secondary rise is much delayed in the presence of amino acid, corresponding to the extended period of motility and fertilizing capacity. 7. The secondary rise is associated with the growth of bacteria as the spermatozoa die and disintegrate. 8. The addition of penicillin (50 units per ml) is efficacious in checking bacterial growth and the secondary rise in oxygen uptake in suspensions of senescing spermatozoa. 9. In the presence of amino acid, motility and fertilizing capacity can be maintained near their original level for many hours after the rate of oxygen uptake has dropped to less than 5% of the initial rate. 10. Previous evidence that the spermatozoa do not metabolically utilize the added amino acid to any appreciable extent is further supported by the results of experiments with carboxyl C^{14}-labelled glycine. 11. The total oxygen consumption of spermatozoa in the presence of amino acid exceeds that of the controls, which means that the death of the spermatozoa under ordinary conditions is not due to substrate exhaustion but rather to the inability to utilize fully their endogenous substrate. 12. While sea urchin spermatozoa in sea water are quickly immobilized by lack of oxygen, the presence of amino acids enables them to remain highly active for as long as 6 hours at room temperature. 13. No significant glycolysis, CO_2 production, production of ammonia or urea formation is found to occur anaerobically in the presence or absence of amino acid and glycolyzable sugar.

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Antibiotics in Mature Fowl Nutrition.* (18387)

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Olcese, Couch and Lyman(1) reported that the hatchability of eggs from hens fed a diet low in vit. B_{12} decreased to zero in 2 to 4 weeks and further that the hatchability of eggs from hens fed this diet, supplemented with APF concentrates, decreased about the ninth or tenth week of the experimental period. Subsequently Olcese and Couch(2) showed that the injection of crystalline B_{12} into eggs from hens fed a diet low in the vitamin promoted normal hatchability from the sixth through the eighth week but failed to do so after the ninth week in a 17-week experiment. The above-mentioned reports indicate that the hens were depleted of an unidentified factor which was required for normal embryonic development about the ninth or tenth week of the experimental period, since B_{12} was supplied either in the form of an APF concentrate or as the crystalline vitamin. Couch *et al.*(3) in a later report showed that liver fraction "L" contained the unidentified hatchability factor since the feeding of this substance maintained normal hatchability for a period of 16 weeks; whereas, hatchability of eggs from hens given weekly injections of B_{12} decreased after the twelfth week of the test. Cravens and Halpin(4) reported earlier that Liver "L" and fish solubles contained an unidentified factor required by the hen for egg production and hatchability. A number

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of workers have also reported that unidentified factors are required in addition to vit. B_{12} for chick growth(5-9). It should be stated further that antibiotics have recently been shown to have a stimulatory effect on chick growth(10,11).

With the latter reports in mind it appeared desirable to determine whether the antibiotics might have a favorable effect on the hatchability and vitamin B_{12} content of eggs from hens fed diets low in B_{12} and low in the unidentified hatchability factor.

Experimental. Single Comb White Leghorn hens that had been used in an earlier experiment were utilized for the present study. For 5 weeks preceding the start of this test these birds were fed a practical all-mash ration. Records of egg production and hatchability were kept during this period and only birds that were laying at a satisfactory rate and that were producing eggs that would hatch were used in the present experiment.

At the end of the pre-experimental period 48 hens were selected and were divided into 12 groups of 4 hens each. Individual egg production and hatchability records were used in making the group assignments. The basal diet (A-15) was composed of 63% sucrose, 5% dried whey, 22% soybean protein (alpha), 3% soybean oil, 2% fortified fish oil (3000A-400D) and 5% salts IV. The following amounts of vitamins were added in mg per kg: thiamine hydrochloride, 40; riboflavin, 6; calcium pantothenate, 15; niacin, 100; pyridoxine hydrochloride, 4; 2 methyl-1, 4 naphthoquinone, 0.5; alpha tocopherol, 3; folic acid, 2; biotin, 0.2; choline chloride, 2000; PABA, 20; and inositol, 1000. In addition 7.5 g methionine and 4 g glycine were added per kg of diet. The crude materials used in this study (liver extract, Lilly Laboratories; APF 4†, Lederle Laboratories; and Liver fraction "L", Wilson and Company) were assayed for B_{12} content by the method of Skeggs *et al.*(12). The liver extract contained 13 μg B_{12} per ml and the APF 4 and liver fraction "L" contained 0.6 and 1.24 μg B_{12} per g respectively.

