

tempt to overcome a copper deficiency induced by a molybdenum-block of a copper dependent metabolic reaction. Perhaps the failure to demonstrate a copper deficiency at the enzyme level is due to this extreme compensation.

The copper appeared to be associated with the particulate fractions of liver containing a high concentration of protein. The converse appears to be true for molybdenum. This suggests that the copper may be bound to protein as it is in blood.

Summary. In the liver of control rats (-Mo) the copper appeared to be concentrated (> 55%) in the supernatant fraction (nuclei and debris < 15%; mitochondria 20%; microsomes 10%), while in the liver from molybdenum-fed rats more copper was found in the nuclei and debris (27%) and mitochondria (27%) fractions and less in the supernatant (< 37%). The percentage of total copper in the microsomes appeared to be unaltered by dietary molybdenum. The molybdenum was usually found to be concentrated (> 60%) in the supernatant (nuclei and debris < 15%; mitochondria 15%; microsomes 10%). The feeding of sulfate had little or no effect on distribution of copper

or molybdenum.

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Normal and Experimental Involution of Rat Mammary Gland.* (26722)

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Previous studies in this laboratory have employed deoxyribonucleic acid (DNA) as a quantitative index of mammary gland proliferation during various experimental conditions (1,2,3,4) and during normal pregnancy and lactation in the rat and mouse (4,5,6,7). It was thought desirable to investigate reversion of DNA during normal involution of the rat mammary gland and to determine the ef-

fect of the ovarian hormones estrogen and progesterone upon this physiological process. These data will serve as a basic reference for further experiments on prevention of mammary gland involution.

Materials and methods. Data were obtained from primiparous Sprague-Dawley-Rolfsmeyer rats. Young were separated from dam on day 1 of lactation and mammary gland DNA was determined on days 5, 10, 14, and 20 after weaning. Experimental animals received subcutaneous injections of 1 μ g E (estradiol benzoate) or 1 μ g E + 3 mg P (progesterone) from day of weaning.

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TABLE I. Total Mammary Gland DNA during Normal and Experimental Involution.

Day of involution or lactation	No. of animals	Treatment	B.W. (g) mean	DFFT (mg) mean	DNA/mg ($\mu\text{g}/\text{mg}$ DFFT)	Total DNA (mg/100 g)
Day 1 of lactation(5)	11	Normal	245	584	$35.6 \pm .95^*$	$8.46 \pm .77^*$
Day 20 of lactation(5)	9	"	305	1210	$28.3 \pm .45$	11.30 ± 1.0
Day 5 of involution	27	"	258	494	32.2 ± 1.01	$6.10 \pm .47^2$
10	19	"	266	347	30.1 ± 1.51	$3.76 \pm .25^4$
14	9	"	277	389	30.2 ± 1.69	$4.02 \pm .19^5$
20	11	"	279	471	$21.4 \pm .93$	$3.47 \pm .25^6$
5	21	$1 \mu\text{g}$ E	259	386	30.1 ± 1.05	$4.48 \pm .13^{1,6}$
10	10	"	264	389	32.4 ± 1.23	$4.95 \pm .42^7$
14	11	"	271	438	36.9 ± 1.11	$5.88 \pm .28$
20	15	"	291	539	32.1 ± 1.3	$5.84 \pm .2$
5	10	$1 \mu\text{g}$ E + $3 \mu\text{g}$ P	273	482	33.4 ± 2.15	$5.64 \pm .30^3$
10	10	<i>Idem</i>	270	566	35.3 ± 1.18	$7.46 \pm .59$
14	10	"	284	517	33.6 ± 1.66	$6.18 \pm .25$
20	19	"	284	725	27.6 ± 1.17	$6.91 \pm .35$

¹ Significant to day 1 of lactation at .1% level. ² Significant to day 1 of lactation at 2% level.

³ Significant to day 1 of lactation at 1% level. ⁴ Significant to day 5 of involution at .1% level.

⁵ Significant to day 5 of involution at 5% level. ⁶ Significant to day 5 of involution at 1% level.

⁷ Significant to day 5 of involution at 2% level.

* Mean $\pm$ S.E.

Six abdominal-inguinal mammary glands were removed from each rat and DNA determined as previously described(6).

