The influence of dietary tea, coffee and cocoa on protein and energy utilization of soya-bean meal and barley in rats

BY B. O. EGGUM, BIRTHE PEDERSEN AND INGEBORG JACOBSEN

National Institute of Animal Science, Department of Animal Physiology and Chemistry, Rolighedsvej 25, DK-1958 Copenhagen, Denmark

(*Received 11 February 1983 – Accepted 15 April 1983*)

1. Two series of balance experiments were performed with growing rats to test the effect of black tea, green tea, coffee and cocoa on protein and energy utilization. In Expt 1 soya-bean meal was fed as a basal diet and supplemented with freeze-dried materials from 1 l black tea, green tea or coffee/500 g dry matter. Cocoa powder, corresponding to 1 l of the beverage, was also added to the basal diet. In Expt 2 the procedure was repeated with a barley-based diet.

2. In both experiments both tea varieties and coffee had significantly negative effects on true protein digestibility and biological value, while digestible energy was only slightly affected in the barley-based diet. Cocoa had no effect on protein or energy utilization in either soya-bean meal or barley diets, although the protein in cocoa powder was completely indigestible.

3. As the tannin concentration in both tea varieties and coffee was very high it is assumed that the observed deleterious effects might, in part, be explained by anti-nutritional effects of tannin.

4. The strongest deleterious effect was recorded for black tea.

Beverages prepared from tea, coffee and cocoa are important constituents of human diets and yet little is known about their effects on nutrient utilization. There is extensive evidence that tea inhibits iron absorption (Stagg & Millin, 1975; Hessling *et al.* 1979; Rossander *et al.* 1979). Naismith *et al.* (1969) showed that tea, coffee, decaffeinated coffee and caffeine had no effect on growth or feed utilization of young rats, when added to a semi-purified diet, whereas results of Sibbald (1982) suggest that tea and coffee induce reduced digestibility of vegetable protein, while enhancing the utilization of animal protein, in cockerels. As described by Derman *et al.* (1977) tea polyphenols complex with the casein of added milk, but do not prevent normal digestion of the protein. However, several investigations (e.g. Eggum & Christensen, 1975) have demonstrated a strong negative relationship between dietary tannin and protein digestibility.

Tea, coffee and cocoa are frequently consumed during or immediately following a meal. Thus, they become immediately mixed with other dietary constituents in a warm, moist environment possibly conducive to chemical interactions. However, according to Stagg & Millin (1975) tea polyphenols are chemically quite distinct from tannic acid or other commercial tannins and do not induce an adverse gastrointestinal reaction. Although they react with proteins, tea polyphenols do not exhibit the strong irreversible effects associated with true tannins.

Protein constitutes 150–230 g dry weight/kg black tea, but less than 20 g hot-water-soluble solids/kg (McCance & Widdowson, 1960; Das *et al.* 1964). According to Brown & Wright (1963) the protein intake from tea alone is unlikely to exceed 70 mg/d and, therefore, it is insignificant. However, added milk might contribute significant amounts of protein, and althouth casein complexes with tea polyphenols, which reduce the astringency of the tea, this does not prevent normal digestion of the protein.

The present work was undertaken to gain more information on the effect of beverages prepared from tea, coffee and cocoa on protein and energy metabolism. Biological trials were performed with rats and true protein digestibility (TD), biological value (BV), net

protein utilization (NPU), digestible energy (DE) and true amino acid digestibility (TAAD) were determined.

EXPERIMENTAL

Diets

Two protein sources of different quality, soya-bean meal and barley (Expts 1 and 2 respectively) were used to study the effect of black tea, green tea, coffee and cocoa on protein and energy utilization. For each of the three beverages 1 l was prepared in a traditional way and freeze-dried. A daily consumption for a man of 1 l of either of these beverages has been considered to be a realistic amount. Black and green tea were prepared from 10 g dry matter (DM) of leaves. For black tea 4·0 g DM were dissolved, while 3·5 g of green tea were soluble. The coffee was prepared from 35·2 g DM of finely ground beans and 9·2 g were dissolved in the beverage. The freeze-dried materials from 1 l of the two tea varieties and the coffee and 24 g cocoa powder were added individually to 500 g DM with soya-bean meal (Expt 1) or barley (Expt 2) as the protein source. This amount of food corresponds to approximately 9·6 MJ (2300 kcal), which should be close to the daily requirement of energy of an adult person. Because of the relatively high amount of nitrogen in cocoa powder a preliminary experiment was performed with cocoa, fed together with casein as the protein source. As the protein in casein is completely digestible it was thus possible to get an estimate of the digestibility of the protein in cocoa powder. In total, ten diets were tested as each experiment also included a control diet without the addition of freeze-dried materials from the beverages. The N content of the diets was adjusted using a basal diet consisting of an N-free mixture according to Eggum (1973). Minerals and vitamins were also added to meet the requirements. The composition of the diets is shown in Table 3 (see p. 201).

