TABLE II. Summary of Hydroxyproline Values for Mature Animals.

| Fraction | Normal as % of total | Days after injury, as % of normal value 2 | 4 | 8 | 14 |
|---|---|---|---|---|---|
| Total hypro | | 85 | 88 | 92 | 94 |
| Total soluble | 2.20 | 112 | 96 | 95 | 93 |
| Water soluble | .00 | .00* | .54* | .45* | .44* |
| Salt soluble | 1.10 | 117 | 71 | 80 | 65 |
| Citrate soluble | 1.10 | 105 | 71 | 68 | 80 |

* Since normal value = 0, these data reported as % of total hydroxyproline.

in both salt-soluble and citrate-soluble fractions with a corresponding increase in the water-soluble fraction (Table II).

*Discussion.* These results are in agreement with the concept that the neutral solvent-soluble fractions of collagen represent the early products of synthesis. It was found that in mature animals, in which connective tissue collagen turnover is extremely slow, the water-soluble fraction was absent in the uninflamed tissue. In both age groups, regeneration was accompanied by increases in neutral solvent-soluble fractions. It also appears that the citrate-soluble fraction is not associated with the synthesis sequence but is more likely a derivative of the mature collagen fiber.

The primary objective of this study was to determine the chemical nature of the collagen fiber disappearance seen histologically in acute inflammation. This phenomenon could be due to disaggregation of the gross fibers to units too small to be seen, to reversal of the synthesis sequence to convert the fibers to soluble forms or to digestion of the fibers to amino acids or peptides with removal of these end-products from the area. The work reported here indicates that the histologic observation of disappearance and reappearance of collagen fibers in acute inflammation in connecive tissue is due to proteolysis and *de novo* resynthesis.

1. Forscher, B. K., Cecil, H. C., *Arch. Biochem. Biophys.*, 1957, v70, 367.

2. ———, *Gerontologia*, 1958, v2, 174.

3. Schultz-Haudt, S. D., Aas, D., *Arch. Oral Biol.*, 1960, v2, 131.

4. Lowther, D. A., Green, N. M., Chapman, J. A., *J. Biophys. Biochem. Cytol.*, 1961, v10, 373.

5. Forscher, B. K., Stanley, H. R., *A.M.A. Arch. Path.*, 1957, v63, 191.

6. Neuman, R. E., Logan, M. A., *J. Biol. Chem.*, 1950, v184, 299.

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## A Simple Rapid Method for Production of Viral Antibody in Mice.* (27723)

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A technic for production of antibody in mouse peritoneal fluid was described by Munoz(1) utilizing intraperitoneal inoculations of egg albumin or bovine serum albumin mixed in Freund's adjuvant. Ascites production was induced, however, in only ½ the animals tested. Both Herrmann and Kasel and their associates(2,3) used ascites fluid from immunized mice as a source of viral antibodies. Considerable quantities were induced by intraperitoneal injection of Sarcoma 180 cells by the first group and bacterial-adjuvant mixture(4) by the latter. A modification of these methods utilizing Ehrlich tumor cells has proved consistently reliable, required less time or fewer injections and is here described in detail.

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TABLE I. Antibody Titers in Mouse Ascitic Fluid and Serum Obtained from Litter Mates Inoculated with a Test Virus and Ehrlich's Ascites Tumor Cells or with Uninfected Control Fluids and Tumor Cells.

| Group No. (4 mice/group) | Immunizing virus | Titer immunizing virus | *In vitro* challenge virus§ | Neutralizing antibody titers‖ Serum | Ascitic fluid |
|---|---|---|---|---|---|
| 1 | Polio 2 | $10^{-7.0}$ | Polio 2† | 1:64 | 1:32 |
| 2 | Control* | | " | <1:2 | — |
| 3 | Cox B2 | $10^{-6.5}$ | Cox B2† | 1:256 | 1:178 |
| 4 | Control* | | " | <1:2 | <1:2 |
| 5 | Cox A9 | $10^{-8.0}$ | Cox A9‡ | 1:256 | 1:128 |
| 6 | Control* | | " | <1:2 | 1:2 |
| 7 | ECHO 1 | $10^{-7.0}$ | ECHO 1† | 1:32 | 1:32 |
| 8 | Control* | | " | <1:2 | <1:2 |
| 9 | Adeno 1 | $10^{-1.5}$ | Adeno 1‡ | <1:16 | <1:16 |
| 10 | Control* | | " | <1:2 | <1:2 |

* Control: Each animal received undiluted fluids from uninoculated human amnion or monkey renal tissue culture I.M. & I.V. and Ehrlich's ascites tumor cells I.P.

† Tests in primary monkey renal cells.

‡ Tests in primary human amnion cells.

§ 100 $TCD_{50}$ of virus.

‖ Serum titers were determined in neutralization tests in monolayer cell cultures utilizing the cytopathogenic endpoint. Final readings were taken 48 hr after complete cellular destruction of control tubes by the test virus.

