faeces and by its use absorption experiments are much simplified and shortened. The faeces also need not be collected quantitatively which facilitates the be

SUMMARY

treatment of the experimental material.

1. The absorption of carotene was examined with three human subjects. As a source of carotene, raw finely grated carrot or a mixture of grated carrot and olive oil was used.

2. The percentage excretions were calculated by the method of quantitative collection of faeces, and also by the quantitative chromic oxide indicator method which gave the same results. 3. Of the different methods for determination of carotene, chromatographic analysis was found to be the most reliable.

4. The excretion of carotene in human beings on a fat-free diet amounted to about 90% when finely grated carrot was consumed and to about 30-50%when carotene dissolved in oil was taken. When the latter was taken in two portions, the excretion was 30-37% of the carotene dissolved, when taken at one time, the excretion was 50%.

The above work on the absorption of carotene belongs to the investigations of NJF's Kommitté för foderkvalitet (Scandinavian Society for Agronomy, Committee on the Quality of Fodder). I wish to express my sincere gratitude to the Committee for granting the means for this work.

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The Effect of Sodium Alginate on the Absorption of Calcium

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Alginic acid behaves as a monobasic acid, forming salts with a number of positive ions and its strength is comparable with that of monochloroacetic acid. Water soluble salts of Li, Na, K, Cl, Rb, NH_4 and Mg are known, and these form viscous colloidal solutions which on evaporation leave transparent films; whereas the Cu, Zn, Ag and Ni salts are insoluble in water but soluble in ammonia. The addition of Ca, Ba, Hg, Pb or Bi ions to a soluble salt results in precipitation, for salts of these metals are insoluble in water and ammonia. If these ions are released slowly from sparingly soluble substances, e.g. calcium citrate and dicalcium phosphate, continuous gels can be formed in the cold.

Because alginic acid and its salts are able to withstand any degree of heating without loss of stabilizing value or development of off flavours, this chemically reactive colloid has diverse application in the food industry to replace gelatin, agar agar, Irish moss, pectin, gum tragacanth, starch and other colloids. Solutions can be kept sterile by pasteurization for $1 \text{ hr. at } 50^{\circ}$, or by treating with preservatives.

Alginic acid is sold in the form of its sodium salt under the trade name of Manucol. Manucol I contains 15-20 % water and a 1 % solution has a viscosity of 15-30 centistokes at 25°. Manucol IV also contains 10-20 % water but a 1 % solution has a viscosity of 80-150 centistokes at 25°. These two products are used widely as stabilizing agents in ice-cream, reconstituted and artificial creams, chocolate milk suspensions, marsh-mallows, fruit squashes, and as thickening agents in custards, jams, marmalades, sauces, soups and water jellies.

Owing to their increased application in the manufacture of foods, it was considered necessary to investigate the possibility of alginic acid or alginates interfering with calcium absorption in man. Calcium alginate is stable to a pH of 3.9, below which the calcium ion is released and the insoluble alginic acid is formed; but it is not known if conditions in the alimentary tract are favourable for calcium release.

EXPERIMENTAL

Experimental subjects. A calcium balance experiment was done with the following six normal healthy adults.

No.	Sex	Body weight (lb.)	Occupation
1	Male	139	University research worker
2	Female	105	University research worker
3	Female	115	University research worker
`4	Female	110	University student
5	Female	129	University lecturer
6	Female	131	University lecturer

Plan of experiment. The subjects were given 4 preliminary days to adjust themselves to the diet. There were two analytical periods of 7 days, the control period and the experimental period when the sodium alginate was taken by mouth. Sodium alginate (Manucol I, 8 g. dry weight) was taken in three approximately equal amounts on each day of the experimental period. It was mixed with some item in the meal such as porridge, soup, meat or dessert. This quantity was thought to be in excess of the amount any person would be likely to consume in manufactured products in one day. On the assumption that the equivalent weight for alginic acid is 176, 8 g. of sodium alginate would correspond to 0.809 g. of calcium.

Diet. All foods were served in the Department of Biochemistry. Lunch and the evening meal were supplied by the Royal Melbourne Hospital diet kitchen. The meals were similar to those provided for the nursing staff except that milk was restricted and cheese was not included. The following is an example of a typical menu.

$\mathbf{Breakfast}$	Lunch	Evening meal	
Oatmeal porridge	Roast lamb and mint sauce and gravy	Scotch broth	
Toasted white bread	Roast potato	Cold brawn	
Butter	Diced carrots	Salad	
Marmalade	Peach sponge and custard	Toasted white bread	
Coffee		Butter	
		Jam	
		Coffee	

Mid-afternoon-lemon (juice) or other raw fruit

To minimize the interference of phytic acid with the absorption of calcium (McCance & Widdowson, 1942-3; Widdowson, 1941), the bread was made of white flour and the oatmeal porridge was soaked overnight. Foods listed by Kohman (1939) as having a high oxalate content, e.g. spinach, New Zealand spinach, beet-tops and rhubarb, were avoided, as not only is the calcium in these foods unavailable, but the oxalate content is sufficient to make unavailable considerable amounts of the calcium in other foods (Fairbank & Mitchell, 1938; Speirs, 1939).

The quantity of foods eaten, except milk, was left to individual choice and calculation from food tables compiled by the Institute of Anatomy, Canberra, showed that all subjects received adequate calories, protein and at least 0.8 g. calcium daily. This amount is recommended by the Food and Nutrition Board of National Research Council of America (1945) as adequate for an adult. To ensure that each person received sufficient vitamin C, 20 ml. of lemon juice were given every second day.

