STUDIES ON THE PROMOTING FACTORS OF GALACTOSE AND LACTOSE METABOLISM IN THE WHEY
IV. CONCERNING SUBSTANCES IN WHEY THAT INHIBIT DIARRHEA AND CONSEQUENT TROPHOPATHY DEVELOPED WITH LACTOSE AND GALACTOSE

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It has been long recognized that severe diarrhea, sometimes followed by death, develops in young albino rats and mice because of hyperlactose and hypergalactose diets and that rat cataract also develops. Similar results have been reported by Guha (1). It was described in our previous paper (2, 3) that symptoms resulted from the activity of Leloir's enzyme system being deranged by the excessive lactose and galactose.

Each rat was given 4 ml of whey/day, or the amount of filtrate corresponding to 4 ml of whey/day.

Fig. 1. Methods of extraction of active factors.
On the other hand, Takuma, Takayama (4) Tamura, and their co-workers (5) confirmed the fact that preventive factors inhibiting death due to diarrhea and cataract are contained in whey. It has also been demonstrated in a previous paper that the factors preventing cataract developed in rats fed on a hyperlactose diet were lower neutral saturated fatty acid. However, the factors to prevent death due to diarrhea and nutritional disorder seem to be different from those to inhibit cataract. Accordingly, we conducted studies on this point.

METHODS AND MATERIALS

As in the previous study, young albino rats of Wistar strain (from Tamura Research Laboratory) weighing from 30 to 50 g and mice of dd-strain weighing from 20 to 25 g were used. Experimental rats were fed on a hyperlactose diet or hypergalactose diet for 5

![Diagram of purification process](image)

**FIG. 2. Purification of active factors by thin-layer chromatography.**
weeks; experimental mice for 10 days, respectively. Composition of trophopathy—developing diet was galactose (or lactose) 70%, casein 21%, dried yeast 5%, and salts (McCullum 185). The galactose was from Merk and the lactose from Wako.

Methods of extraction of active factors were shown as in Fig. 1 and purification of them in Fig. 2.

**RESULTS**

*Experiment A-1:* Growth of rats and mice fed on a hyperlactose diet.

**A-1-1:** Growth of rats

As shown in Table 1, 14 out of 20 rats died and 6 survived. In 4 of the survivors, cataracts developed within 5 weeks. Weight gained by surviving rats was only 20 g.

**A-1-2:** Growth of mice

As shown in Table 2, all 10 mice died within 10 days.

*Experiment A-2:* Concerning the influence of whey on the growth of rats and mice fed on a hyperlactose diet

**A-2-1:** Concerning the influence of whey on the growth of rats

| No. | Beginning | 1 | 2 | 3 | 4 | 5 weeks |
|-----|-----------|---|---|---|---|---------|
| 1   | 32 g      | 37 g | 40 g |  |  | *       |
| 2   | 40        | 44  | 42  | 46 | * |         |
| 3   | 40        | 47  | 44  | 56 | 54 | 55      |
| 4   | 43        | 32  | 37  | 58 | 44 | 44      |
| 5   | 39        | 43  | 42  | 48 | 32 | 43      |
| 6   | 44        | 44  | 42  | 43 | 43 |         |
| 7   | 34        | 30  |  |  |  |         |
| 8   | 26        | 32  | 37  | 35 | 41 | 38      |
| 9   | 50        | 55  | 59  | 62 | 62 | 59      |
| 10  | 35        | 38  | 34  | 40 | 39 | 41      |
| 11  | 31        | 35  | 40  | 47 | 45 | 56      |
| 12  | 37        | 44  | 34  | 49 | 45 | 45      |
| 13  | 34        | 30  |  |  |  |         |
| 14  | 49        |     |  |  |  |         |
| 15  | 49        | 50  | 51  | 46 | 46 |         |
| 16  | 49        | 48  | 54  | 56 | 68 | 65      |
| 17  | 50        | 57  | 59  | 64 | 69 | 57      |
| 18  | 49        | 53  | 56  | 50 | 56 | 58      |
| 19  | 49        | 48  | 46  | 53 | 43 | 43      |
| 20  | 48        | 49  | 52  | 56 | 60 | 53      |
| **Average** | **40.9 g** | **45.3** | **50.7** | **50.6** | **54.1** | **59.8** |
| **± S.E.** | **7.5** | **4.2** | **4.2** | **4.2** | **4.2** | **4.2** |

† Died 0 1 2 1 4 2 2 0 0 0 0 2

* Cataract 0 0 0 0 0 0 0 3 0 0 1

Gained weight $23.1 \pm 9.5$ g
As described in the previous paper, nutritional disorder did not appear in rats fed on a hyperlactose diet when 4 ml of whey/day/rat were added to the diet. Only 2 rats out of 20 died, and the weight of the surviving rats gained about 60 g (Table 3).

