

The effects of variations in the fat and carbohydrate content of the diet on the levels of magnesium and cholesterol in the serum of white rats*

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It has been well established that dietary constituents, such as saturated and unsaturated fats and magnesium, can influence serum cholesterol levels in man and various experimental animals: saturated fatty acids cause hypercholesterolaemia whereas polyunsaturated fatty acids give rise to hypocholesterolaemia (Groen, Tjong, Kamminga & Willebrand, 1952; Donath, Fischer, van der Meulen-van Eysbergen & de Wijn, 1953; Beveridge, Connell & Mayer, 1957).

It has been shown that in population groups consuming a low-fat diet, in which the fat was predominantly of plant origin, the serum cholesterol level as well as the incidence of thrombosis and other circulatory disorders were comparatively very low (Méndez, Tejada & Flores, 1962). Further, it has become common therapeutic practice to replace all saturated fats by unsaturated fats in the diets of patients suffering from atherosclerotic heart disease (Beveridge *et al.* 1957; Beveridge, Connell, Mayer & Haust, 1958; Boyle, Nichaman & Moore, 1962; Beveridge & Connell, 1962).

There appears to be an inverse relationship between the level of Mg and the level of cholesterol in the serum of man (Bersohn & Oelofse, 1957*a*).

Dietary Mg has been shown to influence the deposition of fat in the arterial system in rats (Vitale, White, Nakamura, Hegsted, Zamcheck & Hellerstein, 1957). These authors found that circulatory disorders were commonly associated with low serum Mg levels. It has further been shown that the abnormal lipoprotein pattern in coronary thrombosis patients, as well as their general clinical picture, are greatly improved by parenteral Mg sulphate therapy (Malkiel-Shapiro, Bersohn & Turner, 1956).

Population groups with a high serum cholesterol level show a greater incidence of circulatory disorders than do groups with a low serum cholesterol level (Higginson & Pepler, 1954; Bersohn & Oelofse, 1957*b*; Méndez *et al.* 1962; Walker, 1963).

Higginson & Pepler (1954) indicated that the mortality rate as the result of coronary thrombosis in the Bantu averaged only 1.6% for the 41–60 year age group, and 3.0% for the 61–80 year age group, whereas the figures for the corresponding European groups were 12.8 and 13.3%, respectively.

Basic differences between the diet of the Bantu and that of the European may, however, be a causative factor in this respect: on the average, less than 20% of the

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total calories in the Bantu diet is derived from fat, whereas 40% of the total calories in the European diet is derived from fat (Walker & Arvidsson, 1954; Walker, 1955).

It is well recognized that circulatory disorders cannot be ascribed simply to any single factor and that they should rather be regarded as multiple-factor disorders. It is, however, accepted that factors such as the serum cholesterol level, serum Mg level and the dietary content of saturated and unsaturated fats, *inter alia*, may have an important bearing on the pathogenesis of circulatory disorders. Consequently, this study was carried out, in the first instance to establish the relationship between saturated and unsaturated fat in the diet and the levels of cholesterol and Mg in the serum. In the second instance different levels of dietary Mg were combined with different dietary fats to ascertain whether or not increased Mg intake suppressed hypercholesterolaemia.

EXPERIMENTAL

Rats

White rats weaned at 3 weeks, and given a balanced rat-cube diet until the experiments began, were used throughout this study. The rats were of a pure strain bred at the University of Potchefstroom and the balanced rat-cube diet was obtained from Messrs Lion Bridge Products, Pretoria.

Expt 1, with basal diet adequate in Mg

Composition of diets. The balanced rat-cube diet was used as control diet. The composition of this diet, control diet (a), as regards the content of fat, Mg, cholesterol and protein is shown in Table 1.

Table 1. *Control diet (a)*

	Fat (g/100 g)	Protein (g/100 g)	Magnesium (g/100 g)	Cholesterol (g/100 g)
Maximum	9.12	23.04	0.300	0.245
Minimum	6.0	18.36	0.255	0.220
Mean	8.0	19.87	0.290	0.230
No. of determinations	8	8	8	8

Diets (b), (c) and (d) were similar to the control diet but were supplemented with 25% butter (b), 25% dripping (c), and 25% sunflower-seed oil (d) respectively, calculated on a weight basis.

