The scope of this report does not permit an explanation of the mode of action of SC-16102. In clearance experiments with conscious dogs (unpublished data) the oral or intravenous administration of SC-16102 at a dose of 5 mg/kg results in an increase in Na (5-fold) and K (2-fold) excretion in the absence of any consistent change in glomerular filtration rate or effective renal plasma flow. This indicates a direct renal site of action of this azido pyrimidine derivative.

Summary. SC-16102, an azido pyrimidine derivative, was found to (a) reverse the Na retention induced by exogenous mineralocorticoids (aldosterone and DCA) with a further increase in K excretion in adrenalectomized rats, (b) produce a natriuresis with some additional K loss in non-DCA treated adrenalectomized rats and (c) elicit a pattern of electrolyte excretion qualitatively similar to that of hydrochlorothiazide in the intact rat. SC-16102 was found to be a non-specific antagonist to mineralocorticoids with the ability to reverse the DCA-induced decrease in the urinary Na/K ratio, the compound being at least 6.8 times the potency of spironolactone and 2.6 times the activity of hydrochlorothiazide.

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Effect of Histamine, Histidine, and Some Related Compounds on the Zinc-Deficient Chick.* (31937)

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Previous work in our laboratory(1) has shown that chicks fed a zinc-deficient soy protein diet develop a "perosis-like" or "arthritic-like" leg disorder characterized by swelling of the hock joints and shortening and thickening of the long bones as in perosis. However, it is unlike perosis, as the slipping of the gastrocnemius tendon from its condyles has not been observed in our experiments. This disorder was not present in zinc-deficient chicks fed casein hydrolysate or egg white diets. Because the difference in diets was in protein source alone, and because some amino acids and their metabolites have zinc-binding properties, effects of certain of these natural chelating agents on zinc deficiency in chicks fed soy protein diets were studied. It was found(2) that histidine (0.5% of the diet) alleviated the leg abnormality but did not affect other zinc deficiency symptoms such as poor growth or low zinc content of bone. It appeared, therefore, that histidine did not increase the availability of dietary zinc but acted in some other way to alleviate the leg defect. The present study was designed to assess (a) the dietary level at which histidine exerts its most beneficial effect and (b) the effect of various histidine metabolites and related compounds on the zinc deficiency syndrome in chicks fed soy protein.

Materials and methods. Three experiments were conducted with day-old New Hampshire × Single Comb White Leghorn chicks with-

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out segregation according to sex. The birds were distributed at random into groups of 10 each and placed in a stainless steel battery at 37° to 40°. Feed and distilled water were provided *ad libitum* in aluminum troughs. Feed was mixed every 2 weeks and stored in a refrigerator until fed. The composition of the basal isolated soybean protein diet (soy protein) was that described previously(2). The basal zinc-deficient diet contained 5 ppm supplemental zinc (control diet) and analyzed, on an air-dried basis, 12.4 ppm zinc in Experiments 1 and 2, and 14.8 ppm zinc in Experiment 3. Small amounts of zinc were added to the basal zinc-deficient diet to reduce mortality and to allow sufficient growth so that leg abnormalities were more prominent. In Experiment 1, histidine (free-base)† at levels of 0.1%, 0.5%, 1.0% and 2.0% of the diet was added to 2 diets—the basal zinc-deficient diet and the basal diet plus 80 ppm supplemental zinc. Zinc was always added in the form of zinc oxide.

In Experiment 2, the following were added to the basal diet at 0.1% each: histidine (free-base),† imidazole,† urocanic acid (dihydrate),‡ histamine (free-base),† β-imidazole acetic acid (hydrochloride),‡ and thiolhistidine.§ Histidine and imidazole, but not the other test substances, were also fed at 0.5% of the diet.

In Experiment 3, the amounts of histamine (dihydrochloride)‖ and supplemental zinc shown in Table I were added to the diet. Graduated levels (0.1% histamine dihydrochloride for the first 2 weeks and 0.2% for the next 2 weeks) were fed in 2 groups. For a comparison of the effects of histamine and histidine, 1.0% histidine (free-base) was added to the diet of 2 groups of chicks receiving, respectively, the basal diet and the diet with 80 ppm supplemental zinc.

