higher forms of life which are exacting in their requirements, the minor vitamins being necessary only for the proper balance of the intestinal flora?

Dr. F. Bergel: This may well be the case. As I have mentioned before, the division of the members of the vitamin B complex into major and minor components was first suggested by the brothers Williams.

Dr. F. W. Chattaway (School of Medicine, Leeds): It is an over-simplification to suggest that vitamin B_c, folic acid and the *Lactobacillus casei* factor are one and the same. The following evidence supports the view that these factors are different: (1) Keresztesy, Rickes and Stokes (1943) have described the preparation from liver of a factor active for *Streptococcus lactis* R but almost inactive for *L. casei*; (2) Chattaway, Dolby, Happold, McMillan and Waters have shown in unpublished work that a folic acid concentrate is inactive for *L. casei* unless certain unknown factors are added, while Chattaway, Dolby and Happold (1944) found that a liver preparation active for *L. casei* could be split into at least two components, one of which was obtained in a form having no stimulatory effect on *S. lactis* R; (3) Williams (1944) has recently referred to the "folic acids" and apparently believes that these are multiple factors.

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Mr. F. A. Robinson gave the following replies:

To Miss Copping: Both pyridoxal and pyridoxamine, or a mixture of the two previously known as pseudo-pyridoxin, are as active as pyridoxin when tested on rats. Unfortunately they are far more potent than pyridoxin in stimulating the growth of certain micro-organisms, particularly *Lactobacilli*, and the results of microbiological tests of the vitamin B_6 content of foodstuffs may, therefore, be considerably higher than those of rat tests.

To Dr. Baar: I agree with Dr. Baar that from published work pyridoxin appears to be the only factor the absence of which causes anaemia in pigs. On the other hand it is highly probable that the diet used in the experiments mentioned by Dr. Baar contained folic acid and, until further tests are carried out with a diet known to be free from folic acid, no valid conclusions can be drawn as to the effect of folic acid deficiency on pigs.

Afternoon Session: Chairman, Dr. L. J. Harris

Fermentation and Human Nutrition

Dr. B. S. Platt and Dr. R. A. Webb (Medical Research Council Human Nutrition Research Unit, National Hospital, Queen Square, London, W.C.1)

Fermentation may, in various ways, make a contribution to human nutrition, the full significance of which has scarcely begun to be appreciated. Foodstuffs may be fermented before they are eaten, and again

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after ingestion, when the materials left unabsorbed in the lower parts of the alimentary canal are subjected to the action of micro-organisms. The contribution of fermentative changes to the supply of B vitamins is the aspect with which this communication is mainly concerned.

Foods Produced by Fermentation

Fermented foods are to be found in various forms all over the world. Generally the fermentation is of carbohydrates; some, however, primarily involve proteins. In Table 1 examples of fermented foods are set out according to whether the fermentation involved is of carbohydrates or of proteins. The foods are arranged also according to the part of the world in which they are to be found.

TABLE 1

CLASSIFICATION AND GEOGRAPHICAL DISTRIBUTION OF SOME FERMENTED FOODS AND BEVERAGES

	Carbohydrate basis	Protein basis
Geographical distribution	Cereal grains, roots and tubers, fruits and fruit juices, milk, sap and sugars	Milk, soya beans and oil seed residues, fish
EUROPE	Bread and other dough products, fermented gruels and beers, wines, pickles, fermented milks	Cheeses
ASIA	Cereal doughs, fermented rice products including rice wines, palm toddy, "poi", pickled vegetables	Soya sauce, fermented whole soya beans and bean curd, fish, oil seed press residues
AFRICA	Sorghum, maize, millet, cassava and banana beers and gruels, palm toddy, fermented fruits	Milk curds
N. AMERICA	Spruce and other beers, wines, pickled vegetables	Cheeses
S. AMERICA	Cassava and maize beers, wines	—

Attention may be drawn to three important points: (1) As a process of food preparation fermentation is ubiquitous; (2) the variety of materials used as a basis for fermentation is wide; (3) alcohol is by no means always the object of the fermentation although it is commonly a product.

Production of Kaffir Beer

The processes of fermentation may be illustrated by work which we have carried out recently on Kaffir beer. For convenience, the processes can be considered under three heads:

The methods by which raw materials are, if necessary, converted into a suitable medium for fermentation.

