urinary porphyrin at all, are, like infectious hepatitis, characterized by an increase of the Type I isomer.

Summary and Conclusion. A considerable excess in the excretion of coproporphyrin III has been commonly noted in a series of urine samples from cases of acute poliomyelitis. The total urinary coproporphyrin is usually in the range of 100-500 γ per 24-hour sample, with from 50-90% of Type III isomer, as compared with 20-100 γ and 8-35%, normally. This abnormal excretion is contrasted with the marked increase of Type I isomer previously found to characterize the urine in cases of infectious hepatitis.

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Experimental Evidence of the Secretion of Urine by the Fetal Kidney.*

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In the fetal rat, ligation of the ureter causes hydronephrosis and ligation of the urogenital papilla causes filling of the bladder.[1] The rate of formation of fluid by the fetal kidney is surprisingly rapid and it may be accelerated experimentally by ligating the renal pedicles of the mother or by injecting a highly concentrated solution of urea under the skin of the fetus.[2]

The present study which grew out of the experiments noted above, has been designed to determine whether this fluid is actually urine or merely a transudate of the fetal blood. In order to attain this objective, we have studied the blood and contents of the bladder of the fetus and also those of the mother.

The material for analysis was collected in the Department of Anatomy from rats in which the pregnancies were dated from the moment of observation of coitus. On the 21st day of pregnancy (20 days and 17 hours ± 2 hours), the mother was anesthetized with ether and her abdomen was opened surgically. Each fetus of a litter was transferred to the abdominal cavity of the mother[3] and its urogenital papilla was ligated by means of an unraveled strand of surgical silk. Then the abdominal incision of the mother was closed by suturing and the anesthesia was discontinued. On the 22nd day of pregnancy and about 2 hours before autopsy, the urinary papilla of the mother was ligated by means of braided silk (Champion No. 6). A few hours before the estimated time for normal parturition, the mother and her fetuses were killed by decapitation (killed at about 21½ days after coitus). Blood emerging during decapitation was allowed to drop into small vials which previously had been moistened with heparin solution. The maternal urine was secured by puncturing the bladder with a hypodermic needle (gauge 27) attached to a 10 cc syringe. The fetal urine was obtained by puncturing the bladder with a micro-pipette prepared from glass tubing and equipped with a small rubber bulb (a "policeman," commonly used in chemical laboratories).† By pooling the samples from the fetuses of one mother or, more rarely, from those of several mothers, it was possible to obtain sufficient material for the analyses.

The chemical methods employed were micro-modifications of the following standard

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1 Wells, L. J., *Anat. Rec.*, 1946, **94**, 504.

2 Wells, L. J., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 287.

3 Wells, L. J., *Anat. Rec.*, 1946, **94**, 530.

† The pipettes were prepared by Dr. J. Francis Hartmann.

TABLE I.
Analysis of Fetal and Maternal Fluids.

Tests	Fetal fluids		Maternal fluids	
	No. of observations	Quantities and ratios	No. of observations	Quantities and ratios
Amt of urine in individual bladders (cc)	50	0.05-0.1* (1.013-1.018) †	30	(1.0-1.5) (1.026-1.030)
Specific gravity of urine	9	1.015‡ (11.7-23.0)	4	1.029 (12.1-19.0)
Blood urea N (mg/100 cc)	9	16.73 (61-384)	10	15.9 (454-556)
Urine urea N (mg/100 cc)	8	195.0 (3.6-23.0)	3	497.0 (29.0-35.0)
Ratio urine urea/blood urea	8	11.7 (0.7-1.2)	3	31.0
Blood creatinine (mg/100 cc)	3	1.0		
Urine creatinine (mg/100 cc)	1	42.0		
Ratio urine creatinine/blood creatinine	1	42.0		
Urine chlorides, as NaCl (mg/100 cc)	2	178.0	2	265.0
Apparent glucose in urine (mg/100 cc)	5	40-50	7	50-60

* In the males. (In females the bladder was usually empty since the normal hypospadias militates against blocking the urinary outlet by ligating the urinary papilla.)
† Range.
‡ Average.

methods: blood urea—incubation of blood with equal amount of urease suspension and determination of ammonia by nesslerization on Folin-Wu filtrate thereof;[4] urine urea—determination of ammonia by nesslerization before and after incubation with urease;[4] creatinine in blood;[5] creatinine in urine;[6] chlorides in urine;[7] specific gravity;[8] reducing substances in urine—Benedict's qualitative reagent with raw urine, the degree of reduction being gauged against known standards of glucose.

The suitability of these micro-modifications was established by work with pure solutions and by duplicate recoveries from the urine and blood of known small added amounts of the substances in question.

Table I gives the principal data obtained. That the fetal kidneys are functioning as such and not merely producing a transudate is shown by the ability of the kidney to concentrate certain materials and to reabsorb others.

