Absorption and Gastric Secretion of Iodine-131 and Chlorine-36 in Dairy Calves.* (30761)

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Studies on iodide secretion by the gastric mucosa have been reviewed by Brown-Grant (1). Gastric clearance of iodide has been shown to exceed that of chloride in rats(2) and dogs(3). The present investigation was made to compare the metabolism of iodine and chlorine in milk fed dairy calves. Absorption and excretion of these halides were studied in relation to their binding in blood plasma and gastrointestinal contents.

Procedure. Six calves between 4 and 6 weeks of age were each fed 3 lb whole milk twice daily at 8 AM and 4 PM. They were dosed orally with I^{131} and Cl^{36} for 7 days immediately before feeding. Constant volumes of NaI^{131} and HCl^{36}, initially containing 100 and 40 μc, respectively, per calf, were pipetted from stock bottles into gelatin capsules at each feeding. Portions of each dosing solution were retained for counting standards. During the last 4 days of dosing, 2 calves were each fed 40 mg/kg b.w. daily of acetazolamide (Diamox‡), a gastric secretion inhibitor(4). Two additional calves each received 0.3 mg histamine dihydrochloride§ s.c. at 10-minute intervals for 2 hours before slaughter. The 2 remaining calves were kept as controls. All calves were fed and dosed as usual on the morning of the 8th day. They were then slaughtered during a 1-hour period beginning 4 hours later.

The gastrointestinal tracts of all animals were tied off and removed at slaughter. All tracts were divided into forestomach (rumen, reticulum, and omasum), abomasum, 3 small intestine sections of equal length, cecum, and

2 sections of colon. The contents of each section were weighed and mixed before sampling. Thyroid glands were digested in hot 10% KOH and diluted to volume with water. Ingesta was fractionated into water extractable material, protein-free extracts, and residue to obtain an indication of I^{131} and Cl^{36} binding. Water extracts of gastrointestinal contents were made by mixing weighed amounts of material with measured volumes of water and centrifuging(5). Clear fluids were obtained from the water extracts by precipitation of colloidal solid matter and dissolved protein(5). Radioactivity in the protein-free extracts was assumed to represent the exchangeable (or non-bound) fraction of ingesta.

Three milliliter samples of liquid material, I^{131} and Cl^{36} counting standards, and 2- to 4-g samples of feces and ingesta were placed in plastic tubes for counting in a well-type gamma spectrometer. Liquid samples and water slurries of solid material were dried in 2-inch Petri dishes. Counting standards were mixed with non-labelled materials obtained from other animals. After allowing 10 weeks for decay of I^{131}, these samples were counted in a Geiger-Mueller counter. The first and second countings measured primarily I^{131} and Cl^{36}, respectively. Corrections were made for background and the percentages of I^{131} counts due to Cl^{36} and vice versa(6). Final results were expressed as percentages of the administered doses of the 2 radioisotopes.

Results. Since histamine was injected only during a 2-hour period before sampling, its effects would be manifested only in samples taken during or after the period of administration. Diamox had no significant effect on blood plasma concentrations of I^{131} or Cl^{36}. Fecal and urinary excretions are highly variable in milk-fed calves, making comparisons between animals of doubtful value. However, I^{131} and Cl^{36} could be compared in the same samples. For these reasons, plasma concen-

TABLE I. Concentrations of I^{131} and Cl^{36} in Blood Plasma of Milk Fed Calves During Simultaneous Daily Dosing.

| Day of dosing | % of daily dose/liter, avg $\pm$ SD | | | |
| | I^{131} | | | Cl^{36} |
	Total	Protein bound	Exchangeable	Total*
2	2.18 ± .68	.07 ± .09	2.11 ± .63	5.06 ± 1.47
3	2.81 ± .72	.34 ± .12	2.48 ± .64	10.44 ± 1.50
4	3.40 ± .66	.58 ± .10	2.82 ± .65	15.90 ± 3.22
5	3.46 ± .80	.68 ± .11	2.78 ± .81	18.23 ± 5.31
6	4.23 ± .75	1.04 ± .22	3.19 ± .79	19.66 ± 2.37
7	4.37 ± .93	1.26 ± .23	3.11 ± .91	28.37 ± 1.42

* All assumed to be exchangeable as no Cl^{36} could be detected in the precipitated protein.

