LIVER IRON STORES IN DIFFERENT POPULATION GROUPS IN SOUTH AFRICA*

R. W. CHARLTON AND T. H. BOTHWELL, *MRC Iron and Red Cell Metabolism Unit, Departments of Pharmacology and Medicine, University of Witwatersrand;* F. G. H. MAYET, *Department of Medicine, University of Natal;* C. J. UYS, *Department of Pathology, University of Cape Town;* AND I. W. SIMSON, *Department of Pathological Anatomy, Institute for Pathology, University of Pretoria*

SUMMARY

The hepatic non-haem iron concentrations of 1 143 subjects dying in Durban, Pretoria and Cape Town were determined. The effects of certain disease processes upon liver iron stores were confirmed, and data from subjects dying from such diseases were excluded. No significant differences were revealed between comparable groups from the 3 cities. In Whites and Bantu, the median values were higher in adult males than in postmenopausal females, and lowest in premenopausal females, reflecting the greater iron requirements imposed by menstruation and pregnancy. The figures in the Bantu were significantly higher than those in the corresponding members of each of the other population groups. In addition, suggestive evidence was obtained that a significant proportion of Coloured males have iron stores that are lower than normal.

In contrast to the situation in adults, significantly lower concentrations were found in Bantu children than in White children. The probable explanation for these observations is that the Bantu children are not exposed to the major source of superfluous dietary iron, namely the home-brewed beers.

In states of frank iron deficiency, all the available body iron is incorporated into functional compounds such as haemoglobin and iron-containing enzymes.[1,2] When, however, there is enough iron to satisfy all functional requirements, any surplus is stored as ferritin and haemosiderin

*Date received: 20 October 1970.

in the liver, spleen and bone marrow. It is thus apparent that the amount of storage iron present at any one time reflects the nutritional status of an individual in so far as iron is concerned. Because of this, several methods have been developed for assessing the body-iron stores. These include the histological estimation of the amount of visible iron and the chemical determination of tissue-iron concentrations.[1] Some caution must, however, be exercised in this regard, since various non-nutritional factors may modify either the amount or the distribution of iron within the body. For example, in malignant neoplasms the iron from catabolized haemoglobin is not released from reticulo-endothelial cells at a normal rate; as a result anaemia develops, and an increased proportion of total body iron is present in the stores.[1] Estimation of the storage-iron content of the body in such subjects gives a falsely optimistic picture of their iron nutrition. In a previous study evidence was obtained that distortion of this type is more than merely a theoretical possibility.[3] A digital computer was used to analyse the non-haem iron concentrations in 3 983 specimens of liver obtained from 26 different countries, and the influence of certain non-nutritional factors such as the cause of death was established. At the same time it was apparent that valid information could be obtained with this approach, provided that allowances were made for these distorting factors.

Deficiency of iron is a major nutritional problem in many countries, while excessive stores of iron are encountered only rarely. Iron overload of varying degrees is, however, extremely common in the adult Bantu population of Southern Africa. The condition, which appears to be due to the presence of large amounts of ionic iron in home-brewed alcoholic beverages, has been extensively investigated by a number of workers over the years.[4-14] The iron nutrition of other ethnic groups has also been assessed, but only two studies have been reported in which specimens from different parts of the Republic were compared.[3,15] Moreover, most of the available information on iron nutrition has been obtained by histological methods, and while this approach can undoubtedly be semi-quantitative, it lacks the precision of chemical analysis. For these reasons it was thought worth while to obtain necropsy specimens of liver from different parts of the Republic for estimation of the non-haem iron concentrations.

MATERIALS AND METHODS

Specimens of liver weighing 3 - 5 g were obtained at necropsy from a total of 1 143 subjects, preserved in buffered formal saline, and transported to Johannesburg for analysis. The storage-iron concentrations were estimated on 1-g aliquots by the method of Torrance and Bothwell.[16] The age, sex and cause of death were recorded in each case. The findings in a limited number of children aged between 4 months and 18 years were analysed separately. Women were considered to be premenopausal if they were aged 39 years or less, and postmenopausal if aged 50 years or more; data from female subjects aged 40 - 49 years were discarded. The data were classified into 4 categories on the basis of the cause of death, 2 of which would be expected to influence the hepatic storage-iron content and 2 of which would not. The categories were respectively malignant neoplasms, uraemia and chronic infections, acute trauma, and finally diseases such as cerebral thrombosis, acute pneumonia, etc. All the information was entered onto cards and analysed by means of a digital computer.

