cursors of urinary ester sulphates are products not of bacterial but of endogenous origin.

Summary. 1. It was shown that adult germfree rats did not excrete detectable amounts of phenyl sulphate and indoxyl sulphate. Total urinary excretion of phenol by this type of animal was also low. 2. Urinary excretion of a large number of other ester sulphates was uninfluenced by bacterial activity in the gut. 3. No difference in the activity of liver enzymes sulphurylating phenol and dehydro*epi*androsterone was found in germfree and conventional rats within the same age group. Nor were there differences between these 2 types of rats with respect to the capacity of their sulphate-activating liver enzymes.

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Alteration in Amniotic Fluid Volume and Other Findings in Meclizine Hydrochloride Induced Anomalies. (28786)

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Recently, King(1) reported the teratogenic effects of meclizine hydrochloride in the Sprague-Dawley rat. He established a dosage schedule which induces palatal clefts in 100% of the viable young and very low, if not insignificant, incidence of fetal resorption. Also, a large percentage of the defective young exhibit bilateral glosso-palatine fusion.

Cohlan(2) states that abnormal embryos in rats subjected to teratogenic doses of Vit. A can often be recognized *in situ* by amniotic sacs swollen with excess fiuid, and Gulienetti *et al*(3) have reported significant alterations of amniotic fluid volumes in rats and mice in which the teratogenic procedures of sodium salicylate toxicity and ariboflavinosis were employed. It is also of interest that palatal

clefts can be induced by amniocentesis(4). In view of these facts, and since oligohydramnios and polyhydramnios are associated with human congenital malformations (although not necessarily with clefts of the palate), it was decided to determine the effects of meclizine and/or the palatal clefts induced by it upon amniotic fluid volume.

Materials and methods. Mature virgin female Sprague-Dawley rats, weighing approximately 200 g, were mated overnight with males of proven fertility from the same strain. Light was controlled automatically so that breeding animals were subjected to 13 hours of darkness in every 24 hours. Conception was assumed to have occurred when spermatozoa were found in vaginal smears, at which time such pregnant animals were removed from the breeding cages and housed either singly or doubly in wire mesh cages. The following day was designated the first day of gestation. All animals were supplied with Purina Laboratory Chow and tap water *ad libitum.*

An alcoholic solution of meclizine hydrochloride was prepared freshly each day by pulverizing Bonine tablets (Pfizer) and adding sufficient 50% ethanol (v/v water) to yield a concentration of 50 mg of meclizine per ml.

On each of the 12th, 13th and 14th days of gestation, 50 mg of the drug was fed by gavage to each experimental rat. Control rats were fed equal volumes of the ethanol solvent. Thereafter, treated and control animals were killed on each of the 15th through the 19th gestational days, at which time amniotic fluid weight, specific gravity, and volume, fetal weight, and weight of the placenta plus the fetal membranes, were determined by a method which is a modification of those employed by Draper(5) and by Lefebvres-Boisselot and Dupuis(6). Briefly, the uterine horns were exposed and incised longitudinally to reveal the fetal sacs and placentae, which were gently teased away from the uterine wall. The intact fetal membranes with their contained fetuses, amniotic fluid, and attached placentae were weighed individually to $\pm$ 0.5 mg on an analytical balance. The amniotic fluid was drained into individual tubes. One hundred microliters of each, measured by a previously calibrated micropipette, was weighed to the nearest milligram to determine the specific gravity. The fetuses were gently blotted on gauze sponges and weighed individually, as were the fetal membranes with the attached placentae. Amniotic fluid weight was determined by subtraction of the fetal weight, and the weight of the membranes and placenta from the total weight. Amniotic fluid volume was calculated by dividing its weight by its specific gravity in g/ml.

Results. Palatal clefts were observed in 100% of the experimental young at 17 days of gestation, 80% at 18 days, and 89% at 19 days. Experimental fetuses with normal palates were confined to a portion of a single litter on each of the 18th and 19th gestational days. Palates were normal in all control fetuses.

The specific gravity of amniotic fluid from experimental animals did not differ from that of control animals, and measured 0.101 mg per 100 μl on each day of gestation.

The mean amniotic fluid weights in treated animals were significantly greater than those in controls on the 15th, 16th, and 17th days of gestation. They were not different on the 18th gestational day, and on the 19th day, the mean in treated animals was significantly less than that in controls (Table I).

Mean weights of experimental fetuses did not differ from those of control fetuses, except on the 17th gestational day, when experimental fetuses weighed more than the controls ($p<0.05$, Table I).

The combined weights of the placenta and the fetal membranes did not differ on the 15th day of gestation, but they were significantly smaller in treated animals than in controls on the 16th, 17th, 18th, and 19th days of gestation (Table I).

Coefficients of variations for either experimental or control animals were comparable for each parameter on any one day of gestation (Table I). Specific gravity determinations exhibited negligible variability throughout.

Discussion. Amniotic fluid volumes were also determined by recovering the fluid after

TABLE I. Weights of Amniotic Fluid, Fetus, and Placenta Plus Fetal Membranes.

