BIOCHEMICAL STUDIES ON THE MAMMARY GLAND FUNCTIONS IV. CHANGES IN THE CATALASE ACTIVITY OF THE RAT MAMMARY GLAND

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IV. CHANGES IN THE CATALASE ACTIVITY OF THE RAT MAMMARY GLAND*

By

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Among a number of studies on the biochemical changes of the mammary gland activities during the phases of pregnancy and lactation, enzyme contents have been demonstrated (refer to (6), on the whole). The biochemical significances *in vivo* of the catalase have still been obscure in spite of its wide occurrence, earlier discovery and subsequent fruitful investigations on the chemical properties as an enzyme (13, 25). However interesting evidences have been shown in the field of cancer biology, *i.e.*, catalase activity decreased markedly in the liver of cancer bearing animals (12, 19).

The results of the present experiment demonstrated that catalase contents increased suddenly at the time of lactogenesis (initiation of milk secretion), though they little raised during pregnancy up to the end of the term. It is also indicated that their remarkably sharp drop happened after the seventh day of lactation, and the curve decreased throughout the later half of lactation, keeping the same level as that of late pregnancy.

Materials and Methods

In total, sixty rats of Kasukabe strain were used in this experiment. The mother rats were permitted continuously to suckle *ad libitum* their young until killed by bleeding after being stunned, just before the enzyme estima-

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tion (21, 22). The mammary glands excised from non-pregnant, pregnant and lactating rats were homogenated with four volumes of cold Krebs-Ringer solution. One ml of the homogenate (equivalent to 0.2 g fresh tissue) was diluted with five ml of 15/M phosphate buffer (pH 6.8). Determination of the enzyme activity was carried out by Stern and Battelli's gas-volumetric method (8, 19, 23) shaking the flask at 0°C. The flask contained six ml of the sample above mentioned and ten ml of a solution of hydrogen peroxide (5 per cent). The oxygen evolved was read in five minutes (19).

The mammary glands of the rats in both non-pregnancy and pregnancy both were considerably rich in the contents fat tissue, and consequently the weight measurement of the specimen alone was apt to produce inaccurate results. The concentration of desoxyribonucleic acid (DNA), therefore, was estimated in every samples by Schneider's method (20), and was used for the calculation of O_2 evolution per net tissue except fat to express the true activity of the enzyme (10, 21).

Results and Discussion

The results obtained in the experiment are given in Table 1, and illustrated in Figure 1: the weight of the whole mammary gland, DNA-P content and O_2 ml/0.2 g fresh tissue or 20γ DNA-P five minutes.

i) As reported previously (21), the weight of the whole mammary glands (inguinal and abdominal) showed an increase during pregnancy, particularly, markedly in the later part of pregnancy, and thereafter, it remained at the same level in general. DNA contents indicated, generally, a similar trend to the weight of the wet weight in this experiment, and to the DNA contents obtained in the previous paper (21). Attention should be given to the sudden and temporary decline and rise in wet weight and DNA, respectively, at the 20th day of pregnancy as was noted previously (21). The differences of wet weight and DNA contents between pregnancy and lactation were proven to be significant statistically ($P<0.01$ and 0.05, respectively) (see, Table 1).

ii) Catalase contents on wet tissue basis appeared to show an abrupt increase which started from the initiation of lactation (L-0), and this was maintained till the seventh day of lactation, although the enzyme contents gained slowly to the parturition during pregnancy. A remarkable diminution following the maximum height occurred since the tenth day of lactation, keeping a final level the same as that of pregnancy. Catalase contents expressed per 20γ DNA-P indicated also a similar tendency, except that the values in pregnancy became relatively a little higher. The high values of the enzyme activity during the earlier lactation in both curves were verified to be significant at the level of 99 per cent fidelity comparing to those of pregnancy and later lactation. Accordingly, it was obviously confirmed that the true

augumentation of the catalase activity occurred during the first seven days of lactation.

