II. THE ACTION OF HYPOPHYSIN, ERGAMINE AND ADRENALINE UPON THE SECRETION OF THE MAMMARY GLAND.

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The action of extracts of various organs, especially that of the pituitary gland (hypophysin) upon milk secretion has been frequently investigated. The results of these investigations have been summarised by Hammond [1913], who made an extensive study of the action of hypophysin upon the milk flow in the goat. He found that there was an immediate action and that the effect soon passed off, and he concluded that the action was not upon the muscular tissue of the gland but rather upon the glandular epithelium. The action depended upon the state of nutrition of the animal and upon the stage of lactation. The composition of the milk was not appreciably altered, though that produced by the action of the drug had a higher percentage of fat than other samples. The milk analyses were limited to estimations of fat and total solids and did not extend over long periods.

Hammond and Hawk [1917] added some further details respecting the action in different states of nutrition and tested adrenaline, which was found to have no effect.

As two goats under normal conditions, on a constant diet, but in different stages of lactation were available, there arose the opportunity of once more testing the action of hypophysin upon the stage of lactation and of extending Hammond's results. Complete analyses of the milk of these goats had been previously made, and by continuing this procedure and including extra samples some further details could be ascertained. At the same time it was of interest to compare its action with that of adrenaline and of ergamine or histamine. The latter substances, as is well known, have an action corresponding with that of excitation of the sympathetic or para-sympathetic nervous systems. Their action, if any, would give probably some information upon the innervation of the mammary gland with nerve fibres of the involuntary nervous system. Hammond's results with hypophysin have been confirmed and extended; ergamine and adrenaline, as might have been expected, had no action.

Experimental.

The hypophysin, ergamine as acid phosphate, and adrenaline used for injection were the commercial preparations supplied by Burroughs, Wellcome and Co. The injections were always subcutaneous in the lumbar region of the body. They were made at different times of the day, usually from one to two hours before milking and generally in both goats about the same time. Before the injection of the drugs an injection of 0·9 % saline was made and once again later; it had no effect and thus the effect of the actual injection was excluded.

The experiments were carried out in two periods; during the first period there was an interval of only one or two days between the injections; during the second period there was an interval of about seven days between the injections of the three drugs in order to ascertain if the total volume of the milk differed in the period of injection from that before and after. The injections were then made more frequently, sometimes twice in the day.

The two goats used in these experiments were in different periods of lactation. Goat *A* had two kids on June 14th, goat *N* one kid on Feb. 2nd, 1920. Goat *A* was in a good state of lactation, goat *N* in a poor state.

Goat *A* during the first ten days was outdoors at grass by day and indoors by night; subsequently she was kept indoors permanently, fastened by a chain in a ground floor room of the building. Goat *N* was always kept in the building in the same room, but in a cage in which she could freely move and turn about. Both goats were given 112 g. of oatmeal at about 8 a.m., noon and 5 p.m. This was eaten rapidly. Except for the first ten days, when goat *A* had grass during the day, after the oatmeal both had 454 g. of hay, which they were allowed to eat at their leisure. They were thus under fairly normal conditions and upon a constant diet. Their weights varied very little during the course of the experiment which lasted about three months, and as far as could be noticed they were in excellent health.

The milk was not drawn by suction, but always by hand by the same experienced milker, who had drawn the milk previously over a long period. During this time the goats were milked twice daily, morning and evening; the two samples were mixed and analysed. To observe the effect of the drugs it became necessary to draw the milk more frequently and it was taken at 9.30 a.m., 11.30 a.m. and 5 p.m. and occasionally at 6.30 or 7 p.m., depending upon the time of injection.

Complete daily analyses of the milk of these goats had been previously made. Owing to the number of samples now taken (six to eight) it was not possible to analyse each separately and two or more samples were combined. The combination of two samples was made in such a way that the particular sample after injection was kept separate. The analyses are shown in the table, numbered 1, 2 or 3 according to the milking time of 9.30 a.m., 11.30 a.m. and 5 p.m.

The methods of analysis were those usually employed in milk analysis. Fat was estimated by Soxhlet's method, total protein by Kjeldahl's method: caseinogen by this method after its precipitation from the sample by dilute acetic acid and washing; albumin in the filtrate from the caseinogen by precipitation with tannic acid, washing and Kjeldahl's method. The nitrogen figures by Kjeldahl's method were multiplied by the factor of 6·38 to give the protein. Lactose was estimated in a sample, precipitated by "dialysed iron," by Fehling's method. Ash was determined by incineration in a small crucible. The data are expressed in grams per 100 cc. milk.

