At this stage the preparation resembles a tube with a cannula in each end. The 2 cannulae are now loosely tied together. The preparation is clamped in a vertical position to a ring stand, and connected to a light lever by means of a thread. An end of the ligature about the atrio-ventricular junction is a convenient place to attach the thread to the atrium. In the resulting preparation, oxygenated Locke's solution at 36 to 37°C flows through one cannula and out the other. The various pressure levels are approximately those shown in the diagram; the chief aims in adjusting these levels are to have the atrium moderately distended and to have sufficient Locke's solution flowing through the preparation. (I have used about 10 cc per minute).

The preparation will continue to beat with a rather constant rate and amplitude for several hours. It responds to changes in the ionic composition of the Locke's solution and to epinephrine in the expected manner. Other substances have not been tried. The advantages of this preparation are its simplicity and durability.

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Effect of Diet and Cinchophen on Production of Experimental Gastric Ulcers in Chicks.

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The production of superficial gastric ulcers in young chicks by a diet deficient in greens, whole grains, and certain fats is a relatively simple procedure. The lesions which develop are never in the proventiculous or acid-secreting area of the stomach, but are always in the muscular part and are best described as gizzard erosions. A number of factors which contribute to their development, prevention, and care have been described by Dam,[1, 2] Almquist and his coworkers,[3–6]

* Supported in part by a grant from Eli Lilly and Company.

1 Dam, H., *Biochem. J.*, 1935, **29**, 1273.

2 Dam, H., and Schonheyder, F., *Biochem. J.*, 1936, **30**, 897.

3 Almquist, H. J., and Stokstad, E. L. R., *Nature*, 1935, **135**, 652.

4 Almquist, H. J., and Stokstad, E. L. R., *J. Nutrition*, 1937, **13**, 339.

5 Almquist, H. J., *J. Nutrition*, 1937, **14**, 241.

6 Almquist, H. J., and Mecchi, E., *J. Biol. Chem.*, 1938, **126**, 407.

Cheney,[7, 8] and Esselen.[9] The lesions develop in approximately 50 to 100% of all chicks, the incidence depending upon the severity of the diet and the age of the bird. Chicks raised from hens on a deficient diet show a higher incidence of severe lesions.[10] These lesions occur in a stomach which secretes highly acid juice[8, 11] rich in pepsin[12] and they are usually located where this acid juice first strikes the lining of the muscular non-acid producing portion of the stomach or gizzard.

Histologically the lesions involve the chitinous gizzard lining of the lumen and the underlying mucosa only, and tissue necrosis does not occur. In the examination of over 2000 birds, the writer has never seen any penetrating or necrotizing ulceration from dietary deficiency alone. The dietary substance, an absence of which causes these erosions, has been termed the anti-gizzard erosion factor and was originally thought to be identical with vitamin K. Although it is closely associated with this vitamin, it is a separate substance as shown by Almquist.[4]

During the last 5 years the writer has carried out a number of experiments to try to produce lesions which were more than superficial and which would present the characteristics of true peptic ulcers as seen in the human stomach. These studies were fruitless until the last 6 months when cinchophen was fed to the chicks in their diet. This drug was chosen because of its well known capacity to produce gastric ulcers in dogs[13, 14] and cats.[15] The results of these cinchophen feeding experiments have been eminently successful not only in the production of penetrating and perforatory gastric ulcers typical of human peptic ulcers histologically, but also in demonstrating that a protective factor in the diet can nullify or modify ulcer production by cinchophen.

Some 250 chicks have been given diets containing cinchophen and a like number of controls have been observed. Examples of these experimental studies are shown in Table I. The basal diet (B, D in table) used is the same as that previously described[8] and is deficient in the anti-gizzard erosion factor. Twenty-five percent and 10% sup-

7 Cheney, Garnett, *Arch. Int. Med.*, 1937, **60**, 705.

8 Cheney, Garnett, *Am. J. Digest. Dis.*, 1938, **5**, 104.

9 Esselen, W. B., *Poultry Science*, 1939, **18**, 201.

10 Almquist, H. J., personal communication.

11 Friedman, M. H. F., personal communication.

12 Cheney, Garnett, unpublished work.

13 Wagoner, F. H., and Churchill, T. P., *Arch. Path.*, 1932, **14**, 860.

14 Stalker, L. K., Bollman, J. L., and Mann, F. C., *Arch. Surg.*, 1937, **35**, 290.

15 Schwartz, S. O., and Simonds, J. P., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1133.

TABLE I.