Since the specific gravity of ultrafiltrates of mammalian plasma is approximately 1.010, the average specific gravity of 1.015 for the fluid from the fetal bladder indicates that overall concentration has occurred. Otherwise stated, this is evidence of active reabsorption of water. If the principle of Long's coefficient is applied, the indication is that there has been in the urine a 50% increase in total solids over that of the glomerular filtrate.

The approximately 12-fold concentration of urea is distinctly less than the 31-fold concentration occurring simultaneously in the mother. However, when it is recalled that the placenta is functioning as an excretory organ, definite evidence is provided that the fetal kidney is already assuming a postnatal role. In this connection, it may be pointed out that the average blood urea nitrogen (b.u.n.) of the fetuses was 0.83 mg% lower than that of their mothers. Had the b.u.n. of the fetus been higher than that of the mother, it might have been taken as evidence of beginning separation of the placenta and such elevation of the fetal b.u.n. might have been considered as a possible

[4] Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry*, Williams and Wilkins, Baltimore, 1932, Vol. 2, vii + 957 pp.

[5] Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, **38**, 81.

[6] Folin, O., *J. Biol. Chem.*, 1914, **17**, 469.

[7] Schales, O., and Schales, S. S., *J. Biol. Chem.*, 1941, **140**, 879.

[8] Barbour, H. G., and Hamilton, W. F., *J. Biol. Chem.*, 1926, **69**, 625.

stimulus to the fetal kidney. However, a definite elevation of fetal over maternal b.u.n. was observed in only one of 8 observations (10.6 mg%).

Because of the known back diffusion of urea through the tubules a more active clearance of creatinine than of urea might be expected. This proved to be the case.

In addition to these evidences of selective concentration there are the evidences of selective resorption. The exact amount of glucose in the urine was not determinable. However the reducing power of the fetal urine was less than that of the corresponding maternal urine. (After yeast fermentation, reducing substances were no longer detectable in either urine by the method employed). In the light of the concentration of other substances, it can be safely concluded that active resorption of glucose occurred. In this connection it is of interest that histochemical stains show alkaline phosphatase‡ to be already clearly demonstrable in the proximal portion of the secretory tubules of these fetal kidneys.[9]

The resorption of chloride is shown to be more active in the fetus than in the mother.

In further consideration of our observations, the survival of fetal rats taken by Caesarian section indicates that the kidneys are able to function before term,[9] as in premature infants. But it is a different problem as to whether they actually function during the period when the placental circulation is still intact. The conditions of our experiments were such that the urine we obtained from the bladder of the fetus must have been produced exclusively during this period. This suggests that fetal urine is the fluid which distends the urinary passages of stillborn infants with hydronephrosis.[10,11] Makepeace and collaborators[12] and Jacqué[13] have analyzed fluid from the bladder of fetuses. Although these investigators failed to make it clear that all of the fluid they analyzed was actually produced before the placental circulation had been interrupted, their papers have internal evidence that this was the case.

Our observations indicate that in fetal rats the urine is more or less dilute. To this extent, our findings are in complete accord with those of other investigators.[12,13] That the fetal urine is actually dilute might be expected in view of the fact that the kidneys of infants are not as effective as those of adults.[14]

It is perfectly clear that the work performed by the fetal kidney is not essential for the survival of the fetus and that the placenta (hemochorial) is the essential excretory organ until birth. Recently Potter[15] has reported a series of 20 infants with bilateral agenesis of the kidney.§

Finally, the question arises as to whether the production of urine by the human fetus is a factor in such clinical disorders as hydramnios. An experimental approach to this problem, using the rat, is in progress at the University of Minnesota.

Summary. The bladders of fetal rats were allowed to fill after the urogenital papilla had been ligated. The contents of the bladder and the blood of the fetus were analyzed, as were also those of the mother. The observations indicate that the fetal kidney produces urine which is somewhat dilute.

‡ In fetuses in which hydronephrosis is produced experimentally by ligating the ureter, the phosphatase in their kidneys is being studied by histochemical and microchemical methods.

[9] Wells, L. J., unpublished observations.

[10] Wells, L. J., and Bell, E. T., *Arch. Path.*, 1946, **42**, 274.

[11] Dohrn, *Monatschr. f. Kinderheilkunde*, 1881, **2**, 98.

[12] Makepeace, A. W., Fremont-Smith, F., Dailey, M. E., and Carroll, M. P., *Surg., Gynec. and Obstet.*, 1931, **53**, 635.

[13] Jacqué, L., *Arch. Internat. Physiol.*, 1906, **3**, 463.

[14] Young, W. F., McCance, R. A., and Dobbs, R., *Proc. Roy. Soc. Med.*, 1943, **36**, 219.

[15] Potter, E. L., *J. Pediat.*, 1946, **29**, 68.

§ In such non-placental forms as chick embryos the nitrogenous wastes are eliminated by the mesonephros and deposited in the allantois.[16,17] During the first 16 days of incubation, the allantois is the only "urinary bladder."[17]

[16] Boyden, E. A., *J. Exp. Zool.*, 1924, **40**, 437.

[17] Fiske, C. H., and Boyden, E. A., *J. Biol. Chem.*, 1926, **70**, 535.