Maternal and transplacental kinetics of trimethoprim and sulfamethoxazole, separately and in combination

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Summary: The fate of trimethoprim and sulfamethoxazole and the combination of both these agents was studied in a group of healthy pregnant women who were undergoing therapeutic abortion. Assays were performed on samples of blood, urine, amniotic fluid and fetal tissues, using a standardized protocol for the selection of patients, dose administration, sample collection and assay techniques. A comparative evaluation of kinetics to assess the maternal handling and the distribution of trimethoprim throughout the fetoplacental unit disclosed no significant difference in the concentration within fetal fluids and tissue compartments. On the other hand, the concentration of sulfamethoxazole was lower in fetal tissues than in fetal fluids when relative ratios to trimethoprim were compared. The implications of the difference in behaviour of the two molecules are discussed from both the pharmacokinetic and clinical points of view.

Résumé: Dans une population de parturientes devant subir un avortement thérapeutique nous avons conduit une étude du destin de l'association triméthoprime-sulfaméthoxazole et de l'une ou l'autre des deux molécules individuelles. Suite à un plan d'expérience standardisé pour la sélection des patients, la dose et l'administration, la collection des échantillons et les techniques de dosage, nous avons procédé à la détermination des concentrations sanguines, urinaires, du liquide amniotique et des tissus fétaux. Nous obtenons ainsi pour les trois substances les données de cinétique comparative qui permettent l'évaluation, d'abord du destin maternel des substances, et ensuite de leur distribution à l'intérieur des compartiments fétaux. L'analyse des résultats démontre que le triméthoprime se distribue universellement à l'intérieur de l'unité fétoplacentaire; il ne semble pas y avoir de différence significative entre les niveaux obtenus soit liquidiens soit tissulaires fétaux. Par contre, nous constatons une diminution progressive des concentrations du sulfaméthoxazole, des liquides aux tissus fétaux, lorsque sont comparées les proportions relatives au triméthoprime. La discussion porte sur les implications pharmacocinétiques et cliniques d'une telle différence de comportement entre les deux molécules.

The clinical efficacy and low degree of clinical toxicity of the combination trimethoprim-sulfamethoxazole (TMP-SMX) have been well documented over the past decade throughout the world. Bacteriuria and/or pyelonephritis during pregnancy are recognized as serious threats to mother and fetus[1,2] and since the main action of this antimicrobial is against gram-negative pathogens of the urinary tract, the studies of Williams and colleagues[3] and Brumfitt and Pursell[4] of bacteriuria of pregnancy are highly important to the obstetrician.

Although Brumfitt and Pursell concluded that there is no strong evidence that either TMP-SMX or sulfamethoxazole alone is teratogenic or causes gross hematologic disorder, no study of the fate of these molecules was carried out.[5] In the only attempt to determine the extent of the penetration of TMP-SMX into the fetoplacental unit, the data were gathered mostly after administration of a single dose.[6] In maternal serum, peak levels of both components were reached within 4 hours; in amniotic fluid the time required was 14 hours for TMP and 10 hours for SMX. Unfortunately in this investigation the data for repeated administration were obtained following intravaginal application, which prevents comparison with previous kinetic studies. We therefore undertook to determine the time taken to penetrate the fetoplacental unit when TMP-SMX or its individual components were taken orally.

The fetoplacental unit

Fig. 1 illustrates the several body compartments of the human model, where the general principles of the passage of drugs across biologic barriers apply. Although the presence of additional membranes would appear to protect the fetus against foreign substances, Yaffe declares that the "placental barrier" is a myth.[7] In effect, drugs are thought to cross the placenta