Animals and feeding

The experimental procedure has been described by Eggum (1973). Groups of five Wistar male rats, each weighing approximately 68 g, were used in the experiments with preliminary periods of 4 d and balance periods of 5 d. Each animal received 150 mg N and 10 g DM daily throughout the preliminary and balance periods. The rats were housed in individual metabolic cages. A 5 d collection of urine and faeces from each rat was analysed.

Chemical analyses

Diets were analysed for DM, N, energy and amino acids. Faeces and urine of individual rats were analysed for N and faeces were also analysed for energy and amino acids. DM and N were determined according to standard methods (Association of Official Agricultural Chemists, 1975). Gross energy was determined as described by Weidner & Jakobsen (1962). Amino acid analyses were carried out according to Mason *et al.* (1980). The freeze-dried materials of tea and coffee and the cocoa powder were analysed for DM, N, ash and tannin. The procedure of Eggum & Christensen (1975) was used for the determination of tannin.

Statistical analyses

The results were subjected to one- and two-way analyses of variance and Tukey's HSD test (Gill, 1978). The minimum level of statistical significance accepted was $P < 0{\cdot}05$.

RESULTS

Chemical composition

Table 1 shows the values for protein (N × 6·25), ash and tannin in soya-bean meal, barley, cocoa powder, black tea, green tea and coffee, and in the residues of black tea, green tea

Table 1. *Concentration (g/kg dry matter) of protein (nitrogen × 6·25), ash and tannin in soya-bean meal, barley, black tea leaves, green tea leaves, coffee beans and cocoa powder and their residues after extraction*

	Protein	Ash	Tannin
Soya-bean meal	498·1	69·0	1·7
Barley	118·8	25·0	4·1
Black tea			
Leaves	247·5	59·0	300·0
Residue	256·3	41·3	167·9
Green tea			
Leaves	216·9	61·3	195·5
Residue	237·5	49·1	63·7
Coffee beans			
Finely ground	147·5	43·6	84·1
Residue	113·8	13·8	14·6
Cocoa powder	215·6	84·7	142·4

and coffee after extraction with 1 l boiling water. The cocoa was added untreated to the diets, consequently no residue would arise from this product. Both soya-bean meal and barley had protein and ash concentrations normally found in these food items, while the tannin concentration was very low in both protein sources. The protein content in both tea varieties was quite high, with the highest value in black-tea leaves (247·5 g/kg). In coffee beans the protein content was much lower, and in cocoa powder the protein content was in between that in tea and coffee. The protein concentration in the residues from both tea and coffee was changed very little as a result of extraction.

The ash content was approximately 60 g/kg DM in both tea varieties and somewhat lower in coffee beans; in the cocoa powder it was 84·7 g/kg DM. The concentration of ash in the residues was much lower than in the original materials, indicating that significant amounts of minerals were dissolved in the beverages. Tannin was very high in both tea varieties, with 300·0 g/kg DM black tea. The content in green tea was much lower (195·5 g/kg DM); coffee beans contained 84·1 g/kg DM and cocoa 142·4 g/kg DM. The tannin concentration in the residues was much lower than in the corresponding original material, which shows that a large proportion of the tannin was dissolved in the drinkable portion.

The amino acid compositions of soya-bean meal and barley are presented in Tables 6 and 7 respectively (p. 203), together with the values for amino acid digestibility. Soya-bean meal is low in sulphur-containing amino acids, the content being far below the requirements for rats. Barley is low in lysine, which is the limiting factor for utilization of barley protein.

