastic series. In the presence of adequate supplies of iron, haemoglobinisation in megaloblasts moves backwards towards the pro-erythroblastic stage in varying degrees according to the severity of the deficiency. Descriptions of the individual cells of the megaloblast series have been made (Whitby & Britton, 1946, and others[8]), but it seems to the writer that one cannot adhere too rigidly to such descriptions (around which controversy still exists), as they tend to divorce one's attention from the fact that megaloblasts are simply normoblasts "gone wrong", and if one retains a sound picture of the cells of normal erythropoiesis there should be, when one views the complete blood findings as a whole, a clear conception of the direction of abnormal erythropoiesis. Indeed, in this connection the plea for a sense of proportion made by Davidson, Davis & Innes (1942)[9] is worthwhile quoting - "The heat engendered by discussions on cytological minutiae should not be allowed to obscure a clear appreciation of what is and what is not important in its practical application to diagnosis and treatment of diseases of the blood." Generally speaking, megaloblasts are larger on the whole, their nuclei are younger and less mature, and their cytoplasm more haemoglobinised than the corresponding normoblasts. Perhaps the easiest cell of the megaloblastic series

to distinguish, and one which readily gives a clue to the marrow picture, is the late megaloblast. In this cell the cytoplasm is more or less fully haemoglobinised, the nucleus is larger and the chromatin less dense and more irregular than the corresponding late normoblast.

These points, plus the larger size of the cell, render differentiation relatively easy. When, as in this series of cases, combinations of deficiencies of iron and haemopoietic principle occur, the identification of the cells of the prevailing haemopoiesis becomes complicated. It may, then, become extremely difficult to distinguish individual cells, a fact readily admitted by Davidson, Davis & Innes (1942)[10], Damesheck & Valentine (1937)[11], Scott (1939)[12], and others. In this thesis, no attempt has been made to classify, separately and quantitatively, the megaloblasts from the normoblasts because the writer felt that to do so would be to introduce more of an element of guesswork than of accuracy. However, the legend does contain descriptions of the types of cell seen, whenever such descriptions can be made, as well as observations on the prevailing blood pictures.

Terminology.

B. Leucopoiesis.

The most prevalent opinion regarding the origin of the granular leucocyte is that it is derived from the primitive haemocytoblast (the Monophyletic Theory) which likewise gives rise to the erythropoietic series, the lymphoblast and the monoblast. In the granular leucocyte series the various stages recognised are the myeloblast, myelocyte and granulocyte. In this thesis the

myelocyte is further subdivided into premyelocyte, myelocyte, and metamyelocyte, in accordance with the usage of Ferrata and others and corresponding fairly closely with the Types A, B and C of Doan, Cunningham & Sabin.(13)

Myeloblasts. These cells measure 11-20μ, are round or oval in shape, and have a nucleus which occupies the greater part of the cell. The cytoplasm is non-granular and, when stained supravitally, has numerous mitochondria pinpointed throughout. When Leishman's stain is used the cytoplasm of the myeloblast is less darkly basophilic than the pro-erythroblast. The nucleus is finely reticulated and has not a clearly defined membrane. The nucleoli are indistinct gaps in the chromatin network.

Premyelocytes. These cells vary considerably in size, some being as large as 20μ. Earlier forms usually have a slate blue cytoplasm and a round, oval or slightly indented nucleus. Strictly speaking, there is no sharp definition between premyelocyte and myelocyte, the former merging gradually into the latter. However, it is thought to be convenient to indicate the relative ages of the myelocytes by these subdivisions. As the premyelocyte matures, the cytoplasm shows a progressively deepending eosin, tingeing usually commencing in the crook of the indented nucleus or, at least, in the less convex side of the nucleus and gradually spreading throughout the cytoplasm as the cell merges into the myelocyte class. Pari passu with the gradual alteration in colour of the cytoplasm, reddish granules begin to appear, in my experience, initially at the periphery of the premyelocyte and, very frequently, in that portion of the cytoplasm which lies between the more convex side of the

nucleus and the limiting membrane of the cell wall, i.e., in the narrowest portion of the cytoplasm. Occasionally, small vacuoles may be seen in the cytoplasm, but their significance is not understood.

Myelocytes. These cells are usually between 11-16μ in size, but considerable variation in size may be encountered. The cytoplasm is pinkish in colour, although still retaining a variable amount of bluish tingeing, and contains numerous granules which can be differentiated according to their staining reactions, thus indicating whether the myelocyte will develop into the mature neutrophil, eosinophil or basophil granular leucocyte. The nucleus is large, occupying approximately one-half of the cell, and is usually eccentrically placed. No nucleoli are to be seen in the nucleus, which is round, oval or slightly indented.

Metamyelocytes. These represent the penultimate stage in the development of the mature granular leucocytes. The nucleus is still immature, usually indented, and less deeply staining than the mature leucocyte. The granules in the cytoplasm clearly indicate the final destination of the developing cell whether neutrophil, basophil or eosinophil. The metamyelocyte is, on the average, slightly larger than the mature adult cell.

Granulocytes. Neutrophil, eosinophil, basophil granulocytes are too well known to warrant any description here.

C. Lymphocytes (large and small) and monocytes are too well-known and need not be described here. Megakaryocytes with their numerous nuclei and large size are unmistakable, and can occasionally be seen in marrow smears. No attempt has been made to

distinguish myeloblasts from lymphoblasts and monoblasts. In any case, the latter two types of primitive cells are usually only seen in the acute leukaemias of lymphatic and monocytic types. Most marrow smears, after identification of cells, have a residue which consists of isolated nuclei, smear cells, basket cells and cellular debris. This residue has been excluded in the differential marrow counts in this thesis in accordance with accepted practice.

Table II.