Table 2. Growth of mice fed on a hyperlactose diet.

| No. | Beginning | 1  | 2  | 3  | 4  | 5  | 6  | 7 days |
|-----|-----------|----|----|----|----|----|----|--------|
| 1   | 24.0 g    | 22.5 g | 21.0 g | 20.5 g | 20.0 g | 19.0 g | 18.0 g | †      |
| 2   | 23.5      | 21.5  | 19.0  | 20.0  | 17.5  | 17.0  | †      |
| 3   | 23.0      | 21.5  | 19.5  | 18.5  | 17.0  | †      |
| 4   | 22.5      | 21.0  | 19.5  | 17.5  | 17.5  | †      |
| 5   | 22.0      | 20.0  | 18.0  | 17.0  | 17.0  | †      |
| 6   | 24.5      | 23.0  | 21.5  | 20.0  | 18.5  | †      |
| 7   | 23.0      | 22.5  | 19.5  | 19.5  | 18.5  | †      |
| 8   | 22.0      | 22.5  | 19.5  | 18.0  | 18.0  | †      |
| 9   | 22.5      | 22.0  | 19.5  | 18.0  | 17.0  | 18.0  | 16.0  | †      |
|10   | 22.5      | 23.0  | 21.5  | 17.5  | 17.5  | 16.0  | 15.0  | *      |

Average 22.9 g | 21.9 | 19.8 | 18.6 | 17.8 | 17.5 | 16.5 |

S.E. 0.7 g | 1.0 |

Table 3. The influence of whey on the growth of rats fed on a hyperlactose diet.

| No. | Beginning | 1  | 2  | 3  | 4  | 5 weeks |
|-----|-----------|----|----|----|----|---------|
| 1   | 32 g      | 40 g | 43 g | 46 g | 45 g | 54 g | 61 g | 63 g | 65 g | 70 g | 85 g |
| 2   | 40        | 45   | 50   | 53   | 55   | 58   | 65   | 75   | 85   | 104  | 110 |
| 3   | 43        | 52   | 51   | 52   | 58   | 62   | 65   | 74   | 92   | 103  | 108 |
| 4   | 45        | 43   | 45   | 49   | 45   | 48   | 49   | 58   | 68   | 78   | 82  |
| 5   | 55        | 54   | 63   | 66   | 68   | 74   | 88   | 101  | 121  | 120  | 132 |
| 6   | 53        | 60   | 61   | 60   | 66   | 68   | 71   | 79   | 87   | 87   | 93  |
| 7   | 53        | 62   | 73   | 77   | 79   | 76   | 78   | 88   | 101  | 107  | 111 |
| 8   | 54        | 63   | 72   | 79   | 82   | 88   | 97   | 104  | 115  | 120  | 130 |
| 9   | 41        | 53   | 53   | 48   | 45   | 55   | †    |
| 10  | 40        | 49   | 49   | 53   | 63   | 73   | 78   | 83   | 94   | 105  | 106 |
| 11  | 45        | 51   | 48   | 53   | 52   | 60   | 62   | 78   | 88   | 100  | 102 |
| 12  | 49        | 49   | 53   | 59   | 63   | 67   | 68   | 65   | †    |
| 13  | 42        | 52   | 60   | 66   | 65   | 76   | 80   | 80   | 94   | 94   | 95  |
| 14  | 35        | 43   | 43   | 49   | 43   | 57   | 64   | 65   | 75   | 78   | 88  |
| 15  | 40        | 57   | 60   | 65   | 61   | 88   | 102  | 102  | 107  | 108  | 112 |
| 16  | 36        | 53   | 58   | 63   | 60   | 85   | 98   | 98   | 105  | 104  | 110 |
| 17  | 42        | 46   | 50   | 56   | 58   | 84   | 99   | 99   | 106  | 105  | 108 |
| 18  | 43        | 44   | 48   | 50   | 52   | 59   | 60   | 62   | 78   | 95   | 102 |
| 19  | 40        | 39   | 43   | 44   | 48   | 58   | 62   | 71   | 78   | 93   | 98  |
| 20  | 45        | 44   | 45   | 50   | 55   | 62   | 63   | 72   | 79   | 92   | 99  |

Average 43.6 g | 53.4 | 58.1 | 74.2 | 91.0 | 103.9 |

±S.E. 6.2 g | 13.0 |

† Died 0 | 0 | 0 | 0 | 1 | 1 | 0 |

* Cataract 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Gained weight 60.5±11.0 g
A-2-2: Concerning the influence of whey on the growth of mice

In case 4 ml of whey/day/mouse were added to a hyperlactose diet; 2 mice out of 10 died within 10 days and 8 survived (Table 4).

