ment after centrifugation at 7,000 g for 30 minutes. After washing with 0.58 M NaCl and recentrifugation, the sediments were frozen and dried. In two experiments a titer of 64,000 (1 mg/cc; 2 units heated Lee) was found.

Discussion. The presence in normal human urine of a potent inhibitor of viral hemagglutination makes readily available a rich source of this substance and one essentially free of protein. Separation of the inhibitor from urine can be accomplished without difficulty and yields of purified material, active at 0.0001 μg/cc, are of the order of 17 mg/liter.

The chemical properties of this substance indicate that it has much in common with similar inhibitors found previously in other biological materials and suggests that, like these, it too is a mucoprotein. The striking effects of salt concentration upon the activity of the inhibitor as well as upon its filterability and sedimentability appear to be referable to its solubility properties in electrolyte solutions. This view is supported by the finding that the inhibitor is precipitated in NaCl solutions of 0.6 M. However, there is also evidence to suggest that a heat labile component in urine may combine with the inhibitor in the presence of salt and lead to some of the observed effects.

Summary. Normal human urine contains a highly active inhibitor of viral hemagglutination, effective against PR8, Lee, FM1, swine, mumps, NDV, and GDVII viruses but inactive against PVM. The inhibitor concentration may approach 2 mg%. Considerable amounts of purified material, active at 0.0001 μg/cc, can be obtained readily. The activity of the inhibitor as it occurs in urine is inversely related to the salt concentration; it should be emphasized that in NaCl solutions of moderate strength it precipitates.

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A Feathering Syndrome in Chicks After Feeding Optimal Levels of Lysine in Absence of Arginine. (17826)

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It has been shown that arginine is important for the prevention of an abnormal feathering syndrome caused by a glycine deficiency in chicks(1) and that a lysine deficiency causes an abnormal pigmentation of the feathers in poults(2). During nutritional studies with milo gluten meal (a 44% protein byproduct of starch manufacture from milo and other grain sorghums) a peculiar feathering abnormality developed in chicks fed a diet containing 50% milo gluten meal supplemented with 2% DL-lysine. Milo gluten meal was the only source of protein in the above diet. This syndrome developed consistently in 5 successive groups of experimental chicks. This study was initiated to determine the nature of the abnormal feathering syndrome.

Experimental. New Hampshire chicks were used in these experiments. The chicks were housed in electrically heated battery brooders with raised screen floors. Feed and water were supplied *ad libitum*. The chicks were wingbanded when one day old, observed daily, and weighed weekly for a period of 4 weeks.

The basal diet (MG-1) contained 50% milo gluten meal (calculated to provide 22% crude protein), 4% salts IV, 3% corn oil,

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TABLE I.
A Comparison of the Amino Acid Content of the Basal Diet (MG-1) with the Amino Acid Requirement of the Chick.

	Biologically active amino acids in basal ration, %	Amino acid requirement of chick (% of diet)*
Arginine	0.48	1.2
Histidine	0.35	0.15-0.30
Isoleucine	1.02	0.6
Leucine	3.94	1.4
Lysine	0.32	0.9
Methionine	0.37	0.9
Phenylalanine	1.25	0.9
Threonine	1.22	0.6
Valine	1.20	0.8
Tryptophane	0.18	0.18-0.25

* Almquist, H. J., *J. Nutrition*, 1947, v34, 543.

2% fish oil (3000A-400D), and 41% corn starch. The following vitamins were added in mg per kg of diet: pyridoxine—3.0; biotin —0.2; thiamin—4.0; riboflavin—6.0; calcium pantothenate—15.0; niacin—100.00; alpha tocopherol—3.0; folic acid—2.0; choline chloride — 2000.0; menadione — 0.5; Merck APF No. 3 (calculated B_{12} 27.5 μg/kg)—1000.

Six groups of 15 chicks each were used in each test. Two such experiments are reported herein. The first group was fed diet MG-1. Four other groups were fed diets MG-9 (2% DL-lysine), MG-17 (2% DL-lysine, 0.54% DL-methionine and 1% glycine), MG-18 (2% DL-lysine, 0.54% DL-methionine, 1% glycine and 0.6% L-arginine) and MG-19 (2% DL-lysine and 0.6% L-arginine) respectively while the sixth group received diet MG-4 (18% crude casein and 10% gelatin).

The amino acid content of the milo gluten meal was determined by standard microbiological procedure(3).

Results and discussion. The amino acid content of diet MG-1 is shown in Table I. The percentages of amino acids in this instance were calculated from the microbiological assay data for the 10 essential amino acids listed. These values are given for the purpose of comparison with the amino acid requirements of the chick, according to Almquist(4). It is immediately apparent that the basal diet (MG-1) is deficient in arginine, lysine, and methionine.

Structural feathering abnormalities were not noted in any case where diet MG-1 was fed. There was a slight degree of depigmentation in the feathers of the chicks fed diet MG-1 and this was probably due to the lysine deficiency of this diet since it has been reported that a deficiency of lysine will cause a depigmentation in the feathering of bronze turkeys. It appears probable that the feathering abnormalities observed in this study when the basal diet was supplemented with lysine (diet MG-9) or with lysine, methionine, and glycine (MG-17) might have been due to the deficiency of arginine and that the addition of lysine precipitated an arginine imbalance or deficiency, which resulted in the abnormal feathering observed. Such an assumption is substantiated by the fact that arginine added with either of the above combinations (MG-18 and 19) corrected the feathering abnormalities. The work in this study was concerned only with New Hampshire chicks. However, the feathering abnormalities described have also been observed in crossbred chicks (NH males x SCWL females). When diet MG-9 (DL-lysine) was fed, the feathering of the chicks was decidedly abnormal in appearance (Fig. 1 and 2). A detailed description of the abnormal conditions of the feathers in these birds appears below. Supplementation of the basal diet with lysine, methionine, and glycine (diet MG-17) likewise resulted in the typically abnormal feathering associated with the inclusion of lysine alone in the basal diet. When the basal diet was supplemented with 2% lysine and 0.6% arginine, feathering of the birds was normal in all instances. Supplementation of the basal diet with lysine, arginine, methionine, and glycine (MG-18) likewise resulted in normal feathering. In addition it should be pointed out that the feathering of birds fed diet MG-4 (18% casein and 10% gelatin) was normal in all respects. Growth studies of the birds

