

fection in that a purulent discharge develops and such complications as Bartholin gland abscess, salpingitis and peritonitis may ensue. In males, these organisms have been found thus far only in cases of chronic prostatitis, chiefly in patients with history of previous gonococcal infection. The infection appears to be transmissible by sexual intercourse. Although the strains which we have recovered have bred true through many subcultures, one must bear in mind the possibility that these strains may represent variant forms of bacteria, perhaps of gonococci.^{2, 3, 4}

The incidence of arthritis in these patients is of particular interest because arthritis is a common complication of both the natural and the experimental diseases produced in animals by organisms of this group. We have not been able, however, to recover pleuropneumonia-like organisms from diseased joints, although we have examined a considerable number of specimens of synovial fluids, synovial tissues and subcutaneous nodules from arthritic patients.

The methods used for the cultivation and examination of pleuropneumonia-like organisms have been previously described.⁵

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Effect of Biotin on Reproduction in the Domestic Fowl.

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Numerous studies have shown the importance of biotin in chick nutrition. Hegsted, Oleson, Mills, Elvehjem and Hart¹ described a scaly dermatitis of chicks fed purified diets, which was not cured by pantothenic acid. In further experiments Hegsted, *et al.*,² found

² Dienes, L., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, **44**, 470.

³ Brown, T. M., *J. Bact.*, 1942, **43**, 82.

⁴ Dienes, L., *J. Bact.*, in press.

⁵ Dienes, L., *J. Infect. Dis.*, 1939, **65**, 24.

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¹ Hegsted, D. M., Oleson, J. J., Mills, R. C., Elvehjem, C. A., and Hart, E. B. *J. Nutrition*, 1940, **20**, 599.

² Hegsted, D. N., Mills, R. C., Briggs, G. M., Elvehjem, C. A., and Hart, E. B., *J. Nutrition*, 1942, **23**, 175.

this type of dermatitis was prevented by biotin concentrates and free crystalline biotin. An increase in growth due to biotin was also reported. Ansbacher and Landy³ have reported similar results using a heat-treated ration.

In attempting to develop suitable purified rations for the study of the effect of various members of the B complex on egg production and hatchability in poultry we have observed a marked fall in hatchability of eggs produced by hens fed purified rations of low biotin content. In this paper we wish to report studies on this condition, which is prevented by materials rich in biotin and by a biotin-concentrate.

Single Comb White Leghorn pullets were housed in individual laying cages and were artificially inseminated. The birds were started on the experimental diets November 25, 1941. The basal ration (R-4) had the following composition: sucrose 61, casein 18,[†] gelatin 5, salts IV 5,[‡] soybean oil 5, solubilized liver extract 4,[†] fish liver oil 2 (3000 IU vitamin A, 400 A.O.A.C. units D per gm). Crystalline vitamins[†] were added as follows per kg: riboflavin 5 mg, thiamin 3 mg, calcium pantothenate 15 mg, pyridoxin 8 mg, nicotinic acid 100 mg, and choline 2 g. Crude supplements to the basal ration were added at the expense of sucrose. Groups 1 and 2 received the basal ration for 11 weeks. It was found that eggs produced by hens fed the basal ration would not hatch and biotin supplements were added at this time. Group 1 received 15 μ g of a biotin concentrate (S.M.A. No. 5000) per 100 g ration and 3% of kidney residue was added to the ration of group 2. Group 3 was fed the basal ration plus 5% whey concentrate and group 4 the basal plus 3% kidney residue for the entire period. There were 2 birds in group 1, 7 in group 2, 3 in group 3, and 4 in group 4.

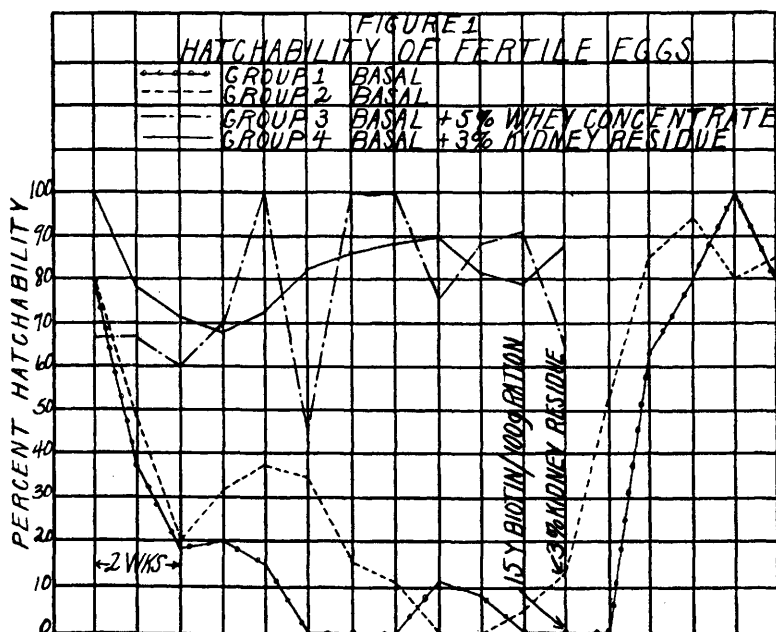
It is apparent from the results in Fig. 1 that the basal ration was inadequate for hens to produce hatchable eggs. A fall in hatchability from approximately 80% of fertile eggs to less than 20% in 6 weeks occurred. That the missing factor was supplied by the whey concentrate or the kidney residue may be seen by comparing the hatch-