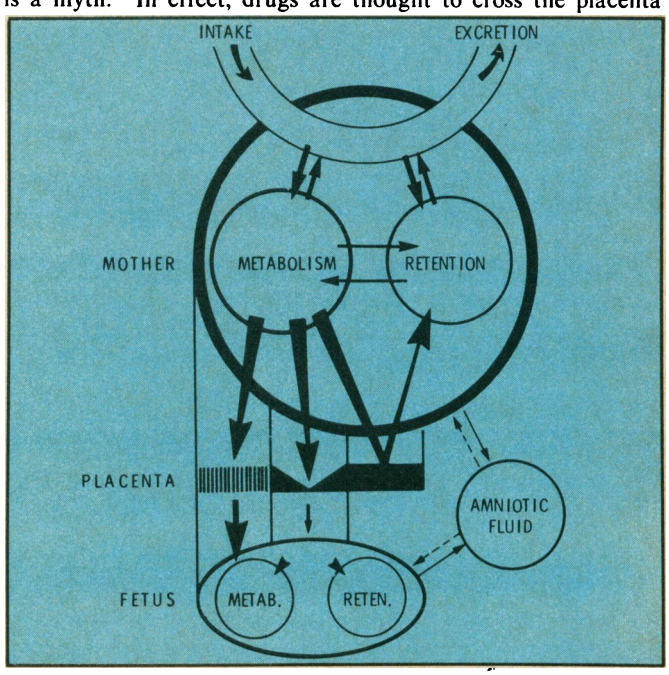

FIG. 1—Diagrammatic representation of the fetoplacental unit.

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essentially by simple passive diffusion, to establish an equilibrium between the maternal and fetal blood, the rate of passage being primarily dependent upon the concentration gradient. This dependence of the fetus on the maternal concentration of a drug renders it highly vulnerable whenever a drug has the capacity to penetrate tissues. The characteristics of trimethoprim are such that Fowle, from pharmacokinetic studies in volunteers, concluded that trimethoprim is distributed in both intracellular and extracellular fluid.[8] Since the distribution volume for trimethoprim exceeds the total body fluid, it must be concentrated in one or more compartments. Although reasonable prediction of plasma concentrations after continued dosage can be made from the knowledge that trimethoprim and sulfamethoxazole have fairly equal half-lives (10.1 and 11.4 hours respectively), similar data have not been obtained for the pregnant woman.

Materials and methods

Kinetic protocol

The kinetic protocol is schematically represented in Fig. 2. The subjects received an identical loading dose of trimethoprim, 960 mg and sulfamethoxazole, 4.8 g, either in combination or separately in identically formulated tablets. The total dose was administered over a 48-hour period and for nearly all patients the regimen consisted of three tablets every 12 hours. Three patients given sulfamethoxazole alone and one given trimethoprim alone received one dose less than the other subjects over a 40-hour period. Because of the difficulty of estimating exactly the time at which operation would be performed (abortion and tubal ligation), the dosage schedule was not strictly identical in all patients, nor was the timing after the last dose of the sampling of body fluids and tissues. Nevertheless the interval after the last dose was within the relatively narrow range of 8 to 12 hours. The 24-hour urine collection extended from the time of administration of the last dose of the loading period. Sampling from other sites was done at the time of operation.

Assays and procedures

The assay procedure for trimethoprim was the microbiologic method described by Bushby and Hitchings,[9] using *Bacillus pumilus* (kindly supplied by Dr. Bushby). The microbiologic assay was first standardized by comparing its results with those of gas-liquid chromatography and spectrofluorometry, using identical samples. The reproducibility of microbiologic and gas-liquid chromatographic methods was found to be highly correlated. By the microbiologic method it is the "active" trimethoprim that is measured.

To assay sulfamethoxazole we used the colorimetric method described by Rieder,[10] which permits the quantitative determination of the potentially bacteriostatic material of sulfonamide type in body fluids and tissues during treatment. This modification of the standard Bratton-Marshall reaction allows the total sulfonamide content in the samples to be calculated.

Drug concentrations were expressed in milligrams per 24 hours of urine collection (mg/24 h), in micrograms per millilitre of fluid (μg/ml) or per gram of tissue (μg/g).

The twin pregnancy in one subject (B.S.) provided an unexpected check on the reproducibility of assays. Table I shows the close correlation for both tissue and fluid sulfamethoxazole concentrations between twins A and B.

All determinations were performed at l'Institut de Recherches Psychiatriques de Joliette.

