sera 17 and 19), capable of reacting with human rather than with rabbit γ-globulin(3).

Summary. A new isoprecipitin for a rabbit γ-globulin allotype was found during attempts to produce rheumatoid factor-like activity in rabbits by immunization with specific typhoid-anti-typhoid complexes. This allotypic specificity, originally designated L, was subsequently found to correspond with Type E of Dubiski and Kelus, Type *b* of Oudin, and and was later reclassified Type A1 of Dray *et al.* This new antiserum facilitated the immunochemical study of allotypic specificities A1, A2, and A3, which are controlled at the *a* locus. The sensitized sheep cell agglutination reactions observed with the sera of some of the immunized rabbits appeared to be due to the isoprecipitin for the allotypic specificity A1 reacting with its antigen in the rabbit amboceptor used. The bentonite flocculation reactions observed with the sera of the immunized rabbits may be due to cross-reactions of the rabbit isoprecipitin and human γ-globulin, or to production of a different anti-γ-globulin antibody which reacts primarily with human γ-globulin.

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In vivo Absorption of Amprolium and Its Competition with Thiamine. (28650)

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The coccidiostat amprolium* [1-(4-amino-2-n-propyl-5-pyrimidinylmethyl)-2-picolinium chloride • HCl] is a weak anti-metabolite of thiamine. It is capable of producing thiamine deficiency in chicks(1), adult chickens, and eggs(2) but only when added at levels in excess of 700 ppm to diets containing approximately 5 to 6 ppm thiamine. Parenteral and oral administration of the vitamin overcomes the vitamin deficiency(1,2). Apparently the primary site of action is in the intestine, because hens administered amprolium orally deposited less thiamine and less drug in yolk than those given comparable doses parenterally, and less thiamine was absorbed in the presence of amprolium from hen's ligated loops, *in situ*(3). Data presented here, obtained by the ligated-loop technic, on absorption of drug in chicks, show that amprolium is absorbed preferentially from the duodenum of the chick and corroborate data from studies with hens showing that amprolium interferes with thiamine absorption.

* Obtained from Merck & Co., Inc., Rahway, N. J.

Methods. Ligated segments of the digestive tract were prepared in male and female White

Leghorn chicks 3 to 5 weeks of age. Chicks were reared in battery brooders using standard husbandry practices. A stock ration was fed *ad libitum* until about 16 hours before operations were performed; water was allowed up to the time chicks were moved from the animal room into the laboratory. Body weight was obtained and chicks excessively heavy or extremely light within a particular age group were discarded. Most chicks were 4 weeks of age and ranged between 250-310 g. In any particular experiment, chicks of the same age were used. Statistical analysis revealed that amprolium was absorbed at the same absolute rate despite differences in sex and age, so data were pooled according to treatment. Ligated sections of digestive tract were prepared in chicks anesthetized with ether. Duodenal loops averaged 11 cm in length. The intestinal sections 12 cm distal to the bile ducts were termed the "upper ⅓" of the remaining small intestine. The "middle ⅓" encompassed the section 5 to 7 cm to each side of the yolk stalk; the "lower ⅓" was the 12 cm section measured proximally from the cecal junction.

Compounds were dissolved in 0.1 M phosphate-buffer, pH 6.5(4), the pH in chick intestine(5). A 1-ml syringe and ½" – 26 gauge needle were used to inject the 1-ml solution at 39°C into ligated loops. The incision was sutured with surgical silk, anesthetic was withdrawn and the chick returned to its cage. After 30 or 60 minutes the chick was sacrificed with CO_2 and the ligated segment removed. It was trimmed of adhering tissue and/or mesentery, and also of intestine beyond the ligatures. Homogenates were obtained according to the procedure described previously(3) and analyzed for amprolium and thiamine(2,6). Solutions prepared for injection were also analyzed, and employed as the standards to reveal actual amount of compound(s) injected. Between 94 and 104% of the drug was recovered at zero time, using doses of 0.8, 8.0 and 80.0 mg in middle ⅓ intestinal sections. Controls injected with 1 ml phosphate buffer were included in all experiments. Data were analyzed according to methods detailed by Snedecor(7) using Dunnett's(8) "t" test to determine significance of differences from control means.

Absorption, *a*, is defined as the amount of compound, *d*, introduced into the ligated segment minus the amount recovered at time of sacrifice. Compound absorbed on the mucosal surface or absorbed within mucosal cells and freed during homogenization was presumably recovered. Thus, absorption refers to net disappearance of compound, and would include degradation if it occurred. No significant degradation of amprolium was detected in 1-hour absorption studies with 6 ligated loops of middle small intestine, injected with 80 mg of amprolium-C^{14}, because 68% of drug was recovered by chemical assay as compared to 66% by C^{14} analysis; and from duodenal loops injected with 6.3 mg, 20% was recovered by chemical assay as compared to 25% by C^{14} analysis.

Results. Absorption of amprolium. Sections of the digestive tract in the chick varied in their ability to absorb in 1 hour a test dose of 8 mg amprolium. The duodenum absorbed 91% of the drug, whereas other sections tested did not absorb significant quantities (Table I).

