from the bile is much smaller in terms of bioactivity than that of estrogen.

Excretion of Estrogen in Bile Following Androgen Injection. Normal dog bile exhibits no estrogenic activity.[6] In 6 of 9 experiments estrogenic activity was demonstrated in the bile following injection of androgens. In 5 of these, the bile was examined for both free and conjugated estrogen, all being found to exist in the free state. We have demonstrated estrogens in the urine of dogs following androgen administration,[11] contrary to earlier reports.[12] It is noteworthy that estrogens appear in the urine during the first 24 hours following androgen administration, whereas, except in one case, they first appeared in the bile *after* 24 hours.

The source of estrogen in the bile and urine after administration of androgen is not known. It does not seem to be produced in the testes or adrenals.[11] The bile fistula dogs employed in the present study were females and the ovaries might have been stimulated by the injected androgen to secrete estrogen. However, the fact that estrogen was present in the urine following injection of testosterone in normal and castrate male dogs militates against this assumption. A portion of the injected androgen may have been converted into an estrogenic compound.[17,18] The total amount of excreted estrogen (bile and urine) was 100 i.u. in Experiment 6 (following the administration of 100 mg testosterone), 57 i.u. in Experiment 10 (following the administration of 50 mg testosterone), and 150 i.u. in Experiment 14 (following injection of 100 mg methyl testosterone). If all of the estrogen was estrone this represents transformation of 0.01 to 0.15% of the administered androgen. Hamilton and Dorfman[17] calculate the recovery from urine in humans as 0.04% of the administered androgen. In view of the probable presence of estrogen in the bile of these subjects, not included in this calculation, it would appear that production of estrogen following administration of androgen (conversion of androgens to estrogen?) is greater in humans than in dogs.

Summary and Conclusions. 1. In bile fistula dogs a single injection of androsterone, testosterone, or methyl testosterone is followed by excretion of androgenic material in the bile. Excretion of small amounts of androgenic material may continue for 72 hours in spite of the fact that all of the androgen-containing bile is removed from the body. 2. The quantities excreted vary considerably in different experiments with either of the 3 compounds studied. 3. A single injection of androgen is frequently followed by excretion of estrogen in the bile. 4. In one instance androgen and estrogen were excreted in the bile following intraduodenal instillation of testosterone.

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Effect of Ascorbic Acid on Chick Growth when Added to Purified Rations.*

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It has long been recognized that chicks do not develop symptoms of scurvy when fed rations low or devoid of ascorbic acid.[1-8] For example, Hart, Steenbock, Lepkovsky, and Halpin[7] reported that chicks fed either a natural or a semi-purified ration did not develop scurvy and had an abundant supply of ascorbic acid in their livers. Carrick and Hauge[6] also found that livers of chicks on a scorbutic diet were high in ascorbic acid. In spite of this work the presence of a "scurvy-like" disease in chicks which was prevented by the inclusion of dried skim milk in the ration and cured by the addition of cabbage

was reported by Holst and Halbrook.[9] This work could not be confirmed by Cribbett and Correll[10] and in the light of recent investigations it is clear that Holst and Halbrook were probably dealing with a deficiency of vitamin K instead of ascorbic acid. Bell, Satterfield, and Cook[11] reported recently that a leg paralysis and other symptoms of a deficiency in 4 hens "under the demands of heavy egg production and fed a diet deficient in ascorbic acid" were cured by subcutaneous injections of ascorbic acid. In this paper we report the growth-promoting activity of ascorbic acid when added to certain *highly purified* chick rations.

Day-old White Leghorn chicks were placed in electrically heated cages with raised screen bottoms, fed the basal ration for 2 to 3 days, and then divided as uniformly as possible into groups of 6. The basal ration, 486K, as previously described in detail,[12] consists essentially of purified ingredients such as dextrin, casein, gelatin, soybean oil, salts, cystine, and known vitamins except ascorbic acid. (This ration has been modified to contain 20 γ of biotin per 100 g instead of 15 γ.) When ascorbic acid was used it was mixed in the ration in the powdered form unless otherwise noted. Water and the experimental ration, which was made up fresh weekly, were supplied *ad libitum*. This ration, 486K, is lacking in at least 2 vitamins, vitamin B_{10} and vitamin B_{11}, and possibly such factors as folic acid[13] (vitamin B_c[14]) which are supplied by solubilized liver when necessary. Without the solubilized liver chicks grow slowly, feather poorly, and become anemic. *p*-Aminobenzoic acid has been found to stimulate growth and feathering when added at high levels to this basal ration, although evidence has been presented that this compound is not a specific growth factor for the chick but acts indirectly by stimulating the production of unknown vitamins through intestinal synthesis.[12]

In Table I the results are given for the addition of ascorbic acid to the basal ration without solubilized liver and modified with various levels of *p*-aminobenzoic acid and other substances. Since the experiments were not performed at the same time, the results for each series of chicks are summarized in terms of "% of control weight," the control weight being that obtained with ration 486K plus 2% of solubilized liver. Neither ascorbic acid nor low levels of *p*-aminobenzoic acid, when fed alone to chicks on the basal ration, promoted growth, but when they were fed together at high levels the growth obtained

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TABLE I.
Results of the Addition of Ascorbic Acid to the Basal Ration without Solubilized Liver Modified with Various Levels of *p*-Aminobenzoic Acid and Other Substances.

