BONE AND VEGETABLE BROTH

BY

R. A. McCANCE, W. SHELDON AND E. M. WIDDOWSON.

(From the Biochemical and Children's Departments, King's College Hospital, London.)

A broth prepared from bones and mixed vegetables appears to be widely used in this country in the feeding of infants, but a search of the literature has failed to reveal any figures giving a complete analysis of such a broth. It was thought, therefore, that an investigation of its manufacture and chemical composition would form a useful study.

The broth is usually prepared as follows:—

The bones are chopped to expose the marrow, covered with water containing a small amount of vinegar, and simmered for 4 to 9 hours. Chopped vegetables such as potatoes, carrots, greens and onions are added, and the simmering is continued for a further hour. The bones and vegetables are then strained off, and the broth is allowed to set to a jelly. Most recipes advise that the final volume should be 1 pint per pound of bones.

Present investigation.

Methods.—The methods of analysis were mainly those described by McCance and Shipp⁷. Sugar was determined by the copper reduction method of Lane and Eynon³ or by Hane's¹ modification of the ferricyanide technique of Hagedorn and Jensen, according to the amount present. Starch was hydrolysed with takadiastase and the resulting sugars estimated (Widdowson⁹). Copper was determined by the method of Callan and Henderson².

Bones.—The first point investigated was the rate of solution of the protein and soluble mineral salts from the bones, and how this was affected by temperature, time of extraction and amount of vinegar added. Two experiments have been performed. In each case 4 lb. of veal bones were purchased, and as much external fat as possible was removed. The bones were chopped as directed and divided into 4 weighed samples of 380-400 gm. These were placed in large conical flasks, and 1,000 c.c. of water were added to each. To the first flask was added 1 tablespoonful of 4 per cent. acetic acid, to the second 2 or 4 tablespoonfuls. The third was not treated with acid. The contents of these flasks were boiled very gently under reflux condensers for 7 hours. The fourth was treated with 1 tablespoonful of 4 per cent. acetic acid and allowed to stand at room temperature for 7 hours. Small amounts of liquid were removed for analysis after 1, 2, 4 and 7 hours.

The pH of the liquid in each flask was followed and it was found that the liquor to which no acid had been added was alkaline throughout. The initial

pH of the liquors to which 1 tablespoonful of acetic acid had been added was 4.4. At room temperature pH rose slowly throughout the experiment and was 6.8 at the end. At 100° C. the pH had risen to 7 in one hour. The pH of the liquor boiled with 2 tablespoonfuls of acetic acid rose gradually from 4.1 but had only reached a value of 6.6 after 7 hours.

Fig. 1 shows the losses of nitrogen and potassium at 100° C. and at room temperature. Since it was found that the addition of acetic acid made little or no difference to the rate of protein extraction, the points plotted for the losses of nitrogen at 100° C. are the mean results obtained from the two experiments (six lots of bones) in which the amount of acid varied as described. The points representing losses at room temperature are also the mean of the two experiments (two lots of bones). The points on the curve showing potassium losses are also means.

FIG. 1.—Rate of loss of potassium and nitrogen from veal bones.

It will be observed that:—

- (a) At 100° C. the loss of nitrogen proceeded at a linear rate for 7 hours.
- (b) At room temperature the loss was much smaller and the rate of loss fell off as time went on (compare loss of salts). It is probable that at room temperature the loss of nitrogen is due only to the diffusion of soluble proteins and extractives from the bones, whereas at 100° C. it is due to the continuous conversion of insoluble collagen to gelatin.
- (c) Temperature made relatively little difference to the loss of potassium.
- (d) The loss of potassium was rapid in the first hour, both at room temperature and 100° C. After one hour the rate of loss fell off rapidly and there was little loss after the second hour.

It has been found that the losses of calcium, iron and magnesium were complete at the end of the first hour, both at 100° C. and at room temperature. The losses per 100 gm. of bones amounted to:—calcium 10 mgm., iron 0.4 mgm., magnesium 2.2 mgm.

It has not been found that the addition of acid, even in larger amounts than those advocated by the recipe, made any difference to the amount of calcium extracted from the bones. This was unexpected, but acetic acid is a weak acid, and the calcium phosphate in the bones is encased in collagen and other protective colloids which probably act as buffers and prevent the acid having any appreciable effect.

