growth occurs under anaerobic conditions unless uracil is added. In the presence of oxygen uracil is non-essential.

The C203S strain of streptococcus is a powerful hemolytic strain. In our experience hemolytic titers on this medium are equivalent to those obtained in broth, provided care is taken to avoid accumulation of acid during growth. The growth and hemolysin titer may be increased by addition of more glucose to the medium and periodic neutralization of the acid formed. However, preliminary work in this direction indicates that some factor, as yet unidentified, becomes the limiting one under these conditions.

Summary. Rapid, heavy growth and hemolysin production of the C203S strain of Group A hemolytic streptococcus have been obtained on a medium of essentially known composition. In addition to factors reported by previous workers, we have found that thiamin, nicotinic acid, adenine or related purines, and an unknown factor which may possibly be biotin are necessary for growth of this strain. The relation of carbon dioxide tension to the purine requirement has been studied.

11558

Modifying Influence of Light on Chick's Comb Response to Androsterone.

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(Introduced by S. R. Haythorn.)

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The assay of androgenic material by biologic methods offers definite advantages over colorimetric determinations when we consider that in measuring the 17 ketosteroids by chromogenic effect we are determining both biologically active and inactive material.[1] In many cases the important consideration is, to what degree is biological activity present in a given specimen. In consequence of this, we made comparisons of colorimetric[2] and biologic determinations in some of our studies. The comb response of one-day-old

[1] Callow, N. H., Callow, R. K., Emmens, C. W., and Stroud, S. W., *J. Endo.*, 1939, **1**, 76.

[2] Neustadt, Rudolph, *Endo.*, 1938, **23**, 711.

chicks, previously reported by Dorfman and Greulich,[3] Emmens,[4] and Frank and Klempner,[5] was employed as the method of studying biologic response. During the preliminary plotting of our curves, to establish normal average response to known dosage, it was recognized by us that light played an extremely important rôle as a modifying factor in the weight response of the chick's comb. Though this factor has been observed in the capon,[6] we have found no mention in the literature regarding the baby chick. It is, therefor, of paramount importance that the influence of light be recognized and taken into account by workers who have adopted the chick comb weight method for the assaying of androgens.

The 309 white leghorn male chicks used in our experiment were procured from the same hatchery on the day of hatching. All chicks used were hatched during the first 3 weeks of April. When the chicks were one day old, daily inunction of the combs was begun and continued for 6 days. The combs were removed and weighed on the day following cessation of treatment and the weights of the birds also were noted. Removal of the comb was accomplished by making lateral and posterior incisions at the base of the comb of the anesthetized chicks and by grasping the anterior end with forceps and stripping the comb off dorsally. All combs were removed by the same individual.

Daily treatment consisted of spreading 0.01 cc of sesame oil over the surface of the comb. This was easily accomplished with accuracy by the use of a micro-titrating pipette calibrated in 0.002 cc divisions and which had the tip bent at a right angle. This amount of oil contained from 0.5 to 50.0 γ of androsterone* and was applied at each inunction to groups of from 5 to 14 chicks. The doses used in the various groups are indicated in Fig. 2 and 3. The oil applied was absorbed within about 10 minutes, during which period the chicks on each dose were kept separated to prevent contamination through contact. Control animals which received only sesame oil were kept in separate cages.

The chicks were divided into 4 groups, each group being exposed to varying degrees of light and darkness. One hundred and five

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3 Dorfman, R. I., and Greulich, W. W., *Yale J. Biol. and Med.*, 1937, **10**, 79.

4 Emmens, C. W., Med. Res. Council "Reports on Biological Standards—V," 1939.

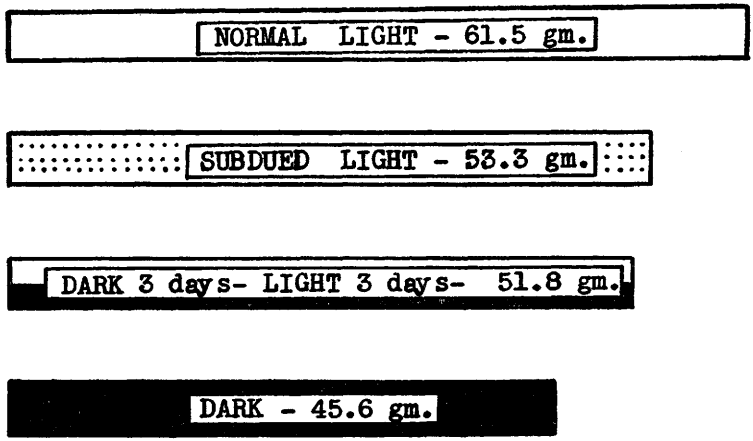
5 Frank, R. T., and Klempner, E., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 763.

