Chapter 15
INTESTINAL MICROFLORA AND LONGEVITY

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INTRODUCTION

Metchnikoff hypothesized that the reason for the diseases which occur during old age is the action of toxic substances produced by putrefaction in the intestinal tract. He noted that the fact that there were many aged people among farmers in the Smoljan region of Bulgaria and in the Caucasus of southern Russia might be related to the fact that they drank large quantities of milk acidified by lactic acid bacteria, and that the secret of long life might be that lactic acid bacteria suppressed the multiplication of harmful bacteria in the intestinal tract. Needless to say this increased the interest in yoghurt and koumiss (alcoholic milk). Although this hypothesis was popular at the beginning of this century it was unsupported by experimental or clinical proof, partly due to the lack of adequate experimental techniques at that time. However, the remarkable developments in techniques for microbiological research and for raising germ-free animals in recent years, have produced data showing the important role played by intestinal bacteria in animals, leading to a fresh reassessment of Metchnikoff's hypothesis.

The average lifespan of the Japanese has increased steadily, and the Japanese are now widely recognized as being the most long lived people in the world. Thinking about the reasons for this increase in longevity, the first reason is a vast decrease in the infant mortality and a decrease in mortality from infectious diseases, but nutrition must be another factor. In trials with animals it has been shown that 'restricted feeding' conditions are associated with long life. '*Ad libitum*' feeding allows animals to eat to full appetite, whereas in restricted feeding food intake is restricted to 60% of *ad libitum* intake. Epidemiological studies have also shown the

effectiveness of food fibre in increasing lifespan through its effect in suppressing the manifestation of 'adult diseases'.

There are many theories as to what is the maximum age for a human being, but at present there is most support for a hypothetical 110–120 years. Scientifically verified data to date shows 3 people in the world who have lived to 113 years. A 120 year-old Japanese recorded in the Guinness book of records is clearly a registration error which has been corrected. Since such errors occur even in a country such as Japan where registration is established, there is a need to be aware of misrepresentation of age in evaluating studies of centenarians in the world. Three areas of the world which are famous for having a lot of centenarians are the Caucasus in the USSR, the Funza region of Pakistan and the Birucabamba region of Ecuador. However, in every case there are many instances of age misrepresentation; for instance in a re-examination of people in the Caucasus claiming to be at least 90 years old, 50% were said to have misrepresented their age[1]. However, it is unquestionable that in this case half of the people were as old as they claimed, and that the mean life spans in these 3 regions are clearly longer than the respective national means; so diet or concern for health must be involved in these long-lived areas.

A. FACTORS INVOLVED IN PROLONGING LIFE

1. Germ Free Environment

a. Prolonging the life of individual organisms

Humans or animals living in a natural environment have many microorganisms on mucous membranes in contact with the outside world, especially the intestine, and on external surfaces, and are affected by them in different ways. The intestinal microflora have a particularly marked effect on the host. Since it has become possible to rear germ-free animals without microorganisms at any site in or on the body, the role of the intestinal microflora and the riddle of host-parasite relationships have become clearer. According to a comparative study on the longevity of conventional animals reared under ordinary conditions and the longevity of germ-free animals, it was evident that germ-free animals lived longer (Fig. 1)[2]. Since this research was on genetically identical mice there was no difference between the conventional and germ-free mice in their potential lifespan, so it seems that the lifespan of animals is mainly shortened by the invasion of the body by intestinal bacteria, etc. from outside.

From such examples it can be deduced that the presence of an indigenous intestinal microflora is not ideal for the host. Certainly the fact that blood ammonia concentration was higher in conventional animals than in germ-free animals would support this. However, on the other hand, there are also many instances of beneficial effects for the host. For example the intestinal bacteria can synthesize vitamins to supply the host, they play a role in increasing the immunology of the host, and they also produce substances which intensify adrenocorticoid hormones.

Whatever the case may be, it is impossible for humans who have lived in a normal environment to be placed in and to live in a germ free environment; so what

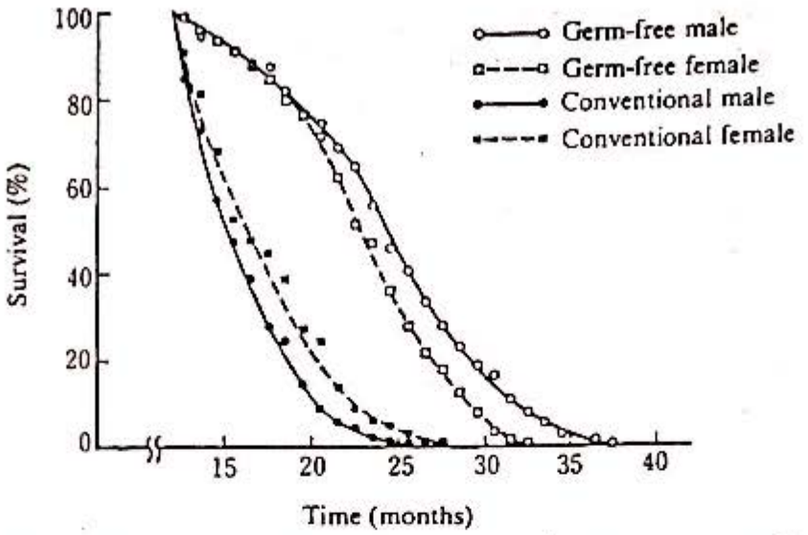


Fig. 1. Natural mortality in normal mice and germ-free mice.[2]

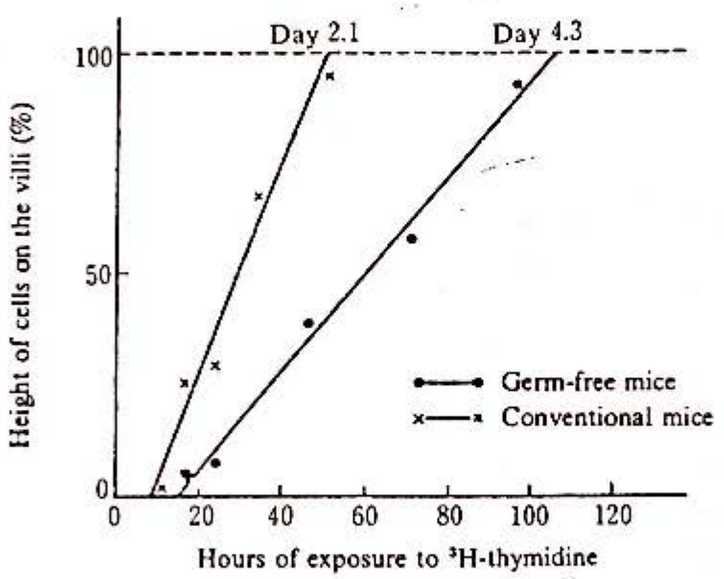


Fig. 2. The migration time of intestinal epithelial cells on the villi.[3]

is needed to prolong life is to ensure that the composition of the intestinal microflora is as far as possible a healthy one.

b. Prolonging the life of the epithelial cells of the small intestine

It has been mentioned above that germ-free animals had a longer lifespan than conventional animals, but the pioneering research of Matsuzawa and Wilson also showed clearly that the lifespan of the epithelial cells of the small intestine, which play the main role in digestion and absorption in germ-free animals, was also twice as long as in conventional animals (Fig. 2).

The lifetime of the cells was measured by noting the fact that epithelial cells were produced in the crypt region, ascended around the villi as on an escalator and finally finished their life by falling off the end of the villi, and using the fact that ^{3}H-thymidine was incorporated into the nucleus of newly produced epithelial cells. It

Table 1
The relationship between survival time after irradiation at 3000 R, and the lifetime of the epithelial cells of the small intestine[5]

Mouse-rearing environment	*Survival after exposure to 3000 R (days)*	*Life of the small intestine epithelium (days)*
Germ-free conditions	7.3	4.3
Infection with a single species in germ-free conditions		
Streptococcus faecalis[a]	6.9	4.1
Escherichia coli	6.2	3.7
Pseudomonas aeruginosa	5.4	3.2
Taken from germ-free conditions to normal conditions	4.6	2.7
Normal conditions	3.5	2.1

[a] Classified as *Enterococcus faecalis* since 1984.

is evident from Fig. 2 that in germ-free mice the life of epithelial cells was 4.3 days whereas in conventional mice it was 2.1 days. It is known that not only is life longer in germ-free mice but the activity of digestive enzymes of the microvilli if the epithelial cells was also approximately 2 times as high[4]. The reason for this seems to be that because the epithelial cells spend a longer time ascending the villi they can become fully mature. In conventional mice the cells fell of the end of the villi without being fully mature.

This leaves the question of which intestinal bacteria shorten the life of the epithelial cells of the small intestine. Table 1 shows the results of an experiment carried out by Wilson. It is evident that *Pseudomonas aeruginosa*, for instance, is a type of bacteria which shortens the life of the epithelial cells of the small intestine, whereas *Streptococcus faecalis* (*Enterococcus faecalis*) has little effect in shortening the life of the epithelial cells. Needless to say, when the capacity of cells to multiply is destroyed by irradiation, the survival time of the epithelial cells of the intestine is greater for longer-lived cells. From results of experiments on correlations of individual lifespans at the cellular level, hints can be obtained as to bacterial species tending to lengthen the life of individuals, and bacterial species tending to shorten it. The lactic acid bacteria appear to have little effect in shortening lifetime.

c. The lactic acid bacteria and longevity

Arai *et al.*[6], reared 3 groups of 90 female mice on a basic feed or feed with pasteurized acid milk added at 15% of feed with dried whole milk added at 1.6% (equivalent to 14% whole milk) from weaning throughout their entire lives, and looked at the effect on lifespan (Fig. 3).