RESULTS

The numbers of specimens from subjects in the different groups, together with the median hepatic non-haem iron concentration for each group, are set out in Table I.

Adults

Effect of cause of death. Storage-iron concentrations have been shown to increase with age in Bantu males. but in White males this does not occur.[3] In women of all races the menopause produces an age-linked effect. Only adult White males could therefore be used to examine the possible effects of the various causes of death upon hepatic storage-iron concentrations, and only the Pretoria group was large enough. Analysis of variance showed that the values in those subjects who died from malignant neoplasms and from chronic infections or uraemia were significantly greater than in those who died from the remaining natural causes or from trauma (Table II). Since there was reason to believe that the values in the first group were higher as a result of the redistribution of body iron rather than an increase in the total amount, they were excluded from the remainder of the analysis of the adult data.

Effect of geographical location. No evidence was obtained that significant differences existed between the values from the 3 different cities, in either White or Bantu subjects (Table III).

Comparison between different races. The data from the 3 cities were pooled to permit a comparison between the different racial groups. In males and in post- and premenopausal females, the storage iron concentrations in the livers from Bantu subjects were significantly higher than those from Whites, Coloureds or Indians ($p<0{\cdot}001$) (Table IV). No significant differences between the last 3 groups were demonstrated, with the exception of the Coloured males. The low median concentration in this group (109 $\mu g/g$) suggested that there might be a significant number of individuals with very low iron stores. Accordingly, a direct comparison between White and Coloured males was undertaken. Of the 133 White males, only 29 had hepatic non-haem iron concentrations lower than 100 $\mu g/g$, compared with 15 of the 34 Coloured males. This difference was significant (Chi squared, $5{\cdot}85: 0{\cdot}02>p>0{\cdot}01$).

Comparison between premenopausal and postmenopausal females. Median values were lower in premenopausal Bantu, White and Coloured women, but the difference was significant only among the Bantu ($p<0{\cdot}01$) (Table V). (There were insufficient data in the case of Indians.)

Children

The number of specimens available for analysis was unfortunately small, and in most cases comparisons were only possible if no data were excluded on the basis of cause of death.

Comparison between males and females. Enough data were available from Bantu children in Durban and in

TABLE I. NUMBER OF SPECIMENS ANALYSED, AND MEDIAN HEPATIC NON-HAEM IRON CONCENTRATIONS AFTER EXCLUDING DATA FROM SUBJECTS DYING FROM MALIGNANT NEOPLASMS, URAEMIA OR CHRONIC INFECTIONS

	Bantu			*White*			*Coloured*			*Indian*		
	All causes of death	*Neoplasms, etc. excluded*	*Median iron ($\mu g/g$)*	*All causes of death*	*Neoplasms, etc. excluded*	*Median iron ($\mu g/g$)*	*All causes of death*	*Neoplasms, etc. excluded*	*Median iron ($\mu g/g$)*	*All causes of death*	*Neoplasms, etc. excluded*	*Median iron ($\mu g/g$)*
Males												
Durban	171	133		—	—		1	0		20	19	
Cape Town	30	27		44	37		45	33		—	—	
Pretoria	110	58		182	96		1	1		—	—	
Total	311	218	845	226	133	198	47	34	109	20	19	188
Postmenopausal females												
Durban	20	15		—	—		—	—		6	6	
Cape Town	3	3		11	8		24	14		—	—	
Pretoria	21	10		91	40		1	1		—	—	
Total	44	28	409	102	48	163	25	15	136	6	6	66
Premenopausal females												
Durban	48	39		—	—		—	—		3	3	
Cape Town	5	4		—	—		16	13		—	—	
Pretoria	59	36		26	15		—	—		—	—	
Total	112	79	154	26	15	89	16	13	101	3	3	—
Children (both sexes)												
Durban	69	47		—	—		—	—		3	3	
Cape Town	7	4		2	2		11	8		—	—	
Pretoria	69	50		44	24		—	—		—	—	
Total	145	101	100	46	26	150	11	8	150	3	3	—

Pretoria to permit a comparison, but in neither case were significant differences in hepatic storage-iron concentrations revealed. In the Durban Bantu children, 25 of the 39 males and 22 of the 30 females had hepatic non-haem iron concentrations less than 200 $\mu g/g$ (Chi squared, $0{\cdot}67: p>0{\cdot}1$), while the corresponding figures in the Pretoria group were 18 of 34 and 26 of 35 (Chi squared, $3{\cdot}41: 0{\cdot}1>p>0{\cdot}05$).

