

were then lyophilized and the resulting powders used in the adsorption isotherm experiments.

Water binding abilities were measured as described by Bull⁶. A 0.1 to 0.5 g sample of lyophilized material was placed into a vacuum dessicator above approximately 100 ml of a saturated salt solution which gave a known relative humidity at the equilibration temperature (20 °C). The salts (humidities) were: LiCl (12.5%), MgCl₂ (33.0%), K₂CO₃ (44.0%), Ca (NO₃)₂ (50.5%), NaNO₂ (65.5%), NaNO₃ (76.0%), KCl (85.0%) and Na₂CO₃ (92.0%)⁷. The dessicators were evacuated and held at 20 °C. After 3 days the vacuum was released and the samples quickly weighed. The samples were then dried at 105 °C, dry weights were measured, and the extent of hydration (g H₂O/100 g sample) was obtained by subtracting the dry from the wet weight.

Results. The adsorption isotherms for several samples are shown in the figure. The shapes of all curves were similar and resembled closely the isotherms published by other workers on purified proteins^{6,8}. Water adsorption values for egg albumin, bovine serum albumin and brine shrimp agree very closely with published values^{7,9}. According to Kuntz and Kauzmann⁸ the region of the adsorption curve below approximately 30% relative humidity is due largely to the penetration of water into the void spaces in the protein crystals, and thus does not reflect accurately protein water affinities. At high humidities (above approximately 80%) small changes in humidity lead to large changes in water adsorption, making accurate comparisons in this range difficult. For these reasons the relative water binding abilities of the samples are better compared using data at intermediate relative humidities, near 50% (table), where the adsorption isotherms are parallel (figure). These data reveal that the water binding abilities of the samples correspond positively with the osmolarities of the fluids in which the proteins occur. By far the strongest water binding ability is found in the case of samples from the 2 salt-tolerant bacteria; lowest water binding ability is by the sample from the freshwater crayfish.

Discussion. These results are consistent with the hypothesis that differences in protein-water affinity are correlated with differences in cellular osmolarity. Thus selection may lead to modification of protein surfaces such that their water binding abilities are always adequate to permit the proteins to remain adequately hydrated within the cell. In view of the very high osmotic concentrations of halophilic bacteria this requirement is perhaps not surprising. It is surprising, however, that apparent differences exist among animal species. Even the approximately 8fold differences in total osmotic concentration between tissues of marine and freshwater crustaceans⁵ appear to be adequate to favor modification in protein water binding abilities.

While these data are consistent with solubility-related adaptations of soluble proteins, they are clearly but a tentative first step in the analysis of this question. Differential contributions of nucleic acid water adsorption and protein denaturation among the samples may have affected our results. Studies of purified, well-defined soluble proteins are an obvious next step in resolving this question. Ultimate resolution of the question may entail accurate amino acid composition analyses of soluble proteins, since the water binding abilities of amino acids differ markedly¹⁰. In this regard it is interesting that halophilic bacterial proteins contain extremely high amounts of aspartate and glutamate⁴, the 2 amino acids having the highest water binding abilities¹⁰.

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Effect of vasectomy on hepatic drug metabolism¹

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Summary. 2 months after bilateral vasectomy the metabolism of aniline but not aminopyrine was increased in rat liver homogenates, whereas vasectomy did not affect the metabolism of either compound in guinea-pig liver homogenates.

Vasectomy is a widely used procedure for fertility control in man. There is, however, a paucity of information concerning possible metabolic consequences of vasectomy. In one recent study, increased drug metabolism was observed in liver homogenates prepared from vasectomized compared to control rats². In view of the importance of an effect of vasectomy on hepatic drug metabolism, the present study was conducted in order to independently examine this phenomenon.

Materials and methods. Adult rats (approximately 350 g) obtained from SASCO, Omaha (NE) and adult English Short Hair guinea-pigs (approximately 600 g) obtained from CAMM Research Institute, Wayne (NJ) were housed individually and maintained on commercial laboratory diets ad libitum except that the animals were fasted 24 h prior to surgery. Under pentobarbital (Nembutal) anesthesia rats

were bilaterally vasectomized by ligation and removal of a portion of each vas deferens essentially as previously described². Guinea-pigs were similarly vasectomized except that the vasa deferentia were exposed via a lower abdominal incision. Control animals of both species were treated the same as the vasectomized animals except that the vasa deferentia were neither ligated nor cut. 2 months after vasectomy the animals were weighed, immediately sacrificed by decapitation and the livers removed for organ weight determination and analysis of in vitro drug metabolism. Aminopyrine and aniline were used as sub-

1 Supported by a grant from the University of Nebraska Medical Center.

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Table 1. Effect of vasectomy on hepatic drug metabolism in rats*

| Drug substrate | Control (nmoles/min g liver) | Vasectomized (nmoles/min g liver) | Change (%) |
|----------------|------------------------------------|---|------------|
| Aniline | 7.52 ± 0.55 (5) | 10.36 ± 0.57 (5)** | + 38 |
| Aminopyrine | 104.9 ± 12.83 (5) | 131.16 ± 4.51 (5)*** | + 25 |

* The results are expressed in terms of the metabolic product formed (see Materials and methods) and the numbers given are the mean ± SEM (number of animals). ** $p < 0.01$ versus control. *** $p < 0.07$ versus control.