Procedure. Rats were divided into four groups. The number of rats and the diet given to each group were:

No. of rats	Diet
23	Control diet (a)
20	Diet (b) containing 25% domestic butter
20	Diet (c) containing 25% dripping
20	Diet (d) containing 25% sunflower-seed oil

The duration of this experiment was 4 weeks.

Method of blood sampling. The method of Burhoe (1940), whereby 2.5 ml blood are withdrawn directly from the heart, was employed throughout. Blood was withdrawn

from six rats out of each group weekly. Blood from the same animal was, therefore, withdrawn at intervals of 3 weeks. Blood samples were allowed to coagulate at room temperature. After centrifuging, the serums were stored in a deep freeze and the determination was done on the following day.

Expts 2A and B, with basal diet low in Mg

Expt 2A

Composition of diets. The low-Mg diet, diet (e), described by Vitale *et al.* (1957), was used except that cellulose acetate was used instead of CellufLOUR and sunflower-seed oil instead of Spry fat. The salt mixture of Jones & Foster (1942) was used except that CaCO₃ and MgSO₄ were omitted from the mixture, and CaCO₃ and MgCl₂ added as shown in Table 2. The mean cholesterol content of this diet was found to be 285.0 mg/100 g. The compositions of diet (e) and of the salt mixture are shown in Tables 2 and 3 respectively. The following vitamin additions were made to each kg of diet: 4 mg thiamine hydrochloride, 8 mg riboflavine, 4 mg pyridoxine hydrochloride, 25 mg calcium pantothenate and 40 mg nicotinic acid. The fat content of maize meal averaged 3.2 g/100 g, the protein content 9.49 g/100 g and the Mg content 98.0 mg/100 g.

Table 2. *Basal diet (e) (g/100 g)*

Casein	10.0	Vitamin mixture*	0.1
Glucose	58.1	Sunflower-seed oil	20.0
Salt mixture (Table 3)	5.0	CaCO ₃	1.5
Cellulose acetate	5.0	MgCl ₂	0.025
Choline chloride	0.3		

* Contained halibut oil 5.0 g, α -tocopheryl acetate 1.0 g, sunflower-seed oil 44.0 g.

Table 3. *Salt mixture (g)*

NaCl	292.5	MnSO ₄ .2H ₂ O	9.35
KH ₂ PO ₄	816.6	ZnCl ₂	0.5452
FeSO ₄ .7H ₂ O	56.6	CuSO ₄ .5H ₂ O	0.9988
KI	1.66	CoCl ₂ .6H ₂ O	0.0476

Procedure. There were twelve rats in the control group and twelve in the experimental group. This experiment lasted 9 weeks. The control group received the low-Mg diet, diet (e), throughout. The diet of the experimental group was varied as follows. They received diet (e) for 3 weeks. At this stage and during the rest of the experiment the sunflower-seed oil in the diet was replaced by the same weight of domestic butter (20 g/100 g). After 6 weeks the glucose in the diet was replaced by the same weight of maize meal (58 g/100 g), and the diet so changed was given until the end of the experiment. Blood sampling was the same as described for Expt 1.

Expt 2B

The same diet as for Expt 2A was used. There were ten rats in the control group and ten in the experimental group. The procedure was the same as in Expt 2A, except that sunflower-seed oil was replaced by butter after 2 weeks and blood for analysis was withdrawn from each animal every week.

Analytical methods

Diets. Fat content was determined by means of the Soxhlet apparatus, cholesterol by the method of Sperry & Webb (1950), protein by the Kjeldahl method and Mg by the method of Orange & Rhein (1951), with the modification that a 50 mg/100 ml titan yellow solution was employed instead of the prescribed 25 mg/100 ml solution. This modification was introduced because it was observed that the relationship between Mg concentration and the extinction was more nearly rectilinear when an increased concentration of titan yellow was used. This effect is illustrated in Fig. 1.

