XCVIII. THE DIETETIC VALUE OF BARLEY, MALT AND MALTED LIQUORS AS DETER- MINED BY THEIR VITAMIN CONTENT.

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THE investigation of malted beverages and their precursors is not new. Harden and Zilva [1918] found bottled ale and stout, as well as beer, to be lacking both in the antineuritic and antiscorbutic factors. These authors considered the water-soluble B factor and the antineuritic factor to be identical, and used pigeons to test for this vitamin, while guinea-pigs and monkeys were employed in the tests for the presence of the antiscorbutic factor. They also found that 40 g. of malt when fed alone to pigeons did not prevent or even delay the onset of symptoms of polyneuritis in the birds. Some earlier work by Cooper [1912, 1914] on the antineuritic properties of the precursors of beer, should be mentioned here. This author [1912] found that a diet containing 13 g. of polished rice, 2 g. of raw beef, and 5 g. of barley (unhusked, 12 % water content) would recuperate pigeons in the early stages of polyneuritis resulting from diets consisting too exclusively of polished rice. Also [1914] as a result of testing out three different samples of malt extract for their efficiency in curing polyneuritis in pigeons, he found that two of the samples had a rapid curative action, whereas the third even in large doses had no effect. Steenbock, Kent and Gross [1918] having experimentally examined the dietary qualities of barley with regard to its effects on the growth of rats, concluded that this cereal contained an abundance of water-soluble B vitamin. When fed on a ration containing barley 40, butter-fat 5, salts 3·7, caseinogen 18, and dextrin 33·3, rats showed entirely normal growth curves, whilst when the barley content was reduced to 20 % growth was not quite normal. With regard to the fat-soluble A vitamin content, however, they reported "The amount of fat-soluble vitamin introduced with 15 % of barley in the ration, so far as improvement in growth is concerned, is negligible, 60 % however improves the ration markedly."

In the course of the present investigation barley, malt (kilned), unfinned and clear ale have been systematically tested for the presence of accessory food factors with the results recorded below.

Fat-soluble A vitamin was tested for in the usual way with rats. The experience of previous workers with cereals and the effect of heat on these

suggested a negative result, except perhaps in the case of the barley. This was borne out by experimental results, and even in the case of the original barley a diet containing 50 % unhusked barley and representing about 7 g. of barley per rat per day failed to produce normal growth. An equal ration of malt in the diet also failed to prevent the onset of symptoms due to lack of vitamin A in the diet or to bring about any amelioration of the symptoms when these had developed on a vitamin A deficient diet. Both fined and unfined beers were tested for the presence of this vitamin. It was calculated that 20–30 cc. of these liquids respectively were consumed per rat per day, given by mixing the liquid with the dry food, or by filling the animals' drinking flasks with it. Some fined beer was also evaporated down under reduced pressure at 60° C. to a quarter the original volume and 20–30 cc. of this were given to rats, but in all cases, whether tested to prevent the onset of symptoms or given in the attempt to cure, the results were negative.

The presence of the antiscorbutic factor was tested for on guinea-pigs with the usual basal diet of oats and bran to which barley, malt, fined or unfined beers were added. A ration containing 60 % of barley or malt neither protected nor cured; and the result which Harden and Zilva [1918] obtained when testing for the antiscorbutic factor in pale ale and malt on guinea-pigs was confirmed by similar experiments in the present series. The residues in 5 g. lots, obtained by evaporating down fined and unfined samples of beer under reduced pressure neither protected nor acted curatively.

The testing for the presence of water-soluble B vitamin in the substances under investigation was carried out on rats of weights 30–35 g. at the commencement of the experiments, the temperature of the rat room being maintained at 21° C. as nearly as possible. Samples of English Standwell, English Chevalier, Californian, Chilian, Czecho-Slovakian and Australian malt, together with some of their original corresponding barley samples were obtained. The former were procured directly after malting and bottled in fruit-jars, the barley samples were also kept bottled. The water content of the malt averaged 3 % and that of the barley about 14 %, so that in the feeding experiments 11 g. of barley were considered equivalent to 10 g. of malt. It should be mentioned here that the malt samples used in this investigation were treated by the following kiln-drying process:

24 hours' drying to	90° F.
24 „ „ „	130° F.
24 „ „ „	180° F.
6–8 hours' final curing at 200° F.	