Compared with the other groups, the group given pasteurized acid milk lived on average approximately 7 weeks longer; an increase in lifespan of approximately 8%. The same result was obtained in a subsequent repeat experiment. There was no

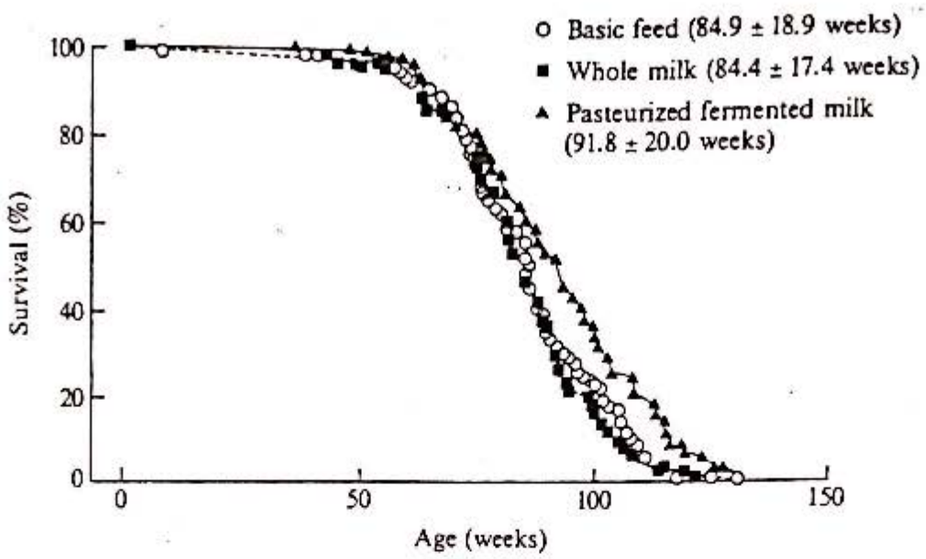


Fig. 3. The survival curves of mice reared on different feeds.[6]

difference among groups in causes of death, which were kidney failure, pneumonia and tumours according to dissections. However, there was a lengthening of time before death from kidney failure in the group given acid milk. Examination of faecal microflora showed that the group given acid milk maintained counts of bifidobacteria 10 times those in the other groups throughout nearly the complete period.

Thus, one way of lengthening lifespan would be a germ-free environment, but it is impossible to create a germ-free environment, and man must of necessity make contact with the normal microflora in the course of life. Accordingly, lifespan can probably be lengthened by at least making the intestinal bacteria as good for health as possible.

2. Restricted Feeding

a. Prolonging life by restricted feeding

McCay *et al.* observed the effect in rats when only energy intake was restricted[7]. Since the overall feed intake of the restricted group was also smaller, protein, minerals and vitamins had to be supplemented to avoid the danger of deficiencies being created by restricting energy. The restricted group were allowed to eat only enough to maintain their body weight (up to 10 g increase in body weight in 100 days). The results showed that by day 1000 all of the control rats were dead, whereas the restricted fed rats were still alive. McCay *et al.* deduced that a slow growth rate and extended growing period was a factor in prolonging life. In the subsequent research of Berg *et al.*[8] too, restricted feeding stopped excessive fat accumulation, and whereas approximately half the males in the *ad libitum* group died within 760 days, both males and females in the restricted feeding group and the females in the ad *libitum* group were still alive on day 760, and in good health (Table 2).

Comparing the presence of tumours and pathological signs in the heart, kidneys and blood vessels at 800 days of age, there was a significant difference between the *ad libitum* and restricted groups, with the incidence being less in the restricted

Table 2
Prolonging life by restricted feeding[8]

Rats		Ad libitum *feeding*	*Restricted feeding*
Body weight	male	400 g	260 g
	female	260 g	163 g
Lifespan	male	800 days	1000 days
	female	1000 days	1300 days
Vascular and kidney problems, etc. (at 800 days)	male	100%	24%
	female	60%	0%
Tumours (at 800 days)	male	58%	26%
	female	41%	12%

group. There is not much data on restricted feeding in humans, but it has been reported to be effective in preventing illness among the elderly.

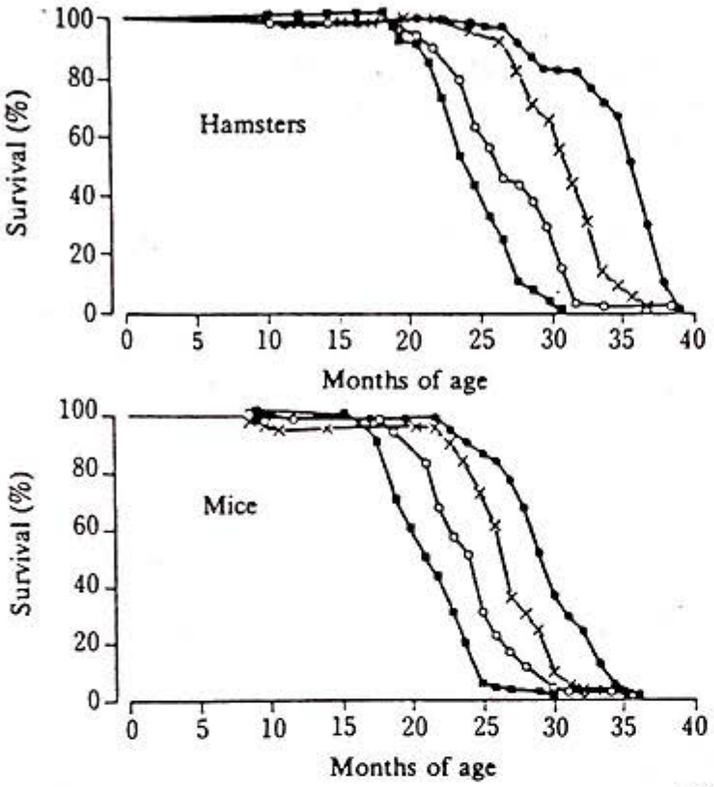
Concerning the period of starting restricted feeding, Stuchliková *et al.*[10] compared the lifespans of rats, mice and hamsters subjected to restricted feeding in their first or second year only, with those of animals restricted throughout their lives (Fig. 4). Restricted feeding in the first year was most effective.

In the experiment of Weindruch *et al.*[11] on the other hand, using low-protein feed under the same conditions of restriction a less marked prolonging of life was noted. From this it is evident that restricted feeding loses its effect when it entails an insufficient intake of nutrients.

b. Prolonging the life of the epithelial cells of the small intestine by restricted feeding

Restricted feeding can prolong the life of the body, and it has also been shown by Koga and Kimura that, like germ-free conditions, restricted feeding also prolongs the life of the epithelial cells of the small intestine[12]. This was of course in experiments using conventional animals. Komai *et al.* tried experiments combining both germ-free conditions and restricted feeding. The results showed that whereas the lifespan of jejunal and ileal epithelial cells in conventional mice was 65–76 h with *ad libitum* feeding and was increased by 33–41 h with restricted feeding (38% restriction) to 98–117 h, it was already increased in germ-free mice fed *ad libitum* to 95–100 h, and was 100–119 h with restricted feeding, which was a smaller increase than expected (Table 3)[13]. The lifespan of the epithelial cells of the small intestine of germ-free mice under restricted feeding is probably the maximum value for these mice.

The above results indicate that restricted feeding is best for animals as long as it does not entail an insufficient energy intake, and that the mechanism by which it slows down the ageing process is by slowing down cell turnover. Restricted feeding had an effect on the lifespan of the epithelial cells of the small intestine whether the normal intestinal flora was present or not; the reason that there was no great



Feeding regime	
1st year	2nd year
unrestricted	unrestricted
restricted	restricted
unrestricted	restricted
restricted	unrestricted

Fig. 4. Survival curves on different feeds (hamsters and mice).[10]

Table 3

Prolonging the life of the epithelial cells of the small intestine in mice by restricted feeding, and the contribution of the normal intestinal microflora[13]

Group	*Jejunum*			*Ileum*		
	Time		(%)	*Time*		(%)
Normal animals						
Ad libitum	75.6	+42.1	(100)	65.2	+32.5	(100)
Restricted	116.7		(154)	97.7		(150)
Germ-free animals						
Ad libitum	109.0	+10.2	(144)	94.8	+5.3	(145)
Restricted	119.2		(158)	100.1		(154)

increase in lifespan in germ-free mice is probably that by becoming germ free the lifespan of the epithelial cells was already near the critical value.

c. Restricted feeding and haematopoiesis and immune functions

Hishinuma and Kimura have shown that restricted feeding (40% restriction) led to many small spherical erythrocytes and shortened the lifespan of erythrocytes in mice; however, despite the shortened lifespan of erythrocytes oxygen transport was maintained at levels as high as or higher than in controls. This was because, in

contrast with the situation for the epithelial cells of the small intestine, etc., the shortening of the lifespan of these active cells made them more active with respect to cell functions. A series of trials[14] has shown that with restricted feeding the number of nucleocytes in bone marrow was approximately twice that in controls, and the number of pluripotent stem cells (CFUs) in the femur was increased approximately 1.4-fold. The proportion of CFUs in the S period was also higher with restricted feeding, at 27% compared with 18% for the control group. Erythropoetin-sensitive cells, which indicate erythrocyte Fe utilization, also showed a value of 15% with restricted feeding as opposed to 11% for the control group.

Hishinuma *et al.* also discovered interesting results when it came to effects on T-cell formation and functions[15]. They showed that with restricted feeding for 8 weeks, body weight was 34% lower for the restricted group than for the control group, and the weight of the spleen was 75% lower. However, when splenocytes were analysed using flow cytometry after forming a cell-enriched fraction by passage through a nylon wool column, the proportion of bright Thy-1,2$^+$ cells (T cells) was considerably increased by restricted feeding. This indicates that the formation of T cells is altered by feed restriction. Then the results of IL-2 response and mixed lymphocyte reaction (MLR) in the presence of IL-2, carried out using the cell-enriched fraction, showed that the T cells of the restricted group were much more responsive to MLR and responsive to IL-2, showing an intensification of T cell function by feed restriction. These facts indicate that restricted feeding not only affects T cell formation, but also affects their functional expression.

B. DIETARY FIBRE INTAKE AND DIGESTIVE TRACT PHYSIOLOGY

1. Dietary Fibre and the Prevention of Adult Diseases and Cancer

In 1971, Burkitt[16] proposed the hypothesis shown in Fig. 5 concerning interrelationships between colon cancer and food, based on American experience to that time and on a great deal of epidemiological data. Thus, when highly refined foods are eaten, fibre intake is decreased because there is less roughage than when non-refined (or semi-refined) foods are eaten, and the intestinal environment is altered. This change affects the intestinal microflora, and promotes the breakdown of bile acids. As a result, the production of cancer-related substances increases, and because fibre intake is small, faecal volume is small, decreasing the frequency of faecal excretion and prolonging passage through the digestive tract, so that the time that cancer-related substances are in contact with the intestinal mucous membrane is also prolonged and the danger of cancer is increased. When a lot of fibre is eaten, on the other hand, the opportunity for cancer is suppressed for the opposite reason. The British worker Trowell furnished detailed evidence at a conference in the USA, showing a relationship (negative correlation) between rectal cancer and food fibre (Fig. 6)[17]. There is epidemiological data showing an inverse correlation between colon cancer and stomach cancer, and the relationship between stomach cancer and food will be discussed below.

