that it is generated during blood coagulation. It would seem an attractive hypothesis that the proteolytic enzymes plasmin or thrombin formed during blood coagulation might be involved in the generation of this serum chemotactic activity. Indeed, it has already been shown that plasmin splits complement components into chemotactically active agents[4,10]. Alternatively, one may assume that analogous to our findings with chromatographed serum, chemotactic factors are already present in plasma, but masked by inhibitors or otherwise, or that they are unmasked by their interaction with the immune complexes[1].

The relation of these findings in vitro to the accumulation of granulocytes and macrophages in inflammatory sites is unknown. However, immune complex induced accumulation of both neutrophils and macrophages does occur in vivo. Since the accumulation of the 2 cell types has been shown to be independent of each other[11,12], it is tempting to speculate that the interaction of plasma with antigen-antibody complexes results in the formation of different cytotaxins specific for both types of leukocytes.

In conclusion, the chemotactic activity of serum differs from that of plasma in the following ways: (1) Normal serum contains chemotactic factors for both neutrophils and macrophages. Normal plasma has no chemotactic activity for either neutrophils or macrophages. (2) If fresh serum is incubated with antigen-antibody complexes, a great rise in neutrophil chemotactic activity is seen but a drop is observed in macrophage chemotactic activity. On the other hand, the incubation of plasma with antigen-antibody complexes causes a rise in activity for both neutrophils and macrophages. Neutrophil granules upon incubation with plasma generate chemotactic activity for macrophages, but not upon incubation with serum[13,14].

Zusammenfassung. Die chemotaktische Aktivität von Kaninchenplasma und -serum für neutrophile Leukozyten und Makrophagen ist verschieden. Normales Serum enthält im Gegensatz zum Plasma chemotaktische Faktoren für Neutrophile und Makrophagen. Inkubation von Serum mit einem Immunkomplex bewirkt einen erheblichen Anstieg der chemotaktischen Aktivität für Neutrophile, dagegen eine Verminderung für Makrophagen. Die Inkubation von Plasma mit Immunkomplex bewirkt einen starken Anstieg für beide Zelltypen. Nach Inkubation einer Granula-Fraktion aus Neutrophilen mit Plasma – nicht aber mit Serum – entsteht chemotaktische Aktivität für Makrophagen. Diese Befunde lassen einen möglichen Zusammenhang zwischen Chemotaxis und Koagulation vermuten.

J. F. Borel and E. Sorkin

Schweizerisches Forschungsinstitut,
Medizinische Abteilung,
CH-7270 Davos-Platz (Switzerland), 18 September 1969.

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Production of Antibody for Rat Tail Tendon Collagen

Evidence for direct antigen-antibody reactions as the auto-immune basis for many connective tissue diseases has been largely circumstantial[1]. Though collagen and related proteins have been considered to be immunologically inert, recent studies[2–4] have shown that even in the absence of accepted antigenic determinants, collagen does possess a degree of antigenicity. The present study was undertaken to produce purified antibodies against specific fractions of collagen as the prelude to determining whether such antibodies might induce changes in newly proliferating connective tissue in experimental animals similar to those noted in human diseases associated with the auto-immune response.

Materials and methods. A total of 9 guinea-pigs (without consideration of sex) were given periodic injections of 0.15 *M* citrate buffer (pH 3.8) extracted rat tail tendon collagen according to the schedule outlined in the Table. Blood was collected retro-orbitally, twice weekly from alternate eyes, about 3 ml from each eye per collection, permitted to clot at 37 °C for 4 h and the serum obtained after rimming and centrifugation at 1000 × g for 20 min.

The collagen used as the antigen was prepared from tail tendons of laboratory rats (175–225 g; Holtzman, Madison, Wisc.). The well-minced tendons were washed at 4 °C for 24 h with 50 ml/g tendon of a 3 g/l sodium chloride solution. The tendons, recovered by decantation, were extracted with 1.0 *M* sodium chloride (50 ml/g tendon) for 24 h at 4 °C. The solution thus obtained contained the neutral salt soluble collagen (NSS). The tendons then were subjected twice to further extractions with 0.15 *M* citrate buffer, pH 3.8 at 4 °C for 24 h. After dialysis against cold running tap water, the precipitated acid soluble collagen (AS) was suspended in 1% saline (2 mg/ml on a dry weight basis). Only the AS fraction was used as an antigen in this experiment.

The sera were qualitatively assayed for the presence of antibody against NSS or AS collagen by the Ouchterlony method[5]. Fractionation of the pooled guinea-pig sera on Sephadex G-200 was according to the general procedure of Killander[6]. Nitrogen determinations on the antigen and antibody fractions were performed with a Coleman Nitrogen Analyzer.