Table 2 shows the total amounts of DM applied to prepare 1 l tea, coffee and cocoa. For black tea 40% was dissolved in the beverage, while the corresponding values for green tea and coffee were 35 and 34% respectively. Relatively less of the protein was extracted during preparation and this would be of no nutritional importance. Approximately 0·3 g of the ash in tea and 1·2 g of the ash in coffee were dissolved into 1 l beverage, which might be of importance in providing some microminerals. However, most of the tannins were extracted during tea and coffee preparation. For black tea, green tea and coffee 1·99, 1·55 and 2·62 g tannin respectively would be consumed with 1 l beverage and cocoa (1 l) would provide 3·42 g tannin.

The amounts of freeze-dried extracts used in the diets are shown in Table 3 together with the other dietary components.

Table 2. *Dry matter (DM) applied (g) and amounts (g) of DM, protein (nitrogen × 6·25), ash, and tannin dissolved when preparing 1 l of the beverages of tea, coffee and cocoa*

	DM	Protein	Ash	Tannin
Black tea				
Leaves	10·0	2·48	0·59	3·00
Residue	6·0	1·54	0·25	1·01
Dissolved in 1 l water	4·0	0·94	0·34	1·99
Green tea				
Leaves	10·0	2·56	0·61	1·96
Residue	6·5	1·41	0·27	0·41
Dissolved in 1 l water	3·5	1·15	0·34	1·55
Coffee beans				
Finely ground	35·2	5·16	1·53	2·96
Residue	23·4	2·66	0·32	0·34
Dissolved in 1 l water	11·8	2·50	1·21	2·62
Cocoa powder	24·0	5·17	1·96	3·42

Expt 1. TD, BV, NPU and DE in soya-bean meal

As the preliminary study with cocoa powder and casein demonstrated that protein in cocoa was completely indigestible, a correction was made for the protein in cocoa. In this way it was possible to get an estimate of any inhibitory components on protein utilization in cocoa powder. Therefore the values for protein utilization in the diet with cocoa are based on protein from the soya-bean meal only. The protein contribution in the diets from tea and coffee was considered negligible and no effort was made to correct for these small amounts.

TD was negatively affected only by black tea, with a significant decrease from 0·880 to 0·847 (Table 4). Both tea varieties and the coffee had a negative effect on BV, with the strongest decrease from 0·753 in the control group to 0·644 in the group with black tea. The effect of coffee was almost the same as for black tea, while the effect of green tea was less, but still significant. Cocoa had no influence on BV. NPU was affected in the same way as BV. DE was negatively affected only in the group given cocoa, with a significant decrease from 0·879 to 0·853.

There was no significant difference in food intake or weight gain of rats given the experimental diets. The starting and final weights of each rat were 68 and 85 g respectively.

Expt 2. TD, BV, NPU and DE in barley

The group with cocoa was corrected in the same way as in Expt 1. TD was negatively affected by both tea varieties and by coffee, while cocoa had no effect on this value (Table 5). The strongest effect was observed for coffee with a decrease from 0·898 in the control group to 0·849. Black tea had nearly the same effect as coffee, while the effect of green tea was much less although significant. Cocoa had no effect on TD. As for soya-bean meal, BV was negatively affected by both tea varieties and coffee, with the strongest decrease for black tea from 0·746 in the control group to 0·700 in the group with black tea. Green tea and coffee had a slightly lower effect; cocoa had no influence on BV; NPU was affected in the same way as BV. DE was negatively affected by both tea varieties; however, the effect was rather small. Coffee and cocoa had no significant effect on DE.

There was no significant difference in food intake or weight gain of rats given the experimental diets. The starting and final weights of each rat were 68 and 83 g respectively.

Table 3. *Formulation of diets (g dry matter from each component)*

Protein source...	Soya-bean meal					Barley				
Diet...	Control	Black tea	Green tea	Coffee	Cocoa	Control	Black tea	Green tea	Coffee	Cocoa
Beverage	0	8·0	7·0	23·6	48·0	0	8·0	7·0	23·4	48·0
Soya-bean meal	192·0	192·0	192·0	192·0	192·0	—	—	—	—	—
Barley	—	—	—	—	—	788·0	788·0	788·0	788·0	788·0
Nitrogen-free mixture*	752·0	744·0	745·0	728·0	704·0	156·0	148·0	149·0	132·6	108·0
Mineral mixture*	40·0	40·0	40·0	40·0	40·0	40·0	40·0	40·0	40·0	40·0
Vitamin mixture*	16·0	16·0	16·0	16·0	16·0	16·0	16·0	16·0	16·0	16·0

* Composition according to Eggum (1973).