The appearance in faeces of mutagenicity, which is closely related to carcino-

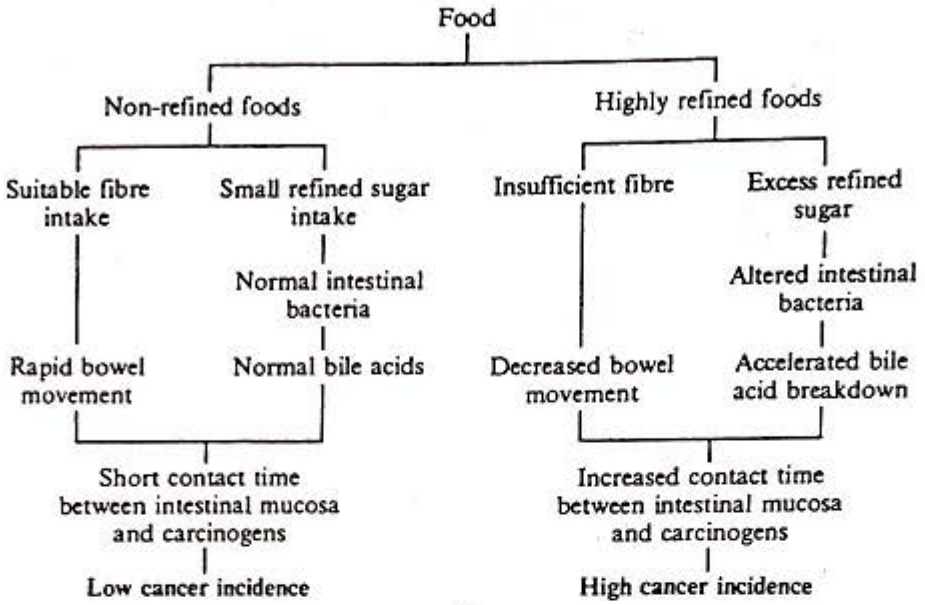


Fig. 5. The relationship between the incidence of colon cancer and food composition.[16]

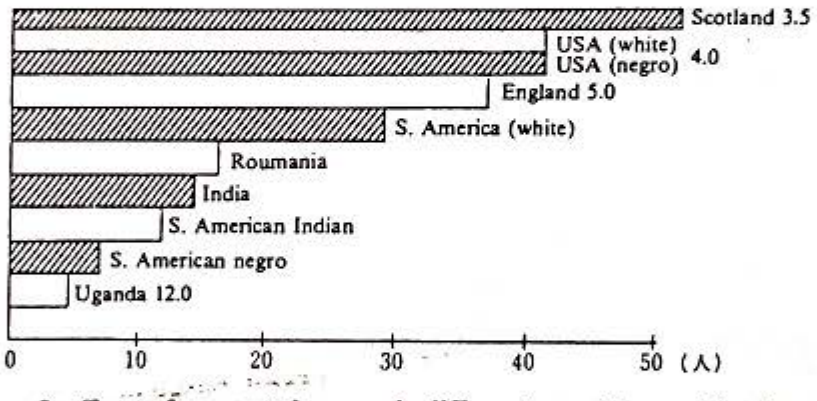


Fig. 6. The number of sufferers from rectal cancer in different countries, and intakes of dietary fibre (DF).[17]

genicity, is also modified by differences in food. Bruce *et al.* produced epoch-making research data on this aspect, being the first people to show the existence of mutagenicity in human faeces. Figure 7 shows that mutagenicity can be seen when eating a normal western diet, and that it is increased if fibre intake is too small or fat or protein intake is too great, and decreased by adding ascorbic acid or α-tocopherol[18]. Bruce *et al.* subsequently reported that the main mutagenic substance in human faeces was 'fecapentaene', cresol with an enol ether side chain having 5 double bonds.

Data from the author's laboratory showed that mutagenicity in faeces from conventional mice with normal intestinal microflora was high with a fibre-free food, and was suppressed by 15% cellulose addition (Fig. 8).

The fact that in this case germ-free mice fed a fibre-free diet gave similar values to mice fed the diet with 15% cellulose indicates that the intestinal bacteria contribute to the increase in mutagenicity that is seen with fibre-free diets under

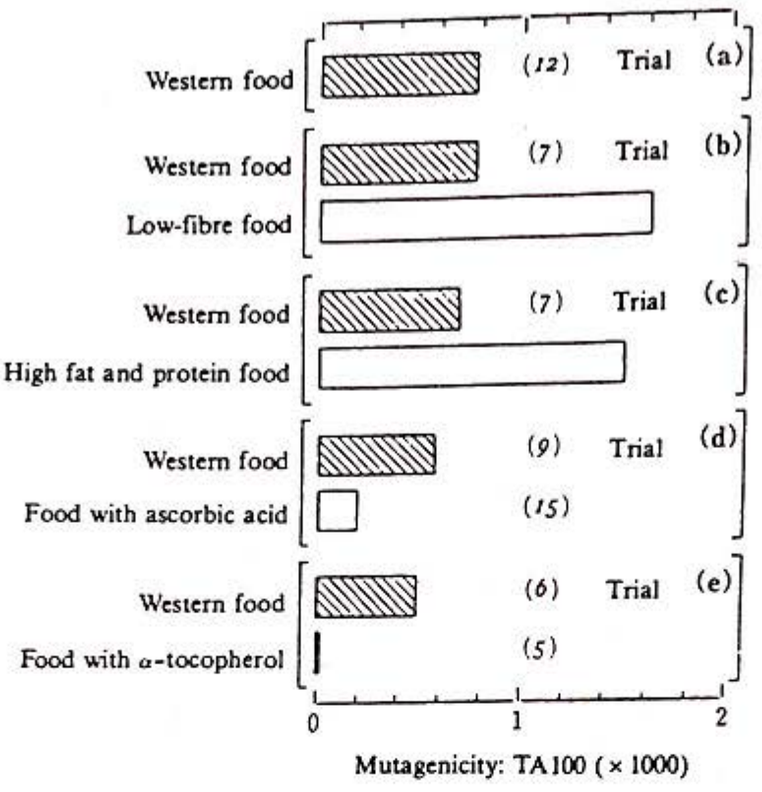


Fig. 7. Modifications in mutagenicity in human faeces with differences in food contents The number of samples is given in brackets ( ). (Ascorbic acid – 4 g/day; α-tocopherol 400 mg/day).[18]

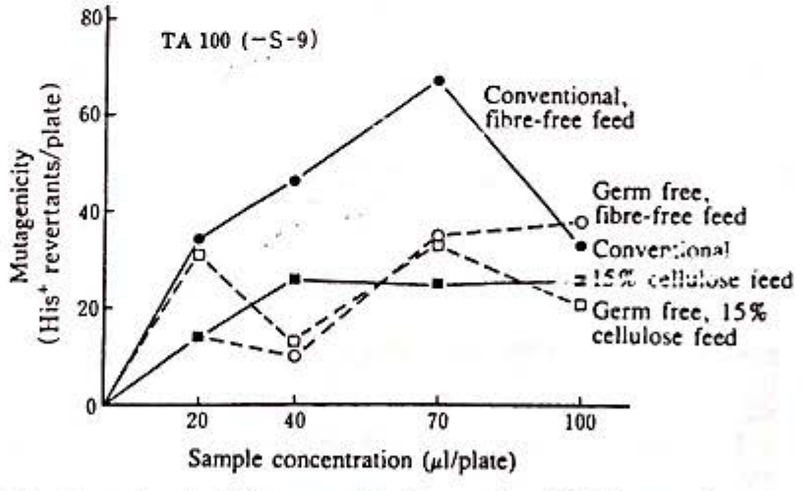


Fig. 8. Mutagenicity in extracts (ether → alkali → ether) of faeces from germ-free and conventional mice (Komai and Kimura, unpublished).

conventional conditions. The possibility can be considered that with fibre-free diets mutagenic substances would be concentrated in the meagre intestinal contents, but the make-up of the intestinal microflora would also be expected to be adversely affected.

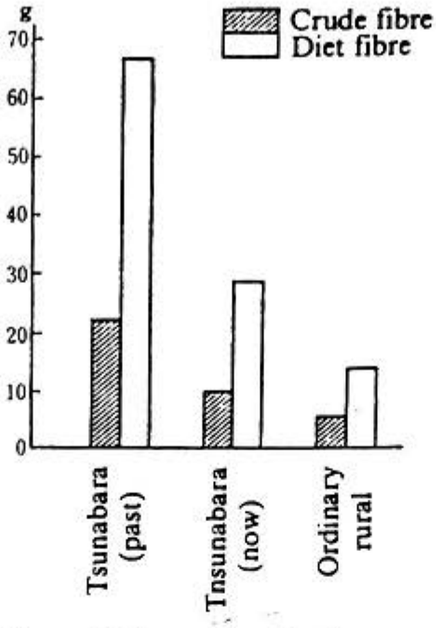


Fig. 9. Comparison of crude fibre and dietary fibre intake among old people.[19] Ref.: Takatsu, T., Oyokawa, K., Akazawa, T., Komori, Y.: Studies on diet and longevity. II *Ann. Rep. Fac. Education, Iwate Univ.* 14 (1981).