Peripheral Blood Findings of 16 Cases with associated Clinical Conditions

Case No.	Age+	Hb. % gms.	Colour Index	R.B.C.	W.B.C.	Eosmophils %	Reticulocytes %	Other Clinical Conditions present
1	20	2.56	0.6	1,420,000	7,800	-*	7.0	Auricular fibrillation: died.
2	25	6.8	0.98	2,400,000	8,800	35.0	10.9	SP +++, L+.
3	25	5.95	0.82	2,500,000	13,100	4.3	14.0	-
4	60	4.25	0.68	2,210,000	9,980	4.75	9.2	Pyorrhoea.
5	30	5.1	1.0	1,710,000	10,925	2.0	6.0	SP+, L+++.
6	55	4.25	0.68	2,200,000	9,000	4.2	9.5	-
9	30	5.95	1.01	1,750,000	5,600	9.7	4.0	Avitaminosis (A).
10	45	2.55	0.81	1,080,000	6,550	3.0	8.0	-
11	45	2.55	0.80	1,100,000	7,000	3.0	6.0	Avitaminosis (A).
12	30	7.65	0.88	3,000,000	13,240	2.4	8.2	Pyorrhoea.
14	25	4.25	0.86	1,670,000	5,600	4.2	14.0	Pyorrhoea SP+++, L+.
15	25	2.55	0.55	1,600,000	7,800	22.0	4.5	Pyorrhoea.
16	?	5.1	0.7	2,550,000	8,800	7.1	3.5	Pyorrhoea. Mossy foot.
17	30	3.4	0.98	1,200,000	4,800	2.5	11.0	Elephantiasis scrotum. Pyorrhoea. Avitaminiosis (A).
19	?	1.7‡	0.74	800,000	6,400	6.25	8.0	Pyorrhoea L+++. Avitaminiosis (A).
20	50	4.25	0.99	1,520,000	11,200	3.0	8.5	Filariasis: pyorrhoea. Elephantiasis: chiggers of feet.

+Ages given are approximate since none of the patients could tell his age.

*Not performed. ‡See explanatory note in legend.

SP = Splenomegaly. L = Hepatomegaly.

Peripheral Blood Findings

Table II gives in summary form the peripheral blood findings of the 16 cases studied together with the concomitant pathological findings of each case. The haemoglobin percentages as recorded on the Sahli haemoglobinometer varied between 15% and 45% except in Case 19 where the percentage was less than 15% and matching of colours appeared to be comparable without the addition of water. These percentages have been adjusted, as explained above, (14.5 gms. Hb. = Sahli 85%) to read in grammes of Hb. %.

It will also be noticed that a definite reticulocytosis existed in each case, varying from 3.5% (Case 16) to 14% (Cases 3 and 14), indicating that the bone marrow appeared to be functioning actively. This general reticulocytosis, when read in conjunction with the low myeloid-erythroblastic ratio in each case (Table III), confirms that the hypoplastic or even aplastic states of the bone marrow sometimes said to be found in severe infestations of ankylostomiasis have not been reached. The existence of a reticulocytosis in all 16 cases is contrary to the findings of Biggam and Ghalioungui (1934)[14], who found no reticulocytosis in over 100 cases of ankylostomiasis. The presence of an actively hyperplastic bone marrow was well illustrated in Case 1 which went to autopsy a few days after admission. In this case, red bone marrow was found to extend throughout both femora, the tibiae, fibulae, and in the upper extremities as far as both wrists.

Three cases (Nos. 2, 5, 14) exhibited palpable spleens which could have been attributed to chronic malaria (subtertian malignant) of the type endemic in and around Tanga. But the

proportionate part played by this chronic malaria in the production of or continuance of the severe anaemia is very difficult to gauge. Nevertheless, it must rank as a factor, not to be discounted, in view of the known fact that severe degrees of dyshaemopoietic anaemia do occur in malignant subtertian malaria. Fairley & Bromfield (1937)(15) and Fairley and others (1938)(16) have described in Macedonia cases of nutritional macrocytic anaemia which they classify as (a) haemolytic, (b) non-haemolytic and distinguished by the presence of hyperbilirubinaemia and urobilinuria in the former. These authors further suggest that the haemolytic variety is caused by malarial toxic action on erythropoiesis and that the non-haemolytic variety may arise from dietary deficiency. The cases described by them are definitely megaloblastic and more severe than the cases studied in this thesis, and many of their patients were females who were pregnant.

Eight cases (Nos. 4, 12, 14, 15, 16,17,19,20) showed pyorrhœa alveolaris and varying degrees of dental caries, and the resulting toxins may be credited with a possible minor role in the maintenance of the anaemia. Filariasis (Cases 17, 20) and "mossy foot" (Case 16) have no direct role since anaemia is not a clinical feature of these conditions. The same may be said of vitamin A deficiency found in four cases (Cases 9, 11, 17, 19). Regarding those cases in which the liver was enlarged, either by itself (Case 19) or in association with splenomegaly (Cases 2, 5, 14), no clear cut diagnosis was apparent to explain the enlargements satisfactorily. It seemed not at all improbable that they resulted from continued mal- or under-nutrition over a long period of time.

To support this tentative hypothesis, the liver in the four cases mentioned was uniformly enlarged and easily palpable under the thin abdominal wall of the patients. No irregularities nor nodules were detected to suggest cirrhosis or malignancy, while the Van den Bergh reaction was negative both direct and indirect. In addition, the liver in Case 1, which went to autopsy, showed evidence of fatty degeneration, although it was not apparently enlarged. These facts, admittedly slender, combined with the dry, soft, lifeless appearance of the hair, the dry, easily scaling skin, the absence of subcutaneous fat and the anaemia presented a picture which seemed to the writer to bear some resemblance to the cases described by Trowell (1940)[17], Trowell & Muwazi (1945)[2], Trowell (1946)[18], Hughes (1946)[19], and Gillman and Gillman (1945)[20]. Indeed, the latter workers (Gillman and Gillman) have stated that "recurrent attacks of subclinical and overt malnutrition result in progressive liver damage and are, in no small measure, responsible for cirrhosis and possibly for primary carcinoma of the liver so frequently encountered in young negroes of South Africa."

