CXLIII. A SPECTROGRAPHIC EXAMINATION OF THE MINERAL CONTENT OF HUMAN AND OTHER MILK

BY HERBERT DINGLE AND J. H. SHELDON

*From the Imperial College of Science and Technology, South Kensington, S.W. 7,
and the Royal Hospital, Wolverhampton*

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THE mineral content of milk has been the subject of many investigations by spectrographic and other methods, and the presence of certain elements has been definitely established. Regarding the less abundant constituents, however, there is still much uncertainty, the findings of different experimenters showing considerable disagreement. This is doubtless due in part to actual differences in the composition of the samples examined, but a review of the methods employed suggests that the technique has often been inadequate for definitive results with respect to substances present only in small traces. Further, most of the work has been done with cow's milk, the mineral content of human milk having been almost entirely neglected.

The present investigation has been undertaken with the object of obtaining information as complete and unambiguous as possible concerning the minerals present in various samples of milk commonly used in the feeding of infants. It was also considered desirable to ascertain whether manufactured milks, both dried and tinned, showed any significant difference in their mineral contents from fresh cow's milk. Quantitative results cannot yet be obtained with sufficient precision to justify publication, but a knowledge merely of the constant presence or absence of an element is of some importance in view of several known instances in which an element exercises a biological influence quite disproportionate to its abundance.

Preparation of the material

Thirty samples of human milk and twenty-six of cow's milk in various forms were examined; details are given below.

(i) *Human milk.* The samples for the main investigation (modifications for subsidiary analyses will be described later) were extracted from 22 patients in normal health at the Royal Hospital, Wolverhampton, at various intervals from 3 to 17 days after confinement. Two quantities were taken at different times from each of 8 of the patients, bringing the total number of samples to 30. The milk was expressed from the breast direct into a clean test tube, the nipple being placed inside the tube so as to avoid contamination from the skin. The test tubes employed were new and were prepared by three preliminary boilings in dilute nitric acid, and washed out in glass-distilled water. The milk was then dried at 100° in a steam oven and the dried material sent to the Imperial College in small glass tubes which had been similarly cleaned. On arrival each was very gently charred in a clean porcelain crucible and returned to its original bottle in the form of a black powder.

(ii) *Other milk.* Three specimens of cow's milk were taken from the pail immediately after milking and treated in a similar manner to the human milk. The manufactured milks were taken from the containers and ashed on Vitreosil

capsules, cleaned by boiling in dilute HCl, the ash being sent to the Imperial College. The milks examined were as follows:

1. Nestlé's Condensed Milk. Fluid milk in a tin.
2. Nestlé's "Ideal" Milk. Fluid milk in a tin.
3. "Secway." Dry milk. Tin with paper wrapping.
4. Mead's "dextri-maltose". Powder in tin. No paper.
5. Allenbury's Humanized Milk Food. No. 1. Dry milk. Tin with paper.
6. Allenbury's Humanized Milk Food. No. 2. Dry milk. Tin with paper.
7. Allenbury's (Malted) Milk Food. No. 3. Dry milk. Tin with paper.
8. Humanized Trufood. Dry milk. Tin. No paper.
9. Ostermilk No. 2. Dry milk. Tin with paper wrapping.
10. Cow and Gate. (Full Cream.) Dry milk. Tin with paper wrapping.
11. Savory and Moore. Dry milk. Tin with paper.
12. Mellin's Food. Dry. Glass with brass top but no paper wrapping.
13. Horlick's Malted Milk. Dry milk. Glass with tin top but no paper.
14. Virol and Milk. Dry milk. Tin with paper wrapping.
15. Lactogen (Nestlé). Dry milk. Airtight tin with paper wrapping.
16. Lacidac (Full Cream) (Cow and Gate). Dry. Tin with paper wrapping.
17. Dorsella (English Full Cream). Dry milk. Cardboard. Paper wrapping.
18. Eledon (Nestlé). Dry milk. Tin with paper wrapping.
19. Bengers Food. Dry milk. Tin with paper wrapping.
20. Dorsella. Humanized Milk. Dry. Tin with paper wrapping.
21. Libby's Unsweetened Evaporated Milk. Tin.
22. Kingsway Condensed Milk. Sweetened. Fluid in tin.
23. Dairy-Man Pure Devon Cream. Fluid in tin.

