dehydrogenase and oxidase systems and it seems possible that interference with the latter by means of porphyrin antagonism is more critical for *T. congolense* because of the demands of its metabolism.

It might be conjectured that the effects on an oxidase system should be readily detectable, if not more profound, in infections with *T. cruzi*, the blood form of which is more sensitive to cyanide than *T. congolense*. Our experiments did not show this to be the case, however, and it will be recalled that not only is *T. cruzi*, in the blood stream, a consumer of less oxygen and much less glucose than *T. congolense* but also, in its intracellular phase, concerning the physiology of which little is known, it is protected from attempts to change its environment.

*Summary.* A number of tetrapyrrole derivatives of both plant and animal origin were tested for ability to influence the course of *Trypanosoma congolense* infections in mice. Of these, chlorophyllins, containing either magnesium or copper, and hematoporphyrin were effective in delaying deaths from trypanosomiasis and with prolonged administration cures could be effected. The non-metallic phaeophorbide relatives of chlorophyllin were ineffective as were deuteroporphyrin, mesoporphyrin, phylloporphyrin, pyrroporphyrin, rhodoporphyrin and uroporphyrin, as well as certain bile pigments. The antitrypanosomal effect of chlorophyllin and hematoporphyrin could be reversed, either completely or partially, by hemin, but not by protoporphyrin. The hypothesis that the mechanism of action of these tetrapyrrole derivatives in suppressing trypanosome infection involves competitive inhibition of a growth factor essential for the parasite (apparently hemin, rather than protoporphyrin) seems to be supported by our experiments. Other species of hemoflagellates and certain other parasites were uninfluenced by compounds active in *T. congolense* infections. It is conjectured that sensitivity of this particular parasite may be attributable to a certain combination of physiological attributes which is not possessed by its relatives.

1. Pfeiffer, R., *Dtsch. med. Wschr.*, 1892, v18, 28.
2. Novy, F. G., MacNeal, W. J., *J.A.M.A.*, 1903, v41, 1266.
3. Granick, S., Giler, H., *J. Gen. Physiol.*, 1946, v30, 1.
4. Lwoff, A., *Zentralbl. Bakt. Parasitenk. I. Orig.*, 1933, v130, 498.
5. ———, *Recherches sur le pouvoir de synthese de flagelle Trypanosomides.* Masson, Paris, 1940.
6. Granick, S., Gilder, H., *Advances in Enzymology*, 1947, v7, 305.
7. Hutner, S. H., Provasoli, L., In *Biochemistry and Physiology of Protozoa.* Edited by S. H. Hutner and A. Lwoff, 1955, v2, 17.
8. Goble, F. C., Boyd, J. L., Singer, I., *J. Immunol.*, 1958, v80, 204.
9. Goble, F. C., Boyd, J. L., *J. Protozool.*, 1957, v4 (Suppl.), 17.
10. Bases, R., Brodie, S. S., Rubenfeld, S., *Cancer*, 1958, v11, 259.
11. Lwoff, A., Lwoff, M., *Ann. Inst. Pasteur*, 1937, v59, 129.
12. Agosin, M., von Brand, T., *Exp. Parasitol.*, 1954, v3, 517.
13. Baernstein, H. D., *Ann. N. Y. Acad. Sci.*, 1953, v56, 982.

Received February 5, 1959. P.S.E.B.M., 1959, v100.

## Induction and Maintenance of Mammary Growth and Lactation in Rats with Acetylcholine or Epinephrine.*† (24766)

JOSEPH MEITES (With technical assistance of C. S. Nicoll and P. K. Talwalker)
*Dept. of Physiology and Pharmacology, Michigan State University, East Lansing*

Mammary growth and lactation can proceed independently of connections to the nervous system, as suggested by experiments involving mammary transplantation or ligation of nerves to the mammary glands(1). The nervous system can influence mammary function, however, as indicated by observations

* Published with approval of Mich. Agr. Exp. Sta. as Jour. Art. No. 2388.
† This work was supported in part by NIH grant.

