SYNOVIAL FLUID CHANGES IN INTRA-ARTICULAR THERAPY

EFFECT OF PREDNISOLONE TRIMETHYLACETATE

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There are many reports on the clinical effects of hydrocortisone and related compounds in intra-articular therapy, but few authors seem to have made a detailed study of the changes in synovial fluid. While undertaking a clinical trial of prednisolone trimethylacetate (Murdoch and Will, 1959) we decided to make observations on the cytological and biochemical characters of the synovial fluid in an attempt to detect changes which might occur, and to establish their possible relationship to clinical improvement.

Method

Knee-joints were investigated. In the course of the trial injections were given at weekly intervals, and were then gradually spaced out and eventually stopped as improvement occurred. Before each injection a small amount (usually 3 to 5 ml.) of joint fluid was removed for analysis. The mucin content was estimated by the acetic acid method (Ropes and Bauer, 1953). Viscosity was assessed clinically into one of three grades: slight, moderate, or marked. A portion of synovial fluid was put into a sequestrene bottle to prevent clotting, and the remainder into a plain glass tube. A total white-cell count was done on the sequestrene specimen within two hours of removal. A thin film was made, fixed with heat, stained with Leishman, and a differential cell count carried out. The total protein was estimated by a modification of the biuret method (Gornall *et al.*, 1949). Electrophoresis was carried out, in a free hanging horizontal tank, using Whatman No. 3 paper and a barbitone buffer of pH 8.6 (Jencks *et al.*, 1955). After staining and drying the strips, ammonia vapour was used to intensify the colour, and they were scanned in a recording photo-densitometer. The total surface area of the densitometer tracing and the individual areas of the protein fractions were estimated with a planimeter, and it was thus possible to obtain the percentage distribution of the various fractions. From the total protein figure the absolute values could be obtained.

Results

Fifty-seven joints were aspirated. In 17 joints there were too few samples owing to rapid resolution of effusion or contamination with blood. In the remaining 40 a sufficient number of suitable serial specimens were available for detailed study of viscosity, mucin content, total and differential cell counts, protein content, and electrophoretic pattern during therapy. These comprised 30 rheumatoid joints, 6 osteoarthritic joints, and 4 joints with hydrarthrosis. The findings are discussed separately under the disease headings.

Rheumatoid Arthritis

In these effusions the fluid was usually watery, pale yellow, and turbid. Fibrin flakes were present, and clotting occurred on standing. The cell content was high and polymorphonuclear cells predominated.

Marked changes occurred in the cell count during treatment with prednisolone trimethylacetate. The initial average cell count was 6,936 per c.mm., with a wide range (1,000 to 36,000 per c.mm.). At the end of treatment there was a fall in every case, the average cell count being 1,163 per c.mm. These results are summarized in Fig. 1.

In performing the differential count the four mononuclear types described by Ropes and Bauer (1953) were identified. For convenience in assessing the results, however, the cells were placed into three groups: polymorphs, lymphocytes, and monocytes. The initial differential count

showed a polymorphonuclear predominance in all but one joint, the average figure being 74.4%. Most of the fall during treatment was due to a sharp diminution in polymorphs, with the appearance of a relative lymphocytosis. Examination of the absolute figures, however, showed a real drop in all three types—namely, polymorphs 84.7%, lymphocytes 42.6%, and monocytes 67.1%.

The initial average protein was 4.4 g. per 100 ml. (range 3.35 to 5.6 g. per 100 ml.). 28 joints showed a fall after treatment with prednisolone trimethylacetate, 1 was unchanged, and 1 showed a rise. The final figure was 3.4 g. per 100 ml. (range 2.3 to 4.9 g. per 100 ml.) representing an average drop of 1.0 g. per 100 ml. These results are summarized in Table I.

