

BIOCHEMICAL INVESTIGATION OF THE NUTRITION STATUS OF URBAN SCHOOL CHILDREN AGED 12 - 15 YEARS: PROTEIN STATUS

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The National Nutrition Research Institute (NNRI)¹ concluded in 1959 that there was no over-all shortage of protein in the total food supplies of the Republic of South Africa. Deficiencies might nevertheless exist through maldistribution of food supplies, and it is desirable that we should be able to recognize both actual and latent protein deficiency so that preventive measures may be instituted where necessary.

With the dual object of evaluating biochemical methods for the assessment of protein nutrition status and assessing the protein nutrition status of the 4 main racial groups in Pretoria, the NNRI carried out nutrition status surveys on the following population groups:

- (a) White children aged 7 - 11 years (April - June 1962).
- (b) Bantu children aged 7 - 15 years (April - June 1963).
- (c) Coloured and Indian children aged 7 - 15 years (April - June 1964).
- (d) White children aged 12 - 15 years (April - May 1965).

Since the survey on the older White children was carried out 3 years later than that on the younger children, it would not have been strictly correct from the point of view of the statistical analysis to treat the data for the 2 surveys as an unbroken sequence of observations. It was therefore decided to analyse the results of the age-groups 7 - 11 years and 12 - 15 years separately. The results for the younger age-group have already been published.² The present paper deals with the age-group 12 - 15 years.

MATERIAL AND METHODS

The survey on the Bantu children was carried out on a statistically representative sample drawn at random from a population of 10,723. For various reasons, including non-response, only 263 of the 320 children drawn were actually included in the survey. The number of children surveyed in each age-sex group varied from 28 - 39 out of the 40 actually drawn.

The surveys on the Coloured and Indian children were done concurrently and were based on 2 randomly-drawn samples of 200 children each, representing a population of 544 Coloured and 944 Indian children. The numbers actually surveyed were

174 Coloured and 160 Indian children. The number of Coloured children surveyed in each age-sex group varied from 19-25 and of Indian children from 19-21 out of the 25 actually drawn.

The survey on the White children was based on a statistically representative sample of 280 children drawn at random from a population of 15,451. Only 248 children were actually surveyed, the number in each age-sex group varying from 28-33 out of the 35 actually drawn.

The statistical planning of these surveys and the sampling procedure used have been fully described by Fellingham.²

The following biochemical determinations were done for the assessment of protein nutrition status:

1. Serum proteins were determined by the fractional salting out of the different protein fractions. Details of the method were given in an earlier paper.²
2. Urea was determined according to the colorimetric method of Coulombe and Favreau¹ on 2-hour urine specimens collected from 9 to 11 a.m.
3. Urinary creatinine was determined according to the alkaline picrate method of Peters.²

The purpose of the statistical analysis was to determine whether children of different age, sex and racial groups came from the same (statistical) population in respect of the different variables and, if not, to determine between what age, sex and racial groups the differences lay. A 5% level of significance was applied in all statistical tests. The statistical methods used for this purpose are given by Du Plessis *et al.*²

RESULTS AND DISCUSSION

The biochemical results are illustrated by frequency distribution curves (Figs. 1-9). The 5th, 10th, 50th, 90th and 95th percentiles are also given. The results of the analysis of variance and of the multiple comparisons in respect of race are given in Table I.

TABLE I. THREE-WAY ANALYSIS OF VARIANCE AND MULTIPLE COMPARISONS TESTS FOR DIFFERENCES DUE TO RACE, SEX AND AGE*

Variable	Race		Sex	Age
	Analysis of variance	Multiple† comparisons	Analysis of variance	Analysis of variance
Total protein	P<0.1%	<u>W B I C</u>	P>5%	P>5%
Albumin	P<0.1%	<u>W B I C</u>	[P>5%	[P>5%
Albumin as % of total protein, α_1	P<0.1%	<u>I W B C</u>	P>5%	P>5%
Total globulin	P<0.1%	<u>B C I W</u>	P>5%	[P>5%
α -globulin	P<0.1%	<u>W B C I</u>	P>5%	P>5%
β -globulin	P<0.1%	<u>W B C I</u>	P>5%	P>5%
γ -globulin	P<0.1%	<u>W B C I</u>	P<5%	P>5%
γ -globulin as % of total protein	P<0.1%	<u>W B C I</u>	P<5%	P>5%
Urea/creatinine ratio	P<0.1%	<u>W B C I</u>	P<1%	P<5%

*P values of 5% or less indicate a significant difference.