TABLE I. Effects of Acetylcholine and Epinephrine on Mammary Growth and Lactation.

| Group No. | Total rats | Treatment | No. rats lactating | Avg lactation rating | Avg % parenchyma |
|---|---|---|---|---|---|
| | | Virgin rats | | | |
| (1) | 10 | Controls, saline only, 15 d | 0 | 0 | 7 |
| (2) | 10 | 10 μg Est.,* 10 d; saline, 5 d | 0 | 0 | 12 |
| (3) | 5 | 25 mg Acetyl.†/kg/BW, 2x daily, 15 d | 0 | 0 | 17 |
| (4) | 5 | 10 μg Est., 10 d; 25 mg Acetyl./kg/BW, 2x daily, 5 d | 4 | 3+ | 27 |
| (5) | 5 | 10 μg Est., 10 d; .25 mg Epin.,‡ 2x daily, 5 d | 5 | 5+ | 41 |
| | | Lactating rats, litters removed 4th day postpartum§ | | | |
| (6) | 5 | Controls, saline | 0 | 0 | 11 |
| (7) | 5 | 25 mg Acetyl./kg/BW, 2x daily | 5 | 3+ | 26 |
| (8) | 5 | 50 mg *Idem* | 4 | 3+ | 32 |
| (9) | 5 | .2 mg Epin., 1x daily | 4 | 2+ | 40 |
| (10) | 4 | .25 mg " , 2x " | 4 | 2+ | 32 |

* Est. = estradiol. † Acetyl. = acetylcholine iodide. ‡ Epin. = epinephrine in oil.
§ Inj. for 10 days after litter removal.

that (a) application of fresh litters to the nipples of virgin rats can initiate mammary development and secretion, and (b) the milking stimulus is essential for maintenance of mammary function after parturition(2). Under any of these conditions, the possibility remains that the mammary gland may be partially under the control of neurohormones, which are released in the brain and stimulate pituitary function. Acetylcholine and epinephrine have been shown to be involved in release of LH during estrous cycle of the rat (3), and it was of interest to determine whether they could also influence mammary growth and secretion.

*Methods.* To study the effects on mammary growth and initiation of lactation, virgin female albino rats (Carworth) weighing 175-200 g were injected subcutaneously as follows: 1) controls, physiological saline only for 15 days; 2) 10 μg estradiol‡ in corn oil daily for 10 days, followed by saline for 5 days; 3) 25 mg acetylcholine iodide kg/BW twice daily for 15 days; 4) 10 μg estradiol daily for 10 days, followed by 25 mg acetylcholine iodide (Eastman) kg/BW twice daily for 5 days; 5) 10 μg estradiol daily for 10 days, followed by 0.25 mg epinephrine in oil (Parke-Davis) twice daily for 5 days. To study the effects on maintenance of mammary structure and secretion, the litters of lactating rats were removed on 4th day after parturition, and the mothers were injected subcutaneously for 10 days as follows: 1) controls, physiological saline; 2) 25 mg acetylcholine iodide/kg/BW twice daily; 3) 50 mg acetylcholine iodide/kg/BW twice daily; 4) 0.2 mg epinephrine in oil once daily; 5) 0.25 epinephrine in oil twice daily. All rats were killed on the day after last injection, and right and left inguinal glands were removed for gross and histological examination. Ovaries, uterus, adrenals and thymus glands from virgin rats were also excised and weighed. Mammary glands were fixed in Bouin's fluid and histological sections were stained with eosin and hematoxylin. Arbitrary lactation ratings of 0 to 5+ were assigned each gland upon microscopic examination. Percentage of parenchymal tissue (ducts, lobules, alveoli) in each gland was also estimated.

*Results.* The data are shown in Table I. In virgin rats, only a duct system and no secretion was present in the mammary glands of rats given saline (Group 1) or estradiol (Group 2), with somewhat greater development of the ducts in the latter group. Acetylcholine iodide alone, without estradiol priming (Group 3), induced some duct growth in only 2 of the 5 rats and no secretion, while Groups 4 and 5, which received estradiol followed by acetylcholine iodide or epinephrine in oil,

‡ Estradiol was kindly furnished by Dr. J. O. Reed of Foundation Labs., N. Y. City.