### Table 4. The influence of whey on the growth of mice fed on a hyperlactose diet.

| No. | Beginning | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10 days |
|-----|-----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|---------|
| 1   | 24.5 g    | 22.0 g | 20.5 g | 20.5 g | 21.0 g | 20.5 g | 20.5 g | 21.0 g | 20.5 g | 20.5 g |         |
| 2   | 22.5      | 21.0 g | 21.0 g | 19.5 g | 19.0 g | 18.5 g | 17.5 g | 18.5 g | 19.0 g | 19.5 g | 20.0 g |
| 3   | 22.0      | 20.5 g | 20.5 g | 20.0 g | 19.0 g | 18.0 g | 18.0 g | 16.0 g | †    |       |         |
| 4   | 21.0      | 19.0 g | 20.0 g | 19.0 g | 19.0 g | 19.0 g | 18.5 g | 19.0 g | 19.0 g | 19.0 g |         |
| 5   | 20.5      | 20.0 g | 19.0 g | 19.0 g | 19.0 g | 18.0 g | 18.0 g | 18.0 g | 17.0 g | 17.5 g |         |
| 6   | 22.0      | 21.5 g | 20.5 g | 20.0 g | 19.5 g | 20.5 g | 20.0 g | 20.5 g | 20.5 g | 21.0 g |         |
| 7   | 23.5      | 21.0 g | 19.5 g | 19.5 g | 20.0 g | 19.5 g | 20.0 g | 19.5 g | 20.0 g | 19.5 g |         |
| 8   | 20.0      | 18.0 g | 19.0 g | 18.0 g | 18.0 g | 18.0 g | 18.0 g | 18.0 g | 18.0 g | 18.0 g |         |
| 9   | 23.5      | 22.0 g | 22.5 g | 20.0 g | 20.0 g | 19.5 g | 18.5 g | 19.5 g | 20.0 g | 20.5 g | 20.5 g |
| 10  | 22.5      | 21.0 g | 19.0 g | 18.5 g | 18.0 g | 17.5 g | 16.5 g | †    |       |       |         |

Average 22.2 g ± S.E. 1.3 g

### Table 5. Effect of ethanol-ether extract.

| No. | Beginning | 1   | 2   | 3   | 4   | 5 weeks |
|-----|-----------|-----|-----|-----|-----|---------|
| 1   | 53 g      | 63 g | 69 g | 59 g | 77 g | 88 g | 98 g | 106 g | 118 g | 113 g | 110 g |
| 2   | 61        | 67   | 59   | 67   | 77   | 74   | 69   | 43    | 43    | †     |       |
| 3   | 44        | 45   | 53   | 49   | 58   | 52   | 43   | 43    | †     |       |       |
| 4   | 66        | 66   | 60   | 60   |       | †     |       |       |       |       |       |
| 5   | 63        | 58   | 73   | 57   |       |       |       |       |       |       |       |
| 6   | 60        | 59   | 64   | 51   | 56   |       |       |       |       |       |       |
| 7   | 60        | 68   | 58   | 66   | 88   | 101  | 112  | 115  | 126  | 123  | 120   |
| 8   | 38        | 50   | 58   | 55   | 57   | 62   | 73   | 79   | 76   | 72   | 68    |
| 9   | 37        | 42   | 48   | 49   | 50   | 52   | 58   | 63   | 77   | 76   | 76    |
| 10  | 39        | 48   | 56   | 61   | 66   | 72   | 81   | 79   | 98   | 105  | 98    |
| 11  | 39        | 45   | 52   | 55   | 54   | 64   | 75   | 76   | 92   | 90   | 91    |
| 12  | 44        | 51   | 56   | 55   | 57   | 65   | 77   | 80   | 89   | 93   | 92    |
| 13  | 43        | 48   | 57   | 60   | 75   | 79   | 90   | 89   | 92   | 98   | 95    |
| 14  | 41        | 38   | 48   | 52   | 63   | 65   | 73   | 73   | 67   |       |       |
| 15  | 56        | 59   | 69   | 74   |       |       |       |       |       |       |       |
| 16  | 62        | 61   | 76   | 90   | 103  | 110  | 114  | 122  | 126  | 124  | 124   |
| 17  | 42        | 45   | 48   | 51   | 48   | 46   | 48   | 53   | 57   | 63   | 64    |
| 18  | 40        | 45   | 47   | 56   | 60   | 57   | 58   | 64   | 63   | 69   | 64    |
| 19  | 31        | 35   | 37   | 42   | 43   | 40   | 36   | 35   | 32   | 31   |       |
| 20  | 29        | 29   | 28   | 30   | 33   | 31   | 30   | 30   | 34   |       |       |

