ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1977, p. 688-694

Vol. 11, No. 4
Printed in U.S.A.

Tobramycin: Maternal-Fetal Pharmacology

BETTY BERNARD,* SALVADOR J. GARCIA-CÁZARES,[1] CHARLES A. BALLARD, LAURI D. THRUPP, ALLEN W. MATHIES, AND PAUL F. WEHRLE

*Departments of Pediatrics and Obstetrics and Gynecology, and the Hastings Infectious Disease Laboratory, University of Southern California School of Medicine, and the Los Angeles County/University of Southern California Medical Center, Los Angeles, California 90033**

Received for publication 5 October 1976

To investigate the maternal-fetal transfer of tobramycin (TBM) and its distribution in the fetus, a single dose of 2 mg/kg was administered intramuscularly to 35 pregnant patients (13 first trimester, 22 second trimester) 0.5 to 34 h before hysterectomy. TBM concentration was assayed microbiologically in maternal serum, fetal tissues (placenta, brain, lung, liver, and kidney), and fluids (amniotic, cerebrospinal fluid [CSF], urine, and serum). Mean maternal serum half-life (1.54 h) and mean peak serum concentration of TBM were within ranges reported for nonpregnant adults. In fetal serum, half-life was 5.2 h, and TBM levels did not exceed 0.58 μg/ml. For intervals up to 34 h, the mean TBM concentration in placental tissues was 1.4 μg/g. Concentration differences related to fetal maturation were found for fetal CSF, amniotic fluid, and fetal kidney. No antimicrobial activity was found in the fetal CSF of >16 weeks' gestation. TBM was present predominantly in the second trimester amniotic fluid specimens. Fetal kidney concentrations reached 7.2 μg/g at 34 h after maternal drug administration. Higher TBM concentrations were related to advanced maturation of the fetal kidney. Second trimester fetal urine concentrations for TBM ranged from 0.1 to 3.4 μg/ml, and the fetal urinary half-life was 3.7 h. Knowledge of fetal pharmacology is essential for weighing the fetal benefits or risks of antimicrobial therapy for the infected gravid patient.

Tobramycin (tobramycin sulfate [Nebcin]; TBM) is one of the more recently identified components of the nebramycin complex introduced by Stark et al. (12) in 1967. Nebramycin is known to have eight factors, of which TBM is factor 6. These are water-soluble, basic compounds that fall into the general class of aminoglycosidic antibiotics produced by fermentation biosynthesis with *Streptomyces tenebrarius*.

TBM sulfate was released for clinical use in the United States in 1975. Its particular effectiveness is in the inhibition of *Pseudomonas* species, including those resistant to other aminoglycosides. The clinical benefits and risks of maternal therapy for the conceptus are often overlooked because of a paucity of human data. This study was undertaken to investigate the maternal-fetal transfer of TBM and its distribution in the human fetus of 9 to 20 weeks' gestation.

(Presented in part at the American Pediatric Society-Society for Pediatric Research, San Francisco, Calif., May 1973.)

[1] Present address: Instituto Nacional de Perinatologia, Vosgos y Montes Urales, Lomas de Chapultepec, Mexico 10, D.F.

MATERIALS AND METHODS

Thirty-five healthy gravid patients were admitted from July 1972 to June 1973 for elective therapeutic abortion and sterilization by hysterectomy. After informed consent was obtained, a single 2-mg/kg intramuscular dose of TBM was administered to the patients before surgery. Thirteen patients were in the first (I) trimester, and 22 were in the second (II) trimester. The time interval between administration of TBM to the patient and the clamping of the uterine arteries at delivery will be referred to as an "interval" in this report. This interval varied from 2 to 20.5 h for the trimester I patients and from 40 min to 34 h for trimester II patients. Thirty-four fetuses, from 9 to 20 weeks' gestation, were available for sampling.

Gestational age was calculated from day 1 of the last normal menstrual period. When the date of the last menstrual period was uncertain, fetal weight and foot length were used to estimate gestational age (13).

Maternal serum samples were taken before the intramuscular administration and at 1, 2, 4 and 8 h after the injection of TBM. A delivery time sample was obtained at the time of uterine artery clamping.