Qualitative colour tests for tryptophane, tyrosine and sulphur, which were carried out after the bones had been strained off, gave negative results. This indicates that the only protein obtained from the bones was gelatin, which is well known to be of limited biological value.

FIG. 2.—Rate of loss of soluble constituents from savoy cabbage at 100° C.

Vegetables.—The second part of the investigation was devoted to a study of the proportion of the constituents contributed to the broth by the vegetables. Four vegetables were chosen for this purpose—potatoes, onions, carrots and savoy cabbage—and each vegetable was investigated separately. The vegetables were cut up into small pieces, the potatoes, for example, into 1 cm. cubes, and the carrots into thin slices. They were then covered with water, and simmered for varying lengths of time up to 2 hours, strained off, and the nitrogen, calcium, iron, potassium, sugars, starch, etc., in the resulting vegetable waters were estimated. Fig. 2 shows the percentage losses of nitrogen, salts, free acid and sugar from savoy cabbage, when boiled in water for 2 hours. The rate of loss of these constituents is very rapid at first,

but slows off after the first 30 minutes. A rapid loss of soluble constituents during the first half-hour was also observed in the cooking of the other three vegetables. It will be noticed that the losses for sugar are greater than those for any of the other constituents, but in the other vegetables this was not necessarily the case. In carrots the losses of potassium and chlorine were both greater than the loss of sugar, and in onions the loss of chlorine was greater, and that of potassium less. The losses of iron, calcium, phosphorus and nitrogen were generally as in cabbage lower than those of the sugars, potassium and chloride which indicates that the former were less freely diffusible. Much of the calcium, for example, was probably in the form of pectate, and only a relatively small proportion of it could be dissolved out with water. The loss of total nitrogen tends to be low because much of the protein is relatively insoluble. For the same reason the percentage loss of starch from potatoes was found to be very small indeed (10 per cent. after two hours).

In contrast with bones (fig. 1), it has been found that vegetables do not lose their soluble constituents when soaked in water at room temperature. This is because the vegetable cells are still alive and their membranes intact, for it had been found that if the cells have been killed by steam at 100° C., the salts diffuse rapidly out when the vegetables are placed in water at room temperature. Meat and marrow cells are not alive when they are prepared for cooking and have lost their semi-permeable membranes. Their salts therefore are readily removed by water at room temperature. Hence it would appear essential to boil vegetables in order to extract their salts, and the present experiments show that one hour's boiling is sufficient to do this.

Broths.—The amounts of the various constituents of a broth contributed by the bones and by the vegetables was investigated in the following way (table 1).

TABLE 1.
COMPOSITION OF BROTHS PREPARED AT ROOM TEMPERATURE AND 100° C.

	MGM. PER. 100 C.C.							
	Bones alone.				Bones and vegetables.			
	100° C. 1	100° C. 2	Room Temp. 1	Room Temp. 2	100° C. 1	100° C. 2	Room Temp. 1	Room Temp. 2
Nitrogen ...	376	382	27	34	455	478	35	38
Carbohydrate—								
Sugar ...	0	0	0	0	705	620	95	116
Starch ...	0	0	0	0	81	75	0	0
Fat ...	—	—	—	—	2750	2220	49·6	46·2
Sodium ...	49	53	40	37	51	53	41	36
Potassium ...	19·3	20·5	15·3	15·4	44·4	39·7	18·5	17·6
Calcium ...	3·2	1·5	4·7	5·4	21·2	9·9	5·5	5·9
Magnesium ...	0·63	0·56	1·6	1·7	2·8	2·2	1·7	2·1
Iron ...	0·08	0·07	0·14	0·18	0·25	0·20	0·19	0·22
Phosphorus ...	3·7	5·2	5·8	5·6	7·1	7·5	5·9	5·0
Chlorine ...	49	59	43	43	46	51	42	42
Copper ...	0·018	0·016	0·019	0·024	0·021	0·023	0·015	0·022

Four 400 gm. samples of well broken-up veal bones were placed in large flasks, and 1,000 c.c. water were added, together with 1 tablespoonful of 4 per cent. acetic acid. Two of the flasks were simmered gently for 7 hours under reflux condensers, while the other two remained at room temperature. At the end of this time 100 c.c. were removed for analysis; 40 gm. of chopped potatoes, of onions, and of carrots were then added to each, and the heating or soaking was continued for a further hour. Finally the broths were strained.