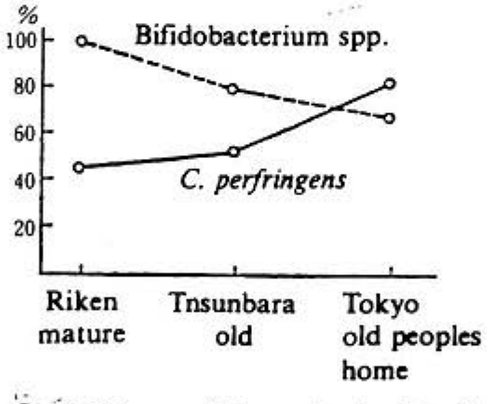


Fig. 10. Bifidobacteria and *Clostridium perfringens* in the intestinal bacteria. [19] Note: From Mitsuoka, T. (1980) Ref.: Komori, Y.: *Climate and people of Tsunabara long-lived rural area*

2. Dietary Fibre Intakes and Intestinal Microflora in Long-Lived Rural Areas

In Japan there are some so-called long-lived rural areas, including the Tsunabara area of Kaminohara-machi in Yamanashi prefecture, made famous by Kondo, Komori, Takatsu *et al.* 'Studies on diet and longevity' and Mitsuoka *et al.* 'Studies on intestinal flora in the aged'. In the past, dietary fibre intake in Tsunabara was 66.8 g; even now it is 28.8 g, which is much greater than the general intake of 13.1 g for rural areas[19] (Fig. 9). The main sources of this fibre intake are cereals and tubers.

It is evident that this rich intake of fibre has its effects on the intestinal microflora. Results of analysis of faeces from aged inhabitants of Tsunabara showed that counts of *Bacteroidaceae*, *Clostridium* spp., *Peptococcaceae* and detection of *Bacillus* spp. were significantly lower, and counts and rates of detection of

Bifidobacterium spp. were higher than in city dwellers, and detection rates of *Clostridium perfringens*, which is known to show a particular increase in old age, were lower than among old people in the city (Fig 10[19].

Various studies have been carried out on how foods containing dietary fibre affect the intestinal microflora, but there are few reports where such considerable changes have been noted as those shown above. This may reflect of the fact that effects differ according to the type and amount of fibre.

3. Different Varieties of Dietary Fibre and their Effects on the Physiology of the Digestive Tract

a. Dietary fibre and the regeneration of digestive tract epithelial cells

The fact that the incidence of colon cancer is generally low in regions which have a high intake of dietary fibre has already been mentioned. However, looking at the incidence of stomach cancer, the reverse is the case, and incidence is highest in areas that have a high dietary fibre intake. Such epidemiological data indicates the possibility that dietary fibre may have different effects on the behaviour of epithelial cells of the stomach and the colon. The few studies that have been carried out to date on the effect of dietary fibre on the epithelial cells of the mucous membrane of the digestive tract have concentrated on the small intestine and the colon, but no definite results have been obtained. Moreover, the questions of whether the effects of dietary fibre are the same at different sites within the digestive tract, and whether the intestinal bacteria are involved, remain unanswered. Komai *et al.* have carried out the following experiment using conventional and germ-free ICR mice, with the aim of answering these questions.

First of all, the effect of eating dietary fibre on regeneration of epithelial cells in the small intestine was measured at appropriate sites within the digestive tract in a 4-week experiment with mice given diets containing cellulose or pectin – two sources of dietary fibre with different characteristics. In the case of conventional mice, increasing diet cellulose content (5, 15, 30%) had no effect in increasing the speed of regeneration of epithelial cells in the small intestine (Fig. 11)[20]. However, when given feed containing pectin at 20%, there was an increase in the number of cells making up the villi and crypts of the small intestine: mitotic activity in the area of multiplication was increased and regeneration was considerably accelerated[21]. Thus it is clear that the effect of 'dietary fibre' differed according to the type and intake of dietary fibre. A trial with germ-free mice gave the reverse result to that with conventional mice: the regeneration of epithelial cells in the small intestine was accelerated by increasing amounts of cellulose, but was not affected by pectin.

Exactly the same phenomenon was noted in the case of the epithelial cells of the colon, but it was evident that the epithelial cells of the integument to the mucous membrane of the stomach were almost unaffected by dietary fibre (Table 4). The reason for the different results obtained in germ-free and conventional mice is thought to be because in germ-free mice the residence time of food residues in the intestinal tract is longer, and large amounts of cellulose present for a long time have the physiological property of speeding up the regeneration of intestinal epithelial cells irrespective of the intestinal bacteria. The results from germ-free mice also

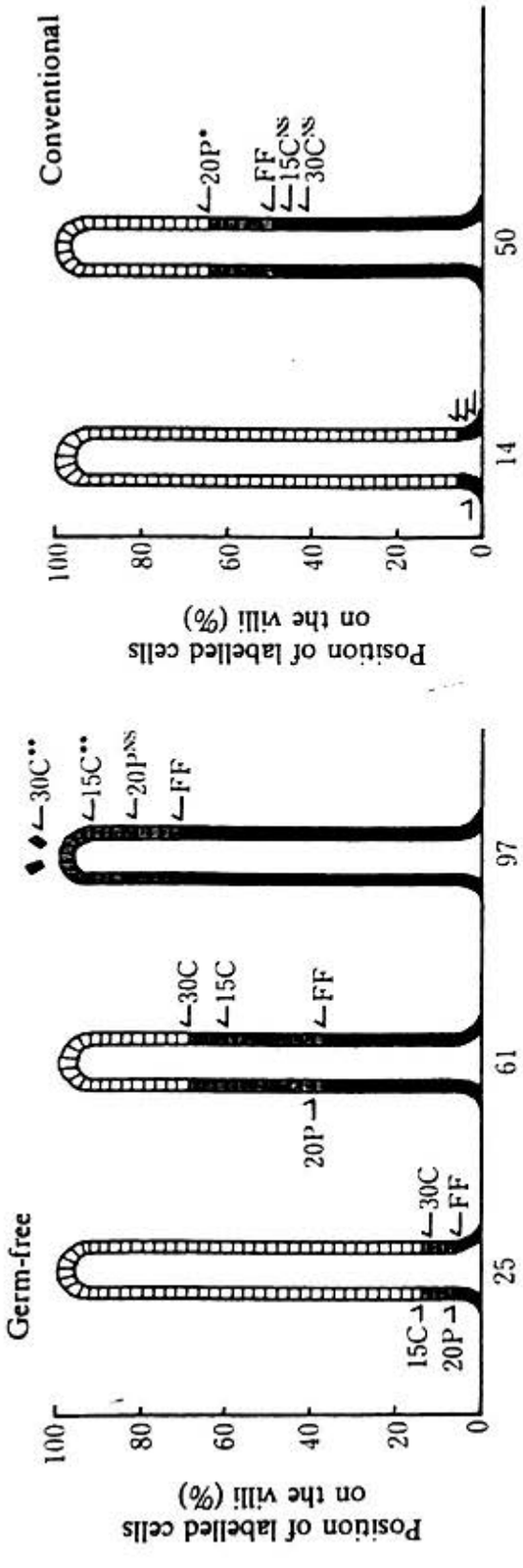


Fig. 11. Positional migration of labelled cells on the jejunal villi in germ-free and conventional mice[20] (* $P < 0.5$, ** $P < 0.01$ and NS compared with group FF).

Table 4
The effect of dietary fibre on the speed of regeneration of epithelial cells at different sites within the digestive tract[21]

	Cellulose feed			*Pectin feed*
	5%	*15%*	*30%*	*20%*
Germ-free mice				
Gastric mucosal epithelium	—	—	*	*
Jejunal epithelium	*	+	++	*
Colon epithelium	*	++	++	*
Conventional mice				
Gastric mucosal epithelium	—	—	*	*
Jejunal epithelium	*	*	*	++
Colon epithelium	*	*	*	++

*: No change compared with a fibre-free diet.
+, ++: Quicker cell regeneration than for the FF group.
—: Not measured.

indicate that pectin in itself does not have the physiological effect of speeding up regeneration, but since it only has the effect of speeding up the regeneration of intestinal epithelial cells in the presence of the intestinal bacteria, it is thought that some of the many products of pectin fermentation by the intestinal bacteria, such as short-chain fatty acids (SCFA) or ammonia, promote cell multiplication[22].

b. Dietary fibre and the production of short-chain fatty acids and ammonia in the intestinal tract

The studies of Sakata and Yajima showed clearly that short-chain fatty acids suppress the multiplication of cell cultures but promote cell multiplication *in vivo*[23]. So how could short-chain fatty acids be produced by intestinal bacterial fermentation in the experimental of Komai *et al.* on conventional mice? Table 5 shows the results. With cellulose feeding, the concentration (μmol/mg faeces) of excreted short-chain fatty acids was low, but with 20% pectin it became high. Daily excretion (μmol/day) was increased by cellulose and by pectin, but the increase was considerably greater with pectin. It is the concentration of shortchain fatty acids in the intestinal tract that is pertinent, given the stimulating effect of short-chain fatty acids on the intestinal mucous membrane, and the reason that 20% pectin feed accelerated the regeneration of intestinal epithelial cells could be the high concentration of short-chain fatty acid.

Ammonia is also said to be a cell growth modulator for intestinal epithelial cells. Table 6 gives data on ammonia production. Since in germ-free mice given fibre-free feed ammonia production was less than 1/10th that in conventional mice, it must have been influenced to a large extent by the intestinal microflora. With dietary fibre, the ammonia concentration was diluted with cellulose feed, but no such

Table 5
The effect of dietary fibre intake on faecal excretion of short-chain fatty acids in conventional mice (Komai *et al.* unpublished)

	FF (5)	*5C (5)*	*15C (5)*	*30C (5)*	*20P (5)*
Acetic acid	75.9%	69.7%	77.1%	89.1%	69.3%
Propionic acid	5.8	7.8	4.8	0	16.4
iso-Butyric acid	0	0.6	5.0	2.9	2.6
n-Butyric acid	1.6	6.1	3.5	2.2	2.6
iso-Valeric acid	10.3	9.9	4.0	0.9	6.2
n-Valeric acid	6.4	6.1	5.7	5.0	3.1
Excreted concentration of SCFA (μmol/mg faeces)	0.093 ±0.002	0.084 ±0.003	0.080 ±0.014	0.063*** ±0.001	0.133** ±0.006
Daily SCFA excretion (μmol/day)	31.4 ±0.5	49.1** ±1.5	80.9* ±14.2	145.2*** ±2.5	192.8*** ±8.2

Numbers are means or means ± standard errors.
*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$ (compared with group FF).

