

CASEIN IN INFANT-FEEDING

THE PREPARATION OF DRY POWDERED PARACASEIN AND PRELIMINARY
EXPERIMENTS CONCERNING ITS USE AS THE PRINCIPAL
PROTEIN CONSTITUENT IN INFANT FOOD *

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In a previous communication¹ we mentioned the preparation of dry powdered paracasein and showed that it was completely digested when fed as a constituent of infant food. It seems desirable to give the details of the method used in preparing this substance, together with additional experiments involving its use.

I. METHOD OF PREPARING DRY POWDERED PARACASEIN

Fat-free milk is curdled by the addition of rennin and when the curd has become so firm that it will make a clean break when the finger is thrust diagonally into it and then lifted up, divide it into small pieces, remove the whey and wash several times with water. If the curd is obtained from a dairy, or for any reason becomes matted together, it must be broken up and then passed through a meat chopper.

The finely divided curd is now placed in a large vessel such as a cheese vat or a clean wash tub, and to it is added five volumes of water for every volume of milk used to produce the curd. The curd is now dissolved by adding about 10 c.c. of concentrated ammonia water for each quart of milk used. As the curd goes into solution rather slowly, more or less continuous agitation will be necessary.

After complete solution the paracasein is precipitated in the following manner: Take 2.5 c.c. of glacial acetic acid for every quart of milk used and dilute it with 25 volumes of water. Add this diluted acid to the solution of paracasein, a few cubic centimeters at a time,

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1. Bowditch, H. I., and Bosworth, A. W.: *AM. JOUR. DIS. CHILD.*, 1913, vi, 394.

and with constant agitation. This addition of acid will precipitate the paracasein and very close attention is required at this point, for an excess of acid will redissolve the precipitated paracasein which should separate out as a large flocculent precipitate quickly settling to the bottom of the vessel. If a very fine precipitate is formed, which does not settle rapidly, it is an insoluble calcium salt of paracasein² and the addition of a little more acid will change it to the desired form, free paracasein. More or less acid than the amount specified may be required to give the proper precipitate, depending on the condition and age of both the milk and the curd and on the amount of ammonia used to dissolve the curd. After the paracasein has settled and the supernatant liquid has been removed, it is washed several times with water and then redissolved, using the same volume of water as before, but using caution at this point so that an excess of ammonia is not added, for this would cause hydrolysis with consequent loss in yield of paracasein. It is best to dilute the ammonia water with fifty volumes of water and use just enough of this diluted ammonia to dissolve the paracasein. After complete solution the paracasein is again precipitated with dilute acetic acid and washed as before.

The procedure from this point on depends on how pure a product is desired. At each precipitation some insoluble calcium paracaseinate is formed and carried down with the large mass of free paracasein and it is only by repeated precipitations that the calcium is all removed. In order to secure practically ash-free paracasein it will be necessary to make six or seven precipitations.² Two precipitations, if properly made, will give a fairly good product.

After the final precipitation and washing the paracasein is placed in a linen bag and allowed to drain for two hours. It is then transferred to a mortar and triturated with 95 per cent. alcohol. After allowing to settle, decant off the alcohol and again triturate with a fresh portion of alcohol, repeating two or three times. The dehydration is completed by allowing the paracasein to remain in contact with a large volume of 95 per cent. alcohol for an hour or two. This alcohol is removed by decantation and filtering through the linen bag and if desired the bag and its contents may be placed in a press to remove most of the remaining alcohol. The preparation is now made to pass through a 20-mesh sieve and finally dried at a temperature between 80 and 90 C.

With proper care as to technic the final product will be a fine powder. If a powder is not obtained it can be secured by grinding in a coffee mill. If grinding is necessary the material should be placed in

2. Van Slyke and Bosworth: *Jour. Biol. Chem.*, 1913, xiv, 203.

the drying oven again in order to remove any alcohol which might be held inside the larger particles.

One product made by us with two precipitations contained 1.7 per cent. moisture, 0.4 per cent. ash and 98 per cent. paracasein. It was insoluble in water, slightly soluble in a 5 per cent. solution of sodium chlorid and completely and quickly dissolved by a dilute solution of sodium carbonate. The substance prepared in this manner is paracasein, not casein. The only difference between paracasein and casein is in the size of the molecule. One molecule of casein is split, by rennin, into two molecules of paracasein and no other substance is produced as was formerly believed.³ This is quite similar to the production of two molecules of dextrose from one molecule of maltose.

II. THE USE OF DRY POWDERED PARACASEIN AS THE PRINCIPAL PROTEIN CONSTITUENT OF INFANT FOOD

1. *Low protein feeding.*—As we wished to secure additional data on the digestibility of dry powdered paracasein and also learn to what extent it might be used to supply the protein in infant food, we secured the data given below. The child selected for this experiment was 8 months old, weighed 4,480 grams and was in good physical condition. He had been receiving 1,470 c.c. each twenty-four hours, of the following mixture:

Sixteen per cent. cream.....	160 c.c.
Buttermilk	800 c.c.
Sterile water	640 c.c.
Milk-sugar	53 gm.