The figures in the following table show the volumes at each milking, and the fat analyses, and the calculations to give the figures for the output of the whole day, the times of injection, etc. The data are so numerous that they are greatly abbreviated, and only those showing the essential features are given. The amounts of lactose, total protein, caseinogen and albumin except on the first two days are omitted and none of the figures of the second period is shown.

Results.

(*a*) *Changes of Volume.*

1. *Hypophysin.* The injection of hypophysin generally about two hours before the milking time produced a flow of milk only in goat *A*, which was in an early stage of lactation. The effect is distinctly marked in the volume of the second sample collected after an interval of two hours from the first sample. The volumes of 250 and 260 cc. were obtained in comparison with normal volumes varying from 35 to 145 cc. The volume of the next milking was then smaller than the normal, 230 and 150 cc. against 255 and 325 cc. An increase was not definitely noticed in the volume of the third sample collected after an interval of about six hours from the previous one. A volume of 350 cc. was obtained between volumes of 325 and 410 cc., but the average volume for this milking time was between 200 and 300 cc. No increase in volume was observed in the first sample collected at 9.30 a.m., *i.e.* about 16 hours after the sample at 5 p.m. The total daily volume of milk was not appreciably altered. A change over the total period could not be observed as the goat *A* gave a gradually diminishing volume of milk.

No change in volume of the samples was observed in goat *N* after an injection of hypophysin. This goat gave a more regular daily volume and the effect was to make a slight general increase of the total volume; previous to the injection the average volume was 423 cc.; during the period it was 448 cc. and after 468 cc. A similar slight increase was again noted later, the volumes being 395, 424 and 509 cc. The increase may however be due to other circumstances, as the goat *N* had at the time of this injection been in lactation for eight to nine months. The injection in goat *A* at the later period did not produce a rapid flow as at the former time, but there was a very slight increase of total volume; the averages of the periods were 710, 724, 730.

PROTOCOLS.

Date Sept.		Goat *A* Vol.	Ash	Fat	Lact.	T. prot.	Cas.	Alb.	Goat *N* Vol.	Ash	Fat	Lact.	T. prot.	Cas.	Alb.
7		1100	0·83	6·22	4·06	3·64	3·00	0·47	450	0·91	5·75	4·55	3·57	2·57	0·75
8		1200	0·80	7·80	4·23	3·59	2·95	0·48	390	0·90	5·07	4·64	3·73	2·75	0·75
*9		1200	0·76	7·02	4·26	3·61	2·95	0·51	430	0·97	5·50	4·60	3·52	2·53	0·77
†10	1	900	0·85	4·20	4·36	3·77	2·90	0·51	315	0·91	5·31	4·60	3·63	2·53	0·75
	2	250	0·80	11·21	4·00	3·61	2·75	0·46	10	—	12·07	4·37	3·68	2·50	0·80
	3	230	0·82	9·12	4·11	3·85	2·90	0·47	140	0·90	6·25	4·64	3·69	2·59	0·70
per total		1380	0·83	6·29	4·26	3·75	2·87	0·49	465	0·91	8·47	4·61	3·65	2·55	0·74
‡11	1	900	0·87	5·75	4·31	4·00	3·07	0·53	308	0·91	5·22	4·70	3·77	2·64	0·75
	3	215							90						
	2	55	0·80	11·35	4·08	3·78	3·02	0·47	12	—	8·67	4·38	3·73	2·26	0·52
		1170	0·87	6·01	4·30	3·99	3·07	0·53	410	0·91	5·28	4·66	3·77	2·63	0·74

* Both goats injected with 1 cc. 0·9 % NaCl after first milking.

† Both goats injected with 0·5 cc. hypophysin after first milking; milk collected one hour after injection (10.25 a.m.).

‡ No injection.

