URINARY TRACT DISEASE IN SPINAL CORD INJURY PATIENTS

1. A Brief Outline of the Problem

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Amelioration of urinary tract disease remains a challenging problem in patients with spinal cord injury. Renal failure due to infection is the most frequent cause of death (Jousse, 1967; Nyquist and Bors, 1967). Antibiotic therapy has not proven adequate in many cases. Studies now appearing in the current literature indicate a resurgence of interest in the role of antibodies and other host immunity factors in urinary tract disease (Quinn and Kass, 1960; Kass, 1965).

We were interested to explore the usefulness of autogenous vaccines in urinary tract disease. We are not aware of any previous clinical trials. The Spinal Cord Injury Service at the Veterans Administration Hospital, Long Beach, California, is well suited for such studies because these patients present an unusually high risk population for urinary infection. Although a long list of bacterial species are involved in urinary tract disease, only four or five Gram-negative bacilli are known to account for the large majority of cases. We hope to develop, eventually, autogenous vaccines that would help circumvent the considerable problem of serological heterogeneity within each bacterial species.

Prior to considering clinical application of vaccines, it was necessary to answer the question: do antibodies play an important role in the pathogenesis of urinary tract infections? We have been studying the natural history of urinary tract infection and antibody response in selected spinal cord injury patients over periods of many months (more than three years in some cases). Our initial purpose was to accumulate data that would enable appraisal of: (1) the diagnostic and prognostic significance of a patient's antibody titre to his own urinary flora, and (2) the reliability of antibody titre versus urine culture as a guide to the etiology of urinary tract disease.

MATERIALS AND METHODS

Repetitive Urine Cultures. Large numbers of patients were examined for predominant urinary bacteria, for degrees of variability of urinary flora in individual patients and also in contrasting groups of patients. Urine cultures were performed weekly on each patient for the first two to six months, and then monthly during the rest of the patient's hospital stay. Semi-quantitative colony counts (Hoeprich, 1960) were appraised against the patient's clinical status. A frequency index was calculated for each bacterial species as follows: number of urine samples divided by the number of samples positive for the respective organism in each patient.

TABLE

Statistical Analysis of Results in Recent Injury versus Long-term Injury Patient Groups

Patient group	Average number of species per urine culture	't'* of the difference	Urine cultures with one species	't'* of the difference
Long-term injury	2·1 ± 0·18**	3·05 (P = 0·0027)	17 ± 6·1%	28·1 (P = much less than 0·001)
Recent injury	1·3 ± 0·06		67 ± 5·7%	

't' * = Difference—standard error of the difference.

(*Note.* Values greater than 3 indicate a chance probability of less than 1:370, and therefore are considered statistically significant.)

** Mean ± standard error of the mean.

Antibody Studies. Initial and monthly blood samples were taken throughout the hospital stay of each patient in the study. Serum antibody was titred by tube agglutination (50°C., 2 and 20 hours) against all strains of autogenous bacteria as isolated. Bacterial suspensions tested were: (1) live, unheated, 18-hour, brain-heart infusion broth (Difco Laboratories) cultures. (For *Pseudomonas sp.* 0·5 per cent. formalin was added to prevent phage lysis) and (2) broth cultures heated at 100C. for one hour to destroy *H* and/or *B* antigen components. Where a bacterial species was repeatedly isolated over a long period of months, it was sometimes purposeful to confirm observed fluctuations in antibody titre by an in-parallel retest, using the entire battery of patient's serum samples against pooled antigen from several isolates of the bacterial species.

Analysis of Data. To circumvent random unpredictability in individual patients it was necessary to use some method of data summarisation that would enable statistical analysis for reliability of observations. One objective method for sorting data was to contrast results in two groups of patients according to the duration of their injury. One group contained 40 'recent injury' patients in whom culture and antibody studies were begun one to six months after injury. The other group contained 11 'long-term injury' patients whose studies were started from $9\frac{1}{2}$ to 206 months after injury. A second but subjective method of sorting data was important but more difficult. It was based on the patient's clinical status. The groups were composed of 'recent injury' patients, free of episodes of urinary disease, versus 'long-term injury' patients with frequent febrile episodes caused by urinary tract infections.

SUMMARY OF RESULTS

Pattern of Urinary Bacterial Flora. Data from 605 urine cultures on 29 patients showed that five bacterial genera accounted for 80 per cent. of the 997

strains isolated: *Proteus mirabilis* (240 ×), *Proteus rettgeri* (171 ×), *Pseudomonas sp.* (143 ×), *Aerobacter aerogenes* (173 ×), and *Escherichia coli* (113 ×). These five organisms plus *Paracolon*, var. *Providence* (57 ×), and *Proteus vulgaris* (41 ×) accounted for 90 per cent. of all isolates. The most frequently encountered five species proved also to be the most important urinary pathogens in this series, and were responsible for 79 of 100 patient-infections.

