latter of these methods introduces errors discussed by Svensson.[16]

Discussion. Our results indicate that for the 16 cases studied, none of the protein components accessible to electrophoresis shows any marked change. Particularly, only 4 of the 16 cases showed a loss of 0.5% in fibrinogen, while as many cases showed a gain of the same magnitude. Witts[17] has published the statement that fibrinogen levels may be reduced 30% before prothrombin time is significantly prolonged. With respect to the earlier claims cited above, our results indicate that the change in clotting time and sedimentation rates following acetylsalicylate ingestion is not due to marked changes in fibrinogen levels. Actually, the presence of any large asymmetric molecule can change sedimentation rate regardless of protein concentration.[18,19] Whether this is due to an effect on protein surface charge or to chemical interaction is not known. Smith[20] claims that salicylates are bound to some plasma proteins.

Ham and Curtis[21] have reported a very careful study of the relation between hepatic function and plasma proteins. These authors concluded that plasma fibrinogen was an especially sensitive index of liver disease or dysfunction. They stated that in damaged livers the plasma fibrinogen was sharply decreased. If these reports are correct then our results indicate that salicylates are not hepatotoxic, and likewise, any effect of salicylates on prothrombin time is not reflected by corresponding changes in fibrinogen levels.

Summary. 1. Blood of normal subjects was examined by electrophoresis before and after ingestion of 4 g of acetylsalicylic acid per day for 7 days. Physical and chemical constants obtained were in good agreement with published values.

2. No marked changes were observed in any protein components accessible to electrophoresis. The conclusion is drawn that the effect of salicylates on blood clotting time and sedimentation rates of erythrocytes cannot be due to a reduction of plasma fibrinogen, and that salicylates are probably not hepatotoxic.

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18 Zozaya, J., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 182.

19 Yardumian, K., *Am. J. Clin. Path.*, 1937, **7**, 105.

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Development of Renal Function in Fetal and Neo-Natal Rabbits Using Phenolsulfonphthalein.*

R. C. WILLIAMSON AND E. P. HIATT. (Introduced by John H. Ferguson.)

From the Physiology Department, University of North Carolina School of Medicine, Chapel Hill, N. C.

At birth mammals undergo tremendous physiological adjustments in their respiration, nutrition, circulation and excretion correlated with the achievement of independence of the material circulation, Much attention has been directed toward the first 3 of the above mentioned adjustments but fewer physiological studies have been made on the development of an independent renal excretion. Most of the work reported indicates that there is some formation of urine in the terminal stages of uterine life.[1,2,3] It has been the purpose of our investigations to try to determine to

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what extent there is renal function in fetal rabbits and to attempt to measure the change in the renal excretion at birth and during the first few days of postnatal life. The rabbit was selected as our experimental animal because of the convenience of its breeding habits, its short gestation period and the fact that, like man, it has only a vestigial allantois.[4]

Methods. Phenolsulfonphthalein was selected as our indicator of renal function because it is rapidly excreted by tubular secretion as well as glomerular filtration.[3] It has been widely used since 1910 as a clinical test of renal function in man and is relatively non-toxic.[2] Finally the method of quantitative estimation of this dye in body fluids is simple and accurate.

A number of does were bred, each by a single copulation, in order that young of known age could be obtained. Some of these does were anesthetized before term and the fetuses exposed while others were allowed to deliver in order that newborn of various ages could be obtained. In this manner a series of fetuses and newborn of known age covering the period from the 26th to the 42nd day after conception was obtained. Deliveries occurred between the 30th and the 33rd days after conception.

A subcutaneous injection of one mg of PSP in a volume of 0.5 ml was adopted as the test dose. The percentage of this dose excreted into the bladder in one hour was determined.

In the fetal series 4 mothers were lightly anesthetized with 1.3 g of urethane per kilogram of body weight augmented with ether during the surgery. The uteri were exposed and a small opening made in each horn. Through these openings the urogenital papillae of 2 fetuses were tied off and the PSP injections made subcutaneously with special care to avoid leakage. Then the uterine incisions were sutured and the abdomen closed during the collection period. At the end of the hour the fetuses were removed, sacrificed and the bladder contents washed out for analysis. The survival of the fetuses as indicated by pulsation of the umbilical artery was taken to be a proof of the continued normal function of the placenta.

In the newborn series there were 11 litters. Tests of the rate of excretion of PSP were made at daily intervals after birth. Each young rabbit was injected subcutaneously with the standard dose of PSP and suspended over a beaker. At the end of an hour each was sacrificed, the bladder contents added to any urine voided during the hour and analysed for PSP.