The dose-absorption curve, *d vs a*, characterizing duodenal capacity for absorbing amprolium is shown in Fig. 1. The *d vs a* relationship was linear up to *d* values of about 12 mg (38 μmoles). Thereafter, the curve plateaued and showed a tendency to reverse itself at *d* values exceeding 38 mg (120 μmoles). Fluid accumulation, "ballooning," occurred within most ligated duodenal loops at values of *d* exceeding 22 mg.

The absorptive capacity of the middle portion of the small intestine in the chick was determined at several dose levels, *d*, which were previously used to characterize the *d vs a* curve in the duodenum. Drug absorption from the middle ⅓ small intestine amounted to a non-significant 14% when *d* was 0.8 mg as compared to the almost complete absorption of this dose from the duodenal loops (Table II). However, as *d* was increased in loops of the middle ⅓ section, *a* increased, both in absolute and relative units. This was in contrast to the relative decrease in *a* observed when comparable amounts of am-

TABLE I. Absorption of Amprolium in 1 Hour from Ligated Sections of Digestive Tract in Chicks.

Section		No. chicks	*d* (mg)	Recovered at 1 hr (mg)	Absorbed in 1 hr *a* (mg)	Absorbed in 1 hr *a/d* × 100
Crop		4	8.0	7.3	.7	9
Duodenum		10	8.0	.7	7.3	91§
Remaining small intestine	Upper 1/3	4	8.0	6.9	1.1	14
	Middle 1/3	4	8.0	7.4	.6	8
	Lower 1/3	10	8.0	7.2	.8	10
Ceca		4	8.0	7.8	.2	3

§ $P \leq 0.01$.

Dunnett's allowance value in a comparison of 10 controls *vs* 4 experimentals = 1.2 mg or 15%; thus absorption, *a*, exceeding 1.2 or 15% is significant at $P \leq 0.05$.

Analysis of variance of mg recovered at zero time and at 1 hr

Source of variation	d.f.	Mean square
Between sections	6	62.5 §
Within "	36	.383

prolium were introduced into duodenal loops (Table II).

Absorption of amprolium in presence of thiamine. The *d vs a* curve for thiamine in the chick duodenum was reported to be linear with values of *d* up to 10 mg, followed by the curving and plateau phase of the curve which are associated with a high incidence of ballooning(9). Thus, thiamine injected simultaneously with amprolium into ligated loops should not exceed 10 mg. This avoids the fluid accumulation and resultant non-specific decrease in intestinal absorption which would mask a competitive effect between amprolium and thiamine. In order to allow for addition of excess thiamine below the 10 mg dose, the absorption rate was determined for .315 mg amprolium. It was constant up to 30 minutes but decreased in the interval between 30 and 60 minutes. Therefore absorption time for studies on competition was reduced to 30 minutes.

The addition of 3.37 mg or more of thiamine to the solution containing .315 mg amprolium significantly reduced absorption of the drug from ligated duodenal loops (Table III). When only 1.38 mg of thiamine was used the effect was just significant at P = .05. Presumably, further experimentation may indicate this dose of thiamine is borderline for an effect.

Absorption of thiamine in presence of amprolium. As shown in Fig. 1, fluid accumulation did not become a problem in absorption studies until the amprolium dose exceeded 22 mg. Thus, doses of amprolium introduced simultaneously with thiamine were maintained within this limit in a 1-hour

TABLE II. Comparison of Absorptive Capacity for Amprolium Between Duodenal and Middle Small Intestine Loops Within Chicks. Absorption time = 1 hour.

Dose (mg) *d*	Duodenum *a* (mg)	Duodenum *a/d* × 100	Middle small intestine *a* (mg)	Middle small intestine *a/d* × 100
.8	(9)* .74(± .03)§	93	(8) .11(± .06)	14
8.0	(10) 7.4 (± .41)	93	(8) 1.38(± .18)	17
20.0	(8) 11.9 (±2.3)	60	(4) 5.8 (±2.8)	29
40.0	(7) 11.9 (±2.7)	29	(4) 16.7 (±2.7)	42

Analysis of variance of absorbed amprolium

Source of variation	d.f.	Mean square
Sections (S)	1	159.0§
Dose (D)	3	429.0§
S × D	3	83.7
Error	50	4.12

* No. chicks.
† Mean (± S.D.).
§ $P \leq .01$.

TABLE III. Interference with Amprolium Absorption by Thiamine from Ligated Duodenal Loops Within Chicks. Absorption time = 30 min. Solution volume = 1 ml.

d (mg)				Amprolium absorption	
Amprolium	Thiamine	T/A*	No. chicks	*a* (mg)	%
.315†	0	0	16	.243	100
.315	1.38	4/1	4	.224‡	92
.315	3.37	10/1	8	.196§	81
.315	6.74	20/1	8	.164§	67
.315	10.11	30/1	8	.159§	65

Allowance value = .016 mg; thus values less than .227 mg for *a* are significantly less than .243.

