tive collagen with a *p*H optimum near neutrality. We have so far been unable to demonstrate activity greater than in trypsin controls with cultures of synovia from three subjects with degenerative joint disease. At temperatures of 20° to 27°C the enzyme appears to split the collagen molecule across the three chains to produce ¾- and ¼-length fragments similar to those resulting from the action of the collagenase from tadpole tissues. We did not identify a further split of the larger fragment to a 62-percent fragment recently observed by Jeffrey and Gross (*4*) using an enzyme from postpartum rat uterus. However, the products of the initial cleavage by synovial enzyme are denatured at 37°C, and the denaturation products are then further degraded to polypeptides of low molecular weight. This latter action is also inhibited by EDTA and is probably ascribable to the same enzyme that causes the initial cleavage, although purification of the enzyme will be necessary to establish this point.

The observations that activity is maximal at neutral *p*H and negligible at *p*H 5.0 and that frozen-thawed tissue does not produce enzyme suggest that lysosomal enzymes are not responsible for the effects observed. It is possible that synovial collagenase plays a role in destruction of collagenous structures which occurs in rheumatoid arthritis.

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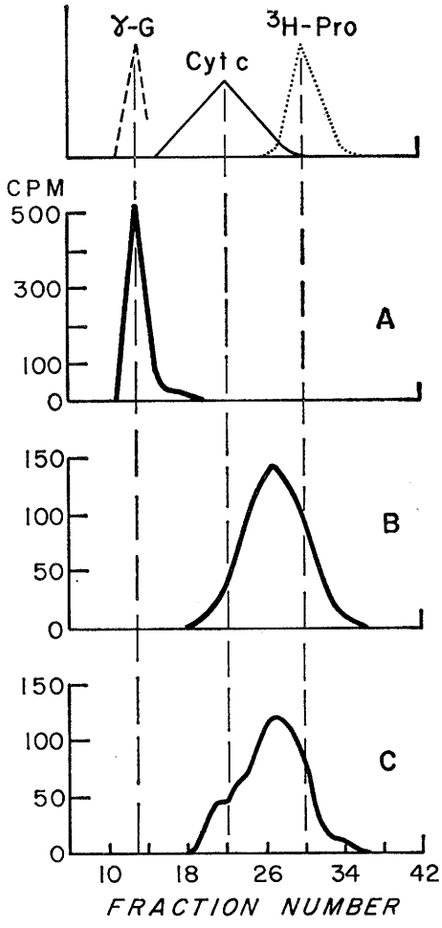


Fig. 4. Elution patterns of enzymatic degradation products of $^{14}C$-labeled collagen on columns of Sephadex G-75. Columns (1 × 35 cm) were equilibrated with 5*M* LiCl containing 0.01*M* tris-HCl, *p*H 7.4, at room temperature. The positions where markers were eluted are shown at the top of the diagram. Reaction mixtures consisted of synovial enzyme and: (A) native collagen in solution at 20° or 27°C; (B) thermally denatured collagen (gelatin) in solution at 27°C; (C) reconstituted collagen fibrils at 37°C.

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## Slow Virus Kidney Disease of Mice

Abstract. *Preliminary observations based on organ weight differential, renal function, and glomerular lesions in mice infected neonatally with lymphocytic choriomeningitis indicate the presence of a slow virus-induced kidney disease of mice. This condition is accompanied by a marked decrease in the size of the kidneys, with progressive diminution of renal function, as shown by measurements of creatinine and urea clearance.*

In spite of increasing interest in recent years in the occurrence of viruria in man and animals, no clear-cut transmissible kidney disease of viral origin has been described (*1*). The closest candidates in this respect appear to be the temporary impairments of renal function reported by clinicians in such human infections as mumps (*2*), measles (*3*), infectious mononucleosis (*4*), acute lymphocytic choriomeningitis (LCM) (*5*), Coxsackie B3 infection (*6*), and vaccinia (*7*).

In the case of acute LCM infection of mice, their ability to excrete virus in urine is well known (*8*). This excretion continues during persistent tolerant infection (PTI) (*9*) which has also been shown to cause a late onset disease of the glomeruli (*10, 11*). This communication reports data which indicate that the kidney is the main target of damage in PTI infection of mice with LCM virus, resulting finally in a shrinkage of the kidneys and impaired renal function.