TABLE II. Excretion of I^{131} and Cl^{36} in Urine and Feces by Milk Fed Calves During Simultaneous Daily Dosing.

| Day of dosing | % of daily dose, avg $\pm$ SD | | | |
| | Urine | | Feces | |
	I^{131}	Cl^{36}	I^{131}	Cl^{36}
2	2.9 ± 2.0	2.0 ± .4	.1 ± .1	.4 ± .2
3	15.2 ± 5.2	3.7 ± 1.2	12.0 ± 15.4	.3 ± .2
4	18.2 ± 4.8	7.4 ± 3.8	47.9 ± 19.1	.3 ± .2
5	11.9 ± 4.7	9.9 ± 6.7	26.8 ± 25.8	.4 ± .2
6	16.7 ± 2.9	9.8 ± 3.8	31.4 ± 18.6	.4 ± .3
7	18.1 ± 6.2	17.0 ± 5.6	36.3 ± 14.4	.3 ± .2

trations (Table I) and urinary and fecal excretions (Table II) are averages for all 6 calves.

Concentrations of Cl^{36} in plasma averaged over 5 times that of I^{131} (Table I). By the 7th day of dosing, 29% of the plasma I^{131} was protein-bound, whereas no Cl^{36} could be detected in the precipitated protein. Despite higher plasma Cl^{36} levels, urinary I^{131} excretion exceeded that of Cl^{36} (Table II). Fecal I^{131} increased to over 30% of the daily dose

while less than 0.5% of the daily Cl^{36} intake was voided in feces. At slaughter, thyroid I^{131} uptake ranged from 10.8 to 30.1% of the cumulative dose with no consistent treatment effect. Thyroid accumulation of Cl^{36} was negligible. Three times more I^{131} than Cl^{36} was recovered from the abomasum (Table III). Recoveries of administered I^{131} from the forestomach and small intestine did not differ from Cl^{36} in the corresponding segments. However, in relation to iodine, chlorine absorption in the lower bowel was much more complete.

Water-extractable I^{131} in ingesta decreased from an average of 82% of the total forestomach I^{131} content to less than 15% in the lower large intestine (Table III). In contrast, Cl^{36} was essentially 100% water-extractable from ingesta throughout the digestive tract. Of the I^{131} that was water-extractable, increasing percentages could be precipitated from samples obtained lower in the tract whereas Cl^{36} was predominantly non-

TABLE III. Distribution and Binding of I^{131} and Cl^{36} in the Milk Fed Calf Digestive Tract at Slaughter.

| Segment | % of daily dose/segment* | | Ratio | H_2O extractable % of total | | Non-precipitable % of H_2O extractable | |
	I^{131}	Cl^{36}	I^{131}/Cl^{36}	I^{131}	Cl^{36}	I^{131}	Cl^{36}
Forestomach†	51.0 ± 35.0	49.9 ± 31.4	1.0	82.1	92.6	76.9	89.8
Abomasum	28.9 ± 9.4	9.3 ± 3.0	3.1‡	58.8	98.7	68.2	137.3
Small intestine 1	4.5 ± 4.2	3.4 ± 2.4	1.3	88.4	93.4	88.2	131.9
" " 2	3.8 ± 2.4	3.4 ± 2.2	1.1	91.1	94.8	72.3	134.3
" " 3	1.9 ± 1.5	1.7 ± 1.7	1.1	83.2	109.9	58.1	120.1
Cecum	16.3 ± 8.4	.8 ± .6	20.4‡	20.0	106.5	43.5	104.6
Large intestine 1	5.1 ± 3.2	.3 ± .2	17.0‡	24.2	150.5	38.6	68.7
" " 2	24.0 ± 16.1	.5 ± .5	48.0‡	14.8	175.8	40.2	82.0

* Avg ± standard deviation.
† Includes rumen, reticulum and omasum.
‡ Significantly greater than a ratio of 1 (P < .05).