Analysis

The arc spectrum of each specimen was photographed. For this purpose a portion was placed in the first instance on graphite electrodes, and the arc was struck at 220 V. The spectrum of the electrodes alone was photographed previously by prolonged exposure in every case, and the spectrum of the milk was photographed in an adjacent position. A comparison spectrum of the iron arc was then photographed on the other side of the milk spectrum to enable the lines to be identified. All the specimens were treated in the same manner, the effective exposure time being about 1 min., during which three successive portions of the material were supplied to the arc at approximately equal intervals.

The graphite electrodes, though fairly pure, contained small proportions of a few impurities, the chief of which were Ti, V, B and Si. For the detection of such elements in the milk, fresh spectra were taken by an identical process, but with copper instead of graphite poles. This was necessary, since the lines of an impurity in the electrodes are often very greatly strengthened when metals are inserted into the arc, even though none of that impurity is added. The presence of an element in the milk was therefore not considered established unless it revealed itself on electrodes which themselves showed no trace of the element. This point will be further discussed later.

Two spectrographs were used for the greater part of the investigation. The region $\lambda\lambda 6720\text{--}3700$ was examined with a glass instrument having separate collimator and camera tubes and giving a dispersion ranging from $25 \text{ \AA./mm.}$ in the red to $3.7 \text{ \AA./mm.}$ in the violet; and the ultra-violet region, from about $\lambda\lambda 3560$ to 2530 , with a quartz Littrow spectrograph (Hilger Type E 1), with dispersion ranging from 6.7 to $2.8 \text{ \AA./mm.}$ These instruments were so disposed as to photograph the spectrum of a single source simultaneously, the source

being situated between the two slits on the common line of collimation. In spite of the large dispersion, therefore, the whole region from $\lambda\lambda 6720$ to 2530 (with the exception of the small gap between $\lambda\lambda 3700$ and 3560 , which is unimportant for the purpose in view) was photographed at once. Almost all elements detectable by spectrographic analysis have sensitive lines falling within this range; the only exceptions are B—best indicated by a close pair of lines at $\lambda\lambda 2498, 2497$ —and Rb which, though it gives a sensitive pair at $\lambda\lambda 4216, 4202$, is best detected by the still more sensitive pair in the infrared at $\lambda\lambda 7948, 7800$, since the violet lines fall in a strong part of a cyanogen band which is almost certain to appear in a graphite arc in air. For these elements, therefore, separate exposures were made on other instruments suitable for the purpose.

The spectra obtained had considerably larger dispersion than has been usual in this work, but not larger than is necessary for certainty with respect to several of the elements concerned. The only comparable dispersion appears to be that employed by Blumberg & Rask [1933] who used a Hilger E 1 spectrograph throughout, and thus obtained similar ultraviolet spectra but a considerably smaller dispersion in the visible region. Blumberg & Rask's investigation, however, was limited to cow's milk, and since only graphite electrodes were used, the results were indefinite with respect to the elements occurring therein as impurities.

Preliminary indications

The spectra were examined for the presence of 51 elements. Each specimen of non-human milk was examined separately for each element, and for most of the elements each specimen of human milk also was so examined. On account of reduction of the material, however, the additional plates on copper poles for the detection in human milk of Ti, V, B and Si, and those in the infrared region for the detection in human milk of Rb, were obtained with a mixture of all the 30 specimens. The almost identical character in most other respects of the various samples of human milk makes it unlikely that there was any marked variation in the content of these elements.

In Plate IX are shown the spectra of two mixtures—one of all the human and the other of all the non-human specimens—taken under the same conditions on carbon electrodes (which, for a reason which will be appreciated later, were chosen on account of their small content of Ti and V, although in other respects they were less pure than the graphite poles used in the actual analysis) which showed identical spectra by themselves. Pronounced differences between the spectra on the plate may therefore with some confidence be attributed to variations in the respective mixtures.