In addition to the milk used in food preparation, each person was allowed 400 ml. daily. The volume was measured accurately every morning and set aside in individual containers. All milk and the water rinsings of the containers had to be consumed.

Sampling of diet. Each person had a plate, cup, etc. which was weighed and numbered. All food except bread, jam, butter, sugar and salt was weighed directly on to the plate just prior to the meal and placed in an electric oven to reheat it before it was eaten. The methods suggested by McCance & Widdowson (1942-3) were adopted to ensure that the sample was representative of the whole. The servings were given in accordance with the subject's appetite and all waste such as bones and extra fat was removed to avoid weighing back after the meal. The weighings were done on a pan balance which weighed to the nearest gram. A fifth of the amount consumed by each person was weighed to the nearest 0.1 g. and placed in glass specimen jars large enough to hold all the samples for the analytical period. The jars were closely covered and no preservative was necessary.

The butter, jam, salt and sugar were weighed out at the beginning of the week and again at the end, and a fifth of the amount eaten was added to the food samples. Each person had a loaf of bread which was weighed and sampled at the beginning of the week and the remainder then had to be used.

All liquids except milk were sampled for analysis by putting aside an equal volume in a liquid sample jar. With milk, a tenth of the quantity allowed was measured by burette and kept in a bottle under toluene for analysis. Tap water was used throughout and, despite its low calcium content of 2.1 mg./l., it was considered advisable to include it for analysis.

Sampling of excreta. Toluene was used as a preservative for the urine. The bladder was emptied after breakfast on the first day and this specimen was discarded. All other specimens were collected, including the one after breakfast on the eighth day.

Carmine (0.4 g.) was taken before breakfast on the first and eighth day to tag the faeces. Glass specimen jars were used to collect all faeces for the week and no preservative was used. Collection began with the specimen which was stained by the dye taken on the first day, and continued until the second dose of carmine appeared. The portion which was stained by the carmine taken on the eighth day

Table 1. The effect of sodium alginate on the retention of calcium

(For the subjects marked A, the control period was the first analytical period and for those marked B, the second analytical period.)

Period	Metabolism of calcium					
	Tutaha	Output (mg./day)				
	(mg./day)	Urine	Faeces	(mg./day)	Absorption (%)	
Control Experimental	938 921	$\begin{array}{c} 258 \\ 244 \end{array}$	658 624	$\begin{array}{rrr} + & 22 \\ + & 53 \end{array}$	30 32	
Control Experimental	883 1002	234 220	636 566	$\begin{array}{r} + 13 \\ + 216 \end{array}$	28 43	
Control Experimental	854 947	108 160	737 713	$\begin{array}{r} + & 9 \\ + & 74 \end{array}$	14 25	
Control Experimental	930	273	585	+ 73	37	
Control Experimental	940 876	$\begin{array}{c} 125\\ 124 \end{array}$	832 771	- 17 - 19	14 12	
Control Experimental	921 962	186 164	681 735	$\begin{array}{rrr} + & 54 \\ + & 62 \end{array}$	26 24	
	Control Experimental Control Experimental Control Experimental Control Experimental Control Experimental Control	Control938Experimental921Control883Experimental1002Control854Experimental947Control—Experimental930Control940Experimental876Control921	PeriodIntake (mg./day)Output UrineControl938258Experimental921244Control883234Experimental1002220Control854108Experimental947160Control——Experimental930273Control940125Experimental876124Control921186	Period Intake (mg./day) Output (mg./day) Control 938 258 658 Experimental 921 244 624 Control 883 234 636 Experimental 1002 220 566 Control 854 108 737 Experimental 947 160 713 Control — — — Experimental 930 273 585 Control 940 125 832 Experimental 876 124 771 Control 921 186 681	$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	

was excluded. The diet was continued until the last collection was completed. No laxatives were permitted or required during the experimental period.

Analytical technique. The analytical arrangements for drying, ashing, and the preparation of the solutions for assay have been described by McCance & Widdowson (1942-3). In the first week the following quantities were taken for analysis: 1/100th of the weight of food and liquids including milk, 100 ml. urine and 50 g. faeces. In the second week smaller portions were found to be more convenient. The calcium was determined by the micromethod described by McCance & Shipp (1933).

RESULTS

The results of the balance experiment are given in Table 1. McCance & Widdowson (1942–3) consider that balances within ± 25 mg./day are not significantly positive or negative.

The results of this experiment suggest that sodium alginate does not interfere with the absorption of calcium in normal healthy adults.

SUMMARY

A calcium balance experiment was done with six healthy adults. The experimental periods were of 7 days—a control period and a test period during which 8 g. sodium alginate were taken daily by mouth. No evidence was obtained to suggest that sodium alginate interferes with calcium absorption from a normal varied diet.

This investigation was carried out during the tenure by one of us (F. B. R.) of a grant for research in Nutrition from the Australian National Health and Medical Research Council. We desire to acknowledge the gift of Manucol I from the Imperial Chemical Industries of Australia and New Zealand Ltd., the help given by the Superintendent of the Royal Melbourne Hospital in making available the services of the diet kitchen, and the willing co-operation of our associates, R. D., K. L., J. D., and B. W., in submitting to the discipline of the experiment.

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