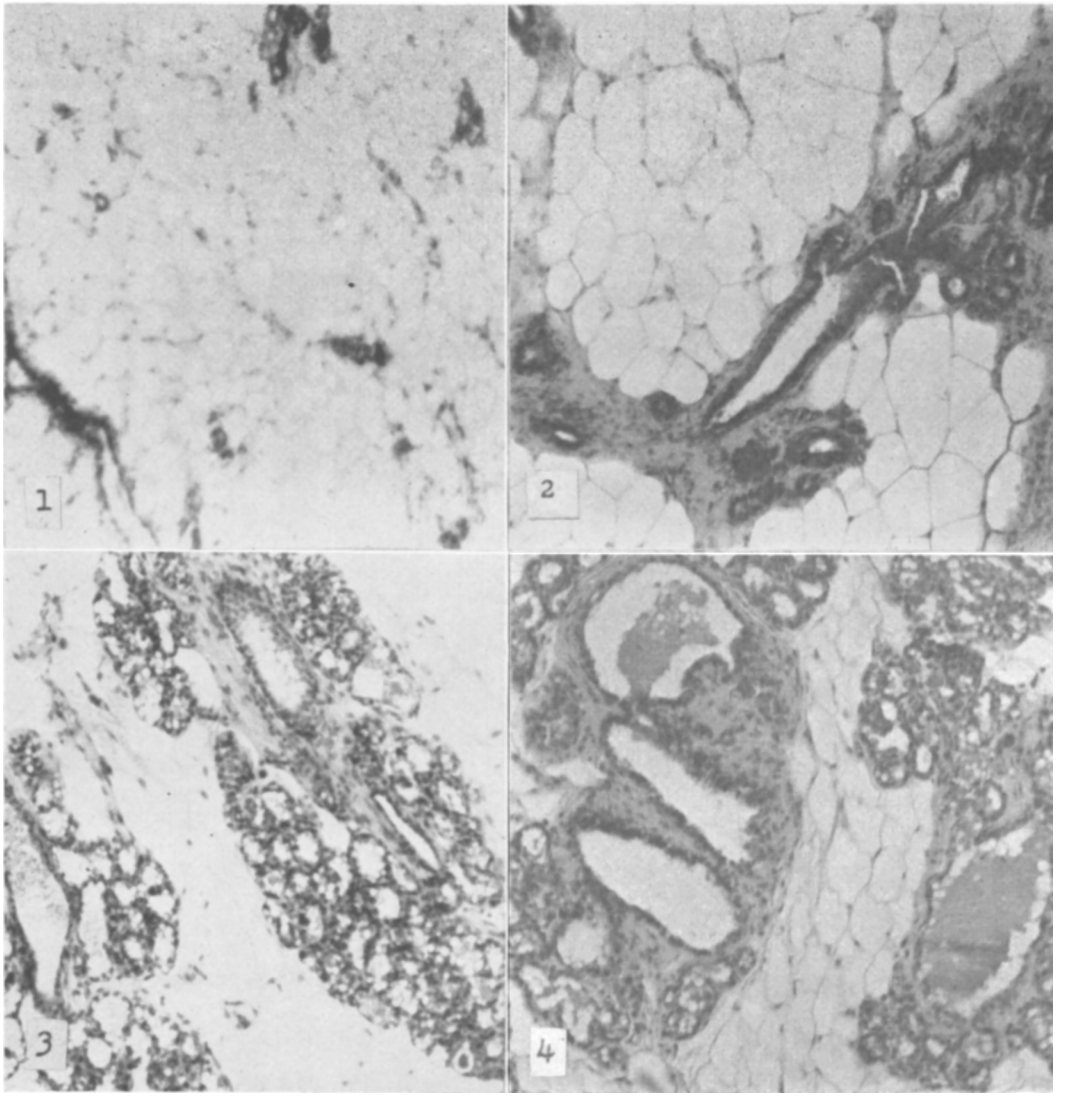


FIG. 1-4. Mammary sections from virgin rats after 15 days treatment: (1) control, saline; (2) estradiol 10 d, saline 5 d; (3) estradiol 10 d, acetylcholine iodide 5 d; (4) estradiol 10 d, epinephrine 5 d. × 100.

showed extensive lobule-alveolar growth and secretion in all except one animal. The effects of these treatments on the mammary glands are shown in Figs. 1-4.