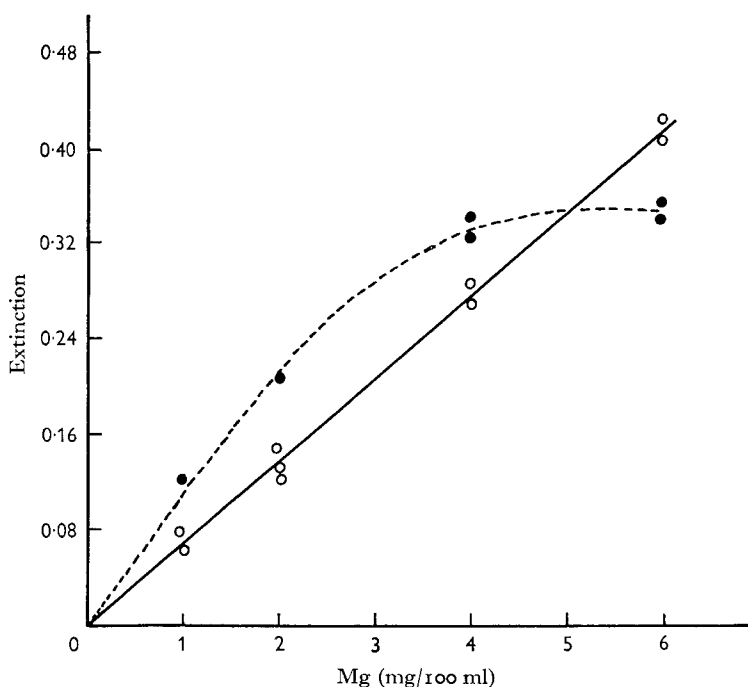


Fig. 1. Relationship between extinction and magnesium concentration in standard solutions of titan yellow. ●—●, 25 mg/100 ml; ○—○, 50 mg/100 ml.

Serum. Serum cholesterol and Mg were determined by the same methods as used for analysis of the diets.

Changes in serum Mg and cholesterol levels were compared by means of *t* tests throughout this study.

RESULTS

Expt 1

Figs. 2*a* and *b* show the mean weekly serum Mg and cholesterol levels after the addition of 25% domestic butter to the diet. The decline in serum Mg level was marked for the first 2 weeks and thereafter stayed the same for the second 2-week period. The decline in the serum Mg level was found to be statistically significant ($P < 0.01$).

The inverse relationship between serum Mg and cholesterol (Bersohn & Oelofse, 1957*a*) is clearly illustrated. As the serum Mg level decreased from 3.9 to 2.5 mg/100 ml the serum cholesterol level showed an increase from 65.8 to 81.6 mg/100 ml over the 4-week period. The latter increase was found to be statistically significant ($P < 0.001$).

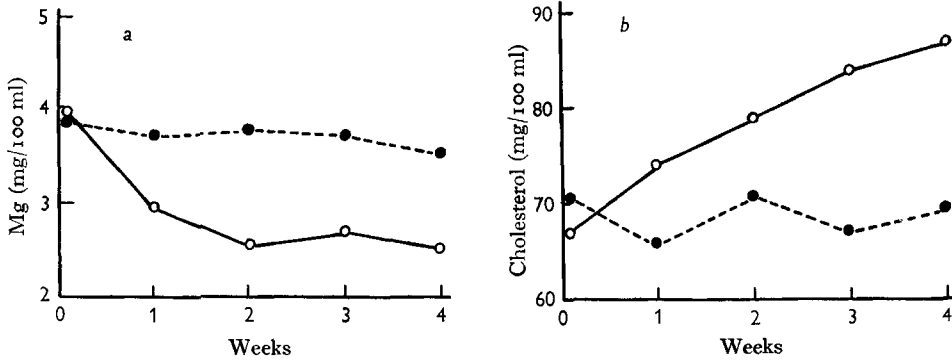


Fig. 2. Expt 1. Effect of supplementing the diet with 25% domestic butter on the concentration of (a) magnesium and (b) cholesterol in the serum of rats. ●—●, unsupplemented diet; ○—○, supplemented diet.

