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.

level of glomerular filtration and that the filtration rate is fairly constant at low and high rates of urine formation.[4]

Alving and Miller have shown that after the intravenous injection of inulin (10 g in adults) the plasma inulin concentration, plotted logarithmically against time, decreased at first in a curvilinear manner, later falling, however, in a linear or almost linear relationship with time. The curvilinear relationship marks, presumably, the period of equilibration between the blood and plasma and the extracellular fluids. The straight line relationship follows because once equilibrium has been established the rate of fall of the plasma inulin level is determined by the rate at which inulin is cleared from the plasma by the kidneys. Since inulin is quantitatively excreted in the urine after intravenous injection, and since, as previously stated, the inulin clearance is independent of the rate of urine formation, the rate of fall of the inulin in the plasma after the straight line relationship has been established should have a direct relationship with the clearance, and it should be possible to relate the slope of this line to the clearance. The determination of the slope of this line is the basis for the method here proposed for the estimation of glomerular filtration rate.

The method is applied as follows: a blood sample is obtained which serves to correct subsequent blood inulin analyses for the non-inulin chromogenic material. 0.15 g of inulin per kilo body weight is injected intravenously. At approximately 2 and 3 hours after the completion of the injection blood samples are drawn. The exact times of the injection and the drawing of the second and third samples are noted. The inulin content of the 2 samples of blood is determined. On semi-logarithm paper the 2 blood samples are plotted on the logarithmic coördinate against time on the linear coördinate. A straight line is drawn between these 2 points, and the slope of the line determined by the formula:

$$\text{slope} = \frac{K - \log C}{t}$$

where K is a constant whose value is determined by extending the line to zero time and taking the log of the concentration at this point; C is the plasma inulin concentration in mg % at any given time, t, in minutes, after the inulin injection.

The relation of the slopes of the lines obtained above to inulin clearance as actually determined is shown in Fig. 1. The dots rep-

[4] Smith, H. W., *The Physiology of the Kidney*, Oxford University Press, New York, 1937.

resent data on children ranging in age from 5 to 15 years. The inulin clearances corrected for body size were done according to the technic described by Alving and Miller[4] and the inulin determinations by the method of Alving, Rubin, and Miller.[5] Each point represents the average of 2 consecutive periods. The open circles represent points calculated from the data of Alving and Miller.[4] A fairly good relationship is shown to exist between the 2 factors.

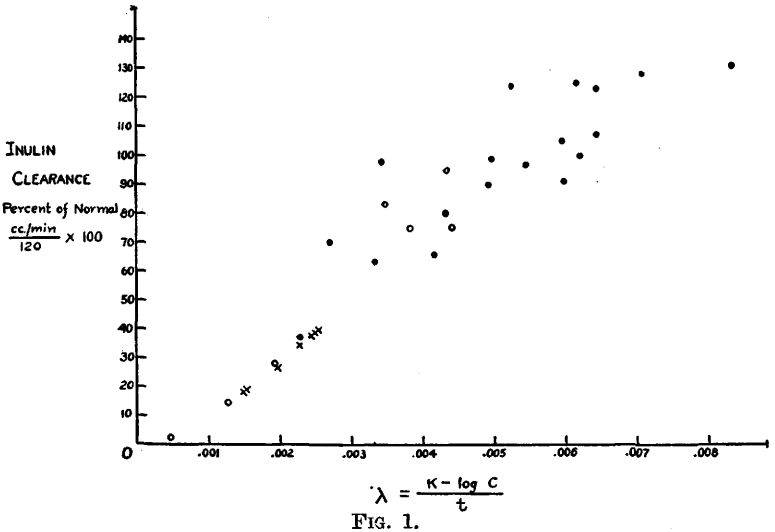

FIG. 1.

Relation of inulin clearances to slopes of lines representing fall in plasma inulin concentration following intravenous injection of inulin. Dots represent data on children from 5 to 15 years. Circles represent points calculated from the data of Alving and Miller. For explanation of crosses see text.

The slope of the line obtained as described appears to offer a fair estimation of the rate of glomerular filtration. The method would seem to be of value in instances in which the collection of urine specimens is very difficult and in which the error in collecting urines is probably greater than the errors inherent in the method. This would apply particularly to very young infants.