6 Womack, E. B., Koch, F. C., Domm, L. V., and Juhn, M., *J. Pharm. Exp. Therap.*, 1931, **41**, 173.

* The androsterone in this study was kindly supplied to us by Dr. Ernst Oppenheimer of Ciba Pharmaceutical Products, Inc.

animals were maintained in a large incubator in total darkness, being brought into daylight only during the period of treatment which did not exceed one hour per day. Thirty-three chicks were placed in a room where they received very little natural light, referred to as "subdued". Ninety-one animals were placed in a large, well-lighted, airy room where both natural and artificial light was adequate and in which the cage was so located that all the chicks received approximately the same amount of light. The last group of 100 chicks was placed in total darkness for the first 3 days of treatment and were then placed in the same room as the chicks of Group 3 for the second 3 days. Although a mortality of approximately 50% was noted among the groups kept under deficient lighting conditions, only 5% died when the animals were kept in adequate light. The numbers of animals referred to above represent the surviving members of each group.

The effect of the various light conditions may be seen in Fig. 1 where the average body weights of each group, taken at the seventh day, have been presented. The inhibition to body growth, due presumably to inadequate light, is clearly seen where a direct correlation between the amount of light the chick received and the body weight is made. The influence of light alone on the body growth of chicks had been previously reported.[7] The body weights of each



AVERAGE BODY WEIGHTS OF CHICKS
UNDER VARIOUS CONDITIONS OF LIGHT
FIG. 1.

[7] Bovie, W. T., *Boston Med. and Surg. J.*, 1925, **192**, 1035.

of our groups were plotted against the dosage of androsterone but failed to indicate any correlation with the treatment. This graph has been omitted.

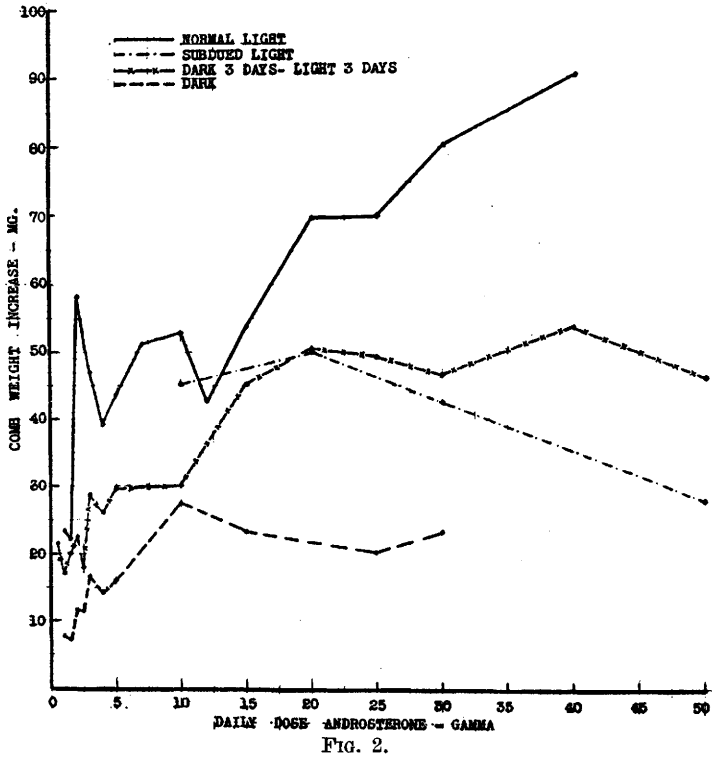


FIG. 2.
Variations in Comb Weight Increase as Influenced by Altered Light Intensity.

In Fig. 2, the average comb weight increase of the treated animals over the control comb weights has been plotted against the daily dosage of androsterone. A comparison of the curves in this figure shows the effect of exposing the chicks to varying degrees of light intensity while employing the same dose of androsterone. There is a slight response of the animals maintained in the dark as compared with those in normal light, with the groups exposed to inadequate light occupying a median position. It is also of interest to observe that the animals kept under deficient light conditions failed to show a corresponding comb weight increase with the application of higher concentrations of androsterone as did the groups in normal light. The curves in the "deficient light groups" tend to reach a plateau. The curve of the groups in normal light is still fairly steep at a daily dosage of 40 γ, a fact rather important if this method is to be considered for assay of unknown extracts.