Table 2. Effect of vasectomy on hepatic drug metabolism in guinea-pigs*

| Drug substrate | Control (nmoles/min g liver) | Vasectomized (nmoles/min g liver) | Change (%) |
|----------------|------------------------------------|---|------------|
| Aniline | 6.55 ± 0.67 (6) | 6.85 ± 0.34 (7) | + 5 |
| Aminopyrine | 43.05 ± 2.25 (6) | 47.07 ± 1.75 (7) | + 9 |

* The results are expressed the same as in table 1.

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strates for drug metabolism reactions in 10,000 × g – 10 min liver supernatant preparations as described by Mazel³. Metabolism of aminopyrine and aniline were determined by assaying the formation of formaldehyde and p-aminophenol, respectively, as previously described³ except that 20% trichloroacetic acid was used to stop the reactions. The significance of difference between means was established by the Student's t-test.

Results and discussion. Vasectomy significantly increased the in vitro metabolism of aniline by rat liver homogenates (table 1). This increase is similar to and confirms the observation of Esterday et al.² who found that vasectomy of rats increased the in vitro metabolism of p-chloro-N-methylaniline. Both aniline and p-chloro-N-methylaniline exhibit type II binding spectra with hepatic cytochrome⁴ even though they undergo different types of metabolism (aromatic hydroxylation and N-demethylation, respectively). By contrast, the metabolism (N-demethylation) of aminopyrine (a type I compound)⁵, although appearing to be increased, was not significantly affected by vasectomy (table 1). These results suggest that vasectomy may affect drug metabolism through a mechanism that alters specific substrate-cytochrome interactions.

The results in table 2 show that vasectomy of the guinea-pig had no effect on the hepatic metabolism of either aniline or aminopyrine. These findings suggest that the effects of vasectomy on hepatic drug metabolism are species specific and raise the question of which laboratory species is an appropriate model for indicating possible metabolic consequences of vasectomy in man. The results of the present study emphasize the need for more extensive studies on the relationship between vasectomy and hepatic drug metabolism in both animal model systems and man.

Radioimmunoassay of polyacrylamide¹

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Summary. Antiserum to polyacrylamide has been produced in rabbits. It can be used in a sensitive radioimmunoassay for quantitation of polyacrylamide at very low concentrations. This novel approach to the analysis of a synthetic, biologically inactive, hydrocarbon polymer has potentially widespread implications, for example, in the downstream measurement of flocculating agents used in water purification treatments.

Radioimmunoassay (RIA) was developed for detection of biochemicals at very low concentrations in biological fluids². Peptide, protein and steroid hormones, digitalis alkaloids, morphine, LSD and other drugs have been measured by RIA. This report illustrates the adaptability of the technic to a different class of substances. We have developed an RIA for polyacrylamide, a nonbiological, nonnaturally occurring compound, one use of which is as a water purifying agent (DowflocTM) on the basis of its flocculant properties. There is a need to determine the downstream concentration of polyacrylamide in the range of parts per million to parts per billion. Conventional analytic methods for polymers in aqueous solution are not sufficiently sensitive or selective to meet this requirement. Polyacrylamide resin 164 (Dow Chemical Company) of mol. wt of approximately 200,000, with 3.4% of the amide groups hydrolyzed to carboxylic acid, was exhaustively dialyzed to remove low molecular weight materials (PAA). Polyacrylamide-bovine serum albumin con-

jugate (PAA-BSA conjugate) was prepared by reacting the carboxylic acid groups on the polymer with the amine groups on the BSA (Miles Laboratories) via the carbo-diimide method³, as follows: Approximately equimolar amounts (2.0 g PAA, 0.7 g BSA) were dissolved in 50 ml of water and the pH adjusted to 4.7 with HCl. Approximately 150 mg of 1-ethyl-3-dimethylaminopropyl carbo-diimide (Aldrich Chemical Co.) was added, and the reaction mixture was stirred for 48 h, then exhaustively dialyzed against deionized water and lyophilized. Approximately 2 g of the reaction product was recovered. Samples taken from the reaction mixture at 0, 4, 8, 24 and 48 h were analyzed via aqueous gel permeation chromatography on Sephadex G-150, eluting with 0.2 N NaCl and detecting with a Waters R-400 refractometer. Both the PAA and the PAA-BSA complex were completely excluded from the gel and eluted at V_0 ; however, BSA was resolved and eluted at $V_0 + 0.4 V_i$. The formation of the product was inferred from the disappearance of the