Table 4. *Expt 1. The effect of tea, coffee and cocoa on true protein digestibility, biological value, net protein utilization and digestible energy in soya-bean meal*

Treatment...	Control	Black tea	Green tea	Coffee	Cocoa	SEM	Statistical significance: *F*
True protein digestibility	0·880^{b}	0·847^{a}	0·866^{b}	0·866^{b}	0·871^{b}	0·004	7·7***
Biological value	0·753^{c}	0·644^{a}	0·720^{b}	0·647^{a}	0·749^{c}	0·004	188·4***
Net protein utilization	0·662^{c}	0·545^{a}	0·623^{b}	0·562^{a}	0·652^{c}	0·005	109·6***
Digestible energy	0·879^{b}	0·868^{b}	0·870^{b}	0·867^{b}	0·853^{a}	0·003	8·9***

$^{a, b, c}$ Mean values that do not share a common superscript letter in the same horizontal row were significantly different ($P < 0·05$).

Values were significantly different: *** $P < 0·001$.

Table 5. *Expt 2. The effect of tea, coffee and cocoa on true protein digestibility, biological value, net protein utilization and digestible energy in barley*

Treatment...	Control	Black tea	Green tea	Coffee	Cocoa	SEM	Statistical significance: *F*
True protein digestibility	0·898^{d}	0·859^{ab}	0·878^{bc}	0·849^{a}	0·890^{cd}	0·005	21·0***
Biological value	0·746^{c}	0·700^{a}	0·721^{b}	0·720^{b}	0·744^{c}	0·004	18·2***
Net protein utilization	0·670^{c}	0·602^{a}	0·633^{b}	0·612^{ab}	0·662^{c}	0·005	31·6***
Digestible energy	0·824^{cd}	0·805^{a}	0·808^{ab}	0·830^{d}	0·818^{bc}	0·003	17·1***

$^{a, b, c, d}$ Mean values that do not share a common superscript letter in the same horizontal row were significantly different ($P < 0·05$).

Values were significantly different: *** $P < 0·001$.

Expt 1. TAAD in soya-bean meal

TAAD was, in general, negatively affected by both tea varieties and coffee, while cocoa had no marked influence on these values (Table 6). The values for the most limiting amino acids in soya-bean protein, methionine and cystine were negatively affected by tea and coffee. There was no consistent effect on other amino acids. Glycine and proline digestibilities were low in both groups with tea, while the corresponding values for coffee and cocoa did not seem to be affected.

Expt 2. TAAD in barley

As for soya-bean protein, black tea, green tea and coffee had a general negative influence on amino acid digestibility, but the effects were more pronounced in barley (Table 7). Cocoa had no marked effect on these values. The digestibility of the most limiting amino acid in barley protein, lysine, was lowered by both tea varieties and by coffee. Alanine, aspartic acid, glycine, isoleucine and threonine also had lower digestibility values.

DISCUSSION

The results confirm that beverages prepared from black tea, green tea and coffee might have a negative effect on protein and energy utilization in rats. The deleterious effect was especially pronounced on the BV. Cocoa did not seem to contain any inhibitor affecting protein utilization, although the protein in cocoa powder was completely indigestible. The

Table 6. *Expt 1. The effect of tea, coffee and cocoa on true amino acid digestibility* in soya-bean meal. The amino acid composition of soya-bean meal (g/kg protein) is also given*

Treatment...	Control	Black tea	Green tea	Coffee	Cocoa	Soya-bean meal (g/kg protein)
Alanine	0·835	0·798	0·796	0·823	0·849	42·2
Arginine	0·947	0·906	0·927	0·944	0·960	71·1
Aspartic acid	0·903	0·876	0·888	0·903	0·901	107·7
Cystine	0·917	0·859	0·905	0·862	0·908	15·1
Glutamic acid	0·928	0·893	0·910	0·915	0·929	178·7
Glycine	0·858	0·801	0·828	0·850	0·820	41·6
Histidine	0·961	0·927	0·925	0·944	0·965	33·8
Isoleucine	0·888	0·853	0·872	0·884	0·900	45·1
Leucine	0·901	0·868	0·882	0·896	0·911	74·9
Lysine	0·902	0·872	0·881	0·901	0·917	59·9
Methionine	0·873	0·810	0·836	0·823	0·872	14·9
Phenylalanine	0·919	0·897	0·904	0·924	0·921	52·2
Proline	0·910	0·838	0·873	0·905	0·920	49·6
Serine	0·923	0·889	0·907	0·928	0·939	49·8
Threonine	0·864	0·825	0·845	0·873	0·867	37·2
Tyrosine	0·895	0·851	0·862	0·900	0·893	30·2
Valine	0·849	0·836	0·852	0·870	0·844	50·2
Protein (nitrogen × 6·25)	0·880	0·847	0·866	0·866	0·871	—

* Determined on single samples of pooled faecal material from five rats.