After surgical removal of the uterus, its contents were dissected under sterile conditions. Total amniotic fluid volume was measured, and an aliquot

was taken for sampling. The placenta was separated, weighed, and sampled. The fetus was weighed and then measured for crown-heel, crown-rump, and foot length. Fetal blood, urine, and cerebrospinal fluid (CSF) samples were obtained by needle aspiration. Fetal brain, lung, liver, and kidney tissue samples were acquired by dissection. Complete sampling of all fetal tissues and fluids was not technically possible in fetuses of less than 12 weeks' gestation. All specimens were frozen at −20°C until time of TBM assay.

TBM concentrations in maternal and fetal tissues and fluids were determined in duplicate by microbiological assay (1) with a modified standard cylinder plate method and *Bacillus subtilis* (ATCC 6633) on AM11 agar (Difco, 0593-01, Detroit, Mich.). The lowest detectable TBM concentrations were the following: 0.06 μg/ml for fetal urine and in maternal and fetal serum; 0.1 μg/ml for fetal CSF and amniotic fluid; 0.4 μg/g for placenta; and 0.08 μg/g for fetal brain, kidney, lung, and liver. Control curves of fetal tissues and fluids were made from a pool of six antibiotic-free fetuses.

STATISTICS

The two-sample rank test (the Mann-Whitney *U* test) was used to measure the significance of differences between any two groups of data, and significance levels were judged at $P < 0.01$. A regression line, determined by the method of least squares, was obtained from individual TBM concentrations in tissues and fluids and extrapolated to theoretical time zero. The time at which the sample concentration was one-half the concentration at theoretical time zero was defined as the half-life ($t_{1/2}$) with the following equation: $t_{1/2} = (-\log_e 2)/\beta$, where β is the rate constant for decrease in concentration in tissues or fluids with time.

RESULTS

Maternal serum. Maternal serum concentrations of TBM at 1, 2, 4, and 8 h, plus those at delivery time only up to an 11-h interval, are shown in Fig. 1. TBM was not detected in the delivery time maternal serum samples of the 15 gravid patients who delivered after the 11-h interval (Table 1). A mean maternal peak serum concentration of 4.0 μg/ml occurred at 1 h and $t_{1/2}$ was 1.54 h. Since no significant difference by trimester was found for $t_{1/2}$ or peak serum concentrations, the 35 patients could be considered as one group and are represented by the regression line in Fig. 2a ($r = 0.93$).

Placenta. TBM appeared in the first placental sample at 40 min and remained detectable until the last sample, taken at 34 h (Table 1). In all but 2 of the 29 samples, concentrations were above 1 μg/g, with an overall mean concentration of 1.4 μg/g. Although not significant, a definite trend toward increasing TBM concen-