Eighty-four mice (NYLAR-A inbred strain) (*10*) were divided into two groups. One was inoculated intracerebrally at birth with 0.02 ml of a $10^{-3}$ dilution of a 20 percent mouse-liver suspension infected with LCM virus (PTI mice); the other group was inoculated intracerebrally at birth with 0.02 ml of a $10^{-3}$ dilution of a 20 percent suspension of normal mouse liver (control mice). The latter was used in order to eliminate the possibility that the disease was due to an autoimmune response to the liver tissue contained in the inoculum, rather than to the LCM virus.

The mice were killed at ages ranging from 10 to 16 months and their kidneys were weighed. Results can be seen in Table 1. The weights for PTI females and males were significantly less than those for the controls. The difference between mean kidney weights

for males and females was significant in the control group but not in the PTI group, which indicates a sex-specific incidence of the disease. Since it was known (*12*) that the PTI mice consistently weigh less than control mice it seemed necessary to compare the relative kidney weights, expressed as percentages of body weights. However, the difference in gross weight between PTI and control animals can largely be explained on the basis of the complete lack of adipose tissue developed by the older control animals. In addition, it is well established (*13*, *14*) that wasting of adult animals is not linearly related to kidney weight, which remains constant. In the adult animal, therefore, absolute changes in kidney weight are of greater significance than relative changes in kidney weights as percentages of body weight.

Table 1. Mean kidney weights (in milligrams) of PTI and control mice, ± S.E. The differences between PTI and control values are significant at the 5 percent level of confidence.

| PTI mice | | Control mice | |
|---|---|---|---|
| Female | 124.1 ± 8.5 | Female | 164.5 ± 8.5 |
| Male | 134.4 ± 8.5 | Male | 223.9 ± 8.5 |

Liver, thymus, and spleen also showed decreases in weight in the PTI's, but of distinctly less magnitude than the decrease for the kidney; this fact suggests a primary lesion of the kidney. Histological study of these mice confirms earlier observations (*11*) and shows diffuse involvement of the capillary tufts, hyaline thickening of the basement membrane of the capillary loops, and perivascular infiltration of lymph and plasma cells (Figs. 1–4). No lesions of this type, nor any other evidence of late disease, were found in any of the control mice. In view of the apparent localization of the LCM late disease process in the kidneys of the affected animals, attempts were made to measure the relative kidney function in similar groups of animals.

No data on renal function tests in mice were found in the literature. Therefore, a simple method of evaluating clearance of blood urea or creatinine was devised, based on human clinical techniques. The mice were starved of food overnight to establish a common base line for all animals tested, by eliminating variable feeding habits immediately prior to the test, and to prepare the animals for anesthesia. Our animals were then given 3700 mg of urea per kilogram of body