In view of the fact that Couch *et al.*(3) had found that dried whey would improve egg production, 5% of this product was used in diet A-15 of the present study. The first group of 4 hens was fed diet A-15 unsupplemented. Groups 2 through 11, inclusive, were fed diet A-15 supplemented as shown in Table I. Supplements fed groups 10 and 11 were added to diet A-15 in place of an equivalent amount of sucrose. The hens in group 12 of this experiment were fed a practical all-mash diet. The practical diet contained: 42% ground yellow corn, 15% ground oats, 10% wheat bran, 10% wheat gray shorts, 2% meat and bone scraps, 5% dehydrated alfalfa leaf meal, 2% fish meal, 12% soybean oil meal, 1% bone meal, ½% salt, and ½% fortified fish oil (3000A-400D). Ten grams of manganese sulphate and 20 g BY-21 (riboflavin supplement, Commercial Solvents Corporation, Terre Haute, Ind.) were also added per 100 lb. All experimental diets were mixed fresh once each week, and were kept before the birds at all times. Oyster shell and tap water were supplied *ad libitum*. The management and experimental procedure with regard to the care and handling of the birds, insemination, incubation and mortality records were the same as that previously reported by Olcese, Couch and Lyman(1).

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† Stated to contain 2-4 mg aureomycin per g by the manufacturer.

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TABLE I. Effect of Vitamin B_{12}, Liver Extract, Antibiotics, Lederle APF, and Liver "L" on Hatchability and Vitamin B_{12} Content of Egg Yolks.

Group	Supplement to basal diet (A-15)	Avg % hatchability		Avg vit. B_{12} content of egg yolks (mμg/g)	
		2-4 wk	5-8 wk	2-4 wk	5-8 wk
1	None	25	0	1.4	0.9
2	2 μg B_{12} inj.*	38.4	14.2	7.5	5.2
3	0.5 ml liver extr. inj.*	50	33.3	7.2	4.1
4	Aureomycin HCl (66 mg/kg)	0	0	2.6	1.1
5	Aureomycin HCl (66 mg/kg) + 2 μg B_{12}*	26.6	10	5.8	5.7
6	Streptomycin dihydrochloride (66 mg/kg)	52.9	4.3	3.0	1.4
7	Streptomycin dihydrochloride (66 mg/kg + 2 μg B_{12}*	50	6.6	7.8	4.0
8	Procaine penicillin (G) (33 mg/kg)	5	0	2.3	1.3
9	Procaine penicillin (G) (33 mg/kg) + 2 μg B_{12}*	62.9	10	3.6	4.5
10	2% APF 4 (24 μg B_{12})	71.4	26.6	7.1	6.1
11	4% liver "L" (49.6 μg B_{12})	95.4	78.3	7.8	6.8
12	Practical	96.2	90.4	2.7	1.2

* Per hen per week.

Eggs were set aside on the first day of the hatching week for the determination of the vit. B_{12} content of the yolks. The egg yolks of individual hens within a group were pooled. Ten grams of the pooled sample were accurately weighed, 190 ml of NaAc buffer (pH 4.5) were added and the sample was blended in a Waring Blendor. An aliquot equivalent to 1 g of egg yolk was pipetted into a 125 ml Erlenmeyer flask and the flask was stoppered with a cotton plug. Vitamin B_{12} was liberated from the egg yolk by autoclaving for 30 minutes in the NaAc buffer at pH 4.5 at 15 lb pressure. After autoclaving, the pH of the sample was adjusted to 7.0 and the volume was made up to 100 ml. This solution was filtered and stored in a refrigerator at approximately 20° C. Dilutions for assay purposes were made from this stock solution. The B_{12} content of the egg yolks was determined by the method of Skeggs *et al.*(13).

