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J. Donald Smiley, … , Charlotte Sachs, Morris Ziff

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Research Article

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In Vitro Synthesis of Immunoglobulin by Rheumatoid Synovial Membrane

J. Donald Smiley, Charlotte Sachs, and Morris Ziff

From the Department of Internal Medicine, Rheumatic Diseases Unit, The University of Texas, Southwestern Medical School, Dallas, Texas

Abstract A technique for the in vitro culture of rheumatoid synovial tissue with ^{14}C-amino acids and isolation and quantitation of the newly synthesized immunoglobulins has been developed. This technique has been used to compare immunoglobulin synthesis of 12 rheumatoid synovia with that of synovia from nonarthritic patients and with that of normal human lymph nodes and spleen. In addition, the spleen of a patient with Felty's syndrome has also been examined. Immunoglobulin synthesis in rheumatoid synovia has been shown to be quantitatively and qualitatively similar to that of normal human spleen and lymph nodes although somewhat less active than the Felty's syndrome spleen examined. 79% of the immunoglobulin produced in rheumatoid synovia was of the IgG type, whereas IgM comprised 10% and IgA, 11% of the total. Less than 10% of the IgM synthesized was found to be rheumatoid factor. A fraction containing approximately 90% of its radioactivity in the form of IgG has been obtained for further studies.

INTRODUCTION

Numerous lines of evidence have emphasized the immunologic aspects of rheumatoid synovitis. The rheumatoid synovial membrane usually shows moderate to dense infiltration of the deeper layers with lymphoid cells. These occasionally form compact nodes that actively are producing antibody (3). By fluorescent staining techniques these lymphoid cells are seen to contain immunoglobulin G (IgG), immunoglobulin M (IgM) (4, 5), and rheumatoid factor (5). Recently, intercellular deposits of IgG (6) and complement components (6, 7) were demonstrated within the synovial membrane by fluorescent staining methods. In addition, inclusion bodies containing IgG, IgM, and complement have been demonstrated within polymorphonuclear cells of the synovial fluid from patients with active rheumatoid arthritis (8, 9), and the level of complement in the synovial fluid has been found to be decreased relative to its protein content (10), which suggests activation by immune complexes (11).

Surgical synovectomy, undertaken for therapeutic reasons (12), provides a ready source of sterile, fresh synovial tissue from actively involved joints of patients with rheumatoid arthritis. Studies to be described have utilized suspensions of this material for an investigation of the synthesis of immunoglobulins by rheumatoid synovial tissue. Comparison of the immunoglobulin formation in rheumatoid synovial tissues with that of splenic tissue from a patient with Felty's syndrome has shown the rheumatoid synovium to have a similar level of immunoglobulin synthesis to that of the lymphoid tissues examined.

METHODS

Tissue incubations. Synovial tissue specimens from 12 joints of 11 different patients with classical rheumatoid arthritis, as defined by the criteria of the American Rheumatism Association (13), and from three patients with other joint abnormalities were collected at surgery

This study was published in part in abstract form (1, 2).

Dr. J. Donald Smiley is Senior Investigator of The Arthritis Foundation. Dr. Charlotte Sachs' present address is the Department of Histology, Karolinska Institutet, Stockholm, Sweden.

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under sterile conditions and dissected free from fat and coarse fascia. Lymph nodes removed from two nonarthritic patients, one undergoing an elective cholecystectomy and the other, herniorrhaphy, a spleen from a patient with rheumatoid arthritis and Felty's syndrome, and a spleen from a patient with splenic rupture from a gunshot wound were also studied.

In individual experiments, the synovial membranes, lymph nodes, and splenic tissues were cut into cubes, 2 to 3 mm on each side, and the mince was added to 5 volumes of Eagle's medium (14) that contained one-tenth of the amount of amino acids usually added, decomplemented normal human serum in a final concentration of 5%, and 1000 U of penicillin G/ml. After adjustment to pH 6.8 with saturated sodium bicarbonate, the incubation medium was sterilized by filtration through a Millipore disc, 0.45 μ porosity. When bubbled with oxygen–carbon dioxide, the final pH of the tissue incubation was 7.4. 50 μc of a mixture of algal protein hydrolysate–^{14}C-amino acids, 500 μc/mg (Nuclear Research Chemicals, Orlando, Fla.), was added to each 30 ml of culture suspension which contained about 8 g of minced synovium or lymphoid tissue. The tissue suspension was bubbled for 6 hr with sterile, water-saturated 95% O_2–5% CO_2, which was introduced at a slow rate through a glass capillary sealed into the bottom of a 40 ml conical test tube that was loosely covered with a Teflon-lined screw cap at the top. The tube was immersed in a water bath maintained at 37°C.

