the excretion of nitrofurantoin administered by constant intravenous infusion in a series of patients with endogenous creatinine clearances between 22 and 160 ml per minute. The average of clearance of nitrofurantoin was greater by a factor of approximately 2.8 than the simultaneously determined clearance of endogenous creatinine. Nitrofurantoin in humans appears, accordingly, to be excreted primarily by tubular secretion. In that event participation of nitrofurantoin in a solute concentration gradient appears to be unlikely in man. Nitrofurantoin may, therefore, be less satisfactory as a chemotherapeutic agent in treatment of pyelonephritis in humans than an analogue that would participate in the solute concentration gradient.

Restriction of water intake to limit urine flow and to provide higher concentrations of the chemotherapeutic agents at least in the final urine may have important clinical drawbacks. Chernew and Braude have recently demonstrated that phagocytosis in urine is decreased in the presence of high solute concentrations(11). In addition, medullary blood flow is less in antidiuresis than in diuresis (12). The relative importance of concentration of the chemotherapeutic agent, of the effect of solute concentration on phagocytosis, and of changes in medullary blood flow in pyelonephritis has not been assessed. The time-honored procedure of water-loading patients with pyelonephritis may be far more effective than has been recognized.

Summary. Increasing renal tissue concentration gradients have been demonstrated in hydropenic albino and Kangaroo rats for sodium, chloride and urea. No significant gradients could be demonstrated for water, potassium, *p*-aminohippurate and nitrofurantoin. Non-participation of nitrofurantoin in the solute concentration gradient may limit its effectiveness as a chemotherapeutic agent in pyelonephritis. However, phagocytosis as affected by solute concentration and medullary blood flow as related to diuresis may be of greater importance clinically.

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Studies on Ester Sulphates. 18. Ester Sulphate Formation in the Germfree Rat.* (28785)

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The occurrence of various phenol sulphates in the urine of mammals has long been known(1,2). These compounds which, under physiological conditions, probably represent the main portion of the ester sulphate fraction are believed to reflect the detoxication in the liver of toxic phenolic compounds formed by bacteria in the gut(3,4,5). Other

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TABLE I. Excretion of Phenol and ^{35}S-Labelled Compounds in Urine of Germfree and Conventional Rats During First 24 Hours After Injection of Radioactive Sulphate.

Rat No.	Type of animal	Wt, g	Isotope excretion: Total urinary ^{35}S-sulphur %	Mean	Isotope excretion: Urinary ester ^{35}S-sulphate %	Mean	Phenol excretion* mg/24 hr	Mean
1	Germfree	420	30.4	32.5	9.8	10.8	.03	.02
2	"	420	34.6		11.7		.01	
3	Conventional	441	32.5	35.8	13.7	13.4	.56	.76
4	"	439	39.0		13.1		.95	

* Includes *m*- and *o*-cresol if present(24).

urinary phenolic sulphate esters might be more closely related to ordinary metabolic processes in the mammalian tissues(6-10). This also applies to many of the steroid sulphates recently shown to be present in mammalian urine(11-18).

Urinary ester sulphate patterns can be easily visualized by means of 2-dimensional paper chromatography, combined with autoradiography, performed on urine specimens from experimental animals given injections of radioactive sulphate(19).

This principle was used in the present study, the scope of which was to investigate in which respects the ester sulphate excretion in germfree rats differs from that in conventional animals. The ability of liver extracts from the experimental animals to sulphurylate phenol and dehydro*epi*androsterone (DHA†) and to form active sulphate (PAPS†) was also determined *in vitro*.

Experimental. Animals. Six germfree male rats and 6 conventional male rats were used. The germfree rats were reared according to the technique of Gustavsson(20,21). Both germfree and conventional rats were of the same strain, inbred in the germfree isolators for 14 generations. Members of the 14th generation were removed from the isolators and kept in a conventional animal room. The conventional rats used in the present study were 3rd generation descendants of these ex-germfree animals. The germfree animals used belonged to the 17th generation of the aforementioned inbred germfree strain.

The weight of the animals is shown in Tables I and II. Both groups of rats were fed the semi-synthetic diet D_7(21) and water *ad libitum*. The diet was sterilized by autoclaving at 121°C for 20 minutes. At 21 days of age juvenile rats were separated from their mothers.