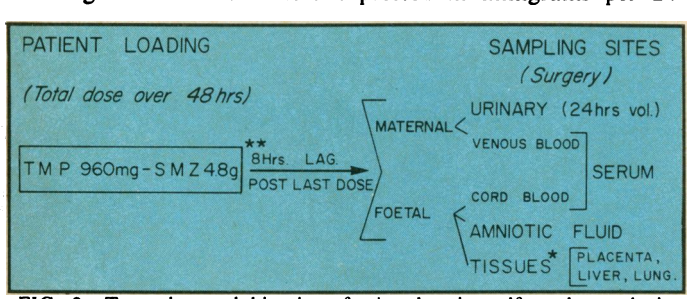

FIG. 2—Transplacental kinetics of trimethoprim-sulfamethoxazole in humans—kinetic protocol. *Available only in the individual component study. **Total doses were the same whether given in combination or individually.

Subjects

In Table II are summarized the clinical characteristics of the women who underwent therapeutic abortion and tubal ligation. Informed consent was obtained prior to the oral loading period. Over the 4 years that the study lasted both the maternal and gestational ages remained fairly uniform. The number of previous pregnancies was generally similar, namely three or more. Later in the course of the study there was a tendency for subjects to present themselves earlier in pregnancy, which rendered more difficult the sampling of amniotic fluid, fetal blood and tissues because of the small fetal size.

Drug dosage

The all-important variable of dose had not previously been

Table I—Assay reproducibility

Mother B.S. Age: 33 Gestational age: 14 weeks On sulfamethoxazole	Fetal fluids (γ/ml)		Fetal tissues (γ/ml)		
	Amnios	Cord blood	Placenta	Liver	Lung
Twin A	*	99.1	3.0	5.5	†
Twin B	34.0	75.5	4.1	6.6	2.7

*Not available
†Insufficient quantity

Table II—Clinical characteristics

Population* N = 31		Maternal age (years)	Gestational age (weeks)
TMP-SMX study (n = 7)	Mean	35.00	14.86
	± SD	± 6.88	± 1.68
	Range	27-40	13-18
Trimethoprim study (n = 11)	Mean	35.64	14.50
	± SD	± 5.39	± 2.85
	Range	22-43	10-19
Sulfamethoxazole study (n = 13)	Mean	34.77	13.69
	± SD	± 4.07	± 3.68
	Range	25-41	09-22

*All subjects were gravida III and above.

Table III—Drug dosages

Population N = 31	TMP	SMX
TMP-SMX study (n = 7)	15.01 mg/kg ± 1.99	74.9 mg/kg ± 9.67
Trimethoprim study (n = 11)	15.27 mg/kg ± 1.78	—
Sulfamethoxazole study (n = 13)	—	71.37 mg/kg ± 15.89

standardized, apart from scheduling a fixed dose either of TMP-SMX or of either of its components. As shown in Table III, when the dose is corrected for body weight the values obtained for doses administered in the individual component studies compared very well with those from the combination study: for TMP, 15.01 mg/kg in combination, as compared with 14.57 mg/kg singly; and for SMX, 74.91 mg/kg in combination, compared with 74.33 mg/kg singly.

Results

Combination study

The concentrations in maternal urine and serum and in fetal serum and amniotic fluid of sulfamethoxazole are listed in Table IV and for trimethoprim in Table V. The seven patients from the combination study provided values for all body fluid sampling sites except fetal cord blood, which in six subjects could not be assayed for sulfamethoxazole because the quantity of liquid was insufficient after the microbiologic assay for trimethoprim. In Figs. 3 and 4 the relative urinary excretion and body fluid distribution of both molecules of the combination are graphically represented. A difference in kinetic behaviour is at once apparent; while TMP is concentrated equally in maternal serum, fetal serum and amniotic fluid, the lower concentration of SMX in amniotic fluid than in maternal serum is significant ($P < 0.05$).