Supplement to ration 486K (per 100 g)	Avg wt of 6 chicks at 4 weeks				Avg % of control weight
	Series 1	Series 2	Series 3	Series 4	
	g	g	g	g	
No supplement (basal only)	140	136	142	127	61
2 g solubilized liver (control)	242	234	214	212	100
100 mg ascorbic acid	150	126			58
20γ *p*-aminobenzoic acid (P.A.B.A.)				134	63
50γ P.A.B.A.		146			62
100γ "		144		164	70
100γ P.A.B.A. + 100 mg ascorbic acid			180		84
500γ "		143	124		60
500γ P.A.B.A. + 20 mg ascorbic acid			196		92
500γ " + 100 " " "		196	151		78
1 mg P.A.B.A. + 100 mg ascorbic acid			164		77
10 " " + 100 " " "			204		95
500 mg sulfasuxidine		83		85	38
500 " " + 100 mg ascorbic acid		104			44
500 mg sulfasuxidine + 100γ P.A.B.A.				101	48
500 " " + 10 mg "				151	71
1 g whole liver substance			217		101
1 " " " " + 100 mg ascorbic acid			253	247	117
10 g whole liver substance			285		133

was nearly equal to that on solubilized liver. Only traces of these compounds are present in liver fractions which provide for normal growth of chicks on this ration.[13] Ascorbic acid counteracted the depressing effect of sulfasuxidine somewhat but *p*-aminobenzoic acid at high levels was most effective. Likewise, ascorbic acid stimulated growth when fed with a low level of whole liver substance, however, whole liver substance at a higher level, without added ascorbic acid, caused chicks to grow at a still higher rate than those on the control ration indicating the absence of unknown factor(s) even in the control ration.

The effect of ascorbic acid upon growth of chicks receiving the control ration was also determined (see Table II). Unexpectedly, it was found that the feeding of ascorbic acid gave a definite and consistent, although small, increase in growth when fed at 100 mg per 100 g of ration. Although administration of ascorbic acid by dropper and by injection as the sodium salt was partly effective, adding it to the ration in solution caused all of its activity to be lost, probably due to destruction in the ration itself.

Several other related substances were fed which, in a few instances, likewise caused growth. Arabinose, when fed at a level of 1%, promoted growth to the same degree as ascorbic acid. Other levels of arabinose have not been tested as yet. It is interesting in this connection that Stokstad *et al.*[15] have shown that certain carbohydrates, especially arabinose and glucuronic acid, were active in promoting growth of chicks receiving a semi-purified ration low in the carbohydrate portion of the "rice factor." It would be interesting to know if ascorbic acid could effectively replace the arabinose and glucuronic acid under the conditions of their experiments.

The growth rate of chicks receiving the control ration was stimulated also by the addition of 500 γ of *p*-aminobenzoic acid per 100 g of ration. When ascorbic acid was fed with *p*-aminobenzoic acid no additional growth was obtained over that observed with either one alone. This may indicate that both of these compounds are acting in the same manner. Sulfasuxidine, as reported previously,[12] caused no depression of growth when fed with the liver. In fact this drug for some reason, appeared to stimulate growth when fed alone

[15] Stokstad, E. L. R., Almquist, H. J., Mecchi, E., Manning, P. D. V., and Rogers, R. E., *J. Biol. Chem.*, 1941, **137**, 373.

TABLE II.
Results of Addition of Ascorbic Acid and Other Substances to the Basal Ration with Solubilized Liver.

Supplement to ration 486K with 2% solubilized liver (per 100 g)	Avg wt of 6 chicks at 4 weeks				Avg % of control weight
	Series 5	Series 6	Series 7	Series 8	
	g	g	g	g	
None (control)	242	248	234	191	100
1 mg ascorbic acid		250			101
50 mg ascorbic acid		251			101
100 " " "	318	281	263	224	118
500 mg ascorbic acid			243		104
1000 " " "			254		109
10 mg ascorbic acid per day by dropper			253		108
5 " " " " " inj. subc.			252		108
100 mg ascorbic acid (in sol. on ration)			233		100
100 " iso-ascorbic acid				194	102
1000 mg glucose				203	106
1000 " arabinose				223	117
500γ *p*-aminobenzoic acid	294		261		117
500γ " " + 100 mg ascorbic acid			257		110
500 mg sulfasuxidine			264		113
500 " " + 100 mg ascorbic acid			275		118
Practical ration (Wis. 45W)				196	103
" " + 100 mg ascorbic acid				202	106

while ascorbic acid when fed in addition to the sulfasuxidine was of little value in promoting further growth. Growth of chicks on a practical farm ration, low in or devoid of ascorbic acid, was not helped by the addition of this vitamin. This was not surprising when we consider that the natural products in the practical ration contain arabinose and certain other carbohydrates, which may have the same effect on growth as the ascorbic acid. Thus, the addition of ascorbic acid itself to the practical ration would be ineffective and unnecessary.