Nine experiments were carried out to determine the placental transmission of PSP from maternal to fetal blood and vice versa. In one series the mother was injected intravenously with 6 mg of PSP in one ml and the fetal blood tested after an interval of one hour for the presence of the dye. In the other series 2 fetuses were injected subcutaneously, each with 3 mg of PSP in 0.5 ml volume. The mother was catheterized and urine collected for a period of one hour during which several maternal blood samples were taken. The maternal and fetal blood samples were obtained by heart puncture.

The concentration of PSP in the centrifuged urine was determined with the aid of a photoelectric colorimeter on the properly diluted urine alkalinized with sodium carbonate. The plasmas were first precipitated with one part of saturated trichloracetic acid to 8 parts of blood, the supernatant portion alkalinized and the color estimated. This method measures only the fraction of the phenol red which is not bound to proteins but avoids the errors accompanying hemolysis.

To test the influence of the anesthetic on the renal excretion of the dye, some of the newborn were given 1.3 g of urethane per kg and the rate of their excretion of PSP simultaneously compared with that of an unanesthetized litter mate.

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[1] Barclay, A. E., Franklin, K. J., and Pritchard, M. M. L., *The Fetal Circulation*, Charles C. Thomas, Springfield, Ill., 1945.

[2] Rowntree, L. G., and Geraghty, J. T., *J. Pharm. and Exp. Therap.*, 1910, **1**, 579.

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

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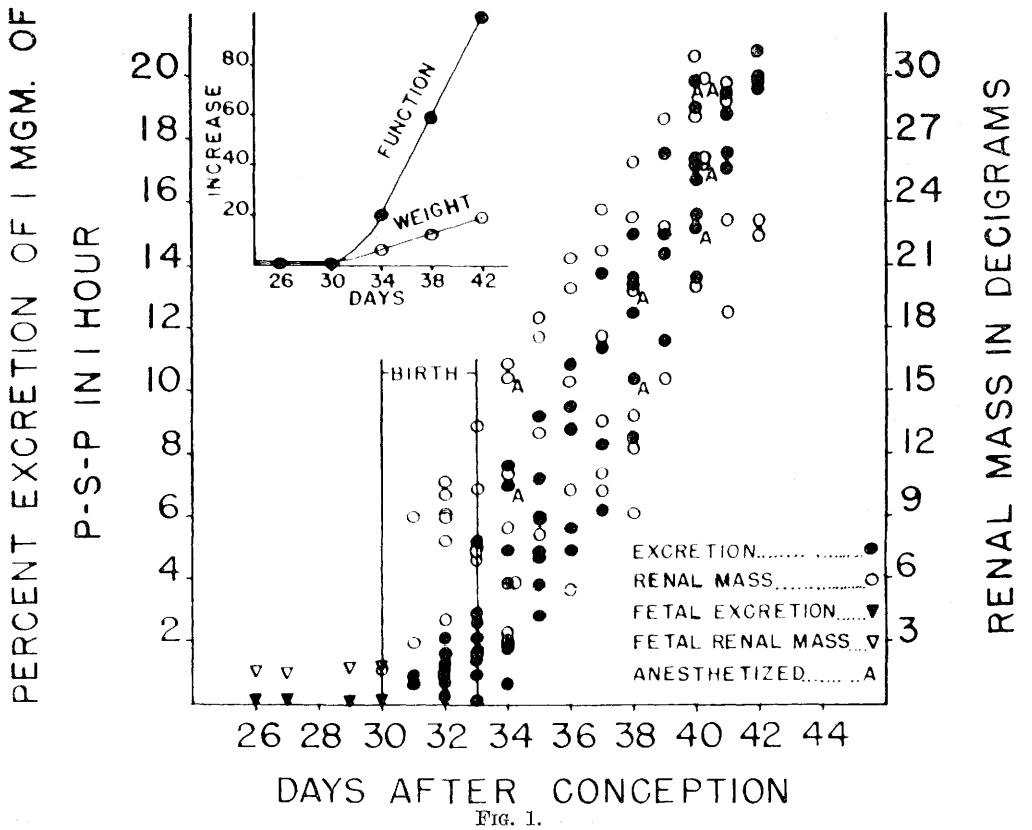

FIG. 1.