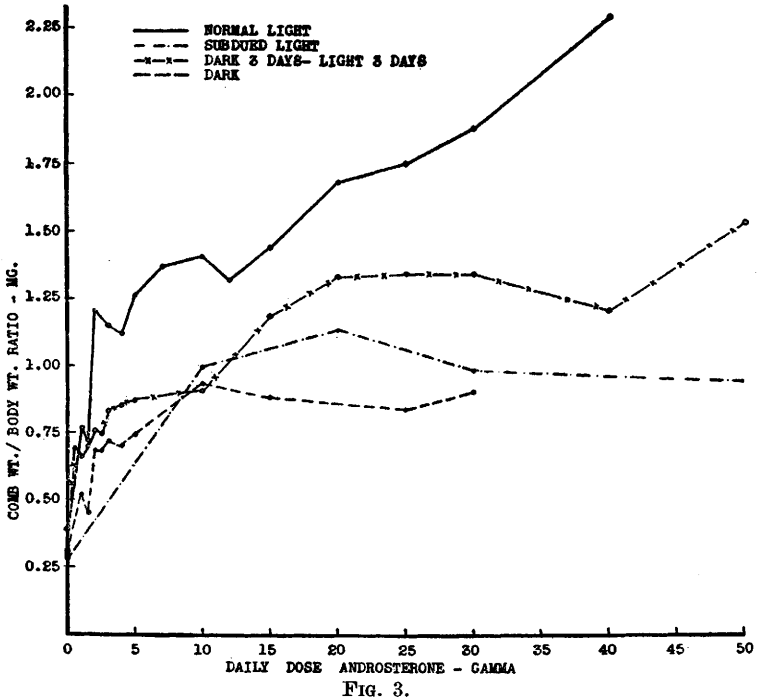


FIG. 3.
Comb Weight Body Weight Ratio Curve as Influenced by Altered Light Intensity.

In Fig. 3, the ratios of average comb weight over the average body weight have been plotted against the daily dose. Consideration of the comb weight in relation to body weight tends to remove some of the variations occurring when the comb weights alone are considered.

It becomes evident that two variable factors influence the growth of the combs of chicks; namely, the amount of androgenic material employed, and the intensity of the light to which the chicks are exposed. The light factor must, therefore, be made constant and of adequate intensity. With the changing seasons of the year, this must be borne in mind and due corrections made if a constant response is to be secured and sources of error are to be reduced to a minimum. Disregard of the amount of light available to the chicks may easily explain the discrepancies in results obtained by various workers. It is not within the province of this report to postulate whether the stimulating influence of androsterone is inhibited by the lack of light, but it is evident there is sufficient physiological inhibition to the animal organism so that it acts as a factor in preventing proper response.

Summary. Exposure to varying degrees of light influenced the

weight response of male chicks' combs to which androsterone had been applied by inunction. The weight response of the combs to androgenic stimulation is considerably greater in birds receiving normal daylight than in those kept in the dark or in inadequate light. Body weights of the various groups indicate a lack of growth which is in direct correlation to the lack of light. The importance of exposing the test birds to an adequate and a constant source of light is emphasized if this method is to be used for the assay of unknown androgens.

11559

Renal Physiology in Infants and Children: I. Method for Estimation of Glomerular Filtration Rate.

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The more exact methods for estimation of kidney function have been quite successfully employed in older children,[1] but they have not been very widely applicable to infants because of the obvious difficulties in obtaining complete urine specimens. Schoenthal and his coworkers[2] studied the urea clearance in 9 normal infants ranging in age from 2 to 11½ months and concluded that the urea clearance corrected for surface area agreed with the values observed by Van Slyke and his coworkers for older children and adults, which indicated to them that renal function measured by the ability to excrete urea is as well developed in infants as in later life. Their studies did not include, however, the investigation of renal function during very early postnatal life. The method to be described here originated in an attempt to study the renal physiology during the newborn period. The single injection inulin clearance test proposed by Alving and Miller[3] seemed especially applicable to this problem. Inulin clearances seemed preferable to urea clearances for this study since it has been well established that the inulin clearance is at the

1 Cullen, G. E., Nelson, W. E., and Holmes, F. E., *J. Clin. Invest.*, 1935, **14**, 563.

2 Schoenthal, L., Lurie, D., and Kelly, M., *Am. J. Dis. Child.*, 1933, **45**, 41.

3 Alving, A. S., and Miller, B. F., *A Practical Method for the Measurement of Glomerular Filtration Rate (Inulin Clearance)*, to be published.