Changeability of Flora. Almost all of the patients showed mixed urinary tract infections, usually with frequently changing bacterial flora. These tendencies were more pronounced in the 'long-term injury' patients than in the 'recent injury' patients. However, even the latter group tended to show mixed, variable flora, so that no short series of replicate urine cultures could serve as a reliable indicator of aetiology or as a guide to proper antibiotic therapy.

Two parameters confirmed the greater tendency toward mixed infections in the 'long-term injury' (*LT*) group than in the 'recent injury' (*R*) group (see the Table). The average number of different species isolated per urine specimen was $2{\cdot}1 \pm 0{\cdot}18$ for *LT* patients, and $1{\cdot}3 \pm 0{\cdot}06$ for *R* patients. The difference divided by the standard error of the difference equalled 3·05 and was statistically significant. The frequency of urine cultures which yielded a single bacterial species was $17 \pm 6{\cdot}1$ per cent. in *LT* patients and $67 \pm 5{\cdot}7$ per cent. in *R* patients. The difference divided by the standard error of the difference equalled 28·1 and was highly significant.

Urine Cultures versus Antibody Titre as a Guide to Etiology. We examined the importance of the patient's antibody titres to answer the following questions on etiology:

(1) Does the antibody titre in mixed infections help to decide which of the several organisms is actually invading tissue and causing disease? The obvious expectation was that the invasive organism would stimulate a more vigorous response than would the non-invasive commensals.

(2) Does the antibody titre remain low when a single bacterial species is repeatedly isolated in pure culture, but with no clinical signs of urinary tract disease?

Analysis of our data gave affirmative answers to both questions. Furthermore, when the data on the infection-free *R* patients were compared with those of the *LT* patients with repeated exacerbations of urinary disease, it became apparent that antibody response provided the clearer guide to the etiological agent than did the frequency of isolation for any given bacterial species. For example, 'high' antibody titres (greater than 1 : 100) were general in the *LT* group (27 of 38 patient species), but very exceptional in the *R* group (1 of 18). Conversely, 'low' antibody titres (1 : 40 or less) were almost the rule in the *R* group (14 of 18 patient-species), but were rarely found in the *LT* group (3 of 38). The average antibody titre in the *LT* group was actually 1 : 266—well above the arbitrary 'high' of 1 : 100. In contrast, data on the frequency of isolation (*FI*) of a bacterial species failed completely to distinguish between these *R* and the *LT* groups of patients. The average *FI* in the former group was 0·28, and was 0·27 in the latter. The *FI* of individual species showed no correlation to antibody response in either patient group. A low *FI* (0·2 or less) occurred fifteen times with

high antibody response, and twelve times with low antibody response. A high *FI* (0·5 or more) occurred in four of five *R* patients, none of whom showed any signs of urinary infection; only one of these four high *FI* species was accompanied by a high antibody titre.

We therefore believe that antibody testing could serve as a useful adjunct to repeated urine cultures for pin-pointing the significant invading bacterium. Our data thus far indicate that high antibody titres frequently appeared in clinical bacteriuria, but rarely in 'recent injury' patients free from symptomatic disease.

Animal Experiments. Rabbits, guinea-pigs and mice were injected with acetone-killed vaccines (Walter Reed Army Institute of Research, 1964) of *Escherichia coli*, *Aerobacter aerogenes*, *Proteus rettgeri* and *Proteus vulgaris*. The vaccines showed uniformly high immunogenicity. Toxicity, pyrogenicity and safety tests were mostly well within acceptable limits (U.S. Dept. Health, Education and Welfare, Public Health Service Regulations, 1965).

A small series of 14 rabbits vaccinated with each of the above four bacteria were challenged by surgical implantation of live organisms into the kidney pelvis. Proved instances of specific protection were very few, but these did seem related to antibody level.

DISCUSSION

Our studies have shown that antibody does play an important role in urinary tract disease and encourages us to continue our efforts toward an eventual clinical trial of autogenous vaccines in selected spinal cord injury patients.

The top five Gram-negative bacteria which accounted for 79 per cent. of our patient infections are notorious for their serotype multiplicity. Nine patients yielded repeated isolates of *Escherichia coli* or *Aerobacter aerogenes* over long time periods. These isolates were serotyped and in all nine patients the replicate isolates were found to be of a single serotype for each patient, although different from patient to patient. This observation suggests that the problems of antigen heterogeneity might be circumvented in the autogenous vaccine.

We have yet to learn whether repeated parenteral injection of monovalent autogenous vaccines will stimulate antibody titre to significantly raised levels; furthermore, whether this level will prove effective against the risk or repeated intercurrent reinvasion by the indigenous micro organism.

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