tetrazolium" method (*5*). The estriol substrate did not react during measurement by this method. The 16-ketoestradiol was identified by chromatographic comparison with standard material in toluene-methanol (75 percent) and chloroform-formamide systems, as well as by thin-layer chromatography in a system of ethyl acetate, *n*-hexane, and ethanol (80:15:5) (*6*). The metabolite, separated as a phenolic substance (reduced blue tetrazolium), gave a characteristic Kagi-Miescher reaction (*6*) and, upon reduction with borohydride, was converted to 16-epiestriol (*6*).

The enzyme activity occurred in the whole homogenate from kidneys of female rats and resulted in the conversion of 85 percent of 200 μg of estriol to 16-ketoestradiol in the presence of 10 μmole of NAD. From three to ten rats were used in each experiment. Upon further fractionation, the entire activity was found to reside in the supernatant fraction obtained by centrifugation at 105,000*g*. A system of, for example, lactic dehydrogenase and pyruvic acid was provided to oxidize any reduced NAD that might be formed, and thus to insure completion of the reaction when the supernatant fraction was used alone. Most of the studies (see Tables 1 and 2) were performed with the 800*g* supernatant fraction of the crude homogenate, for which no exogenous system for reoxidizing the reduced NAD was necessary.

When compensation had been made for dilution, mixing the extracts from kidneys of male and female rats gave activities which were simply additive—a finding which suggests that no inhibitors or activators were operative. The enzyme system metabolized 16-epiestriol only slightly, suggesting relative stereospecificity for the 16α-hydroxy configuration. Details of the specificity for other types of substrates are not available; however, the pattern for steroid dehydrogenases revealed so far has shown a high degree of substrate specificity. The enzyme reaction was barely demonstrable in liver extracts prepared and tested under conditions similar to those described for rat kidney.

The time course for the appearance of the enzyme activity suggests that this activity may well be another example of enzyme formation induced by steroid hormones, although until further data are available this cannot be proved conclusively. This enzyme provides a system for studying the mechanism of hormone action in metabolic adaptation. There is a clear-cut, almost absolute, sex difference, which can be affected by castration and hormone administration. Since slight activity occurs in castrated females, the enzyme may well be influenced by other factors, in addition to the hormones used in this study.

The conversion of estriol to 16-ketoestradiol may have a quantitative significance heretofore unrecognized. In addition, the data suggest that the extent of estriol metabolism may well be different in male and female rats (*7*).

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Biodegradation of Alkylbenzene Sulfonate in a Simulated Septic Tank and Drain Field

Abstract. *A straight-chain alkylbenzene sulfonate was 90 to 95 percent biologically degraded in a laboratory bench-scale septic tank and drain field. Under the same conditions, only about 35 percent of a highly branched alkylbenzene sulfonate was degraded.*

Straight-chain alkylbenzene sulfonates are more rapidly degraded than highly branched alkylbenzene sulfonates in sewage treatment and in surface waters (*1*). However, one of the principal causes for concern over the stability of detergents after disposal is the contamination of ground waters, and consequently wells, in suburban areas using septic tanks for waste disposal. The relative rates of biological degradation of various detergents under these less aerobic conditions are therefore of considerable interest.

A simulated septic tank and drain field system was used to study the biodegradation of two alkylbenzene sulfonates from (i) a mixture of secondary phenylalkanes with straight chains containing 10 to 13 carbons and (ii) a typical alkylate derived from propylene tetramer and pentamer. Each septic tank consisted of two cylindrical compartments, the first having a liquid capacity of 2 liters and the second 1 liter. Each drain field was a series of columns packed with soil to a level just below the inlet, then gravel past the inlet, topped off with more soil. These were connected so that any excess over the capacity of the first column would pass through the gravel into the second, and so forth. After the septic tank had been seeded with sludge from an operating full-scale septic tank, sterilized raw whole sewage containing 10 parts per million of alkylbenzene sulfonate, tagged with sulfur-35, was fed into the septic tank automatically for 4 minutes once each hour for 16 consecutive hours each day, with an average residence time of 5 days. Effluent samples from the septic tank and soil columns were acidified with HCl, extracted with ether, deposited on activated carbon, and counted by the technique of House and Fries (*2*).

The efficiency of the system for removing organics was followed by measuring chemical oxidation demand (*3*). During the period of the tests, oxidation demand removals were 60 to 65 percent in the septic tank and about 80 percent over-all. Weekly averages of the percentage of alkylbenzene sulfonate remaining in the septic tank effluent are plotted in Fig. 1 Removal in the septic tank alone averaged about 15 percent for the branched-chain sample and 30 to 40 percent for the straight-chain compound. Almost all of this branched-chain removal and about one-third of the straight-chain removal were by adsorption on new sludge formed throughout the run. Biodegradation accounted for the other two-thirds of the straight-chain compound removed. Figure 2 shows the analyses of the soil column effluents. The overall removal in the system was about 35 percent for the polypropylene alkylbenzene sulfonate and 90 to 95 percent for the straight-chain sulfonate.

A typical household effluent contains 10 to 20 parts of alkylbenzene sulfonate per million. A full-scale septic tank drain field system operating with the same efficiency as the laboratory system and receiving polypropylene benzene sulfonate might discharge an

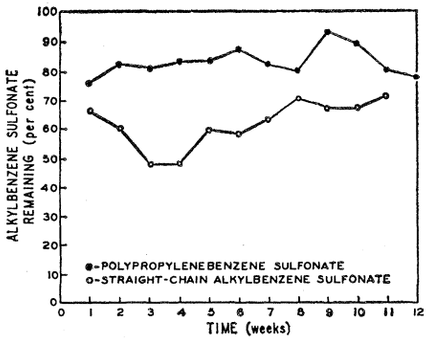

Fig. 1. Removal of alkylbenzene sulfonates in a simulated septic tank (no drain field).