Fractionation of soluble protein. At the end of the incubation period the mixture was frozen and thawed once, then spun at 105,000 *g* for 1 hr in a Spinco Model L ultracentrifuge to remove insoluble particles. Casein hydrolysate-amino acid mixture (Difco, Laboratories, Detroit, Mich.) was then added to the supernatant to a final concentration of 1% and the solution dialyzed at 0°C for 36 hr against 1000 volumes of 0.01 M Na_2HPO_4. By a stepwise elution method (15) the dialysate was then chromatographed on diethylaminoethyl cellulose (DEAE), which had a capacity of 0.86 meq/g. Four protein peaks were obtained (Fig. 1) that contained approximately 60–70% of the applied radioactivity. Because the initial peak contained relatively more radioactively labeled γ-globulin in its first portion, this peak was subdivided into two parts, as illustrated; each was redissolved in 2–3 ml of water, centrifuged to remove any denatured protein, and frozen at -10°C until further studies were carried out. Peaks II, III, and IV were dialyzed overnight against 0.01 M sodium phosphate buffer, pH 7.0, then lyophilized, dissolved, centrifuged and the clear supernatants frozen as above.

As a control for nonspecific radioactive contamination of the various soluble protein peaks, three separate synovial samples were divided into two parts. To one part, ^{14}C-amino acids were added at the beginning of the incubation period, while to the other the ^{14}C-amino acids were added at the end of incubation. In the latter instance, less than 5% of the radioactivity incorporated by experimental samples was present in each fraction of the control samples after chromatographic separation of the soluble protein fraction as described above. On the other hand, more than 95% of the radioactivity present in aliquots of the dialyzed supernatant of the experimental samples was precipitated routinely at a final concentration of 5% trichloroacetic acid.

In two experiments, the soluble proteins of fraction Ia were further fractionated by passage of a 0.2 ml aliquot through a column, 1.3 cm diameter and 90 cm in height, containing Sephadex G-200 (Fig. 2). The Sephadex G-200 column was equibrated and eluted with a buffer adjusted to 0.01 M NaF, 0.4 M NaCl, and 0.02 M sodium phosphate, pH 7.2. Elution was carried out at room temperature and after the fractions that formed two protein peaks were collected, they were pooled separately and dialyzed overnight against 0.01 M sodium phosphate buffer at pH 7.0, then concentrated to 1.0 ml by dialysis against 20% polyvinylpyrrolidone in 0.01 M sodium phosphate, pH 7.0. The first protein peak was eluted from the same column by the same void volume and in the same location as that of a similar amount of purified normal human IgG.

Immune precipitation of immunoglobulins. To define the distribution of immunoglobulins, tube precipitations with specific antisera to IgG, IgA, and IgM were carried out with aliquots of each of the protein peaks from DEAE. Goat antiserum to human IgG (Hyland Laboratories, Los Angeles, Calif.), rabbit antiserum to human IgA, and rabbit antiserum to human IgM were used. IgA was prepared from the serum of a myeloma patient, according to the method of Heremans and Vaerman (16). IgM was prepared from the serum of a patient with Waldenström's macroglobulinemia by a modification of the method of Flodin and Killander (17). Antisera to IgA and IgM were prepared in rabbits by two injections, respectively, separated by a 1 wk interval, of 5–7 mg of immunoglobulin in 0.5 ml of complete Freund's adjuvant divided into all four foot pads. Animals were bled 10 days after the last injection. The antisera were adjusted to 40% saturation at 0°C with ammonium sulfate and the resulting precipitate dissolved in 0.01 M sodium phosphate, pH 7.0, and subsequently dialyzed against this buffer. The anti-IgA and anti-IgM preparations were absorbed with human IgG at equivalence before use.