Experiments were first directed to ascertain the minimum amount of each of these substances required to be added to the basal ration to give a normal growth curve for the rats on a basal diet of 24 % caseinogen, 47.5 % starch, 14.5 % cane sugar, 10 % purified butter-fat, 4 % dry salt mixture. Rats having 0.5 g. of malt added to their diet or the corresponding weight of barley did not show normal growth (Fig. 1). Those having 1 g. of malt or 1.1 g. of

barley (which at the beginning of the feeding represented about 30 % of the total ration) showed normal growth curves (Fig. 2), but no pregnancies. When the amount given daily was 1.5 and 1.7 g. respectively there were good growth curves with pregnancies. Fig. 3 shows the average growth curves up till pregnancy for the six samples (English Standwell, Chevalier, Chilian, Californian,

Fig. 1. Curves 1-3 show the effect of adding 0.5 g. of malt to the rats' basal diet. Curves 4-6 the effect of adding 0.5 g. of barley to the basal diet. Curves 7 and 8 for rats on basal diet only.

Fig. 2. Curves 1 and 2 the effect of adding 1 g. malt to basal diet protectively. Curves 3 and 4 the same quantity of malt added when growth had ceased on basal diet. Curves 5 and 6 1.1 g. barley added to basal diet protectively. Curves 7 and 8 the same amount of barley added to the basal diet when growth had ceased on this, indicated by arrowhead.

Fig. 3. Curve 1 is the average growth curve of twelve rats, pairs of which had 29 % of the six samples of malt respectively in their dry ration. Curve 2 is the average growth curve of the twelve rats, pairs of which had 29 % of the corresponding six samples of barley respectively in their dry food ration. Curve 3 is the average growth curve for four rats on the basal diet only.

Australian, Czecho-Slovakian) of malt and their corresponding barleys, fed protectively to six pairs of rats along with two pairs of controls on basal diet only. In this case the rats were drawn from four families. The malt or barley in this experiment always formed a definite proportion (29 %) of the dry ration. These curves have been averaged to save space since the result in each case was the same—the rats on malt showing a slightly better growth curve than those on the corresponding barley. It seemed possible at first that these

results indicated a definite synthesis of water-soluble B vitamin on germination of barley, just as the antiscorbutic vitamin is formed under these conditions. However, the difference is so small that a more probable explanation for the small difference would seem to be in the increased ease of assimilation of malt due to the fact that the process of malting increases the solubility of the total solids of the barley kernel.

These results, therefore, show that the water-soluble B vitamin content of barley as determined by its influence on the growth of rats is not affected by the process of malting.

As already stated, Harden and Zilva [1918] assuming the identity of water-soluble B vitamin with the antineuritic factor found in the case of one sample of malt that 40 g. of this did not even delay the onset of polyneuritis in pigeons, whereas Cooper [1914] found that of three samples of malt extract two of them in quantities of 5 and 7 g. respectively and therefore corresponding to about 6 and 8 g. of actual malt cured pigeons with polyneuritis following an exclusive diet of polished rice, whereas the third sample in 10 g. doses

Fig. 4. Curve 1 shows the growth curve of pigeon A. Curve 2 that for pigeon B. Curve 3 the growth curve for pigeon C.

(corresponding to about 11 g. of malt) had no curative effect. In the present investigation some of the malt from the English Chevalier sample of barley which brought about growth in rats in 1.5 g. doses daily was tested curatively on three pigeons. The birds were fed on the same basic diet as was used in the rat experiment till they showed early symptoms of polyneuritis and then malt was added to their diet. At first they were fed naturally with the dry mixture, made up into pellets with about an equal weight of water. At the commencement of the experiment it was judged that about 30 g. of the moist basal food was eaten daily per bird, but as the experiment progressed loss of weight and corresponding decline in appetite had to be recorded. Pigeon A (Fig. 4) after 24 days developed very marked symptoms of polyneuritis during the night and died next day. Pigeon B (Fig. 4) on the 20th day showed twitchings of its wings and was forcibly fed with 10 g. of malt and 5 g. of dry basal food daily. Pigeon C (Fig. 4) showed retraction of the neck on the 24th day and was then fed forcibly a similar malt ration to B. The symptoms showed rapid improvement while the weights in both cases improved also. Thus the same sample of malt which when added to the basal diet of rats gave normal growth was found to have curative effects when added in 10 g. doses (the minimum was not determined in this case) to the diet of pigeons, in which