Results. The presence of anti-rat tail tendon collagen antibody in guinea-pig serum was indicated after 24 h by the typical Ouchterlony reaction as evidenced in the Figure (a–f). A strong reaction was given against the AS tendon collagen whether the collagen antibody was at the pH of extraction (3.8), near neutrality or at pH 5.5. The best response was given at pH 5.5. (d). A positive antigen-

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antibody reaction still was obtained when the pH of the guinea-pig antiserum was varied (c, d, e). Only minimal reaction was evidenced with NSS collagen after 120 h (b) when the pH of the antigen or antibody were adjusted with citrate to each pH as stated above for AS collagen. Acid precipitation of serum protein as the source of a pseudo-positive response is removed from consideration by the absence of any reaction between various citrate buffers (pH 3.8–7.0) in the antigen wells and guinea-pig antiserum. No reaction was obtained with neutral salt or citrate extracted collagen against normal guinea-pig or normal rat sera. Small differences favoring citrate were obtained when the AS collagen was redissolved in citrate, acetate or HCl buffers at pH 3.8, 5.5. or 7.0 prior to running the Ouchterlony assay.

When each guinea-pig antiserum fraction obtained from Sephadex G-200 chromatography was assayed against AS collagen (2 mg/ml) at a fixed nitrogen concentration (1.5% protein nitrogen; 0.02 ml in each well), the antibody activity was localized between pooled peaks 1 and 2.

During the second week of the injection, large welts appeared at the sites of injection which persisted through the test period.

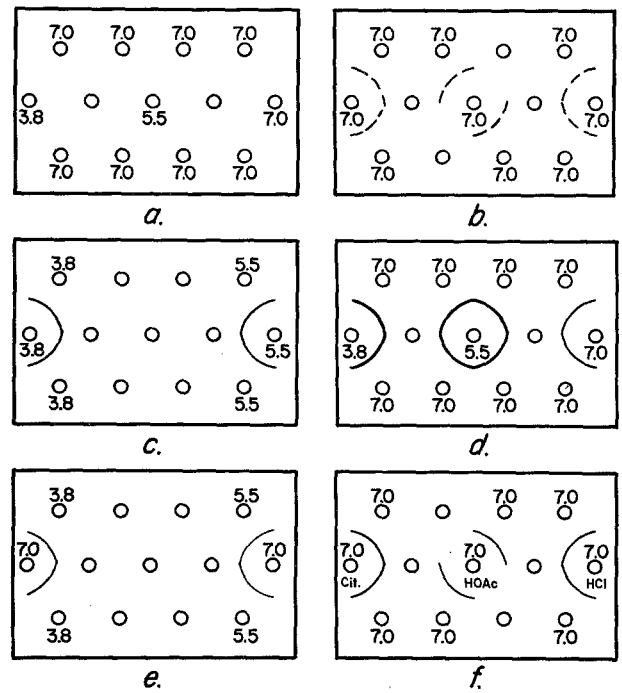
Discussion. Antisera have been produced in rabbits to a number of collagen fractions and derivatives[2–4,7]. Immunization generally was by way of repeated s.c. injection of the protein and the appearance of antibody based on often equivocal evidence of a skin reaction[7–10], the precipitin reaction[4] or complement fixation[2,3].

Cross-reaction with insoluble collagen as the antigen has been demonstrated with the serum of rheumatoid arthritis patients[11] and an autoimmune basis has been proposed[1] for several connective tissue diseases. In no case, however, has an antibody against foreign protein been isolated or has the specific antigen been characterized.

The present study makes use of a homogeneous collagen fraction of rat tail tendon to induce antibodies in the guinea-pig. Fractionation of the antiserum by Sephadex G-200 chromatography results in purified antibody amenable to the quantitative study of the role of antibodies in connective tissue degeneration.

Contrary to the suggestion of O'DELL[10] that the antigenicity of the collagen molecule lies in its rigidity rather than in the presence of aromatic determinants, it is evident that NSS collagen differs sufficiently in its primary structure from the AS fraction so that these can be differentiated by an antibody prepared specifically against one of them. Thus the nature of the antigenic determinants in collagen remains unknown.

An increased Ouchterlony response was noted when the assay of AS collagen was performed below pH 7.0. Further enhancement was manifested when the pH of the citrate-extracted collagen was maintained with citric acid rather



Summary of the ouchterlony microdiffusion assay. In all cases, the 2 outermost rows of holes contained guinea-pig antiserum at the pH indicated by the numerals. The antiserum was obtained against the citrate soluble fraction (AS) of rat tail tendon collagen. In general, the center row of holes contained AS collagen (antigen). The unmarked holes were not used. Except as noted, all results were obtained at 48 h. In slide (a), the center row contained no collagen but only 0.15 *M* citric acid adjusted to the indicated pH. In slide (b) the antigen was the neutral salt soluble collagen fraction. The positive reaction was minimal only after 120 h. Slides (c)–(e) show the effect of varying the pH of the antiserum or of the AS collagen preparation. Slide (f) indicates the results of using different acids to redissolve the citrate soluble collagen fraction.

Injection and blood collection protocol for antibody production

Week	Protocol
1–3	S.c. injection, 3 times weekly, 0.25 ml acid soluble collagen (AS) with 0.25 ml complete Freunds adjuvant (cFa).
4–5	No injections.
6–7	S.c. injection, 3 times weekly, 0.50 ml AS with 0.50 ml cFa.
8	No injections.
9–11	Collect blood retro-orbitally, average 3 ml from each eye per collection.
12–14	No injection or blood collection.
15	Single s.c. injection, 0.50 ml with 0.50 ml cFa.
16–18	Collect blood retro-orbitally.
19	No injection or blood collection.
20–22	Collect blood retro-orbitally.