†W=White, B=Bantu, I=Indian, C=Coloured. For the multiple comparisons test the convention of underlining with a common line all groups which did not differ significantly was followed.

The analysis of variance showed no influence of age on any of the serum protein fractions. Since this was also the case in the age-group 7-11 years,² the findings for the Pretoria surveys fully substantiate the work of Oberman *et al.*⁶ who found that after the age of 3 years the serum protein fractions were not influenced by age.

Total Serum Protein

The values for total serum protein concentration obtained in the age-group 12-15 years were similar to those found in the age-group 7-11 years in so far as more than 90% of the values for each race in both age-groups fell into the 'high' range as judged by the ICNND (Inter-

departmental Committee on Nutrition for National Defense) classification.⁷ In the age-group 12-15 years only one child (a Bantu) fell into the 'deficient' range and only one (an Indian child) into the 'low' range (Table II). The

TABLE II. DISTRIBUTION OF TOTAL SERUM PROTEIN VALUES ACCORDING TO THE ICNND CATEGORIES

ICNND categories	% of total White children	% of total Bantu children	% of total Indian children	% of total Coloured children
Deficient < 6.00 G/100 ml.	0	0.4	0	0
Low 6.00-6.39 G/100 ml.	0	0	0.6	0
Acceptable 6.40-6.99 G/100 ml.	6.0	3.0	6.9	3.4
High ≥ 7.00 G/100 ml.	94.0	96.6	92.5	96.6

analysis of variance showed a significant influence of race ($P<0.1\%$) but not of sex or age on the total serum protein values. The multiple comparisons technique used to test for significant differences between all pairs of races showed that all races differed significantly from each other (at a 5% level of significance) with the exception of Coloureds and Indians.

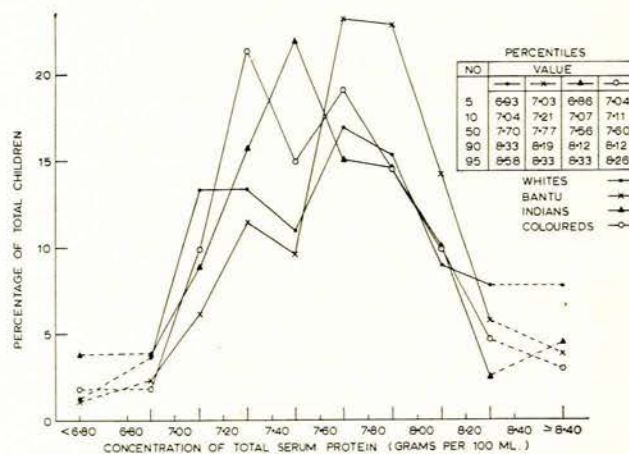


Fig. 1. Frequency distribution of total serum protein values in Pretoria children of 12-15 years.

The findings for total serum protein on the whole confirm the conclusion of Du Plessis *et al.*² that total serum protein has limited value as a criterion for protein nutrition status.

Serum Albumin

The albumin values for the 4 racial groups differed less from one another in the age-group 12-15 years than in the age-group 7-11 years.² This was due to a decrease of 0.42G/100 ml. in the mean albumin value (Table III) for the White children of 12-15 years as compared with the value obtained for the younger children. The mean albumin values for the 2 age-groups did not differ by more than 0.02 G/100 ml. in the 3 non-White races (Table III). The analysis of variance showed no effect of age in either age-group.

The method used for the determination of albumin was tested against a reference serum at regular intervals and yielded consistently accurate results throughout the 4 surveys. The difference between the albumin values for the 2 White age-groups is therefore a real one and probably reflects a change which took place in the protein nutrition status of the children during the interval between the 2 surveys. The sharp increase in the price of meat towards the end of 1964 and the beginning of 1965 may have influenced the albumin values in the latter survey.