Day of gestation	Control N	Control Mean (mg) ± S.E.	Control C (%)	Meclizine treated N	Meclizine treated Mean (mg) ± S.E.	Meclizine treated C (%)	P
			Amniotic fluid weight				
15	32	395 ± 7.2	9.7	17	440 ± 8.4	7.9	<.001
16	30	460 ± 9.2	9.1	22	538 ± 14.6	12.7	<.001
17	34	628 ± 10.1	9.4	19	680 ± 21.0	13.5	<.050
18	38	803 ± 14.6	11.2	25	784 ± 23.3	14.9	>.500
19	31	832 ± 18.0	12.0	28	755 ± 19.3	13.5	<.010
			Fetal weight				
15	32	313 ± 4.9	11.3	17	300 ± 7.1	9.8	>.100
16	30	497 ± 7.8	11.6	22	518 ± 10.0	9.0	>.100
17	34	889 ± 17.0	11.2	19	980 ± 36.1	16.1	<.050
18	38	1652 ± 22.4	8.4	25	1676 ± 44.9	13.4	>.500
19	31	2367 ± 39.9	9.4	28	2312 ± 50.8	11.6	>.200
			Weight of placenta + fetal membranes				
15	32	334 ± 6.9	8.6	17	310 ± 8.6	11.4	>.050
16	30	387 ± 7.7	9.2	22	337 ± 15.6	21.6	<.010
17	13	451 ± 10.0	8.0	19	407 ± 14.7	15.8	<.025
18	38	620 ± 9.1	9.0	25	553 ± 17.8	16.1	<.005
19	31	656 ± 16.5	14.0	28	617 ± 16.6	14.2	<.025

N, No. of determinations; S.E., standard error of mean; C, coefficient of variation; P, probability values for differences between means.

amniotomy and measuring the volume directly, and by a dilution technique employing radioiodinated human serum albumin. However, the weight method described, was employed because it yielded results with greater reproducibility and smaller coefficients of variation than did the other methods tried. Also, the variability observed is less than that reported in studies of amniotic fluid volume by others.

Figures for the incidence of palatal clefts are given only for the 17th day of gestation and beyond because the palatine shelves of the Sprague-Dawley rat normally fuse on the 16th day of gestation.

Amniotic fluid volume in microliters was less than its weight in milligrams by a factor of 0.01 in both experimental and control animals. Therefore, the amniotic fluid weights are directly comparable as close estimates of the volume.

Polyhydramnios in humans is frequently attributed to inability of the fetus to ingest amniotic fluid. Palatal clefts, particularly if associated with glosso-palatine fusion, would be expected to interfere with swallowing of amniotic fluid, but the excess fluid exists in meclizine treated animals prior to the normal time of palate closure, and lower than normal values are observed shortly after the normal time of palate closure. Therefore, if swallowing is indeed impaired, as would be expected from the morphologic defects described, such impairment is apparently not a factor in the genesis of polyhydramnios unless such an effect is more than offset by decreased production of amniotic fluid following withdrawal of the drug.

These findings suggest that meclizine has a direct effect upon amniotic fluid volume which is independent of the palatal defect, and that the reversal of the initial effect following withdrawal of the drug is a rebound phenomenon. The amniotic fluid response to meclizine may be mediated by the drug's antihistaminic action on the cardiovascular effects of histamine. Histamine dilates arterioles and elicits edema in humans, but in rodents, histamine constricts arterioles(7). Therefore, antihistamine in the rat would be expected to induce a relative arteriolar dilation with resultant elevation of capillary blood pressure, which could favor amniotic fluid production and/or inhibit its reabsorption.

The progressive decrease in the magnitude of excess amniotic fluid in treated animals and its decline to a lower than normal value on the 19th day of gestation may have resulted from the parallel relative reduction in

weights of the placenta and fetal membranes, since they are believed to be a primary source of amniotic fluid.

Summary. Meclizine, administered to pregnant Sprague-Dawley rats on the 12th, 13th and 14th days of gestation in a dosage (50 mg per day), which induced palatal clefts in almost one hundred percent of the fetuses, resulted in a significantly elevated volume of amniotic fluid on the 15th day of gestation. Thereafter, progressive decline to a normal value on the 18th gestational day and a significantly depressed amniotic fluid volume on the 19th day of gestation occurred. Fetal weights were approximately the same in experimental and control animals. The weights of the placenta and fetal membranes combined were significantly less than normal on the 16th, 17th, 18th, and 19th days of gestation. Possible mechanisms by which the alterations in amniotic fluid were effected are discussed.

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Experimental Rubella in Rhesus Monkeys.* (28787)

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The role of maternal rubella in the production of congenital malformations of the human fetus has long been recognized(1), and the recent isolation and identification of the rubella agent(2,3) lends importance to the development of an *in vivo* system in which to study this phenomenon. Many years ago Hess reported that inoculation of defibrinated blood from rubella patients failed to produce a rash in 4 rhesus monkeys so tested(4). In 1942, however, Habel described the production of a light, scattered, macular rash with leukopenia in 12 of 41 rhesus monkeys inoculated with nasal washings or blood from patients in the early stages of rubella. Another 15 animals responded with leukopenia, but no exanthem (5). Utilizing similar techniques, Krugman *et al* were unable to induce rubella in 6 cynomologous monkeys(6), while a recent communication by Sigurdardottir *et al* indicates that rubella with rash can be produced experimentally in African green monkeys(7). The possibility that laboratory animals during the various stages of their procurement may contract human diseases and develop immunity thereto is exemplified by the experience with rubeola in rhesus monkeys(8). Since such an occurrence might explain the irregular response of monkeys challenged with the rubella agent, a study was designed to test the thesis that rhesus monkeys recently trapped from a colony isolated from contact with man and his microbial complement, are susceptible to infection with rubella virus.

Materials and methods. Rhesus monkeys. The source of presumed susceptible animals was the isolated colony of rhesus monkeys maintained by the Laboratory of Perinatal Physiology, Nat. Inst. of Neurological Diseases and Blindness, on Cayo Santiago, a

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