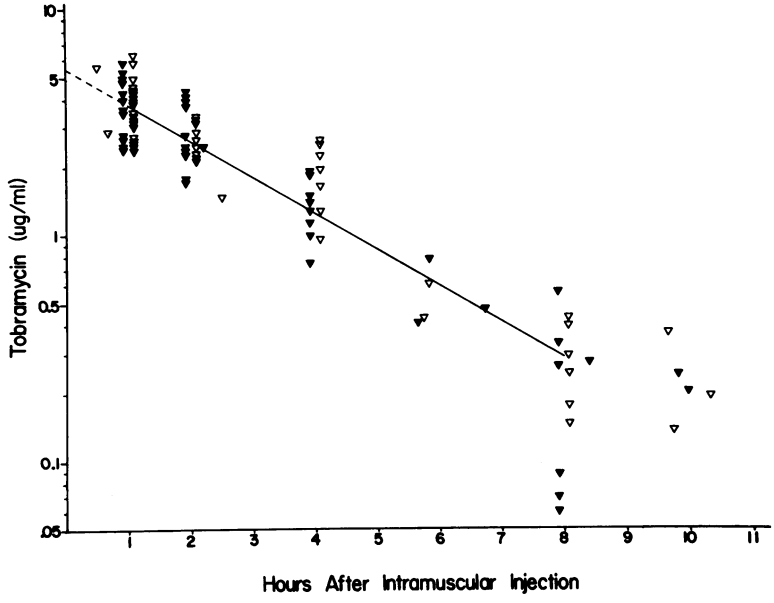

FIG. 1. *Maternal serum concentrations after a single 2-mg/kg injection of tobramycin to 35 healthy gravid patients who were 9 to 20 weeks' gestation. The symbols represent individual TBM concentrations of individual maternal serum samples at 1, 2, 4, and 8 h, plus the scattered samples taken at the time of clamping the uterine artery. The solid line represents a linear regression of the mean maternal serum concentration, and the dotted line represents the extension of this line to a theoretical time zero (r = 0.93). Symbols: (▼) Trimester I patients, (▽) trimester II patients.*

TABLE 1. *TBM concentrations after a single intramuscular dose of 2 mg/kg administered to 35 healthy gravidas 9 to 20 weeks' gestation*

Gestational age (weeks)	Interval[a] (h)	TBM concn in: Maternal serum at delivery (μg/ml)	Fetal tissues and body fluids: Placenta (μg/g)	Fetal serum (μg/ml)	Kidney (μg/g)	Urine (μg/ml)	Amniotic fluid (μg/ml)	CSF (μg/ml)
Trimester I patients								
12	2:00	2.8	1.04		1.2		0	0
11	2:13	2.05					0	0.2
11	4:00	1.0					0	0.3
12	4:00	1.45	1.92	0.56	2.4		0	0.2
12	5:40	0.42	1.6	0.28			0	0.2
9	5:50	0.8						
9	6:45	0.48					0.2	
11	8:25	0.28	1.6	0.25			0	0.7
11	9:50	0.25	1.5	2.56	0		0	
11	10:00	0.21	1.2	0	0.8			0.5
13	16:35	0	1.6	0	1.2	0	0.2	0
13	19:35	0	1.0	0	0	0.1	0.1	0.1
9	20:35	0		0				
Trimester II patients								
20	0:40	2.9	1.16	0	0	0.27	0	0
15	2:30	1.5		0.58	1.0	3.4	0	0
20	4:00	1.3	1.52	0.28	1.9		0.3	0
17	5:45	0.44	1.6	0.14	2.4	2.3	0.3	0
17	5:50	0.62	1.2	0.34	2.2		0.1	0
14	8:05	0.15	2.0	0.17	0.8		0.2	0
17	9:05	0	0.9	0.32	1.8		0.1	0
20	9:40	0.38	1.3	0.2		2.2	0.2	0
19	9:45	0.14	1.0	0.1	3.0	1.8	0.3	0
20	10:20	0.2	1.2	0.1	4.6	1.0	0.5	0
15	11:45	0	1.3	0	1.7	1.0	0.2	0
16	12:00	0	1.8	0.08	2.6	0.7	0.5	0.2
20	12:00	0	0.5	0.07	4.8	1.3	0.5	0
20	16:00	0	1.1	0.06	2.0	0.3	0.3	0
17	16:22	0	1.7		3.3	0.6	0.3	0
20	17:00	0	1.5	0.1	6.6	0.6	0.6	0
20	18:30	0	1.1		3.7	0.4	0.4	0
15	19:25	0	1.6	0	1.5	0.2	0.2	0.1
14	20:00	0	1.1	0	2.2	0.1	0.2	0
20	20:00	0	1.0	0	4.0		0.2	0
19	21:35	0	1.6	0	3.2	0.2	0.4	0
20	34:00	0	2.8		7.2	0	0.13	0

[a] Interval = hours between maternal TBM administration and clamping of uterine arteries.

trations with an increasing interval can be seen in Fig. 2b. There was no correlation of placental TBM concentration with duration of gestation, maternal serum concentration, or fetal tissue or fluid levels.

Fetal serum. TBM was detected in 19 fetal serum samples at delivery time. These concentrations are represented in Fig. 2c. The time when TBM first appeared in fetal serum, 2.5 h after maternal injection, was also the time when the highest TBM concentrations were observed. All fetal serum concentrations were less than 0.6 μg/ml and undetectable by a 17-h interval. The $t_{1/2}$ determined from these individual samples is 5.25 h ($r = 0.69$). No difference in TBM concentrations in fetal serum could be related to gestational age.