TABLE III. MEAN SERUM ALBUMIN VALUES FOR THE AGE GROUPS 7-11 AND 12-15 YEARS

Age-groups	Mean serum albumin concentration (G/100 ml.)			
	White children	Bantu children	Indian children	Coloured children
7-11 years	4.46	3.84	3.95	3.80
12-15 years	4.04	3.86	3.93	3.82

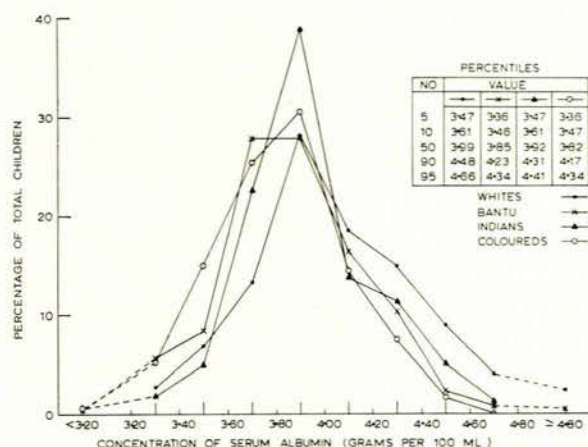


Fig. 2. Frequency distribution of serum albumin values in Pretoria children of 12-15 years.

When the ICNND classification for albumin values⁸ was applied to the results obtained for the children of 12-15 years, it was found that only 0.4% of the Bantu children and none from the other races fell into the 'deficient' range, while 6.9, 11.8, 5.6 and 12.1% of the White, Bantu, Indian and Coloured children respectively fell into the 'low' range. From 88 to 94% of children of all races were therefore in the 'acceptable' and 'high' ranges. The difference in albumin values between the White children of 7-11 years (Table IV) and those of 12-15 years² was evident in the fact that 82.9% of the former fell in the 'high' range while for the latter the figure was only 25.4%.

TABLE IV. DISTRIBUTION OF SERUM ALBUMIN VALUES ACCORDING TO THE ICNND⁸ CATEGORIES

ICNND categories	% of total White children	% of total Bantu children	% of total Indian children	% of total Coloured children
Deficient (<2.80 G/100 ml.)	0	0.4	0	0
Low (2.80-3.52 G/100 ml.)	6.9	11.8	5.6	12.1
Acceptable (3.53-4.20 G/100 ml.)	67.7	77.2	80.6	79.9
High (>4.20 G/100 ml.)	25.4	10.6	13.8	8.0

The analysis of variance showed a significant influence of race, but not of sex or age, upon the albumin values. The multiple comparisons test showed significant differences (at a 5% level) between all pairs of races with the exception of Bantu and Indians and Bantu and Coloureds.

Albumin as a Percentage of Total Serum Protein

To eliminate the influence of factors such as haemo-concentration and oedema, Oberman *et al.*⁶ proposed that the serum albumin concentration be expressed as a percentage of the total serum protein concentration. These authors considered the normal range to be from 50-65%.

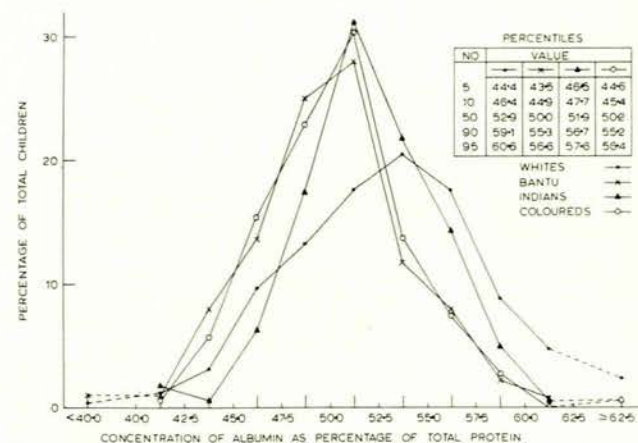


Fig. 3. Frequency distribution of serum albumin values expressed as a percentage of total serum protein in Pretoria children of 12-15 years.

The present results for White children support this view, since the mean values for albumin as a percentage of total serum protein were 58.9% for the children of 7-11 years and 52.8% for those of 12-15² (Fig. 3).

The range for normal values suggested by Oberman⁶ could not be applied to the other 3 racial groups because, although their albumin concentrations were lower than those of the White children, there was virtually no difference in the total serum protein concentrations of the White and non-White groups. This was due to an increase in α - and γ -globulin concentration in the 3 non-White groups, which raised their total serum protein values by 5-9%. Since the increase in α - and γ -globulin is not necessarily related to the protein nutrition status of these groups, Du Plessis, *et al.*² suggested that, to allow for this difference, a figure of 46% instead of 50% be adopted as the lower limit of normal for albumin percentage in the case of the Bantu, Coloured and Indian children.