It would be illegitimate, however, to regard the strength of a line as giving a direct indication of the abundance of the corresponding element, chiefly for the following reason. A certain proportion of the atoms of most elements become "ionized" in the arc (i.e. the atom is deprived of an electron), and an ionized atom gives a different spectrum from an ordinary neutral atom. Consequently, the more an element is ionized the weaker will the lines of its neutral atom appear. Now the spectra show that the degree of ionization is greater for the human than for the non-human milk.¹ This may be seen by comparing lines of neutral and ionized atoms, respectively, of a single element. Fe provides a striking example: the line of neutral Fe at $\lambda 2742$ is only slightly weaker in the spectrum of non-human milk, while the neighbouring line (marked Fe⁺) of ionized Fe at $\lambda 2739$

¹ This is doubtless due to the obvious excess of K in the non-human milk. K is an element which is very easily ionized, and the electrons which are set free by its ionization tend to recombine with ionized atoms of other elements and increase the proportion of neutral atoms of those elements.

is scarcely visible in that spectrum. A similar effect is shown by other elements—e.g. Ba and Sr (compare $\lambda 5536$ of Ba with $\lambda 4554$ of Ba^+ , and $\lambda 4607$ of Sr with $\lambda 4077$ of Sr^+). It follows that if we are to get a true estimate of the relative abundance of the elements from the strength of the spectrum lines, we must suppose the neutral lines (including all marked lines which have no + sign attached to the chemical symbol) of each element in the spectrum of human milk to be somewhat strengthened.

Absolute results cannot, of course, even then be obtained, owing to unavoidable differences in time of exposure, quantity of material used etc. We may, however, choose an element as standard and obtain results relative to that. P is suitable for this purpose, since its lines (at $\lambda\lambda 2555, 2553$) are approximately equal in strength in the two spectra when the spectrum of human milk is suitably augmented as already suggested. The only striking differences which then appear are an excess of K and Sn in the spectrum of non-human milk, and of Si, Cu and Fe in that of human milk. Of these, the Sn may be attributed to the containers, and it is doubtful if the excess of Cu and Fe in human milk has much importance since each of these elements showed wide variations from one specimen to another of each kind of milk. Their occurrence is discussed in more detail below. The results for K and Si, however, appeared to be significant. Each of these elements also varied in abundance in the individual specimens, but on the whole their excess in their respective kinds of milk seemed at first to be quite definite.

It was noticed, however, that none of the three specimens of fresh cow's milk showed Si, and this fact suggested that possibly the apparent excess of this element in human milk was due to solution from the glass in which it was collected. Examination of further specimens collected in Si-free vessels confirmed this suggestion, and in order to avoid false conclusions with regard to other elements, a subsidiary series of experiments was made.

Subsidiary experiments. For these experiments fresh samples of human milk were obtained in various containers, the object being to obtain evidence of the presence of as many elements as possible under conditions which precluded the possibility of such elements having been introduced outside the human body. Since human milk, in the normal process of breast feeding, passes direct from the mother to the child, it is clearly important to take into account every possible source of contamination.

It may be presumed that solution from the atmosphere is negligible. The only elements detectable spectroscopically which, so far as is known, could have been introduced in this way, are Na, C and Hg: the first two are known to be present in milk, and the last was not detected in any of the spectra. Care was, of course, taken to avoid dust. The remaining possible sources of impurity are three in number, viz. the vessels in which the milk was collected, the crucibles used for the preliminary charring, and the electrodes used for the arc.

Specimens were obtained, by the process already described, in vessels of copper, rubber, zinc, and platinum. They were charred in crucibles of porcelain, glass, copper, zinc and platinum, and examined with electrodes of graphite, copper and zinc. At least three specimens were obtained in each kind of vessel (except platinum, only one tube of this material being available), and all the vessels, crucibles and electrodes were subjected to separate spectroscopic investigation to ascertain their composition. In order to ensure that these subsidiary specimens were in no way abnormal, milk from the same woman was collected in every case, as nearly as possible at the same time, in glass tubes and treated exactly as in the main investigation. Comparison with the earlier spectra showed that the new material was identical with the old. It should be

added that the collecting vessels, crucibles and electrodes used in the main investigation were also separately analysed.

It was hoped in this way to determine definitely whether each element revealed in the main experiment was actually contained in human milk or introduced after extraction. The evidence obtained appears to be conclusive except for four elements, B, Cu, Fe and Pb, for which, for reasons stated below, the results are less certain than those for the other elements.