Table 6
The effect of dietary fibre intake on ammonia production in the large intestine (colon and caecum) (Komai & Kimura, unpublished)

	Intestinal contents (mg dry wt.)	*pH*	*Ammonia (N)*	
			(μg/intestine)	*(μg/mg dry wt.)*
CV-FF (6)	27.6±2.6	7.50±0.09	20.7±3.6	0.72±0.09
CV-5C (6)	46.8±8.3	7.22±0.05	31.4±3.3	0.73±0.06
CV-15C (6)	103.5±15.5	7.38±0.05	36.2±2.4**	0.39±0.05*
CV-30C (6)	160.5±26.2	7.11±0.05	42.3±3.0**	0.00±0.07**
CV-20P (6)	74.2±15.2	6.84±0.32	49.1±5.1*	0.73±0.13
GF-FF (5)	219.2±20.3	7.62±0.04	12.4±0.4*	0.06±0.00**

Numbers are mean values ± SE; numbers in brackets are number of animals.
*: $P < 0.05$; **: $P < 0.01$ compared with CV-FF, CV: conventional, GF: germ-free.

concentration was seen with pectin and the concentration was 2 times greater than with the fibre-free diet, even after taking body weight into account. This result also indicates that feeding pectin leads to an abundant intestinal fermentation. In fact, when the apparent digestion of cellulose and pectin was measured, the values were 32.4% for the 5%-cellulose diet and about 20% for the 15%- and 30%-cellulose diet, whereas a higher value of 48.2% was obtained with the 20%-pectin diet.

The amounts of fibre given here were probably excessive, but nevertheless, it is clear that the effect of dietary fibre in the lumen of the digestive tract differ according to the type of fibre. However these experiments leave unanswered the question as to the make up of the intestinal bacterial flora in these cases.

Table 7
The effect of cellulose-containing feeds on faecal steroid excretion in mice (Komai & Kimura, 1987)

Group	*Intake* (*g/day*)	*Faecal excretion* (*mg/day*)	*Neutral sterol excretion*		*Bile acid excretion*		*Free acid* (%)
			(*μg/day*)	(*μg/mg*)	(*μg/day*)	*μg/mg*)	
Germ-free mice							
Basic feed (5)	3.9	202	49.4	0.245	542.0	2.683	11.2
5% cellulose (4)	4.9	784	138.7	0.176	989.3	1.262	19.3
15% cellulose (4)	4.5	1110	167.2	0.151	1012.4	0.912	24.4
30% cellulose (4)	5.7	2013	327.2	0.163	1325.5	0.658	49.0
Conventional mice							
Basic feed (5)	4.4	357	71.7	0.201	749.8	2.100	53.9
5% cellulose (4)	4.6	584	82.2	0.140	949.0	1.625	48.5
15% cellulose (4)	4.6	1013	140.9	0.137	1413.7	1.396	38.9
30% cellulose (5)	6.4	2308	328.1	0.142	1427.3	0.618	47.6

Numbers of animals in brackets. Mean values only given.

c. Dietary fibre and faecal steroid excretion
Bile acids are synthesized in the liver from cholesterol and enter the hepatic circulation. In the intestinal tract they are mainly responsible for emulsifying food fat, playing the role of a surface-active agent. The vast majority (95%) is reabsorbed by the time it reaches the ileum, so that under normal conditions faecal excretion is confined to the remaining 5%. In themselves the bile acids are not carcinogenic, but they are auxiliary carcinogens, with carcinogenicity being heightened by chemical substances in cancer of the large intestine. Accordingly, the presence of large concentrations in the colon and rectum after accomplishing their main role in fat digestion and absorption presents problems. Hitherto it has been taken that food fibre hindered the resorption of bile acids from the lumen of the intestinal tract, and increased their faecal excretion[24], but the way in which this occurs and the involvement or otherwise of the intestinal bacteria are still not clear. These points are beginnning to be elucidated by the use of germ-free animals.

The effect of cellulose feed Here we give the results of a series of experiments conducted by Komai *et al.* using germ-free and conventional animals.

In germ-free mice, as feed cellulose content increased daily excretion of neutral sterols (*μg*/day) and excretion of bile acids (*μg*/day) increased; with group 30C, the increase in excretion of neutral sterols was 6.6-times that with the basic feed without added fibre, and the excretion of bile acids reached 2.4-times that with the basic feed (Table 7). However, the faecal concentrations (*μg*/mg) both of neutral sterols and of bile acids were diluted by the cellulose. In conventional mice a similar result was obtained to that in the germ-free mice. However, there was a difference in the proportions of free bile acids. Thus, in germ-free animals, bile acids are generally

Table 8
The effect of pectin feed on faecal steroid excretion in mice (Komai & Kimura, 1987)

Group	*Intake (g/day)*	*Faecal excretion (mg/day*	*Neutral sterol excretion*		*Bile acid excretion*		*Free bile acids (%)*
			(μg/day)	*(μg/mg)*	*(μg/day)*	*(μg/mg)*	
Germ-free mice							
Basic feed (5)	3.7	380	76.1	0.201	327.8	0.863	7.4
20% pectin (5)	4.3	1511	314.0	0.209	431.8	0.286	12.3
Conventional mice							
Basic feed (5)	4.9	337	72.7	0.221	223.8	0.664	54.3
20% pectin (4)	5.5	1449	118.4	0.089	1066.2	0.736	90.7

Numbers of animals in brackets. Mean values only given.

present in the intestinal tract in conjugated form, but it was interesting that whereas with the basic feed this was the case, the amount of free bile acids increased as cellulose content increased.

The effect of high pectin feed In an experiment similar to the one described above, daily excretion of neutral sterols (μg/day) was increased in germ-free mice by a high-pectin feed to 4.1-times that with a fibre-free diet, but daily excretion of bile acids (μg/day) was only increased slightly, to 1.3-times that with the fibre-free feed (Table 8). As far as the concentration excreted was concerned, there was no change in the case of neutral sterols, but the concentration of bile acids was diluted about 3 times. In conventional mice on the other hand, daily excretion of neutral sterols was increased only slightly to 1.6 times that with the fibre-free feed, whereas daily excretion of bile acids was increased to 4.8 times that with the fibre-free feed. As far as concentrations were concerned, neutral sterol concentration was diluted 2.5 times by 25% pectin feed, but there was no dilution of bile acids, which were if anything slightly more concentrated. The proportion of free bile acids was hardly affected at all in germ-free mice, where it was less anyway, but there was a steep increase in conventional mice due to feeding pectin.

Summarizing the above results for cellulose and pectin feeds: in experimental groups in which both an increase in daily bile acid excretion and an increase in the proportions of free bile acids were seen (e.g. germ-free 30C, conventional 20P, etc.), the possibility must be considered that bile acids detected were from epithelial cells passed into faeces, since the regeneration of intestinal epithelial cells was being promoted (Table 4) and there is abundant sloughing off of intestinal epithelial cells. This can no doubt be clarified in the future by analysing the bile or bile acids contained in isolated epithelial cells. Whatever the case may be, from data has which emerged showing that free bile acids speed up the regeneration of the epithelial cells of the small intestine[25], free bile acids seem to be the most important factor in shedding light on the phenomena described above.

d. Conclusions

This section has outlined the effects of different types of dietary fibre on the physiology of the digestive tract, concentrating on fundamental aspects. It does not apply directly to human life, but nevertheless it gives various hints which we would like to emphasize.

Firstly: the regeneration of epithelial cells, particularly in the lower digestive tract, can be speeded up by eating dietary fibre. This is possible when large amounts of readily-fermentable dietary fibre are consumed. Dietary fibre does not have an effect in accelerating the regeneration of the surface epithelia capping the fundic glands of the stomach, but it is unclear whether or not there is any effect on the various secretory cells. It could be that the effect of dietary fibre is greater when it comes to the functions of secretory cells.

Secondly: although large amounts of dietary fibre are needed to have an effect on the regeneration of epithelial cells in the lower digestive tract, even small amounts are effective at influencing the environment within the lumen. For instance even with 5% cellulose, the concentration of bile acids was decreased compared with a fibre-free feed by 77% in conventional mice, and 47% in germ-free mice, and even smaller quantities seem to have an effect. When there is a lot of sloughing off of epithelial cells, one would expect large energy losses, and besides increasing free bile acids, there is a danger of disturbances to other metabolic systems with high fibre intakes (this may be desirable in the case of pathological states such as obesity or hyperlipidaemia); so although it is recommended that a lot of dietary fibre is eaten in normal life, there needs to be attention that intakes are not excessive.

Thirdly: different types of dietary fibre have different effects on the physiology of the digestive tract. Thinking about the human diet, there is not much opportunity to eat dietary fibre as a single product. However, it this was done then probably the phenomenon described above (increased sloughing off of epithelial cells in the digestive tract) would occur. Therefore, the best way is probably to combine foods which contain different types of dietary fibre, rather than to eat large amounts of a single type of dietary fibre.

Whatever the case may be, it is clear that the intestinal bacteria contribute to the expression of the beneficial physiological effects of dietary fibre; therefore by controlling the normal intestinal microflora sensibly it should be possible to create a more desirable intestinal environment.

C. DIET AND INTESTINAL BACTERIA IN THE AGED

We have already mentioned aspects of diet and intestinal bacteria in a long-lived village in Japan (Tsunabara) (Fig. 9, 10); here we will concentrate in research in long-lived areas of the Soviet Union, which is not often cited. One thing that the world famous long-lived areas, such as the Caucasus in the USSR, Birucabamba in Ecuador, Hunza in the Karakoram region of Pakistan and the Smoljan region of Bulgaria, have in common is the quality of the diet: cereals such as wheat, barley and maize, and also pulses and potatoes are eaten, and they do not over eat – total

Table 9
The main components of food eaten in 1 day by aged inhabitants of the Abkhaz Autonomous Region of the Soviet Caucasus[28] (g/day)

Age	*Cereals*	*Maize*	*Kidney beans*	*Cleaned barley*	*Vegetables*	*Fruit*	*Milk*
60–74 (68)	171.4	159.2	47.5	23.1	196.5	144.3	444.1
75–89 (64)	151.6	122.5	29.6	15.9	140.6	163.2	418.9
90– (46)	145.9	118.4	47.5	14.7	160.0	174.5	472.6

Age	*Fermented milk*	*Concentrated fermented milk, cream*	*Creamy oil, butter*	*Meat*	*Fish*	*Sugar*	*Plant fats*	*Eggs*	*Wine*	*Vodka*
60–74 (68)	95.5	3.6	17.0	67.2	5.5	33.3	5.4	20.0	114.8	5.5
75–89 (64)	117.0	3.9	16.7	38.5	2.2	30.7	3.6	13.2	53.7	7.3
90– (46)	107.4	2.7	9.5	35.1	2.4	32.2	4.8	14.8	18.2	4.1

intakes of energy and protein are not excessive. The diet is balanced, and is also rich in fruit and vegetables. They also have in common that they live in climates with clean air, go to bed early and get up early, and do sufficient work, without stress. Moreover, the Caucasus, the Hunza region and the Smoljan region are characterized by the consumption of fermented milks such as yoghurt as part of the normal diet.