Fetal kidney. The highest and most persistent TBM concentration was demonstrated in the fetal kidney (Fig. 2d). TBM was present as early as 2 h and as late as 34 h in 24 of 27 samples analyzed. At more than 9 h after maternal antibiotic injection, 53% of the trimester II fetal kidney samples had TBM concentrations of 3 μg/g or more, a clinically therapeutic concentration. Two 20-week fetal kidney samples had 6.6 and 7.2 μg of TBM per g at 17 and 34 h, respectively. A significant correlation be-

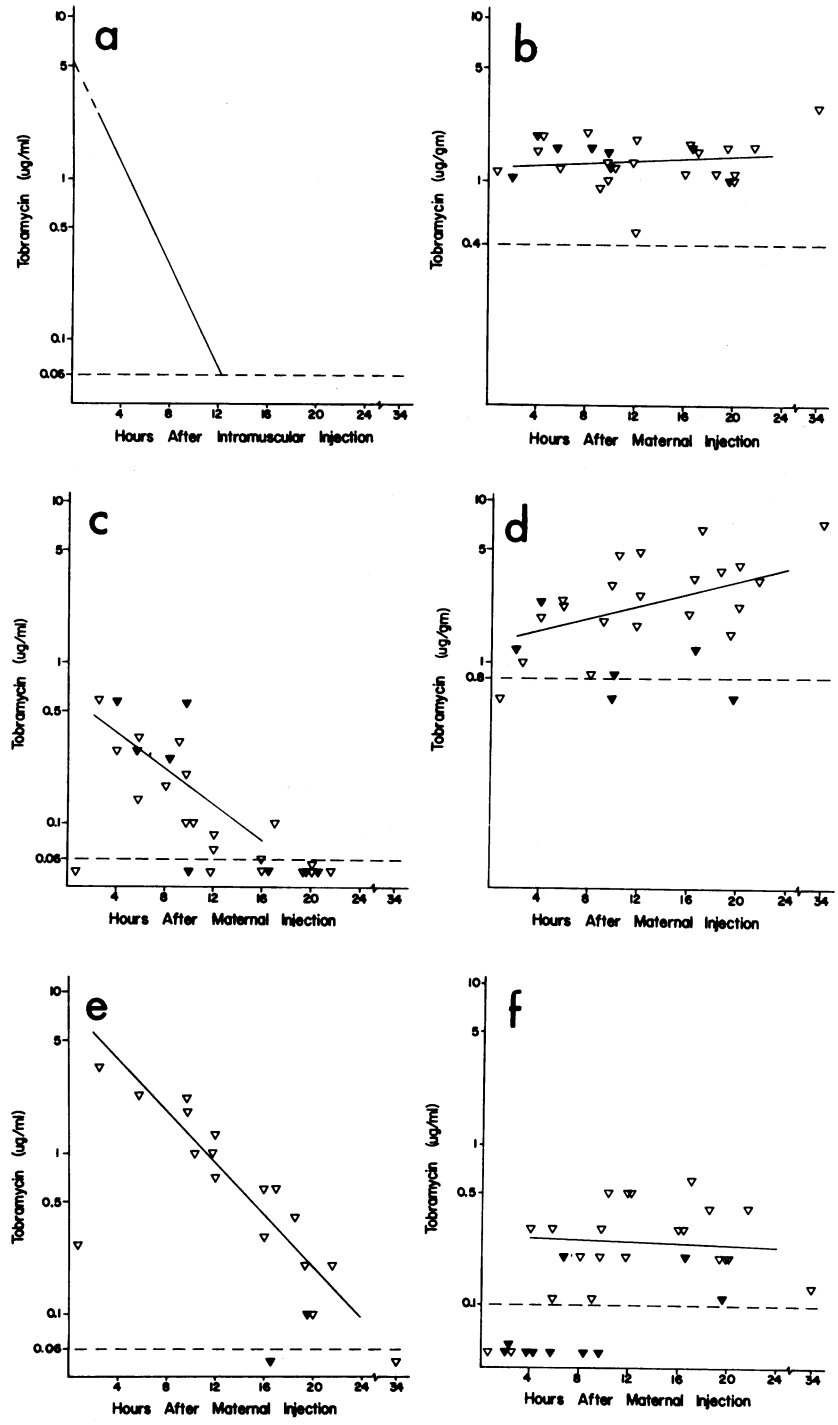

FIG. 2. *Tobramycin concentrations in: (a) maternal serum ($r = 0.93$; $t_{1/2} = 1.54$ h); (b) placenta (mean, 1.4 μg/g); (c) fetal serum ($r = 0.69$; $t_{1/2} = 5.25$ h); (d) fetal kidney ($r = 0.54$); (e) fetal urine ($r = 0.92$; $t_{1/2} = 3.7$ h); (f) amniotic fluid (mean, 0.25 μg/ml), after a single intramuscular dose administered to 35 gravid patients 9 to 20 weeks' gestation. The solid lines represent the regression line or mean concentration as determined from the individual values represented by the triangular symbols in figures (b) through (f). The interrupted horizontal lines indicate the lowest detectable concentrations of tobramycin for that tissue or fluid. Symbols: (▼) Samples from trimester I of pregnancy, (▽) samples from trimester II of pregnancy.*

tween the interval from maternal injection and fetal kidney concentration can be seen in Fig. 2d ($r = 0.54$). When gestational age was related to the fetal kidney TBM concentration, the correlation coefficient improved to $r = 0.79$. An even more significant correlation coefficient of $r = 0.82$ was found when multiple regression analysis was used to relate (i) TBM concentration in fetal kidney, (ii) the interval after maternal TBM injection, and (iii) the weeks of gestation (Fig. 3).