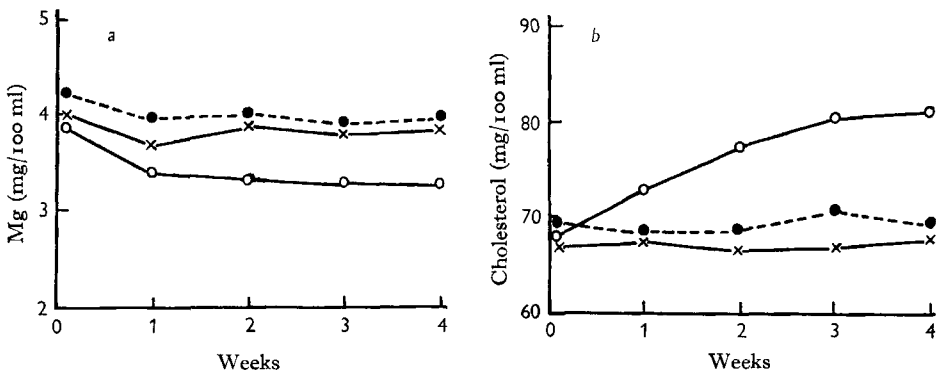


Fig. 3. Expt 1. Effect of supplementing the diet with 25% dripping or 25% sunflower-seed oil on the concentration of (a) magnesium and (b) cholesterol in the serum of rats. ●—●, control diet (a); ○—○, diet (c) (25% dripping); ×—×, diet (d) (25% sunflower-seed oil).

Figs. 3*a* and *b* show the mean weekly serum Mg and cholesterol levels with the experimental diets supplemented by 25% sunflower-seed oil and dripping respectively. The addition of sunflower-seed oil caused no statistical change in either serum Mg or cholesterol level. In contrast, supplementation with dripping caused a decrease in the serum Mg level from 3.96 to 3.08 mg/100 ml, which was found to be statistically significant ($P < 0.01$), and an increase in the serum cholesterol level from 68.3 to 80.3 mg/100 ml which was also found to be statistically significant ($P < 0.001$).

Expt 2

Expt 2A. Figs. 4*a* and *b* show the mean serum Mg and cholesterol levels respectively when, in the low-Mg diet, sunflower-seed oil was replaced by domestic butter after 3 weeks and glucose by maize meal after 6 weeks.

The mean serum Mg levels in both the control and experimental groups decreased from 4.5 to 1.5 mg/100 ml and remained stable at this level in animals receiving the low-Mg diet.

The replacement of sunflower-seed oil by domestic butter did not significantly affect the serum Mg level; replacement of glucose by maize meal, however, caused a marked increase from 1.2 to 3.0 mg/100 ml when the level had become stable. This increase was found to be statistically significant ($P < 0.001$).

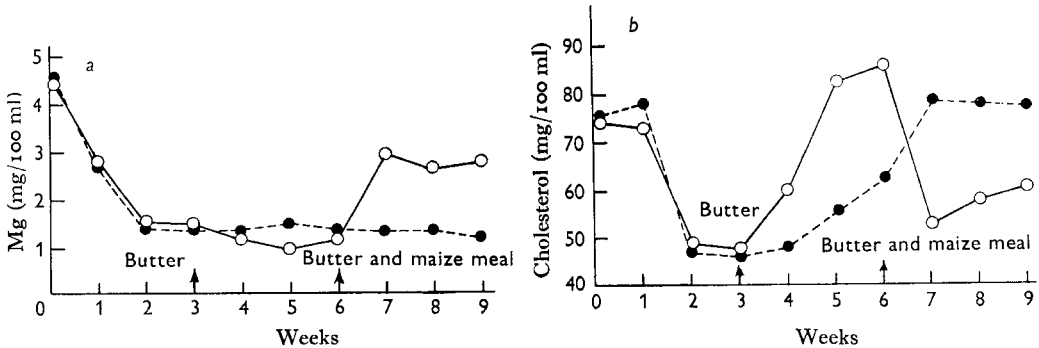


Fig. 4. Expt 2A. Effect of the replacement of sunflower-seed oil by domestic butter, and glucose by maize meal, on the concentration of (a) magnesium and (b) cholesterol in the serum of rats. ●-●, control; ○-○, experimental.

The serum cholesterol level of both the control and experimental groups decreased sharply when the low-Mg diet was given. However, after 3 weeks the serum cholesterol level of the control group, in contrast to the serum Mg, began to increase and attained the earlier level after 7 weeks. This may be ascribed to an increased endogenous cholesterol production (Gould & Taylor, 1950; Bronte-Stewart, Antonis, Eales & Brock, 1956).