Analysis of variance of a *(mg)*

Source of variation	d.f.	Mean square
Groups	4	.0139 §
Remainder	39	.000467
	S.D. = .0216	

* Molar ratio.
† .315 mg = 1 μmole.
‡ P ≤.05.
§ P ≤.01.

absorption study. As shown in Table IV, amprolium significantly reduced thiamine absorption at the amprolium/thiamine ratios studied.

Discussion. Chicks eating approximately 60 g of medicated feed containing 125 ppm amprolium consume 7.5 mg of drug during their 14 hours of daylight. When an amount equal to the drug intake for 1 day, 8 mg, was introduced into various sections of the digestive tract, only the duodenum showed a preferential ability to absorb the drug; other sections absorbed very little, or none at all. One of the intestinal sections, the middle ⅓, with practically no capacity for amprolium absorption at the 8 mg dose, also showed no capacity to absorb the drug even though the dose of 0.8 mg amprolium approached the physiological range of about 0.54 mg/hour. However, when amprolium was introduced in non-physiological amounts which produced a "saturation effect" or plateau in the duodenal dose-absorption curve, then the middle intestinal segment absorbed, both relatively and absolutely, an increasing amount of drug. This apparently indicated a "break-through" phenomenon.

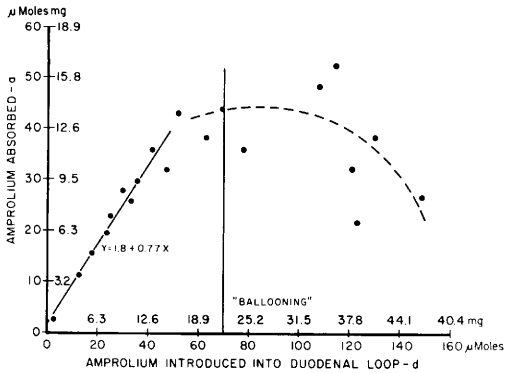

FIG. 1. Dose-absorption curve for amprolium introduced into ligated duodenal loops of chicks. Absorption time = 1 hr. Values to right of vertical line at 70 μM dose are influenced by accumulation of fluid in intestinal lumen, "ballooning." Each point represents 4 to 10 chicks.

Amprolium and thiamine apparently compete for absorption. This was demonstrated *in situ* in laying hens(3), and in chicks when an excess of one was present over the other. The quantities of drug and vitamin used for studying their interference may seem excessive or non-physiological in terms of compound per unit body weight or in relationship to quantities of either compound that may be present in the digestive tract at a given time. However, their quantities are not excessive when viewed in relationship to dose-absorption curves which directly measured the ability of the duodenum to absorb these compounds. The competitive effect was demonstrated in each situation well within the absorptive capacity of the duodenum and amounts used occurred on the linear portion of the *d vs a* curve. Excessive fluid accumulation did not occur at these doses. Also important was that physical measurements†

† Nuclear resonance study by Dr. N. R. Trenner and a study on dialysis rate through cellophane membrane by Dr. C. Rosenblum of Merck Sharp & Dohme Research Laboratories.

TABLE IV. Preventing Thiamine Absorption from Chick Duodenal Loop with Use of Amprolium. Absorption time = 1 hr. Volume of solution = 1 ml.

d (mg)				Thiamine absorbed	
Thiamine	Amprolium	A/T*	No. chicks	*a* (mg)	%
1.35†	0	0	4	1.14	100
1.35	7.5	6/1	4	.60§	53
1.35	10.1	8/1	4	.50§	44
1.35	14.8	12/1	4	.52§	46
1.35	21.4	16/1	4	.38§	33

Analysis of variance

Source of variation	d.f.	Mean square
Groups	4	.3568§
Remainder	15	.0141

* Molar ratio. † 1.35 mg/ml = 4 mM § $P \leq .01$.

have ruled out the possibility that a chemical complex of the two compounds within the intestinal lumen would account for the interference effect of thiamine on amprolium or *vice versa*.

It was previously shown that amprolium produced a thiamine deficiency in chickens and their eggs when added at excessively high levels to breeder rations(1,2,6,10). On this basis, one can infer from the data in this study that the decrease in absorption of thiamine plays a major part in accounting for the thiamine deficiency produced by excessive use of amprolium in diets.

Summary. Ligated intestinal sections of the digestive tract in chicks were injected *in vivo* with 8 mg of the coccidiostat, amprolium. Only the duodenum absorbed significant amounts of the drug (91%) in a 1-hour absorption period. A dose-absorption curve for the duodenum revealed a linear response to about 12 mg and a plateau portion associated with fluid accumulation in loops when higher doses of the drug were introduced. Thiamine introduced simultaneously with .315 mg of amprolium (an antimetabolite of thiamine) decreased amprolium absorption significantly when thiamine/amprolium ratios exceeded 4, and amprolium in excess over thiamine decreased thiamine absorption at molar ratios exceeding 7.5.

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