Source of Secretion of Milk "Let-Down"* Hormone in Domestic Mammals. (19568)

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Early anatomists, failing to observe secretory cells in the neurohypophysis, were not inclined to consider this organ an endocrine gland. When physiologists showed that extracts of the posterior lobe possessed a high degree of pharmacological activity on the uterus, blood vascular system, kidneys and mammary gland, workers were stimulated to determine the origin of the active principles. Herring(1) suggested that the cells of the pars intermedia invaded the posterior lobe and became transformed into hyaline bodies (Herring-bodies) which represented the secretion. That Cushing(2) still held this view up to 1933 is implied by the following quotation: "The active principles of the posterior lobe and its several fractions must under all circumstances primarily be derived, not from the pars nervosa, but from its epithelial investment, the pars intermedia." In 1935 Geiling and associates(3) commenced the publication of data which proved that in a number of species of animals, although the pars intermedia was lacking, the vasopressor (antidiuretic) and oxytocic hormones were present in posterior lobe extracts. Recently Bargmann and Scharrer(4) suggested that the hormones of the posterior lobe were secreted by neurosecretory cells of the hypothalamus and were transported down the axons to the pituitary stalk and pars nervosa.

Using the lactating sow as an assay animal, Whittlestone(5) has shown that highly active vasopressin as well as oxytocin will cause milk let-down. It seemed of interest therefore to determine whether the theory of hypothalamic secretion applied to the milk let-down factors of the posterior lobe.

Experimental. A number of calves, heifers and lactating cattle, a few ewes and one sow, were slaughtered during the course of other experiments at Ruakura. Within 30 minutes the entire neurohypophysis was dissected out and dropped into cold acetone. Later the median eminence, the stalk and the posterior lobe were separated, further defatted and dehydrated in changes of acetone, dried *in vacuo* and weighed. The dried material was ground up with sand, extracted with a known volume of 0.05% acetic acid, centrifuged and the supernatant liquid heated in a water bath. The resultant precipitate was centrifuged off and the solution stored in a refrigerator until assayed. The extract of the median eminence was sometimes difficult to clarify but the cloudy extract was found to produce no ill effects in the assay animals. The activity of the extracts was estimated by injection into the lactating sow and determining by hand milking the duration of action of the "let-down" hormone(5). Comparisons were made with a standard solution of oxytocic hormone prepared from lyophilized Pitocin kindly supplied by Parke, Davis & Co., Detroit. (This preparation is checked occasionally for loss of potency against an International Standard powder.) In this series of assays, the sows were first injected with 0.5 I.U. of solution and the time during which milk could be removed was noted. This was followed by an injection of the extract to be assayed. The response of the latter was then matched by a further injection of the standard. Milk let-down may be detected in the sow following

* Recently Cowie, et al., (1951) suggested that the term milk "ejection" be used in preference to milk "let-down" to indicate the effect of alveolar and ductal contraction of the mammary gland. Since the term milk "ejection" connotes the forceful passage of milk to the exterior, which does not necessarily occur, the term milk "let-down" as a prelude to the act of milk removal seems more appropriate. (Cowie, A. T., Folley, S. J., Cross, B. A., Harris, G. W., Jacobsohn, D., and Richardson, K. C., *Nature*, 1951, v168, 421.)

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TABLE I. The Distribution of Milk Let-Down Activity in the Mammalian Neurohypophysis.

				Median eminence			Stalk			Posterior lobe		
	No.	Age	Body wt, lb	Wt (mg)	Total activity (I.U.)	Specific activity (I.U./mg)	Wt (mg)	Total activity (I.U.)	Specific activity (I.U./mg)	Wt (mg)	Total activity (I.U.)	Specific activity (I.U./mg)
Calves		Wk										
	102	4	81	523	—	—	19	.7	.035	129*	14	—
	104†	4	82	614	—	—	10	.6	.06	116*	20	—
	12	4	93	378	.1		9	.5	.06	20	22	1.1
	105	8	113	121	—	—	9	.23	.025	Lost		
	106	8	123	132	.15		10	.1	.01	31	31	1
	11	8	132	477	Trace		8	.7	.09	25	28	1.1
	32	12	190	230	—	—	10	.1	.01	31	59	1.9
	30	12	148	60	—	—	2‡	1	.5	22	55	2.5
	10	12	187	246	—	—	9	Lost		26‖	16	.6
	107	16	207	217	—	—				32	74	2.3
	101	16	182	101	Trace		4	.6	.15	26	52	2
	1	18	156	152	Trace		3	.6	.2	26	23	.9
	2	18	205	292	—	—	10	.6	.06	49	49	1
	3	18	175	212	—	—	13	1.3	.1	25	28	1.1
	18	18	127	299	—	—	7	.7	.1	29	75	2.6
	100	20	251	484	—	—	12	—	—	60	108	1.8
	33	20	301	179	Trace		11	.55	.05	33	58	1.75
	26	20	291	168	—	—		Lost		32	51	1.6
	17§	64	560	354	—	—	16	.8	.05	71	178	2.5
	18	67	460	262	.5		13	.9	.07	44	79	1.8
Cows		Yr										
	608	2	662	264	—	—	21	.3	.01	57	37	.6
	73	3	580	330	—	—	12	.6	.05	48	23	.5
	89	3	597	322	—	—	24	.6	.025	59	22	.4
	598	3	763	267	.3	.001	21	.8	.04	82	31	.4
	563	5	708	341	—	—	25	.2	.01	23	13	.6
	T5	5	797	279	—	—	21	.08	.004	79	40	.5
	T6	5	756	311	Trace		20	.08	.004	83	58	.7
	45W	5	1009	328	—	—	18	1	.055	41	21	.5
	48W	5	1026	306	—	—	8	.6	.07	95	38	.4
Sheep		Yr								Whole pituitary		
	P147	5		125	.15		9	.18	.02	423	9	
	C372	"		233	—	—	5	.05	.009	139	5.5	
	P 13	"		146	—	—	14	1.4	.1	235	1.8	
	C384	"		261	—	—	5	.15	.03	189	1.7	
	P 45	"		389	—	—	Lost			297	4.8	
										Posterior lobe		
Pig				262	—	—	2	—	—	16	13	.8