Rats 1-4 (Table I) were given 4 mc of ^{35}S-labelled sodium sulphate by intraperitoneal injection. The animals were kept in metabolic cages, and the first 24-hour urine portions from each of them were collected. *Urine analyses. Total radioactivity* excreted was estimated (in quadruplicate) as follows: 100 μl of urine were pipetted into a 15-ml conical centrifugation tube. Ten ml of distilled water were added, and after careful mixing 100 μl of the solutions were plated and measured in a Geiger-Müller apparatus (mica end-window tube; 1.9 mg/cm^2).

Additions of 0.5 ml of 0.1 M HCl, 1 ml of 1 M $BaCl_2$ and 0.5 ml of 1 M Na_2SO_4 were made to the remaining contents of the centrifugation tubes. The tubes were stoppered and shaken vigorously, and placed overnight in a refrigerator at 4°C. They were then centrifuged at 1000 *g* for 15 minutes, after which aliquots of the supernatants were plated and measured as stated earlier. *The ester sulphate fraction* of the urinary radioactivity was calculated from the values obtained (after correction for volume of reagents added) and from the total radioactivity of the urine.

The standard deviation (s) of a single determination of the urinary ester sulphate excretion was estimated from the deviation (d) from the mean by the following formula:

$$s = \sqrt{\frac{\Sigma d^2}{n}}$$ where n = number of observations.

† The following abbreviations are used: DHA, dehydro*epi*androsterone; EDTA, ethylenediamine tetraacetic acid; PAPS, 3′-phosphoadenosine-5′-phosphosulphate; ATP, adenosine triphosphate.

TABLE II. Ability of Liver Extracts of Germfree and Conventional Rats to Sulphurylate Phenol and Dehydro*epi*androsterone and to Form Active Sulphate (PAPS), Expressed as μmoles of Ester Sulphate or PAPS Formed per Hour per Gram (Wet Weight) Liver. For experimental conditions, see text.

				Sulphurylation of Phenol		Sulphurylation of DHA		Formation of PAPS	
Rat No.	Animal	Age, days	Wt, g	μmoles/g liver/hr	Mean	μmoles/g liver/hr	Mean	μmoles/g liver/hr	Mean
5	Germfree (juvenile)	30	65	.96	.95	.95		1.81	1.74
6	*Idem*	30	60	.94		.72		1.67	
7	Conventional (juvenile)	26	54	.92	.91	.59	.58	1.80	1.79
8	*Idem*	26	54	.90		.57		1.78	
9	Germfree (adult)	319	387	1.67	1.63	.06	.09	2.54	2.42
10	*Idem*	319	427	1.58		.12		2.29	
11	Conventional (adult)	317	428	1.24	1.34	.11	.10	1.88	2.01
12	*Idem*	317	415	1.43		.8		2.13	

From 8 determinations of the ester sulphate fraction of one urinary specimen giving a mean of 13.2%, s was calculated to be 0.78%.

The urinary ester sulphate patterns were visualized as described elsewhere(19,22). Five and 25 μl of each of the undiluted urines were subjected to 2-dimensional paper chromatography on Whatman No. 1 paper. Two pairs of solvents, phenol-water + butanol-ammonia (I + II) and phenol-water + butanol-acetic acid-water (I + III) prepared as described earlier, were used(22).

High-voltage paper electrophoresis (0.075 M sodium acetate-acetic acid buffer, pH 5.5, 30–40 V/cm, 1 h) was performed on 5- and 10-μl samples of each urine specimen.

All paper chromatograms and electropherograms were subjected to autoradiography on Gevaert Curix X-ray films, as described elsewhere(19). The autoradiograms were examined and compared in a viewing box.

Phenyl sulphate was identified by comparison of chromatographic and electrophoretic data of the suspected urinary spots with those of biosynthetically prepared phenyl sulphate (22).

Indoxyl sulphate was identified on paper chromatograms according to Decker(23), as reported earlier(22).

Total urinary phenol excretion was determined by the method of Tompsett(24). The determinations included *m*- and *o*-cresol, if present. *Enzyme studies.* Particle-free supernatant fluids of the livers from animals 5–12 were prepared by separate homogenization in 4 vol. of ice-cold 0.15 M KCl containing 0.001 M EDTA (pH 7.0), followed by centrifugation at 105,000 *g* for 60 minutes, as described elsewhere(25).

