

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which induced mammary growth and lactation following estrogen administration in virgin female rabbits(7) and rats (unpublished), and retarded mammary involution and prolonged secretory activity in parturient rats after litter removal(8,9).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can also stimulate mammary growth and initiate secretion in the rat following estrogen administration (unpublished).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 μ g 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.

TABLE I. Glucose Concentration in Thoracic Lymph, Arterial Plasma and Venous Plasma of Rats.

Group	Body wt, g	Gavage	No. of animals	Lymph flow, cc/hr	Lymph glucose	Arterial plasma glucose mg %	Venous plasma glucose	Total lymph glucose, mg
Intact rats								
I †	349	None	9	.9 ± .09*	161 ± 8*	211 ± 14*	136 ± 7*	
II	240	"	12	.62 ± .01	113 ± 5	146 ± 8	100 ± 7	.7
III	221	H ₂ O, 5 cc	10	.78 ± .05	110 ± 6	98 ± 9	85 ± 4	.86
IV	246	250 mg glucose in 5 cc	6	.83 ± .13	173 ± 5	129 ± 5	102 ± 3	1.43
V	262	1,000 mg glucose in 5 cc	10	1.12 ± .14	242 ± 14	182 ± 12	161 ± 11	2.70
Adrenalectomized rats								
VI	291	None	7	.95 ± .23	60 ± 5	59 ± 7	55 ± 4	.57
VII	254	H ₂ O, 5 cc	6	1.10 ± .17	78 ± 6	67 ± 10	59 ± 7	.87
VIII	282	1,000 mg glucose in 5 cc	9	.97 ± .18	94 ± 6	66 ± 6	58 ± 4	.91
Adrenalectomized rats pretreated with hydrocortisone								
IX	264	None	8	.74 ± .01	111 ± 11	122 ± 15	111 ± 13	.82
X	294	H ₂ O, 5 cc	8	.89 ± .01	104 ± 15	103 ± 8	104 ± 12	.90
XI	266	1,000 mg glucose in 5 cc	12	2.10 ± .28	182 ± 20	172 ± 21	160 ± 20	3.82

* Mean values ± stand. error.

† Group I non-fasted, lymph collection for 90 min. period.

Lymph was collected for one hour periods from jugular lymph sac in the neck(4) while animals were maintained under nembutal anaesthesia. Lymph collections were started 15 minutes after gavage in those animals receiving fluids (water or glucose solutions) by stomach tube. At termination of lymph collection, the abdominal cavity was opened and blood samples taken from inferior vena cava and abdominal aorta. Lymph and blood samples were heparinized. Adrenalectomized animals were used 7-10 days after operation and were maintained on 1% NaCl for drinking purposes during that period. Normal rats were fasted overnight (18-20 hours) before use. Adrenalectomized rats fasted overnight had lymph flows so low that they could not be measured. Therefore, all adrenalectomized rats were fasted only 4-6 hours before use. Glucose determinations were made on lymph, and arterial and venous plasma using Nelson's modification of the Somogyi technic(5). Intestinal contents of a few animals were analyzed to estimate amount of glucose absorption. Hydrocortisone acetate in saline suspension (Merck)† was injected subcutaneously 15 mg/day for 3 days, the animals being used 4-5 hours after last injection.

Results of the experiments are presented in Table I. Lymph glucose concentration of fasted normal rats (Group II) is between that for arterial and venous plasma, suggesting that lymph glucose levels are in equilibrium with blood plasma glucose, probably at the capillary level. These same relationships prevailed in normal non-fasted rats (Group I). This observation essentially confirms findings of other workers(1,2) who used other animal species.

Administration of glucose solutions by stomach tube to normal fasted rats resulted in higher glucose concentrations in both blood plasmas and lymph. However, total amount of glucose in lymph was small. Less than 0.3% of absorbed glucose could be accounted for by direct passage to lymphatic vessels, regardless of manner of calculation. A portion of this small amount can probably be accounted for by passage to blood vessels first with subsequent filtration to the lymphatic stream in the general lymph-forming capillary beds.