Average 47.4 g ± S.E. 10.3 g

† Died 0 0 0 0 3 1 0 0 2 1 2
* Cataract 0 0 0 0 0 0 0 0 0 0 0

Gained weight 45.9 ± 13.9 g
### Table 6. Effect of alkaline extract.

| No. | Beginning | 1 | 2 | 3 | 4 | 5 weeks |
|-----|-----------|---|---|---|---|---------|
| 1   | 58 g      | 61 g | 68 g | 70 g | 75 g | 78 g | 82 g | 91 g | 91 g | 93 g |
| 2   | 53        | 52   | 61   | 62   | 68   | 72   | 75   | 84   | 102  | 113  | 118  |
| 3   | 45        | 42   | 45   | 48   | 44   | 48   | 46   | 48   | 58   | 69   | 82   |
| 4   | 65        | 64   | 73   | 76   | 78   | 84   | 98   | 111  | 131  | 130  | 142  |
| 5   | 63        | 60   | 61   | 70   | 76   | 78   | 81   | 89   | 97   | 97   | 103  |
| 6   | 63        | 62   | 73   | 78   | 80   | 78   | 88   | 98   | 111  | 117  | 121  |
| 7   | 64        | 63   | 73   | 79   | 82   | 88   | 97   | 104  | 115  | 120  | 130  |
| 8   | 41        | 53   | 61   | 70   | 82   | 72   | 89   | 94   | 105  | 106  | 104  |
| 9   | 40        | 40   | 49   | 53   | 65   | 55   |      |      |      |      |      |
| 10  | 35        | 50   | 46   | 53   | 52   | 60   | 62   | 76   | 86   | 99   | 101  |
| 11  | 41        | 49   | 53   | 59   | 62   | 67   | 68   |      |      |      |      |
| 12  | 40        | 50   | 58   | 64   | 63   | 74   | 78   | 78   | 92   | 92   | 93   |
| 13  | 32        | 40   | 40   | 46   | 40   | 54   | 61   | 62   | 60   | 62   | 87   |
| 14  | 36        | 53   | 56   | 61   | 57   | 84   | 98   | 98   | 105  | 103  | 105  |
| 15  | 41        | 46   | 50   | 55   | 57   | 68   | 77   | 89   | 98   | 99   | 106  |
| 16  | 41        | 48   | 51   | 57   | 62   | 69   | 80   | 82   | 93   | 102  | 109  |
| 17  | 40        | 40   | 49   | 53   | 65   | 55   | 72   | 77   | 83   | 85   | 94   |
| 18  | 40        | 44   | 48   | 50   | 50   | 57   | 58   | 62   | 78   | 82   | 95   |
| 19  | 30        | 31   | 35   | 36   | 39   | 45   | 48   | 50   | 45   | 58   | 66   |
| 20  | 40        | 45   | 49   | 52   | 53   | 57   | 68   | 78   | 103  | 103  | 105  |

Average 45.5 g    54.9  62.3  74.9  91.0  103.0
+ S.E. 10.9 g     17.1

† Died 0 0 0 0 0 0 1 1 0 0 0
* Cataract 0 0 0 0 0 0 0 0 0 0 0

Gained weight 57.0 ± 12.2 g

### Table 7. Effect of ethanol-ether extract.

| No. | Beginning | 1 | 2 | 3 | 4 | 5 weeks |
|-----|-----------|---|---|---|---|---------|
| 1   | 59 g      | 67 g | 59 g | 58 g | 63 g | 68 g | 70 g | 72 g | 73 g | 75 g | 88 g |
| 2   | 58        | 58   | 65   | 56   | 59   | 55   |      |      |      |      |      |
| 3   | 62        | 57   | 72   | 68   | 62   |      |      |      |      |      |      |
| 4   | 65        | 65   | 61   | 63   | 65   | 77   | 78   | 83   | 82   | 78   | 89   |
| 5   | 54        | 55   | 53   | 59   | 69   | 78   | 79   | 86   | 98   | 103  | 109  |
| 6   | 42        | 50   | 59   | 56   | 58   | 63   | 72   | 80   | 88   | 94   | 100  |
| 7   | 40        | 46   | 53   | 55   | 54   | 65   | 76   | 75   | 71   | 79   | 83   |
| 8   | 40        | 49   | 55   | 56   | 58   | 63   | 72   | 68   |      |      |      |
| 9   | 38        | 43   | 49   | 55   | 55   | 55   |      |      |      |      |      |
| 10  | 39        | 49   | 57   | 62   | 62   | 62   |      |      |      |      |      |