After further consideration, we have decided that the application of separate criteria for different races is not an altogether satisfactory procedure. We therefore now suggest that the lower limit for the normal albumin percentage be adjusted in accordance with the γ -globulin concentration and progressively decreased according to the scale given in Table V as the γ -globulin increases above 1.3 G/100 ml.

When this new procedure was applied to the results obtained for the 12-15-year-old children, it was found that 19.4, 20.9, 17.2 and 6.3% of the White, Bantu,

Coloured and Indian children respectively had albumin percentages which did not meet the required standards. At this stage no explanation is available of the fact that such a large proportion (19.4%) of the White children had low albumin percentages despite the relatively high mean value. (The albumin and total serum protein values of the White children were, however, somewhat more widely dispersed about the mean than those of the Indian children). The grading for the 3 non-White races agree reasonably well with that based on the serum albumin values and with that obtained for the Bantu, Coloured and Indian children of 7-11 years by the original procedure.²

TABLE V. SUGGESTED LOWER LIMITS FOR ALBUMIN CONCENTRATION AS A % OF TOTAL PROTEIN

<i>γ</i> -globulin concentration G/100 ml. serum	Albumin concentration as % of total protein
< 1.30	50
1.30—1.34	49
1.35—1.39	48
1.40—1.44	47
≥ 1.45	46

The analysis of variance showed a significant influence of race but not of sex or age upon the values for serum albumin expressed as a percentage of total serum protein.

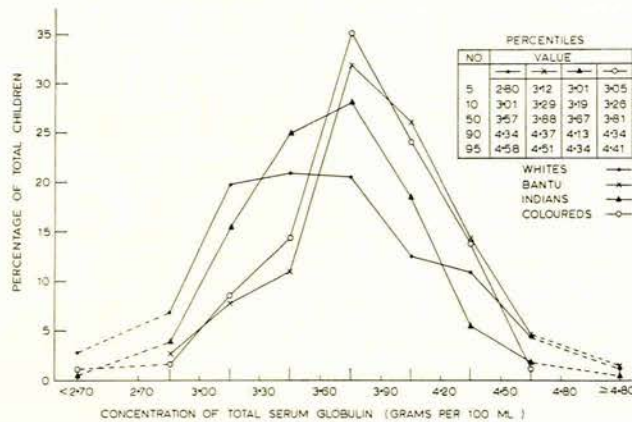


Fig. 4. Frequency distribution of total serum globulin values in Pretoria children of 12-15 years.

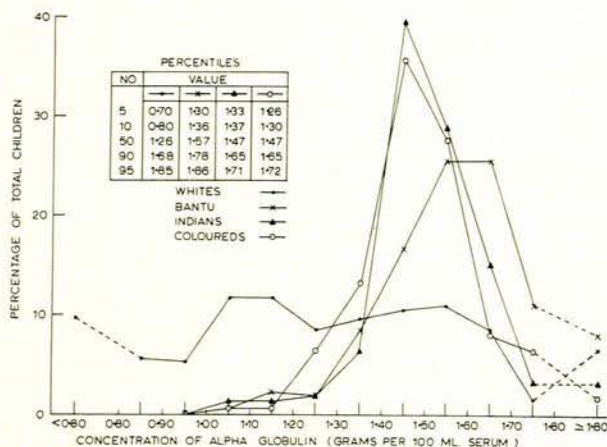


Fig. 5. Frequency distribution of serum α-globulin values in Pretoria children of 12-15 years.

Application of the multiple comparisons test showed that all races differed significantly from each other except (a) White and Indian and (b) Coloured and Bantu.

Serum Globulin

The findings for total serum globulin and the α-, β- and γ-globulin fractions are given in Figs. 4-8.

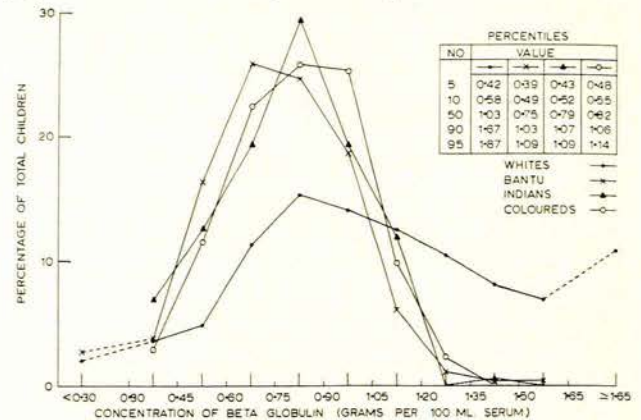


Fig. 6. Frequency distribution of serum β-globulin values in Pretoria children of 12-15 years.