TABLE IV. Titratable Acidity (ml 0.01 N NaOH/g ingesta) and Ratios of I^{131} and Cl^{36} Concentrations in Abomasal Content to Concentrations in Blood Plasma at Slaughter.

Component ratios	Diamox	Histamine	Control
Abomasum I^{131}/ plasma total I^{131}	16.9[b]	32.8[a,b*]	20.0[a]
Abomasum I^{131}/ plasma exchange-able I^{131}	29.8[a]	55.2[a]	36.8
Abomasal Cl^{36}/ plasma Cl^{36}	.51[b]	.01[b]	.78
Titratable acidity (ml)	8.4	8.7	8.6

* Values in the same line bearing the same superscript differ significantly (a, P $<$.10; b, P $<$.05).

precipitable. Titratable acidities of abomasal contents were unaffected by administration of Diamox or histamine (Table IV). However, treatment differences were apparent in the ratios of I^{131} and Cl^{36} concentrations in abomasal content to the concentrations in blood plasma (Table IV). Histamine increased gastric secretion of both I^{131} and Cl^{36} while Diamox reduced I^{131} secretion.

Discussion. High retentions of both I^{131} and Cl^{36} as indicated by low excretions (Table II) may have resulted from low iodine and chlorine intakes from the all milk diet. Decreasing percentages of water-extractable I^{131} in ingesta from more posterior segments of the digestive tract (Table III) indicate an association of a portion of the iodine with undigested residue. This non-extractable form of iodine, probably an organic form excreted into the digestive tract, was then progressively concentrated by continuing iodide absorption in the lower bowel. Recovery of Cl^{36} in the same extracts averaged near 100%. Values greater than 100% in the lower tract most likely were due to errors associated with determining the very low Cl^{36} levels in the ingesta as well as to concentration of Cl^{36} by removal of inert matter during the extraction. The negligible association between chlorine and ingesta could be involved in the high net Cl^{36} absorption (Table II).

The lack of treatment differences in titratable acidity of abomasal contents (Table IV) most likely was due to buffering action of ingesta since samples were taken less than 5 hours after milk was fed(7). Abomasal/

plasma Cl^{36} ratios averaged less than one. However, the gastric juice was diluted by ingesta. If undiluted gastric juice had been collected, it is likely the ratio would have exceeded unity. Plasma I^{131} concentrations averaged only $\frac{1}{5}$ those of Cl^{36} (Table I) whereas 3 times more I^{131} than Cl^{36} was recovered from the abomasum at slaughter (Table III). Thus, although Cl^{36} and I^{131} both responded in the same direction to gastric stimulation or inhibition (Table IV), net gastric secretion of iodine exceeded that of chlorine by 15 times. If only exchangeable (non-bound) plasma I^{131} is available for gastric secretion, the ability of the abomasum to concentrate I^{131} over Cl^{36} is even greater.

Apparently, the accumulation of iodine by the gastric stomach is not entirely incidental to secretion of chloride in gastric juice(3). A recent report supports the view that iodide and acid secretion are separate and distinct processes(8). It has been suggested that gastric iodine concentration may be connected with origin of both the thyroid gland and glandular epithelium of the stomach from a common endodermal layer during embryonal development(2). The concentrating mechanism may serve to create an iodide pool for subsequent reabsorption and recycling(9). This is supported by observations on total gastrectomy patients(10). Despite a normal dietary iodine intake, urinary excretion and plasma inorganic levels were low(10), indicating a malabsorption of iodine.