In all experiments, control groups of chicks were fed the basal soy protein diet and the basal diet plus 80 ppm supplemental zinc.

During the course of each experiment, the chicks were weighed weekly and observed for abnormalities. When the chicks were 4 weeks of age, leg scores(1) and body weights were taken. Then the chicks were killed by cervical vertebrae fracture. Tibias and femurs were removed for analysis and stored at −8° until they were analyzed for zinc. Legs were scored for the "perosis-like" or "arthritic-like" condition using a scale of 1 to 5(1). Length : width measurements were made on tibias and femurs, and tibias were analyzed for zinc as described previously(1). Statistical analysis was by Duncan's multiple range test for unequal replication(3).

Results and discussion. In Experiment 1 (Table I) supplemental histidine at 0.1% and 0.5% of the diet had no significant effect on the zinc-deficient chick, except that 0.1% histidine appeared to increase the leg score. In previous work, a lowering of the leg score by histidine at 0.5% of the diet was noted(2). In the present study histidine at 1.0% and 2.0% of the diet markedly alleviated the leg abnormality caused by zinc deficiency. Body growth and zinc content of tibias were not significantly affected, whereas the leg scores were significantly improved ($p<0.05$). The legs of zinc-deficient chicks fed 1.0% or 2.0% supplemental histidine were essentially free of abnormalities and the scores were not significantly different from those fed 80 ppm supplemental zinc, with or without supplemental histidine.

Having repeated the observation that histidine did alleviate the "arthritic-like" defect of zinc-deficient chicks, and with the observation that its maximal effect occurred when histidine was present at a high level in the diet, we decided to feed various histidine metabolites and related compounds to see if the alleviation of the leg disorder was due to a metabolite of histidine and whether some related substances would exert a similar effect. In Experiment 2 (Table I) histamine, the product of decarboxylation of histidine by histidine decarboxylase, was shown to have an effect similar to that of histidine at the 1.0% level in Experiment 1. Histamine at 0.1% of the diet did not significantly affect growth or zinc content of tibias, but did significantly improve leg scores ($p<0.05$). However, 0.1% histamine was not as effective as zinc

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in overcoming leg defects, as the leg scores of chicks fed histamine were significantly higher than those of chicks fed 80 ppm supplemental zinc ($p<0.05$). In contrast to histamine, the related compounds (histidine, imidazole, urocanic acid, β-imidazole acetic acid and thiolhistidine) at 0.1% of the diet had no significant effect on the zinc-deficient chick. However, imidazole at 0.5% of the diet significantly depressed growth ($p<0.05$). It is of interest that imidazole, which has been shown to be an inhibitor of histidine decarboxylase *in vitro*(4), also appeared to aggravate other zinc deficiency symptoms as evidenced by much poorer feathering, greater amounts of dermatitis, and more severe leg abnormalities.

Experiment 3 was conducted to confirm the effect of histamine on the zinc-deficient chick and to assess what level of histamine was most beneficial. From the data in Table I, it appears that histamine at 0.2% of the diet had approximately the same effect on zinc-deficient chicks as did 1.0% histidine. At 0.2% histamine, growth of the zinc-deficient chicks was not significantly depressed.

TABLE I. Body Weights, Leg Scores and Zinc Content of Tibias at 4 Weeks of Age in Chicks Fed a Soy Protein Diet with Supplemental Histidine, Histamine, or Related Compounds.