Effect of Diet, Cinchophen Feeding, and Certain Protective Supplements Added to the Diet, on Grade of Ulceration in Stomachs of Chicks.

Series No.	Diet	No. of chicks in experiment		Cinchophen		Protective supplement	Age at autopsy days	Avg wt at autopsy g	Grade of ulceration
		Beg.	End	% of diet	Days fed				
S-1, 1 and 2	R.F.S.M.*	12	10	—	—	—	28	104.5	0.20
S-1, 4	"	12	6	1.5	14	—	28	64.0	2.67
S-1, 3 and 5	"	24	17	1	14	—	28	66.1	0.84
S-1, 7	" + 25% alfalfa	12	0	1	14	—	28	—	—
S-5, 7	B.D.†	8	8	—	—	—	21	152.0	0.75
S-5, 6	"	10	5	2	14	—	20	55.6	1.80
S-2, 4	"	12	8	1.5	14	—	29	106.0	2.90
S-2, 6	"	8	8	1.5	14	Fresh milk	28	131.6	1.63
S-3, 5	"	12	8	1	10	—	21	98.3	2.20
S-3, 8	"	12	9	1	10	Fresh milk + 1% M.G.‡	20	110.4	0.89
S-2, 9	" + 25% alfalfa	6	6	—	—	—	28	102.5	0.17
S-5, 8	" " "	10	1	2	14	—	19	—	—
S-2, 8	" " "	9	8	1.5	14	—	29	94.75	4.0
S-3, 6	" " "	12	12	1	10	—	21	96.7	2.67
S-3, 7	" " "	7	7	0.5	10	—	21	108.7	2.0
S-4, 1	" + 10% "	7	7	—	—	—	21	173.14	0.07
S-4, 3	" " "	10	8	1.5	11	—	22	78.25	4.0
S-4, 8	" " "	10	6	1.5	11	5% Cerophyl	22	96.0	1.75
S-4, 6	" " "	10	8	1.5	12	1% "	23	80.75	2.6
S-4, 7	" " "	10	10	1.5	8	5% MG‡	23	110.6	2.35
S-4, 5	" " "	10	8	1.5	11	1% "	22	89.5	2.7
S-4, 4	" " "	10	7	1.5	10	0.5 cc bile daily	21	86.6	3.2
S-3, 4	" " "	10	10	1.0	10	—	20	154.5	2.55

*R.F.S.M.—Equal parts rice flour and skimmed milk powder.
†B.D.—Basal diet (see text).
‡M.G.—Dried canned mixed greens (Heinz).

plements of dry alfalfa were added to it to give bulk. The larger percent of alfalfa ordinarily gives moderate protection against erosions, but interferes with the nutritive value of the diet, while the 10% level offers only slight protection. A diet of equal parts of rice flour and dried skimmed milk powder was originally used (R. F. S. M. in table) which contained the anti-gizzard erosion factor, but it did not prove satisfactory as the control birds failed to gain weight satisfactorily and ultimately developed "crazy chick disease" reported as vitamin E deficiency.[16]

Cinchophen (Merck) was added to the diet at levels from one-half to 2%. Dietary supplements containing the anti-gizzard erosion factor were added at levels of one and 5% in the form of dried canned mixed greens (asparagus, lettuce, and kale)† and cerophyl (dried grass stalks)‡ and as fresh whole pasteurized milk substituted for drinking water, as these substances have previously been shown to be excellent sources of the factor.[12] Bile was added in one experiment in accordance with Almquist's findings that it is highly protective against gizzard erosions.[6]

The birds were raised as already described;[8] and the various special diets were started from the third to the fourteenth day after hatching.§ Cinchophen feeding was not started until 5 to 7 days later, except in the fourth series of birds (S-4 in the table) when it was started simultaneously with the supplemental diet. Six to 12 chicks were utilized for each experiment and the survivors were autopsied during the fourth week of life as a rule. Post-mortem examination was carried out on birds dying while under observation and on those which were well preserved. The average weight of the chicks at the time of autopsy is recorded in the table as an indication of their state of nutrition at the time of death.

As an indication of the severity of the lesions which were produced, they are scored in the table on a basis of depth as follows: Grade 1, hemorrhage in the mucosa but without any break in the overlying chitinous layer. This is the earliest change which occurs. Grade 2, an erosion of the superficial gastric lining and mucosa but without evidence of any reaction in the underlying structures. Grade 3, similar to Grade 2 but the lesion has a punched-out appearance often with indurated margins and with a flat base on the submucosa

[16] Pappenheimer, H. M., Goettsch, Marianne, and Jungherr, Erwin, Bulletin 229, Connecticut State College, Storrs, Conn.