Fetal urine. Fetal urine was available from 19 fetuses of more than 12 weeks' gestation. TBM appeared promptly in the first sample taken at 40 min. The highest concentration, 3.4 μg/ml, occurred at an interval of 2.5 h. After this peak, a steady decline in concentration was observed. A $t_{1/2}$ of 3.7 h was calculated ($r = 0.92$; Fig. 2e). No relationships could be established between fetal urine and fetal kidney TBM concentrations or gestational age.

Amniotic fluid. In fetuses older than 13 weeks' gestation, antimicrobial activity in amniotic fluid was first detected at a 4-h interval. An overall mean TBM concentration of 0.25 μg/ml was sustained until the 21.6-h interval. In trimester II samples, TBM was found in fetal urine before it appeared in amniotic fluid samples (Fig. 2f). At or beyond a 4-h interval, only three of eight trimester I amniotic fluid samples revealed TBM activity, whereas all of 20 trimester II samples had aminoglycoside present, indicating a significant relationship to gestational age (Table 1).

CSF and other tissues. Of 27 fetal brain samples, none contained TBM in detectable concentrations. In the fetal CSF, very low TBM concentrations (0.1 to 0.7 μg/ml) were found at the 2- to 20-h interval in 9 of 15 fetuses of less than 17 weeks' gestation. No TBM was detected in the 16 fetuses of 17 weeks' gestation or more (Table 1). TBM could not be detected in any of the 32 fetal lung or 33 fetal liver specimens.

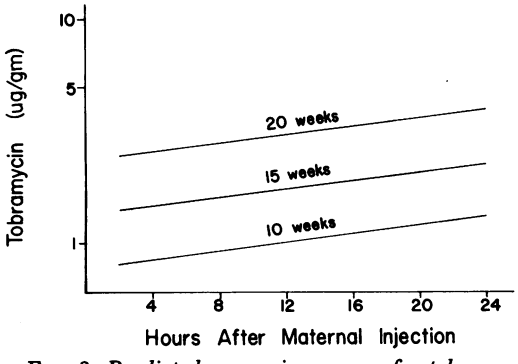

FIG. 3. *Predicted regression curves for tobramycin concentrations in fetal kidneys of 10, 15, and 20 weeks' gestation after a single 2-mg/kg intramuscular injection to 35 gravid patients, 9 to 20 weeks' gestation. ($Log_n Y' = -1.3525 + 0.024X + 0.11037$, where X = time in hours after TBM administration to the gravid patient and Y = gestational age in weeks, $Rx \cdot Yz = 0.82$.)*

DISCUSSION

Our data, from the single sampling of 34 different fetuses in the early weeks of pregnancy, demonstrated enough consistency to establish biological curves and provide a meaningful indicator of the kinetics and distribution of TBM in the conceptus.