Specific precipitation of the individual immunoglobulins was carried out by adding 0.5 ml of the specific antiserum to 0.1 ml of each radioactive DEAE peak. The tubes were incubated for 1 hr at 37°C, chilled at 0°C overnight, and then centrifuged at 10,000 rpm in a Servall SS-1 centrifuge. Each supernatant was checked by capillary precipitation with additional antibody to demonstrate that antibody excess had been obtained. It was possible to obtain significant precipitates in the respective peaks because the original incubation mixture contained nonradioactive serum present in the unwashed tissues used, and because the culture medium itself contained 5% normal human serum.

Antigen-antibody precipitates were washed three times with 0.9% saline and dissolved in 0.5 ml of 1.0 M sodium hydroxide. This was then diluted to 1 ml; 15 ml of Bray's phosphor containing 40 g/liter of thixotropic gel (Cab-

O-Sil, Packard Instrument Co., Inc., Downers Grove, Ill.) was added, and liquid scintillation counting performed.

When additional IgA or IgM was added to peak Ia, equivalent amounts of appropriate antibody added, and the specific precipitate isolated as above, less than 1% of the total cpm in the fraction was coprecipitated, which emphasized the specific nature of this immune precipitation method.

Quantitation of rheumatoid factor produced by tissue incubations. To determine the rheumatoid factor content of the IgM–^{14}C produced by the various tissues studied, an immune coprecipitation technique was used. Pooled serum from 11 patients previously shown to have a high titer of rheumatoid factor was titrated with heat-aggregated human IgG derived from pooled human serum Fraction II (American Red Cross) to determine the region of immunological equivalence. Sufficient rheumatoid factor and heat-aggregated IgG were then added to 0.1 ml aliquots of the DEAE peak IV to produce 5 mg of immune precipitate. The rheumatoid serum made up 95% of the volume of the final mixture. After centrifugation, the supernatant contained a rheumatoid factor titer of 1:160 by the tube latex method (19), compared with a titer of 1:10,240 for the pooled rheumatoid sera before the addition of the aggregated IgG. Parallel coprecipitations of IgM, as described above, were also carried out.

RESULTS

Incorporation of ^{14}C-amino acids into soluble protein. When the five fractions (Fig. 1) of the soluble protein from DEAE-cellulose chromatography of supernatants obtained after incubation of rheumatoid synovial suspensions were analyzed, it was found that significant radioactivity had been incorporated into all fractions (Table I). Of the 12 synovia examined, all but 4 (Patients 6, 7, 9, and 10) demonstrated more radioactivity in peak Ia than in the other protein peaks. In Patients 9 and 10, the total counts in peak Ia were low. This was associated with a lower percentage of total counts represented as IgG and a higher percentage represented by IgA and IgM. Considerable variation in total incorporation of ^{14}C-amino acids occurred among the patients, even though approximately 8 g of tissue was used in all instances except with Patients 10, 11, and 12. Smaller amounts of tissue, 4.0, 1.3, and 5.4 g, respectively, were used in these experiments and proportionately less radioactive label was added.

Distribution of immunoglobulin in newly synthesized soluble protein fractions. Per cent of total cpm precipitated by specific antisera to each of the three common immunoglobulins in each of the DEAE protein fractions is given in Table II. As shown, most of the radioactive, newly synthesized IgG was found in Fraction Ia, most of the newly synthesized IgA in Fractions II and III, and most of the newly synthesized IgM in Fraction IV. It is of interest that this distribution of precipitable radioactivity, representing immunoglobulin newly synthesized in the synovial membrane, parallels the distribution of these immunoglobulin types in serum separated by the same technique.[1] Considerable variability in the per-

[1] LoSpalluto, Joseph. Personal communication.