Individual component studies

In Tables VI and VII are listed the concentrations of sulfamethoxazole and trimethoprim for all seven sampling sites. These are graphically represented in Figs. 5 and 7 for body fluids and in Figs. 6 and 8 for fetal tissues. Here again the behaviour of the two molecules is different. While TMP concentrations from all the various sampling sites are comparable, SMX concentrations are significantly lower in the fetus than in the mother. The value in fetal serum is lower than in maternal serum ($P < 0.001$) and the concentration in amniotic fluid is lower still ($P < 0.001$). On the other hand, in fetal tissues differences in concentrations could not be found, as demonstrated by paired *t* tests, for either molecule at the three sampling sites—placenta, liver and lung. However, when ratios of the mean concentrations of SMX to TMP are examined (Table VIII) a further reduction in fetal tissue penetration by SMX is suggested; the ratio for fetal lung is 5:1 as compared with 9:1 for placenta and 10:1 for fetal liver.

Table IV—Sulfamethoxazole* concentrations

N = 7	Urinary† (mg/24 h)	Serum (γ/ml) Maternal	Serum (γ/ml) Fetal	Amniotic fluid (γ/ml)
G.K.	222.0	23.40	‡	17.03
M.S.	309.4	34.55	61.10	30.48
Z.H.	318.0	64.03	‡	14.95
J.F.	623.2	29.90	43.74	9.75
F.Z.	668.3	78.57	‡	20.63
Z.D.	852.8	42.58	‡	21.61
D.R.	252.3	7.67	‡	11.53
Mean	463.7	40.10		18.16
±SD	247.3	24.24		7.60

*Bratton-Marshall colorimetric assay (modified by Rieder[10])
†Sampling extends from the end of the 48-hour loading period.
‡Not enough serum remaining after TMP assay.

Table V—Trimethoprim* concentrations

N = 7	Urinary† (mg/24 h)	Serum (γ/ml) Maternal	Serum (γ/ml) Fetal	Amniotic fluid (γ/ml)
G.K.	124.32	1.50	0.82	1.03
M.S.	145.60	0.60	1.36	0.90
Z.H.	218.40	3.85	1.10	2.50
J.F.	136.80	1.80	0.48	1.30
F.Z.	163.00	1.60	1.30	1.40
Z.D.	92.56	2.23	1.60	1.40
D.R.	17.98	0.79	0.38	0.85
Mean	128.38	1.77	1.01	1.34
±SD	62.13	1.08	0.46	0.56

*Microbiological assay method
†Sampling extends from the end of the 48-hour loading period.

Discussion

The fetoplacental unit as a pharmacokinetic human model

The present study supports concepts recently expressed in a review by Yaffe of the relationships between administration of drugs to the pregnant woman and fetal outcome.[7] Because of the widespread use of drugs, often in high dosage, during pregnancy, the physician should have available precise kinetic data on the handling by mother and fetus of the agent prescribed. Although one might assume the presence of barriers to the transference of drugs to the fetus, namely the placenta and amniotic fluid, we could detect no such limitation with respect to trimethoprim. While there appeared to be some restriction on the transference of sulfamethoxazole, this has yet to be quantitated, since it is inferred only from a twice daily

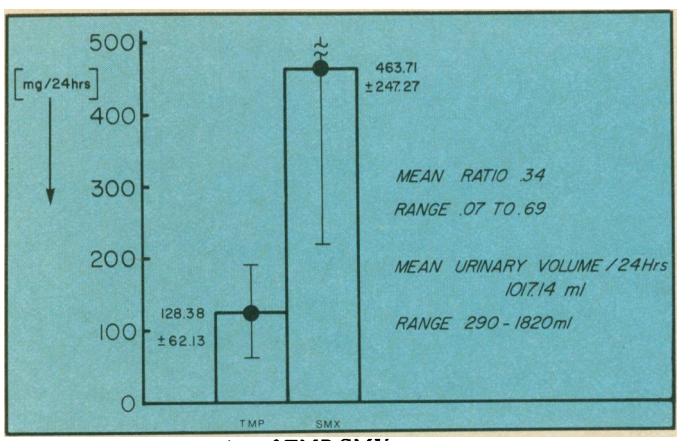

FIG. 3—Urinary excretion of TMP-SMX.