The question arises whether or not glucose actively enters the blood capillaries of the gut at a more rapid rate than it would enter lymph capillaries. On the other hand, both blood and lymph capillaries may be equally

† Kindly supplied by Merck & Co.

capable of taking up glucose at the same relative rate, after it has passed through the intestinal mucosa, the difference being accounted for on the basis of rapidity of blood flow as compared to the sluggishness of lymph formation. From the data obtained in fasted normal rats where lymph glucose would seem to be in free equilibrium with blood plasma glucose in the general lymph-forming capillary beds, the latter possibility would seem to be the best one.

In the period after gavage (75 min) about 90% or 900 mg of glucose was absorbed (Group V). On the basis of data from Reininger and Saperstein(6) the blood flow through the gut in fed rats of 250 g B.W. is 9.4 cc/min which would equal about 388 cc plasma 75 minutes ($9.4 \times .55 \times 75$). Therefore each cc of plasma circulating through the intestine must have taken up about 2.3 mg of glucose on the average. One cc of lymph (Group V) calculates to contain 2.4 mg of glucose. These calculations are, of course, estimates. Some of the lymph glucose must have come from other lymph-forming capillary beds. However, it would seem that one need not assume a more specific role of the blood capillaries in the uptake of absorbed glucose from the gut than can be accounted for on the basis of the difference in rates of blood flow and lymph formation. It would, therefore, appear that the blood carries about 400 times more glucose away from the gut than does lymph.

It is well known that blood sugar is low in adrenalectomized animals(3). In all adrenalectomized groups (VI, VII, VIII), both plasma and lymph glucose values were low. Gavage of glucose solution failed to elevate these values, probably because of poor intestinal absorption. Pretreatment of adrenalectomized rats with hydrocortisone returned plasma and lymph glucose values toward normal levels (Groups IX, X and XI). It is of interest that lymph flow in Group XI (hydrocortisone treated, adrenalectomized rats, gavaged with glucose) was increased more than

100% over control groups. In group X, an equivalent gavage with water did not have the same effect and in Group VIII, glucose gavage in untreated adrenalectomized animals also failed to increase lymph flow. The increased lymph flow in Group XI represents a large percentage of amount of fluid administered and, therefore, probably cannot be accounted for by direct transfer from the gut to the lymphatic stream. An alternate explanation is that hydrocortisone "expanded" the extracellular space, causing a transient edema and, therefore, an increased lymph flow, this effect being apparent only in the case of glucose gavage.

Summary. 1. It is confirmed that in the fasted state lymph glucose is in equilibrium with blood plasma glucose, probably at the capillary level. 2. Less than 0.3% of absorbed glucose appears in the lymphatic stream. 3. The greater quantity of glucose which enters the blood capillaries in normal fasted rats is explained on the basis of difference in rates of blood flow and lymph formation rather than endothelial selectivity. 4. Plasma and lymph glucose values are low in adrenalectomized rats but are returned toward normal by pretreatment with hydrocortisone. 5. Lymph flow in hydrocortisone pretreated rats is increased by 100% after gavage of glucose solution but not after water gavage.

Acknowledgment is made to Chester Wong for technical assistance.

1. Yoffey, J. M., Courtice, F. C., *Lymphatics, Lymph and Lymphoid Tissue*, Harvard Univ. Press, Cambridge, Mass. 1956.

2. Heim, G. W., Thompson, R. S., Barter, F. C., *Am. J. Physiol.*, 1935, v113, 548.

3. Verzar, F., McDougall, E. J., *Absorption from the Intestine*, Longmans, Green and Co., London, 1936.

4. Reinhardt, W. O., *PROC. SOC. EXP. BIOL. AND MED.*, 1945, v58, 123.

5. Nelson, N., *J. Biol. Chem.*, 1944, v153, 376.

6. Reininger, E. G., Saperstein, L. A., *Science*, 1957, v126, 1176.

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