Results and discussion. The average percentage hatchability of eggs from hens fed diet A-15 unsupplemented was 25 for the second, third, and fourth weeks (Table I). No live chicks were obtained from these hens after the fourth week of the experiment. This observation is in agreement with the work of Couch *et al.*(3) where diet A-15 was fed under similar conditions. It should be pointed out that the vit. B_{12} content of egg yolks from hens fed diet A-15 alone was lower than that of any other group in the test (Table I). The hatchability of eggs from hens injected with B_{12} (Group 2) was higher than that of those where no B_{12} was injected. There is little question but that the B_{12} content of egg yolks was increased by the injection of the vitamin into the birds (Table I). A further increase in hatchability was apparent when the hens were each injected with .5% liver extract (reticulogen) per hen per week (Group 3). The vit. B_{12} content of the egg yolks from these birds is approximately the same as that of those from Group 2 (Table I). The feeding of aureomycin and penicillin apparently assisted in the depletion of the birds of vit. B_{12} and possibly of the unidentified factor (Groups 4 and 8), since in the latter 2 cases the hatchability from the second through the fourth weeks, inclusive, is lower than that of birds not fed the antibiotic (Table I). When vit. B_{12} was injected and aureomycin was fed (Group 5) the hatchability was only slightly lower than that of Group 2. Hatchability of

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eggs from birds fed penicillin and injected with B_{12} (Group 9) is higher than that of Group 2 for the second through fourth weeks, inclusive. The feeding of streptomycin (Groups 5 and 6) appeared to maintain a somewhat higher level of hatchability during the second through the fourth week, inclusive, than that of comparable groups (1 and 2, Table I). The feeding of 2% APF 4 (Group 10) produced a higher percentage hatchability than did the injection of vit. B_{12} (Group 2). Couch *et al.*(3) have reported earlier that this APF, which is a by-product from the manufacture of aureomycin, probably contains some of the Liver "L" hatchability factor, although these workers fed 5% of this APF to demonstrate this fact. The hatchability of eggs from birds fed 4% Liver "L" (Group 11) is approximately the same as that of those fed the practical all-mash diet during the second and fourth weeks, inclusive, and slightly lower during the fifth through eighth week, inclusive. From these data it is quite evident that Liver "L" contains a factor necessary for hatchability and embryonic development, which further substantiates the work of Couch *et al.*(3).

It should be pointed out that the injection of vit. B_{12} into the birds (Group 2, 5 and 7) produced egg yolks that contained approximately the same amount of vit. B_{12} (Table I). Attention is also directed to the fact that egg yolks from birds fed APF 4 (Group 10) and Liver "L" (Group 11) contained about the same quantity of vit. B_{12} as did egg yolks from hens injected with vit. B_{12} (Groups 2, 5 and 7). It is not possible to explain the decreased B_{12} content of egg yolks from birds fed penicillin and injected with B_{12} (Group 19); particularly during the second through the fourth week, inclusive.

The data on the B_{12} content of egg yolks from birds fed the practical diet is of considerable interest, since the B_{12} content of yolks from these birds is low when compared to that of egg yolks of birds injected with vitamin B_{12} (Groups 2, 5 and 7) or fed APF 4 (Group 10) or Liver "L" (Group 11). Yet the percentage hatchability of eggs from birds fed the practical diet was above 90% throughout the test. This practical all-mash diet has been used for the production of depleted chicks to use in B_{12} chick work. It is believed that the data obtained on the vit. B_{12} content of the egg yolks points to the fact that vit. B_{12} is not the limiting factor in this diet, although the egg yolks obtained were low in B_{12}. It does point to the fact that even though the egg yolks are low in B_{12} that the diet contains an adequate amount of the Liver "L" factor or provides a favorable media where such factor can be synthesized in the intestinal tract of the bird.