Each group of 8 mice, 4 test and 4 control animals from the same litter, was kept together from birth to 21 days of age when the experiment was begun. Undiluted viral infected fluids removed from monkey renal cell cultures maintained on BAF medium(5) with 5% horse serum served as antigens. Each mouse was injected intramuscularly in the hind-quarter with 0.25 ml of viral antigen mixed with a rapid to and fro movement in the same syringe with 0.25 ml of Freund's complete adjuvant (Difco Laboratories). A second dose was given in the same manner one week later. One week after the second inoculation each animal received 0.25 ml of the viral antigen without adjuvant intramuscularly and 0.1 ml was administered slowly by the intravenous route. At the same time each mouse was given intraperitoneally 0.1 ml of undiluted ascitic fluid from mice previously inoculated by the same route with Ehrlich's ascites tumor cells. Approximately 1-5 $\times 10^7$ cells were injected and 7 days later the ascitic fluid was harvested aseptically with needle and syringe. The yield varied with a maximum of 10 ml from a single animal. Pooled fluids were spun twice at 2000 rpm for 10-minute periods. Cells were discarded, and the cell-free supernate, inactivated at 56°C for ½ hour, was used in neutralization tests in monolayer tube cultures.

Table I summarizes the results with several different viruses. Following immunization it is evident that neutralizing antibodies developed to virtually the same titer in both the serum and ascitic fluid. This has been observed by others for both neutralizing and HI antibodies(2,3,6). It will be recalled that by immunoelectrophoretic technics mouse peritoneal fluid has been found to be similar in composition to mouse serum and that egg albumin antibodies in ascitic fluid have been found in the gamma globulin fraction(7). It should be noted that the pooled ascitic fluids from one group of control animals (Group 6) contained a very low level of neutralizing activity. No explanation for this finding is apparent. The failure to induce formation of adenovirus antibodies probably was due to the low concentration of the viral antigen.

1. Munoz, J., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 757.

2. Herrmann, E. C., Jr., Engle, C., *ibid.*, 1958, v98, 257.

3. Kasel, J. A., Lieberman, R., Smith, H. A., *Virology*, 1959, v9, 702.

4. Lieberman, R., Douglas, J. O. A., Humphrey, W., Jr., *Science*, 1959, v129, 775.

5. Milovanovic, M. V., Enders, J. F., Mitus, A., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 120.

6. Wagner, R. R., *J. Immunol.*, 1955, v74, 446.
7. Anacker, R. L., Munoz, J., *Bact. Proc.*, Abstracts of 60th Annual Meeting, 1960, p88.

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## Observations on the Course of Fibrinolysis.* (27724)

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The existing methods for measuring various components of the fibrinolytic system suffer from uncontrollable variables and effects of multiple factors. For example, timed clot lysis methods of measuring activator are affected by the amounts of plasminogen and fibrinogen. Fibrin plate methods are qualitative rather than quantitative. Where casein is used as a substrate for fibrinolytic enzymes, care must be taken in the interpretation of the results since the physical properties of fibrin differ from those of casein, and plasmin is known to have different affinity for different substrates(1). The studies by Sherry and his co-workers(2) using $I^{131}$ labelled fibrinogen give the most clearcut results, but preparation of the labelled fibrin is tedious.

The method described below has many of the features of the $I^{131}$ labelled fibrinogen method without the necessity of labelling the fibrinogen, and gives information about the various phases of clot digestion, whether the time period under investigation be minutes or hours.

*Methods.* Five-ml samples of 0.3% human fibrinogen (Pentex) in borate buffer (0.18 M, pH 7.4) containing 0.04 M NaCl were used, and a suspension of human plasminogen‡ was added in most determinations. The mixture was clotted for 30 minutes at room temperature with 1.0 ml of serum, used immediately after preparation from recalcified fresh human plasma, as a source of thrombin since commercial thrombin preparations, bovine in origin, contain large quantities of bovine plasminogen. During clotting the flask was gently shaken to form a discreet, tight clot. The liquid was decanted and the clot washed twice by shaking for 5-10-minute periods in buffer, then incubated in a total volume of 5.0 ml of borate buffer, containing activator (in the studies to be reported human urokinase‡ was used). Lysis of the clot was followed by taking serial 0.5-ml aliquots, which were diluted to 5.0 ml with borate buffer and filtered immediately to remove insoluble fibrin. The amount of solubilized protein was then determined by reading the optical density at 280 mμ. The change in optical density was converted to amount of fibrin lysed by comparison with a fibrin preparation standardized using the Folin-Ciocalteu reagent by the method of Ratnoff & Menzie(3). The data reported are expressed as mg fibrin lysed per 100 ml of incubation solution.

*Results and discussion.* The plot of fibrin lysed with time is S-shaped (Fig. 1). The initial lag period was found to be due to the physical properties of the clot. Clots which are formed by allowing the mixture to gel without shaking were found to lyse without the lag period, but were not satisfactory because it was impossible to wash out contaminating protein. Flattening of the top of the curve occurred as fibrin dissolution neared completion. Only traces of ultraviolet absorbing material leaked out of the washed clots during incubation in the absence of added activator. On replicate observations, individual points on the curve differed from the mean by an average of 7.6% (± SEM 1.4%).

The maximum rate of lysis (the slope of the straight part of the curve) was found to be

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