In general, the colour index was below unity, except in two instances where it was 1.0 and 1.01 and in three instances where it approximated to unity. Unfortunately, in the absence of adequate apparatus, neither the mean corpuscular volume (M.C.V.) nor the mean corpuscular diameter (M.C.D.) of the cells could be determined, and the decision as to size of cell could only be judged by comparison with normal films from healthy individuals. As regards the colour index and the mean corpuscular haemoglobin (M.C.H.) little importance was attached

to them except to indicate in a rough way the prevailing trend of the anaemia. The peripheral blood films stained with Leishman's stain showed anisocytosis, poikilocytosis, and normoblasts in small numbers. The erythrocytes, for the most part, appeared deficient in haemoglobin, but there were present a varying number of macrocytes fully haemoglobinised. However, the general appearance of the blood films could very well mislead one into labelling them, without fuller investigation, as typically iron deficiency hypochromic anaemia. As expected in parasitic diseases, eosinophilia was present and, in one case (No. 2), rose as high as 35% of the total W.B.C. count corresponding with an increase in eosinophil myelocytes, metamyelocytes and polymorphonuclears in the sternal marrow films.

Assuming 10,000/cmm. to be approximately the upper limits of normality, the moderate polymorphonuclear leucocytosis found in four cases (Nos. 3, 5, 12, 20) may be readily explained in two cases (Nos. 12 and 20) by the presence of septic foci, viz., pyorrhoea (No. 12) and pyorrhoea and chiggers (No. 20), but in the remaining two cases (Nos. 3 and 5) no such simple explanation is forthcoming.

In the few cases in which the Cooke count (Cooke, 1930) was performed, the general tendency was a shift to the left, the leucocytes appearing younger than those found in normal blood films.

Table III

Case Number	Myeloblasts	Premyelocytes	Myelocytes	Metamyelocytes	Granulocytes	Haemocytoblasts	Proerythroblasts	Early Normoblasts	Intermediate Normoblasts	Late Normoblasts	Lymphocytes	Monocytes	Myeloid-Erythroblastic Ratio
1	1.9	4.7	15.0	3.3	32.3	1.0	1.4	13.6	7.6	13.8	4.3	1.1	1.5 : 1
2	4.9	4.5	7.2	10.0	28.7	-	2.6	9.3	16.9	11.5	4.0	0.4	1.3 : 1
3	1.5	2.5	3.8	10.0	37.7	1.6	1.5	6.8	12.0	16.3	3.7	2.6	1.5 : 1
4	2.8	4.0	7.0	5.6	25.0	-	2.6	11.0	17.4	19.6	3.0	2.0	0.8 : 1
5	0.7	2.3	6.3	13.7	30.2	-	1.8	5.3	9.3	23.4	5.5	1.5	1.3 : 1
6	1.4	2.0	5.0	9.2	27.2	-	2.2	5.8	17.0	25.0	3.4	1.8	0.9 : 1
9	1.8	1.8	4.7	6.6	35.3	1.4	0.8	6.3	11.0	23.2	5.6	1.5	1.15: 1
10	1.1	3.7	6.5	7.5	30.1	-	1.7	11.0	15.0	19.5	2.7	1.2	1.04: 1
11	0.4	0.6	2.2	3.8	52.4	-	1.2	2.4	5.8	10.4	18.4	2.4	3.0 : 1
12	1.8	2.0	5.0	11.4	46.0	-	1.6	3.0	8.8	16.0	3.2	1.2	2.25: 1
14	0.8	3.0	7.8	16.4	23.8	2.0	2.8	8.0	15.8	18.0	1.0	0.6	1.1 : 1
15	1.0	2.3	10.5	18.3	30.5	-	0.5	1.5	6.9	23.9	3.8	0.8	1.9 : 1
16	1.0	2.0	8.4	12.5	44.5	-	0.8	2.2	5.8	16.6	6.2	0.0	2.7 : 1
17	2.4	5.9	7.7	14.6	18.2	-	2.9	7.3	18.7	18.4	3.7	0.2	1.3 : 1
19	2.8	3.5	7.1	11.9	23.7	-	2.8	2.8	8.0	17.0	14.8	5.6	1.6 : 1
20	1.6	6.5	9.3	20.9	27.5	-	1.5	4.1	7.6	15.8	4.1	1.1	2.27: 1

Table IV

Case No.	Myelocytes			Metamyelocytes			Polymorph Granulocytes		
	E.	B.	N.	E.	B.	N.	E.	B.	N.
1	0.9	0.3	13.8	0.3	0.0	3.0	0.0	0.3	32.0
2	1.3	0.2	5.7	3.8	0.0	6.2	4.5	0.0	24.2
3	0.8	0.0	3.0	0.5	0.5	9.0	2.0	0.5	35.2
4	1.0	0.4	5.6	2.4	0.0	3.2	3.6	0.2	21.2
5	0.3	0.0	6.0	1.0	0.0	12.7	1.6	0.0	28.6
6	0.6	0.0	4.4	1.2	0.0	8.0	2.4	0.0	24.8
9	0.1	0.0	4.6	1.3	0.0	5.3	3.0	0.0	32.3
10	1.2	0.3	5.0	0.8	0.0	6.7	1.3	0.5	28.3
11	0.6	0.0	1.6	0.4	0.0	3.4	4.8	0.0	47.6
12	0.5	0.0	4.5	0.8	0.0	10.6	1.3	0.5	44.2
14	2.0	0.2	5.6	1.8	0.0	14.6	1.4	0.0	22.4
15	4.5	0.0	6.0	2.5	0.0	15.8	4.8	0.6	25.1
16	1.2	0.0	7.2	1.5	0.0	11.0	4.5	0.2	39.8
17	1.5	0.2	6.0	2.2	0.0	12.4	2.3	0.2	15.7
19	0.7	0.0	6.4	1.4	0.0	10.5	4.2	0.0	19.5
20	0.8	0.1	8.4	0.1	0.0	20.8	2.4	0.1	25.0

E = Eosinophil B = Basophil N = Neutrophil

Table V

Comparison between Normal Ranges of Differential Marrow Counts and the Ranges of Counts of the present 16 Cases.