RESULTS

In estimating the mineral content of milk, two points need consideration: (1) the universality, (2) the certainty, of the presence of each element. The main investigation gave evidence on the former, and the subsidiary experiments on the latter of these points. In the following statement, the various elements concerned are dealt with separately in the first instance, and the conclusions are afterwards summarized.

(1) In all the specimens examined, of both human and non-human milk, the following elements were detected, and confirmed by the subsidiary experiments as constituents of human milk: Ca, Mg, Na, P, Ba, Mn. Rb may safely be included among these elements, for it appeared in every specimen of non-human milk and in the mixture of all human milks. As already stated, it was not looked for in the separate samples of the latter.

It is surprising to find Ba in this list. It was not found by Wright & Papish [1929], or in the Indian milks examined by De [1935], but was present in all of 19 samples of cow's milk examined by Blumberg & Rask [1933]. Its constant presence in the present series suggests that it may be an essential element in nutrition. There are certain observations in support of this, for Ramage & Sheldon [1931] found it regularly in the choroids of cattle in amounts increasing with age, though it was not found in other species. Gerlach & Muller [1936], using a different spectrographic technique, found it in the choroid of every specimen of all the species examined, and also found that the greatest amounts were present in cattle. Rb is found so constantly in tissues that its constant presence in milk is not surprising, and the observation is in accord with that of Ramage *et al.* [1933] that the amount of Rb in the infant's liver appears to rise slightly during the nursing period.

(2) The following elements were found and confirmed in almost every specimen: K, Sr, Li, Zn, Al. The character of the exceptions, indicated in the following notes, suggests that these elements may be included with those mentioned in the previous paragraph as normal constituents of milk.

(a) K was found in all non-human milks and in all except two specimens of human milk. These two came from the same woman, 3 and 8 days after confinement, respectively. Both spectra were generally weak, but when, by prolonged exposure and use of additional material, they were brought up to average strength, there was still no certain evidence of K. If the extreme red region had been examined the element would possibly have shown itself, but there is no doubt that the milk of this woman was abnormally deficient in K. On the whole, K was decidedly stronger in non-human than in human milk.

(b) Sr was found in all non-human and in all but one specimen of human milk. A sample taken from the same woman 3 days later, however, showed this element.

(c) Li was never strong enough to reveal its presence by more than one line—at $\lambda 6708$ in the red. This is not an easy region to observe, and in a few specimens

of both types of milk the line could not be detected with certainty. The most probable interpretation of the results is that Li is normally present in milk in very small quantity.

(d) Zn was found in very small traces in all specimens except two of non-human and three of human milk. Each of the three human specimens, however, came from a woman in earlier samples of whose milk Zn was found. This suggests that the Zn content of human milk might decrease as lactation proceeds, a possibility which is supported by the fact that the specimens in which this element was most pronounced were obtained during the first few days. This observation is in complete agreement with that of Sato & Murata [1932]. Using a chemical method they found that human, cow's and ewe's milks showed high figures for Zn immediately after parturition, with a marked fall to a steady level after 10–15 days.

(e) Al was very weak in all specimens except one of non-human and two of human milk, which failed to show the element at all; it appeared, however, in a later sample from the same source as one of the exceptional human specimens.

(3) Cu appeared in every spectrum examined, but we did not succeed in obtaining electrodes spectroscopically free from this element. It was present also in slight traces in all collecting vessels, and the greatly increased strength of its lines in the spectra of milk collected in copper tubes shows that milk is able to dissolve copper in (spectrographically) large amounts. This element must therefore be considered as possibly present in small quantity in milk.

(4) Fe is subject to uncertainty for the same reason as Cu. The most Fe-free material obtained was zinc, which showed the presence of Fe by only one line, at $\lambda 2483$, and in the specimens collected, treated and examined in zinc this line showed no clear enhancement in strength, and no other Fe lines appeared. It may be said, therefore, that there is no unquestionable spectrographic evidence of the presence of Fe in milk. In the spectra obtained in the main investigation, however, Fe showed greater variation than most elements, though all specimens were treated similarly. It is possible, therefore, that Fe is an irregular constituent of milk.

