TABLE II.
Effect of Dibuline (10 mg I.M. at 9:30 p.m., 1:30 and 5:30 a.m.) on 12-Hour Nocturnal Gastric Secretion in Man.

	Control			During Dibuline			% change		
Case	Vol. (cc)	Free HCl (Cl units)	Free HCl (mg)	Vol. (cc)	Free HCl (Cl units)	Free HCl (mg)	Vol.	Free HCl (conc.)	Total output HCl
1	435	75	1191	744	54	1456	+71	—28	+
2	998	89	3200	1456	79	4170	+46	—	+30
3	401	49	719	506	34	624	+26	—30	—
4	1188	42	1833	1047	69	2616	—	+64	+43
5	553	67	1352	671	76	1851	+	+	+37
6	922	59	1988	1103	65	2593	+	+	+30
7	583	60	1282	671	72	1761	+	+	+37
8	1188	90	3892	1074	94	3690	—	+	—
9	1026	83	3103	1207	78	3416	+	—	+
10	598	56	1211	616	35	790	+	—38	—35
11	740	58	1556	622	39	885	—	—33	—43
12	1008	33	1208	757	14	387	—25	—58	—68
13	1232	63	2802	726	33	878	—41	—48	—69
14	1098	48	1908	591	33	491	—55	—31	—74
15	572	42	875	214	68	526	—63	+64	—40

mg) seems comparable to 1.0 to 2.0 mg of atropine. The decrease in gastric secretion, when it occurs, persists usually for 2 to 3 hours only. The repeated administration of the drug 4 hours after the initial dose is no more effective than the initial injection.

Dibuline is tolerated better than atropine. The parenteral injection of the latter in 1.0 mg doses every 4 hours, very frequently produces marked dryness of the mouth, blurring of vision, and tachycardia. Repeated injections of 10 mg of dibuline every 4 hours very seldom produce side effects; when they do occur, however, the symptoms are mild and of brief duration.

Conclusions. The effect of dibuline on gastric secretion is variable, transitory, and unpredictable. Further trial of the drug in the treatment of peptic ulcer seems to us unwarranted.

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Possible Sources of the Androgenic Factor in Cow Manure. (17384)

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The bacterial flora of the stomach of a ruminant is known to synthesize a number of compounds: proteins from urea;[1] vitamin B complexes,[2,3] etc. It was felt desirable to determine changes in 17-ketosteroids on incubated feces.

Turner[4] finds an androgenic factor in fecal material of pregnant cattle and goats. Longwell and Gassner[5] report increased size of comb when chicks are fed dried fecal material as part of their diet. They have made some attempt at characterization of the compound but have not reached a definite conclusion as to its chemical nature.

It seems desirable to investigate two pos-

1 Smith, J. A. B., and Baker, F., *J. Biochem.*, 1944, **38**, 496.

2 Hunt, C. H., Burroughs, E. W., Bethke, R. M., Schalk, A. F., and Gerlaugh, Paul, *J. Nutrition*, 1943, **25**, 207.

3 Burkholder, Paul R., and McVeigh, Ilda, *Proc. Nat. Acad. Sci. U. S.*, 1942, **28**, 285.

4 Turner, C. W., *J. Dairy Science*, 1947, **30**, 1.

5 Longwell, Bernard B., and Gassner, F. X., *Fed. Proc.*, 1947, **6**, 272.

sible sources of the androgens found in the feces of pregnant cattle and to study the properties of the material extracted: first, its production by bacterial flora upon incubation of fecal material; and secondly, its presence in demonstrable amounts in the bile of pregnant cows. Immature rats, ovariectomized female rats and single comb White Leghorn baby chicks, both sexes, served as experimental subjects.

Experimental. Collection and disposition of samples of feces. The cow manure, free of urine, was collected within 2 hours after dropping from a pregnant cow of 7½ months duration. The sample was thoroughly mixed and divided into 3 portions which were placed in sterilized fermentation jars. Sample No. 1 weighed 3333 g; No. 2, 3379 g; No. 3, 3751 g. Sample No. 1 was immediately covered with absolute alcohol in such amounts that with the water present, the mixture was about 70% alcohol. The untreated samples No. 2 and No. 3 were placed in an incubator at 37.5°C, the former remaining for one week, and the latter for 2 weeks.

Extraction of feces. The feces, which was placed in absolute alcohol as indicated above, was extracted in the cold 3 times with agitation, using calculated amount of 95% alcohol to give about 70% concentration. The combined extracts were allowed to stand overnight, centrifuged and decanted. This solution was evaporated to dryness under reduced pressure to a small volume. It was then boiled with norite and filtered through super sal to remove colored matter. The alcohol solution was evaporated to dryness under reduced pressure and this procedure repeated until all the water was removed. The residue was taken up in alcohol so that 1 ml contained the equivalent of 15 g of fresh manure. After their respective periods of incubation, samples No. 2 and No. 3 were treated in a like manner. All were very dark in color, which boiling with norite and filtering through super sal did not remove.

Such high dilutions had to be made in order to be able to apply the Zimmermann test, that the following further attempt was made to remove the chromogenic material. An aliquot of each sample was evaporated to dryness under diminished pressure, taken up in water and extracted 3 times with carbon tetrachloride. These were then washed twice with 2N NaOH, and once with 10 cc of Lycopon (1N NaOH with 10 g of sodium hydrosulfite). The CCl_4 was evaporated to dryness under diminished pressure and the residue dissolved in alcohol. Samples No. 1 and No. 3 still were a dark brown color and No. 2 was a bright yellow color. Later tests with the Zimmermann test indicate that for some reason No. 2 gave no definite absorption band at 508 mμ.