The area that has been most studied, the Caucasus in the southern Soviet Union, is an intercontinental bridge linking Europe and Asia, sandwiched between the Black Sea and the Caspian Sea. Various races live in this area, but the general principles governing the diet are surprisingly fixed[26]. For example, there are fixed and unchangeable food contents, an aversion to over-eating and a strict atmosphere surrounding meal times, and the food is all fresh, etc. One food that can be found throughout the Caucasus is the milk product matsuni or kefir; this is a fermented milk with a flavour resembling that of yoghurt. Matsuni is slightly more watery than yoghurt, and kefir is more watery still. In practice matsuni or kefir are eaten after every meal, particularly when something has been eaten that is difficult to digest. In the Soviet Union, kefir as a fermented milk has a high reputation, and accounts for 70% of the total amount of fermented milk consumed[27]. The above facts have been dealt with in many books; here, we report recent insights into diet and intestinal bacteria, etc. among aged people in the Caucasus.

1. Food and Intestinal Bacteria in the Case of Aged People in the Abkhaz Autonomous Region of the USSR

a. Food and intestinal bacteria in the case of aged people

Kvasnikov *et al.*[28] investigated food and intestinal bacteria, using elderly (68 aged 60–74 and 64 aged 75–90) and aged (46 aged over 90 years) subjects of the Abkhaz Autonomous Region in the Caucasus region of the Soviet Union, which is reputed

Table 10
The chemical composition of daily food eaten by aged people in the Abkhaz Autonomous Region[18]

Main food component	*Age group*			*Recommended quantity*
	60–74 years	*75–89 years*	*≥ 90 years*	
Protein (g/day)	98.4	83.0	80.8	75.5–71.5[a]
Animal protein	47.7	44.1	43.1	45.5–43.0[a]
Essential amino acids (g/day)				
Trp	1.44	1.33	1.30	1[b]
Leu	8.54	7.14	7.05	4–6
Ile	4.55	3.92	3.86	3–4[b]
Val	5.32	4.59	4.50	4
Thr	3.95	3.47	3.40	2–3[b]
Lys	5.84	4.95	4.87	3–5[b]
Met	2.08	1.83	1.80	2–4[b]
Phe	4.80	4.16	4.07	2–4[b]
Non-essential amino acids (g/day)				
His	3.22	2.70	2.84	2
Arg	5.14	4.38	4.28	6
Cys-Cys	1.38	1.18	4.16	2–3[b]
Tyr	5.53	4.83	4.82	3–4[b]
Ala	4.41	3.57	3.48	3
Ser	5.12	4.41	4.35	3
Glu	19.45	16.33	16.13	16
Asp	6.59	5.02	4.98	6
Pro	7.24	6.45	6.34	5
Gly	3.34	2.63	2.57	3
Carbohydrate (g/day)	304.4	247.6	243.6	304–288.5[a]
Lipid (g/day)	79.2	70.1	64.9	73.5–67.0[a]
Vegetable fat	26.6	16.2	17.5	25.0–23.5[a]
Polyunsaturated FA	11.2	8.2	8.9	3–6[b]
Saturated FA	25.8	21.9	19.4	
Linoleic acid	10.3	7.3	8.1	2–6[b]
Monosaturated FA	22.7	15.9	15.2	
Cholesterol	0.290	0.230	0.230	0.3–0.6[b]
Phospholipid	3.69	2.75	2.80	5
Triglyceride	57.1	44.1	41.7	
Minerals				
Calcium	972	881	927	800–1000[b]
Phosphorus	1881	1549	1560	1000–1500[b]
Sodium	2992	3017	2905	4000–6000[b]
Potassium	3369	2668	2811	2500–5000[b]
Chlorine	2457	2098	2169	5000–7000[b]
Magnesium	494	397	401	300–500
Iron	22.6	17.1	16.9	15
Zinc	15.8	11.9	11.7	10–15[b]
Manganese	7.51	5.97	6.00	5–10[b]
Copper	3.1	2.3	2.2	2

Table 10—*contd.*

Main food component	*Age group*			*Recommended quantity*
	60–74 years	*75–89 years*	*⩾ 90 years*	
Selenium	0.021	0.015	0.016	0.5
Fluorine	0.449	0.552	0.358	0.5–1.0[b]
Iodine	0.120	0.101	0.109	0.1–0.2[b]
Vitamins (mg/day)				
Ascorbic acid	65.4	51.3	57.2	88–75; 69–75[a]
Thiamine	1.54	1.23	1.24	2–1.8; 1.6–1.4[a]
Riboflavin	1.84	1.63	1.70	2.6–2.4; 2.3–1.9[a]
Nicotinic acid	19.0	14.7	14.8	21–19; 18–15[a]
Pantothenic acid	5.85	4.79	5.02	5–10[a]
Pyridoxine	2.58	2.02	2.02	2.4–2.0; 1.9–1.6[a]
Folic acid	251.3	210.6	205.5	0.2–0.4[b]
Choline	230.0	191.1	201.1	500–1000[b]
Vitamin E	22.9	16.6	17.4	10–20[b]
Calorie content (kcal)	2606	2187	2095	2425–2100[a]

[a] First numbers show values for 60–70 years, 2nd numbers show values for ⩾ 70 years.
[b] The minimum levels and optimum levels in food are given, calculated using the food/nutrition equation for balanced foods on the basis of the fact that the energy content of the daily food investigated was relatively low, and assuming that amino acids and minerals were not particularly deficient up to this age. Therefore there are no intermediate values between these values.

to have many aged people. Table 9 shows the main foods and amounts eaten by the aged subjects in 1 day.

It is clear that compared with 60–74-year-olds, aged subjects of 90 and older had lower intakes of cereals, butter, meat, fish, eggs, and wine, but did not have lower intakes of cow's milk, fermented milk and vegetable fats, etc. They also investigated the chemical composition of the daily intake, and the results are in Table 10.

According to this, the aged subjects over 90 had a mean energy intake 511 Kcal lower than the 60–74-year-olds, but there was no remarkable change in nutrient intakes with increasing age, and the diet showed a good balance.

Kvasnikov *et al.* also investigated the normal intestinal microflora in each age stratum including the aged in a series of studies. The results are shown in Table 11. In aged people aged 90–105, *Lactobacillus* spp. were particularly common, and counts of *Bifidobacterium* were not very different from those in younger age groups.

Table 12 lists species of lactobacilli, lactococci and bifidobacteria isolated from different age groups. Even in aged people of 90 and over there was a rich variety of lactic acid bacteria. When the antibiotic activity of the isolates towards *E. coli*, *Shigella flexneri*, *Proteus mirabilis* and *Staphylococcus aureus* was investigated, many of the isolated strains showed strong antibiotic activity.

It seems that in this long-lived region, the structure of food – based on milk products and plant foods – in terms of quantity and quality created conditions in which circulatory diseases and cancer did not readily occur. It can be deduced that

Table 11
Structure of the normal intestinal bacterial flora in inhabitants of the Abkhaz Autonomous Region by age group[28]

Age (years)	*Counts per 1 g faeces*							
	Lactobacillus		Enterococcus		Bifidobacterium		Bacillus	
	range	*mean*	*range*	*mean*	*range*	*mean*	*range*	*mean*
1–16	10^4–10^9	6×10^6	10^3–10^9	4×10^7	10^8–10^9	5×10^6	10^2–10^7	2×10^6
17–55	10^4–19^9	1×10^8	10^4–10^9	1.7×10^7	10^6–10^8	5×10^7	10^7–10^8	3×10^7
60–74	10^5–10^{10}	7×10^8	10^6–10^{10}	5×10^8	10^5–10^9	4×10^8	10^6–10^8	1×10^8
75–89	10^6–10^{10}	3×10^8	10^6–10^9	1.4×10^8	10^5–10^9	1×10^8	10^6–10^7	3×10^7
90–105	10^7–10^{12}	4×10^9	10^7–10^{10}	8×10^8	10^6–10^9	5×10^8	10^5–10^8	1×10^7

Table 12
Lactobacilli and bifidobacteria in the intestinal tract of inhabitants of the Abkhaz Autonomous Region[28]

Age (years)	*Species detected*
1–3	*Lactobacillus casei* var. *casei*, *L. casei*, *Streptococcus* faecium*, *S. thermophilus*, *Bifidobacterium bifidum*, *B. longum*
4–8	*L. cellobiosus*, *L. acidophilus*, *S. thermophilus*, *S.* faecium*
9–14	*L. casei* var. *alactosus*, *L. buchneri*, *L. acidophilus*, *L. cellobiosus*, *S. thermophilus*, *S.* faecium*, *B. bifidum*, *B. longum*
17–25	*L. plantarum*, *L. acidophilus*, *S.* durans*
35–55	*L. plantarum*, *L. viridescens*, *S.* faecalis*, *S.* durans*
60–74	*L. casei*, *L. cellobiosus*, *L. plantarum*, *S.* faecium*, *S.* durans*, *S. thermophilus*
75–89	*L. plantarum*, *L. acidophilus*, *L. salivarius*, *L. casei* var. *alactosus*, *L. buchneri*, *L. cellobiosus*, *S.* durans*, *S.* faecium S.* faecalis*, *S. thermophilus*, *Leuconostoc mesenteroides*, *L. curvatus*, *B. bifidum*, *B. longum*
90–105	*L. fermenti*, *L. cellobiosus*, *L. plantarum*, *L. acidophilus*, *L. buchneri*, *L. casei* var. *alactosus*, *L. plantarum* var. *arabinosus*, *L. salivarius*, *L. curvatus*, *B. bifidum*, *B. longum*, *S.* durans*, *S.* faecium*, *S. thermophilus*, *Leuconostoc mesenteroides*

* Since 1984 changed from genus *Streptococcus* to genus *Enterococcus*.

the good make-up of the intestinal bacterial flora, with its complex associations, plays an important part in the overall prevention of ageing changes, and results in prolonged longevity.

b. Plasma lipid level in the aged

Working independently from Kvasnikov, Khechinashvili *et al.*[29] measured blood pressure and plasma lipids in the Abkhaz Autonomous Region in 12 people aged below 90 (81–89) and 39 aged people of 90–106.