In parturient rats after litter removal, the saline-treated controls (Group 6) showed extensive involution of the mammary system, with only collapsed islets of closed alveoli or masses of epithelial cells, and no secretion. Injections of acetyl choline iodide (Groups 7 and 8) or epinephrine in oil (Groups 9 and 10) retarded mammary involution and maintained lactation in most treated rats. The larger doses of these drugs appeared to be no more effective than the smaller doses in prolonging mammary function. Representative histological sections of the mammary glands from these groups are shown in Figs. 5-7.

In all animals which received acetylcholine iodide, there was some salivation and appearance of a porphyrin-colored secretion on the surface of the eyes within a few minutes after injection, which disappeared shortly thereafter. No obvious symptoms were noted fol-

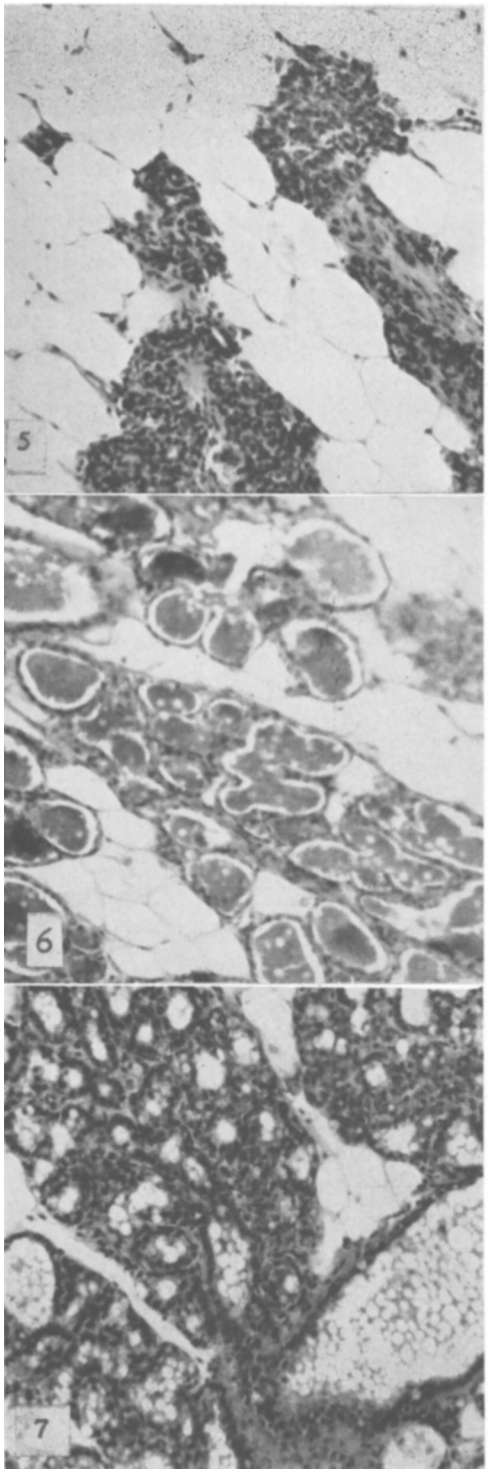


FIG. 5-7. Mammary sections from parturient rats, 10 days after litter removal: (5) control, saline; (6) acetylcholine iodide; (7) epinephrine. × 125.

lowing epinephrine injections. None of the rats given the drugs died or lost body weight, and there was no apparent alteration in weights of ovaries, uterus, adrenals or thymus glands in virgin rats. When acetylcholine iodide was given alone to normal cycling rats (Group 3), no changes were observed in the regularity of the estrous periods, as judged by examinations of vaginal smears for 2 weeks before and during the 15-day period of treatment.

*Discussion.* The mechanism(s) by which acetylcholine or epinephrine induced or maintained mammary development and lactation in these rats remains to be elucidated. It is possible that they elicited a release of prolactin and perhaps other hormones favorable to mammary growth and secretion. Since weights of the ovaries, uterus, adrenals and thymus glands remained unchanged, nor were estrous cycles disturbed in the rats given acetylcholine iodide alone, it seems unlikely that the neurohormones acted through altering the secretion of pituitary gonadotropins (FSH and LH) or ACTH.