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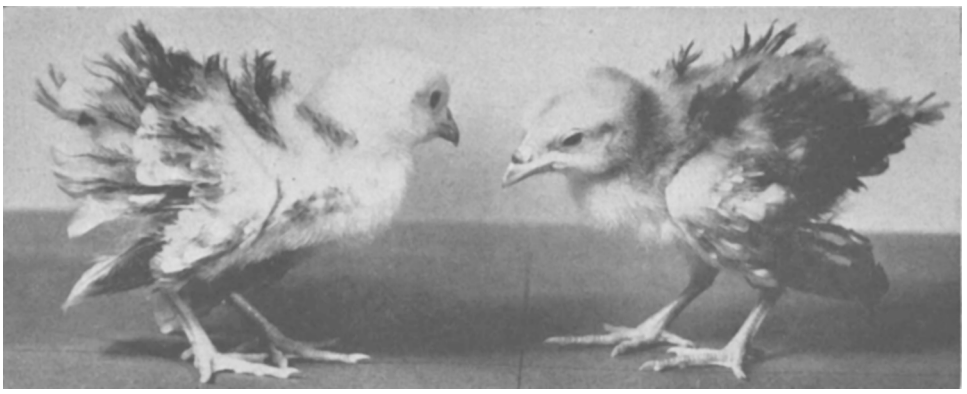

FIG. 1.
Two chicks fed diet MG-9 (2% DL-lysine). Note ruffled feathers.

FIG. 2.
Wing feathers of a chick fed diet MG-9 (2% DL-lysine).

in these tests and others, in which milo gluten meal was used, will be reported elsewhere.

The chicks fed diet MG-9 and MG-17 exhibited an extremely abnormal feathering at 4 weeks (Fig. 1 and 2). All of the feathers were spoon-like in appearance. This was apparently caused by the retention of an abnormally long feather sheath which covered the proximal third or more of the feather shaft. The spoon-like appearance was accentuated by limp and degenerate barbs and barbules along the proximal 4/5 of the feather not covered by the abnormally long sheath. The integrity of the vane was maintained only at the tip by normal barbs and barbules. The abnormally small and flexible rachis followed the normal feather contour for about one-half the length of the feather and then was bent sharply outward. The primary wing feathers were turned so that the arc described by the rachis pointed downward and the distal ends of the primaries almost dragged on the bottom of the cages. The secondary wing feathers were turned in the opposite direction and the feather tips were placed well above the level of the back.

Attention is called to the fact that DL-lysine only was fed in these tests. The question as to whether the abnormal feathering reported herein would have occurred with the feeding of D- or L-lysine in a diet low in

arginine remains unanswered.

Summary. The addition of lysine to a chick diet deficient in lysine, arginine, and methionine caused an abnormal feathering which was characterized by curved and degenerate feathers. A detailed description of the feathering abnormalities is given. When arginine was added, along with the lysine, the feathering of the birds was normal. The addition of the lysine without arginine apparently created a severe deficiency or imbalance of the latter amino acid which resulted in the abnormal feathering.

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Determination of Inulin by Means of Resorcinol. (17827)

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Despite numerous difficulties encountered in its use, inulin has remained the substance of choice for the determination of the glomerular filtration rate in man. The chief disadvantage has been the necessity of yeasting plasma and urine in the diphenylamine method(1), a step which is both cumbersome and a potential source of variable errors. Any method that avoids yeasting would be advantageous to renal physiologists and clinicians. Several investigators have examined the applicability of the reaction of resorcinol with fructose(2-4) to the determination of inulin(5-8) but differences in technic imply that methodological errors have not been reduced to a minimum. Accordingly it was decided to investigate the resorcinol method further and to compare it with the diphenylamine method under clinical conditions. Since this work was initiated, Roe(9) has reported an adaptation of the resorcinol method and we have included a comparison of our final method with this later procedure. Since completion of this work, Rolf *et al.*(10) have presented a modified diphenylamine procedure for fructose and inulin determinations. Their method advantageously eliminates the yeasting step but maintains the disadvantages of the tedious purification of diphenylamine, long heating period, and danger of crystallization of diphenylamine after cooling. Moreover, the method herein described yields a higher inulin/glucose color ratio and consequently lower blank values than the method of Rolf *et al.*

Conditions for the determination of inulin. Since the determination of inulin by the resorcinol reaction depends upon the hydrolysis of inulin to fructose, the strength of acid employed in the hydrolysis and the time and temperature of heating will all be critically important.

As judged by color development in a standard procedure using known amounts of inulin, we have found that 15 to 18% hydrochloric acid in the final reaction mixture gives maximal color development. It would appear that at this concentration maximal hydrolysis is achieved, and at the same time optimal conditions are set up for the reaction of fructose with resorcinol(3). As the concentration of acid is reduced color development becomes progressively less, while at higher concentrations slightly increased color

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