Summary. Data have been obtained on the effect of feeding aureomycin, penicillin, streptomycin and APF concentrate and Liver "L" in a diet low in vit. B_{12}. Hatchability of eggs from birds fed this diet unsupplemented decreases to zero after the fourth week. Injection of vit. B_{12} into the hens improved hatchability somewhat. The feeding of aureomycin and penicillin alone apparently assisted in the depletion of birds of vit. B_{12} and possibly of the unidentified factor. Liver "L" contains a factor necessary for normal hatchability and embryonic development. The B_{12} content of the egg yolks was determined by a standard microbiological assay. Injection of vit. B_{12}, feeding of an APF concentrate and the feeding of Liver "L" produced egg yolks that contained approximately the same amounts of vit. B_{12}. Hatchability of eggs from hens fed the practical all-mash diet was about 90% during the test period. Yet, the vit. B_{12} content of egg yolks from these birds was lower than that of similar birds injected with vit. B_{12} or fed a source of vit. B_{12} in the form of an APF concentrate or Liver "L."

Aureomycin hydrochloride, streptomycin dihydrochloride, penicillin, crystalline vitamins, and one APF concentrate (APF 4) used in this study were generously supplied by Merck and Company, Rahway, N. J. and Lederle Laboratories, Pearl River, N. Y.

Soybean protein (alpha) was obtained from Glidden and Co., Chicago, Ill.

Soybean oil was contributed by Dr. J. E. Hunter, Allied Mills, Inc., Libertyville, Ill., and Buckeye Cotton Oil Company, Cincinnati, O.

Liver fraction "L" was contributed by Dr. David

Klein, The Wilson Laboratories, Division of Wilson and Company, Chicago, Ill.

The methionine and glycine were obtained through the courtesy of Dr. Julius Johnson, The Dow Chemical Co., Midland, Mich.

The dried whey was supplied through the courtesy of the Western Condensing Co., Appleton, Wisc.

The liver extract (reticulogen) was supplied by Lilly Laboratories, Indianapolis, Ind.

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Effects of Oxygen, Analeptics, and Artificial Respiration on the Toxicity of Procaine.* (18388)

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The introduction into the blood stream of procaine in sufficient amount to cause a sudden high concentration is thought to cause circulatory collapse or "cardiac death," while a gradual increase in blood procaine produces first a central stimulation which is followed by depression with eventual respiratory failure(1-4). If this be true the most frequent cause of death from slow intravenous infusions of procaine should be respiratory failure. Such has been reported to be the case (5-8), although cardiac and respiratory failure may occur almost simultaneously(2,9). Experiments designed to study the effect of anesthesia and the rate of injection on blood levels and toxic reactions during intravenous infusions of procaine permitted preliminary observations on the cause of death from such administrations. In 81 out of 83 experiments on dogs, the heart continued to beat for from 30 seconds to 8 minutes after respiratory movements ceased. In the other 2, heart apparently stopped at the same time as respiration. It is of interest that these 2 dogs were anesthetized with ether, which has been shown to sensitize animals to procaine(10,11). These observations stimulated us to study in more detail the cause of death from slow intravenous infusions of procaine and to evaluate experimentally the therapeutic effectiveness of analeptics, oxygen, and artificial respiration.

Experimental. Controls. Eleven experiments were run in which the dogs breathed air. Blood pressure and respiratory movements were recorded on a kymograph by means of a mercury manometer and a Manning pneumograph. The condition of the heart was also checked with a stethoscope and respiratory movements by close observation of the animal. Ether was administered to produce light anesthesia for from 30 to 45 minutes before the procaine infusion was started. The procaine hydrochloride was injected as a 1 to 5% solution in normal saline by way of the right femoral vein at a constant rate of 2 mg/kg/min. The infusion of procaine was continued until respiratory movements ceased, at which time it was stopped. The ether was discontinued when the procaine infusion was started and thereafter was administered in the minimal amount

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