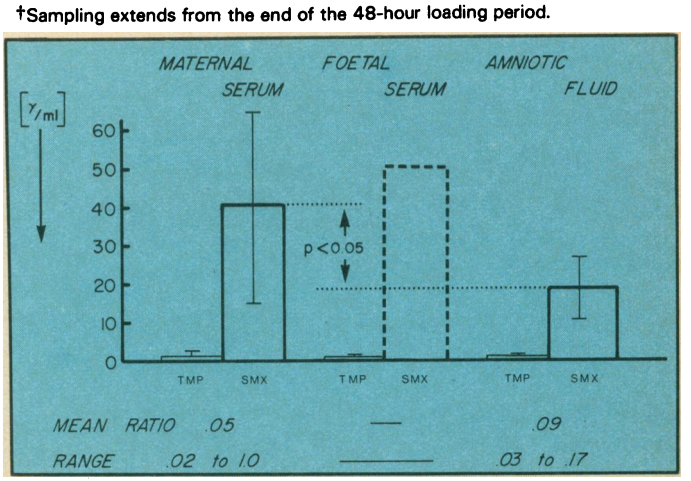

FIG. 4—Distribution of TMP-SMX in body fluid compartments.

Table VI—Sulfamethoxazole study (concentrations)

n = 13	Total dose/kg	Sampling sites: Maternal fluids: Urine	Maternal fluids: Blood	Fetal fluids: Amnios	Fetal fluids: Cord blood	Fetal tissues: Placenta	Fetal tissues: Liver	Fetal tissues: Lung
V.M.	50.28	128.25	88.7	*	*	1.9	2.19	†
B.D.	53.33	894.6	64.2	*	43.4	1.4	†	*
L.L.	61.02	587.6	130.2	30.2	*	3.3	2.9	6.3
T.E.	69.57	823.3	*	*	67.9	4.9	7.7	4.7
R.D.	69.98	1088.0	92.5	12.3	*	2.3	3.2	4.8
S.M.	70.59	77.6	111.3	39.6	81.2	5.1	*	*
D.P.	73.28	1178.1	98.10	17.0	*	2.5	†	†
B.S.	74.77	1003.5	96.23	A*	99.1	3.0	5.5	†
				B 34.0	75.5	4.1	6.6	2.7
S.A.	76.19	1188.0	88.7	16.0	69.8	3.3	*	4.9
M.E.	80.0	428.4	113.2	*	*	2.3	*	*
M.C.	85.87	*	98.1	*	82.1	2.3	2.7	2.7
D.E.	97.96	855.7	90.6	*	*	*	*	*
B.J.	103.45	915.6	112.3	*	*	2.2	2.2	†

*Not available

†Insufficient quantity

Table VII—Trimethoprim study (concentrations)

n = 11	Total dose/kg	Sampling sites: Maternal fluids: Urine	Maternal fluids: Blood	Fetal fluids: Amnios	Fetal fluids: Cord blood	Fetal tissues: Placenta	Fetal tissues: Liver	Fetal tissues: Lung
G.J.	15.34	102.0	2.15	2.8	0.58	0.48	0.15	*
B.D.	18.05	70.6	0.96	6.3	0.79	0.35	0.19	0.80
L.A.	13.24	128.3	*	1.5	*	0.13	0.68	0.21
T.C.	13.12	51.04	*	*	†	0.25	0.40	0.68
W.R.	15.12	49.5	1.02	5.3	1.0	0.15	0.64	1.21
F.C.	15.48	68.9	0.49	0.47	0.64	0.36	0.11	0.25
L.J.	15.68	24.0	*	1.75	†	0.19	0.45	0.99
M.R.	15.14	38.2	0.98	*	*	0.23	0.60	1.90
K.V.	14.12	*	1.52	*	*	0.12	0.99	0.26
L.I.	12.52	70.4	*	0.49	0.6	0.56	0.37	0.96
R.L.	12.47	47.56	1.20	†	0.79	1.35	0.15	0.21