The main points are:—

(a) The losses of nitrogen and potassium from the bones at 100° C. and at room temperature are what is to be expected from a study of fig. 1.

(b) The losses of iron, calcium and magnesium from the bones are actually greater in the cold than they are at 100° C. This unexpected result had been noted in the experiments described on page 253, but it was thought at that time that the differences must have been technical in origin. The present results, however, agree so closely with those previously obtained that it seems that they must be accepted. The amounts of these three metals in the broths are increased by the addition of vegetables, but only if the broths are heated.

(c) The vegetables contribute little or nothing to the broths at room temperature. At 100° C. their presence increases the nitrogen, potassium, iron, calcium and magnesium found in the final product.

(d) All the carbohydrates are derived from the vegetables.

(e) Most of the phosphorus and copper and the whole of the sodium and chlorine comes from the bones, whether the broths are heated or not.

(f) Determinations of fat were not made before the vegetables were added but practically all of it must have come from the bones.

It is evident that except for the gelatine, and perhaps the fat, broths could equally well be prepared by allowing the bones to soak in cold water for several hours, adding the vegetables and boiling for another hour.

Composition of broths.

Analyses have been carried out on a number of broths which had been prepared in the kitchen in iron, enamelled and aluminium vessels, according to the standard recipe. No differences could be detected in their iron content, and the mean of all these results is found in table 2, column 1. Column 2 shows the mean results for the two broths which had been prepared at 100° C. in glass vessels in the laboratory, and which have already been discussed. These results are all calculated on the basis of 1 pint per lb. of bones.

Broths of a similar nature are prepared commercially. Two samples of these have been purchased from one firm on different dates. The results of their analyses are shown in column 3, and are given as mgm. per 100 c.c. of the broths as purchased.

For comparative purposes analyses of cow's milk and human milk have been made, and these results are given in columns 5 and 7. Milk analyses obtained by other workers are given in columns 4 and 6.

It will be seen that the nitrogen content of the broths is very variable. The laboratory broth contains more nitrogen than cow's milk. The kitchen broth contains less nitrogen than cow's milk, and rather more than human milk. It must be remembered, however, that the nitrogen in milk is derived from casein and lactalbumen, proteins of acknowledged biological value. On the other hand, none of the broths gave colour reactions for tyrosine, tryptophane or sulphur. Therefore the protein in them must have been almost entirely gelatin, the biological value of which is not to be compared with that of milk proteins.

TABLE 2.
COMPARATIVE ANALYSES OF BROTHS AND MILK.

MGM. PER 100 C.C.							
	Kitchen broth (mean of 6). 1 pt. per lb. bones.	Lab. broth (mean of 2). 1 pt. per lb. bones.	Commer- cial broths (mean of 2).	Cow's milk (Sherman).	Cow's milk (Present analysis).	Human milk (Sherman).	Human milk (Present analysis).
Nitrogen ...	312	875	705	560	480	240	190
Carbohydrate—							
Sugar ...	735	1240	272	5000	4400	6500	6130
Starch ...	92	146	48	0	0	0	0
Fat ...	—	4620	—	3000	—	3000	—
Sodium ...	51.0	97.5	48.5	51.0	43.0	—	43.0
Potassium ...	58.0	69.5	42.0	143.0	156.0	47.0	74.5
Calcium ...	5.2	28.6	10.2	120.0	119.0	34.0	22.6
Magnesium ...	1.9	4.5	3.9	12.0	13.5	5.0	4.1
Iron ...	0.15	0.41	0.28	0.24	0.11	0.3**	0.096
Phosphorus ...	6.1	13.7	6.96	93.0	94.0	15.0	14.0
Chlorine ...	58.0	91.0	56.5	106.0	93.0	35.0	37.0
Copper ...	—	0.041	0.028	0.015*	0.023	0.05†	0.03

* From Lindow, Elvehjem, and Peterson⁴.

** From Mackay⁶.

† From Zondek and Bandmann¹⁰.