A study of his nitrogen metabolism was made during four days while he was receiving this food and the figures given in Table 1 under the heading "Buttermilk Period," are averages representing the average figures for a twenty-four-hour period. The food was then changed to one made according to the following formula:

Sixteen per cent. cream.....	161 c.c.
Distilled water	1,439 c.c.
Lactose	89 gm.
Paracasein	25 gm.

It will be noticed that the mineral constituents in the last formula are supplied by the whey carried in the 161 c.c. of 16 per cent. cream. We will call attention to this point in another paper, simply stating here that the low sodium chlorid excretion while the child was on this food was due to the small mineral intake.

The protein in the above formula is not all paracasein for 161 c.c. of 16 per cent. cream will contain about 4 gm. of protein of which 2.8 gm. will be casein and 1.2 gm. albumin. The 2.8 gm. of casein

3. Bosworth: Jour. Biol. Chem., 1913, xv, 231.

is equivalent to an equal amount of paracasein, so that out of the 29 gm. of protein in the formula all but 1.2 gm. can be considered paracasein. Just what favorable influence this 1.2 gm. of albumin might have on the utilization of the paracasein we are unable to state at this time.

The child took the food well after the first few feedings and seemed to maintain his general good condition during the nine days he received it. The nitrogen metabolism while on this food is represented by the figures in Table 1 under the heading "Paracasein Period." These are average figures for a twenty-four-hour period.

TABLE 1.—LOW PROTEIN FEEDING. URINE ANALYSIS; AVERAGE FIGURES FOR TWENTY-FOUR-HOUR PERIODS

	Buttermilk Period	Paracasein Period
Volume in c.c.	832.0	1110.0
Total acidity as N/10	128.4	46.9
P ₂ O ₅ , gm.	1.002	0.395
Chlorids as NaCl, gm.	1.100	0.165
Total nitrogen, gm.	2.020	1.335
N as NH ₃ , gm.	0.311	0.205
N as urea, gm.	1.544	0.782
Uric acid, gm.	0.073	0.056
Creatinin, gm.	0.020	0.020
Creatin, gm.	0.028	0.025
Nitrogen in feces, gm.	0.365	0.173
Gain in weight per twenty-four hours.....	41.2	13.3

A study of Table 1 indicates no marked change in the general nitrogen metabolism with the change of food and no toxic effect was observed. It will be noticed that the amount of nitrogen in the feces during the paracasein period is less than half that found during the buttermilk period, indicating better digestion and absorption of the paracasein. The average daily increase in body weight during the paracasein period was less than during the buttermilk period. The low phosphorus and chlorin eliminations during this period would indicate that this was due to a lack of sufficient inorganic material in the food. We hope to publish work on this before long.

2. *High protein feeding.*—It occurred to us that the dry, powdered paracasein might be combined with whey, or milk, and cream to make preparations quite similar to Finkelstein's formula. We made several experiments along this line and will report two of them here because they seem to possess special significance. One child was fed a high protein food containing plenty of whey and the other child a

high protein food containing an extremely small amount of whey. The two formulae are as follows, each being a twenty-four-hour quantity:

Formula 1.—Fat, 1.5 per cent.; sugar, 6 per cent.; protein	.25 whey
	6.5 dry casein
Twenty per cent. lactose solution.....	337 c.c.
Thirty-two per cent. cream.....	44 c.c.
Whey	355 c.c.
Water	662 c.c.
Lime-water	21 c.c.
Paracasein	93.0 gm.

Formula 2.—Fat, 1 per cent.; sugar, 6 per cent.; protein	0.4 whey
	6.5 dry casein
Twenty per cent. lactose solution.....	384 c.c.
Thirty-two per cent. cream.....	68 c.c.
Water	967 c.c.
Paracasein	96.0 gm.

In each case a study of the nitrogen metabolism was made during a preliminary period on a "normal" diet and also during the period of paracasein feeding. In no case was all the paracasein indicated in the formula ingested during a twenty-four-hour period, for it was quite impossible to manipulate the feeding-bottle so that the infants received all the paracasein; more or less always remained adhering to the walls of the bottle. The figures for total nitrogen in the urine, however, indicate the ingestion of large amounts of protein. The urines were collected in twenty-four-hour quantities and the analytical data obtained are given in Tables 2 and 3.

CLINICAL REPORT (RÉSUMÉ)

Experiment 1 (High Protein and Whey).—A male child 7 months old weighing 11 pounds 1 ounce. Diagnosis, rachitis, otitis media chronica. His digestion was normal and he was gaining weight. With the administration of special formula no untoward effect was seen until the last day or so, when diarrhea set in. The child's disposition and general condition remained good. He took his food fairly well. Although calorically feeding 120 calories per kilo, he lost during the experiment 9 ounces. (Unfortunately he died ten days later of bronchopneumonia.)