Date Sept.		Goat *A* Vol.	Ash	Fat	Goat *N* Vol.	Ash	Fat	Remarks
12	1	920	—	—	310	—	—	No injection.
	2	100	—	—	35	—	—	
	3	255	—	—	85	—	—	
		1275	0·84	7·80	430	0·90	4·99	
13	1	840	0·83	5·00	300	0·91	4·89	Both injected with 1 cc. hypophysin after first milking; milk collected one hour after injection.
	2	260	0·74	14·09	37	0·82	9·29	
	3	150	0·90	7·37	83	0·94	4·77	
		1250	0·82	7·09	420	0·91	5·25	
14	1	940	0·85	4·13	335	0·96	4·31	No injection.
	3	325			118			
	2	95	0·74	10·35	42	0·90	10·74	
		1360	0·84	4·57	495	0·95	4·86	
15	1	680	0·86	4·65	325	0·92	4·50	Goat *A* injected with 1 cc. 0·9 % NaCl at 3 p.m. Goat *N* injected with 1 cc. hypophysin at 3 p.m.
	2	70	0·86	10·62	10	—	9·65	
	3	350	0·82	9·00	130	0·90	6·29	
		1100	0·85	6·41	465	0·91	5·11	
16	1	520	0·84	4·00	280	0·96	4·10	No injection.
	2	95	0·80	6·30	40	0·90	7·52	
	3	410	0·84	6·39	115	0·96	4·69	
		1025	0·84	5·37	435	0·95	4·57	
17	1	800	—	—	305	0·94	3·89	Both goats injected with 1 cc. hypophysin at 8.15 a.m.; milk collected one hour later.
	2	145	—	—	45	—	5·98	
	3	265	—	—	95	0·92	4·00	
		1210	0·83	5·54	445	0·93	4·12	
18	1	750	—	—	325	0·95	4·12	No injection.
	2	35	—	—	40	0·88	7·40	
	3	210	—	—	110	0·88	4·69	
		995	0·81	5·51	475	0·93	4·53	
19	1	680	0·80	5·15	315	—	—	No injection.
	2	50	0·80	9·30	35	—	—	
	3	230	0·80	8·40	110	—	—	
		960	0·80	6·15	460	0·95	4·15	
	4	30	0·83	9·40	35	0·94	5·53	
20	1	660	0·85	5·50	300	—	—	Goat *A* injected with 1 cc. hypophysin at 8.15 a.m.; milk collected one hour later.
	2	65	0·82	8·10	30	—	—	
	3	175	0·88	6·52	105	—	—	
		930	0·85	6·00	470	0·94	4·39	
21	1	620	0·87	5·82	305	0·93	3·94	Goat *N* injected with 1 mgm. ergamine phosphate at 9.45 a.m.; milk collected one hour later.
	3	145			45	0·75	8·21	
	2	70	0·82	10·25	100	0·94	4·97	
		835	0·87	6·19	450	0·93	4·60	