symptoms of polyneuritis had been developed through feeding with the same basal diet as the rats were having. The negative result which Harden and Zilva obtained with 40 g. of malt may have been due to variation of the vitamin content with sample—one of the malt extract samples tested by Cooper [1914] did not cure in 10 g. lots. The experience in the present investigation has however been that the vitamin B content of malt as determined with rats has been constant for the eleven different samples tested. Nor does it seem that the reason for the conflicting results should be looked for in the possible non-identity of the water soluble B and antineuritic factors. Funk [1914] on feeding pigeons with the same balanced basal diet as rats were having produced symptoms of peripheral neuritis both in the birds and animals, a result which has been corroborated in the present investigations. Levene and Muhlfeld [1923] have sought to revive the controversy as to the identity or non-identity of the above-mentioned factors, but their claim "to have obtained additional evidence in support of the view that the antineuritic and growth-promoting principles are not identical" would seem doubtful on the simple ground of their experimental results, for the two samples of yeast which gave the "less satisfactory" results with rats are the two (of the four under test) which did not give good results when tested on pigeons. But a more serious objection to their claim seems to lie in the planning of their experiments, for while they are careful to give their rats a well-balanced basal diet they are content to feed their pigeons artificially with polished rice which gives an ash of about 0.5 % (calcium content very small) and fat about 0.25 %. The paper of Steenbock and his collaborators [1918] to which reference has already been made contains several interesting charts which illustrate the need for balancing the diet of rats. These authors showed that barley alone fed to these animals would not give normal growth, that barley fortified with fat-soluble vitamin improves the growth curves, that salt additions improve barley as a food more than the addition of either fat-soluble vitamin or protein and so on. E. Mellanby [1921, 1922] as a result of his work on rickets insists on the need for a balanced diet when testing out a particular ingredient. His results led him to the conclusion that "the minimum of any substance essential for perfect health and development is a variable, dependent on the other factors of the diet eaten." His later work on cereals [1924] further led him to the conclusion that there is in cereals a positive toxic factor tending to produce rickets, and that a larger amount of the antirachitic vitamin is therefore required to neutralise the added effect of this agent. Thus, as a result of feeding oatmeal, barley, rice, rye and white flour respectively in equal amounts, other constituents being the same, to a litter of puppies, he found that oatmeal produced by far the worst rickets and white flour least—a result not to be explained by the difference in inorganic salts content or carbohydrate. The worst rickets could be prevented by the addition to the diet of small quantities of cod liver oil. In the light of these results and those of Steenbock and his co-workers one may well ask whether it is a fair experiment to test for the presence of

one essential ingredient of the diet of an experimental animal or bird whose diet has consisted wholly of barley or of polished rice. It is here that the critical analysis by Mitchell [1919] of the experimental evidence in favour of the identity or non-identity of the water-soluble growth-promoting vitamin with the antineuritic vitamin would seem to lose much of its value; for after stating that "polished rice, however, is defective in several factors beside the antineuritic factor. It is, therefore, probable that foods containing comparable amounts of this vitamin may be unequally effective in preventing or curing the polyneuritic symptoms, depending upon the extent to which they supplement polished rice in these other respects. Conversely foods containing unequal concentrations of the antineuritic principle may have their relative values as sources of this vitamin distorted for the same reason," and then illustrating this by reference to the experience of Campbell and Chick [1919] who found that a ration of 60 cc. of autoclaved milk added to the basal diet of oats, bran and water delayed by one to three weeks the normal onset of scurvy in guinea-pigs, he then proceeds to compare results obtained with unequal unbalanced diets.