*Not available

† Insufficient quantity

Table VIII—Comparative analysis of drug concentrations

	Sampling sites: Maternal fluids: Urine	Maternal fluids: Blood	Fetal fluids: Amnios	Fetal fluids: Cord blood	Fetal tissues: Placenta	Fetal tissues: Liver	Fetal tissues: Lung
Sulfamethoxazole study (n = 13) 71.37 mg/kg	823.56	98.24	24.85	69.98	3.38	4.62	4.24
Trimethoprim study (n = 11) 15.27 mg/kg	62.79	1.13	3.07	0.72	0.37	0.46	0.75
Ratio of means 4.67:1	13:1	87:1	8:1	97:1	9:1	10:1	5:1

dosage for a 2-day loading period. As has been shown by Ylikorkala and colleagues[6] as well as by ourselves, it is possible, though difficult, to study the fate of drugs in the fetoplacental unit. Our study had one deficiency, namely that the experimental unit could not be used as its own control by furnishing repeated assays to establish the function of time and dose. The use of multiple samples according to the same protocol when these two variables are altered could provide a baseline for establishing the dosage of continuous treatment with TMP-SMX for infections during pregnancy.

The clinical setting

The management of subjects for whom a therapeutic abortion is planned presents a challenge to experimental methodology. The greatest difficulty we encountered was in timing correctly both the oral loading phase of the drug and the surgical procedure. The attrition rate did not exceed 39% (14 of 38 subjects) in the individual component study. Failure to procure specimens for assay from all seven sampling sites reduced the number of experimental subjects still further. Inability to obtain amniotic fluid, cord blood or fetal tissues because of the small size of the fetus accounted for most of these losses. In a few instances omission, breakage or improper handling was responsible. However, in view of the many impediments the harvest of cases was sufficient to allow determination with reasonable accuracy of the concentrations of trimethoprim and sulfamethoxazole at the various sampling sites.

Data analysis

All tests of difference used were paired *t* tests, with one exception, viz. amniotic fluid compared with fetal blood SMX levels, since in only four subjects were assays available at both sites. Student's *t* test was done on the maximum number of available values for each sampling site. Since the study consisted of two phases, for the TMP-SMX combination first and for the individual components subsequently, both sets of data were not compared.

Because of the lack of complete data in the studies of individual components a compromise was reached to utilise the maximal number of values from the raw data tables. Pairing the results from two or three sites having reference to body fluids or tissues within the maximal number of subjects available was the method employed.

Transplacental kinetics

In order to assess tissue penetration of trimethoprim and sulfamethoxazole, a steady state should have been reached. It is our claim that the data presented were collected under such conditions. Since for normal adults with normal disposal mechanisms the administration of two tablets of TMP-SMX every 12 hours achieves equilibrium phase concentrations in less than six doses,[11] our loading procedure, dosage and time interval appear to ensure the steady state level.

Having this assurance we can calculate from our data the

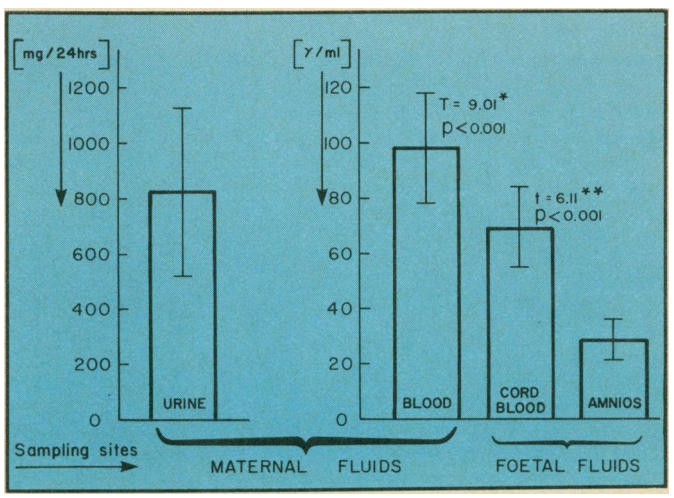

FIG. 5—Concentration of sulfamethoxazole in body fluids. *Paired sample of five subjects. **Independent samples of six subjects each.