Date Sept.		Goat *A* Vol.	Goat *A* Ash	Goat *A* Fat	Goat *N* Vol.	Goat *N* Ash	Goat *N* Fat	Remarks
22	1	570	0·85	5·60	250	0·94	4·36	Goat *A* injected with 1 mgm. ergamine phosphate at 9.45 a.m.; milk collected one hour later.
	3	120	0·88	6·30	100			
	2	110	0·74	9·38	35	0·90	7·45	
		800	0·84	6·23	385	0·94	4·64	
23	1	730	0·90	5·72	320	0·94	4·83	Both goats injected with 1 mgm. ergamine phosphate at 3.45 p.m.; milk collected at 5 p.m.
	2	140	0·82	10·02	18	—	7·82	
	3	160	0·91	5·80	142	0·90	5·26	
		1030	0·89	6·41	480	0·93	5·07	
24	1	660	0·85	5·15	315	0·92	4·73	No injection.
	2	80	0·83	9·22	30	0·86	9·85	
	3	200	0·86	6·50	105	0·93	4·95	
		940	0·86	5·78	450	0·92	5·12	
25	1	640	0·90	6·05	300	0·92	4·56	Goat *A* injected with 0·5 mgm. ergamine phosphate at 8.15 a.m. Goat *N* injected with 1·0 mgm. ergamine phosphate at 8.15 a.m. Milk of both goats collected one hour later.
	2	50	0·80	9·54	20	—	8·54	
	3	140	0·87	7·40	100	0·91	5·18	
		830	0·89	6·49	420	0·92	4·90	
26	1	570	—	—	295	—	—	
	2	70	—	—	15	—	—	
	3	205	—	—	120	—	—	
		845	0·89	6·73	430	0·98	4·96	
	4	33	lost	7·43	33	0·93	6·57	
27	1	622	0·88	5·03	282	0·94	4·78	Both goats injected with 1 mgm. ergamine phosphate at 3.30 p.m. Milk collected at 5 p.m.
	2	20	—	—	10	—	—	
	3	225	0·87	7·04	130	0·96	6·46	
		900	0·88	5·67	455	0·94	5·40	
28	1	600	0·87	5·68	300	0·92	4·67	Both goats injected with 1 mgm. ergamine phosphate at 6.15 p.m. Milk collected at 9.15 a.m. next day.
	2	50	—	—	15	—	—	
	3	130	0·86	7·32	125	0·93	7·94	
		780	0·87	6·66	440	0·92	5·71	
29	1	630	0·88	7·64	295	0·95	5·15	No injection.
	2	30	0·86	8·76	10	0·93	5·72	
	3	220			130			
		880	0·87	7·96	435	0·94	5·33	
30	1	540	0·87	6·80	295	0·93	5·62	Both goats injected with 1 mgm. adrenaline at 1.15 p.m.; milk collected at 5 p.m.
	2	45	—	—	20	—	—	
	3	110	0·87	7·63	100	0·93	8·01	
		695	0·87	7·21	415	0·93	6·31	
Oct.								
1	1	620	0·90	6·92	285	0·97	5·28	Both goats injected with 1 cc. 0·9 % NaCl at 1.30 p.m.; milk collected at 5 p.m.
	2	40	—	—	35	—	—	
	3	210	0·86	8·02	110	0·94	6·51	
		870	0·89	7·24	430	0·96	5·69	
2	1	590	0·81	5·63	300	0·93	5·00	Both goats injected with 1 mgm. adrenaline at 9.45 a.m.; milk collected at 11 a.m.
	2	30	—	—	15	—	—	
	3	200	0·86	8·28	100	0·91	7·01	
		820	0·84	6·37	415	0·92	5·56	
	4	24	—	9·24	20	—	8·34	
3	1	536	0·87	6·60	265	0·94	5·46	No injection.
	2	40	—	—	35	—	—	
	3	140	0·87	8·38	100	0·94	6·66	
		740	0·87	7·12	420	0·94	5·98	
4	1	650	0·86	5·61	315	0·91	4·87	Both goats injected with 1 mgm. adrenaline at 1.15 p.m.; milk collected at 5 p.m.
	2	30	—	—	10	—	—	
	3	170	0·88	8·90	110	0·92	6·21	
		850	0·86	6·27	435	0·91	5·24	
5	1	480	0·91	6·00	260	0·95	4·75	No injection.
	2	75	—	—	10	—	—	
	3	190	0·89	7·49	115	0·94	5·70	
		745	0·90	6·53	385	0·95	5·06	

2. *Ergamine.* The injection of ergamine had no pronounced effect on the separate volumes of the samples of either goat and the average volume of the total milk per day was about the same as when no injection was made. A general effect over the whole period was not noticeable in the case of goat *A* which was giving a diminishing flow of milk, but a slight diminution can be made out in the case of goat *N*. During the second period of injection the flow of milk in goat *A* was more constant, the average daily volumes being 645, 656, 657 cc. for the pre-, actual, and post times; the average figures of goat *N* at this time were 532, 493, 576. In both cases the changes are not essentially different from the ordinary daily volumes.

3. *Adrenaline.* During the first period no effect could be seen in the case of either goat, both the separate volumes and the average daily volumes remaining unchanged. During the second period there was an apparent increase in goat *A*, but the increase occurred at a time when the daily flow was lessening; the reverse effect can be made out in goat *N*. The general effect is not sufficient to indicate any definite action of adrenaline.

(*b*) *Composition of the Milk.*

No real change in the chemical composition of the total milk per day occurred in either goat after the injections of hypophysin, ergamine and adrenaline. If any change did occur, it was not greater than the normal variations in composition. These normal daily variations are most marked in the fat content. Possibly the fat content was lowered over the total period during which hypophysin was injected and raised during the ergamine period, but again the alterations were not more than those which occur daily.