Average 49.7 g    58.3  60.5  74.5  82.4  93.8
± S.E. 10.2 g     9.4

† Died 0 0 0 0 0 0 2 2 0 1 0 0
* Cataract 0 0 0 0 0 0 0 0 0 0 0

Gained weight 42.4 ± 12.7 g
Experiment A-3: Effect of ethanol-ether extract of active charcoal in acidified whey on the growth of rats

As shown in Fig. 1, substance A extracted with ethanol-ether (3:1) mixture and B with alkaline solution. On the growth of rats fed on a hyperlactose diet, the alkaline extract (B) was more effective than the ethanol-ether (3:1) extract for inhibition of diarrhea and death (Tables 5 and 6).

Experiment A-4: Effect of alkaline extract of active charcoal which was extracted with ethanol-ether (3:1) mixture

According to Fig. 1, substance C was extracted. As shown in Tables 7 and 8, the active factors were also extracted. It became clear that the alkaline extract (C) contains

| Table 8. Effect of alkaline extract of active charcoal which was extracted with ethanol-ether (3:1) mixture. |
|---|---|---|---|---|---|---|
| No. | Beginning | 1 | 2 | 3 | 4 | 5 weeks |
| 1 | 41 g | 46 g | 48 g | 53 g | 54 g | 58 g | 69 g | 79 g | 89 g | 104 g | 106 g |
| 2 | 42 | 46 | 50 | 52 | 52 | 59 | 60 | 64 | 80 | 84 | 97 |
| 3 | 40 | 47 | 50 | 56 | 56 | 61 | 68 | 79 | 81 | 92 | 101 |
| 4 | 33 | 41 | 41 | 46 | 46 | 40 | 55 | 62 | 63 | 61 | 76 |
| 5 | 36 | 43 | 46 | 60 | 58 | 68 | 78 | 89 | 97 | 97 | 104 |
| 6 | 37 | 52 | 48 | 55 | 54 | 62 | 64 | 78 | 88 | 101 | 103 |
| 7 | 40 | 52 | 60 | 69 | 81 | 71 | 88 | 93 | 104 | 105 | 103 |
| 8 | 60 | 59 | 69 | 75 | 78 | 84 | 93 | 101 | 111 | 116 | |
| 9 | 46 | 43 | 46 | 49 | 45 | 39 | † | | | | |
| 10 | 62 | 61 | 72 | 77 | 79 | 77 | 87 | 97 | 110 | 116 | 120 |
| Average | 43.7 g | 53.0 | 60.2 | 75.5 | 92.4 | 104.7 |
| † Died | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Castrated | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |

Gained weight: 61.3 – 7.7 g

Table 9. Growth of rats fed on a hypergalactose diet.

| No. | Beginning | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 days |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 34.0 g | 40.0 g | 40.0 g | 35.0 g | 40.0 g | 32.0 g | † |
| 2 | 32.0 | 40.0 | 40.0 | 33.5 | 35.0 | † |
| 3 | 30.0 | 40.0 | 37.0 | 31.5 | 30.0 | † |
| 4 | 34.0 | 37.0 | 35.0 | 30.0 | † |
| 5 | 32.0 | 32.0 | 33.3 | 33.5 | 32.0 | † |
| 6 | 36.0 | 42.0 | 41.5 | 40.0 | 36.5 | 35.0 | 32.0 | 32.0 | 30.5 | 31.0 | 30.5 |
| 7 | 40.0 | 39.5 | 38.0 | 38.0 | 37.0 | 35.0 | 31.0 | † |
| 8 | 34.0 | 42.0 | 40.0 | 31.5 | 32.0 | † |
| 9 | 39.0 | 41.0 | 40.0 | 34.5 | 34.0 | † |
| 10 | 38.0 | 39.0 | 36.0 | 35.0 | | | | | | | |
| Average | 34.9 g | 39.2 | 38.0 | 34.2 | 34.5 | 34.0 | 31.5 | 32.0 | 30.5 | 31.0 | 30.5 |
| ∆S.E. | 3.1 g |
| † Died | 0 | 0 | 0 | 0 | 2 | 5 | 1 | 1 | 0 | 0 | 0 |
significantly more nutrition-promoting factors in comparison with the ethanol-ether (3: 1) extract (A).

**Experiment B-1:** Growth of rats and mice fed on a hypergalactose diet.

**B-1-1:** Growth of rats

As shown in Table 9, young rats fed on a hypergalactose diet produced 90% mortality within 10 days.