Mammary growth and secretion in virgin rats were induced only after priming with estradiol, while acetylcholine iodide alone apparently had little or no effect on the mammary system. Epinephrine without estradiol priming has not yet been tested.§ Estrogens alone have been shown to induce mainly duct growth(4) and increase pituitary prolactin content(5), but initiate secretory activity only if the rats are ovariectomized(6). It is remarkable therefore, that injections of acetylcholine or epinephrine produced lobule-alveolar growth and initiated lactation in 5 days in the intact, estrogen-primed rats. More recently, we have observed lobule-alveolar growth and lactation when neurohormones were injected together with estradiol rather than in sequence.

The results of the present study can be compared with those previously reported with res-

§ ADDENDUM. In subsequent experiments, injections of epinephrine alone into intact rats for 15 days failed to induce mammary growth or lactation, and did not alter the estrous cycle. Estrogen priming therefore appears to be essential for the mammary-stimulating action of epinephrine.

erpine, which induced mammary growth and lactation following estrogen administration in virgin female rabbits(7) and rats (unpublished), and retarded mammary involution and prolonged secretory activity in parturient rats after litter removal(8,9). Reserpine also increased prolactin content of the rabbit pituitary(10). We recently observed that serotonin, which is released from the brain following reserpine administration(11), can also stimulate mammary growth and initiate secretion in the rat following estrogen administration (unpublished) These studies suggest that acetylcholine, epinephrine and perhaps other neurohormones such as serotonin may be involved in normal mammary development and lactation This aspect of the problem is currently being investigated

*Summary.* 1. When virgin rats were injected subcutaneously for 10 days with 10 μg estradiol daily, followed by twice-daily injections of 25 mg acetylcholine iodide/kg/BW or 0.25 mg epinephrine in oil for 5 days, extensive lobule-alveolar growth and lactation occurred. Rats given estradiol or acetylcholine iodide alone showed little or no mammary growth, and no secretion. Weights of ovaries, uterus, adrenals and thymus glands were not changed by treatment with either neurohormone, nor was the estrous cycle disturbed in rats given acetylcholine iodide alone. 2. When the litters of lactating rats were removed on 4th day after parturition, and the mothers injected with acetylcholine iodide or epinephrine in oil for 10 days, mammary involution was retarded and secretion was maintained. Rats given saline during this period showed extensive disintegration of the mammary system and no secretion. These experiments suggest that the 2 neurohormones may induce release of prolactin and perhaps other factors from the pituitary favorable to mammary growth and lactation.

1. Folley, S. J., *Brit. Med. Bull.*, 1947, v5, 142.
2. Selye, H., *Am. J. Physiol.*, 1934, v107, 535.
3. Everett, J. W., Sawyer, C. H., Markee, J. E., *Endocrinology*, 1949, v44, 234.
4. Turner, C. W., Chap. XI in *Hormone Assay* 1950, p261, Academic Press, N. Y.
5. Meites, J., and Turner, C. W., *U. Mo. Agr. Exp. Sta. Res. Bull.*, No. 415, 1948.
6. Reece, R. P., Turner, C. W., *idem.*, No. 266, 1937.
7. Meites, J., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 728.
8. ———, Talk at 49th Ann. Meet. Am. Soc. Animal Production, Chicago, Nov. 29, 1957.
9. Benson, G. K., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 550.
10. Meites, J., *ibid.*, 1958, v97, 742.
11. Page, I. H., *Physiol. Rev.*, 1958, v38, 277.

Received February 9, 1959. P.S.E.B.M., 1959, v100.

## Effect of Adrenalectomy and Hydrocortisone on Lymph Glucose in Rats.* (24767)

GERALD F. HUNGERFORD
*Dept. of Anatomy, University of S. California, Los Angeles*

That glucose concentration of peripheral lymph is in equilibrium with blood plasma glucose has been established by experiments on dogs and cats(1,2). It is also generally stated that glucose absorbed through the intestinal mucosa is carried away by the blood vascular system almost entirely, the lymphatics playing a minor role(3). However, direct observations have been few and often contradictory. The purpose of this research was to reevaluate the role of the lymphatic vessels in transporting absorbed glucose in the rat; and to determine whether or not endocrines may be able to influence this mechanism.

*Materials and methods.* Male Albino rats, 200-350 g body weight were maintained on stock diet of laboratory pellets until use.

* Aided by USPHS Grant, work performed at George Washington Univ., Washington, D. C.