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FATE OF INGESTED HISTAMINE IN SHEEP

II. FAECAL AND URINARY EXCRETION*)

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High concentrations of histamine were found in rumen of sheep subsequent to ingestion of silage (*Sjaastad* 1967 a). In a previous paper (*Sjaastad* 1967 b) disappearance of orally administered histamine from the rumen and inactivation of histamine by rumen contents were examined. In order to obtain further information on the absorption of histamine from the digestive tract of sheep, examinations of histamine in urine and faeces subsequent to oral administration of histamine diphosphate or feeding of silage were undertaken.

METHODS

Experimental animals, feeding regime and the method for histamine loading were the same as used in the previous study (*Sjaastad* 1967 b). Urinary histamine was estimated as described in details elsewhere (*Sjaastad* 1967 c). The recoveries of histamine diphosphate and N-acetylhistamine added to urine were 88.8 ± 5.8 (s) and 77.3 ± 9.6 %, respectively.

Histamine determination in faeces

Free histamine. Faeces was well mixed and an aliquot of 80 g was homogenized with 400 ml of distilled water for 10 min. N-HCl in amounts sufficient to keep pH at approximately 2 was added in portions during homogenization. The volume of the homogenate was measured in a volumetric cylinder, and after gently mixing, aliquots corresponding to 10 g of faeces were pipetted off and centrifuged at

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3000 × g for 10 min. at room temperature. After adjusting the pH to 6.5 (Merck's indicator paper), the supernatant fluids were passed through Amberlite IRC-50 columns (60 × 10 mm). From this point on the procedure was identical to that described for determination of histamine in rumen liquor (*Sjaastad* 1967 a). The recovery of histamine diphosphate added to faeces before homogenization (10—100 μg/10 g faeces, 20 expts.) was 33.9 ± 3.9 (s) %.

Conjugated histamine. Faeces was homogenized and centrifuged (as for free histamine) and conjugated histamine determined in the supernatant fluid (each aliquot corresponding to 10 g of faeces) by the method described for conjugated histamine in rumen liquor (*Sjaastad* 1967 b).

Biological histamine assays were carried out as previously described (*Sjaastad* 1967 a). The values for both free and conjugated histamine are in this study expressed as histamine diphosphate and represent the mean of duplicate analyses. Corrections for losses during the extraction procedures were not carried out.

Radioactive measurements

1) Urine. For the determination of C^{14}-activity, 100 μl of urine (diluted 10 times) and a small drop of detergent (to reduce surface tension) were plated and left at 60—70°C until completely dry.

2) Faeces. Aliquots of 100 g were homogenized with 600 ml of distilled H_2O and centrifuged at 3000 × g for 10 min. Of the supernatant fluid 100 μl was plated.

The radioactive samples were counted on a Beckman flow-counter (Lowbeta II) for at least 200 counts and never for less than 3 min. The background was about 1.5 c.p.m. and the geometrical efficiency of the counter approximately 27 %. Plates used for faeces were occasionally weighed before and after plating. The density of mass was of an order which was found not to cause any substantial selfabsorption. The figures given for radioactivity are corrected for background.

MATERIALS

Histamine diphosphate (termed HiDP) from Nutritional Biochemicals Corp., Cleveland, Ohio was used.

C^{14}-histamine dihydrochloride (labelled in the ring-2 position) was purchased from the Radiochemical Centre, Amersham. The specific activities in the two batches used were 116 and 166 μCurie/mg.

Ion-exchange resin. Ion-exchange was performed with Amberlite IRC-50, Standard Grade. Batches of the resin were prepared according to *Bergstrøm & Hansson* (1951).

RESULTS

When histamine was given by mouth the urinary excretion of both free and conjugated histamine increased the following 24 hrs. (Table 1). The increments accounted for only a small

Table 1. Urinary excretion of free and conjugated histamine in three sheep after oral administration.

Sheep no.	Dose of histamine (g)	Extra histamine in urine the first 24 hrs. after giving histamine (as μg HiDP)		Extra histamine as per cent of administered dose		Ratio Extra conjugated histamine / Extra free histamine
		Free	Conjugated	Free	Conjugated	
4	0.2	15.7	1274	0.008	0.64	81.1
„	1.0	226	10830	0.023	1.08	47.9
„	1.0	46.0	5720	0.005	0.57	124.3
5	0.2	12.7	206	0.006	0.10	16.2
„	0.4	5.3	337	0.001	0.08	63.6
„	1.0	238	8700	0.024	0.87	36.6
„	1.5	250	9400	0.017	0.63	37.6
7	0.2	28.1	345	0.014	0.17	12.3
„	1.0	177	2660	0.018	0.27	15.0
„	1.0	420	4700	0.042	0.47	11.2
„	1.5	420	14100	0.028	0.94	33.6
„	10.0	647	38000	0.006	0.38	58.7
			Mean	0.016	0.52	

fraction of the dose given. The mean increase in conjugated histamine was approximately 30 times larger than that in free histamine (Table 1). In the second 24-hr. period after administration of histamine, the urinary excretion of neither free nor conjugated histamine deviated much from the control level.