Treatment, % of diet	Zinc supplemented, ppm	Body wt, g	Leg score	Zinc in tibias, ppm*
		Experiment 1		
None	5†	192 A‡	§3.8 B	36 A
.1% Histidine (free-base)	5	195 A	4.6 A	35 A
.5% "	5	214 A	3.7 B	36 A
1.0% "	5	210 A	1.9 C	36 A
2.0% "	5	177 A	1.7 C	50 A
None	80	348 B	1.3 C	160 BC
.1% Histidine (free-base)	80	335 B	1.3 C	150 B
.5% "	80	348 B	1.4 C	169 C
1.0% "	80	315 B	1.3 C	233 D
2.0% "	80	322 B	1.4 C	248 E
		Experiment 2		
None	5†	192 BC	3.8 ABC	36 AB
"	80	348 D	1.3 E	160 C
.1% Histidine (free-base)	5	195 BC	4.6 A	35 A
.5% "	5	214 C	3.7 BC	36 AB
.1% Imidazole	5	172 AB	4.6 A	30 A
.5% "	5	161 A	4.7 A	35 AB
.1% Urocanic acid (dihydrate)	5	177 AB	4.6 AB	29 A
.1% Histamine (free-base)	5	190 BC	2.6 D	42 B
.1% β-imidazole acetic acid (hydrochloride)	5	167 AB	3.3 CD	35 AB
.1% Thiolhistidine	5	171 AB	4.3 AB	29 A
		Experiment 3		
None	5‖	178 BCD‡	§4.0 A	32 A
.1% Histamine (dihydrochloride)	5	171 BCD	2.9 B	39 AB
.2% "	5	166 BC	1.6 CD	46 B
.4% "	5	125 A	1.4 CD	75 C
.1%→.2% "	5	167 BC	2.1 C	51 B
1.0% Histidine (free-base)	5	197 CD	1.7 CD	39 AB
None	80	289 E	1.4 CD	150 D
.1% Histamine (dihydrochloride)	80	261 E	1.3 D	163 DE
.2% "	80	203 D	1.2 D	152 D
.4% "	80	155 B	1.2 D	167 E
.1%→.2% "	80	208 D	1.1 D	159 DE
1.0% Histidine (free-base)	80	279 E	1.4 CD	226 F

* Fat-free, dry basis.

† Diet contained a total of 12 ppm zinc by analysis.

‡ For each experiment, values within a column followed by the same letter are not significantly different ($p>0.05$) from each other.

§ 1 = normal; 5 = very abnormal.

‖ Diet contained a total of 15 ppm zinc by analysis.

The leg scores were significantly lower ($p<0.05$) than those of the zinc-deficient controls, and were not significantly different from the groups fed 80 ppm zinc, with or without histamine. However, in apparent contrast to histidine, histamine at 0.2% or 0.4% of the diet did significantly increase the zinc content of tibias ($p<0.05$). Nonetheless, the zinc content still was much lower than in chicks fed 80 ppm zinc. It was found that to overcome abnormal legs in chicks fed soy protein diets by increased dietary zinc, a concentration of zinc in tibia of approximately 70 to 80 ppm in the fat-free dry bone was needed(1). The chicks fed 0.2% supplemental histamine had much less than this and thus appeared to have the low tibia zinc content characteristic of zinc-deficient chicks. It is likely that the increase in zinc concentration in the tibias was related to the slow rate of growth of chicks fed histamine. Chicks with less total body weight gain, within groups, had higher tibia zinc concentrations. This was especially apparent in the chicks fed histamine at 0.4% of the basal diet. Here the values for tibia zinc ranged from 115 ppm for a chick which weighed 105 g and had gained a total of 63 g to 56 ppm for a chick which weighed 143 g and had gained a total of 107 g. These observations suggest that the greater amount of newly formed zinc-deficient bone in the larger chicks dilutes the zinc present in bone at the outset of the experiment, and that the effect of the higher level of histamine in increasing bone zinc concentration is the result of the growth depression and not of increased zinc availability.

In all experiments, the longest length to narrowest width ratios of femurs and tibias were also determined. These measurements, in general, gave results similar to the leg scores. Since they measured only gross dimensions, and did not take into account the amount of swelling of the hock, twisting of the leg, or the walking ability of the chick (which the leg score did), and because interpretation of the length:width ratio was complicated by an effect of body weight on this ratio, the results are not reported here.