The accessory substances or essential nutrients which are produced in the course of fermentation.

The end products of the process.

Changes in the Raw Material prior to Fermentation. Cereal grains are often allowed to germinate and it has been observed that in the process a considerable increase may occur in the amount of B vitamins as is shown in Table 2.

Sometimes the treatment of cereal grains prior to fermentation is arrested short of actual germination. Nevertheless, a similar synthesis of B vitamins, as well as of other food factors, occurs. We recognize

TABLE 2

SYNTHESIS OF B VITAMINS DURING GERMINATION OF MAIZE: BASED ON BURKHOLDER (1943)

Vitamin	Ratio of amount in germinated grain, to original taken as 1
Riboflavin	3·5
Nicotinic acid	3·7
Biotin	2·3
Pantothenic acid ..	1·7
Pyridoxin	1·1
Folic acid	4·0
Inositol	1·9
Aneurin	0·94

that, in the early stages of fermentation, some of the changes are incidental whereas others are an essential part of the process. Micro-organisms may well assist at this stage without being necessary for the fermentative process itself. Micro-organisms may be associated with the raw material; these may be contaminants which have to be eliminated from this first phase in fermentation. On the other hand, there are accompanying organisms which proliferate in the early stages, later playing a role in fermentation, and in other ways contributing to the sum total of the changes required.

Synthesis of Vitamins during Fermentation. In the stage of fermentation which corresponds with the inoculation of the wort and the proliferation of the yeast in European brewing, further amounts of B vitamins are synthesized. In the European process this has recently been worked out for riboflavin with the results shown in Table 3.

TABLE 3

RELATIVE INCREASE IN QUANTITY OF RIBOFLAVIN IN FERMENTATION DURING EUROPEAN BEER BREWING: BASED ON TULLO AND STRINGER (1945)

Serial no. of brew	Increase		
	In beer (wort = 1)	In yeast (pitching yeast = 1)	Total (original = 1)
1	2·0	10·8	2·36
2	2·1	13·8	2·55
3	1·4	21·7	2·49

Importance of the End Products of the Process. The terminal phase of fermentation in which there may be accumulation of organic acids, alcohol and gaseous products of fermentation, especially carbon dioxide, is important in relation to the subject under consideration because, as the end products accumulate, a chemical and micro-biological equilibrium is achieved, which tends to preserve the B vitamins developed earlier in the process.

Outline of Kaffir Beer Manufacture. The beer is made from malted grain and unmalted starchy material. Grain for "malt" is soaked and germinated and then dried in the sun and pounded. During the germination the micro-organisms which are already present on or in the grain multiply to some extent and produce hydrolytic enzymes. Various proportions of malted and unmalted material are mashed in cold and boiling water, respectively, and mixed. The enzymes, developed microbiologically or produced by the grain during germination, saccharify the starch. Bacteria acting on the sugars formed produce lactic acid and certain B vitamins. The beer is sometimes consumed at this stage, after only one day's fermentation; it is then called sweet beer. For an alcoholic beer, the brew is heated after the first day and subsequent