Sulphurylating activities of liver extracts with respect to sulphate conjugation of phenol and DHA were determined as described elsewhere(25). The supernatant fluid was incubated together with one of the aforementioned acceptors in a buffer solution containing ^{35}S-labelled inorganic sulphate and ATP. After incubation, the inorganic sulphate and ester sulphates formed were separated, and the amount of the latter was calculated on the basis of radioactivity of this fraction.

PAPS formation in liver extracts was determined according to Wengle(25), essentially as follows. During incubation of the enzyme system outlined above, active sulphate (PAPS) accumulates in the absence of sulphate acceptors. After incubation for a certain period, sulphate activation is stopped by the addition of EDTA(26). The labelled sulphate group of PAPS is then transferred to added phenol by further incubation. The

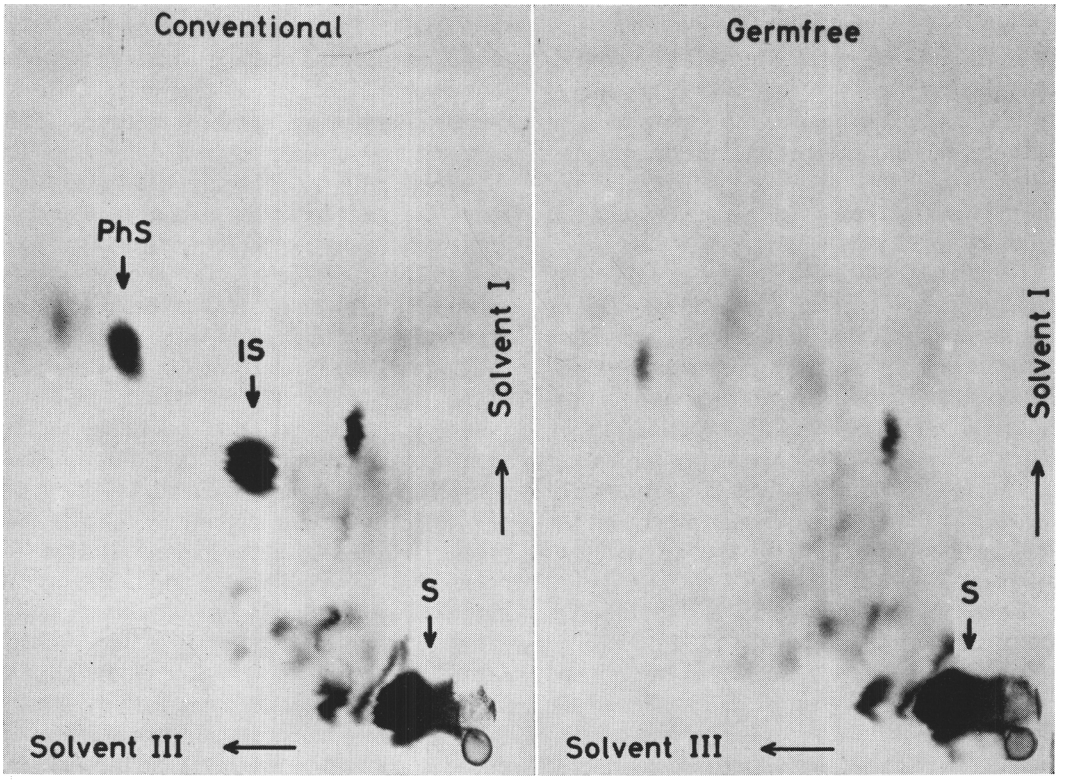

FIG. 1. Autoradiograms of paper chromatograms performed on 25-μl urine samples from a conventional and a germfree rat during the first 24 hr after intraperitoneal injection of ^{35}S-labelled sulphate. Position of phenyl sulphate (PhS), indoxyl sulphate (IS), and inorganic sulphate (S) is indicated in the figure.

amount of phenyl sulphate formed, which thus reflects PAPS formation, was then calculated on the basis of the radioactivity of this fraction.

Results. The results of the *in vivo* studies are summarized in Table I and illustrated in Fig. 1. Urinary excretion of labelled ester sulphate as a percentage of total radioactivity excreted was lower in the 2 germfree rats. This difference in ester sulphate excretion seemed to be closely correlated to a highly characteristic difference between the ester sulphate patterns in the 2 groups of rats (Fig. 1). Thus, the 2 most dominant spots in the pattern of the conventional rats (PhS and IS in Fig. 1) were lacking in the ester sulphate patterns of the germfree rats.