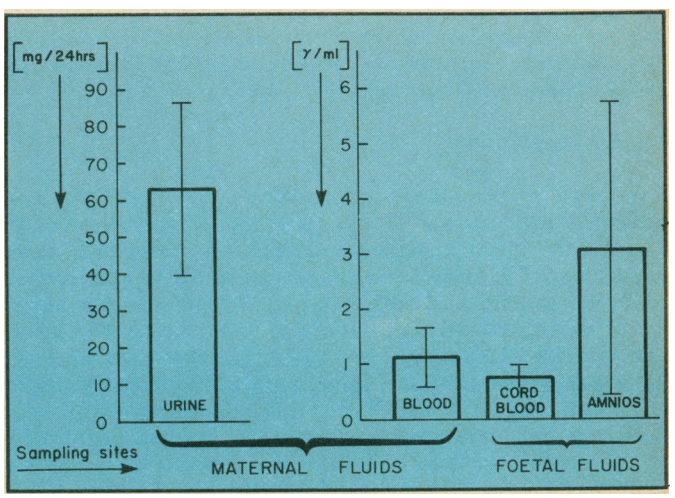

FIG. 7—Concentration of trimethoprim in body fluids. All P values indicated nonsignificance (paired *t* test).

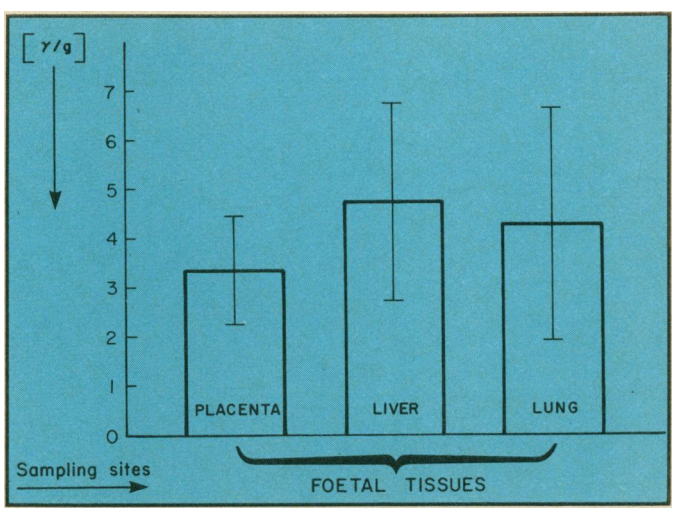

FIG. 6—Concentration of sulfamethoxazole in fetal tissues. All P values indicated nonsignificance (paired *t* test).

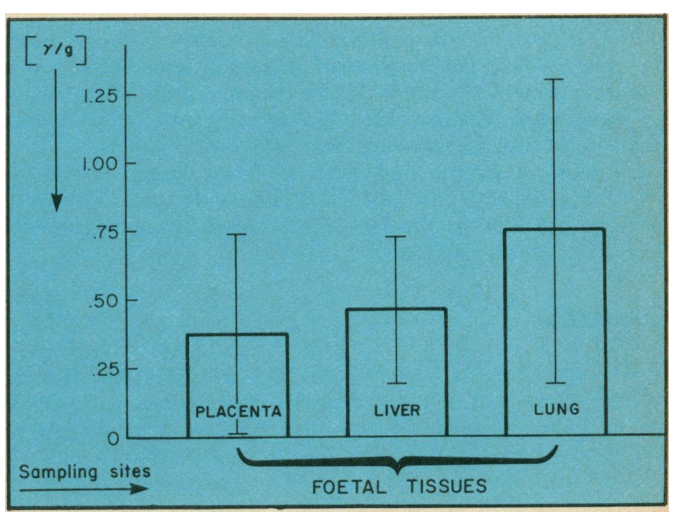

FIG. 8—Concentration of trimethoprim in fetal tissues. All P values indicated nonsignificance (paired *t* test).

extent and degree of the penetration of TMP into the fetoplacental unit. Previous experience has shown that TMP can be assayed in bronchial secretions, prostatic fluid, pus from osteomyelitis and, as reported by Stamey and Condy,[12] vaginal fluid. These examples differed from the present situation in one respect: there was only one tissue barrier between the circulation and the secretory cells involved. Only one other example is comparable with the fetoplacental unit from a distribution point of view, the biliary system, where trimethoprim passes from the general circulation into the enterohepatic circulation. Newman reported a similar unlimited transfer of trimethoprim into bile and some impediment to the passage of sulfamethoxazole, although this was less pronounced than in the present instance of the placenta.[13]