In the experimental group, replacement of sunflower-seed oil by butter caused a statistically significant increase, from 47 to 86 mg/100 ml, in the serum cholesterol level ($P < 0.05$). Replacement of glucose by maize meal caused, after 1 week, a significant decrease in the serum cholesterol level, from 86 to 56 mg/100 ml ($P < 0.05$).

At the end of the 6th week the difference in serum cholesterol level between the control and experimental groups amounted to 23.5 mg/100 ml. This difference was found to be statistically non-significant ($P > 0.05$).

After 9 weeks the serum cholesterol levels of the control and experimental groups did not differ statistically although the mean value attained for the experimental group, 61 mg/100 ml, was considerably lower than that of the control group, 78 mg/100 ml.

Expt 2B. Figs. 5*a* and *b* show the serum Mg and serum cholesterol levels. As with Expt 2A, the serum Mg level of the control and experimental animals decreased during the first 2 weeks when the low-Mg diet was given. Thereafter the serum Mg level of the control group remained stable throughout the experimental period of 8 weeks. Replacement of the sunflower-seed oil by butter after 2 weeks did not cause any significant change in the serum Mg level of the experimental group. This result is similar to that for Expt 2A. Further, replacement of glucose by maize meal in the 5th week caused a significant increase in the serum Mg level of the experimental animals during the following 3 weeks ($P < 0.001$), and the serum Mg levels of the control and experimental animals were found to differ significantly at the end of the experiment ($P < 0.001$).

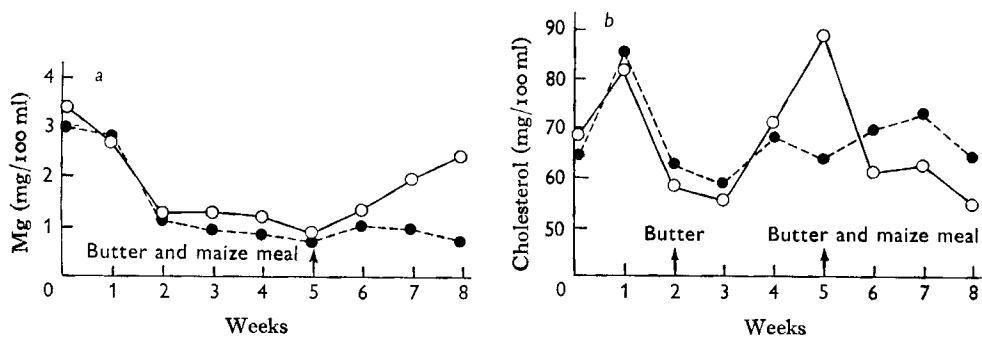


Fig. 5. Expt 2B. Effect of the replacement of sunflower-seed oil by domestic butter, and glucose by maize meal, on the concentration of (a) magnesium and (b) cholesterol in the serum of rats. ●--●, control; ○—○, experimental.

Similarly, serum cholesterol values, although slightly different as far as the absolute figures were concerned, were found to be similar at various stages in this experiment as compared with those in the preceding one. It is clear therefore that neither the serum Mg nor the cholesterol levels were affected by the 3-weekly withdrawal of blood for analysis.

DISCUSSION

The determination of Mg by the method of Orange & Rhein (1951), employing titan yellow, has been subjected to criticism by Vallee (1954) and Wacker & Vallee (1957). According to these authors, the flame photometric method would be more reliable. The latter method was, however, also criticized by Hanna, MacIntyre, Harrison & Fraser (1960). In our investigation the titan yellow method was found reliable provided that a 50 mg/100 ml instead of a 25 mg/100 ml titan yellow solution was used.

The findings that hypercholesterolaemia follows the ingestion of large quantities of saturated fats and that hypocholesterolaemia is associated with the ingestion of unsaturated fat (Groen *et al.* 1952; Beveridge *et al.* 1958; Beveridge & Connell, 1962; Boyle *et al.* 1962), as well as the demonstration of an inverse relationship between serum Mg and serum cholesterol levels (Bersohn & Oelofse, 1957*a*; Malkiel-Shapiro

et al. 1956), have been confirmed by the results obtained in Expts 1 and 2. In the latter, however, it was also shown that the hypercholesterolaemia which follows the ingestion of saturated fat is effectively countered by the replacement of glucose by maize meal in the diet. The relative hypocholesterolaemia which followed the addition of maize meal to the diet was closely associated with a corresponding increase in the serum Mg levels (Figs. 4*a* and *b*; 5*a* and *b*). It has been shown that the Mg content of maize meal is high (Crawford, Hamersma & Marloth, 1942). Consequently the protective hypocholesterolaemic action afforded by maize meal can, at least partly, be ascribed to this fact. This supposition is in line with the results of Malkiel-Shapiro *et al.* (1956), Parsons, Butler & Sellars (1959) and Cradock (1960), who indicated the hypolipaeamic action of parenterally administered magnesium sulphate.