Comparison between children aged 4 months - 10 years and those aged 11 - 18 years. In neither girls nor boys among the Bantu children could a significant difference be demonstrated between those aged 10 years or younger and those aged 11 years or older. Among the girls, the numbers with hepatic non-haem iron concentrations less than 200 $\mu g/g$ were 13 out of 21 in the younger Durban group and 5 out of 9 in the older (Chi squared, $0{\cdot}1: p>0{\cdot}1$), while in the corresponding groups from Pretoria the figures were 17 out of 25 and 6 out of 10 (Chi squared, $0{\cdot}2: p>0{\cdot}1$). In the younger Durban boys, 14 of 26 were below 200 $\mu g/g$ compared with 5 of 13 older boys (Chi squared, $0{\cdot}8: p>0{\cdot}1$), while the figures in the Pretoria boys were 13 out of 26 and 4 out of 8 (Chi squared 0·0).

Comparison between Bantu children from Pretoria and those from Durban. Since no effect of either age or sex had been established, the observations were pooled, and a comparison was made between the Bantu children from Durban and those from Pretoria. However, no significant difference was demonstrated. Of the 69 Durban children, the hepatic non-haem iron concentration was lower than 200 $\mu g/g$ in 37, compared with 40 out of 69 in the Pretoria children (Chi squared, $0{\cdot}26: p>0{\cdot}1$).

Comparison between Bantu and White children. There were unfortunately not enough observations on Indian or Coloured children to permit a comparison between all 4 ethnic groups, but there were 44 specimens from White Pretoria children which could be compared with 69 Bantu children from Pretoria (Table VI). There was a highly significant difference ($p<0{\cdot}001$), the Bantu values being *lower* than those in Whites. Inspection of the data showed, however, that a somewhat higher proportion of the White specimens had come from subjects dead from malignant neoplasms, uraemia or chronic infections than was the case with the Bantu children. Since the difference in storage-iron concentrations between the two groups might possibly have been due to this rather than to nutritional factors, a second comparison was made after excluding such observations from both groups. When this was done, a significant difference was still present, but it was considerably smaller ($p<0{\cdot}05$). The median values in the White and Bantu groups were 357 $\mu g/g$ and 117 $\mu g/g$ respectively when all the observations were included, and only 150 $\mu g/g$ and 100 $\mu g/g$ after the neoplasms, etc. had been omitted.

DISCUSSION

Ever since Strachan[4] reported that iron overload was common in the adult Bantu population of South Africa, the condition has been studied by a number of investigators in different parts of the Republic. In several of the earlier reports the quantity of iron present in the liver was assessed histologically. However, the criteria used to judge the degree of siderosis varied from observer to

TABLE II. EFFECT OF CAUSE OF DEATH ON HEPATIC NON-HAEM IRON CONCENTRATIONS

		Analysis of variance			
Group	*No.*	*Trauma*	*Cardiovascular, etc.*	*Neoplasms*	*Chronic infections*
Pretoria White males	182	—	−2·58	+1·56	+1·61
Pretoria Bantu males	110	—	−0·02	+0·58	−1·16
Durban Bantu males	171	−1·68	+0·71	−0·04	+1·30

TABLE III. EFFECT OF GEOGRAPHICAL LOCATION ON HEPATIC NON-HAEM IRON CONCENTRATIONS (NEOPLASMS, URAEMIA AND CHRONIC INFECTIONS EXCLUDED)