In numerous published reports estimates have been made of the tissue penetration of TMP-SMX and its components.[14,15] It has been generally inferred that trimethoprim becomes concentrated in tissues. However, from the present study we must conclude that it is the penetration of sulfamethoxazole that is limited, at least when the drugs are administered separately. The trimethoprim levels remained comparable, not only within the maternal and fetal fluid compartments but also within the fetal tissues. It is to be noted that of the three tissues assayed the lung, which is the fetal tissue least perfused, registered the lowest sulfamethoxazole concentrations relative to trimethoprim. Inasmuch as the present model shows restricted movement of trimethoprim across membranes and limited transfer of sulfamethoxazole, accumulation of trimethoprim in tissues has not been found. Perhaps continued administration over a prolonged period could provide the data necessary to decide between conflicting conclusions on the true fate of TMP-SMX and its components in tissues.

Clinical implications

In view of the ability of trimethoprim to reach the fetoplacental unit, its use in the treatment of certain types of intrauterine infection deserves consideration, especially those occurring at the time of delivery and affecting the amniotic sac and fluid. At the same time the toxicity of trimethoprim due to its antifolate activity must be kept in mind. As Herbert points out, subjects who are sensitive to folic acid deprivation, e.g. the pregnant woman and the fetus, are at risk from prolonged treatment with TMP-SMX.[16] Although Navarro has been unable to find true dose-related toxicity in children aged from 3 to 24 months, the dose administered was much lower and the drug was not given during the postnatal period.[17] Messer, in a review of pregnancy anemias, directed attention to the increased need for folates in acute infection, during anticonvulsant therapy and with increased age and parity.[18] It has been suggested that a fetomaternal folate gradient is maintained during pregnancy. The cause of folate deficiency in pregnancy appears to be a combination of increased fetal demand and inadequate maternal intake. Hence the importance of the advice of Spector and colleagues that "if treatment with TMP-SMX in patients at risk is necessary over prolonged periods of time, hematological indices and tests of folate status should be checked at regular intervals".[19] It is possible that TMP-SMX should be added to the list of antimicrobials with potential hazard to mother and fetus.[20]

Conclusions

We report for the first time the total transplacental kinetics of TMP-SMX and its components in human subjects. During a steady state both maternal and fetal handling of the drugs was analysed. After an oral loading of either sulfamethoxazole, 4.8 g and/or trimethoprim, 960 mg, given in combination form or separately in three or four doses over 36 to 42 hours, samples were taken from mother and fetus 8 to 12 hours after the last dose. Body fluids and tissues were taken for drug assays from 31 subjects from 9 to 22 weeks pregnant at the time of hysterotomy and tubal ligation.

When given in combination, sulfamethoxazole levels were lower in amniotic fluid than in maternal serum; the difference was significant ($P < 0.05$). Trimethoprim levels in fetal and maternal sera and in amniotic fluid were similar.

When the drugs were given individually, sulfamethoxazole levels were lower in fetal serum than in maternal serum; the difference was significant ($P < 0.001$). At the same time the level of sulfamethoxazole in amniotic fluid was lower than in fetal serum. No differences were found between the concentrations in the three fetal tissues assayed, placenta, liver and lung. In regard to trimethoprim, no significant difference was discovered between the concentrations within the body fluids compartment and the fetal tissues compartment.

The relative fates of sulfamethoxazole and trimethoprim appear different when ratios of their respective levels are compared at each sampling site. Very high SMX:TMP ratios are found in maternal (87:1) and fetal (97:1) serum. Much lower ratios are shown for amniotic fluid (8:1), placenta (9:1) and fetal liver (10:1). In fetal lung the very low ratio of 5:1 is found. Therefore trimethoprim appears freely mobile within the compartments studied. Sulfamethoxazole, on the contrary, is not as mobile, as shown by the decreased ratios of blood to tissue. Our data suggest that the previously reported greater tissue concentrations of trimethoprim are in fact accounted for by decreased tissue penetration by sulfamethoxazole. In view of the ability of trimethoprim to cross all the fetoplacental membranes the issues of drug dosage and clinical toxicity for mother and child must be seriously considered when prolonged treatment with TMP-SMX is contemplated during pregnancy.

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