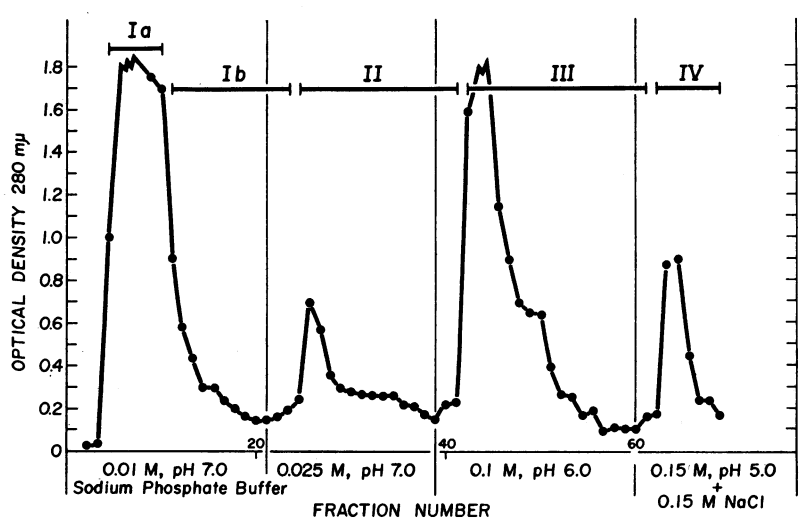

FIGURE 1 DEAE-cellulose chromatography of the soluble supernatant fraction from incubation of rheumatoid synovial tissue.

TABLE I

Incorporation of ^{14}C-Amino Acids into Soluble Protein by Rheumatoid Synovial Membrane Suspensions

DEAE-cellulose peak	Ia	Ib	II	III	IV	Total
			cpm × 10^{-3}			
1. M.C.	1760	195	189	802	697	3643
2. L.D.	1710	555	238	226	246	2975
3. H.R.	1650	58	85	642	521	2956
4. W.C.-I*	640	208	210	302	369	1729
5. O.B.	651	343	225	120	284	1623
6. V.M.	444	46	161	460	344	1455
7. L.W.	211	42	113	512	299	1177
8. W.C.-II*	364	220	44	69	162	859
9. H.C.	53	52	439	84	64	742
10. M.S.	121	44	59	128	6	359
11. W.H.	131	33	17	32	12	225
12. E.S.	49	3	9	27	11	99

* W.C.-I and W.C.-II represent two synovial membranes removed at separate operations from different joints of the same patient.

centage of cpm in each peak precipitated by specific antisera was noted (Table II).

When the total cpm of all protein peaks precipitable by specific antiserum to IgG were added together and compared with the total cpm recovered in the soluble protein eluted from DEAE, it was found that an average of 28.8% of newly synthesized soluble protein was present as IgG (Table III). Similar calculations for IgA and IgM showed 4.3 and 3.5%, respectively, of total cpm present in each. When only the radioactivity of the immunoglobulin was considered, IgG represented 78.6%, IgA, 11.7%, and IgM, 9.7% of the total.

Ia was purified further with a Sephadex G-200 column (Fig. 2). Two protein peaks were obtained, and the tubes containing each were pooled and concentrated. Aliquots were then precipitated with goat anti-human IgG. In the two Ia fractions studied, 92 and 95%, respectively, of the radioactivity present in the first peak was removed by such immunoprecipitation, whereas only 20 and 25% of the radioactivity of the second peak was so removed. This radioactivity in the second peak, precipitated by anti-IgG, may represent some trailing of the IgG of the first peak and may also consist of low-molecular-weight fragments of IgG synthesized in vitro.

Synovial immunoglobulin synthesis in conditions other than rheumatoid arthritis. It was not possible to secure normal human synovial tissue for comparison with the rheumatoid synovia. However, synovial tissue was obtained from patients

TABLE II

*Newly Synthesized Immunoglobulin in Soluble Protein Fractions**

DEAE fraction	% of cpm precipitated				
	Ia	Ib	II	III	IV
IgG	52 ± 14	26 ± 19	19 ± 10	10 ± 5	4 ± 4
IgA	1 ± 1	2 ± 2	8 ± 6	7 ± 4	2 ± 2
IgM	0 ± 1	1 ± 1	2 ± 1	5 ± 3	12 ± 5

* Expressed as per cent of total cpm precipitated by specific goat antiserum to human IgG and rabbit antiserum to human IgA or IgM. Each value presented is the mean ± SD of 12 synovial incubations.