	Neutrophil		Eosinophil		Basophil	
	Normal Range	This Series	Normal Range	This Series	Normal Range	This Series
Polymorphonuclear	20-50.	15.7-47.6	0-4	0-4.8	0-1.0	0-0.6
Meta-myelocytes	2.5-12.	3-20.8	0-2.5	0.3-3.8	-	0-0.5
Myelocytes	2-8.	1.6-13.8	0-1	0.3-4.5	-	0-0.4
Premyelocytes	0.5-5.0.	0.6-6.5				
Myeloblasts	0-2.5	0.4-4.9				
Late normoblasts	7-19	10.4-25.0				
Intermed. normoblasts	4-15	5.8-18.7				
Early normoblasts	0-2	1.5-13.6				
Pro-erythroblasts	0-4	0.5-2.9				
Haemocytoblasts	0-1	0-2				

Table VI

Comparison between the Range of Erythroblastic Marrow Counts of this Series and the Ranges of Counts of Simple Hyperplasia, Pernicious Anaemia and Normal.

	Normal	Simple Hyperplasia	Pernicious Anaemia	This Series of 16 Cases
Haemocytoblasts	0-1	1-3	0-3	0-2
Proerythroblasts	0-4	1-10	1-8	0.5-2.9
Early normoblasts	0-2	2-12	0-2	1.5-13.6
Intermediate ,,	4-15	10-30	0-8	5.8-18.7
Late ,,	7-19	10-40	0.5-12	10.4-25
Early megaloblasts	0	0	4-40	Present
Intermediate ,,	0	0	4-12	Present
Late ,,	0	0	2-10	Present

Sternal Marrow Findings

Table III gives the percentage number of cells counted in sternal marrow smears. Table IV gives further percentages on subdivision of myelocytes, metamyelocytes and polymorphs into their eosinophil, basophil and neutrophil components. Table V shows a comparison of the results recorded in Table III with the normal range of bone marrow cell counts suggested by Whitby and Britton (1946)(21).

As has already been noted above (see page 15.) differential counts of megaloblasts as distinct from normoblasts have not been made because of the difficulty of accurate differentiation in many instances, but indication is made in cases where the megaloblastic series is definitely represented.

It will be noted (Table III) that, in this series of cases, the myeloid-erythroblastic ratio varies from 0.8 : 1 to 3 : 1, with an average ratio of 1.53 : 1. The myeloid-erythroblastic ratio observed in normal cases varies between 8 : 1 and 2 : 1. It is, therefore, apparent in this series of cases that there is greater activity on the erythropoietic side than on the leucopoietic side, although there would appear to be some degree of increased productivity in the latter as judged by (a) the younger appearance of the leucocytes in the peripheral blood and (b) a tendency to greater numbers of myelocytes and premyelocytes in the marrow counts as compared with the normal ranges (Table V). Nevertheless, it is not at all clear what significance, if any, can be placed on this slight leucopoiesis. Possibly, in a number of cases, it may be due to minor degrees of sepsispresent, but,

on the other hand, the possibility cannot be dismissed that it may be part of the prevailing blood picture. In this connection, it may be emphasised that, in cases of true iron and haemopoietic principle deficiencies, a leucocytosis and leucopoietic marrow reaction is not, characteristically, to be found.

The study of the individual myeloid cells of the marrow revealed little or no departure from generally accepted descriptions. In connection with the developing premyelocyte it was observed that granules usually made their first appearance in that portion of the cytoplasm lying between the more convex side of the nucleus and the cell periphery, while, at the same time, a progressively deepening eosin tingeing appeared in the less convex side of the nucleus. Vacuoles were occasionally to be observed in some myelocytes, but what their precise significance was, was not understood. Eosinophil myelocytes and meta-myelocytes were noted in greater numbers than found in normal marrow films coinciding with the eosinophilia present in the peripheral blood counts. Basophil myelocytes were observed in a few cases but only in one case (No. 3) were basophil meta-myelocytes discovered.

In contrast to the myeloid series, considerable variations from normal were observed in the erythroid series. The numbers of haemocytoblasts and pro-erythroblasts observed in marrow counts did not differ from the ranges accepted for normal, for simple hyperplasia and for pernicious anaemia (Table VI), although, on the whole, the pro-erythroblasts appeared to be slightly fewer than in the two latter conditions. The percentage numbers of early, intermediate and late normoblasts showed wide variations in

different cases. In three cases (Nos. 1, 4, 10) the early normoblasts were prevalent and constituted over 10 per cent. of the total cell counts, while, in six cases (Nos. 2, 4, 6, 10, 14, 17) the intermediate normoblasts were mostly predominant. The percentage numbers of late normoblasts showed a fairly general increase as compared with normal counts, and showed considerable variations in size and shape and in degree of haemoglobinisation. Pyknotic nuclei were commonly seen as well as nuclei in the process of extrusion or fragmentation prior to extrusion. The cytoplasm of many of the normoblasts was fully haemoglobinised, but in many others varying degrees of eosinophilia were observed, while an appearance of punctate basophilia was not uncommonly seen. Micronormoblasts were present in varying proportions, while, of great interest, was the appearance of late megaloblasts in all cases. Sometimes there were more in some cases (Nos. 2, 4, 5, 9, 10, 19) than in others (Nos. 1, 6, 12), and they could be readily recognised by their larger size, their eosinophilic cytoplasm and their nuclei with the stippled arrangement of the irregularly disposed chromatin material. But, taken by and large, the numbers of normoblasts predominated over the numbers of late megaloblasts. The evident warped nature of many of the late normoblasts both as regards size, shape, degree of haemoglobinisation and nuclear appearance, is a characteristic feature of iron deficiency anaemia and does not occur, as a rule, in the anaemias of the haemolytic or haemorrhagic type when the bodily iron reserve is adequate to meet the needs of the rejuvenating erythrocyte (Scott, 1939). In the latter conditions the normoblasts develop in a regular fashion. So far as

differentiation between the late normoblasts and late megaloblasts was concerned, little difficulty was experienced, but the same could not be said when it came to differentiation of the earlier stages of normoblasts and megaloblasts. The chief cause of confusion lay in the fact that the normoblasts themselves were developing abnormally after a similar design to that of true iron deficiency anaemias, and haemoglobinisation was insinuating itself into the intermediate and even into the early normoblasts. The greatest difficulty in this connection lay in the differentiation of the intermediate megaloblast from the earlier normoblast and of the early megaloblast from the pro-erythroblast. It was because of these difficulties that no quantitative estimate of the relative proportions of megaloblasts to normoblasts was attempted. Nevertheless, in a number of cases, the earlier megaloblasts were distinguishable. In Cases 2 and 4 early megaloblasts with partially haemoglobinised cytoplasms were observed. In some films, particularly those of Case 10, early erythroblasts were noted with partially or fully haemoglobinised cytoplasm, and it is almost certain that two at least of the cells were intermediate megaloblasts because of their larger size, their evenly stippled chromatin nuclear make up and the large size of the nucleus. The others of this particular case apparently belonged to the early normoblast group. Again, in Case 10, early normoblasts fully or almost fully haemoglobinised were observed extruding their nuclei which were definitely very immature and leaving behind very large macrocytic cells. Side by side with this early extrusion of immature nuclei from early normoblasts could be seen other early normoblasts whose