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than when other acids were used to resolubilize the same protein preparation. Thus it is possible that unknown chemical determinants in collagen assume 'more favored' antigenic conformations under acidic pH as a function of the acid anion. Hence highly specific differences in connective tissue proteins can be discerned immunologically, permitting evaluation of collagen molecules containing unusual amino acids produced through tissue culture[12] in autoimmune pathogenesis[13].

Zusammenfassung. Im Meerschweinchen wurde erfolgreich Antikörper gegen Ratten-Kollagenextrakt erzeugt. Dieser reagiert mit der säurelöslichen Kollagenfraktion, nicht aber mit neutralsalzlöslichen Substanzen. Die Spezifität des Antikörpers soll für die weitere Erforschung von degenerativen Autoimmun-Krankheiten benützt werden.

S. B. NEEDLEMAN and N. STEFANOVIC

Radioisotope Service, V. A. Research Hospital, Chicago (Illinois 60611, USA), 19 June 1969.

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Mutagenic Effect of Isopropyl Methane Sulphonate in Mouse

The mutagenic effect of sulphonates has been investigated in several biological materials. For some of them, chromosome breaking ability was demonstrated[1].

However, there is still a great need for experiments on mammals. The effects of Myleran, a difunctional sulphonate, were observed on mouse spermatogenesis[2]. A similar investigation was carried out with a monofunctional compound: methyl methane sulphonate in which we attempted to correlate the cytological effect with its genetical consequences[3]. More recently, experiments were designed to detect dominant lethal mutations induced in mouse by alkylating agents. Some mutagenic effect of the compound tested here were mentioned for the mouse[4]. This sulphonate has been known for its mutagenicity in higher plants[1,5]. Early works in mouse (unpublished data) showed that isopropyl methane sulphonate (IsoPMS) is able to induce chromosome aberrations which incited us to test its mutagenic power. The results of the first experiments, dealing with dominant lethal mutations, are reported here.

Material and methods. Male mice of C_3H (4 months old, weighing approximately 30 g) previously controlled during 4 years for spontaneous chromosome aberrations, were injected i.p. with IsoPMS. Buffered solutions were prepared immediately before use. Injected males were mated with 4 $C_{57}BL$ females of a completely unrelated strain. Vaginal plugs were checked every day and fresh females were added. They were sacrificed at 14 days' pregnancy. Dominant lethal mutations were recorded following classical procedure[6]. The percentage of dead implants is for deciduomata.

Early losses represent both deaths of zygotic origin and unfertilized eggs. 2 criteria were used to measure dominant lethality. One was the ratio live embryo/corpus luteum. The other criterion is the live embryos after treatment expressed as percentage of the control,

$$\text{Dominant lethal mutants} = 100 - \left(\frac{\text{Live embryos in treated group per } ♀}{\text{Live embryos in control group per } ♀} \times 100 \right)$$

This technique does not yield any specific information on the nature of mutations involved.

However, early deaths should have a higher probability of gross chromosome abnormalities which would not be the case of deciduomata[7]. This would help to distinguish the 2 classes of dominant lethals.

Results. Toxicity tests were first realized to select doses useful in mutagenesis. LD_{50} ranged from 60–120 mg/kg for 4-month-old animals (according to the strain).

From these data the dose of 100 ml/kg was chosen for further experiments. The total period (60 days) during which dominant lethal mutations were scored was divided in 4 subperiods: (1) mature spermatozoa from vas and epididymis; (2) post-meiotic stages from testes; (3) meiotic stages (spermatocytes); (4) pre-meiotic stages (spermatogonia). This estimate is from OAKBERG's[8] data on the duration of the different stages of spermatogenesis.

The effects on the pre-meiotic stages could not be recorded owing to the sterile period which lasted from 37 days to about 60 days after injection. Data for the 3 first periods are given in the Table. They show clearly that IsoPMS is an efficient mutagen in inducing dominant lethal mutations.

There is no significant difference between the amount of total mutations induced during the 3 periods. In the third period (spermatocytes), however, there are significantly less dead implants and more early losses as compared with the 2 first periods.

$\chi^2 = 6.05$ 2 df $P < 0.05$ for dead implants,
$\chi^2 = 10.06$ 2 df $P < 0.01$ for early losses.

Discussion. Some daily differences in the amount of induced dominant lethal mutations seem to exist but the data reported here are still too scanty to ascribe a more precise sensitivity to a specific stage. As far as we can see, the sensitivity of spermatogonia to IsoPMS was so high that it resulted in a complete sterility. The present data are in agreement with previous ones in at least 2 respects (1) for a high mutation rate induced by IsoPMS (the higher dose of 200 mg/kg resulted in 80–90% dominant lethal mutations)[4] and (2) the occurrence of a sterile period (after 50 mg/kg males were sterile from 31–56

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