TABLE III

Fraction of Newly Synthesized Soluble Protein Present in Individual Immunoglobulins
(Mean and SD of 12 Experiments)

Immunoglobulin	% of total protein radioactivity*	% of total γ-globulin radioactivity‡
IgG	28.8 ± 9.0	78.6 ± 10.8
IgA	4.3 ± 2.3	11.7 ± 8.9
IgM	3.5 ± 1.5	9.7 ± 4.8
Total	36.6 ± 8.4	100.0

* Sum of cpm attributable to each immunoglobulin type in all DEAE peaks compared with total cpm in all peaks.
‡ Sum of cpm in each immunoglobulin type compared with total cpm of IgG, IgA, and IgM.

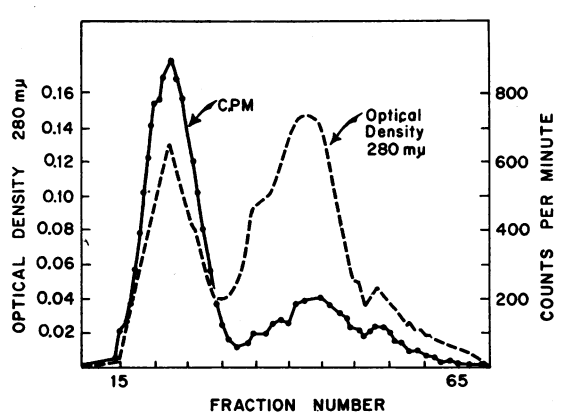

FIGURE 2 Elution pattern of DEAE-cellulose fraction Ia on Sephadex G-200.

TABLE IV

Comparison of Immunoglobulin synthesis in the Rheumatoid Synovium with That in Other Conditions

Procedure or diagnosis	% of total soluble protein radioactivity present as IgG	IgA	IgM
Fracture revision of hip	2.0	0.9	1.8
Fracture revision of hip	3.1	1.2	2.2
Arthrodesis of hip, Legg-Perthes' disease	9.0	1.5	3.6
Rheumatoid synovitis (Mean of 12 specimens)	28.8	4.3	3.5

undergoing joint surgery for revision of fractures in two cases and Legg-Perthes' disease in a third. These synovial membranes were incubated under conditions similar to those used for rheumatoid synovia. The results of these three experiments are shown in Table IV. It is seen that very little, if any, immunoglobulin was produced by synovia obtained during fracture revision of the hip. The small amounts of radioactivity precipitated may represent adherence of nonimmunoglobulins to the immune precipitates utilized for isolation of the immunoglobulins. However, the third synovium from the patient with Legg-Perthes' disease showed small but significant synthesis of IgG, 9% of total soluble protein radioactivity being present in this form. Symptoms had been present for 2 yr before operation. The much greater synthesis of immune globulins by the rheumatoid synovia is emphasized by the presence of 28.8% of soluble protein radioactivity present in IgG, the dominant immunoglobulin formed.

Comparison of synovial immunoglobulin synthesis with that of human lymphoid tissues. The large amount of immunoglobulin synthesis observed in the rheumatoid synovial membrane prompted a comparison of the activity of rheumatoid synovial tissues with those of human lymph nodes and spleen. It was observed in different experiments that on a weight basis synovial membrane incorporated the same order of magnitude of cpm as two lymph nodes studied (Table V). Both the normal and the Felty's spleen were considerably less active in ^{14}C-amino acid incorporation into soluble protein than were comparable weights of synovial tissue. Much of this difference

TABLE V

Incorporation of ^{14}C-Amino Acids on a Weight Basis by Rheumatoid Synovia and Other Lymphoid Tissues

Tissue	Weight incubated	cpm recovered in soluble protein	cpm in tissue
	g		*cpm/mg*
Rheumatoid synovia*			
L.W.	10.0	1,175,000	118
H.R.	10.0	2,950,000	295
M.C.	9.3	3,640,000	392
V.M.	4.2	1,450,000	690
Lymph node-1‡	0.20	33,000	165
Lymph node-2§	0.05	53,000	1070
Normal spleen‖	16.50	1,440,000	87
Felty's spleen	9.70	108,000	11

* These specimens were accurately weighed after mincing to permit possible comparison with other tissues.

‡ Abdominal node obtained at elective surgery for cholecystectomy.