As shown in Table 13, compared with elderly people aged below 90, the aged

Table 13
Pulse rate, blood pressure and plasma lipids in elderly people aged less than 90 and in aged people in the Abkhaz Autonomous Region[29] (mean ± s.d.)

Measured item	*Elderly people < 90*		*Aged people*	
	Male (81–88)	*Female (86–89)*	*Male (90–106)*	*Female (90–102)*
Subjects	6	6	15	24
Age	85.7 ± 2.4	87.5 ± 1.3	95.7 ± 5.4	93.2 ± 3.5
Pulse rate/min	82.7 ± 10.5	82.0 ± 5.5	77.3 ± 9.5	82.9 ± 13.2
Blood pressure				
Systolic (mmHg)	153.3 ± 16.0	173.3 ± 18.9	160.0 ± 28.5	160.6 ± 22.8
Diastolic (mmHg)	83.3 ± 12.5	101.7 ± 15.7	87.0 ± 11.8	95.6 ± 14.3
Total cholesterol (mg/dl)	156.2 ± 23.8	184.2 ± 24.1	153.3 ± 31.4	184.7 ± 42.5
Triglyceride (mg/dl)	64.8 ± 42.5	58.7 ± 20.7	82.3 ± 41.9	111.9 ± 67.3
Lipoprotein cholesterol (mg/dl)				
HDL-chol	48.8 ± 6.4	60.2 ± 11.2	44.8 ± 5.9	48.5 ± 12.8
LDL-chol	94.5 ± 25.1	112.3 ± 20.9	93.9 ± 25.5	113.8 ± 34.3
VLDL-chol	12.8 ± 8.5	11.7 ± 4.3	16.6 ± 8.3	22.4 ± 13.4
HDL-chol/total cholesterol	32.2 ± 7.9	33.0 ± 7.1	29.8 ± 5.9	27.3 ± 8.2

people of 90 and above showed unchanged blood pressure, and plasma total cholesterol and HDL-cholesterol showed very little change compared with those under 90 of the same sex. The proportion of HDL-cholesterol in total cholesterol was not much different in the aged compared with the elderly people under 90 (males 29.8%, females 27.3%). Taken together the above data point to low incidences of ischaemic heart disease and atherosclerosis.

2. Plasma LDH Activity in Aged People in the Soviet Republic of Azerbaizhan

There is also a report on the plasma lactate dehydrogenase (LDH) activity in aged people in the Kedabeksky area of the Republic of Azerbaizhan in the same Caucasus region where there are reputed to be many aged people. Mamedov *et al.*[30] carried out an investigation into total plasma LDH activity and isozyme patterns in people aged from 20 to 120 (Table 14).

Looking at Table 14, in 30 aged people of 90–120, total plasma LDH activity was still at about the same level as in 50–59-year olds, showing no real increase. The isozyme pattern was characterized by more LDH_1 and little $LDH_4 + LDH_5$. The fact that there was little of the $LDH_4 + LDH_5$ fraction, which is most common in skeletal muscle and liver, no doubt reflects the regression of skeletal muscle with age. Assuming this to be the case, Mamedov *et al.* concluded from the fact that there was no marked decrease in LDH activity, that overall intracellular energy

Table 14
Plasma LDH activity and isozyme patterns in subjects of different age groups[30] (Kedabeksky area of the Republic of Azerbaizhan) (mean ± s.e.)

Subjects	*Age*	*Total LDH activity (Wróblewski U/mg N)*	*Isozyme pattern (%)*			
			LDH1	*LDH2*	*LDH3*	*LDH4+LDH5*
20	20–25	6.60±0.13	22.8±5.6	31.8±5.8	28.8±4.7	17.0±6.7
20	50–59	4.07±0.37	26.8±8.0	36.3±6.6	25.0±4.8	11.9±3.4
20	60–69	3.01±0.36	25.7±7.3	32.4±4.6	29.5±3.9	12.9±4.7
20	90–120	4.17±1.21	35.3±4.3	35.9±4.3	27.1±4.5	1.8±1.0

consumption was at a similar level to that in the prime of life, and that long life is possible through the healthy functioning of the energy producing pathways.

D. FUTURE DIET AND THE MANAGEMENT OF THE INTESTINAL MICROFLORA

1. Fermented Milk Products and Health

Recently, Shiomi *et al.*[31] at the Japanese National Institute of Health reported that when mice with transplanted Ehrlich ascites cancer or Sarcoma 180 tumours were given feed with polysaccharide extracted from kefir grains added at 0.1%, the multiplication of these tumours was suppressed by 59% and 81% respectively.

Toba *et al.*[32] investigating kefir grains observed that there were many yeasts in the surface layers covered with short rods thought to be *Lactobacillus kefyr*, long non-encapsulated rods, and yeasts. There were many yeasts towards the surface, but they decreased in numbers towards the inside of the grains. Inside the grains there were no short rods, but it was occupied by long encapsulated polysaccharide-producing rods. It is thought that the last mentioned encapsulated polysaccharide-producing bacteria hold the key to the physiological activity of kefir grains.

There are reports of aged people over 100 years old in Japan who have drunk a lot of fermented milk[33]. Probably because consumption of meat, which readily causes intestinal putrefaction, is traditionally low among the Japanese the intestinal bacteria are purged even without consuming much in the way of fermented milk to fulfil this role. However, when one thinks of the westernization of the Japanese diet (tendency towards a meat-based diet) which is going on at the present time, the time has come to take fermented milks seriously as a means of producing a beneficial intestinal microflora.

a. The beneficial physiological effects of lactic acid bacteria

A decrease in the activity of enzymes associated with the production of harmful substances and of cancer-causing substances in the intestinal tract has been noted in practice when yoghurt or lactic acid bacteria are administered[34]. Consumption of yoghurt or lactic acid bacteria also serves to maintain a healthy balance in the

Table 15
Immunoglobulin levels in gnotobiotic mice given feed with liberal addition of live yoghurt or heated yoghurt (Wade, S. *et al.*)[38]

Diet	IgG_{2a}	IgG_{2b}	IgG_1	IgG_M	*IgA*
Live yoghurt	27.3	12.4	24.7	48	trace
Thermized yoghurt	9.5	4.7	10.0	32	trace

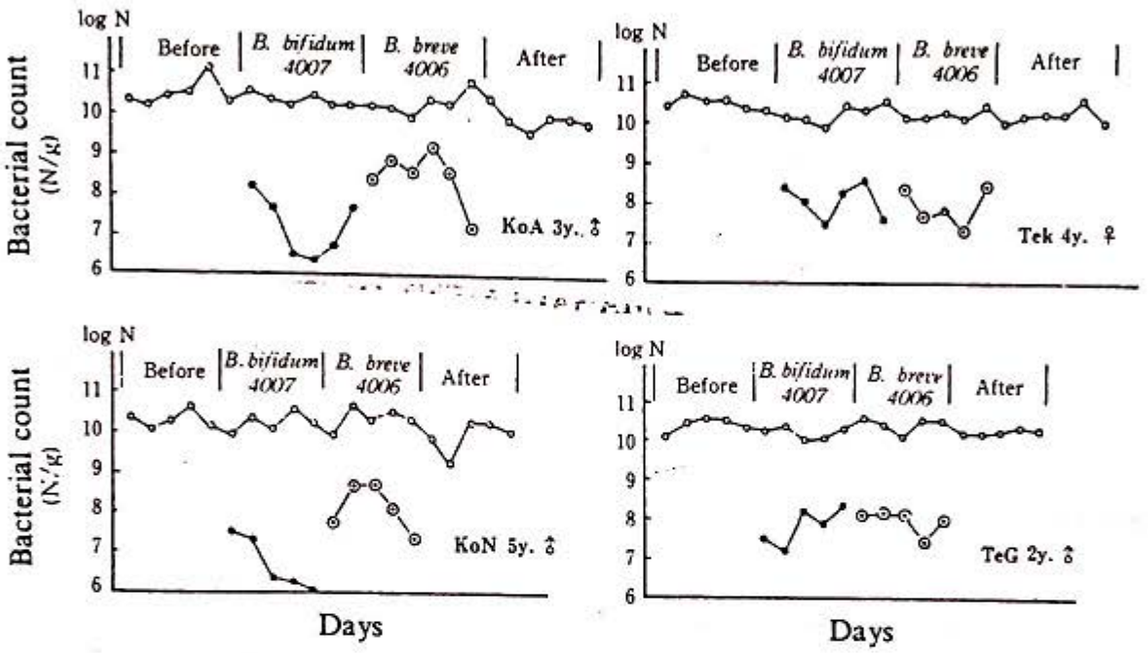


Fig. 12. Level of recovery and behaviour of administered bifidobacteria.[40]

intestinal microflora, and works to eliminate harmful bacteria such as *E. coli*, and make a healthy intestinal environment. In many animal experiments, effects have been noted from administration of yoghurt or lactic acid bacteria which might contribute to the prevention of ageing, such as immunoactivating actions due to heightening macrophage and T-cell function[35], anti-cancer activity, and an anti-hyperlipidaemia action[36], etc. The physiological effects of administering yoghurt or lactic acid bacteria appear to differ between types with live and killed bacteria. Besnier and Bourlioux showed that intestinal lactase activity was raised in germ-free mice given live yoghurt[37]. However, this phenomenon is not seen with dead yoghurt. Moreover, when Wade *et al.* measured immunoglobulins in germ-free mice given liberal amounts of live yoghurt or heat-treated yoghurt, there was a larger increase in immunoglobulins with the live yoghurt (Table 15).[38].