Table 7. *Expt 2. The effect of tea, coffee and cocoa on true amino acid digestibility* in barley protein. The amino acid composition of barley protein (g/kg protein) is also given*

Treatment...	Control	Black tea	Green tea	Coffee	Cocoa	Barley (g/kg protein)
Alanine	0·840	0·781	0·799	0·787	0·850	40·1
Arginine	0·920	0·872	0·886	0·896	0·919	47·1
Aspartic acid	0·853	0·779	0·803	0·786	0·829	53·7
Cystine	0·936	0·882	0·905	0·914	0·942	21·1
Glutamic acid	0·947	0·913	0·929	0·929	0·953	259·9
Glycine	0·839	0·774	0·794	0·777	0·791	37·9
Histidine	0·951	0·899	0·910	0·922	0·962	20·7
Isoleucine	0·907	0·855	0·871	0·859	0·913	39·0
Leucine	0·928	0·885	0·896	0·895	0·933	70·8
Lysine	0·848	0·818	0·829	0·827	0·850	32·9
Methionine	0·913	0·851	0·872	0·872	0·919	17·8
Phenylalanine	0·925	0·889	0·907	0·915	0·919	51·8
Proline	0·910	0·870	0·897	0·910	0·903	116·6
Serine	0·921	0·874	0·889	0·901	0·931	42·2
Threonine	0·858	0·790	0·817	0·813	0·854	33·1
Tyrosine	0·909	0·864	0·886	0·882	0·922	30·1
Valine	0·879	0·844	0·864	0·851	0·886	50·9
Protein (nitrogen × 6·25)	0·898	0·859	0·878	0·849	0·890	—

* Determined on single samples of pooled faecal material from five rats.

slight decrease in energy digestibility with cocoa powder in the diets is assumed to be due to a very low digestibility of the energy as well as of protein in cocoa. The results indicate that tannin (as well as protein) in cocoa was unavailable and thus passed through the

digestive tract without ill effects. As cocoa had no measurable effect on the criteria applied in the present study, the discussion will deal with tea and coffee only.

Our results support those of Sibbald (1982), which indicated that tea and coffee induce reduced digestibility of vegetable protein in cockerels. As the chemical analyses show a very high concentration of tannin in both tea varieties and in coffee, it is tempting to assume that tannin is at least one of the factors causing the reduced protein utilization when tea or coffee are fed together with soya-bean meal or barley. This assumption is in agreement with the earlier work of Eggum & Christensen (1975). However, this contradicts the results of Derman *et al.* (1977) that tea polyphenols complex with protein but do not prevent normal digestion of the protein. The findings of Stagg & Millin (1975) support the hypothesis of Derman *et al.* (1977) that tea polyphenols have no adverse gastrointestinal reaction. However, as discussed by Fenwick & Hoggan (1976), methods of analysis for tannin determination are rather non-specific and different types of phenolic compounds, including phenolic choline esters, might be included in the values obtained.

Fuller *et al.* (1967) reported that supplementation with methionine, choline and arginine reduced the toxicity of 1% dietary tannic acid and completely alleviated the adverse effect of 0·5% tannic acid. This is believed to result from the need for methyl groups for the O-methylation of gallic acid derived from tannic acid (Potter & Fuller, 1968), a general phenol-detoxifying mechanism (Williams, 1959), which has also been demonstrated in the rat by Booth *et al.* (1961). The strong reduction in digestible methionine in soya-bean meal, when given together with tea or coffee, could support this hypothesis. As methionine and cystine are the limiting amino acids in soya-bean protein, the corresponding decrease in BV indicates further a reaction between gallic acid and methionine. Whether this is the main reason for the reduction in BV, when soya-bean meal is given together with tea or coffee, is not known. However, more tannin was extracted from black tea and coffee than from green tea and the two first-mentioned beverages also had the strongest negative influence on protein utilization.