* Whole pituitary gland. † Male calf. ‡ Incomplete stalk. § Pregnant heifer, No. 18 non-pregnant identical twin. ‖ Posterior lobe not completely separated.

injections of as little as 0.02-0.03 unit of oxytocin.

Results. In the dissection of the neurohypophysis there are no clearly defined boundaries of the median eminence of the hypothalamus. In the present study the area immediately superior to the attachment of the stalk surrounding the third ventricle was included as the "median eminence." Since its boundaries were poorly defined, a considerable amount of adjacent tissue was included.

It was noted in the dissections that the fibers of the superior end of the stalk are attached to the tissue surrounding the third ventricle. In most of the dissections the line of demarcation was arbitrarily set by cutting the superior end of the stalk close to the median eminence. Under these conditions the

fibers of the stalk attached to the surface of the third ventricle would be included as part of the median eminence. In a few cases the fibers of the stalk were dissected free of their attachment to the surface of the third ventricle.

It will be observed that the median eminences of most cattle contain little or no milk "let-down" hormone (Table I). In a few cases there were traces to measurable amounts. Such activity is probably due to stalk fibers retained by the median eminence after dissection.

These data indicate that the suggestion of Bargmann and Scharrer regarding the origin of the posterior pituitary hormones in the hypothalamus apparently does not hold for the milk "let-down" hormone. If the hormone is secreted in that division of the neurohypophysis it must be in an inactive form, becoming active in its passage down the stalk.

In this connection it is interesting to note that the stalk in every case contained measurable amounts of hormone. However, the amount of hormone in the stalk per mg of acetone-dried tissue (specific activity) was roughly of an order of one-tenth or less that of the posterior lobe. These observations suggest that the movement of hormone is from the posterior lobe to the stalk rather than from the median eminence to the stalk.

Summary and conclusions. 1. The neurohypophyses of a series of animals were separated into the median eminence, stalk and posterior lobe. The acetone-dried tissue in each division was extracted and assayed for milk "let-down" hormone content, using lactating sows as assay animals. 2. The median eminence usually contained an insufficient amount of hormone to cause milk "let-down" in the sow. In a few cases some activity was observed. 3. It was concluded that the milk "let-down" hormone is either not secreted in the hypothalamus or, if secreted there, exists in an inactive form which becomes active only as it passes down the stalk into the posterior lobe. Since the posterior lobe contained roughly 10 times the amount of hormone present in the stalk per unit of dry matter, it seems more probable that the posterior lobe is the source of the milk "let-down" hormone.

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1. Herring, P. T., *Quart. J. Exp. Physiol.*, 1908, v1, 121.

2. Cushing, H., *Am. J. Path.*, 1933, v9, 539.

3. Geiling, E. M. K., *Bull. Johns Hopkins Hosp.*, 1935, v57, 123.

4. Bargmann, W., and Scharrer, E., *American Scientist*, 1951, v39, 255.

5. Whittlestone, W. G., *J. Endocrinology*, 1952, in press.

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Effect of Epinephrine and Insulin on Tissue Electrolytes in Normal and Demedullated Rats. (19569)

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A reduction in the blood potassium level has been reported following the administration of insulin and epinephrine in normal animals and man(1-4) Considerable evidence in the literature(5,8), however, indicates that either epinephrine or insulin reflexly releases the other humoral agent apparently through the mediation of altered glycemic levels. In view of the interaction between these two endocrine substances it appeared desirable to investigate further the effect of insulin and epinephrine on the ionic content of plasma and selected tissues.

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