The rate of excretion of phenolsulfonphthalein and the combined weights of the 2 kidneys in fetal and newborn rabbits of varying age. The rate of excretion (solid dots) is plotted as the percentage of a 1 mg subcutaneous dose excreted in one hour. The kidney weights (circles) are plotted in decigrams against the ordinate on the right. The absolute rate increase of excretion and renal mass is plotted in the small graph at the upper left, using earliest noted fetal excretion and renal mass values as bases.

The kidneys of fetuses and newborn were removed and weighed after the excretion rate had been determined.

Results. The excretion of PSP by the fetuses in one hour was practically negligible in spite of high plasma concentrations of the dye and showed very little change between the 26th and the 30th day of gestation. (Fig. 1) The weight of the fetal kidneys also showed little change during this period.

Following birth there was a regular increase in the rate of excretion of PSP and in the kidney weight with increasing age. The increase in function was about 5 times greater than the increase in renal mass, *i.e.* the rate of dye excretion at the end of 10 days postnatal life was 100 times greater than the prenatal rate while the renal mass was increased less than 20 fold.

In the experiments comparing litter mates, one of which was anesthetized, there was virtually no effect of the anesthesia on the rate of dye excretion. (Fig. 1)

There was no appreciable transmission of the dye across the placental barrier in either direction during one hour.

Discussion. It is well established that some renal excretion occurs in mammalian fetuses as they approach term.[4,5,6] Gersh has defined qualitatively the beginning of renal excretion in rabbit and other mammalian fetuses by histochemical technique.[7] He found evidence

[5] Daly, Harriet, Wells, L. J., and Evans, Gerald, PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 78.

[6] Smith, C. A., *The Physiology of the Newborn Infant*, Charles C. Thomas, Springfield, Ill., 1945.

of glomerular filtration and tubular secretion even in the mesonephros, but his work does not indicate the relative quantity of materials excreted in the urine. That this avenue of excretion is not important so long as the placenta functions normally is indicated by the observation that fetuses can survive to term without kidneys or with obstructed urinary passages.[4,6,8]

In considering the cause of the rather abrupt change in renal function which we have observed at the time of birth, one must consider both physiological and morphological factors. Hamilton, Woodbury and Woods[9] have measured the blood pressures of fetal and newborn rabbits. They find some increase in the systemic arterial pressure at the time of birth and a gradual continuing increase to the adult level. This seems to be true of other mammalian fetuses as well.[1,4,6] †

On the morphological side there are reports that the glomeruli undergo changes at the time of birth which render them more capable of filtration.[6,10] Gruenwald and Popper suggest that the fully developed visceral layer of Bowman's capsule impairs filtration in the embryonic kidney.[10] They believe that a rupture of this epithelial layer occurs at birth with an expansion of the capillary loops of the glomerulus.

The combined effect of the increase in blood pressure and the increase in filtering surface may cause the sudden increase in renal function observed at birth. The dye then excreted would represent the sum of that filtered at the glomeruli and that secreted by the tubule cells, the dye secreted by the tubule cells being flushed out by the fluid filtered at the glomeruli.

Our failure to find any appreciable placental transmission of PSP in one hour is at variance with the finding of Lell and Liber.[11] These investigators found that 15 to 30% of a dose of PSP injected into rabbit fetuses could be recovered from the urine of the mother in a 6-hour period. This discrepancy with our results may be due to the difference in the time interval if it is not due to differences in the technique of injecting the fetuses.

Summary. 1. Renal function in the rabbit as measured by phenolsulfonphthalein excretion increases rapidly and regularly in the first 10 days after birth but does not exist to an important degree before birth. 2. At the end of 10 days of postnatal life the rate of dye excretion is 100 times greater than the prenatal rate while the renal mass is increased less than 20 fold. 3. In one hour PSP does not traverse the rabbit placenta in either direction in appreciable amounts after the 26th day of gestation. 4. The effect of urethane anesthesia on the excretion of PSP by newborn rabbits was found to be negligible.

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8 Potter, E. L., *J. Pediat.*, 1946, **29**, 68.

9 Hamilton, W. F., Woodbury, R. A., and Woods, E. B., *Am. J. Physiol.*, 1937, **119**, 206.

† Another possibility is suggested by the work of Truetta *et al.* (*Lancet*, 1946, **251**, 237) who have described a physiological mechanism for diverting the renal blood flow from its normal course in such a way as to render the renal cortex ischemic.

10 Gruenwald, Peter, and Popper, Hans, *J. Urol.*, 1940, **43**, 452.

11 Lell, Wm. A., and Liber, K. E., *Anat. Rec.*, 1928, **38**, 53.