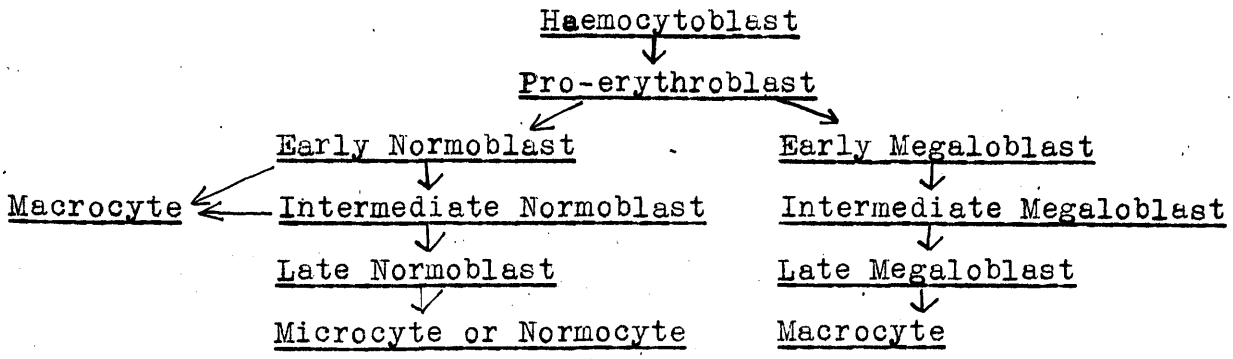
nuclei were in a state of division. In other films (Cases 2, 4, 9, 19 particularly) groups of intermediate normoblasts of apparently the same age and arranged in clusters were observed with their cytoplasm exhibiting varying degrees of haemoglobinisation indicating the irregularity of this process under the abnormal conditions prevailing in the haemopoietic system of the cases under examination. In one particular film from Case 4 an early normoblast was seen, partially haemoglobinised, but its predominantly eosinophilic cytoplasm displaying a mottled or blotchy basophilic appearance: the nucleus of this cell was in a state of mitotic division. In another film an early normoblast was observed with a definitely eosinophilic cytoplasm and possessing a nucleus of a peculiar rosette shape, having a central less dense and somewhat eosinophilic area, contrasting with the dense appearance of the lobes of the rosette - an obvious nuclear division. This cell would suggest that nuclear division is not necessarily antecedent to haemoglobinisation, but that it can take place irrespective of the process of haemoglobinisation. The numerous instances found in other films of fully or partially haemoglobinised early normoblasts undergoing nuclear division supported this suggestion. Another appearance which was not infrequently present was that in which an intermediate normoblast exhibited fragmentation of its nucleus. As an illustration of this, in one particular instance (Case 11) an intermediate normoblast was observed in which the major portion of the nucleus was in the process of extrusion, leaving behind a smaller, eccentric, fringelike nuclear remnant. In this latter case, it was, by no means, beyond doubt that the cell was in fact

an intermediate normoblast and not a late megaloblast in the process of extrusion of its nucleus prior to becoming a macrocyte. Punctate basophilia in late normoblasts and occasionally in the earlier normoblasts was not uncommonly seen, and once (Case 17) a pro-erythroblast, apparently normal in size and structure, was observed undergoing mitotic division. It is perhaps interesting to record in passing that a "ring stage" malarial trophozoite was observed in an intermediate normoblast whose cytoplasm was almost fully haemoglobinised.

Generally speaking, the prevailing impressions derived from the study of the sternal marrow films of this series of 16 cases and others examined but not listed here were -

(a) The Irregularity of Maturation of the Erythrocyte. While the majority, judging by the size and appearance of the resulting erythrocyte, seemed to be derived from normoblasts, a varying minority appeared to be derived from megaloblasts - these being, in fact, macrocytes. Furthermore, a small number appeared to be derived irregularly from earlier stages of normoblasts, giving rise to very large macrocytes. Two hypotheses at least might be advanced to explain the latter gross irregularity - either some inimical agent acting upon the earlier developing normoblasts and causing premature death and expulsion of the nucleus or an absence of the necessary concentration of some factor responsible for the full and healthy maturation of the earlier to later normoblasts. The latter hypothesis is in accord with the generally accepted views regarding the deficiency anaemias (e.g., haemopoietic deficiency anaemia, iron deficiency anaemia). It is generally held by most

haematologists that, on the one hand, late normoblasts give rise to erythrocytes even under conditions of severe iron deficiency and that, on the other, late megaloblasts produce macrocytes when the haemopoietic principle is deficient. But it appears to the writer that it is possible for an erythrocyte or, rather, a macrocyte to arise from an earlier stage of erythropoiesis under conditions of stress. What the actual fate of such an irregularly formed cell would be is not understood. Schematically the stages of development of the red cells were -



(b) The Irregularity of Haemoglobinisation. Considerable variations in the stage at which haemoglobinisation took place were evident not only in different cases but also in the same films. There were late normoblasts with fully eosinophilic cytoplasm or with partial eosinophilic cytoplasm, there were early and intermediate normoblasts with degrees of eosinophilia varying from none to full, and there were groups of earlier normoblasts of the same age in the same film but showing varying degrees of haemoglobinisation.

(c) The Dimorphic (22) Character of the Bone Marrow Blood Films. The predominant cells present were undoubtedly normoblastic but megaloblastic cells were obvious in all cases in varying proportions.