Silage histamine usually produced increments in urinary free and conjugated histamine of the same magnitudes as when equivalent amounts of histamine diphosphate were given. In a

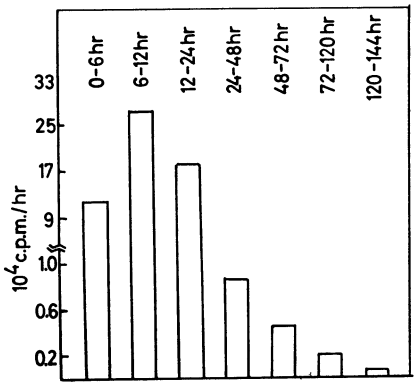

Figure 1. Radioactivity in urine after giving 50 μCurie C^{14}-histamine and 1 g histamine diphosphate by mouth.

Table 2. Urinary excretion of free and conjugated histamine and radioactivity after giving C^{14}-histamine and carrier by mouth. Under the conditions used for counting, 100 μCurie corresponded to 5.9×10^7 c.p.m.

Sheep no.	Dose of histamine (g)	Dose of C^{14}-histamine (μC)	Extra histamine in urine (as μg HiDP) Free 0-24 hrs.	Free 24-48 hrs.	Conjugated 0-24 hrs.	Conjugated 24-48 hrs.	Radioactivity in urine (10^6 c. p. m.) 0-24 hrs.	24-48 hrs.	Per cent of administered C^{14}-histamine recovered as radioactivity in urine the first 48 hrs.
7	0.2	60	28	3.1	345	—*)	1.33	0.04	3.9
„	1.0	80	177	1.6	2660	3.7	3.29	0.13	7.2
„	1.0	50	420	45	4700	54	4.49	0.31	16.6
4	1.0	60	45	5.6	5720	66	5.60	0.38	17.6
1	0.2	50	—	—	—	—	2.90	0.40	11.1
„	0.2	50	—	—	—	—	1.94	0.32	7.6
3	0.2	50	—	—	—	—	3.60	0.43	13.7
„	0.2	50	—	—	—	—	3.51	0.17	12.5

*) — = not examined.

few experiments, however, little or no free histamine could be demonstrated. In these instances the urine extracts strongly counteracted the response of the guinea-pig ileum to internal histamine standards. The inhibiting substance was easily washed out of the organ bath.

When C^{14}-histamine was given by mouth together with carrier-histamine, between 4 and 18 % of the administered radioactivity was excreted with the urine during the first 48 hrs. after administration (Table 1). Most of the radioactivity recovered in the

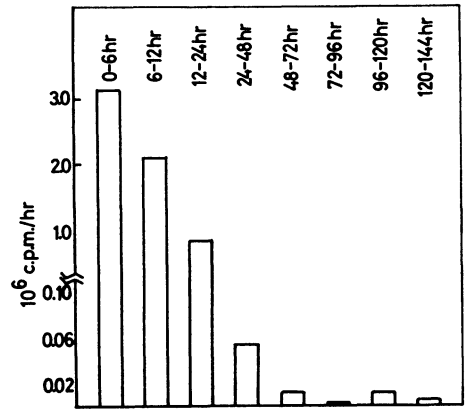

Figure 2. Radioactivity in urine following subcutaneous injection of 100 μCurie C^{14}-histamine.

Table 3. Excretion of histamine in faeces after oral administration.

Sheep no.	Dose of HiDP (g)	Dose of C^{14}-histamine (μ Curie)	Histamine in faeces (as μg HiDP) Free 0-24 hrs.	Free 24-120 hrs.	Conjugated 0-24 hrs.	Conjugated 24-120 hrs.	Radioactivity in faeces 0-120 hrs. after giving histamine (10^6 c. p. m.)	Per cent of administered radioactive dose recovered in faeces 0-120 hrs.
4	0.2	0	n.d.*)	n.d.	145	233	—**)	—
5	0.2	0	n.d.	n.d.	n.d.	n.d.	—	—
„	0.4	0	11.1	n.d.	48	112	—	—
7	0.2	60	n.d.	n.d.	n.d.	n.d.	1.87	5.3
„	1.0	80	n.d.	n.d.	253	n.d.	1.41	3.0
„	1.0	50	n.d.	n.d.	116	153	0.73	2.4
4	1.0	60	669	n.d.	603	n.d.	4.08	11.2

*) n.d. = not detectable.
**) — = not examined.

urine was excreted during the first 24-hr. period (Table 2, Fig. 1). The radioactivity in the urine reached a maximum after a shorter interval of time when C^{14}-histamine was given by mouth than when injected subcutaneously (Figs. 1 and 2).

Faecal excretion of histamine

When sheep were fed hay or concentrates, neither free nor conjugated histamine could be demonstrated in faeces. After giving histamine by mouth, small amounts of conjugated histamine could usually be demonstrated whereas free histamine was demonstrated in only two out of seven experiments (Table 3).