As far as the authors know, no work has been reported on the changes

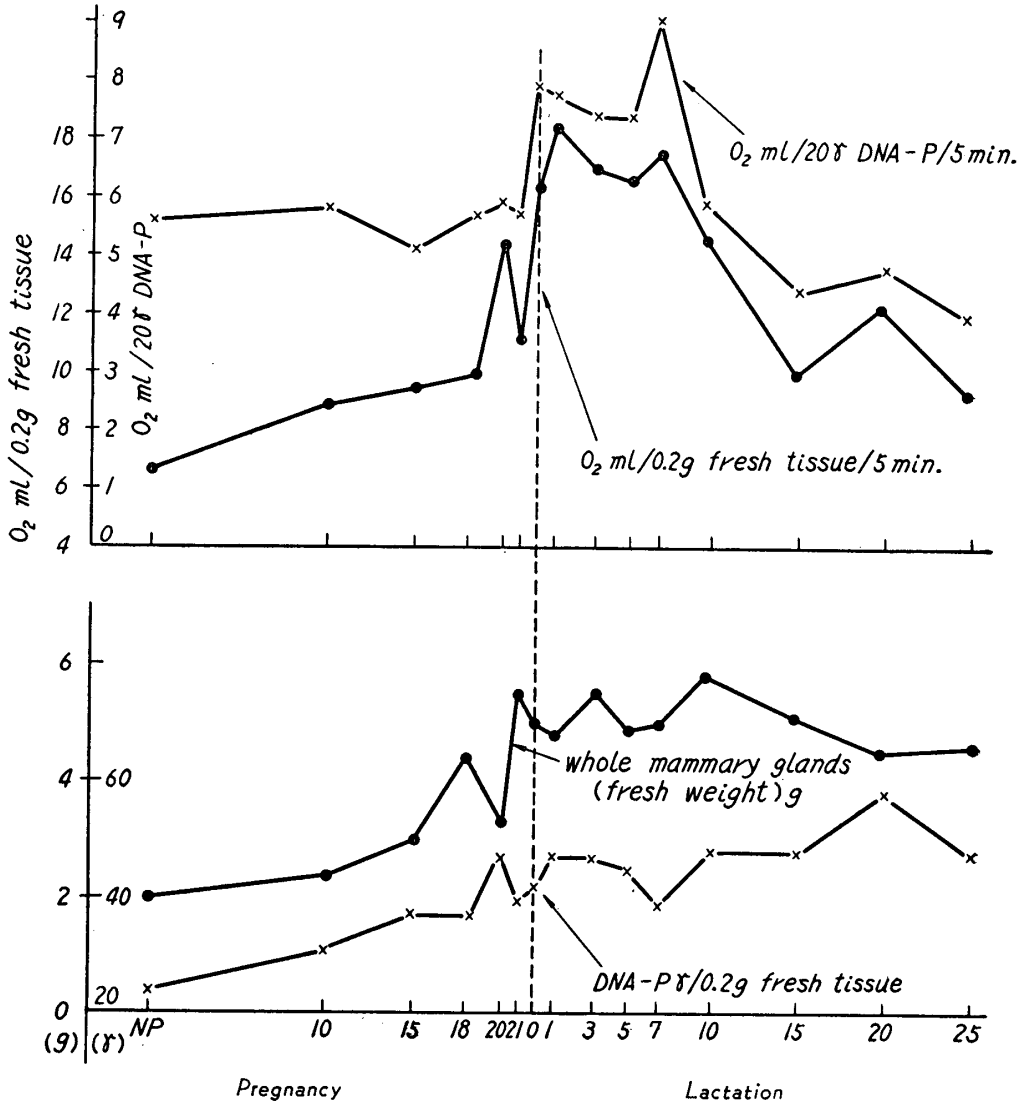

Fig. 1. Changes in fresh weight, DNA contents and catalase activity of rat mammary gland.

in catalase contents in the mammary glands throughout the time from pregnancy to weaning. Referring to the changes in the contents of various enzyme systems of the mammary gland during the phases of pregnancy, parturition and lactation, most of them so far investigated raised up precipiously from the last part of pregnancy or the beginning of lactation. They maintained the highest values during the tenth to 20th day of lactation (6, 7, 9–11, 17, 18), when milk formation seemed to be most intense (4).