Discussion

The picture presented in the foregoing pages is that of a severe anaemia - the true extent of which could only be appreciated by prolonged and careful studies of the bone marrow blood films. Investigation of the peripheral blood alone gave little clue to the underlying pathology excepting, perhaps, a greater number of macrocytes present than one would customarily expect in pure iron deficiency anaemia. The bone marrow was shown to be hyperplastic and erythropoiesis to be essentially normoblastic, but with a very definite, if variable, megaloblastic admixture. Trowell (1943)[22] working in Uganda, and to whose work the writer had no access until 1944, found a similar mixed normoblastic and megaloblastic marrow reaction in cases of malarial and ankylostomiasis infestation and labelled his cases as dimorphic anaemia. Mention has also been made of the work of Fairley and others (1937[15] and 1938[16]) in Macedonia who were able to demonstrate mixed megaloblastic and normoblastic marrow reactions in males and females. These latter workers divided their cases, which they labelled as nutritional macrocytic anaemia, into two classes - (a) haemolytic and (b) non-haemolytic distinguished by the presence of urobilinuria and hyperbilirubinaemia in the former cases which were malarial in origin. Although the 16 cases forming the basis of this thesis were resident in an endemic malarial area and 3 of them had palpably enlarged spleens which could be attributed to chronic malaria, the Van den Bergh reaction in all cases was negative. The cases reported by Fairley and others were much more severely megaloblastic than this present series and, in addition, striking changes were reported in the

leucocytes - changes not observed by the writer. These changes consisted of morphological alterations in megakaryocytes, precocious nuclear polymorphism, atypical eosinophilia, amphophil and azure granules, perforation of the nucleus and cytoplasmic vacuolation as well as a tendency to leukopenia. Cytoplasmic vacuolation was, however, observed in some myelocytes in the writer's cases. Mixed megaloblastic and normoblastic erythropoiesis has been noted by other workers mainly in connection with pregnancy and with the megaloblastic anaemia known as tropical macrocytic or nutritional anaemia. Wolff and Limarzi (1945)[23] observed three cases of macrocytic anaemia in 30 cases of anaemia of pregnancy, but did not say whether megaloblastic and normoblastic erythropoiesis could be present together. Davidson, Davis et alia (1942)[24] observed the presence of both types of marrow reaction and further noted that the colour index could be below unity despite the presence of a megaloblastosis. Napier (1940)[25] pointed out also that the intermixing of macrocytosis and microcytosis in the microcytic anaemia of pregnancy is apt to be misleading in that one might mask the other and thus conceal the dual nature of the anaemia. Israels (1941)[26] also recognised the possibility of both megaloblastic and normoblastic erythropoiesis existing side by side.

It seems probable from the writer's experience in East Africa that many cases of this dual type of anaemia are missed, especially if too much reliance is placed on peripheral blood findings with a colour index below unity on the Sahli haemometer. The misleading fact of a colour index below unity was pointed out by Davidson, Davis et alia (1942)[24], by Trowell (1943)[22], and by the

writer (1944)[27]. Indeed, Trowell (1943)[22] goes so far as to say that "With the Sahli scale any C.I. over 0.85 should suggest hyperchromia and pernicious anaemia." It is presumed that by pernicious anaemia he means a blood picture resembling true pernicious anaemia since he states in the same communication that he "has never seen a true case of pernicious anaemia in an African." Nor has the writer seen or heard of a true case of pernicious anaemia in an African in East Africa. In the present series of cases, all the patients had a readily demonstrable ankylostomiasis infestation, the presence of which in addition to colour indices below or in the region of unity, the low haemoglobin percentages and the apparently hypochromic peripheral blood pictures, tended to be misleading as to the true nature of the underlying dual type of the anaemia. The question naturally arises as to the role of ankylostomiasis in the production of the mixed megaloblastic and normoblastic erythropoiesis demonstrated in this thesis. According to Stitt (1945)[28] and other investigators, the anaemia produced by ankylostomiasis is hypochromic and usually microcytic with a colour index of less than unity in severe cases. Biggam and Ghalioungui (1934)[14] in Egypt observed a microcytic type of anaemia with a low colour index in over 100 cases, but they failed to examine the marrow. On the other hand, Dick & McCarthy (1946)[29] working in East Africa stated that, given a good diet, a native with hookworm infestation need not develop anaemia. In the writer's experience this latter statement has a good deal of truth in it, but should be interpreted with caution. Wright (1946)[30] in Kenya warns against acceptance of Dick & McCarthy's statement

without question, and adds that there seems to be no constant relationship between the number of worms and the degree of anaemia. He also suggests the possibility of dietary deficiency or of some unknown factor lacking in diet to explain the variations of degrees of anaemia. The dietary deficiency aspect has also been emphasised by De Langen (1936)[31], Manson-Bahr (1940)[32], Trowell (1944)[33] and others. In East Africa, ankylostomiasis would appear to produce severer cases of anaemia in the coastal and low lying areas than in the upland areas, and the writer has frequently observed that it is the poorer peasant type of native who suffers most, while his more highly paid and more educated compatriot seldom shows the severer degrees of anaemia, even although he carries a heavy worm load. The dietary aspect is well emphasised by the fact that the writer has noticed that a poorer class patient with severe anaemia and ankylostomiasis will very often exhibit a reticulocytosis on hospital mixed diet alone without the addition of iron, and it would seem to be that the combination of hospital diet and iron with the usual steady improvement in most cases is sufficient in itself to deceive the busy clinician as to the pathological changes occurring in the marrow. This does not imply, of course, that all cases of severe ankylostome anaemia have a mixed megaloblastic and normoblastic bone marrow reaction, but it does imply that the majority of cases have an underlying dietary deficiency which, in an unknown minority, may be severe enough to produce a megaloblastic erythropoiesis of greater or lesser degree.

From the foregoing it appears that the underlying basis of these tropical macrocytic or megalocytic anaemias, whether the

haematology is mixed megaloblastic and normoblastic or megaloblastic alone, lies in a dietary deficiency. They differ principally from pernicious anaemia in that they recover under appropriate treatment, as first pointed out by Osler (1919)[34], and once the nutritional element is improved the anaemia improves. For the most part, therefore, the tropical megalocytic (or macrocytic) anaemias are the result of nutritional errors, and the characteristic blood picture may develop only when some added factor such as pregnancy, malaria, ankylostomiasis, etc., is superimposed on an already weakened haemopoietic system.