The application of this method to 7 apparently normal full-term newborns ranging in age from 4 to 9 days revealed slopes corresponding to inulin clearances ranging from 20 to 40% of normal (120 cc per minute). The slopes of these lines are plotted on Fig. 1 as crosses along the general line of the graph. The lines are shown as Group I in Fig. 2. That a straight line relationship in the decrease of the plasma inulin concentration, plotted logarithmically against time, does exist in newborns after an intravenous injection

5 Alving, A. S., Rubin, J., and Miller, B. F., *J. Biol. Chem.*, 1939, **127**, 609.

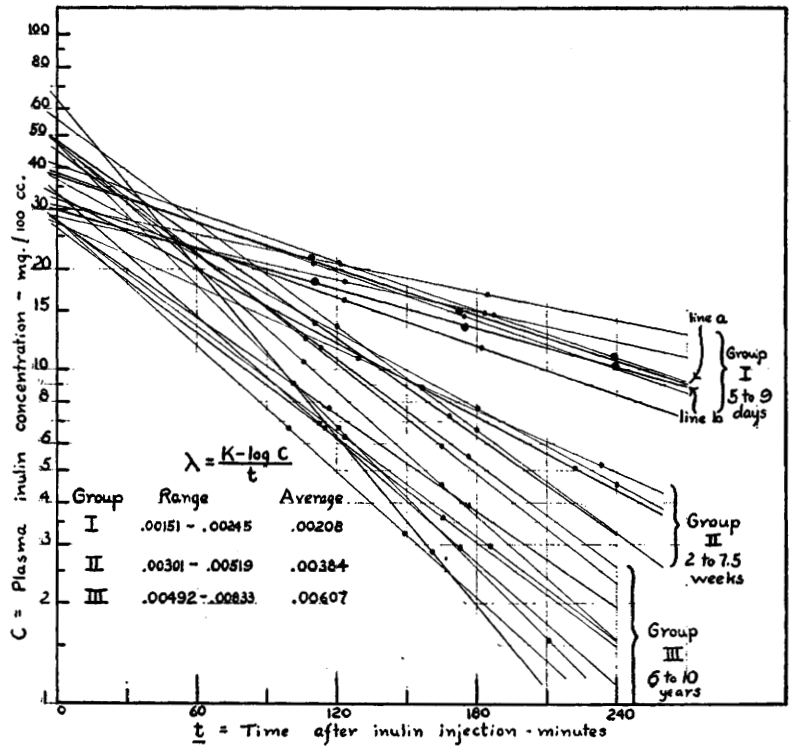

FIG. 2.

Lines obtained in different age groups by plotting plasma inulin concentrations logarithmically against time following intravenous injection of inulin.

of inulin is shown in Fig. 2 in which 3 points on the line were obtained in 2 instances (lines *a* and *b*). The lines obtained in 4 older infants ranging in age from 14 days to 7½ weeks and apparently without kidney disease (Group II) and the lines obtained by repeated determinations on 2 children 6 and 10 years of age (Group III) are also plotted on Chart 2. The slopes of the lines obtained on Group II correspond to clearances ranging from 50 to 90% of normal, and the slopes of the lines of Group III correspond to normal clearances. These results suggest a definitely diminished inulin clearance in newborns, which rapidly disappears during early infancy, perhaps in some cases as early as the 14th day. These results correlate with the histological characteristics of the renal glomerulus in early postnatal life, a recent study of which is reported by Gruenwald and Popper,[6] who showed that in embryonic life there exists a resistance against filtration due to a matting together of the glomerular loops which are invaginated in a sac of high columnar epithelium.

[6] Gruenwald, P., and Popper, H., *J. Urol.*, 1940, **43**, 452.

In early postnatal life the peaks of the loops are still covered by this type of epithelium, while in the second year the histological appearance is similar to that of the adults.

Further studies are needed to prove the validity of this observation and to further elucidate its mechanism.

11560

Chromodacryorrhea, a New Criterion for Biological Assay of Acetylcholine.

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The peculiar phenomenon of the shedding of bloody tears by rats was reported in connection with the studies of dacryorrhetin, a compound prepared from muscle.[1, 2] This phenomenon is so unique and easily distinguishable from ordinary lacrimation that the term, chromodacryorrhea, is proposed for it. It has served as a very convenient criterion for the biological assay of dacryorrhetin.

When Selye[3] published a paper in which he quotes Freud's observation[4] that acetylcholine causes rats to shed tears tinged red by blood,† one of us (T) examined chromodacryorrhetic and other properties of acetylcholine to see if dacryorrhetin could be in reality acetylcholine.[5] The results of these investigations showed that these two compounds are not identical and at the same time suggested a possibility of using chromodacryorrhea as a new criterion for a biological assay of acetylcholine. We have thus determined how small amounts of acetylcholine can be detected accurately by this criterion under different conditions.

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1 Tashiro, Shiro, and Stix, Helen, *Biol. Bull.*, 1935, **64**, 327.

2 Tashiro, Shiro, *Proc. Am. Soc. Biochem.*, 1937, **8**, xcviii.

3 Selye, Hans, *Canadian Med. Assn. J.*, 1937, **36**, 200.

4 Freud, J., *Acta Brevia Neerl.*, 1933, **3**, 159.

† Although no casual observer would question the presence of blood in tears, and it gives a positive benzidine reaction and its bands are much like those of oxyhemoglobin when examined with a hand spectroscope, yet Tashiro and Badger have evidence that the red pigment in the bloody tears is not oxyhemoglobin.

5 Tashiro, Shiro, Kongressbericht. II. des XVI Internat. Physiologenkongress 1938, 46.