§ Inguinal node obtained during herniorrhaphy.

‖ Removed after abdominal trauma.

may be explained by the much greater erythrocyte content of spleen compared with synovium or lymph node.

In view of the variability introduced by the presence of components such as fibrous tissue and erythrocytes in any comparison of immunoglobulin synthesis between synovial and lymphoid tissue on a weight basis, the ratio of immunoglobulin synthesis to total soluble protein synthesis was chosen as a better measure of the relative rate of immunoglobulin synthesis in synovial and lymphoid tissues, respectively.

TABLE VI

Comparison of Immunoglobulin Synthesis of Human Lymphoid Tissues and Rheumatoid Synovial Membranes

Tissue	% Total radioactivity* IgG	IgA	IgM
Rheumatoid synovia (Mean of 12 specimens)	28.8	4.3	3.5
Lymph node-1	16.7	2.2	1.7
Lymph node-2	10.0	4.8	5.8
Normal spleen	29.9	2.0	3.2
Felty's syndrome spleen	63.4	5.5	2.7

* Radioactivity in total newly synthesized soluble-protein.

Relative to over-all protein synthetic activity, as reflected by the percentage of total radioactivity incorporated into newly synthesized soluble protein, the rheumatoid synovia were as active in the synthesis of immunoglobulin as the normal spleen, and somewhat more active than the two lymph nodes studied (Table VI). The spleen from the patient with Felty's syndrome showed approximately twice the immunoglobulin synthesis that was observed in the rheumatoid synovia.

Quantitation of rheumatoid factor produced during rheumatoid synovial tissue incubation. The proportion of IgM-^{14}C of DEAE Fraction IV coprecipitable with rheumatoid factor and heat-aggregated human IgG is shown in Table VII. Since Fraction IV contains all of the detectable rheumatoid factor in rheumatoid sera (20), only this fraction was analyzed. As can be seen, a mean of only 1.2% of the radioactivity present in Fraction IV from the rheumatoid synovia was coprecipitated with rheumatoid factor, although a mean of 14.7% of this peak represented IgM-^{14}C. From Felty's spleen supernatant, 2.5% of the total cpm in Fraction IV coprecipitated with rheumatoid factor and 15% precipitated as IgM-^{14}C, respectively. When a normal spleen was examined, only 0.6% of the total cpm in Fraction IV coprecipitated with rheumatoid factor, and 4.2% of the fraction precipitated as IgM-^{14}C. Since, by this separation technique, a small amount of IgG is carried over into DEAE Fraction IV, it is possible that some IgG counts were also coprecipitated with the added rheumatoid factor. Therefore, this procedure provides only an upper limit to the percentage, representing rheumatoid factor, of the IgM-^{14}C coprecipitable from synovial membrane suspensions, i.e., an average of 9% of the IgM-^{14}C in the seven patients studied. Patient E. S., who showed no detectable rheumatoid factor in DEAE peak IV by this technique, was rheumatoid factor–negative by the latex fixation and sensitized sheep cell agglutination techniques (21, 22), which thus lends support to this method for quantitation of rheumatoid factor.

TABLE VII

Proportion of IgM-^{14}C of DEAE Fraction IV Coprecipitable with Rheumatoid Factor and Heat-Aggregated IgG

Source of DEAE fraction IV	% of Total radioactivity	
	IgM–anti IgM	IgG–rheumatoid factor precipitate
Rheumatoid synovia		
D.P.	13.5	0.8
D.F.	25.2	2.4
M.F.	7.4	1.1
V.M.	25.0	1.9
H.R.	11.2	0.8
E.S.	10.9	0.0
M.W.	10.0	1.1
Average	14.7	1.2
Rheumatoid spleen	15.0	2.5
Normal spleen	4.2	0.6

DISCUSSION

Incorporation of ^{14}C-amino acids into soluble protein by rheumatoid synovial membrane suspensions. There was a very active level of protein synthesis in the rheumatoid synovial tissue suspensions. All of the isolated fractions separated on DEAE-cellulose contained large amounts of radioactivity that had been incorporated during the in vitro incubation period, presumably into soluble cytoplasmic proteins, as well as into cell products such as immunoglobulins. It is to be expected that differences in the proportions of various cell types would alter the distribution of radioactivity among the various protein fractions obtained with DEAE chromatography. This would also be influenced by any differences in the intensity of antigenic stimulation of these cells, and it is possible that the observed variations in protein synthetic activity from patient to patient may at least, in part, be accounted for on this basis. It is of interest that the two samples of synovium obtained from different joints of the same patient, W. C., showed only small differences in the pattern of uptake of radioactive label into the soluble protein fractions. However, the differences noted probably reflect mainly variability in the amount of lymphoid infiltration of synovia from one joint to another.