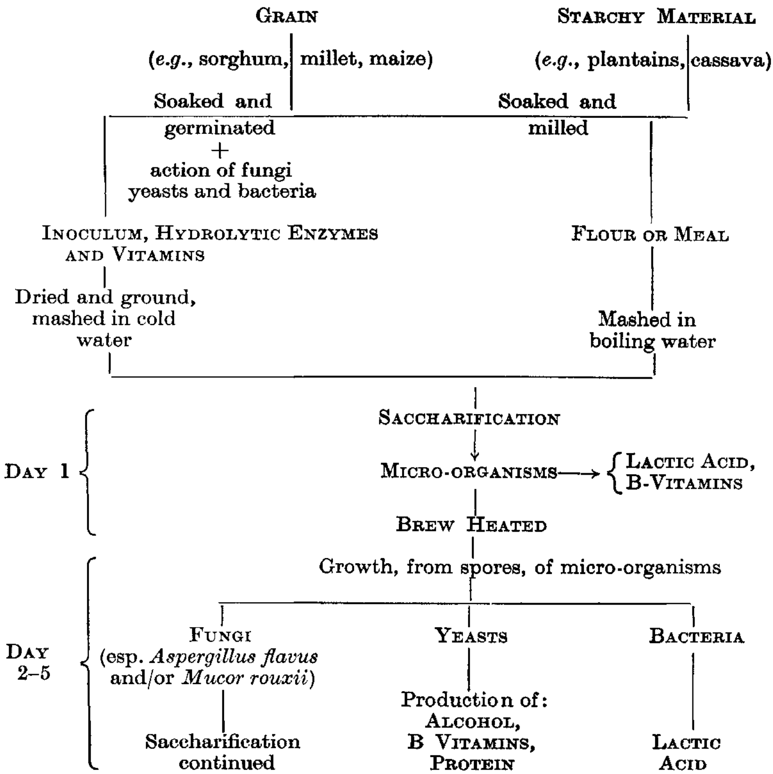

FIGURE 1. OUTLINE OF THE PROCESSES WHICH TAKE PLACE IN THE PREPARATION OF KAFFIR BEER.

proliferation of specific fungi from spores leads to the production of hydrolytic enzymes. Yeasts, using hydrolytic products of fungal enzymes, multiply, producing alcohol, B vitamins and new proteins, and the bacteria continue to produce lactic acid. After about the fifth day the brew is generally ready to be consumed. It may in some cases have a further quantity of malted grain added 24 hours before consumption. An outline of the process is given in Figure 1.

The manufacture of Kaffir beer is a good example of the "biological ennoblement" of plant foodstuffs; the contribution to a simple diet of certain B vitamins which may be made from this source is shown in Table 4 where estimates are given of the intake of aneurin, riboflavin and nicotinic acid from an African maize diet, and from another diet in

TABLE 4

THE EFFECT ON A DAY'S INTAKE OF B VITAMINS OF SUBSTITUTING IN AN AFRICAN MENU, 5 PINTS OF MAIZE BEER FOR THE MAIZE USED IN ITS MANUFACTURE

Food item	Amount of food item	
	Diet without beer	Diet with maize beer
Maize, wholemeal	350 g.	137·5 g.
Maize, 60 per cent. extraction	350 g.	137·5 g.
Maize beer	—	5 pints (2840 ml.)
Vegetables, leafy green, and yellow	130 g.	130 g.
Sweet potatoes	470 g.	470 g.
Kidney beans	30 g.	30 g.
Nutrient values of diets		
Calories	3060	2979
Vitamin B_1	2·00 mg.	1·95 mg.
Riboflavin	1·13 mg.	2·32 mg.
Nicotinic acid	11·70 mg.	20·30 mg.

which some of the maize is assumed to have been converted into maize beer, drunk at the rate of 5 pints a day, which for many is a conservative figure of consumption.

Although in fact a maize diet is notoriously associated with the development of pellagra, this disease is never seen in the maize beer drinker. For many years now it has been thought that fermented brews of the Kaffir beer type have special nutritive qualities (see, for example, Bryant, 1907).

In the West Indies where descendants of African stock are to be found, a recent survey showed a high incidence of many minor evidences of nutritional deficiencies which might be attributed to insufficiency of B_2 vitamins. In these territories fermented cereal beverages are no longer brewed, fermented sugar drinks being taken instead; these may add something to the diet, but it is unlikely that they have a nutritive value comparable with that of Kaffir beer, nor are they consumed regularly in such considerable quantities (Table 5).

Food Yeast

Here we would direct attention to the value of the fermentation process which is now being developed on a commercial scale by the cultivation of an organism, *Torula utilis*, which has many desirable properties, some of them unique amongst micro-organisms even in its

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own group. The process is described in detail in a pamphlet, "Food Yeast—A Venture in Practical Nutrition", published by Colonial Food Yeast Limited, London, May, 1944. In Table 6 the B vitamin content of food yeast is set out.