**B-1-2:** Growth of mice

As shown in Table 10, young mice fed on a hypergalactose diet produced 70% mortality within 10 days.

**Experiment B-2:** Concerning the influence of whey on the growth of rats and mice fed on a hypergalactose diet

### Table 10. Growth of mice fed on a hypergalactose diet.

| No. | Beginning 1 (g) | 2 (g) | 3 (g) | 4 (g) | 5 (g) | 6 (g) | 7 (g) | 8 (g) | 9 (g) | 10 days (g) |
|-----|----------------|-------|-------|-------|-------|-------|-------|-------|-------|--------------|
| 1   | 25.0           | 22.5  | 22.5  | 22.0  | 23.0  | 21.0  | †     |       |       |              |
| 2   | 24.4           | 22.0  | 23.5  | 23.5  |       |       | †     |       |       |              |
| 3   | 22.5           | 21.0  | 20.5  | 22.0  | 21.0  | 22.0  | 20.5  | 21.0  | 21.0  | 21.0         |
| 4   | 22.0           | 20.5  | 20.0  | 20.0  |       |       | †     |       |       |              |
| 5   | 20.0           | 18.0  | 17.5  | 16.0  | 16.0  | 16.5  | 17.0  | 18.0  | 18.0  | 17.0  17.5 |
| 6   | 22.0           | 19.5  | 19.5  | 19.0  |       |       | †     |       |       |              |
| 7   | 20.0           | 18.5  | 18.0  | 17.5  |       |       | †     |       |       |              |
| 8   | 24.0           | 21.5  | 22.5  | 22.5  | 21.5  |       | †     |       |       |              |
| 9   | 21.5           | 21.5  | 21.0  | 22.0  | 21.5  | 20.0  | 19.5  | 19.5  | 19.0  | 18.5  18.0 |
| 10  | 22.0           | 21.5  | 21.5  | 21.0  | 20.0  | 20.0  |       |       |       |              |

Average: 22.4 g ± S.E.: 1.6 g

† Died: 0 0 0 0 4 1 2 0 0 0

### Table 11. The influence of whey on the growth of rats fed on a hypergalactose diet.

| No. | Beginning 1 (g) | 2 (g) | 3 (g) | 4 (g) | 5 (g) | 6 (g) | 7 (g) | 8 (g) | 9 (g) | 10 days (g) |
|-----|----------------|-------|-------|-------|-------|-------|-------|-------|-------|--------------|
| 1   | 31.0           | 42.0  | 43.5  | 40.5  | 40.5  | 42.0  | 44.0  | 44.0  | 41.5  | 42.0         |
| 2   | 30.0           | 45.5  | 44.0  | 39.0  | 42.5  | 42.5  | 40.0  | 43.0  | 43.0  | 46.0         |
| 3   | 30.0           | 40.0  | 36.0  | 41.5  | 45.0  | 44.0  | 43.0  | 45.0  | 45.0  | 49.0         |
| 4   | 29.5           | 40.0  | 35.0  | 37.5  | 37.0  |       |       |       |       |              |
| 5   | 28.0           | 40.0  | 39.0  | 38.5  | 41.0  | 40.5  | 42.5  | 41.5  | 44.0  | 43.5  43.0 |
| 6   | 39.0           | 50.0  | 51.5  | 48.5  | 51.0  | 48.5  | 50.0  | 52.0  | 52.0  | 51.5         |
| 7   | 40.0           | 55.5  | 54.0  | 49.0  | 52.5  | 52.0  | 50.0  | 53.0  | 53.0  | 56.0  56.5 |
| 8   | 38.0           | 39.0  | 39.5  | 41.0  | 42.0  | 38.5  | 37.0  | 33.0  |       |              |
| 9   | 38.0           | 50.0  | 49.0  | 48.5  | 61.0  | 58.5  | 60.0  | 62.0  | 62.0  | 61.5  62.0 |
| 10  | 35.0           | 45.0  | 41.0  | 46.5  | 51.0  | 49.0  | 48.0  | 50.0  | 50.0  | 54.0  53.0 |

Average: 33.8 g ± S.E.: 4.3 g

† Died: 0 0 0 0 0 1 0 1 1 0
B-2-1: Concerning the influence of whey on the growth of rats

In young rats, death due to diarrhea and nutritional disorder was inhibited by feeding on a hypergalactose diet containing 4 ml of whey/day/rat. In the rats, the death rate decreased from 90 to 20% in days (Table 11).

B-2-2: Concerning the influence of whey on the growth of mice

In young mice, death due to diarrhea and nutritional disorder was inhibited by feeding on a hypergactose diet containing 4 ml of whey/day/mouse. Their death rate also decreased 70 to 0% in days (Table 12).