Considering from the significant augumentation of catalase activity only

during earlier lactation, it may be assumed that this enzyme did not play a direct or close part in the synthesis of the milk constituents. However, taking the evidences into consideration, that catalase stimulates the oxidative

Table 1. Weight of whole mammary gland, DNA-P concentration and catalase activity.

Gp.	Stage	Rats no. used	Mammary gland wt. g	DNA-P γ/0.2 g fresh tissue	Catalase activity O_2ml/0.2 g wet tissue/5 min.	Catalase activity O_2ml/20γ DNA-P/5 min.
I	N-P	4	2.0	24	6.7	5.9
II	P-10	4	2.4	31	8.9	5.8
	P-15	4	3.0	37	8.2	5.1
	P-18	5	4.4	37	10.0	5.7
	P-20	3	3.3	47	11.2	5.9
	P-21	4	5.5	39	11.0	5.7
	(Av.)		(3.72)	(38.2)	(9.86)	(5.62)
III	L- 0	6	5.0	42	16.4	7.9
	L- 1	4	4.8	47	18.1	7.8
	L- 3	5	5.5	47	17.0	7.4
	L- 5	4	4.9	45	16.7	7.4
	L- 7	3	5.0	39	17.6	9.1
	(Av.)				(17.16)**	(7.92)**
IV	L-10	4	5.8	48	14.6	5.9
	L-15	3	5.1	48	10.0	4.4
	L-20	3	4.5	58	12.3	4.8
	L-25	5	4.6	47	9.3	4.0
	(Av.)		(5.10)**	(46.8)*	(11.40)	(4.82)

*, ** indicates difference to be statistically significant at 0.05, 0.01

processes of alcohls and others by oxidases (5, 14, 15), the results of the present experiment might be explained to be concerned to a specific biochemical reaction which would happen only during the earlier stage of lactation. It is also of interest to point out here that adrenalectomy and castration depressed the activity of liver catalase (1, 2). Since it is well established (3, 18) that all mammary gland activities are controlled by almost all hormones, especially sex and adrenal hormones. The abrupt raise of mammary catalase activity at early lactation may be related to some hormonal changes.

Let us consider the index by which the activity was expressed. Folley and his coworker noticed (7) the gross increase in weight of the mammary gland to be due to milk retained in the gland. In the present experiment and in Goto and Ugami's paper (10), the pups were left to suck milk immediately before the death of mother rats, and in our previous works (21, 22) the lactating glands were shown to contain about three mg lactose per one g of the fresh tissue throughout all lactational phases. Presuming the lactose concentration of rat milk to be 3.0 per cent in average (4, 7), our previous data indicated that

the milk retained was about 10 per cent of the whole mammary gland weight, and this figue appeared to be much less than that of Folley *el al.* (cf. 40–50%). Our results on the change of DNA contents show the same tendency as Folley and Greenbaum's total weight changes of the mammary gland corrected for milk retained in the tissue. Measurement of DNA seemed to be much simpler than that of lactose. As more reliable critersons for expression of biochemical activity, Smith examined (23) some indices and showed DNA as a more stable one in the guinea pig mammary glands though there was considerable variation during pregnancy. So that conclusively the enzyme activity should to be expressed on DNA basis, besides on wet weight. In the present experiment, an increase in appearance of the catalase activity expressed on wet tissue basis during pregnancy turned to be proven as a inexact one when the activity was given as DNA basis (Fig. 1).

Further studies on the significance of the catalase in milk formation are required for a clear explanation.

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Summary

1. Changes in the catalase activity of the rat mammary gland throughout pregnancy, lactogenesis and lactation were studied to seek a relation between the enzyme activity and mammary functions.

2. The enzyme activity was measured gas-volumetrically by Stern-Battelli method. DNA concentration in the gland was determined for estimating parenchymal except fat tissue.

3. The catalase activity on DNA basis showed a signifcant increase ($P<0.01$) in the earlier stage of lactation alone. *i.e.*, from initiation of lactation to the seventh day of lactation. However, slight difference was demonstrated between the activity of pregnancy and the later phase of lactation.

4. The biochemical significance of mammary catalase was not sufficiently clarified by this experiment, but was discussed as compared with the changes of other enzymatic activities which is probably relating to milk formation.

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