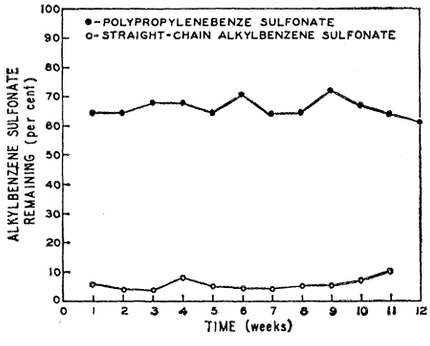

Fig. 2. Overall removal of alkylbenzene sulfonates in a simulated septic tank and drain field.

effluent containing 6 to 14 parts of detergent per million. Replacement with straight-chain alkylbenzene sulfonates could lower this concentration to 0.5 to 2.0 ppm. These concentrations are further reduced in the ground water by subseqeunt dilution with water from other sources, such as rain and agricultural or horticultural water (*4*).

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Ornithocholanic Acids and Cholelithiasis in Man

Abstract. *Ornithocholanic acids and small soft gall stones were found in the bile of patients from which* Klebsiella pneumoniae *was also isolated. Cholelithiasis may be initiated by metabolic disturbances in the conjugation of bile acids in liver tissue and the subsequent precipitation of cholesterol and bile pigment.*

In the guinea pig, suppurative lesions due to *Klebsiella pneumoniae,* as well as the parenteral injection of toxic products of this microorganism, led to the excretion in bile of ornitholanic acids and the rapid formation of cholesterol and pigment precipitates in the gall bladder (*1*). The specificity of the klebsiella organism or its products in the induction of this process was not evaluated. However, ornithine was a metabolic byproduct of these microorganisms. When C^{14}-labeled ornithine was injected into the guinea pig, some of it was found to conjugate with the three cholanic acids in the bile (*2*). Studies in vitro also indicated that ornithine conjugates with cholanic acids in the liver tissue of the guinea pig and rat.

The correlation between ornithocholanic acids, recent gall stones, and bacterial infections, other than in the gall bladder, has now been studied in man. Autopsies were performed on 62 unselected patients, and bacterial cultures were made from cardiac blood and areas of grossly discernible exudative inflammation. The presence of small soft stones, measuring 1 to 3 mm in diameter, was noted, and the presence of conjugated bile acids in fluid bile was determined by column and paper chromatography (*2*). The soft biliary stones, when present, contained 78.6 ± 6.8 percent cholesterol, 16.3 ± 4.7 percent bile pigment and no bile acid, thus being very similar in composition to the material found in the guinea pig (*3*).

The bacterial cultures, from 26 of the patients, were negative; these patients had no significant inflammatory lesions, and neither ornithocholanic acids nor soft stones (with one exception) were found (Table 1). *Klebsiella pneumoniae* was found in cultures from pulmonary tissue from ten patients which had suffered from pneumonia; cultures from the blood of eight of these patients were also positive for *K. pneumoniae.* Small soft gall stones and ornithocholanic acids were found in all ten patients which had pneumonia, and also in two patients which had pyelonephritis due to *K. pneumoniae.* The incidence of Klebsiella infections in our patients is higher than reported elsewhere, but has been confirmed by type-specific antisera in doubtful cases. No gall stones or ornithocholanic acids were found in two out of three patients which had positive post-mortem blood cultures but showed no signs of disease due to this organism. Except for two patients which had pneumonia due to *Escherichia coli,* the other organisms recovered from tissue and blood were unassociated with soft biliary stones or ornithocholanic acids (Table 1). It is of interest that Bacmeister in 1908 produced biliary precipitates upon addition of bile to the culture media of gram-negative bacteria (*4*).

The average relative amount of ornitho- as compared to tauro- and glycocholanic acids in human bile at autopsy is indicated in Fig. 1. However, Wiggins and Wootton (*5*) and Sjövall (*6*) have reported that human bile contains more glycine than taurine conjugates. Although L-ornithine, unlike taurine and glycine, is a diamino acid, the correlated results of acidometric and gravimetric determinations of isolated ornithocholanic acids suggest that the ornithine is linked by a single amino group to the cholanic acids. Furthermore, a positive ninhydrin reaction is given by ornithocholanic acids from the bile of guinea pig, rat or man, and from the liver incubated in vitro.

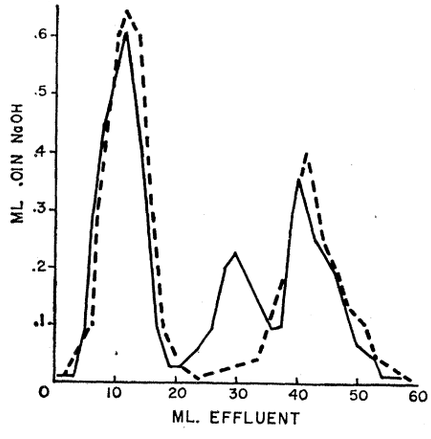

Fig. 1. When conjugated bile acids from patients with klebsiella infections are analysed by column chromatography, ornithocholanic acids appear between bile acids conjugated with taurine (left) and with glycine (right). The dotted line indicates the presence of only taurine and glycine conjugates in normal human bile.