It was proved that cataract-inhibiting substances were different from the trophopathy-inhibiting factors in those experiments. In consideration of the above results, experiment C was conducted.

| No. | Beginning | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  |
|-----|-----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1   | 23.5 g    | 23.5 g | 24.0 g | 23.5 g | 24.0 g | 24.5 g | 25.0 g | 24.5 g | 25.0 g | 24.0 g | 25.0 g |
| 2   | 22.5      | 22.0 | 23.0 | 23.5 | 23.5 | 23.5 | 23.5 | 23.0 | 23.0 | 23.0 | 23.0 |
| 3   | 22.0      | 20.0 | 20.0 | 21.0 | 20.0 | 20.0 | 20.0 | 19.0 | 19.0 | 19.0 | 19.0 |
| 4   | 21.0      | 21.5 | 22.0 | 21.5 | 21.5 | 21.5 | 21.0 | 21.0 | 23.0 | 21.5 | 21.0 |
| 5   | 21.0      | 20.0 | 20.0 | 20.0 | 20.0 | 19.5 | 19.0 | 20.0 | 19.0 | 19.0 | 19.0 |
| 6   | 24.0      | 23.5 | 24.0 | 24.5 | 24.0 | 24.5 | 25.0 | 25.5 | 25.5 | 25.0 | 26.0 |
| 7   | 22.5      | 22.5 | 23.0 | 22.5 | 23.0 | 23.5 | 24.0 | 23.5 | 24.0 | 23.5 | 24.0 |
| 8   | 20.5      | 19.5 | 19.5 | 19.5 | 20.0 | 19.5 | 19.0 | 20.0 | 19.0 | 19.5 | 19.5 |
| 9   | 23.0      | 23.0 | 23.5 | 23.0 | 23.0 | 23.0 | 24.0 | 24.0 | 24.0 | 24.0 | 24.5 |
| 10  | 22.0      | 21.0 | 21.5 | 22.0 | 22.0 | 22.5 | 22.5 | 23.0 | 23.5 | 23.5 | 24.0 |

Average 22.2 g | 21.6 | 22.0 | 22.1 | 22.1 | 22.2 | 22.3 | 22.5 | 22.1 | 22.5 |

± S.E. 1.0 g | 2.5

† Died 0 0 0 0 0 0 0 0 0 0 0

| No. | Beginning | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  |
|-----|-----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1   | 29.5 g    | 29.3 g | 27.5 g | 27.2 g | 27.2 g | 28.5 g | 28.4 g | 28.3 g | 28.9 g | 27.5 g | 28.5 g |
| 2   | 28.5      | 27.6 | 27.0 | 26.8 | 26.2 | 27.1 | 27.8 | 28.2 | 28.0 | 27.0 | 27.8 |
| 3   | 28.3      | 27.8 | 26.5 | 26.6 | 26.2 | 26.2 | 26.6 | 27.6 | 27.1 | 24.5 | 24.5 |
| 4   | 27.0      | 26.5 | 25.7 | 26.4 | 24.3 | 24.1 | 24.2 | 24.0 | 23.3 | 22.3 | 22.5 |
| 5   | 26.2      | 25.6 | 25.5 | 25.0 | 22.0 | 22.8 | 22.2 | 23.0 | 22.9 | 21.5 | 22.0 |
| 6   | 23.0      | 22.5 | 21.5 | 21.0 | 21.0 | 22.2 | 22.2 | 21.8 | 21.0 | 20.8 | 21.2 |
| 7   | 27.4      | 26.4 | 25.6 | 25.0 | 24.3 | 25.5 | 25.6 | 25.3 | 25.3 | 23.9 | 23.9 |
| 8   | 26.2      | 26.3 | 25.9 | 25.5 | 25.0 | 25.0 | 25.3 | 25.9 | 25.2 | 24.3 | 24.8 |
| 9   | 27.1      | 26.8 | 25.4 | 26.0 | 24.8 | 25.9 | 25.3 | 25.5 | 25.0 | 23.0 | 24.0 |
| 10  | 26.8      | 26.5 | 25.3 | 24.0 | 24.2 | 24.8 | 25.0 | 25.6 | 25.1 | 24.5 | 24.8 |

Average 27.0 g | 26.5 | 25.5 | 25.3 | 24.5 | 25.2 | 25.2 | 25.5 | 25.1 | 23.9 | 24.4 |

± S.E. 1.6 g | 2.2

† Died 0 0 0 0 0 0 0 0 0 0 0
Table 14. Effect of spot having Rf 0.08.