Davidson & Leitch [1934] report a conflict of chemical evidence concerning the amount of Fe in milk, though there appears to be general agreement that it is present. The estimates range from about 0.17 to 0.02 mg. per 100 ml. for human milk, and the variation is somewhat greater for cow's milk. Considerable changes during lactation are reported. This is not qualitatively inconsistent with our results. We have made experiments to determine the effect on the spectrum of adding known small quantities of Fe to specimens of milk, and find that, while the addition, to amounts equal to those used in the investigation, of Fe in the proportion 0.02 mg. to 100 ml. produces a definite strengthening, the change is less than the variation from one unadulterated specimen to another.

While the evidence is inconclusive, therefore, its general tendency is towards a low, and possibly a zero, Fe content for human milk. The results indicate that it would be worth while to look for chemical evidence of Fe in samples of milk collected in and treated with materials in which Fe is not detectable chemically. Owing to our failure to obtain electrodes spectroscopically free from Fe, we cannot say with certainty whether or not glass is such a material. Experiments we have made, however, strongly suggest that glass taken from test tubes is not free from Fe, while fused silica probably is.

(5) B and Si appeared in most specimens of non-human milk and in the mixture of all human milks in the main investigation. As already remarked, however, Si did not appear in the spectra of human milk in the subsidiary

experiments in which it could not have appeared as an impurity. There is thus no evidence of the presence of Si in milk.

It was thought that B might also have been introduced from the glass containers, since the spectrum lines of this element are well known to occur as impurity lines in vacuum tube spectra, but the evidence on this point is not conclusive. In the main investigation B was absent from only two specimens of non-human milk, and it appeared in the spectrum of the mixture of human milks, but in no instance was more than a slight trace detectable. It appeared in fresh cow's milk, which showed no Si, and there appeared in general to be no relation between the strengths of the B and Si lines in the various spectra. In the subsidiary experiments, B was found in none of the spectra where its appearance would have been conclusive, but in view of the lower sensitivity of the test with copper and zinc electrodes, and the fact that under the best conditions it was only just detectable, it cannot be said with confidence to be absent. The irregular strength of the B lines in the various spectra suggests that this element is sporadically present in minute traces in milk.

(6) In the main investigation Pb appeared faintly in all but five specimens of human milk, but in only eight samples of non-human milk, all of which had been commercially prepared. Seven of these samples of non-human milk showed Sn also; the other (a dry milk packed in glass with brass top) showed Pb as strongly as any sample, but no trace of Sn. It would be unsafe, therefore, to conclude that the Pb in non-human milk came from an impurity in the containers though it may, of course, have been introduced in the process of preparation.

No container used for human milk was found spectrographically free from Pb, though in some it was only just detectable. It appears unlikely that such minute amounts would be dissolved selectively in sufficient quantity to be observed, and the non-appearance of Pb in the spectra of some specimens collected in vessels containing this element supports the conclusion that Pb is a possible constituent of milk. If this be accepted, the evidence suggests that the Pb, like the Zn, content of human milk tends to decrease with time after confinement. Of the five Pb-free samples of human milk in the main investigation, three came from women in earlier samples of whose milk the element was found. It appears also that Pb occurs more consistently in human than in generally available non-human milk.

(7) Mo was very weakly represented in all but five samples of non-human milk, but in none of the human milks examined separately, though it was just detectable in the mixture of all of them. Its relative strength in non-human milk may, of course, be attributable to acquisition of this element during the process of preparation.

(8) A trace of Sn was found in only one specimen of human milk, extracted 8 days after confinement. A sample extracted from the same woman 12 days after confinement did not show this element: its presence in the former sample may therefore probably be considered accidental. The non-human milks showed great variations in the abundance of Sn, doubtless due to the manner of packing. As would be expected, milks packed in contact with the container showed Sn lines most strongly, fluid milks being decidedly richer in Sn than the dry samples. None of the specimens of fresh cow's milk showed this element.

The final conclusions reached may be summarized as follows:

(I) The following elements, roughly in order of spectrographic prominence, may be regarded as normal constituents of both human milk and the commercial milk foods examined: Ca, Mg, Na, P, K, Rb, Li, Ba, Sr, Mn, Al, Zn.

(II) Pb and B are probably normal constituents of both kinds of milk, which occur in minute and irregular quantity.