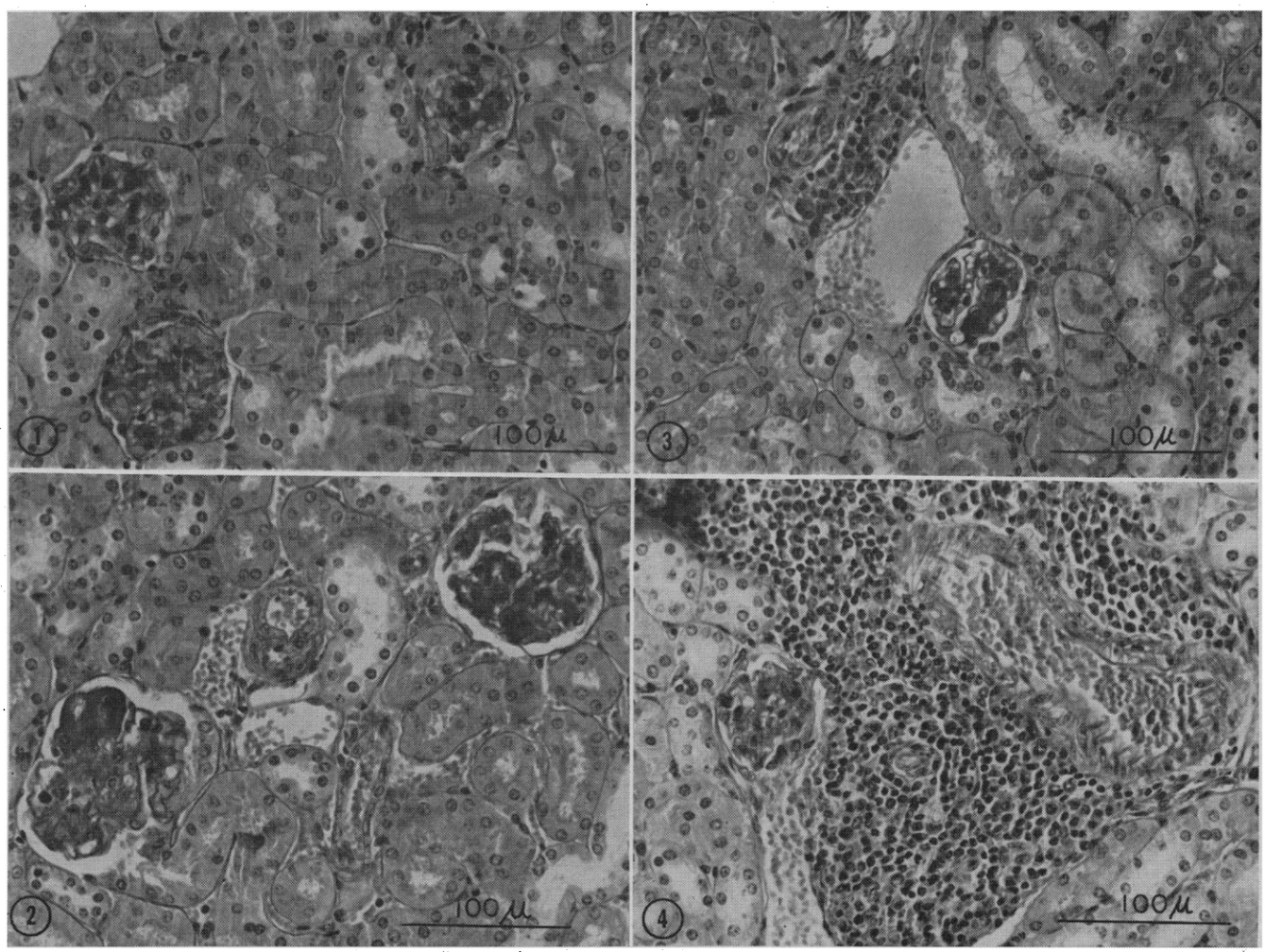


Fig. 1. Glomeruli in a kidney 5 months after infection. Note the smudging of the glomerular capillary loops without cellular infiltration. This material is periodic acid-Schiff (PAS) positive. Fig. 2. Glomeruli in kidney 16 months after infection. Severe intracapillary hyalinization (PAS positive), both focal and general. No cellular infiltration. Fig. 3. Glomerulus in kidney 16 months after infection. Same slide as Fig. 2, but different area, showing mononuclear, cellular infiltration around a small artery, plus PAS-positive casts in some tubules. Fig. 4. Severe mononuclear cellular perivascular infiltration in infected kidney. Abnormal alteration of glomerulus similar to that in Figs. 1–3 is also present.

Table 2. Urea clearance in control and PTI mice after intraperitoneal injection of urea. The dose was 3700 mg/kg. The mice were orbitally bled 2½ hours after inoculation. The assay was read at 480 mμ in the Beckman D.U. spectrophotometer.

| Group | Age (mo.) | No. of mice | BUN* (Mean ± S.E. |
|---|---|---|---|
| Control | 15 | 9 | 53.9 ± 1.7 |
| Control | 19 | 4 | 59.9 ± 5.6 |
| Control | 23 | 7 | 63.4 ± 2.9 |
| Mean | *18.6* | | *58.5 ± 1.9* |
| PTI without L.D.† | 10 | 19 | 74.8 ± 7.6 |
| PTI with L.D. | 15 | 14 | 90.4 ± 8.6 |
| PTI with L.D. | 23 | 3 | 106.7 ± 14.2 |
| Mean | *16.4* | | *93.2 ± 7.5* |

* Blood urea nitrogen (mg/100 ml of plasma). † L.D., late disease.

weight intraperitoneally and 2½ hours later were bled orbitally. Urea analysis was carried out by the diacetyl monoxime procedure (*15*). Table 2 shows the results, which indicated that 10-month-old mice without significant late disease had impaired ability to clear urea from their blood. The PTI mice with late disease, although slightly younger than the controls, had more severely impaired renal function, with blood urea levels 62 percent higher than the control group (93.2 versus 58.5 mg of blood urea nitrogen per 100 ml of plasma).