Group	*City*	*No. in group*	*No. with hepatic iron <200 μg/g*	*Chi squared (corrected)*	*P*
White males	Cape Town	37	17		
	Pretoria	96	52	0·72	>0·1
White postmenopausal females	Cape Town	8	4		
	Pretoria	40	25	0·46	>0·1
Bantu males	Cape Town	27	7		
	Pretoria	58	10		
	Durban	133	19	2·26	>0·1
Bantu postmenopausal females	Pretoria	10	3		
	Durban	15	3	0·35	>0·1
Bantu premenopausal females	Pretoria	39	23		
	Durban	36	20	0·10	>0·1

observer, so that only approximate comparisons can be made between the results obtained in different studies. In addition, the material was often selected in one way or another, so that it is not possible to obtain an accurate picture of the incidence of siderosis in the population as a whole. Moreover, while it has been shown that the size of the stores can be satisfactorily judged by histological methods in subjects with iron overload,[5,7,11] the technique is not sensitive enough to provide an assessment of iron nutrition in non-siderotic individuals. The alternative, and more satisfactory approach of quantitative chemical analysis has been applied in several studies.[5,7,11-15]

Bothwell and Bradlow[11] determined the iron concentrations in the livers of 147 Bantu subjects (16 females) dying of acute trauma in Johannesburg. Only 35% of these individuals had hepatic iron concentrations within or slightly above the range regarded as normal in other populations, i.e. less than 0·2% dry weight (approximately 400 μg/g wet weight). Bothwell and Isaacson[13] carried out a similar study on 318 Bantu males and 265 Bantu females dying in hospital in Johannesburg from unspecified causes. Values less than 0·2% dry weight were found in 29·6% of the males. In the present investigation concentrations below 400 μg/g wet weight were found in 30·7% of the Bantu males.

The similarity of these figures might suggest that undue preoccupation with the theoretical effects of non-nutritional factors upon hepatic iron concentrations is unwarranted; however, this is probably only true when the majority of the population has abnormally large stores of iron, as in the Bantu.[3] Under such circumstances the effects of pathological processes upon the size or distribution of the body-iron stores are masked. That the influence of such factors cannot be ignored in non-siderotic populations was illustrated in the present investigation. In the 44 White children from Pretoria, the median hepatic storage-iron concentration was 357 μg/g, but when the 20 observations obtained from individuals who had died from neoplasms, chronic infections or uraemia were excluded, the median figure was only 150 μg/g (Table VI). In Bantu children the effects of excluding this group were similar but less marked. Estimating the hepatic storage-iron concentration in subjects with such diseases gives a falsely high picture of the iron nutritional status because the iron from catabolized haemoglobin is not released into the plasma at a normal rate, and accumulates in reticulo-endothelial cells in the liver and elsewhere.

The sex of the subjects is another factor which must obviously be taken into consideration when iron nutrition is assessed. In women the increased iron requirements associated with menstruation and pregnancy mean that more iron has to be obtained from the diet if the individual is to stay in balance. Since the amount of available iron in the diet is nearly always limited, it is to be expected that iron stores in premenopausal women will be smaller than those in men, and also smaller than those in postmenopausal women. This was confirmed in the present study, and it is therefore obvious that valid comparisons between groups of women cannot be made unless this factor is taken into account.

The effect of age must also be considered. Older women have greater concentrations of storage iron than younger women, but this is due rather to the menopause than to a direct effect of age itself. Age has previously been shown not to influence storage-iron concentrations in males from a number of different countries.[3] The excretion of iron from the body in healthy males varies within relatively narrow limits,[17] and the regulation of iron balance is achieved by adjusting absorption to meet requirements. Since it is known that the average western type of diet contains more available iron than is needed by adult males,[18] the finding that body-iron stores in old men are no larger than in young men testifies to the efficiency of the intestinal mucosal mechanisms for excluding super-

TABLE IV. COMPARISON BETWEEN HEPATIC NON-HAEM IRON CONCENTRATIONS IN DIFFERENT ETHNIC GROUPS

Racial Group	*Males*				*Postmenopausal females*				*Premenopausal females*			
	No. in group	*Median non-haem iron (μg/g)*	*No. with liver iron <400 μg/g*	*Chi squared (corrected)*	*No. in group*	*Median non-haem iron (μg/g)*	*No. with liver iron <200 μg/g*	*Chi squared (corrected)*	*No. in group*	*Median non-haem iron (μg/g)*	*No. with liver iron <200 μg/g*	*Chi squared (corrected)*
Bantu	218	845	67	All groups, 96·7 ($p<0·001$)	28	409	7	All groups, 11·5 ($p<0·01$)	79	154	44	Bantu, White and Coloured groups, 7·86 ($p<0·01$)
White	133	198	105		48	163	29		15	89	13	
Coloured	34	109	27	Bantu excluded, 0·17 ($p>0·10$)	15	136	10	Bantu excluded, 0·26 ($p>0·10$)	13	101	11	Bantu excluded, 0·16 ($p>0·10$)
Indian	19	188	15		6	66	4		3	(Insufficient)		

fluous iron. The mucosa is not, however, able to reject all unwanted iron if very large amounts are ingested. This has been demonstrated in experimental animals[1] and in the clinical situation it is manifested both by acute iron poisoning and by the development of siderosis in subjects such as the Bantu who are continually exposed to excessive amounts of ionic iron in the diet.