TABLE 5

INCIDENCE OF SIGNS OF NUTRITIONAL ILL HEALTH ATTRIBUTABLE TO INSUFFICIENCY OF B VITAMINS IN THE DIET IN 192 BARBADOS SCHOOLCHILDREN, DECEMBER, 1944

Sign of ill health	Incidence per cent.
Hair of head: dry staring	7·8
hypochromotrichia present	22·4
Scleral conjunctiva: hyperaemia	34·9
"excess tissue," grade I	36·0
grade II	27·1
grade III	2·1
Lachrymation	20·3
Blepharitis	0·5
Skin of face: folliculosis	19·3
dyssebacia	0·5
Gums: blue, congested	3·1
Tongue: blue colour change (including magenta)	42·7
filiform papillae lost	6·3
fissures (geographism)	4·7
Lips: cheilosis	7·3
Skin of limbs: xerosis	51·9
loss of elasticity	55·8
"crackled"	14·1
"permanent goose flesh"	6·8
ulcers and ulcer scars	30·2
defective hairs	5·7

In Table 7 is shown the effect on the intake of B vitamins, in a diet providing 2400 Calories per head daily, of the addition of 3 per cent. of food yeast to flour of 80 per cent. extraction, which might form the basis

TABLE 6

CONTENT OF B VITAMINS IN FOOD YEAST (*Torula utilis*), EXPRESSED IN mg. PER 100 g. DRY WEIGHT: BASED ON STUBBS, NOBLE AND LEWIS (1944)

Aneurin	0·7 to 4·0	Biotin	0·1 to 0·16
Riboflavin	2·4 to 4·7	Pyridoxin	2·8 to 4·7
Nicotinic acid	37·5 to 61·0	Inositol	250 to 285
Pantothenic acid	10·6 to 15·9	*p*-Aminobenzoic acid	1·4 to 4·0

of a flour for the peoples of the West Indies. It is suggested that this flour with added food yeast be called "ennobled" flour.

This proposal may be regarded as an attempt to recover by a sophisticated procedure something of what the consumer lost when he was no longer able to brew and drink his traditional beers.

Fermentation in the Alimentary Canal

Fermentation in the alimentary canal may be examined according to a plan parallel to that used for investigating food fermentation outside the body.. The nature of the materials left over for the growth of intestinal organisms is determined by the nature of the diet, and by the extent to which digestion and absorption of the foodstuffs contained in the diet have occurred before they reach the part of the alimentary canal

TABLE 7

THE EFFECT ON THE INTAKE OF B VITAMINS IN A BARBADIAN DIET OF REPLACING WHITE FLOUR OF 70 PER CENT. EXTRACTION, RICE AND SOME STARCHY ROOTS BY 8 OZ. OF 80 PER CENT. EXTRACTION CANADIAN FLOUR "ENNOBLED" WITH 3 PER CENT. OF FOOD YEAST

Vitamin	Average daily intake calculated from data for total food supply in 1944	
	Without inclusion of "ennobled" flour mg.	After inclusion of "ennobled" flour mg.
Aneurin	0·78	1·11
Riboflavin	0·76	1·19
Nicotinic acid ..	7·20	9·98

where proliferation takes place. In turn, the nature of the food residues determines not only the amount of bacterial growth but also the composition of the intestinal flora. From work on the effect of the nature of the culture medium on the products formed by various bacteria, it would appear that even with a given flora, the products of bacterial

TABLE 8

CONTENT OF B VITAMINS IN 48-HOUR CULTURES OF INTESTINAL MICRO-ORGANISMS AND IN HUMAN FAECES

Source	Vitamin B_1 mg.	Riboflavin mg.	Nicotinic acid mg.	Biotin mg.
	Per 100 g. fresh cells			
*Bact. coli**	11·5	10·6	6·2	0·23
*Prot. vulgaris**	9·5	5·7	? 0	0·32
*Bact. aerogenes**	4·3	4·1	8·9	0·11
*Bact. faecalis alkaligenes**	13·2	7·8	7·7	0·05
*B. mesentericus**	5·3	1·4	20·4	? 0
*B. vulgatus**	7·2	8·2	70·9	0·08
	Per 24 hours			
Human faeces	0·25†	0·46‡	—	—

* From Burkholder and McVeigh (1942).
† Average of results of Najjar and Holt (1943).
‡ From Najjar, Johns, Medairy, Fleischmann and Holt (1944).

action may be considerably altered. Possibly of special importance for man is the pronounced effect of the addition of fermentable carbohydrate to a medium containing protein (see Kendall, 1923).

There is evidence that intestinal bacteria can synthesize B vitamins and that such synthesis occurs in the alimentary canal of man. Some available data on the synthesis of several of the B vitamins are given in Table 8; values for the vitamin B_1 and riboflavin content of human faeces are quoted also.

