mercial lactogenic hormone† daily for 4 days. As shown in the table, 13 of the 15 animals, sacrificed on the 5th day, showed microscopically confirmed deciduomata, together with large robust-appearing corpora lutea. Eight uninjected control animals showed small regressive corpora and no deciduomata at the sites previously stimulated. The results are interpreted as further evidence of corpus luteum stimulation by the lactogenic hormone.

Presumably this gonadotropic function is mediated by the continued production of progestin in the corpora in sufficient concentration to permit the growth of the deciduomata well past the time when they can normally be produced.

† Acknowledgment is made of the donation of commercial lactogenic hormone of the anterior pituitary gland to Schering Corporation, Bloomfield, N.J.

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## Rôle of Inositol and p-Aminobenzoic Acid in Normal Lactation.

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The fact that several members of the vitamin B complex are required for lactation in the albino rat has long been recognized. Almost every member of the complex has at one time or another been suggested for a crucial role. The list includes thiamin,[1] riboflavin,[2] pantothenic acid,[3] choline,[4] p-aminobenzoic acid,[5] and inositol.[5] Less well defined factors which have been reported as essential are factor W[6], and the filtrate factor.[7] In most cases these reports lack confirmation from other workers.

The pioneer work of Sure[5] indicated that p-aminobenzoic acid is essential for lactation in the albino rat and that inositol may be.

The conclusions drawn from the work were evidently not intended to be final, and it has seemed worthwhile to examine critically the role played by each of these substances.

*Methods.* Young female rats from our own breeding colony were divided into groups of 12 and placed on the following dietary regimes shortly after weaning:

*Group A.* Normal breeding diet* containing all known dietary elements. We have found over a period of 5 years that this diet is capable of meeting all the requirements of breeding rats.

*Group B.* Deficient diet† plus B-complex supplement.‡

| | % |
|---|---|
| * Corn | 28.0 |
| Wheat | 28.0 |
| Whole milk powder | 22.5 |
| Linseed meal | 8.0 |
| Alfalfa | 3.0 |
| Crude casein | 4.0 |
| Calcium carbonate | 0.5 |
| Sodium chloride | 0.5 |
| Brewer's yeast | 3.0 |
| Cod liver oil | 2.5 |
| | g |
| † Casein[6] | 25.0 |
| Agar agar | 2.0 |
| Cystine | 0.2 |
| Butter fat | 10.0 |
| Dextrin | 58.8 |
| Salt mixture 351 | 4.0 |
| Cod liver oil | 2.5 |
| Mixed toxopherols | 0.25 |
| 2-methyl,1,4-naphthoquinone | 0.01 |

(This diet was made up at 5-day intervals and kept under refrigeration until used.)

| | |
|---|---|
| ‡ Thiamin[5] | 120 γ |
| Riboflavin | 120 γ |
| Pyridoxine | 120 γ |
| Calcium pantothenate | 600 γ |
| Nicotinic acid | 6 mg |
| Choline hydrochloride | 15 mg |

(The amounts indicated were the average daily supplement per rat.)

1 Evans, H. M., and Burr, G. O., *J. Biol. Chem.*, 1928, **76**, 263.

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3 Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 625.

4 Sure, B., *J. Nutrition*, 1940, **19**, 71.

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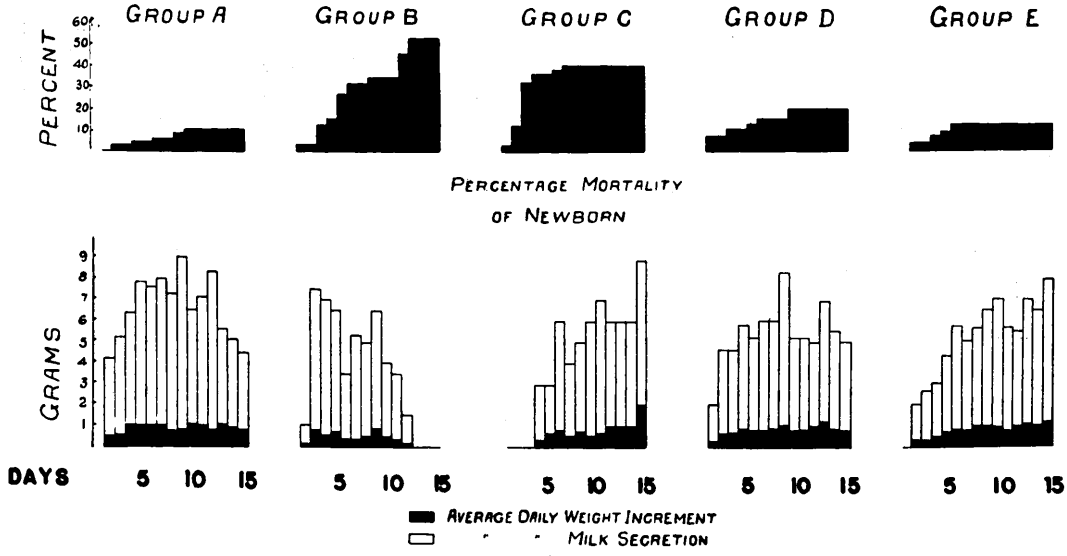


FIG. 1.