Newly synthesized immunoglobulin in soluble protein fractions. Consideration of the amount of newly synthesized immunoglobulin must take into account the fact that only 60 to 70% of the radioactivity applied to the DEAE columns could be recovered by this fractionation. In addition, the IgG, IgA, and IgM from synovial cultures were spread into several protein peaks (Table II),

which suggests considerable heterogeneity. The values presented for each chromatographic fraction, therefore, reflect relative recoveries from DEAE of the individual soluble proteins, including the immunoglobulins. LoSpalluto [1] has shown that the loss of immunoglobulin on DEAE is not uniform among IgG, IgA, and IgM, because he could recover approximately 80% only of applied IgA, 50% of applied IgG, and only about 30% of applied IgM on DEAE chromatography of serum containing known amounts of these immunoglobulins. Thus, it is possible that somewhat more IgG and two or three times as much IgM may have been synthesized than was recovered in the present experiments.

The values obtained (Table III) represent the first quantitative data on the magnitude of immunoglobulin synthesis by rheumatoid synovium. They complement the qualitative localization of IgG and IgM previously reported by others (4–6). Since many cell types other than lymphocytes and plasma cells are present in the synovium, the high proportion of total protein synthesis noted, which is devoted to immunoglobulin synthesis in this tissue, indicates that active antibody synthesis takes place in the rheumatoid synovium and supports the impression held by many investigators (23, 24) that rheumatoid arthritis has an immunologic basis. Whether these immunological phenomena involved are primary or secondary remains an entirely open question.

It is of interest that 78.6% of the immunoglobulin synthesized was IgG. The antigen responsible for the elaboration of various rheumatoid and rheumatoid-like factors has been shown to be IgG (25), and it has been suggested that the formation of these factors results from alteration of the IgG as a consequence of combination of antibody with its specific antigen (26). It is, therefore, likely that synthesis of rheumatoid factor in the synovium, which has been shown to occur in the synovial membrane (5), results from stimulation by antigen-antibody complexes derived from combination of this newly formed IgG with, as yet unidentified, antigens.

The local immune response of the synovial membrane might be anticipated to give rise to the mononuclear cell proliferation noted in rheumatoid synovitis without the necessity for an accompanying more generalized immune response. Recent studies in man of IgA in nasal secretions after a repeat challenge with adenovirus have shown specific IgA antibody to be produced almost entirely by cells localized within the nasal mucosa (17). In addition, oral challenge of monkeys immunized against cholera vibrio lipopolysaccharide has shown the most intensive antibody formation to occur within the intestinal lymphatic tissue, as well as in the mesenteric lymph nodes (28). These observations emphasize the possibility of local stimulation of an immune response in nonlymphoid connective tissue structures when the antigen is presented to a localized anatomical site.

Immunoglobulin synthesis by nonrheumatoid synovia. It was of interest to determine whether normal human synovial tissue also synthesizes gamma globulin. Since normal synovia were not available for study, three synovia from hip joints of patients who had elective orthopedic procedures were evaluated. Two of these, obtained during fracture revision of the hip, showed insignificant synthesis of IgG, IgA, and IgM. A third patient, who had experienced 2 yr of pain in the hip associated with aseptic necrosis due to Legg-Perthes' disease, synthesized about one-third as much IgG and equal amounts of IgM as the mean values noted in the rheumatoid synovia studied. Although histologic sections were not available, the small amounts of immunoglobulin synthesized in the tissues obtained during fracture surgery probably represent base line levels of synthesis which occur in normal synovial tissue. The somewhat increased level of synthesis found in the synovium from the patient with Legg-Perthes' disease presumably represents secondary chronic inflammatory changes.

