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## The Hormonal Basis for "Witches' Milk."

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It has been reported that about two-thirds of all babies show some breast hyperplasia for a short time after birth, and that about two-thirds of this number, regardless of sex, secrete colostrum which is usually followed by a small amount of milk.<sup>1, 2</sup> Topographically and histologically these breasts are miniature lactating glands,<sup>3</sup> and in the light of recent studies on mammary physiology such development is most likely attained through the influence of oestrin and the hypophyseal mammatropic hormone. Since oestrin is formed or stored in the placenta in considerable amounts, it was not surprising that it could be found in the blood and urine of babies for about 5 days after birth.<sup>4-7</sup> Abraham<sup>8</sup> waited until the breast swelling in new-born babies had regressed and then by injecting oestrin caused the hypertrophy and secretion to return. Brühl<sup>5</sup> caused mammary growth in infantile male guinea pigs by injecting 100 mouse units of oestrin daily for 18 days. These animals lactated following cessation of treatment. v. Dobszay<sup>9</sup> by injecting 100,000-150,000 mouse units of oestrin for 5 to 7 days into new-born babies, caused mammary growth (apparently over and above what could normally be expected) and then induced colostrum secretion by injecting an hypophyseal mammatropic preparation.

Since it is possible to detect the hypophyseal mammatropic hormone in the urine of lactating women,<sup>10</sup> and even of normal men,†

\* This investigation was made possible by the cooperation of Dr. F. S. Smyth, Professor of Pediatrics, and his staff, and by a grant from the Board of Research of the University of California.

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8 Abraham, G., *Med. Klin.*, 1930, **28**, 164.

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10 Lyons, W. R., and Page, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1049.

† In an earlier, unpublished investigation, Lyons and Vasquez obtained strong crop reactions with 100 cc. equivalents of urines from each of 7 normal men.

it seemed likely that it could also be found in babies' urine, especially in connection with the secretion of "witches' milk". The urines of 4 new-born babies (B, a female, A, C, and D, males) were, therefore, assayed for mammotropin. Case A showed breast hypertrophy at birth, and began lactating on day 3. Case C began to show slight breast hypertrophy on day 7, while B and D showed no detectable breast growth or secretion during the week of observation.

To each daily 50 cc. sample of urine an equal volume of acetone was added and this mixture stored at 0°C.‡ The acetone concentration was increased to 83% and a sufficient number of drops of 10 N HCl added to adjust the pH to 1.5-2.0 (thymol blue). After centrifuging and discarding any insoluble material, 2 volumes of acetone were added to the supernatant; then, after vigorous agitation, the sample was allowed to stand in the refrigerator overnight. After centrifuging, the supernatant was discarded, and the precipitate dried of acetone, and dissolved in 5.0 cc. of water, the pH being adjusted with NaOH to approximately 7.6 (phenol red).

The local, intradermal, crop test<sup>10, 11</sup> was carried out on squabs, one crop sac area being used for 3.2 cc. (= 32 cc. urine) of each specimen. This amount was given over a period of 4 days with necropsy on the 5th. When a strong reaction was obtained, smaller fractions of the remaining samples were injected into other birds. The reactions obtained were evaluated in accordance with a set of standards made by injecting a series of squabs in a similar manner with different levels of purified mammotropin prepared from sheep pituitaries. This preparation contained a micro-unit (1 M.E.D.) in 0.01  $\gamma$ . Four arbitrary qualitative grades from 1 to 4 plus were established, and it was found that they could be reproduced in the majority of tests by at least 1, 4, 16 and 64 units respectively of

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Estimates made by duplicating these reactions with definite amounts of purified mammotropin showed that one might expect to find a micro-unit in from 1.5-3.6 cc. of unmodified urine. Since the completion of the work reported herein, positive crop reactions have been obtained with as little as 1.0 cc. of male urine. It is therefore suggested, that in tests for mammotropin, the urine be simply neutralized, and injected intradermally for 4 days; and, depending upon the results, concentration or dilution may be resorted to, in order to determine unitage. To detect minimal reactions, the muscle must be stripped from the reaction sites, and the inflammatory exudate must be scraped away before a reading can be properly made.

‡ Ordinarily the urines are worked-up and tested immediately, but these were unavoidably left in storage at 0°C. for 2 years. From what is known regarding the stability of mammotropin it seems unlikely that any great loss of potency occurred.

<sup>11</sup> Lyons, W. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **35**, 645.

mammotropin.§ Due to the small amount of urine available for each assay it was not possible to carry out a strictly quantitative assay, and therefore, the data are submitted in Table I as a qualitative contribution. The figures given represent the smallest amount of urine containing a micro-unit of mammotropin, the largest equivalent tested being 32 cc.

TABLE I.  
Approximate Number of cc. of Urine Containing a Squab Micro-unit\* of Mammotropin.

Day	Baby A	Baby B	Baby C	Baby D
1	8†	32	No assay	No assay
2	8‡	32	" "	" "
3	2§	<32	32	8
4	No assay	32	32	<32
5	0.5§	2	32	<32
6	8§	8	32	<32
7	32§	32	2†	8

\*Minimal amount, which, when injected, in 4 daily doses, within the skin covering the crop sacs of squabs (one month from hatching) produces a typically positive reaction, by the 5th day, in the majority of 5 sacs from 5 different squabs.

†Breasts enlarged.

‡Colostrum secreted.

§Milk secreted.

As may be seen in the table, all of the babies were excreting mammotropin. In only 4 of 23 samples was it impossible to detect a micro-unit in the 32 cc. equivalent, while in lactating Baby A, a relatively high level of excretion (1 unit in 0.5 cc.) was found. The urine obtained from Baby C on the day his breasts began to enlarge (7th) contained one unit in 2 cc. Since the babies left the hospital after the first week, no further clinical observations were made. If it may be assumed that the results obtained from a random choice of 4 cases may be applied to most babies, it can be concluded that the second necessary hormone (mammotropin) is at least available, just as is the first (oestrin) for breast stimulation in the new-born. And if these 2 hormones were assayed in the same urines it would probably be shown that the relative as well as absolute quantities of each must determine whether or not lactation occurs.

*Conclusions.* Mammotropin was detected in the urine of 4 new-born babies. One of the babies, a boy, lactated, and his urine showed the largest amount of hormone (1 unit in 0.5 cc.). It is suggested that "witches' milk" is formed in the infantile breast after successive stimulation with oestrin and mammotropin.

§ Although our test birds were all Silver Kings of both sexes, one month from hatching and weighed 400-450 gm., they varied considerably in their crop reactions.