Regarding the nature of nutritional factor responsible for the production of a megaloblastic marrow reaction, the writer has, as many other observers have also noted (Trowell, Wills, Clutterbuck Davidson, etc.) the accelerated response of the bone marrow erythropoiesis when crude liver extracts are injected parentally in addition to the use of iron medication. Wills (1938)[35] noted that cases of nutritional macrocytic anaemia in monkeys and humans failed to respond to purified liver extract, and suggested that some other factor other than the liver principle might either be lacking or inhibiting the action of the liver principle. Unpurified liver extract (Campalon) was stated to contain two factors - (a) a factor soluble in saturated ammonium sulphate, and (b) a factor insoluble in saturated ammonium sulphate. The soluble factor was curative for nutritional macrocytic anaemia and the insoluble one resembling anahaemin was curative for P.A. only. A possible relationship of the soluble fraction to the vitamin B_2 complex (viz., B_6) was suggested, and it was noted as being present in crude liver and

autolysed yeast products (Wills, Clutterbuck & Evans, 1937,[36] Wills & Evans, 1938).[37] Spies (1946)[38] reported that cases of nutritional macrocytic anaemia treated with riboflavin, thiamine, neocin (nicotinic a), calcium pantothenate, pyridoxamine and pyridoxal, inositol, para-amino benzoic acid and choline failed to respond whereas ascorbic acid might cause a slight reticulocytosis. Responses occurred to the exhibition of beef muscle, 80% alcoholic extract of beef muscle or crude liver extracts. It was deduced that the extrinsic factor was not responsible, for by incubating the gastric juice of pellagrins with macrocytic anaemias together with ground beef and feeding this mixture to pernicious anaemia patients remission of symptoms occurred. Trials with folic acid synthetically prepared were successful in the treatment of macrocytic anaemia, and from these trials it was concluded that folic acid was a definite anti-anaemic factor belonging to the vitamin B_2 complex. This important work of Spies and his collaborators is reviewed in the B.M.J. (1946).[39]

Working from another angle in the study of nutritional deficiencies principally among pellagrins in South Africa, Gillman & Gillman (1945)[20] found that symptoms of acute nutritional deficiency were not improved and were even in some cases worsened by treatment with vitamin preparations. The principal symptoms and signs in these cases consisted of loss of weight, retarded development, oedema, mental backwardness, various manifestations of dermatosis, a softness and brownness of the hair and a diminution of the normal black pigmentation of the skin amounting to pallor in some cases. Very frequently associated with these clinical features is

an anaemia which is often macrocytic. Gillman & Gillman have also demonstrated that a characteristic feature of this syndrome is the invariable presence of fatty degeneration of the liver, and they have recently shown by liver biopsy that remarkable improvement occurs in the liver and in the other clinical features when desiccated hog's stomach is used in treatment. Trowell (1946)(18) has confirmed the effectiveness of treatment with desiccated hog's stomach in some 30 cases of kwashiorkor in Uganda. This disease, kwashiorkor, also a nutritional deficiency disease, was first described in children by Williams (1933)(40) in the Gold Coast, and it presents many features in common with the nutritional deficiency disease syndrome described in negro children of South Africa by Gillman & Gillman and with the syndrome described by Trowell (1940)(17) in East Africa and called by him "malignant malnutrition".

The clinical features of the 16 cases here presented exhibit many points in common with the syndrome described by Trowell (1946)(18). The patients were listless and apathetic, mentally somewhat dull and slow in answering questions. There was oedema, loss of weight, marked absence of subcutaneous fat, an unhealthy appearance of the mouth and gums and albuminuria in addition to a definite megaloblastic erythropoietic tendency. There was also the dry, soft, lifeless appearance of the hair, and skin changes were evident, and taking the form of excessive dryness and lack of oiliness, a tightly drawn, thin, shining appearance over the tibiae in which situation the colour appeared lighter and brownish compared with elsewhere. Certain other signs noted by Trowell in his syndrome of malignant malnutrition were not examined, viz.,

X-ray examination of the bowel, the presence of undigested foodstuffs in the stool, and the albumen-globulin ratio, but there is little doubt in the writer's mind that his 16 cases bear a strong resemblance to the cases described by Trowell in Uganda. Their degree of severity, however, was probably less marked, and had it not been for the superimposition of ankylostomiasis and/or malaria they would probably have continued to exist in their malnourished state and never approached a hospital.

In the 16 cases described and from his experience of others, the writer is of the opinion that their fundamental pathological basis is a dietary deficiency or deficiencies which, in the milder manifestations, is not easy to recognise and to diagnose, differentially, from such diseases as pellagra, beriberi and other complicated mixtures of vitamin deficiency conditions. As has been shown, the diet of this series of cases was a very impoverished one in almost all respects; in calories, first-class proteins, fats, minerals and vitamins, and it seems almost incredible that so many natives of Tanganyika and Uganda can even subsist on such poor diets, but it is not possible to judge this by European standards, for we do not know what changes in the physiological make-up of the African so many years of this impoverished type of diet have wrought. It is equally certain that a European accustomed to a reasonable mixed diet would soon, very soon, suffer ill effects from a diet solely consisting of cassava, a small handful of beans and very little else. In this connection, the effects of chronic starvation in Europeans subsisting on a daily diet mainly of rice (14 ozs.), vegetables (6 ozs.), meat (3 ozs.),

cooking oil (1 oz.), sugar (1 oz.), have been demonstrated in Japanese returned prisoners of war (Gupta 1946 (41) and Price 1946 (42)). Examination of approximately 1,000 of the internees, male, female and children, revealed malnutrition in all cases, due to hypoproteinaemia. Macrocytic anaemic was shown to be present in many cases and colour indices were sometimes as high as 1.5.

Many of the more severely ill natives with hookworm and anaemia improve rapidly with iron and hospital diet, some respond slowly to iron and hospital diet, but show an accelerated response to liver injected parentally, others show little response to vitamins and iron medication alone unless reinforced by liver parentally, and a few die despite all forms of treatment. It does not seem to make much difference whether the syndrome is called malignant malnutrition, acute malnutrition, kwashiorkor or tropical malnutrition. They all possess many pathological features in common derived from an underlying nutritional defect, and it is only the more advanced cases who ever reach hospital. As Stannus (1946) (43) pointed out at a conference on nutrition in the Colonial Territories, "unknown deficiency diseases greatly outnumbered those already known."