There does not appear to be any *a priori* reason why iron nutrition should vary in the different centres of the Republic. Examination of the results obtained in the histological study carried out in Cape Town by Uys and co-workers[10] and that performed by Wainwright[9] in Durban suggests that there are no striking differences between the Bantu in the two cities, once allowance has been made for the varying histological criteria. The only previous studies in which any direct comparison was made between specimens from different parts of the Republic were those of Mayet and Bothwell[15] and Charlton and co-workers.[3] Mayet and Bothwell measured non-haem iron concentrations in liver specimens from Durban and Johannesburg; the majority of the subjects in their study had been killed by acute trauma. In that investigation the median value in the 81 Bantu males from Johannesburg was 966 μg/g, and in the 239 from Durban 786 μg/g, compared with 845 μg/g in the 218 Bantu males from all 3 centres analysed in the present investigation. Charlton and co-workers[3] obtained a median hepatic iron concentration of 946 μg/g in 79 Bantu males from Johannesburg, and 776 μg/g in 234 from Durban. When the wide range of hepatic non-haem iron concentrations found in the Bantu is taken into account, these figures are remarkably close.

Very few observations on subjects belonging to other racial groups have been reported. Mayet and Bothwell[15] found a median figure of 268 μg/g in White males from the two cities combined, compared with 198 μg/g in the present study, while in the investigation by Charlton and co-workers[3] the value was 258 μg/g in 73 subjects from Johannesburg. The median figures for Indian males (Durban) were very similar in all three studies, namely 173 μg/g, 188 μg/g and 183 μg/g respectively. These figures may be compared with those found in males from other parts of the world.[3] The median concentrations were 126 μg/g in Swedes, 186 μg/g in subjects from Seattle, USA, and 113 μg/g and 143 μg/g in individuals from two different hospitals in London. In Indians from New Delhi the median concentration was only 93 μg/g. From these observations it appears that the iron nutrition of White and Indian male South Africans is at least as good as that in a number of other countries. Unfortunately not enough observations were made on Coloured subjects in the present study for any firm conclusions to be reached. However, it may be noteworthy that a significantly larger proportion of Coloured males had hepatic iron concentrations less than 100 μg/g than did White subjects. In a previous histological survey Uys and co-workers[10] showed that a portion of Coloured males had increased iron stores. The present results suggest that this population group may also include significant numbers with decreased iron stores. No final conclusions can, however, be reached until a more definitive study has been done on a larger number of subjects.

TABLE V. COMPARISON BETWEEN PREMENOPAUSAL AND POSTMENOPAUSAL FEMALES

	Premenopausal		*Postmenopausal*		
Racial group	*No. in group*	*No. with liver iron <200 μg/g*	*No. in group*	*No. with liver iron <200 μg/g*	*Chi squared (corrected)*
Bantu	79	42	28	7	7·29, p<0·01
White	15	13	48	29	3·57, p>0·05
Coloured	13	11	15	10	1·11, p>0·10

TABLE VI. HEPATIC NON-HAEM IRON CONCENTRATIONS: A COMPARISON BETWEEN BANTU AND WHITE CHILDREN FROM PRETORIA

	All causes of death				*Neoplasms, etc. excluded*			
Racial group	*No. in group*	*Median hepatic non-haem iron (μg/g)*	*No. with hepatic iron <150 μg/g*	*Chi squared*	*No. in group*	*Median hepatic non-haem iron (μg/g)*	*No. with hepatic iron <150 μg/g*	*Chi squared*
White	44	357	12	10·2	24	150	11	4·52
Bantu	69	117	40	(p<0·001)	50	100	35	(6<0·05)