The three separate samples of milk taken at different times of the day after different intervals had normally a distinctly different composition. The fat content was always highest in the second sample taken two hours after the first, and the early morning sample had the lowest amount of fat; it was taken after an interval of 16 hours. The higher fat content was observed in both goats and is thus independent of the period of lactation. The second sample had less protein and lactose than the other samples. On the average the first sample at 9.30 a.m. had more protein and lactose than the third sample, but the differences are not distinctly marked off.

If allowance be made for the ordinary daily variations, the separate samples do not show any appreciable difference from the normal.

Discussion.

Since a marked increase in the volume of milk was only observed in goat *A* at the time of the second milking, and since it was followed by a diminished volume at the third milking, it appears that the action of hypophysin is powerful, rapid and of short duration. The phase of hyper-secretion after injection is followed by a compensatory phase of hypo-secretion. This effect

explains the absence of an increase in volume at the time of the first milking and third milking. The interval between the milkings was here 16 hours; an increase followed by a decrease would not be noticed. The interval between the first and second milkings was two hours. The gland secretes normally (Sept. 12) about 50 cc. every hour; after hypophysin the secretion was 125 cc. (Sept. 10) and 130 cc. (Sept. 13) per hour. Hypophysin had no effect in goat *N* and at the later period in goat *A*. The action of hypophysin is therefore probably not directly upon the gland. If the smooth muscle were stimulated, a flow of milk should follow at either period of lactation. If the secreting cells were affected a flow of milk should also follow in any case. It is most probable that it may act indirectly through the reproductive organs, which have been proved to contain substances acting upon the secretion of the mammary gland. At the earlier period of lactation the reproductive organs will be in a state of activity, whereas in the later stage their state of activity will have disappeared. Hypophysin thus may act upon an active organ which produces the galactogogue; further work will be required to determine whether this active organ be the ovary, corpus luteum, uterus, or placenta.

In practice, the injection of hypophysin will only be of value at early stages of lactation and it may be able to bring a gland into activity when it is not already in that state. It must be remembered that the total volume produced by the gland is not appreciably increased, as shown above and previously described by other investigators.

The composition of the total milk per day is not altered by the injection of hypophysin. It has been previously considered that hypophysin causes a flow of milk with a higher fat content, but, as the data show, it is followed by a milk with a lower fat content. The high fat content of a sample taken at a short interval between milkings is normal and is not really due to the action of hypophysin.

As milk contains more fat if collected at a short interval after a previous longer interval, it appears that the secreting mechanism of the mammary gland is of a two-fold character; a mechanism producing fat and a mechanism producing protein and lactose. The fat-producing mechanism begins to act first and a milk of high fat content results; the protein and lactose mechanism acts later and dilutes the fat content to the normal value. The fat mechanism is probably more easily influenced by other conditions, since the amount of fat in milk is very variable, whilst those of protein and lactose are fairly constant.

Former investigators have observed that adrenaline has no action upon the mammary gland; these results confirm their work.

Ergamine also has no action. If these substances have a positive or negative action, it is rapidly followed by compensation.

The inaction of adrenaline points to the absence of sympathetic nerve fibres from the mammary gland, if the general law of the relationship between the action of adrenaline and the presence of sympathetic fibres is here correct.

It has been suggested from analogy to other glands (stomach) that ergamine perhaps acts on para-sympathetic fibres. The inaction points to the absence of such fibres from the mammary gland.

SUMMARY.

Hypophysin produces a flow of milk only in the early stages of lactation. Its action is rapid and powerful, but of short duration. The flow (hyper-secretion) is followed by a smaller quantity than normal (hypo-secretion). The total volume of milk per day is not altered.

Since hypophysin does not act at later stages of lactation, it is probable that its action is indirect through the organs of reproduction.

The quality of milk secreted after the action of hypophysin is not different from the normal. Normal milk has a high fat content, if it be collected at a short interval after the last milking.

The secretory activity of the mammary gland is not influenced by the subcutaneous injection of adrenaline or ergamine. The gland probably does not contain sympathetic or para-sympathetic nerve fibres for the secretory mechanism. If they be present, the inaction of adrenaline and ergamine is exceptional.

REFERENCES.

Hammond, J. (1913). *Quart. J. Exp. Physiol.* **6**, 311.
Hammond, J. and Hawk, J. C. (1917). *J. Agric. Sci.* **8**, 147.