Preliminary investigations were made on the ascorbic acid content of the livers.† In 13-day-old chicks which had received 100 mg of ascorbic acid per 100 g of ration there were 35.9 mg per 100 g fresh liver and 20.0 mg in chicks that had received the control ration only. Livers of chicks at 4 weeks of age showed less difference, averaging 19.6 mg per 100 g fresh weight for the group receiving the ascorbic acid and 15.3 mg for the control group. This suggests either that excess ascorbic acid is being stored in the liver or, since growth was improved, that the rate of synthesis may not be fast enough in our chicks.

Discussion. It is difficult to determine from these data just how ascorbic acid stimulates the growth of chicks but one possibility is that it acts by means of a true vitamin mechanism. It is conceivable that ascorbic acid cannot be synthesized fast enough by the chick on a highly purified ration, such as has been used in these experiments, to give a maximum rate of growth.

A second possibility is that ascorbic acid stimulates growth indirectly. It may alter the conditions within the intestinal tract to cause more effective utilization of necessary nutrients or to cause greater synthesis of unknown vitamins by intestinal organisms. We know, for example, that feathering is improved by the addition of ascorbic acid to chicks in several of the groups receiving the basal ration with *p*-aminobenzoic acid, indicating that some of the vitamin B_{10} is being synthesized. Further evidence for the indirect action of ascorbic acid is the fact that this compound and *p*-aminobenzoic acid do not give additive effects when fed together on the ration containing solubilized liver.

Another possible mechanism of action is that ascorbic acid in the ration acts as a detoxifying agent. This possibility is unlikely since doubling the levels of solubilized liver or of any of the crystalline vitamins did not depress the growth rate. Also, when the con-

† We are grateful to Miss Marie Zepplin for performing the ascorbic acid assays on the livers.

trol ration was fed with and without ascorbic acid to 2 groups of 3 rats (21 days old) both groups grew equally well for a 5-week period and appeared normal in all respects.

Until we know the mechanism of action of ascorbic acid and other compounds that stimulate growth when fed to chicks receiving such rations as used in these experiments they should be regarded as growth stimulants and not necessarily as specific growth factors. Further studies on these compounds and their mode of action are in progress at our laboratory.

Summary. Ascorbic acid, when fed to chicks receiving various *purified* rations consistently stimulates the growth rate to a small but definite extent. The exact mechanism of action is unknown at present. The relationship of growth effects obtained with ascorbic acid to those obtained with *p*-aminobenzoic acid is compared and discussed.

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A Cross-Protective Reaction Between Moccasin Venom and the Endotoxin of *Salmonella typhimurium*.

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Introduction. We have reported elsewhere[1,2] that mice immunized against the endotoxin of *Salmonella typhimurium* are to a limited extent protected against both lethal and tumor-hemorrhage doses of the endotoxin of *Rhodospirillum rubrum* and *Shigella paradysenteriæ* Flexner, and that a similar cross-protection in respect to the other two organisms is found when the immunization is performed with *Rhodospirillum* or *Shigella*. Further, a characteristic of the endotoxin of gram-negative bacteria generally has been shown to be the ability to induce hemorrhage in transplanted mouse tumors.[3,4] Analogous hemorrhage-induction has been described for venom of the water-moccasin.[5]

The present study was undertaken to determine whether a cross-protection occurs in mice immunized with the endotoxin of a gram-negative bacterium and mice immunized with the ostensibly unrelated hemorrhage-inducing snake venom.

As a representative gram-negative organism, *Salmonella typhimurium* was selected for the source of endotoxin to be compared with the lyophilized venom of the moccasin *Agkistrodon piscivorus*.* The antigenicity of this venom is recognized.[6,7,8]

Experimental. For 10 days several groups of white Rockland male mice were given daily sublethal intraperitoneal injections (about 0.5 LD_{50}) of an aqueous dispersion of the venom. At the end of this period the animals had developed sufficient immunity to survive about 2 LD_{50} amounts of venom. Other groups of mice were immunized with the endotoxin of *Salmonella*. The preparation of this bacterial endotoxin and the dosage schedule employed for the immunization are described elsewhere.[2]

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