There are, however, further aspects that have a bearing on the hypocholesterolaemic action of maize meal: it has been found that, if cereals with a high content of fibre are included in the diet, fat absorption in the small intestine is decreased (McCance & Glaser, 1948-9; Walker, 1951; Coleman & Baumann, 1957). Further, maize meal has a high fibre content (Crawford *et al.* 1942). It seems likely, therefore, that the fibre content of the maize meal of experimental diets used was also a contributory factor in the observed hypolipaeamia.

It has also been shown that the activity of the microflora in the large intestine, where cholesterol and other sterols are broken down, is increased specifically by maize starch (Gofman, 1958). This action of maize meal must, therefore, be regarded as a further contributory factor to its hypocholesterolaemic action.

Finally, attention has been drawn to a non-fatty stimulant of bile secretion contained in cereals (Christensen, Dam & Prange, 1952; Christensen, Dam & Kristensen, 1956); it has been shown that the bile salts are actively hypercholesterolaemic (Swell, Field & Treadwell, 1953; Phil, 1955), and that their addition to the diet causes experimental hypercholesterolaemia (Member, Bruger & Oppenheim, 1944; Vitale *et al.* 1957). The microflora, however, in the large intestine breaks down the conjugated biliary acids to less absorbable compounds (Norman, 1955). The action of maize meal in increasing bile secretion, therefore, is overshadowed by its stimulating action on intestinal microflora, thereby effecting an increased cholesterol secretion, first, by rendering the dietary cholesterol in the intestine less absorbable and, secondly, by breaking down the cholesterol contained in the bile to less absorbable compounds. This action of maize meal may consequently play an important part in its hypocholesterolaemic action.

The close correlation between dietary fat content, hypercholesterolaemia and atherosclerosis has repeatedly been pointed out (Gordon, Bland & White, 1939; Méndez *et al.* 1962; Walker, 1963). The relatively low incidence of atherosclerosis in the Bantu as compared with the European has been attributed to various factors including, *inter alia*, the fact that the staple diet of the Bantu consists mainly of cereals (Higginson & Pepler, 1954; Walker & Arvidsson, 1954; Walker, 1955). The results of this study therefore, support this theory.

SUMMARY

1. Two experiments, with 147 rats as experimental animals, were carried out. Rats were given a balanced diet alone or supplemented with butter, dripping or sunflower-seed oil, or a low-magnesium diet with sunflower seed oil or butter as the fat component, or the low-Mg diet with sunflower-seed oil and either glucose or maize meal.

2. In the first experiment the contents in the diet of saturated and unsaturated fat were varied. It was found that the addition of saturated fats caused hypercholesterolaemia accompanied by low serum Mg levels, whereas the addition of unsaturated fat caused hypocholesterolaemia accompanied by an increased serum Mg level.

3. In the second experiment unsaturated fat in a low-Mg diet was replaced by saturated fat, and glucose by maize meal. It was found that the hypercholesterolaemia and decreased serum Mg level which followed replacement of unsaturated by saturated fat was countered by the replacement of glucose by maize meal.

4. The hypocholesterolaemic effect of maize meal is attributed to (1) its high Mg content, (2) its stimulating effect on the intestinal microflora, thereby increasing the excretion of cholesterol, and (3) its high fibre content, which also enhances the excretion of cholesterol.

5. It is concluded that the above attributes of maize meal may partly account for the relatively low incidence of circulatory diseases in the Bantu.