In fine therefore it may be urged that it is impossible to determine the food value of any constituent of diet where the animal or bird under experiment is not obtaining a balanced diet, and that where it is desired to compare the effect of a constituent on two species these should be fed with the same balanced diet. Thus it seems probable that the different conclusions that have been arrived at by various investigators both as to the question of the identity of the antineuritic and water-soluble B vitamins and as regards this particular investigation, the question of the presence of vitamin B in barley and malt, would disappear if the basal diets used were strictly comparable in composition. The growth stimulant effect and the curative effects of barley and malt in the above described experiments, when used in conjunction with the same basal diet, suggest the identity of the active agents.

The results of testing watery extracts both fermented and unfermented of malt will now be given and then discussed. Fig. 5 shows the result of giving rats bottled beer of a well-known brand purchased from a local shop. Those being fed protectively were given 10 cc. at the start which was afterwards increased to 17 cc. per day per rat. Those tested for a cure were given 17 cc. from the commencement. The results of giving similar amounts of wort of original gravity 1053 and of the fined beer from this are shown in Fig. 6. Fig. 7 shows the result of feeding 5 g. (wet) of brewer's grains, ground up in an Excelsior mill, replacing 1 g. of the dry basal food. In Fig. 8 are compared the growth curves of rats given 10 cc. increasing to 17 cc. of unfined beer (original gravity 1053) with those of rats on a similar quantity of this unfined beer after it had been centrifuged for 10 minutes at 4000 r.p.m. to remove yeast cells.

These results for the water-soluble B vitamin content of malt, wort and beer show that in the process of brewing some of the vitamin present in the

malt disappears before the fined beer stage is reached. For to take the case of the results illustrated in Fig. 5 an original gravity of about 1055 may be assumed for this beer. This corresponds to 1 g. of malt for every 4.8 cc. of the fined product, so that 17 cc. of this should give an excellent growth curve, whereas only subnormal results were obtained. It is true that in this case the protective figure for the actual malt sample could not be obtained, but this

Fig. 5. Curves 1-4, 10 cc. increased to 17 cc. of Bass added daily to the basal diet. Curves 5 and 6 17 cc. of Bass were added daily from the point indicated by the arrowhead. Curves 7 and 8 those for rats on the basal diet only.

Fig. 6. Curves 1-3 show the effect of giving 10 cc. increasing to 17 cc. of wort (gravity 1053) with the basal diet. Curves 4-6 the effect of giving similar amounts of the fined beer from this wort. Curves 7 and 8 rats on basal diet only.

Fig. 7. Curves 1-4, 5 g. (wet) of brewer's grains ground up and replacing 1 g. of the dry basal food. Curves 5 and 6 rats on basal diet only.

Fig. 8. Curves 1-3, rats had 10 cc. increasing to 17 cc. of unfined beer (original gravity 1053) added to their basal diet. Curves 4-6 rats had a similar quantity of this unfined beer after centrifuging. Curves 7 and 8 rats on basal diet only.

figure (1.5 g.) was found to hold for the malt sample from which the beer was made which was given to the rats whose growth curves are shown in Fig. 8. As the original gravity was 1053, every 17 cc. of the beer corresponded to 3.5 g. of malt, which would give an excellent growth curve if no vitamin were lost. From the results with the brewer's grains it would seem that only small amounts of vitamin are retained at this stage. There is the possibility that some of this factor may be absorbed and carried down during the process of hopping [cf. Drummond, 1917], or that the yeast cell itself during the fermentation process may actually absorb it. (Experiments are being carried

out to test this latter possibility.) The usual process of fining does not appear to be the stage at which the vitamin is lost, for samples of fined ale and centrifuged unfined ale brewed from a wort of original gravity of 1045 and therefore corresponding to 3 g. of malt per 17 cc. of beer show no difference in their effects on the growth of rats. The experimental results therefore show:

(1) That both barley and malt contain water-soluble B, as judged by the effect on rats, in considerable and equal amounts.

(2) Malt was also found to prevent and cure readily polyneuritis in pigeons when used in conjunction with the same basal diet as was used in the experiments on growth in rats. The probable explanation of the discrepancies in the results obtained by different investigators on the subject is discussed, as well as the related subject, the identity of vitamin B with the antineuritic factor.

(3) Beer also contains vitamin B independently of its yeast content but to a much less extent than that in the corresponding amount of malt used in its manufacture.

(4) There is no evidence of the presence to any appreciable extent of the other two accessory food factors in barley or its derivatives.

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