(III) The presence of Cu and Fe in milk is doubtful.

(IV) Mo in minute traces is possibly a normal constituent of milk.

(V) No evidence has been obtained of any of the following elements in pure milk: Sb, As, Be, Bi, Cd, Cs, Cr, Co, F, Ga, Ge, Au, In, Ir, La, Hg, Ni, Nb, Os, Pd, Pt, Rh, Ru, Sc, Si, Ag, Ta, Tl, Sn, Ti, W, V, Y, Zr.

The complete absence of Co is somewhat unexpected, for it is an element with active biological properties. Not only is it capable of causing polycythaemia, but recent Australasian work [cf. Askew & Dixon, 1936] suggests that, at any rate in the sheep, it is a necessary element in nutrition.

(VI) The human and non-human milks are in most respects very similar so far as mineral content is concerned; the differences between them are, in general, smaller than the variations between different specimens of either kind. On the whole the human milk specimens resemble one another rather more closely than the others, as might have been expected. There may, of course, be quantitative differences, of which only the roughest indication can be obtained from these observations; nevertheless, one or two general conclusions can be drawn. The most conspicuous difference is an excess of K in non-human milk, but there is no evidence as to whether this is introduced in the process of preparation. Smaller differences are a relative excess of Mo and defect of Pb in non-human milk, in the state in which it was examined. From the practical point of view, however, it is immaterial how the elements present are acquired, and it must therefore be recorded that the baby fed on commercially prepared non-human milk receives Sn and Si in varying amount, neither of which elements is absorbed by the breast-fed baby.

(VII) Fresh and commercially prepared cow's milks are very similar in mineral content. The only noticeable differences are that none of the three specimens of fresh cow's milk showed evidence of Si, Sn or Pb, which elements were irregularly present in many of the 23 specimens of prepared milk, and that in every specimen of fresh cow's milk Fe was much weaker than in the average of the prepared milks. Si and Sn are undoubtedly, and Pb and Fe possibly, introduced into the prepared milk after extraction from the cow.

Comparison with previous observations

No useful purpose would be served by comparing these results in detail with those obtained by other investigators because the samples examined and the conditions of experiment vary so widely. The only other spectrographic analysis of human milk that we have found was made by De [1935], who compared the spectra of five samples of milk from Indian women with an equal number of samples of each of cows', goats' and buffaloes' milk, respectively. De gives a table comparing the results of five analyses, by separate investigators, of various kinds of milk, and the disagreement in the results is indicated by the fact that, out of 21 elements included, only 8 were found by all five. These were Ca, Mg, P, Na, K, Cu, Ti and Zn. B and V may perhaps be added, since these elements were found in four cases and were not tested for in the fifth.

The most striking divergence of the present results from these is concerned with the elements Ti and V. No evidence has been found that these elements occur in milk, though their lines appeared strongly on all spectra taken with graphite poles, even when the poles themselves, by prolonged running of the arc, had been so cleared of impurities at their extremities that no metallic lines appeared in their spectra. That this treatment is insufficient to permit safe

conclusions to be drawn with regard to Ti and V was proved by adding to poles so treated a little "speepure" CaCl_2 known to contain neither of these elements; Ti and V lines nevertheless appeared in strength almost incredible without actual experience of the phenomenon. In Plate IX Ti and V are well represented, although in the spectra of the carbons alone they were barely detectable. In the light of these experiments it appears invalid to deduce the presence of Ti and V from the occurrence of their lines in spectra taken with graphite poles, which have generally been used for this work. No sign of these elements appeared in spectra taken with copper poles. The copper arc is admittedly less sensitive than the graphite arc in the detection of small traces, yet it showed Sr and Ba quite definitely, although those substances were much less prominent than Ti and V in the graphite spectra. We are forced to the conclusion that if Ti and V occur in milk of any of the kinds examined, their abundance is too small for ordinary spectrographic detection.¹

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¹ Webb & Fearon [1937] have called attention to this effect, and they include Mo among the elements which should be suspected on this account. We have, however, found no trace of Mo in the graphite used in this investigation, and the marked difference between human and non-human milks with respect to this element makes it very unlikely that the Mo lines observed arise from the electrodes.

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