REFERENCES

- Bersohn, I. & Oelofse, P. J. (1957*a*). *Lancet*, i, 1020.
 Bersohn, I. & Oelofse, P. J. (1957*b*). *S. Afr. med. J.* **31**, 1172.
 Beveridge, J. M. R. & Connell, W. F. (1962). *Amer. J. clin. Nutr.* **10**, 391.
 Beveridge, J. M. R., Connell, W. F. & Mayer, G. A. (1957). *Canad. J. Biochem. Physiol.* **35**, 257.
 Beveridge, J. M. R., Connell, W. F., Mayer, G. A. & Haust, H. L. (1958). *Canad. J. Biochem. Physiol.* **36**, 895.
 Boyle, E., Nichaman, M. Z. & Moore, R. V. (1962). *Amer. J. clin. Nutr.* **10**, 443.
 Bronte-Stewart, B., Antonis, A., Eales, L. & Brock, J. F. (1956). *Lancet*, i, 521.
 Burhoe, S. O. (1940). *J. Hered.* **31**, 445.
 Christensen, F., Dam, H. & Kristensen, G. (1956). *Acta physiol. scand.* **36**, 329.
 Christensen, F., Dam, H. & Prange, I. (1952). *Acta physiol. scand.* **27**, 315.
 Coleman, D. L. & Baumann, C. A. (1957). *Arch. Biochem. Biophys.* **66**, 226.
 Craddock, A. L. (1960). *Lancet*, ii, 1348.
 Crawford, D. C., Hamersma, P. J. & Marloth, B. W. (1942). *Department of Agriculture and Forestry, Chemical Series no. 171, Science Bulletin no. 20*. Pretoria: Government Printer.
 Donath, W. F., Fischer, I. A., van der Meulen-van Eysbergen, H. C. & de Wijn, J. F. (1953). *Voeding*, **14**, 153.
 Gofman, J. W. (1958). *Amer. J. Cardiol.* **1**, 271.
 Gordon, W. H., Bland, E. F. & White, P. D. (1939). *Amer. Heart J.* **17**, 10.
 Gould, R. G. & Taylor, C. B. (1950). *Fed. Proc.* **9**, 179.
 Groen, J., Tjong, B. K., Kamminga, C. E. & Willebrand, A. F. (1952). *Voeding*, **13**, 556.
 Hanna, S., MacIntyre, I., Harrison, M. & Fraser, R. (1960). *Lancet*, ii, 172.
 Higginson, J. & Pepler, W. J. (1954). *J. clin. Invest.* **33**, 1366.
 Jones, J. H. & Foster, C. (1942). *J. Nutr.* **24**, 245.
 McCance, R. A. & Glaser, E. M. (1948-9). *Brit. J. Nutr.* **2**, 221.
 Malkiel-Shapiro, B., Bersohn, I. & Terner, P. E. (1956). *Med. Proc.* **2**, 455.
 Member, S., Brugger, M. & Oppenheim, E. (1944). *A.M.A. Arch. Path.* **38**, 210.
 Méndez, J., Tejada, C. & Flores, M. (1962). *Amer. J. clin. Nutr.* **10**, 403.
 Norman, A. (1955). *Acta physiol. scand.* **33**, 99.
 Orange, M. & Rhein, H. C. (1951). *J. biol. Chem.* **189**, 379.
 Parsons, R. S., Butler, T. & Sellars, E. P. (1959). *Lancet*, ii, 487.

- Phil, A. (1955). *Acta physiol. scand.* **34**, 206.
Sperry, W. M. & Webb, M. (1950). *J. biol. Chem.* **187**, 97.
Swell, L., Field, H. Jr. & Treadwell, C. R. (1953). *Proc. Soc. exp. Biol., N.Y.*, **84**, 417.
Vallee, B. L. (1954). *Nature, Lond.*, **174**, 1050.
Vitale, J. J., White, P. L., Nakamura, M., Hegsted, D. M., Zamcheck, N. & Hellerstein, E. E. (1957). *J. exp. Med.* **106**, 757.
Wacker, W. E. C. & Vallee, B. L. (1957). *New. Engl. J. Med.* **257**, 1254.
Walker, A. R. P. (1951). *S. Afr. J. Sci.* **47**, 267.
Walker, A. R. P. (1955). *Lancet*, **i**, 565.
Walker, A. R. P. (1963). *Amer. Heart J.* **66**, 293.
Walker, A. R. P. & Arvidsson, U. D. (1954). *J. clin. Invest.* **33**, 1358.