Using the detection in expired air of hydrogen produced by the breakdown of lactose by intestinal bacteria, Kolars *et al.* studied the metabolism of lactose, cow's milk and yoghurt (lactose content of all 3 about 18 g) in the intestinal tract, and found that more hydrogen was expired by subjects given lactose or cow's milk (about 3 times the amount with yoghurt)[39].

Many years have passed since it was noted definitely that lactic acid bacteria in

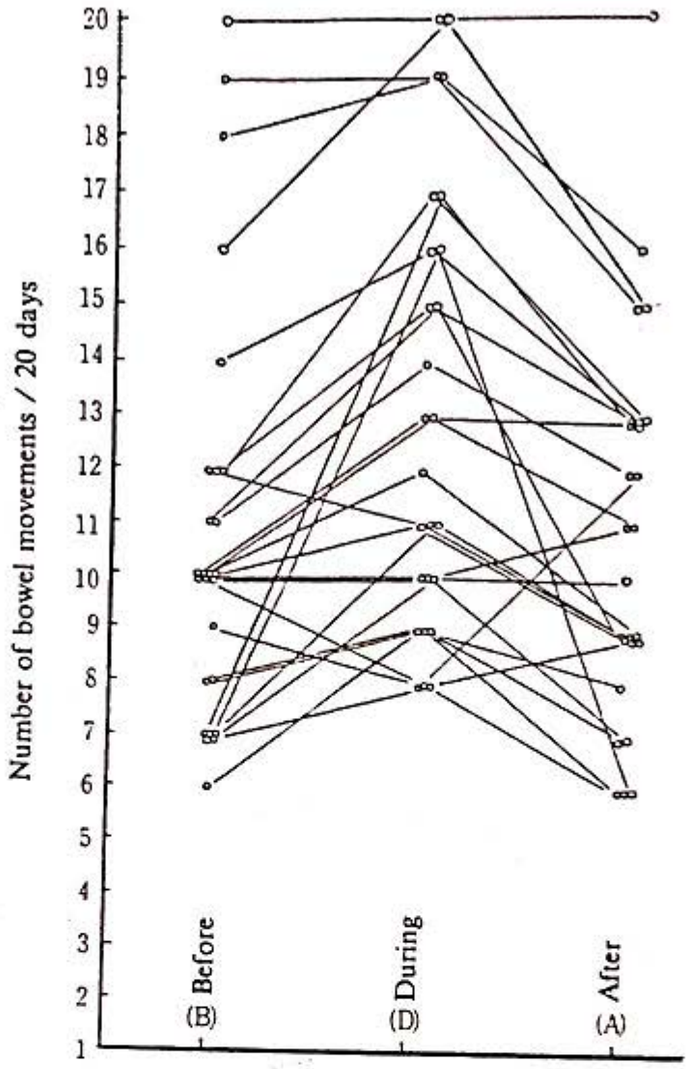


Fig. 13. The effecting bifidus fermented milk on the number of bowel movements (26 subjects, natural bowel movements) D A, B ($P < 0.001$).[41]

yoghurt are transported live along the digestive tract, and this has been confirmed not only in experimental animals, but also in man[38].

Bifidobacterium spp. (bifidobacteria), formerly classified in genus Lactobacillus, have been reported to have various beneficial effects such as suppressing digestive complaints such as diarrhoea or constipation, infectious diseases, and intestinal putrefaction, and improving vitamin metabolism. Tanaka *et al.* gave bifidobacteria (*B. bifidum* 4007 and *B. breve* 4006) at 10^{10} daily to healthy infants and adults and investigated recoveries in faeces; the administered bacteria were recovered at 10^{7-8}/g, but disappeared quickly after ceasing administration (Fig. 12). At this time, a significant increase was noted in the moisture content of faeces when *B. breve* was administered, and there was a decrease in the ammonia content of faeces and the concentration of indican excreted in urine[40].

b. The effect of drinking fermented milk in aged people

It is well known that constipation problems are common in old people, and this fact should be seen against the background of diet or a general decline in activity with advancing years, various basic problems, and the unstable mental constitution of

old people, etc. Decreases in bifidobacteria (representative beneficial bacteria), and increases in *Escherichia coli* (representative putrefactive bacteria), are characteristic of the intestinal microflora in elderly people. Constipation means that products of putrefaction in the intestinal tract are held in the intestinal tract for a longer period, increasing the opportunity for cancer-related substances to make contact with the intestinal mucous membrane as mentioned in Section 2.

Tanaka and Kudasaka investigated bowel movements in nearly bedridden old people (av. 70 years old), and showed that drinking bifidus fermented milk tended to improve bowel movements[41] (Fig. 13). From their data, there was a clear improvement in bowel movements without any side-effects even when the bifidus milk was administered over a long period. This, and the fact that over 90% of the old people liked drinking it, indicates that drinking bifidus milk could be a useful method for preventing constipation in the elderly.

2. Implications for Diet and Health

The previous section introduced recent insights into diet and health with reference primarily to long-lived areas of the Soviet Union. This gives hints as to how the Japanese might also live to a healthy old age, although there are many differences in weather, climate, social and economic conditions from these regions.

Some time ago, Kondo published the results of an investigation into the actual relationships between food habits and the longevity of the inhabitants of 875 areas of Japan over 31 years[42]. His results confirmed the following causal relationships.

(1) Villages with rice-based diets had a shortened life, with a higher incidence of early death from strokes, and generally premature ageing.
(2) Villages with insufficient vegetables and a preponderance of fish in the diet had a shortened life, with a higher incidence of early death from heart disease.
(3) Long-lived villages ate plenty of fish and soyabeans in their normal diet.
(4) A lot of vegetables were eaten in long-lived villages.
(5) There was a low incidence of stroke in villages where seaweed was part of the daily diet.

It is a happy thing that as Japan has become one of the long-lived nations of the world, the above mentioned bad points have improved slightly. However, Japan is still in the top rank in the world when it comes to death from stroke; and in agricultural areas such as the northeast of Japan, some people still have quite a high salt intake[43].

Shibata *et al.* of the Department of Public Health of Akita Prefecture, Japan investigated the correlation between the salt intake of the Japanese and their intakes of different food groups on the basis of national nutrition survey results, and cited miso and vegetables (except green vegetables) as having high positive correlations, and eggs and meat as having high negative correlations. This indicates that salt intake is higher when there are fewer proteinaceous foods. Kimura *et al.* have been able to confirm this in experimental animals[44,45]. They carried out a preference trial

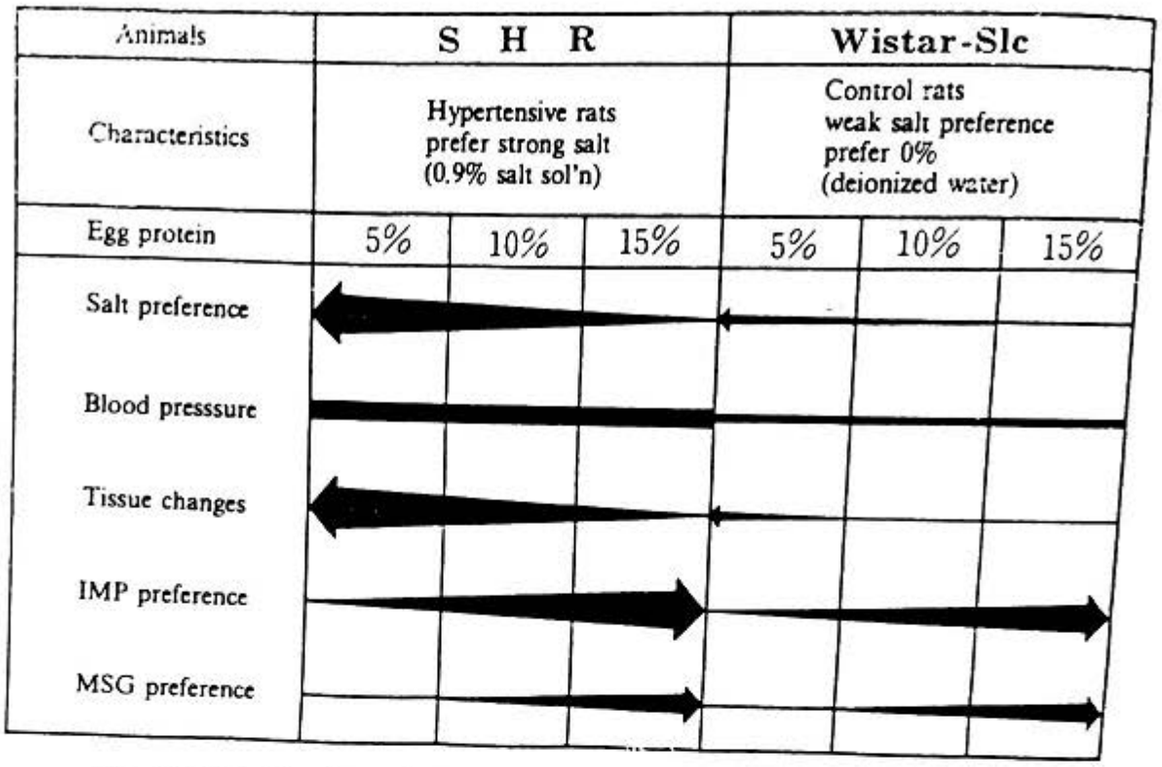


Fig. 14. Modification of salt preference in rats due to food protein level.[43]

with salt solution and diets with different protein contents, using spontaneously hypertensive rats (SHR) and control rats (Wistar-Slc), and observed that preference for salt was weakened at higher protein levels (Fig. 14).

In the SHR rats, which are a basic model for hypertension, giving diets high in good-quality protein weakened salt preference and was significant in improving pathological changes in tissues (arteriosclerosis, etc.). When low-concentrations of a flavour-enhancing solution (sodium inosinate and sodium glutamate at 5 mM each) were placed alongside salt solutions of different concentrations, preference for the flavour-enhancer was strongest among the group calculated to have excess protein intake, and preference for salt solution was further weakened.

From epidemiological data and data from experimental animals such as that described above, it is evident that a suitable intake of good quality protein is desirable for preventing stroke. This should be supplied in a diet which also provides a suitable intakes of food fibre; and so much the better for health if the diet can also maintain a predominance of bifidobacteria and lactic acid bacteria in the intestinal microflora.

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