In a second experiment using different mice, a similar technique was used to measure the rate of disappearance of creatinine from the blood (*16*) 1½ hours after intraperitoneal injection of 1850 mg of creatinine per kilogram of body weight. These results are shown in Table 3. Again the older PTI mice were clearly higher (54 percent) in the blood level of creatinine than the controls, establishing further evidence of renal insufficiency.

Table 3. Creatinine clearance in control and PTI mice after intraperitoneal injection of creatinine. The dose was 1850 mg/kg. The mice were orbitally bled 1½ hours after inoculation. The assay was read at 520 mμ in the Beckman D.U. spectrophotometer.

| Group | Age (mo.) | No. of mice | Creatinine* (Mean ± S.E.) |
|---|---|---|---|
| Control | 16 | 9 | 36.2 ± 3.2 |
| Control | 20 | 5 | 30.4 ± 2.7 |
| Mean | *17.4* | | *34.4 ± 2.4* |
| PTI without L.D.† | 5 | 4 | 19.7 ± 2.5 |
| PTI without L.D. | 10 | 8 | 41.5 ± 5.8 |
| Mean | *8.3* | | *3.42 ± 5.0* |
| PTI with L.D. | 16 | 7 | 62.9 ± 4.5 |
| Labtrol‡ | | | 1.5 |
| Pathotrol‡ | | | 3.5 |

* In mg/100 ml plasma. † L.D., late disease. ‡ Commercial creatinine control from Dade Reagents, Division of Scientific Products, Miami, Florida. Both standards were assayed by the Reference Laboratory, Division of Laboratories and Research. Labtrol (normal) 1.2 ± 0.1 mg; Pathotrol (pathological) 3.3 ± 0.3 mg. Reagents obtained from Dr. Charles Fasce, Division of Laboratories and Research.

It is felt that these data establish a strong basis for concluding that long continued "tolerant" LCM virus infection of the mouse causes increasing damage to the kidney, as revealed by histological study, and deteriorating renal function which may be responsible for the early death of these animals. If these conclusions are correct, this condition appears to qualify as a new slow virus kidney disease, offering a potential model for the study of chronic degenerative renal failure. All data at present indicate that neonatal inoculation with LCM virus is necessary to cause this disease; the role of such auxiliary agents as streptococci is at present only hypothetical, but the possibility of this bacterium causing such a disease in this system appears to be unlikely, since a similar or identical renal lesion has been observed in germ-free mice with congenital persistent tolerant LCM infection (*17*). Similar renal lesions apparently occur in other virus-induced or associated diseases. These lesions have also been observed in NZB mice (*18*) [in which a virus-like particle (*19*) has been seen], AKR mice inoculated with Friend or Rauscher virus (*20*), and the BALB/c/DM/Tex mice (*21*) inoculated with these viruses. The relationship, if any, between all these renal lesions is not yet clear. Similarly, the observation that the renal disease process in B/W mice is mitigated by cyclophosphamide therapy (*22*) is of great relevance in case the LCM glomerular pathogenesis should prove to involve a virus-induced autoimmune mechanism, as has been postulated (*10, 11*).

The LCM virus-induced renal disease appears to fulfill the criteria laid down by Sigurdsson (*23*) in 1954 for the group of diseases he named "slow virus infections." The importance of this group has been emphasized by Gibbs and Gajdusek (*24*), and it is relevant to repeat the criteria here:

"(1) A very long initial period of latency lasting from several months to several years.

"(2) A rather regular protracted course after clinical signs have appeared usually ending in serious disease or death.

"(3) Limitation of the infection to a single host species and anatomical lesions in only a single organ or tissue system."

Sigurdsson admitted that the last statements might have to be modified as knowledge increased; in the main these criteria have remained valid. In terms of the possible role of immunological tolerance in the slow virus infections it is of interest that Sigurdsson stressed that "in chronic infections the immunity mechanism of the body never gets a good grip on the pathogenic microbe and therefore a long and dubious battle ensues."

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