BIOCHEMICAL STUDIES ON THE MAMMARY GLAND FUNCTIONS III. FORMATION OF LACTOSE WITH THE RAT MAMMARY TISSUE IN VITRO

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III. FORMATION OF LACTOSE WITH THE RAT MAMMARY TISSUE IN VITRO*

By

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(Received July 31, 1959)

One of the important problems in the field of lactation biochemistry is the formation of lactose in the mammary gland cells. In this sort of studies, the guinea pig has mainly been used hitherto as a small experimental animal rather than the rat. However, the rat seems to be more advantageous because of its shorter pregnancy term, longer lactational period and in addition, probably greater lactation efficiency considering from the larger rate of litter growth and size of litter.

As regards the lactose formation in the rat mammary slice *in vitro*, the following apparently opposite results have been so far reported, *i.e.*, Hills and Stadie (1) showed a positive result with fairly good yield of lactose comparing with similar experiments of other workers with guinea pigs (2, 3, 4). While Heyworth and Bacon failed to obtain any positive lactose production (5).

Our experiment was aimed to yield an information whether rats mammary gland could be used as a substitute for guinea pigs' in an investigation of lactose formation *in vitro*, and if so, how much efficiency could be expected. The results of this experiment showed that lactose could be produced with rat mammary tissue from glucose as a sole source, although the efficiency of the yield was not so good. In this investigation a special and close attention

* The paper was presented to the Annual Meeting of the Kanto Branch of the Japanese Society of Zootechnical Science, in Tokyo, October, 1951.

** This experiment was carried out at the Scientific Research Institute during one (H.S.) of the authors belonged to the Laboratory of Zootechnical Science (Professor K. Sasaki), Faculty of Agriculture, Tokyo University.

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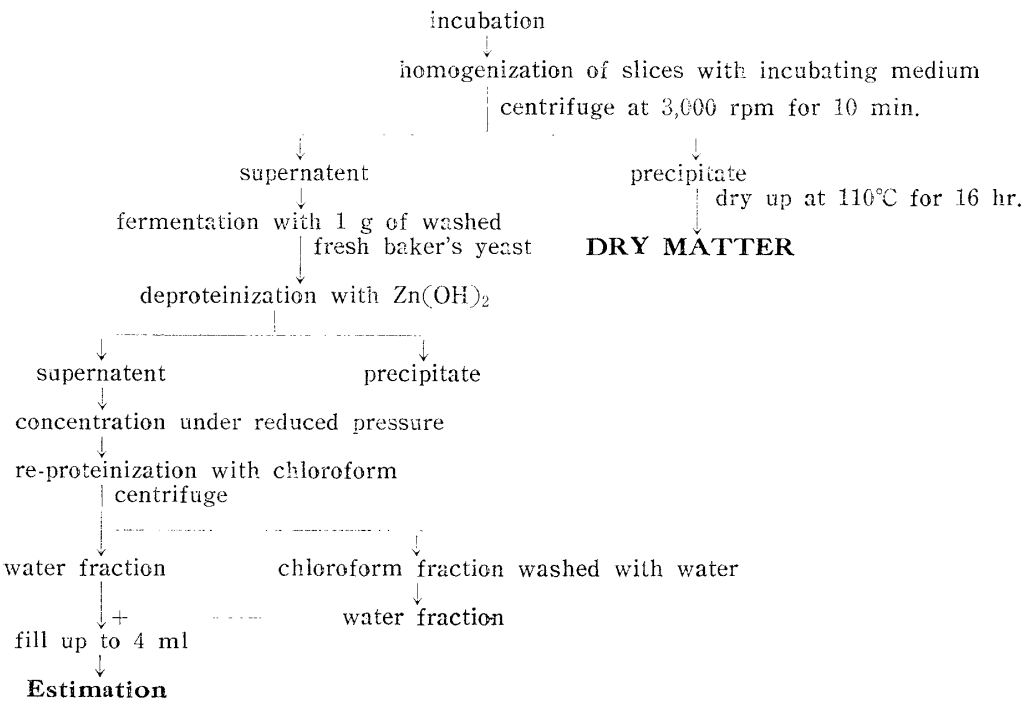
was paid to the selection of the analytical method, since the disparty in the results so far reported seemed to be due to the method employed and to the successive interpretation of the data, as Heyworth *et al.* (4) pointed out. Consequently, Malpress and Morrison's method (6) was adopted.

Materials and Methods

i) *Preparation of slices*; The mammary tissue excised from Kasukabe strain rats at about the middle of the lactation period (from the nineth to thirteenth day) was used. Mother rats were allowed to suckle their young till immediately before death by bleeding after being stunned. Two rats with the same pupping date were used for a set of experiments to get a sufficient amount of slices. Slices were made from the abdominal and inguinal glands by cutting the tissue blocks held between two frosted slide glasses with a razer blade as thinner as possible (from 0.5 to 1.0 mm). The first slice was always discarded. The slices were immensed and washed three times with Krebs-Ringer bicarbonate buffer (pH 7.4) by changing the buffer to eliminate the milk retained (7, 8). Finally they were blotted on filter paper and divided into equal portions (about two g each) and placed in incubating flasks of which contained ten ml Krebs-Ringer bicarbonate solution. These processes were carefully performed on crushed ice to keep the slices cold. The time required from slaughter to the beginning of incubation was not over one hour.