During the first 20 weeks of pregnancy, the maternal plasma volume increases 30% (2), and renal plasma flow and glomerular filtration rate increase 30% to 50% (6). In spite of these changes, the mean maternal peak serum concentration and serum $t_{1/2}$ were found to be within the ranges reported for healthy, nonpregnant adults when similar doses were used (3, 11).

In the placental tissue samples from fetuses in the first half of pregnancy, a mean tissue TBM concentration of 1.4 μg/g was maintained for at least 24 h; this concentration bore no relationship to presence of TBM in maternal serum. If we can assume that micrograms per grams of tissue are equivalent to micrograms per milliliter of body fluids for bacteriological inhibition, then TBM may prove useful in the treatment of placental infections, since in vitro bacterial inhibitory concentrations of TBM range from 0.4 to 0.8 μg/ml for *Staphylococcus aureus* and 0.8 to 3.1 μg/ml for *Pseudomonas* species, *Escherichia coli*, and *Klebsiella pneumoniae* (9, 10). Investigations addressing the question of whether higher placental concentrations can be produced by larger or repetitive doses may define a limited or linear capacity to handle TBM.

After it had crossed the placenta to the fetus, TBM was cleared from the fetal serum within 17 h, with a $t_{1/2}$ of 5.25 h. Kaplan and co-workers (5) reported that the TBM serum $t_{1/2}$ for small premature infants is 8.7 h. If the longer fetal serum $t_{1/2}$ of the premature infant is attributable to immature renal function, then the shortened fetal serum $t_{1/2}$ may reflect the role of the placenta in the fetal clearance of TBM.

TBM was not found in the CSF of the fetuses older than 16 weeks' gestation. A mechanism limiting the entry of this aminoglycoside into the CSF seems to be established at this stage of maturation (4).

Fetal renal tissue concentrations of TBM were high and were found to increase significantly for as long as 34 h, whereas both fetal serum and urine concentrations steadily declined to nondetectable levels by 17 and 21.6 h, respectively. Luft and Kleit (7) reported prolonged renal parenchymal accumulation in adult rats after a single dose of several aminoglycosidic antibiotics. Dissection of the adult rat kidneys revealed 85% of the TBM to be in the cortex, which consists mostly of proximal tubules and where histological evidence of gentamicin toxicity has been described. With advancing gestational age, the human fetal kidney has a more defined cortical structure (14). This may explain the significant differences in TBM concentration in the fetal kidneys of 10 weeks' gestation as compared with those 20 weeks of age. The absolute or relative tissue concentration of TBM that would cause fetal renal toxicity is not known for any stage of fetal maturation. One wonders whether TBM, a potentially nephrotoxic aminoglycoside, could be used more safely in trimester I because of the lower concentrations found in the younger fetal kidney or whether it could be equally or more toxic because of the greater degree of renal immaturity. If, in our studies, the fetal kidney could attain such high and sustained levels after administration of a single therapeutic dose to the gravid patient, then might higher renal tissue concentrations be reached when multiple doses of TBM are administered? This has been shown to occur with other antibiotics by Philipson and co-workers (8). They reported a considerably higher increase in fetal kidney concentration of both erythromycin (500 mg) and clindamycin (450 mg) after multiple doses compared with single doses of the same agents administered to pregnant patients.

Thirty percent of the human fetal glomeruli appear to be relatively mature histologically by 16 weeks' gestation (14). Fetal urine concentrations of TBM were 5 to 10 times greater than those found in simultaneous fetal serum samples. If TBM is primarily excreted by glomerular filtration, our results are consistent with active renal glomerular TBM excretion in the fetus of 13 or more weeks' gestation.

TBM did not appear in the amniotic fluid until it was present in the urine; this is consistent with the premise that the presence of the antibiotic in amniotic fluid is primarily related to renal excretion. One might expect TBM to accumulate in amniotic fluid; however, rather inexplicable static levels were found in amniotic fluid, which is known to undergo a dynamic turnover.