The influence of tea and coffee on protein utilization in barley was slightly different from that in soya-bean meal. TD was relatively more affected, while the influence on BV was less pronounced than for soya-bean meal. As discussed by Eggum & Christensen (1975), tannin has a negative effect on TD and the amounts of tannin dissolved in 1 l of the tea and coffee beverages might explain the changes in TD observed in the groups with tea and coffee extracts in the diets. The group given green tea had the lowest dietary tannin content and the TD in this group was also less affected. The BV was also negatively affected by both teas, as well as by coffee. This may be explained by the lower digestibility of the most limiting amino acid, lysine, in barley protein, when these beverages were given together with barley. There is no general phenol-detoxifying mechanism involving lysine as is the case for methionine. The slight decrease in energy digestibility in both tea groups might also be explained by the tannin extracted from the tea leaves. As discussed by Swain (1965), interference with the action of digestive enzymes by dietary tannin can be expected in view of the general protein-binding properties of tannin. On the other hand, the high content of tannin in the coffee group had no effect on energy digestibility. This indicates that components other than tannin in tea and coffee might be involved in the effects on protein and energy metabolism in rats. To this must also be added that methods of analysis for tannin are rather non-specific and different types of phenolic compounds , including choline esters, might be included in the values obtained (Fenwick & Hoggan, 1976).

REFERENCES

Association of Official Agricultural Chemists (1975). *Official Methods of Analysis*. Washington DC: Association of Official Agricultural Chemists.

Booth, A. N., Robbins, D. J. & DeEds, F. (1961). *Journal of Nutrition* **75**, 104–106.

Brown, P. J. & Wright, W. B. (1963). *Journal of Chromatography* **11**, 504–514.

Das, D. N., Ghosh, J. J. & Guha, B. (1964). *Indian Journal of Applied Chemistry* **27**, 6–10.

Derman, D., Sayers, M., Lynch, S. R., Charlton, R. W. & Bothwell, T. H. (1977). *British Journal of Nutrition* **38**, 261–269.

Eggum, B. O. (1973). A study of certain factors influencing protein utilization in rats and pigs. *Beretning fra Forsøgslaboratonèt*, no. 406.

Eggum, B. O. & Christensen, K. D. (1975). *Breeding for Seed Protein Improvement Using Nuclear Techniques*, 229 pp. Vienna: International Atomic Energy Agency.

Fenwick, R. G. & Hoggan, S. A. (1976). *British Poultry Science* **17**, 59–62.

Fuller, H. L., Chang, S. I. & Potter, D. K. (1967). *Journal of Nutrition* **91**, 477–481.

Gill, J. L. (1978). *Design and Analyses of Experiments in the Animal and Medical Sciences*, vol. 1, 409 pp. Iowa: Iowa State University Press.

Hessling, P. B., Klopper, J. F. & Van Heerden, P. D. R. (1979). *South African Medical Journal* **55**, 631–632.

McCance, R. A. & Widdowson, E. M. (1960). *The Composition of Foods* (MRC Special Reports Series No. 297). London: HM Stationery Office.

Mason, V. C., Bech-Andersen, S. & Rudemo, M. (1980). *Zeitschrift für Tierphysiologie, Tierernährung und Futtermittelkunde* **43**, 146–164.

Naismith, D. J., Akinyanju, P. A. & Yudkin, J. (1969). *Journal of Nutrition* **97**, 375–381.

Potter, D. K. & Fuller, J. L. (1968). *Journal of Nutrition* **96**, 187–191.

Rossander, L., Hallberg, L. & Bjørn-Rasmussen, E. (1979). *American Journal of Clinical Nutrition* **32**, 2484–2489.

Sibbald, I. R. (1982). *Nutrition Reports International* **25**, 133–137.

Stagg, G. V. & Millin, J. (1975). *Journal of the Science of Food and Agriculture* **26**, 1439–1459.

Swain, T. (1965). *The Tannins in Plant Biochemistry*, 552 pp. [J. Bonner and E. V. Varner, editors]. New York: Academic Press.

Weidner, K. & Jakobsen, P. E. (1962). In *Øvelsesvejledning for Landbrugs-, Mejeribrugs- og Licentiatstuderende* [D. S. R. Forlag, editor]. Copenhagen, Denmark: Royal Veterinary and Agricultural University.

Williams, R. T. (1959). *Detoxification Mechanisms*, 2nd ed. New York: Wiley.

Printed in Great Britain