Percentages were calculated after subtracting the base of 2.83 mg DNA/100 g from experimental values(5).

Results. Mammary gland DNA was reported as 8.46 mg/100 g BW (body weight) on day 1 of lactation(5). During course of normal involution, DNA decreased to 6.10 mg/100 g BW by day 5 of involution and at days 10, 14, and 20, DNA values were 3.76, 4.02, and 3.47 mg (Table I). DNA/100 g BW of experimental animals which received E alone was 4.48, 4.95, 5.88, 5.84, and with E + P, values were 5.64, 7.46, 6.18, and 6.91 at days 5, 10, 14 and 20, respectively, of involution. Significant differences exist between day 1 of lactation, and all days of normal and early experimental involution. During normal involution days 10 ($P < .001$), 14 ($P < .05$), and 20 ($P < .01$) were significantly reduced in relation to day 5 of involution, but marked differences were not observed between other days. At day 5 of involution, E group was markedly decreased below both normal ($P < .01$) and E + P groups ($P < .001$). By day 10, E ($P < .02$) and E + P

($P < .01$) values were greater than observed at same period of normal involution. This was evident throughout the remainder of experimental period. E + P DNA values were also consistently greater than obtained with E alone.

Discussion. Early morphological studies on rat mammary glands indicated that by day 5 of involution, only a small number of alveoli remained, the majority of glandular tissue being replaced by adipose tissue. Mammary glands resembled those of a normal virgin animal by day 9 of involution(8).

Present data agree with earlier studies. DNA decreased approximately 42% by day 5 of involution when compared to day 1 of lactation. By day 10, an 85% decrease is evident which then remains essentially constant throughout the remainder of the period. These values are comparable to those of normal virgin animals or ovariectomized controls(3). Preliminary data indicate when young are weaned after a normal lactation period (20 days), mammary glands involute to essentially the same values by days 10 and 20. DNA values for day 10 were 3.84 and day 20, 2.98 mg/100 g BW. This may be due to a faster rate of involution since

DNA values during lactation are significantly higher than observed at day 1 of lactation.

E was found to augment involution during the initial period after weaning. DNA was decreased 71% from day 1 of lactation, and 50% below the normal controls. By day 10, growth effects of E were evident with a 79% increase above control levels by day 20.

E + P did not prevent the initial decrease of DNA. Involution progressed at same rate as under normal conditions. P in combination with E appears to prevent more rapid decrease in DNA as found when E was injected alone. P may have a "masking" effect upon E, since it has been reported to retard involution at 1 mg/day for 6 days(8). E + P growth effects were evident by day 10, with an 82% increase over normal values by day 20.

Ovarian hormones may not retard involution to any significant degree, because DNA values determined at day 5 of involution were as low or lower than observed during normal period. Higher values observed with hormones during later stages of present experiment may be due entirely to growth promoting activity of these hormones upon the mammary gland.

Summary. With DNA as a measure of mammary gland involution, a 42% decrease in DNA between day 1 of lactation and day 5 of involution was noted. By day 10, DNA decreased 85% and remained essentially constant throughout the remainder of the period. E alone appeared to augment retrogression of DNA during initial days of involution, but with E + P this effect was not apparent. With both E and E + P, mammary gland DNA was increased during the later days of experimental period.

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Biologically Active Peptides in *Physalia* Toxin.* (26723)

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Several previous communications from this laboratory have detailed the activity of various kinds of crude *Physalia* toxin preparations(1,2,3). Recently whole toxin has been fractionated chromatographically into a series of peptides. Even after this rigorous treatment, some biological activity persists. This communication presents the results of tests for biological activity of crude toxin on

various microorganisms and protozoa, and describes the distribution of toxicity among the component peptides of the crude toxin of *Physalia*.

Materials and methods. Surviving nematocysts were separated from tentacular tissue by controlled autolysis, and were homogenized to liberate their toxic contents as described by Lane and Dodge(1). Nematocysts were homogenized for 20 to 30 minutes in an all-glass homogenizer. The residue was diluted with 2 to 3 ml of distilled water and centrifuged for 20 minutes at 2°C and 10,000 rpm. The residue was resuspended in a

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