Scheme of pre-estimation preparation



ii) *Incubation*; Our incubation method was essentially the same with that of Malpress and Morrison (4) with but slight modification. A set of experiments consisted of two flasks: a) control, about two g of slices alone with ten ml of the incubating medium, b) experimental, adding 50 mg of glucose, otherwise the same as the control. Incubation was carried on for six hours in a water bath at 37°C with continuous bubbling of a gass mixture (O_2 95% + CO_2 5%).

iii) *Pre-estimation procedure*; Dry matter preparation, fermentation of the remaining glucose, deproteinization and lactose extraction was carried on as shown in the preceding scheme.

iv) *Estimation*; Lactose was determined by Malpress and Morrison's method (4) specific for lactose, but the determination was carried on with four ml instead of eight ml in the original lactose-containing solution (see above scheme). Assuming the differences between the control and experimental is to be the net lactose produced, the results were expressed with lactose mg per dry matter weight or fresh weight per six hours.

Results and Discussion

The results are summarized in Table 1.

Lactose yield in the experimental and control groups was 5.3 and 4.0 mg/g dry weight/six hrs., and the difference between these two groups was statistically significant at a level of 95 per cent fidelity. The net lactose synthesized from glucose was 1.3 mg in average, ranging from 0.2 to 3.2 mg. On the fresh weight basis, lactose produced in the experimental and control, and the net yield were 1.32, 0.98 and 0.34 mg per six hours, respectively. The difference between the experimental and control was verified significant at a level of 99 per cent fidelity.

In a preliminary experiment, it was demonstrated that the remaining glucose in the incubating medium could be completely eliminated by baker's yeast fermentation. Total lactose determined was not high, but it was measured in a half scale, *i.e.*, twice in its concentration to get higher reading, so that the lactose estimation in this experiment was likely to be accurate.

In view of the fact that the values in the control appeared moderately high and the possibility that at least a part of the lactose in this group might be newly synthesized during six hours incubation period, however, the net yield of lactose formed in the experimental seemed to exceed the above difference.

As was described in the preceding experiment, it is clearly demonstrated that lactose could be produced with rat mammary glands *in vitro*. The net lactose synthesized came up to 1.3 mg per dry weight per six hours in average, although this figure was inferior to those either of Hills and Stadie with the

Table 1. Lactose synthesized *in vitro* with or without glucose

Case No.	Lact. stage	Tissue weight used wet g	dry g	ratio (dry/wet)	Lactose mg/g dry ♯ weight/6 hrs. glucose +	−	difference	Lactose mg/g wet weight/6 hrs. glucose +	−	difference
1	12	2.3	0.45 0.45	0.20	8.0	6.0	2.0	1.80	1.35	0.45
2	10	2.1	0.55 0.60	0.27	7.6	4.4	3.2	1.10	1.35	0.75
3	13	1.8	0.55 0.55	0.31	7.0	5.0	2.0	1.40	1.00	0.40
4	12	2.3	0.40 0.30	0.16	6.9	6.3	0.6	1.20	0.95	0.25
5	10	2.0	0.45 0.45	0.23	5.8	4.4	1.4	1.30	1.00	0.30
6	10	2.0	0.45 0.45	0.23	5.6	4.7	0.9	1.25	1.05	0.20
7	12	2.0	0.55 0.45	0.25	5.6	4.6	1.0	1.25	1.25	0
8	14	2.6	0.60 0.60	0.23	5.2	2.7	2.5	1.60	0.80	0.80
9	13	1.9	0.55 0.55	0.29	5.1	3.8	1.3	1.40	1.00	0.40
10	9	2.0	0.50 0.50	0.25	5.0	4.0	1.0	1.15	1.00	0.15
11	12-13	1.9	0.50 0.50	0.20	4.6	4.0	0.6	1.25	0.90	0.35
12	10	2.5	0.70 0.60	0.26	4.4	3.5	0.9	1.55	1.05	0.50
13	9	2.0	0.45 0.55	0.25	4.2	3.2	1.1	0.95	0.85	0.10
14	11-12	2.1	0.60 0.65	0.29	3.3	3.3	0.2	1.05	1.00	0.05
15	10	2.8	0.65 0.70	0.25	3.2	2.0	1.2	1.05	0.70	0.35
16	9	2.0	0.60 0.55	0.29	2.9	1.7	0.8	0.80	0.50	0.30
Av.				0.25	5.3*	4.0	1.3	1.32**	0.98	0.34
±S.d.				±0.06			±0.8			±0.23

*, ** means significant at level of P = 0.05, 0.01

♯ as to dry weight, see text, scheme in *Methods*.

rat (1), or of others with slices and homogenate of guinea pigs (2–5, 9–11). But Hills and Stadie estimated lactose only in the incubating medium (not included that in slices), therefore, the difference between the experimental and the control in their results would be inproperly higher, as Heyworth and Bacon pointed out.

Although no explanation could be offered from this investigation why the present results were so low in production of lactose, this might be in part

attributed to the relatively high values in the control values. In regard to the high values in the control group, it seemed to suggest the possibility that at least most of the lactose might be newly synthesized of cellular glucose or other substance(s). In other words, the net lactose produced in the experimental group might exceed the difference between the two groups. On this point, the works of Malpress and Morrison (4), and Reithel *et al.* (10, 11) was of interest, and they indicated that glycogen would be utilized for lactose formation in the guinea pig mammary glands.

The authors wish to express their sincere thanks to Drs. K. Sasaki, W. Nakahara and M. Naito for their interest in this experiment.

This investigation was supported in part by a Grant in Aid for Fundamental Scientific Research from the Ministry of Education.

The baker's yeast was donated from the Oriental Yeast Co., Tokyo.

Summary

An experiment was carried out to see if lactose could be synthesized with the rat mammary gland slices, since contrary evidences had been proposed on this subject. The results demonstrated that lactose could be actually produced with rat slices *in vitro*.

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