| No. | Beginning | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10 days |
|-----|-----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|---------|
| 1   | 28.8 g    | 27.5 g | 27.5 g | 26.5 g | 27.5 g | 26.0 g | 27.2 g | 27.8 g | 25.0 g | 25.2 g | 25.6 g |
| 2   | 28.0 g    | 26.8 g | 26.5 g | 25.5 g | 26.0 g | 25.5 g | 25.2 g | 24.3 g | 24.5 g | 24.3 g | 23.9 g |
| 3   | 27.8 g    | 26.5 g | 25.5 g | 25.0 g | 25.5 g | 24.8 g | 25.0 g | 23.0 g | 22.8 g | 22.2 g | 22.9 g |
| 4   | 26.5 g    | 24.3 g | 25.0 g | 25.0 g | 24.6 g | 24.5 g | 23.2 g | 22.8 g | 21.7 g | 21.1 g | 22.6 g |
| 5   | 25.4 g    | 23.5 g | 23.8 g | 24.5 g | 22.2 g | 23.2 g | 22.4 g | 19.8 g | †     |     |         |
| 6   | 24.5 g    | 22.8 g | 22.5 g | 22.8 g | 20.9 g | 21.7 g | 20.5 g | 19.7 g | 19.2 g | 20.3 g | 19.9 g |
| 7   | 27.0 g    | 26.0 g | 25.5 g | 25.3 g | 24.8 g | 24.7 g | 24.0 g | 23.0 g | 22.8 g | 22.7 g | 22.8 g |
| 8   | 26.5 g    | 25.5 g | 25.6 g | 24.9 g | 24.5 g | 24.3 g | 23.8 g | 22.8 g | 22.4 g | 22.5 g | 22.3 g |
| 9   | 27.2 g    | 24.8 g | 24.6 g | 24.3 g | 24.0 g | 24.1 g | 23.9 g | 22.7 g | 22.6 g | 22.6 g | 23.8 g |
| 10  | 26.8 g    | 24.3 g | 24.5 g | 24.5 g | 24.3 g | 24.2 g | 23.9 g | 23.0 g | †     |     |         |
|     | Average   | 26.8 g | 25.2 g | 25.1 g | 24.8 g | 24.4 g | 24.3 g | 23.9 g | 22.9 g | 22.6 g | 22.9 g |
|     | S.E.      | 1.1 g  |     |     |     |     |     |     |     |     | 1.5     |
| † Died | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 2    | 0    | 0       |

**Experiment C**: Effect of two spots having Rf, values of 0.24 and 0.08 on the death of mice

As in Fig. 2, thin-layer chromatography was conducted on the active substances. Each mouse was given an amount of this substances corresponding to 4 ml of whey/day/mouse. It was demonstrated in Tables 13 and 14, that the substance having Rf of 0.24 has more powerful inhibitory action for nutritional disorder.

**Experiment D**: The chemical character of the active substance

The ultraviolet spectra of the solution of two spots diluted to 1/100 concentration were showed the same absorption peak at around 280 mp. The inhibitory dose of this substance was 4–6 μg/day/mouse. A yellow color developed with ninhydrin reagent, and the substance showed positive reaction to Lowry's phenol reagent (6). Therefore it is probably a kind of protein. (Fig. 3).

**DISCUSSION**

A hyperlactose diet developed diarrhea in rats, which either died of nutritional disorder or suffered from cataract in the cases of most survivors. It also developed diarrhea in mice, many of which died of nutritional disorder. Furthermore, a similar syndrome was developed by means of a feeding on a hypergalactose diet to rats and mice. As
a result of studies conducted by Day (7), and our laboratory staff, it is considered that the lactose absorbed into the body decomposed to glucose and galactose, the glucose is utilized but unutilized surplus galactose hinders the activity of Leloir's enzyme system and produces an advance effect. Whey contains a factor that inhibits this nutritional disorder. The trophopathy-inhibiting factor, which differs from the cataract-inhibiting factor in the case of rats, was extracted by means of alkaline solution from the active charcoal in acidified whey after the removal of the cataract-inhibiting factor with an ethanol-ether (3: 1) mixture. This substance was further separated into two substances by means of thin-layer chromatography, and the one having Rf value of 0.24 had a more powerful preventive action than the another one with Rf value of 0.08. These substances had same absorption peak at 280 m\textmu. They were protein-like substances, developing a yellow color with ninhydrin reagent and showing positive reaction to Lowry's phenol reagent. Identification studies are scheduled.

**SUMMARY**

Whey contains protein-like substance that inhibit diarrhea and death caused by nutritional disorder resulting from feeding on a hyperlactose or hypergalactose diet to rats and mice. This substance was separated into two substances and had same absorption peak at 280 m\textmu and differed from the cataract-inhibiting factor.

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