³ Ansbacher, S., and Landy, M., *PROC. SOC. EXP. BIOL. AND MED.*, 1941, **48**, 3.

[†] We are indebted to Borden & Co., Special Products Division, New York, New York, for the fish oil and whey concentrate; to Merek & Co., Rahway, New Jersey, for the crystalline vitamins; and to Wilson Laboratories, Chicago, Ill., for the solubilized liver extract.

[‡] Hegsted, D. M., Mills, R. C., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1941, **138**, 459.

[§] Borden's H5P. Unobtainable during present emergency.



ability of eggs from groups 3 or 4 with that of the hens fed the basal ration. The results for groups 3 and 4 are not presented beyond the 11th week because the supplements had proven effective by that time and thus were withdrawn so as to deplete these birds for further study.

The addition of kidney residue or the biotin concentrate to hens in groups 1 and 2 respectively brought about a rapid increase in hatchability of eggs produced by these birds. In 3 weeks the percent of hatchability had risen from approximately 10% to at least 80% in both groups.

It might be questioned whether factors other than biotin are involved but this seems unlikely inasmuch as Hegsted, *et al.*,² have shown that biotin concentrate and free crystalline biotin replaced kidney residue in chick rations similar to that used in these studies. The cost of the crystalline biotin prohibited its being used in these studies. Whether biotin is the heat labile factor reported by Bauernfeind and Norris⁵ to be necessary for avian reproduction cannot be determined from the present study.

The egg production data are not presented inasmuch as there seemed to be little effect of this deficiency on egg production. It is possible that sufficient biotin was present in the basal ration to sup-

⁵ Bauernfeind, J. C., and Norris, L. C., *J. Nutrition*, 1939, **18**, 579.

port adequate egg production. No dermatitis was observed during the experiment.

Summary. Evidence is presented to show that biotin is essential for normal embryonic development in the hen's egg. A satisfactory synthetic ration for use in studies of the vitamin requirements of laying and breeding hens is also presented.

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Studies on Inhibition and Activation of Atropinesterase.*

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In an earlier communication dealing with the enzymatic hydrolysis of atropine by rabbit serum a study was made of the kinetics of the reaction, its enzyme-substrate affinity relations, and effects of pH and temperature.¹ Subsequently the occurrence of atropinesterase in rabbits, its distribution in blood and organs, and certain of its specificity characteristics were reported.² The present study extends these observations to include the influence on activity of certain salts, dyes, sulfhydryl compounds, substances affecting sulfhydryl groups, and physostigmine.

Rabbit serum, used as a source of enzyme, was dialyzed in 10 ml portions against distilled water for 3 days at 5-10° in cellophane tubes. After dialysis the volume was about 15 ml and the serum was centrifuged to remove precipitated globulins. Each batch, kept in a refrigerator, was used within a week. A loss in activity could be observed when the material was used after the first week.

The enzyme activity was determined at 30° and pH 7.4 by the manometric method employing the Warburg apparatus^{1, 2} with the substitution of 0.20% NaHCO₃ for bicarbonate-Ringer solution in order to eliminate possible effects of KCl, NaCl, and CaCl₂. Three ml of substrate solution, containing 10 mg atropine sulfate dissolved in the NaHCO₃ solution, were placed in the main chamber; 0.5 ml of the dialyzed serum preparation and 0.5 ml of a solution of the compound to be tested, dissolved in NaHCO₃ solution, were placed in the side

* Aided by a grant from the Sidney C. Keller Research Fund.

¹ Glick, D., *J. Biol. Chem.*, 1940, **134**, 617.

² Glick, D., and Glaubach, S., *J. Gen. Physiol.*, 1941, **25**, 197.