Experiment 2 (High Protein and Very Small Amount of Whey).—A male child 7 months old weighing 13 pounds, 13½ ounces. Diagnosis, normal child and feeding; well developed and nourished. This patient took his food well at all times, showed but little regurgitation and no indigestion nor diarrhea. There was no fever at any time. Although getting 120 calories per kilo he lost 1 pound in seven days during the experiment. (This patient was discharged well.)

The high protein feeding resulted in a greatly increased elimination of ammonia in both cases. In Table 3 the creatin figures are fairly constant, while in Table 2 quite a marked change is noticed in the creatin excretion with the beginning of the high protein period. We are unable to suggest any explanation for this.

TABLE 2.—HIGH PROTEIN FEEDING WITH ABUNDANCE OF WHEY

URINE ANALYSIS

Twenty-Four-Hour Period Ending	Body Weight, lbs.	Volume, c.c.	Specific Gravity	Total Acidity as N/10	Chlorids as NaCl, gm.	Total N, gm.	N as NH ₃ , gm.	Uric Acid, gm.	Cre-atinin, gm.	Creatin, gm.
August 3	540	1.007	*	0.434	2.147	*	0.0864	0.0210	0.0454
August 4	500	1.005	48.0	0.684	1.624	0.328	0.0603	0.0216	0.0313
August 10 ...	11½	270	1.013	*	0.210	1.663	*	0.0951	0.0150	0.0095
August 11	510	1.015	*	0.410	5.483	*	0.0704	0.0180	0.0069
August 12	620	1.015	38.4	0.149	5.034	0.521	0.0992	0.0190	0.0064
August 13	560	1.018	39.2	0.320	5.457	0.533	0.1066	0.0218	0.0049
August 14	430	1.015	*	0.189	5.058	*	0.0813	0.0164	0.0054
August 15	650	1.018	6.5	0.286	7.826	0.874	0.0819	0.0188	0.0100
August 16 ...	10½	620	1.017	3.7	0.355	8.575	0.868	0.0174	0.0061
August 17	600	1.017	8.467	0.622	0.0212	0.0072

* Alkaline.

TABLE 3.—HIGH PROTEIN FEEDING WITH SMALL AMOUNT OF WHEY

URINE ANALYSIS

Twenty-Four-Hour Period Ending	Body Weight, lbs.	Volume, c.c.	Specific Gravity	Total Acidity as N/10	Chlorids as NaCl, gm.	Total N, gm.	N as NH ₃ , gm.	Uric Acid, gm.	Cre-atinin, gm.	Creatin, gm.
August 1	420	1.008	52.9	0.341	1.705	0.219	0.0643	0.0200	0.0130
August 2	520	1.005	54.4	0.327	0.895	0.267	0.0688	0.0208	0.0169
August 10 ...	13 lbs., 13½ oz.	950	1.008	79.8	0.794	6.118	0.798	0.0950	0.0241	0.0186
August 11	960	1.014	61.4	0.483	8.602	1.317	0.0658	0.0230	0.0121
August 12	800	1.016	89.6	0.378	8.823	0.560	0.1240	0.0240	0.0192
August 13	840	1.015	67.2	0.091	7.762	0.592	0.1091	0.0247	0.0220
August 14	730	1.018	29.2	0.000	9.403	1.226	0.0898	0.0234	0.0167
August 15	530	1.020	63.6	0.091	9.834	0.564	0.1219	0.0225	0.0151
August 16 ...	12 lbs., 13½ oz.	630	1.017	44.1	0.025	7.444	0.494	0.0209	0.0175

In Table 3 on August 14 it will be noticed that the urine was chlorin-free, chlorin appearing in the next twenty-four-hour specimen. This appearance of chlorin-free urine was not accompanied by symptoms of any kind.

The most surprising feature of these experiments in high protein feeding was the fact that no toxic or other unfavorable condition was manifested by the child receiving the small amount of whey. The extremely small amount of whey in the formula used for this experiment would lead one to expect the appearance of some such condition as that noticed by Holt, Levene, et al.,⁴ when they fed their high protein "synthetic" food. The non-appearance, in our experiments, of the symptoms noted by them would suggest that some constituent other than the protein was responsible for the results obtained by them; possibly the 250 c.c. of tenth-normal sodium hydroxid (equal to 1 gm. NaOH) used to dissolve the curd, a constituent of their high protein "synthetic" food.

SUMMARY

1. A method is given for preparing dry powdered paracasein for use in infant-feeding.

2. It is shown that this paracasein is very easily digested and absorbed, and judging from the elimination of creatinin and creatin, it seems possible to maintain normal nitrogen metabolism by its use in infant-feeding.

3. It is shown that disturbances (fever, toxic symptoms, etc.) do not result from feeding this paracasein in exceptionally large quantities for seven days, even if fed with only the small amount of whey carried in 68 c.c. of 32 per cent. cream.

4. Holt, Levene et al.: *AM. JOUR. DIS. CHILD.*, 1912, iv, 265.