The sugar content of the broth is much less than that of milk, and the commercial broth contains by far the least. The broths contain a little starch, which comes from the potato, but commercial broth contains considerably less than the others. It is clear from the small amount of sugar and starch in this preparation that only very small amounts of vegetables were used in its manufacture. All the broths contain about as much potassium as is found in human milk. The calcium and phosphorus contents of the commercial and kitchen broths are extremely low, and compare unfavourably even with human milk. The laboratory broth is rather better in this respect, and contains about the same amounts of calcium and phosphorus as human milk, but it cannot be compared with cow's milk as a source of these elements.

The figures for the iron contents of the commercial and kitchen broths are comparable with the figures given by Sherman⁸ for human and cow's milk. The iron content of the laboratory broth is a little higher. The figures obtained for the milks which were analysed in this laboratory are appreciably lower than those given by Sherman⁸ or Mackay⁶. There seem to be no reason, however, to doubt the accuracy of the present figures. The iron in the cow's milk was determined (a) by a thiocyanate method after dry ashing (McCance and Shipp⁷), and (b) by a thioglycollic acid method (Lyons⁵) after wet ashing, and both gave essentially the same results. It is noteworthy how closely some of the new figures for milks agree with those given by Sherman. There is little worthy of comment in the results for sodium, magnesium and copper.

Conclusions.

As judged by chemical standards, bone and vegetable broths are not of great nutritional value. Their protein is mainly gelatin, and they contain only small amounts of starch and sugar. For technical reasons fat was only determined in the laboratory broth (see tables 1 and 2). The amount found was about the same as that in milk, but in practice the fat will vary (a) with the amount of fat left on the bones, (b) with the type of marrow, and (c) with the amount skimmed off during the preparation of the broth.

Assuming the broth to contain as much as 3 per cent. fat, its calorie value will be far below that of milk, and its inorganic salt composition in most respects also very inferior. Broths and milk contain approximately the same amounts of iron, but the latter is admittedly a poor source of this metal.

Apart from the question of nutrition, broths, especially the home-made type prepared with potato, may be of value in that they help to introduce a little starch into the infant's diet in a very digestible form. Broths may also help to educate the infant's palate to more varied tastes, but the present experiments show that their nutritive value is low, and that in prescribing them, this should be taken into account.

Summary.

1. On boiling veal bones with water or dilute acetic acid the loss of nitrogen is linear for at least the first seven hours. The loss of salts is complete in from one to two hours.
2. On boiling vegetables with water the losses of nitrogen, sugar and salts are very rapid at first, and are almost complete in one hour.
3. There is only a small loss of nitrogen when bones are soaked in water at room temperature, but the loss of salts is comparable with that at 100° C.
4. Vegetables lose little of their constituents unless they are boiled.
5. Figures are given for analyses of human and cow's milk, and for broths made in different ways.
6. Broths compare most unfavourably with milk in calorific value and salt content.

The authors wish to thank Mr. L. R. B. Shackleton and Mr. A. W. Haynes for their help with some of the analytical work. Miss Widdowson is indebted to the Medical Research Council for a part-time grant.

REFERENCES.

1. Hanes, C. S., *Biochem. J.*, Camb., 1929, XXIII, 99.
2. Callan, T., & Henderson, J. A. R., *Analyst*, Cambridge, 1929, LIV, 650.
3. Lane, J. H., & Eyon, L., *J. Soc. Chem. Ind.*, Lond., 1923, XLII, 32.
4. Lindlow, C. W., Elvehjem, C. A., & Peterson, W. H., *J. Biol. Chem.*, Baltimore, 1929, LXXXII, 465.
5. Lyons, E., *J. Am. Chem. Soc.*, Easton, 1927, XLIX, 1916.
6. Mackay, H. M. M., Goodfellow, L., & Bradford-Hill, A., *Med. Res. Council, Spec. Rep.*, No. 157, London, 1931.
7. McCance, R. A., & Shipp, H. L., *Med. Res. Council, Spec. Rep.*, No. 187, London, 1933.
8. Sherman, H. C., *The Chemistry of Food & Nutrition*, 4th Ed., New York, 1932.
9. Widdowson, E. M., *Biochem. J.*, Camb., 1931, XXV, 863.
10. Zondek, S. G., & Bandmann, M., *Klin. Wchnschr.*, Berlin, 1931, X, 1528.