In all other respects, great similarities were observed between the ester sulphate patterns of the conventional and the germfree rats. In paper chromatography in all 3 solvents used, as well as in high-voltage paper electrophoresis, compound PhS was found to migrate in the same manner as phenyl sulphate. On paper chromatograms, compound IS exhibited color reactions and migration characteristics of indoxyl sulphate.

The germfree rats excreted significantly smaller amounts of phenols during a 24-hour period than did conventional rats (Table I).

The results of the *in vitro* studies are listed in Table II. No significant differences were observed between juvenile germfree rats and juvenile conventional rats with respect to phenol- and DHA-sulphurylating activity of their livers. Furthermore, no significant difference was present between the two groups as to the sulphate-activating enzymes of the liver. Nor did liver preparations from adult germfree rats differ from conventional rats with respect to phenol- and DHA-sulphurylating or sulphate-activating enzymes.

The adult rats of both the germfree and conventional types showed, however, higher

activity of phenol-sulphurylating enzymes than the juvenile rats. The DHA-sulphurylating activity was, on the other hand, higher in the liver preparations of young rats of both types than in those of the adult ones. Finally, sulphate-activating liver enzymes did not differ markedly in young and adult animals.

Discussion. All animals used in this study were of the same strain, and had been kept on the same diet during their lives. It therefore seems reasonable that all differences observed between the germfree and conventional rats in the present study might be ascribed to absence of bacterial infection in the former.

The most striking finding in these experiments was the specific differences between the ester sulphate patterns in the 2 groups of animals (Fig. 1).

Thus, in agreement with earlier observations in various mammals(19,22), 2 ester sulphates, tentatively identified as phenyl sulphate and indoxyl sulphate, dominated the urinary ester sulphate pattern of the conventional rats. These compounds could not be detected in the urine of germfree rats.

The absence of the aforementioned major components of the urine of the germfree animals might well account for the differences between the urinary ester ^{35}S-sulphate excretion in germfree and conventional rats shown in Table I. Moreover, the higher excretion of radioactivity observed in the urine of conventional rats (Table I) might to some extent be secondary to the phenyl sulphate and indoxyl sulphate excretion by these animals.

There are at least 2 possible explanations of the aforementioned findings. First, phenol and indoxyl formation might be markedly reduced in germfree animals. Second, their phenol-sulphurylating activity might be deficient. Nor can it be ruled out that a combination of these 2 factors might be responsible for the absence of phenyl sulphate and indoxyl sulphate in the urine of germfree rats.

The present study showed that total phenol excretion in the germfree animals was extremely low in comparison with that in the conventional animals (Table I). This finding is in agreement with other reports on the influence of the intestinal flora on phenol excretion(3,4,5). The almost complete absence of phenol in the urine of the germfree rats would thus explain the lack of phenyl sulphate in their urinary ester sulphate pattern. This suggestion is further supported by the fact that practically no difference was present between the activity of the phenol-sulphurylating enzymes in germfree and conventional rats (Table II). That germfree rats do not excrete indoxyl sulphate has been reported previously(27).

The agreement between the values for phenol-sulphurylating liver activity in germfree and conventional rats belonging to the same age group indicates that this enzyme activity is not directly related to the level of phenol production in the gut. This is surprising, since exogenous administration of phenol has been shown to produce a great increase in the capacity of the phenol-conjugating enzyme systems of liver and kidney slices(28). If the maintenance of the normal level of phenol-sulphurylating activity is partially substrate-induced, endogenously formed phenolic compounds rather than phenol absorbed in the gut might be inducing agents. However, genetic factors are probably more important than substrate induction for achievement and maintenance of normal levels of these enzyme activities. That the activity of the sulphate-activating liver enzymes did not differ in germfree and conventional animals within the same age groups supports this assumption.

With respect to sulphurylation of DHA as well, great similarities were found between the 2 types of rats in the same age group. These results are compatible with the view that steroid sulphurylation is uninfluenced by environmental factors, such as bacterial infection of the gut.

The differences observed between the various enzyme activities in juvenile and adult rats (Table II) are in agreement with results recently reported by Wengle(29).

The urinary ester sulphate patterns (Fig. 1) showed that excretion of a large number of ester sulphates was not influenced by the bacterial activity in the gut. This finding, and the quantitative data on ester sulphate excretion in Table I, indicate that several pre-

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