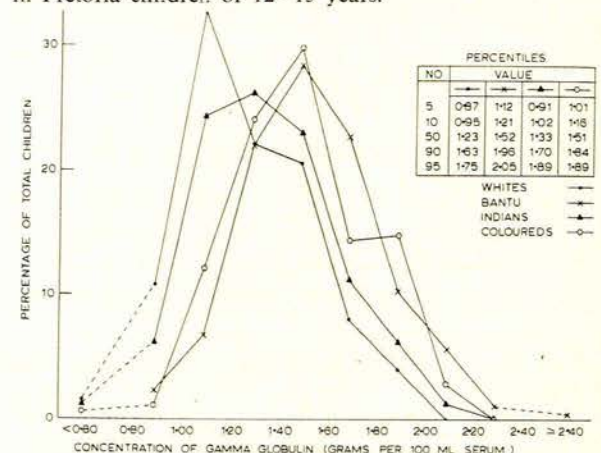


Fig. 7. Frequency distribution of serum γ-globulin values in Pretoria children of 12-15 years.

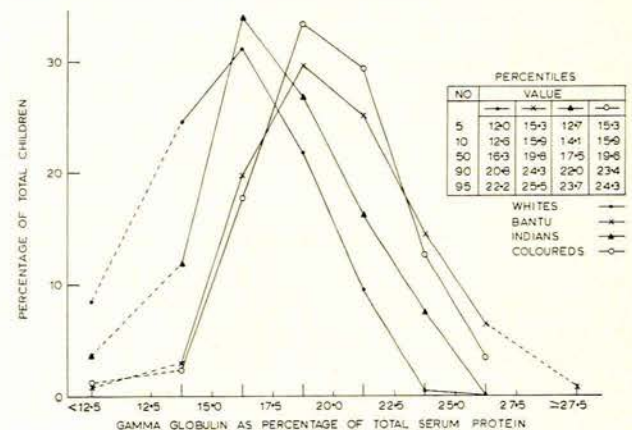


Fig. 8. Frequency distribution of serum γ-globulin values expressed as a percentage of total serum protein in Pretoria children of 12-15 years.

Since very little is known about the nutritional implications of the serum globulins in latent protein deficiency, no attempt will be made to evaluate the findings for the serum globulin fractions in terms of protein nutrition status. In a previous article, however, Du Plessis *et al.*² discussed the possible significance of variations in the albumin and globulin fractions.

From Figs. 5, 7 and 8 it is clear that the α - and γ -globulin concentrations were higher in the Bantu, Coloured and Indian groups than in the White children. The analysis of variance showed a significant influence of race ($P < 0.1\%$) on all the globulin fractions but no influence of sex or age except in the case of γ -globulin, where a significant influence of sex ($P < 5\%$) was found.

Urea/Creatinine Ratio

The possibility that urea output could be used to assess the quantity and quality of the protein intake has long been appreciated.⁹ On the basis of a publication by Arroyave,¹⁰ Du Plessis *et al.*² suggested that children of 7-11 years excreting urea and creatinine in a ratio of less than 18:1 be considered to exhibit sub-optimal protein nutrition. At that stage no data for White children were available* and the results obtained by the application of this criterion to the non-White children seemed very promising.

When the survey on White children of 12-15 years was completed in 1965, comparable results in another age range became available for all 4 racial groups. The statistical analysis of these results showed that the urea/creatinine ratios of White children differed significantly (at a 5% level) from those of the other 3 racial groups but that the Bantu, Coloured and Indian groups did not differ signi-

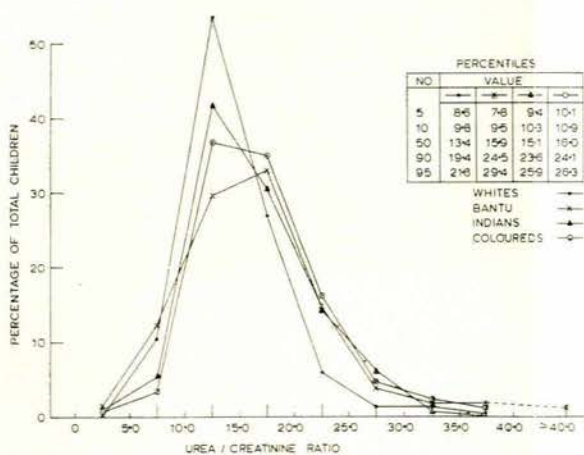


Fig. 9. Frequency distribution of urinary urea/creatinine values in Pretoria children of 12-15 years.

ficantly from one another. A significant influence of sex ($P < 1\%$) as well as of age ($P < 5\%$) was found.