† Supplied through the courtesy of Heinz and Company.

‡ Supplied through the courtesy of the Cerophyl Company.

§ All birds were hatched and supplied through the courtesy of Poehlman's Hatchery in Petaluma, California.

or underlying muscle layer. Grade 4, definite penetrating ulcers with muscular tissue invaded. Grade 5, a perforated ulcer with localized or generalized peritonitis. Grades 1 and 2 are found in dietary deficiency alone; grades 4 and 5 only when cinchophen has been added. Grade 3 is rare in dietary deficiency alone, but very common with cinchophen feeding. Multiple lesions of varying depth were not infrequently encountered. They were graded according to the deepest lesion.

Experimental Results. It is evident from the data presented in the table that the diets devoid of roughage were not very satisfactory as severe grades of ulceration were unusual. Chicks fed on this type of mash diet often do not gain well[17] and the yellow liquid "mustard water" droppings which they passed indicated that the cinchophen might have been eliminated too rapidly for complete absorption to take place. The diet with 25% roughage proved unsatisfactory because the general nutrition of the birds was interfered with. The group of chicks receiving the basal diet plus 10% roughage not only gained weight satisfactorily, but showed a high percent of lesions of the peptic ulcer type when cinchophen was added.

It is also evident that the level of 2% cinchophen in the diet was too toxic for satisfactory experiments, as many of these birds died within 24 to 48 hours with loss of appetite and profound weakness and prostration. Very few survived the period of the experiment. A number of birds in Groups S-5, 6, and S-5, 8 apparently died hepatic deaths. At autopsy the livers were tan to yellow ochre in color and histologically showed marked infiltrations of fat. No definite evidence of hepatitis was found. The only other group of birds which may have suffered liver damage was S-2, 3 which received 1.5% cinchophen and 2% bile salts.¶ Five of these birds were satisfactory for autopsy study and showed small tan livers, huge distended gallbladders and ascites. Histologically the livers showed no evidence of hepatitis.

In general the evidence of severe gastric lesions diminished in direct proportion to the amount of cinchophen fed and when the drug was at 0.5% level, the lesions were only slightly more pronounced than those obtained on a deficient diet alone. With the higher concentrations of cinchophen, the initial lesion was evident in the first 24 to 48 hours but with the lower levels not until the third or fourth day of feeding. Perforations occurred as early as the fifth day but more often between the seventh and fourteenth days.

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17 Hart, E. B., Halpin, J. G., and Steenbock, H., *J. Biol. Chem.*, 1920, **43**, 421.

¶ This group has not been included in the table as only two of seven birds survived the experiment. They ate very little of the mash and failed to gain weight.

When protective supplementary substances containing the anti-gizzard erosion factor were added to the diet a definite diminution in the severity of the lesions was noted even when these substances were fed at only a one percent level. This was particularly noticeable in Group S-3, 8 when whole milk was also added to the diet. The very low grade of ulceration obtained in this group of chicks receiving one percent cinchophen should be compared with the control group S-3, 7 which received 0.5% cinchophen, as the chicks receiving milk consumed only a little over one-half as much feed as this group and consequently proportionately less cinchophen. The results of protective feeding in Group S-4, 8 are particularly worthy of note. The grade of lesions for this whole group including the 5 birds which succumbed from perforation before the experiment was completed was 2.95, but for the 5 survivors was only 1.75. As this group of birds did not receive the protective substance before cinchophen feeding was started, the results indicate that protection was beginning to develop after the first 5 to 7 days and that the good end results were due to healing of previously formed lesions.

The failure of fresh bile to show a beneficial effect on the gastric lesion is difficult to explain. Further studies of a similar nature must be carried out. Possibly hepatic function is impaired by the cinchophen so that the bile cannot be satisfactorily utilized. Perhaps bile simply facilitates the absorption of the anti-gizzard erosion factor and this function is impaired by impaired liver function. In this connection it is of interest to note that dogs with biliary fistula and complete loss of bile develop a high incidence of gastric ulceration even when maintained on a normal diet.

Conclusions. 1. Feeding cinchophen to chicks produces gizzard lesions with the characteristics of peptic ulcers. 2. These gastric ulcers vary in severity with the amount of cinchophen fed. 3. The early lesions are similar to those produced by a dietary deficiency alone. 4. The lesions are less severe in chicks fed a dietary supplement containing the anti-gizzard erosion factor.