Mortality rates of newborn, average daily milk secretion for mothers, and average daily weight increments of pups on various vitamin B-complex regimes (see text).

*Group C.* Deficient diet plus B-complex supplement plus p-aminobenzoic acid (15 mg per day).

*Group D.* Deficient diet plus B-complex supplement plus inositol (15 mg per day).

*Group E.* Deficient diet plus B-complex supplement plus p-aminobenzoic acid and inositol (each 15 mg per day).

All rations were supplied *ad libitum* and the various supplements were administered by stomach tube. Supplements were administered daily except Sundays but this deficiency was overcome by giving 50% greater doses on Saturdays and Mondays.

The B-complex supplement was so prepared that it was contained in 0.5 cc of water. The p-aminobenzoic acid and inositol supplements were so prepared that 0.25 cc of the aqueous solution contained 15 mg. (The former was present as the sodium salt.)

Weights were followed carefully for each animal. When they reached a weight of about 180 g they were placed for a period of 10 days in groups of 3 with an adult male of proven fertility. At the end of this time the males were returned to stock and the experimental animals were placed in individual cages. Litter weights were obtained daily from the date of birth.

*Results.* The experimental animals all grew at satisfactory rates. However, it was noticeable that group A grew somewhat better than any of the animals receiving the deficient diet. This phenomenon strongly resembles that observed by Troescher-Elam and Evans[8] in mice, and the growth curves in general closely resembled those presented by Sure.[5] Fig. 1 shows the average daily milk secretion for the mothers, as indicated by the total weight increment of the litter, and the average daily weight increments for the pups. The cumulative infant mortality rates, as percentages of the total births, appear at the top of the figure.

*Conclusions.* The results indicate that the B-complex supplement without p-aminobenzoic acid or inositol will not support normal lactation, that under these conditions the initiation of lactation is slow, and that it never achieves a normal level. The rapid decline in milk production beginning with the tenth postpartum day is also noteworthy; it culminates in almost complete cessation of lactation on the 14th day.

The addition of p-aminobenzoic acid to the B-complex supplement appears to result in

8 Troescher-Elam, E., and Evans, H. M., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 549.

delayed initiation of lactation, there being no significant amount of milk until the 5th postpartum day. After this time, however, secretion was maintained at a normal level.

With the addition of inositol to the B-complex supplement there was a prompt initiation of lactation which was maintained at a level only slightly less than that of the controls. Further supplementation of this regime with p-aminobenzoic acid resulted in no significant improvement in milk secretion over that in the group which received the B-complex supplement and inositol alone.

These findings are in general supported by the infant mortality rates for the various experimental groups. From these data we may conclude that the B-complex supplement alone or with the addition of p-aminobenzoic acid will not support normal nutrition of young rats. Supplementation with inositol results in mortality rates only slightly greater than those observed in the control animals. Supplementation with both inositol and p-aminobenzoic acid results in mortality rates quite comparable to those of the controls.

*Summary.* The critical role of inositol in the maintenance of normal lactation in the albino rat has been confirmed. P-aminobenzoic acid does not seem to increase lactation directly but when given to animals also receiving inositol it slightly decreases mortality rates of the newborn.

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## Action of Various Steroids on the Hypophysis of the Thyroidectomized Rat.

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The typical modification in the hypophysis of the thyroidectomized rat, namely the degranulation of the eosinophiles and the appearance of large basophilic vacuolated "thyroidectomy cells" are known to be prevented by treatment with thyroid extract,[1-3] but the effect of steroid hormones on the development of thyroidectomy changes has not been definitely established as yet. Zeckwer[2] reported that estrone does not prevent the occurrence of thyroidectomy cells, while Nelson and Hickman[4] claimed that it completely inhibits their development. Severinghaus[3] concluded—partly on the basis of similar experiments—that there is: "sufficient evidence not only to identify these thyroidectomy cells as basophiles, but also as modified basophiles similar in characteristics to castration cells"; it was pointed out, however, that the modifications of the acidophiles are not affected by folliculoid compounds. Subsequently Nelson[5] stated that the prevention of thyroidectomy cells requires three times more estradiol benzoate than the inhibition of castration cell formation.

More recently Clarke *et al.*[6] found that all hormonally active steroids (including folliculoids, testoids, luteoids and corticoids) inhibit the development of castration cells to some extent. In view of these observations it appeared of interest to establish whether the ability to prevent the formation of thyroidectomy cells is also a morphogenetic action common to all steroid hormones.

Table I summarizes our relevant experiments. It will be noted that male albino rats weighing 130-150 g were used throughout. These were thyroidectomized the day before the initiation of treatment and killed 22 days later. Each group consisted of 6 rats. All

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2 Zeckwer, I. T., *Am. J. Path.*, 1938, **14**, 773.

3 Severinghaus, A. E., *Sex and Internal Secretion*, Baltimore, 1939, 3rd Ed., p. 1045.

4 Nelson, W. O., and Hickman, J., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 828.

5 Nelson, W. O., *Federation Proc.*, 1942, **1**, 63.

6 Clarke, E., Albert, S., and Selye, H., *Anat. Record*, 1942, **83**, 449.