Comparison of immunoglobulin synthesis of rheumatoid synovial membranes and human lymphoid tissues. Comparison on a weight basis of total soluble-protein synthesis in rheumatoid synovia with that of other lymphoid tissues of normal and rheumatoid patients indicates that the levels of synthesis in lymphoid tissues and synovia were similar in amount. They were smaller in the spleen, presumably, because of the absence of γ-globulin synthesis by the erythrocytes within this organ. The relatively large variations among the rheumatoid synovia, between the two lymph nodes, and between the two spleens could reflect a difference in antigenic stimulation of these tis-

sues, but also must reflect, in large part, the relative densities of lymphoid cells.

Synovia, lymph nodes, and spleen all showed similar levels of IgA and IgM synthesis. This striking similarity of immunoglobulin synthesis in rheumatoid synovia and in the lymph node tissue emphasizes the immunologic nature of the rheumatoid process and calls attention to a basic similarity between chronic inflammatory tissue and lymphoid tissue.

An interesting aspect of the present work was the observation of a lower-than-anticipated level of IgA synthesis in spleen, lymph node, and synovial tissues. Turnover studies of radioactively labeled IgA (29) and IgG (30) have shown that total body IgA is produced in amounts equal to half that of IgG in most normal subjects. Since both the normal and pathological tissues examined in the present experiments produced predominantly IgG, it may be assumed that IgA is synthesized in other anatomical sites. The recent demonstration of predominantly IgA synthesis in the lymphoid collections of salivary glands (31), nasal mucosa (27), and intestine (32) provides evidence for the existence of these alternate sites of IgA synthesis which could account for the quantitatively similar levels of synthesis of IgA and IgG in the whole body. Studies by Van Furth (33), using radioautographs of normal human lymphoid tissues cultured in vitro, also showed IgG to be the predominant immunoglobulin formed in lymph node and bone marrow cells, even though he observed that peripheral blood lymphocytes and spleen appeared to make equal amounts of IgG and IgA.

Proportion of IgM-^{14}C of DEAE Fraction IV coprecipitable with rheumatoid factor and heat-aggregated IgG. Knowledge of the antibody specificity of the immunoglobulins produced in the rheumatoid synovium would be of great interest. As an initial step, the proportion of IgM present as rheumatoid factor was evaluated. The technique of coprecipitation of radioactively labeled IgM with carrier rheumatoid factor has the limitation that the nonspecific radioactivity coprecipitated has not been accurately defined. However, the technique does permit assignment of an upper limit to the per cent of IgM that represents rheumatoid factor, which appears to be less than 10% of the IgM-^{14}C formed by the rheumatoid synovial membrane.

The small amount of radioactivity, 0.6% of the total counts, which was coprecipitated in the supernatant from the normal spleen, is probably indicative of the magnitude of the nonspecific coprecipitation obtained with this method. The one synovial membrane studied that fell below this value was obtained from a patient, E. S., who showed negative tests for rheumatoid factor by both the slide latex and sensitized sheep cell agglutination methods. However, although latex fixation titers were available in six of the seven rheumatoid patients reported in Table VII, there was no consistent quantitative relationship between the serum titer of rheumatoid factor and the level of rheumatoid factor formation or IgM synthesis in their synovial cultures. The variable intensity of lymphoid infiltration of different areas, even within the same synovium, complicates the interpretation of quantitative comparisons of the histology with the over-all rheumatoid factor or immunoglobulin synthesis.

The necessity for continued antigenic stimulation to maintain active gamma globulin production is well recognized, since germ-free animals fail to develop typical lymphatic tissue and have low levels of immunoglobulins in their serum. This suggests that the rheumatoid synovial tissue, in view of its high level of immunoglobulin production, is being subjected to active stimulation on a chronic basis. Whether this active immunoglobulin synthesis results from local antigenic stimulus by a component of that tissue or whether the inflamed synovial tissue merely provides a repository for already stimulated lymphocytes derived from the circulation remains to be determined. However, the decreased levels of a number of complement components in the synovial fluid (11) argue for the presence of antigen as well as antibody, at least in the synovial extracellular compartment.

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