Although it has been traditional to sample body fluids for evidence of drug concentrations in a toxic range, our findings in the fetal kidney give rise to a serious skepticism about the equatability of such an approach since, with the longer intervals, the fetal kidney had increasing mean concentrations of TBM, whereas fetal serum and urine levels were decreasing to undetectable amounts. Tissue sampling for drug levels may be a more appropriate, although usually inaccessible, approach to extending the understanding of the pharmacology of certain therapeutic agents. Further investigation of the perinatal pharmacology of this and other drugs is imperative to obtain the data necessary for treating the infected gravid patient and her fetus with the increasing number of available antimicrobials.

ACKNOWLEDGMENTS

We wish to express our sincere appreciation and gratitude to Lorraine Wittman and Paul F. Thielen for technical assistance, and to Michaeline Abate for statistical analysis.

This study was supported by a grant from Eli Lilly & Co., Indianapolis, Ind.

LITERATURE CITED

1. Bennett, J. V., J. L. Brodie, E. J. Benner, and W. M. M. Kirby. 1966. Simplified, accurate method for antibiotic assay of clinical specimens. Appl. Microbiol. 14:170-177.
2. Berlin, N. I., C. Goetsch, G. M. Hyde, and R. J. Parsons. 1953. The blood volume in pregnancy as determined by p^{32} labeled red blood cells. Surg. Gynecol. Obstet. 97:173-176.
3. Black, H. R., and R. S. Griffith. 1974. Comparative pharmacology of tobramycin and gentamicin in adult volunteers, p. 24-30. *In* Tobramycin: selected proceedings from the Eighth International Congress of Chemotherapy, Athens, September 8-15, 1973. Excerpta Medica, New York.
4. Evans, C. A. N., J. M. Reynolds, M. L. Reynolds, N. R. Saunders, and M. B. Segal. 1974. The development of a blood-brain barrier mechanism in foetal sheep. J. Physiol. 238:371-386.
5. Kaplan, J. M., G. H. McCracken Jr., M. L. Thomas, L. J. Horton, and N. Davis. 1973. Clinical pharmacology of tobramycin in newborns. Am. J. Dis. Child. 125:656-660.
6. Lindheimer, M. D., and A. I. Katz. 1973. Pregnancy and the kidney. J. Reprod. Med. 11:14-18.
7. Luft, F. C., and S. A. Kleit. 1974. Renal parenchymal accumulation of aminoglycoside antibiotic in rats. J. Infect. Dis. 130:656-659.
8. Philipson, A., L. D. Sabath, and D. Charles. 1973. Transplacental passage of erythromycin and clindamycin. N. Engl. J. Med. 288:1219-1221.
9. Ries, K., M. E. Levison, and D. Kaye. 1973. In vitro evaluation of a new aminoglycoside derivative of kanamycin, a comparison with tobramycin and gentamicin. Antimicrob. Agents Chemother. 3:532-533.
10. Schoutens, E., and E. Yourassowsky. 1974. Tobramycin: clinical and microbiological evaluation, p. 94-101. *In* Tobramycin: selected proceedings from the Eighth International Congress of Chemotherapy, Athens, September 8-15, 1973. Excerpta Medica, New York.

11. Simon, V. K., E. U. Mösinger, and V. Malerczy. 1973. Pharmacokinetic studies of tobramycin and gentamicin. Antimicrob. Agents Chemother. 3:445–450.
12. Stark, W. M., M. M. Hoehn, and N. G. Knox. 1968. Nebramycin, a new broad spectrum antibiotic complex. I. Detection and biosynthesis, p. 314–323. Antimicrob. Agents Chemother. 1967.
13. Trolle, D. 1947. Age of the foetus determined from its measures. Acta Obstet. Gynecol. Scand. 27:327–332.
14. Vernier, R. L., and F. G. Smith, Jr. 1968. Fetal and neonatal kidney, p. 225–260. *In* N. S. Assali (ed.), Biology of gestation, vol. 2. Academic Press Inc., New York.