It is clear from Fig. 9 that the urea/creatinine ratios of the White children were substantially lower than those of the Bantu, Coloured and Indian children. If this finding is an indication of sub-optimal protein nutrition, it directly contradicts the results of the dietary survey^{11,12} conducted

on these children. The implications of the urea/creatinine ratio had therefore to be reassessed.

The level of urea excretion is dependent on 3 main factors, viz.:

- The quantity of protein fed. Urinary urea excretion increases (i) when the intake of a protein of 100% assimilability increases beyond the optimal requirements and (ii) with an increase in intake at all levels when the assimilability of the proteins is less than 100%. The latter situation always applies under normal dietary conditions.
- The quality of the protein. The lower the quality of the protein the greater is the non-assimilable fraction. This fraction is metabolized and most of the nitrogen excreted via the urea cycle.
- Calorie intake. Protein is metabolized to furnish energy when the non-protein calorie intake is too low to meet the requirements.

The dietary survey^{11,12} showed that the animal protein intake of the 3 Coloured races was considerably lower than that of the White children. The protein intake of the Bantu, Coloured and Indian children was made up largely of protein of vegetable origin. Since the biological value of vegetable protein is invariably lower than that of animal origin, this factor would tend to raise the urea/creatinine ratios of the non-White children and would account, in part at any rate, for the apparently contradictory results obtained. Calorie deficiency in some individuals may also have helped to increase the figures for urea excretion in the 3 Coloured races. In addition, intakes of protein relative to the requirement were probably lower in the White children of the 1965 survey than in the younger White children, as judged by the difference in serum albumin concentration.² These factors probably account for the lower urea/creatinine ratios found for the White than for the Bantu, Coloured and Indian children of 12-15 years.

The above considerations indicate that in populations where the basic dietary habits differ widely (as is the case among the 4 racial groups in Pretoria) the ratio of urea to creatinine excretion cannot be used as a criterion of protein nutrition status. There is, however, a possibility that this ratio may prove useful in populations that are homo-

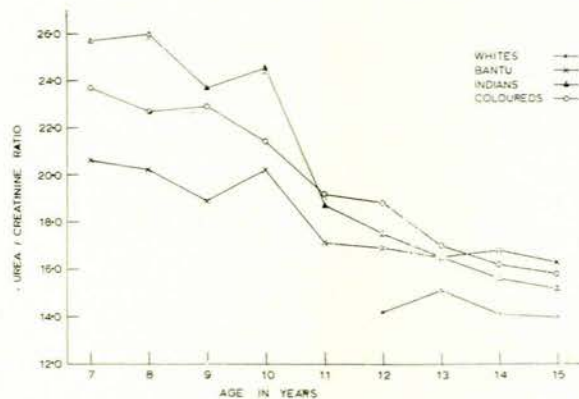


Fig. 10. The relationship between age and mean urea/creatinine ratio's in Pretoria children of 7-15 years.

*The method for the determination of urinary urea was not yet in use in our laboratories in 1962 when the White children of 7-11 were surveyed.

geneous in respect of their dietary habits. If it is used, the influence of age and sex should be taken into account. The influence of age on the present findings is shown in Fig. 10. The influence of sex is evident from the mean values, viz. 22.4 for boys and 20.68 for girls of 7 - 11 years, and 16.66 for boys and 15.44 for girls of 12 - 15 years.

CONCLUSION AND SUMMARY

It would appear that serum albumin concentration and the percentage of albumin in the total serum protein still constitute the most effective criteria for recognizing latent protein deficiency in a population. The ratio of urea to creatinine in the urine proved unsuitable for application to the Pretoria population because of the wide differences in the dietary habits of its 4 racial groups. This parameter may, however, have value in a more homogeneous population.

The serum albumin concentrations and the values for serum albumin as a percentage of total serum protein indicate that

from 5 to 20% of children of all racial groups in Pretoria, would benefit from a higher protein intake.

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