From these data, it appears that there is a zinc-histidine-histamine interrelationship and that the relationship between histidine and histamine may be directly the result of histidine being converted into histamine, possibly in the gastrointestinal tract, in the blood stream by the blood basophil, or in other body tissues.

Since it is known that (a) salicylates effectively control the symptoms of pain and stiffness in most mild or moderate cases of rheumatoid arthritis(5), and also cause an increase in histamine content of the small intestine in rats(6); (b) a high protein (high histidine?) diet exerts a protective action against "adjuvant" induced arthritis in rats (a type of immune response)(7), and (c) from the present data, histamine protects against an "arthritic-like" syndrome in zinc-deficient chicks fed soy protein, one may postulate that histamine may be an important alleviator of some arthritic syndromes.

The cause of the "arthritic" syndrome in zinc-deficient chicks and the protective effect of histamine are matters of pure speculation. Some possibilities are that zinc deficiency may cause (a) a circulating rheumatoid factor to be formed, such as an abnormal plasma protein causing an antigen-antibody inflammatory response in the peripheral joints, (b) large amounts of mucoprotein to build up in the joint area due to the lack of a zinc enzyme needed to synthesize normal mucopolysaccharides, thereby causing an autoimmune inflammatory type of response, (c) an increase in uric acid, such as that found in zinc-deficient rats(8), and the precipitation of urate crystals (such as in gout) in the joint area causing an inflammatory-type response, (d) a defect in histamine synthesis or storage which would result in a lack of histamine needed in some way for normal development of the hock of the chick. Histamine could be (a) complexing or preventing the synthesis of a rheumatoid factor or other protein causing an inflammatory type reaction, (b) causing the transport of zinc to an important enzymatic site, or (c) causing vasodilation and increased connective tissue permeability so that inflammatory substances are removed readily from the peripheral joint area.

From the preceding, it is obvious that

more research is needed to (a) find why zinc deficiency causes an "arthritic" type syndrome in chicks fed soy protein, and how histamine protects against it, and (b) determine if histamine will counteract other "arthritic-like" or "perosis-like" defects.

Summary. Histidine at 1.0% and 2.0% of the diet, or histamine at 0.2% of the diet prevented the "arthritic-like" or "perosis-like" syndrome in zinc-deficient chicks fed soy protein diets, while having little or no effect on other symptoms of zinc deficiency. Other histidine metabolites and related compounds such as urocanic acid, β-imidazole acetic acid, imidazole and thiolhistidine had little or no effect on the zinc-deficient chick. A direct histidine-histamine relationship is postulated.

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Effect of Mercuhydrin Alone and in Conjunction with Ammonium Chloride On Radiostrontium Excretion in Man.* (31938)

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As the time after ingestion or inhalation of radiostrontium increases, an increasing fraction of radiostrontium that entered the body is deposited in the skeleton, where it becomes less accessible for removal. Orally administered ammonium chloride which produces both acidosis and diuresis has been shown to increase urinary excretion of calcium and radiostrontium at time of injection of the tracer(1-3) and as late as 2 weeks thereafter(2). To determine the effect of enhanced diuresis on radiostrontium excretion, the diuretic agent mercuhydrin, which potentiates the diuretic action of ammonium chloride(4) but does not cause acidosis, was used alone and in conjunction with ammonium chloride in studies performed under constant conditions in man.

Materials and methods. Six patients were studied on the Metabolic Research Ward where they received a constant, analyzed low calcium diet containing an average of 200 mg calcium and 750 mg phosphorus per day. Four of the patients received tracer doses of Sr^{85} intravenously (Patients 1-4) and 2 patients orally (Patients 5 and 6). Nine studies were performed on the effect of mercuhydrin on Sr^{85} excretion and 5 on the effect of mercuhydrin used in conjunction with ammonium chloride (Table I). Mercuhydrin, 2 ml, was injected intramuscularly on 1-3 days at time intervals ranging from 1 to 21 days following Sr^{85} administration. Ammonium chloride and mercuhydrin were used together at time intervals ranging from 4 to 27 days following Sr^{85} administration; 9 g ammonium chloride were given orally in divided doses per day

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