Summary. Metabolism of I^{131} and Cl^{36} was investigated by simultaneous daily administration of the two radioisotopes to milk fed dairy calves. Plasma levels of I^{131} averaged $\frac{1}{5}$ and abomasal I^{131} contents 3 times corresponding Cl^{36} values. Thus, net gastric secretion of iodine exceeded that of chlorine by 15 times. Percentages of daily administered I^{131} and Cl^{36}, respectively, excreted during the last 3 days of dosing averaged 15.6 and 12.2% in urine and 31.5 and 0.4% in feces. Chlorine-36 in ingesta from the large intestine was completely water-extractable compared to less than 20% of the I^{131} in the same samples.

1. Brown-Grant, K., Physiol. Rev., 1961, v41, 189.

2. Magas, S., Rozynkowa, D., Acta Biochim. Polonica, 1964, v11, 465.

3. Howell, G. L., Van Middlesworth, L., Proc. Soc. Exp. Biol. and Med., 1956, v93, 602.

4. Birnbaum, D., Hollander, F., ibid., 1952, v81, 23.

5. Hyden, S., Kungl. Lantbrukshogskolans Annaler, 1955, v22, 139.

6. Miller, J. K., Miller, G., J. Dairy Sci., 1965, v48, 988.

7. Ash, R. W., J. Physiol., 1964, v172, 425.

8. Brown-Grant, K., Cumming, J. D., Haigh, A. L., Harries, E. H. L., ibid., 1965, v177, 337.

9. Barua, J., Cragle, R. G., Miller, J. K., J. Dairy Sci., 1964, v47, 539.

10. Harden, R. M., Adams, J. F., Metabolism, Clin. Exp., 1964, v13, 843.

Metabolism of Fats III: Absorption of Hydroxy Acids *via* the Lymph.* (30762)

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Castor oil contains the unsaturated hydroxy fatty acid, ricinoleic acid (12-hydroxy-cis-9-octadecenoic acid), which is found in the glyceride form to the extent of 90% of the total fatty acid composition. Although castor oil acts as a purgative agent in man, considerable amounts may be absorbed and utilized when fed as part of the diet of many animals. It has been found to be 98% digestible in the rat, and when fed to adult animals at the level of 48.4% in the diet was readily metabolized(3).

When fed as a dietary component at a 10% level, ricinoleic acid, 12-hydroxystearic acid and their corresponding triglycerides changed both the carcass and the fecal fat composition of the rat(4). Dietary hydroxy acids were deposited and influenced the character of the normal mixed fatty acid composition of the carcass fat; both saturated and unsaturated hydroxy fatty acids were converted to monoenoic acids in the rat. Large amounts of oleic and hexadecenoic acid seemed to be deposited with a preferential excretion of stearic and linoleic acids in animals fed a source of hydroxy fatty acids in comparison with those fed a source of linoleic acid. No ricinoleic acid has been found stored in either the phospholipids of the small intestine, in the liver and muscle or in liver triglycerides (5).

Recent studies on the digestion and absorption of ricinoleic acid indicated that ricinoleic acid was absorbed from the alimentary tract of the rat and could be detected in the chyle(6). The *in vitro* experiments of Watson and Gordon(6) showed that castor oil was hydrolyzed as well as and perhaps better than olive oil. In the small intestine castor oil appeared to be easily and rapidly hydrolyzed; activation and absorption of the free acid proceeded at a low level of efficiency, resulting in a rapid accumluation of free ricinoleic acid and its mineral salts.

Fats heated in the presence of oxygen have been shown to yield oxidation products which decrease the nutritional value of the fat(1,2). These oxidation products are composed of carbonyl-containing and hydroxylated compounds as well as highly polar polymerized fatty acids which may have been responsible for the loss in nutritive value which has been observed when heated fats are fed to animals.

The present study is concerned with the absorption through the lymph of hydroxylated fatty acids such as ricinoleic acid and its analogs. Comparisons were made with results obtained when corn oil was administered to lymph cannulated rats. A comparison between the lymph lipid composition of rats fed corn oil, triricinolein and ricinoleic acid was also made.

Materials and methods. Preparation of compounds. The triricinolein and free ricino-