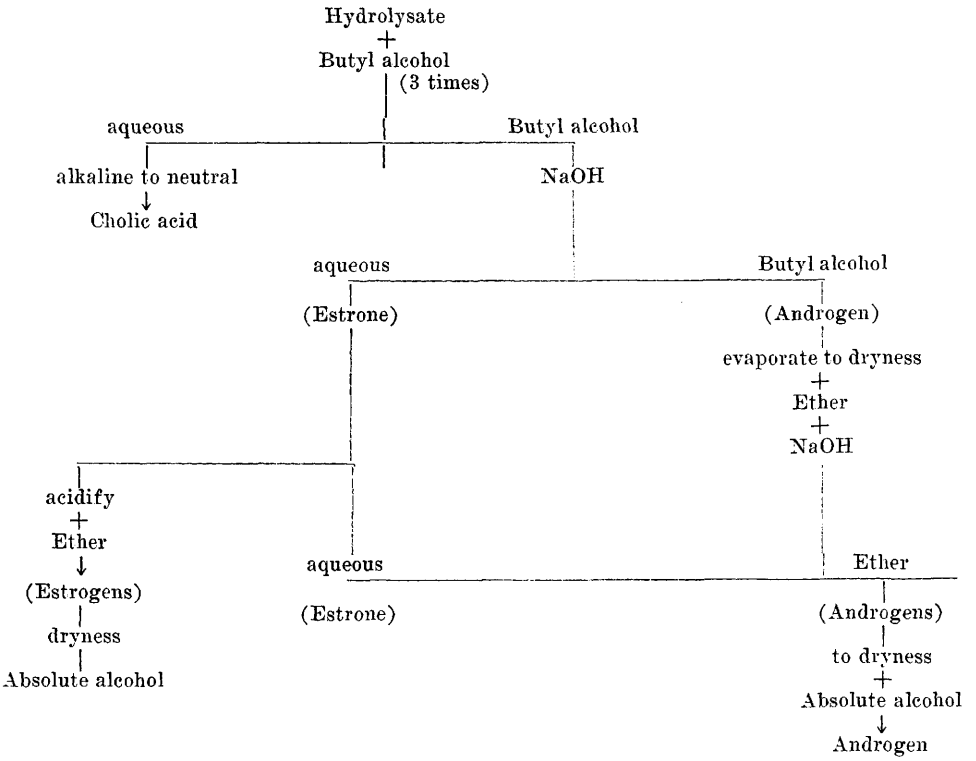
Following the alcoholic extraction of the feces, some of the extract from sample No. 1 was then evaporated to dryness and extracted 3 times with ether. The ether was evaporated to dryness under diminished pressure, and the residue dissolved in absolute alcohol. This will be referred to as sample No. 4.

Extraction of bile. Bile was obtained from pregnant cattle of 3-4 months duration. To it was added one-fourth its volume of concentrated hydrochloric acid, and the mixture boiled for 15 minutes. The hydrolysate was then treated as shown on following page.

Test for androgenic factor using baby chicks; application of extract of feces. Two-day-old single comb White Leghorn chicks of both sexes were used in the test. The extract, equivalent to 3 g fresh manure, was applied to the comb each day for about 4 weeks. This was done only on the fresh extract and not on incubated fecal material in order to demonstrate the presence of androgens in the sample under consideration. Increased comb growth was noted in both male and female chicks, but it is not of mathematical significance.

Application of extract of bile. The extract of bile was applied topically exactly as described for application of fecal extract. An equivalent of 3 ml of fresh bile was applied daily on each chick's comb. An increase in comb growth of both male and female chicks was demonstrable.

Spectrophotometric studies. Samples before and after attempt to remove chromogens were equilibrated and tested for 17-ketosteroids by the Zimmermann reaction. K-values (where $K = \log I_o/I$) for these before



and after the removal of the chromogens are shown below at 508 mμ for the original sample (No. 1) and the 2 which were incubated for one and 2 weeks (Nos. 2 and 3, respectively).

The K-value obtained is a comparative measure of the quantity of the 17-ketosteroids present. Since there was no increase in the K-value, it is conclusive that no increase of 17-ketosteroids was experienced on incubation and that therefore no increase of androgens was evident upon incubation as carried out. Since there was no increase in 17-ketosteroids, no biological androgenic assay was considered necessary to prove that the bacterial flora of the feces was not responsible for the increased androgenic content of pregnant cow manure.

TABLE I.

Sample	Col. I Before	Col. II After
1	1.524	1.533
2	1.164	no peak
3	1.255	1.322

Results and conclusions. 1. The Zimmermann test showed that there was no significant difference in the amount of 17-ketosteroids before and after incubation periods up to 2 weeks. Since the material tested in column II has been extracted thoroughly with 2N NaOH, little estrogenic substance can be present. Hence the figures obtained are believed to indicate androgens.

2. It is therefore believed that the bacterial flora of the ruminant stomach is not concerned with the increased androgenic content of the feces of a pregnant cow. If any changes are obtained, it appears that there is a loss of androgens during incubation, or a loss by the second treatment.

3. The bile from pregnant cows showed a demonstrable amount of an androgenic substance.

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