The fractions of the administered radioactivity recovered in faeces were small (Table 3). It should be noted that the highest radioactivity observed in faeces was associated with the highest faecal excretion of free histamine (Table 3).

DISCUSSION

When histamine is injected subcutaneously to sheep, 2—4 % of the administered dose is excreted unchanged in the urine (*Sjaastad*, unpublished). The present study showed that following oral administration of histamine, on the other hand, the increment in urinary free histamine corresponded to less than 0.05 % of the dose given. A substantial part of orally administered C^{14}-histamine was, however, recovered as radioactivity in the

urine (Table 2). These results indicate that histamine is absorbed from the digestive tract, but is efficiently inactivated before reaching the systemic circulation. It is possible, however, that most of the radioactivity appearing in the urine originate from histamine metabolites being formed within the lumen of the digestive tract.

Whereas absorption of unchanged histamine from the rumen was not clearly demonstrated in a previous study (*Sjaastad* 1967 b), some absorption of unchanged histamine probably takes place from the small intestines. This assumption is based on the facts that high biological activity is found in abomasum after giving histamine by mouth (*Sjaastad* 1967 b) and further that histamine is rapidly absorbed from intestinal loops of sheep (*Sjaastad & Kay,* unpublished). In the present study maximum radioactivity in the urine following oral administration of C^{14}-histamine occurred between 6—12 hrs., whereas the corresponding peak when C^{14}-histamine was injected subcutaneously was found between 0—6 hrs. (Figs. 1 and 2). Since orally administered C^{14}-histamine disappears rapidly from the rumen (*Sjaastad* 1967 b), the above mentioned difference gives additional support to the belief that most of the radioactivity appearing in the urine following oral administration of C^{14}-histamine originates from radioactive substances absorbed from the intestines.

When C^{14}-histamine was given by mouth less than 30 % of the radioactivity was recovered in faeces and urine (Tables 2 and 3). The present experiments offer no basis for explaining the fate of the remaining activity. Possibly, some histamine might be bound to faecal particles and thus escape detection. It is, however, not likely that such a binding, if occurring, should be large enough to account for the remaining radioactivity. It is also possible that histamine in appreciable amounts might be retained in the body for a long time although this has not been found in experiments carried out with other species (*Schayer* 1952, *Halpern et al.* 1959). Still another possibility is that much of the radioactivity is excreted by other routes than those examined in the present study.

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SUMMARY

When histamine was given by mouth to sheep, the urinary increase in free and conjugated histamine on an average corresponded to 0.016 and 0.52 % of the administered dose, respectively. Between 4 and 18 % of C^{14}-histamine given by mouth was recovered as radioactivity in the urine.

Free and conjugated histamine was only occasionally detected in faeces after oral loading with histamine diphosphate, and the quantities were small. Following oral administration of C^{14}-histamine 2.4—11.2 % of the administered radioactivity was excreted with faeces. This study gave no clue to explain the fate of the radioactivity not excreted by faeces or urine.

ZUSAMMENFASSUNG

Die Umgestaltung von Histamin an Schafe verabreicht. II. Die Ausscheidung in Kot und Harn.

Wo Histamin per os an Schafe verabreicht wurde, wurden 0,016 bzw. 0,52 % der administrierten Dosis als eine erhöhte Ausscheidung von freiem und konjugiertem Histamin im Harn wiedergefunden. Wurde dagegen C^{14}-Histamin auf entsprechende Weise verabreicht, wurden zwischen 4 und 18 % der Radioaktivität im Harn wiedergefunden.

Im Kot liessen sowohl freies als auch konjugiertes Histamin sich nur gelegenlich nachweisen, und dann auch nur in sehr kleinen Mengen. Wo C^{14}-Histamin verabreicht wurde, wurden zwischen 2,4 und 11,2 % der Radioaktivität mit dem Kot ausgeschieden.

Die Experimente gaben keine Grundlage für eine Erklärung über die Radioaktivität, die nicht im Kot oder Harn wiedergefunden wurde.

SAMMENDRAG

Skjebnen til histamin gitt per os til sau. II. Utskillelse i fæces og urin.

Når histamin ble gitt per os til sau ble henholdsvis 0,016 og 0,52 % av den administrerte dose gjennomsnittlig gjenfunnet som øket utskil-

lelse av fri og konjugert histamin i urinen. Når C^{14}-histamin ble gitt på samme måte ble derimot mellom 4 og 18 % av radioaktiviteten gjenfunnet i urinen.

I fæces kunne både fri og konjugert histamin bare påvises leilighetsvis etter histaminbelastning per os, og da i meget små mengder. Når C^{14}-histamin ble gitt, ble mellom 2,4 og 11,2 % av radioaktiviteten utskilt med fæces.

Eksperimentene ga ingen basis for å forklare den radioaktivitet som ikke ble gjenfunnet i urin eller fæces.

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