PRELIMINARY OBSERVATIONS ON CERTAIN SEASONAL
VARIATIONS IN THE PHYSICAL PROPERTIES AND
NUTRITIVE VALUE OF COW’S MILK SERUM

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In our work on the problem of zinc in the nutrition of the rat (unpublished data) considerable difficulty was encountered in supplementing the basal synthetic ration with preparations which were potent in the water-soluble vitamin fraction. The basal ration, because of its high purity with respect to zinc, was deficient in all of the water soluble vitamins except B₂, which was sufficiently supplied in 15 per cent of dried egg white. When alcoholic extracts of yeast or liver together with an extract of pork muscle, rich in vitamin B₁, were used to supply the remaining water-soluble factors, poor growth of the animals resulted. It was found that when 2 cc. of whole milk were administered daily to the animals a favorable growth response was obtained. This amount of milk, however, introduced considerable zinc, so an attempt was made to obtain the vitamin B complex from milk and at the same time remove the zinc.

A procedure was developed for the preparation of this fraction from skimmed milk. The skimmed milk was obtained from the University dairy and was an average grade of market milk coming from about twenty herds of cattle. Six gallons of the skimmed milk were placed in a porcelain jar, the temperature brought to 40° C. and 10 cc. of rennet added. After ten minutes the curd was carefully cut and the temperature slowly raised to 46° C., thereby producing a tough curd which settled to the bottom. The resulting whey, which was clear and free from casein particles, was drawn off and allowed to stand at room temperature for 24 hours, permitting the lactic acid organisms to lower the pH to the isoelectric point of the albumin (pH 4.5). It was then placed in a two-liter Erlenmeyer flask and heated in a boiling water bath with frequent stirring until the temperature reached 88° C., which required about 15 minutes. The hot material was cooled on ice and the albumin permitted to settle. The clear, cool serum was siphoned off and concentrated ammonium hydroxide slowly added until a precipitation of the phosphates occurred (pH 9). This precipitate, which contained all but a trace of the remaining zinc, settled to the bottom. The supernatant liquid was withdrawn, and by addition of acetic acid the pH lowered

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The serum was a clear yellow solution which exhibited a green fluorescence. It had a pleasing odor and taste and was readily consumed by the animals.

About the first of February, 1934, a change had occurred in the milk from which the serum was prepared. At this time it became impossible to obtain a clear yellow fluorescent solution upon the precipitation of the albumin. When ammonium hydroxide was added the resulting phosphate precipitate was obtained with more difficulty and was somewhat slower in settling to the bottom. The serum, when brought to a pH of 6, retained a cloudy appearance in contrast to the clear yellow solution which was obtained before the milk had undergone this change. The milk remained in this condition until about the first week of June, 1934, or until the cows had been placed on pasture. The change to green feed had imparted to the milk some change which allowed the preparation of a clear zinc-low serum.

Another series of experiments was started the first week of October, 1934, with the hope of finishing the experiment before the milk changed to the winter condition. It, however, happened that in this year the milk underwent the change to a winter condition in the last week of October, or about 3 months earlier than in the preceding year. The severe drought of the summer of 1934 had limited very seriously the amount of green pasture for the herds.

The nutrition experiments were continued for 12 weeks on the winter serum. It was therefore possible to compare the growth of the control animals, which had received zinc in the summer serum experiments, with the control animals which received zinc in the winter serum experiments. Since the rations were the same in each case any differences in the rate of growth of the animals could therefore be attributed to the serums from the two seasons.

There were 8 animals on the control ration which received summer milk serum—4 males and 4 females, 9 animals on the winter serum experiment—6 males and 3 females. The animals on the summer serum made an average gain of 111 grams for the first 8 weeks, while those receiving the winter serum gained 89 grams in the same period. See Chart 1.

The fact that the winter milk serum was lower than the summer milk serum in certain nutritional factors was also exhibited in another way. Animals which were placed on the zinc low diet for 6 weeks practically ceased growing. If zinc was then administered there was a strong impulse to grow. Animals on summer milk serum made gains of 16 to 22 grams per week for the 4 weeks following the administration of zinc, while those on winter milk serum made gains of 7 to 12 grams for the same period. See Chart 1. These results are in agreement with those reported by Elvehjem, Hart and others (1), who found that rats on a whole milk diet supple-
mented with copper, iron, and manganese failed to grow as well on milk produced in the late winter months as on milk produced in the early fall.

Each year when the commercial milks showed the change to the winter condition, milk was obtained from animals of the University herd, which received choice rations of alfalfa hay and corn silage. This milk also changed in its physical behavior at the same time as the commercial milks from the University dairy. Milk from animals receiving artificially dried roughages also exhibited the same physical behavior as the winter commercial milk.

Numerous attempts were made to obtain from these winter milks a clear serum which would resemble that from summer milk. Experiments were made where different salts were added to the whey in varying amounts before the precipitation of the albumin. The salts tried were potassium phosphate (dibasic), sodium citrate, magnesium sulfate, and calcium lactate. One preparation of clear serum was obtained upon the addition of 0.2 gram of calcium lactate per liter of whey. This, however, could not be repeated with the same beneficial effects. It was found later that if the pH of the whey were reduced from 4.5 to 4 by allowing the whey to stand at room temperature for 48 hours instead of 24 and then increased to 4.5 by the addition of ammonium hydroxide, good precipitation resulted. This procedure has not always been found to work satisfactorily for the production of a clear serum from winter produced milk.

It is not possible for us at the present time to state definitely what constituents have been altered in winter milk causing this change in its physical
properties and the decrease in the nutritive value of the serum. The poor growth promoting qualities of winter serum are in accordance with the results obtained when rats are fed mineralized winter milk as the sole diet. This work indicates that the decrease in growth promoting properties of winter milk is due to a deficiency of the water-soluble vitamins in the serum.

It is generally, but not universally, accepted that vitamin B₁ is constant in cow's milk due to its synthesis by intestinal bacteria (2). In respect to B₂ Hunt and Krauss (3) have shown that its level in cow's milk varies with the ration and is highest when the animal is on green pasture. There are no data available in respect to B₄.

**SUMMARY**

1. The serum of winter produced milk showed physical characteristics at variance with that of summer produced milk. It was clarified with greater difficulty. An adjustment of the salt balance and pH improved somewhat the technique used for the preparation of the serum from winter milk. The time at which these changes occur varies from year to year.

2. The serum of winter produced milk showed lower nutritive value than that of summer produced milk as measured by its use in supplementing a highly purified diet as a source of the vitamin B complex.

3. The significance of these studies lies in the relation of fresh plant tissue as contrasted with field dried material to subtle changes in the milk secreted.

**REFERENCES**