A limited amount of information is available from previous studies with regard to the amounts of storage iron in the livers of women in the Republic. As with the males, most of the studies have been concerned with the incidence and severity of siderosis in the Bantu, and it is generally agreed that while marked iron overload undoubtedly occurs in women, it is less common than in men. For example, Bothwell and Isaacson[13] found that hepatic iron concentrations were within or just above the normal range (up to 0·19% dry weight) in 75·4% of 265 Bantu females dying in hospital, while the comparable figure in males was 29·6%. A tendency for the concentration to rise with age was noted. In Wainwright's study[9] only 2 - 3% of Bantu women below 40 years had severe hepatic siderosis compared with 24 - 31% of males, but in females over 50 years the figure was 48%. Severe siderosis is less common in Bantu women than in men because the major source of the excessive dietary iron is the home-brewed beers, which are less often consumed in quantity by the females. The median hepatic non-haem iron concentration in both pre- and postmenopausal Bantu women was nevertheless found to be significantly higher than the equivalent figures in other races (Table IV). There are almost no data from previous studies with which these values can be compared. Charlton and co-workers[3] found a median hepatic iron concentration of 496 μg/g in 19 postmenopausal Bantu women, a figure similar to that found in the present study (409 μg/g). The women in Mayet and Bothwell's study[15] were not divided into pre- and postmenopausal groups.

The median value for postmenopausal White females in the present study (163 μg/g) may be compared with the figures for Sweden (120 μg/g), Czechoslovakia (170 μg/g), Seattle, USA (133 μg/g), and St George's Hospital, London (119 μg/g).[3] No data for premenopausal women are available for comparison.

Very few observations on children have been reported; however, Wainwright[9] found a mean hepatic non-haem iron concentration of approximately 0·06% dry weight in 13 Bantu children aged 6 months to 7 years, a figure not dissimilar from that found in the present investigation (100 μg/g wet weight). In view of the high storage-iron concentrations in Bantu adults, the fact that the median value in Bantu children was significantly *lower* than that in White children (150 μg/g) is striking. It seems reasonable to conclude that the diet consumed by Bantu children contains less available iron than is present in the White children's diet.

This study was supported in part by grants from the South African Atomic Energy Board and the Wellcome Trust, United Kingdom.

REFERENCES

1. Bothwell, T. H. and Finch, C. A. (1962): *Iron Metabolism*. Boston: Little, Brown & Co.
2. Charlton, R. W. and Bothwell, T. H. (1970): Seminars Hemat., **7**, 67.
3. Charlton, R. W., Hawkins, D. M., Mavor, W. O. and Bothwell, T. H. (1970): Amer. J. Clin. Nutr., **23**, 358.
4. Strachan, A. S. (1929): M.D., thesis, Glasgow University.
5. Gillman, J., Mandelstam, J. and Gillman, T. (1945): S. Afr. J. Med. Sci., **10**, 109.
6. Gillman, J. and Gillman, T. (1951): *Perspectives in Human Malnutrition*. New York: Grune & Stratton.
7. Higginson, J., Gerritsen, T. and Walker, A. R. P. (1953): Amer. J. Path., **29**, 779.
8. Walker, A. R. P. and Arvidsson, U. B. (1953): Trans. Roy. Soc. Trop. Med. Hyg., **47**, 536.
9. Wainwright, J. (1957): S. Afr. J. Lab. Clin. Med., **3**, 1.
10. Uys, C. D., Van der Walt, J. J., Potgieter, G. M. and Golby, H. H. (1960): *Ibid.*, **6**, 1.
11. Bothwell, T. H. and Bradlow, B. A. (1960): Arch. Path., **70**, 279.
12. Bradlow, B. A., Dunn, J. A. and Higginson, J. (1961): Amer. J. Path., **39**, 221.
13. Bothwell, T. H. and Isaacson, C. (1962): Brit. Med. J., **1**, 522.
14. Buchanan, W. M. (1967): University College of Rhodesia, Faculty of Medicine, Research Lecture Series No. 1.
15. Mayet, F. G. H. and Bothwell, T. H. (1964): S. Afr. J. Med. Sci., **29**, 55.
16 Torrance, J. D. and Bothwell, T. H. (1968): *Ibid.*, **33**, 9.
17. Green, R., Charlton, R., Seftel, H., Bothwell, T., Mayet, F., Adams, B., Finch, C. and Layrisse, M. (1968): Amer. J. Med., **45**, 336.
18. Moore, C. V. (1961): Harvey Lect., **55**, 67.