Conclusion

The writer has attempted, by drawing from his observations based on seven years' experience, backed by the presentation of 16 completed cases, to refute a belief all too widely held in East Africa that ankylostomiasis is, per se, a major cause of much of the severe anaemia found there. However, when ankylostomiasis is superimposed upon an impoverished physical background produced by long continued subminimal nutrition, the body is unable to bear this added strain and severe degrees of anaemia often result. More especially prone to the development of the anaemia are those who, in addition to existing under the burden of years of ill-balanced and under-feeding, suffer from the diseases endemic in East Africa, prevalent diseases such as malaria, tropical ulcers, bilharzia, elephantiasis, sepsis and others. But the precise aetiology is, on the whole, obscure and so many factors enter into the ultimate haematological findings. According to Spies (1946)[38] the pathogenesis of none of these anaemias (macrocytic anaemias) is thoroughly understood, and there is much confusion regarding diagnosis and treatment. The writer, while he may have done little in this thesis to contribute to a clearer understanding of the causes, trusts that he has given some indication of the complex nature of many of the so-called "hypochromic" anaemias all too commonly and erroneously ascribed solely to the effects of ankylostomiasis infestation.

The 16 cases here presented may be taken as representative of many more of a similar type to be found in any of the hospitals of many districts of East Africa, and the writer is convinced, from his experience in Tanganyika, that there are many more cases outside

the hospitals than inside. The treatment usually exhibited in the wards is effective up to a point and, being effective, has tended to obscure the underlying and true aetiology. The reasoning is simple. The patient has a haemoglobin percentage below 50% (Sahli) and the eggs of ankylostomiasis are demonstrated in the stools, therefore give him iron medication and a balanced hospital diet. His haemoglobin percentage responds by rising to 60-70 per cent. Therefore it follows that the anaemia is due to iron deficiency, and there, interest ceases as a rule, except in so far as the observance of routine "deworming" and discharge of the patient, when his haemoglobin is approximately 70%, is concerned. The patient himself, much improved, passes out from the hospital into that no-man's land between positive health and hospital ill-health, and, in East Africa, the extent of this no-man's land is great, much greater than it is in Britain or in any of the more advanced countries, and of an extent perhaps not fully appreciated even by those of us practising medicine in this part of the world.

The writer has also attempted to show that the underlying pathological basis responsible for morbid conditions described in his cases has been nutritional deficiency and that, had this nutritionally defective background not been present, the degree of anaemia would have been mild and would have conformed to the normoblastic pattern characteristic of simple iron deficiency anaemia. Furthermore, the cases presented in this thesis bear a close resemblance to those described by Trowell in Uganda under the name of malignant malnutrition and to those of Williams and Hughes in West Africa and of the Gillmans in South Africa.

Although the majority of the cases of anaemia attributable to ankylostomiasis are undoubtedly normoblastic, there are an unknown minority which may be either definitely megaloblastic or mixed megaloblastic and normoblastic, and which present, in varying degree, the signs and symptoms of underlying nutritional deficiency. The exact nature of this nutritional deficiency is, as yet, unknown but the recent work of Spies and others in the exhibition of folic acid in the treatment of nutritional macrocytic anaemia suggests that this compound may constitute one of the missing factors of the Vitamin B_2 complex and be able to supply a part, at least, of the nutritional deficiency. However, it is not clear what is the relationship between the active agent in desiccated hog's stomach used so effectively by Trowell and the Gillmans, the soluble fraction of crude liver extract used with good results by Wills and others, and folic acid in the treatment of macrocytic anaemias. It may well be that there is a close relationship, or it may be that there is more than one deficiency factor involved in the syndrome of acute or malignant malnutrition and macrocytic anaemia (B.M.J., 1946).(44)

Summary.

1. Attention is drawn to the customary type of hospital treatment received by the poorer type of East African native suffering from ankylostomiasis and to his impoverished physical and nutritional background.

2. The prevailing type of diet used by the poorer native is outlined together with some remarks on his economic status.

3. The clinical features of the 16 cases of severe anaemia comprising the body of this thesis are presented in detail.

4. The technique used for peripheral and sternal marrow examinations is outlined.

5. The terminology followed in this thesis is detailed, and emphasis is placed upon the difficulty of differentiation between certain cells of the early normoblastic and megaloblastic series.

6. The peripheral blood findings of the 16 cases together with their concomitant pathological conditions are discussed in detail, and the possible role played by malaria, sepsis, filariasis, mossy foot and avitaminosis (A) in the production of the severe anaemia present, is mentioned.

7. The sternal marrow findings are considered qualitatively, and the outstanding impressionsderived from their study are (a) the irregularity of maturation of the erythrocyte, (b) the irregularity of haemoglobinisation, and (c) the dimorphic character of the anaemia, i.e., the marrow exhibits a mixed normoblastic and megaloblastic reaction.

8. The tendency to overlook the possibility of a mixed normoblastic and megaloblastic marrow, if too much reliance is placed on the colour index and on the peripheral blood findings only, is emphasised.

9. Attention is drawn to the fact that the fundamental pathological basis of this mixed normoblastic and megaloblastic anaemia is a nutritional deficiency or deficiencies, and that the superimposition of such conditions as malaria, pregnancy, ankylostomiasis and other debilitating diseases throws an excessive strain on an already too delicately poised erythropoietic system, so much so that it breaks down and the resulting abnormal erythopoiesis may take the form either of a mixed normoblastic and megaloblastic reaction or of a frank megaloblastic reaction.

10. The nature of the nutritional deficiency or deficiencies responsible for the dual nature of the anaemia is discussed, and reference is made to the possible link up between the factor isolated by Wills from crude liver extract, the factor (so far not identified) in the dried hog's stomach used in pellagrins by the Gillmans in South Africa, and folic acid synthesised by Spies and his collaborators and used so effectively in macrocytic anaemias in America.

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