It is uncertain whether vitamins synthesized in the gut are available to man. The claims of Najjar and his colleagues, that some subjects can subsist for long periods without showing deficiency symptoms on what are considered to be inadequate vitamin intakes, suggest that, at least in some cases, intestinal synthesis of vitamin B_1 and riboflavin does contribute to the nutrition of the human organism (Najjar and Holt, 1943; Najjar, Johns, Medairy, Fleischmann and Holt, 1944). On the other hand, Alexander and Landwehr (1945), from measurements of urinary excretion of vitamin B_1, concluded that intestinal synthesis makes no contribution. The inadequacy of present technical methods which do not account for more than a very small proportion, about 10 per cent., of ingested vitamins, imposes severe limitations on the interpretation of data from experiments on excretion. This must be taken into account in considering Alexander and Landwehr's data for vitamin B_1 and those of Ellinger and Benesch (1945) on the availability of "nicotinamide" synthesized in the human gut.

For doubting the amount of bacterial activity in the alimentary canal, there are, however, no grounds. Estimates of the amount of bacterial cells excreted are generally about 8 g. of dry matter, that is approximately 80 g. of wet material, daily. There are other well known evidences of bacterial action, such as the occurrence of putrefaction.

In some work on the utilization of carbohydrates in the alimentary canal, we looked for alcohol in the circulating blood, suspecting that it might appear there if fermentable carbohydrate escaped absorption before descending to the level at which bacterial action occurs. The results of preliminary experiments on the amount of "alcohol" thus found in the blood are summarized in Table 9.

TABLE 9

CONTENT OF "ALCOHOL" IN THE BLOOD OF HUMAN SUBJECTS AFTER A MEAL HIGH IN CARBOHYDRATE: EXPRESSED AS mg. ETHYL ALCOHOL PER 100 ml. WHOLE BLOOD

Subjects	Hours after meal			
	0	3	9	13
Hi.	5·4	—	8·6	20·1
Tr.	6·4	—	9·2	21·6
He.	—	13·2	16·7	0·0
Ro.	2·6	0·0	4·6	1·1
Pl.	3·2	0·0	74·0	0·0
We.	0·0	1·7	11·4	1·2
Li.	1·4	—	3·4	—

All the results except those for the last subject were obtained with a non-specific technique (Winnick, 1942). The specific method of Gettler and Umberger (1942) was used for the last subject. We hope to pursue these observations further in exploring the extent to which intestinal bacterial activity occurs in man.

There is evidently scope for speculation on the possible contribution made to human nutrition by micro-organisms, particularly those proliferating in the gut. The interrelationships of bacterial activities and the nature of the diet are obviously complex and we may be only at the beginning stage of what may prove ultimately to be a very important aspect of nutrition. An illustration of the importance of these changes is found in a recent publication of Krehl and Elvehjem (1945), who concluded that intestinal synthesis of folic acid was a factor influencing the ultimate recovery, when given nicotinic acid, of dogs which had been deprived of it. Another interesting example is furnished by a recent claim of Milhorat and Bartels (1945) that inability to synthesize a complex of vitamin E with inositol in the human gut is responsible for the development of muscular dystrophy. We can agree with Mitchell and Hamilton (1929) in a way which, perhaps, they did not fully appreciate at the time when they wrote: "It is a matter of first importance in practical nutrition to determine the effect of different foods and classes of foods on the intestinal flora".

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The Vitamin B_2 Complex and Anaemia

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It is not proposed to deal in detail with all the different types of anaemia that have been produced by a deficiency of the components of the vitamin B_2 complex in man and experimental animals; a brief summary will indicate the field that has been covered. A deficiency of riboflavin produces a microcytic, hypochromic anaemia in dogs (Spector, Maas, Michael, Elvehjem and Hart, 1943), and in monkeys (Waisman, 1944) but doubtful and contradictory results have been obtained in human subjects. A pyridoxin deficiency produces a microcytic, hypochromic anaemia in pigs which is not haemolytic in origin (Cartwright, Wintrobe and Humphreys, 1944). A vitamin B_c deficiency produces in chicks a macrocytic anaemia which may be either hyperchromic (Hogan and Parrott, 1940), or hypochromic (Campbell, Brown and Emmett, 1944), and a deficiency of the folic acid fractions gives varying results. The effects of diets deficient in nicotinic acid will be discussed later.

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