TABLE I.—Initial and Final Proteins (g. per 100 ml.) in 30 Rheumatoid Joints

	2-	2.5-	3-	3.5-	4-	4.5-	5-	5.5-6
Initial	—	—	3	5	9	7	5	1
Terminal	4	9	5	6	3	3	—	—

TABLE II.—Initial and Final Albumin Percentage in 30 Rheumatoid Joints

	30%—	40%—	50%—	60%—	70+%	Total
Initial	18	5	6	1	—	30
Final	6	8	11	4	1	30

In studying the electrophoretic changes it is of value initially to add the globulin fractions together so that the main trends in the albumin:globulin ratio can be observed. The average initial albumin proportion was 40% (range 30.5 to 67.6%). During treatment 33 of the joints showed a rise in albumin content, while 8 showed

FIG. 1.—White-cell count before and after treatment with prednisolone trimethylacetate in 30 rheumatoid joints.

a drop, which was usually small. The final average albumin percentage was 50.8% (range 30.7 to 74.5%) (Table II). Detailed analysis of the globulin fractions showed that in three joints the diminution was due largely to a fall in α_2 content, in three joints to a fall in γ -globulin, and in a further three there was a fall of both α_2 - and γ -globulin. In the remainder the diminution was spread through all globulin fractions. A fast-moving component ahead of the albumin band was found in one case on one occasion only.

There was usually a rise in viscosity following treatment. Thus viscosity increased in 11 joints by two grades, and in 15 by one grade. There was no change in three joints, and in one there was a fall by one grade.

Interpretation of the mucin test by the acetic acid method proved unsatisfactory, and it was often difficult to classify the clot in the grades suggested by Ropes and Bauer (1953). In contradistinction to most authors who show a rise in mucin content after treatment, we found a fall of two grades in 10 joints and of one grade in 17 joints. In three joints there was no change.

A typical response to a course of injections with prednisolone trimethylacetate was progressive reduction in the volume of the effusion, accompanied by an increase in viscosity. The marked decrease in the total cell count and the changes in the differential count usually occurred after the first two injections. The changes in total protein and the albumin content were more gradual, however. By the end of treatment only a few drops of clear viscid fluid could be obtained. Fig. 2 (a) is an example.

Globulin Fraction and Duration of Disease in Rheumatoid Arthritis.—Several authors have reported a direct relationship between the γ -globulin content and duration of disease (Ropes *et al.*, 1949; Ohagen, 1950; Ropes and Bauer, 1953). The initial electrophoretic

patterns in 40 rheumatoid joints are grouped according to duration in Table III. There would appear to be no direct increase of γ -globulin with duration.

Osteoarthritis

The joints in this and the succeeding group are too small in number for detailed analysis. Six were available for serial study. The fluid obtained was usually clear, pale yellow in colour, moderately viscous, and did not clot on standing. The initial average cell count was 2,316 per c.mm. (range 200 to 4,000 per c.mm.). Before treatment there was a polymorphonuclear predominance, with the appearance of a relative lymphocytosis after therapy. The absolute figures indicated a fall in all three cell types.

The protein content was lower than in rheumatoid arthritis, the average being 3.3 g. per 100 ml. The final figures show a fall to 2.3 g. per 100 ml. The initial albumin content was 48.9% which is higher than in rheumatoid arthritis. A rise to 59.8% occurred after treatment. There was a slight rise in viscosity.

Hydrarthrosis

There were two patients with bilateral effusions. The synovial fluid of the two cases showed marked differences. In one, the knees showed a high cell count of 14,000 and 40,000 per c.mm. respectively, with a lymphocytosis of 80%. The total protein was high: 4.5 and 4.2 g. per 100 ml., with an albumin of 65.2% and 68.9%. In response to treatment there was an increase of viscosity. The cell counts fell to 400 and 800 respectively, but the differential was unchanged. Total protein dropped to 3.3 and 3.35 g. per 100 ml., with a diminution of approximately 10% in the albumin percentages of both.

In the other case the findings and response to treatment resembled the rheumatoid group.

Control Group

Ten patients with bilateral effusions of comparable severity were studied. Injections were given at weekly intervals, the control knee receiving an equal volume of lignocaine on two occasions in seven of the cases, and once in the remaining three, instead of the active agent. Thereafter both knees were given prednisolone trimethylacetate. In contradistinction to the treated knees there was no change in mucin and viscosity content in the controls. The cell counts rose in six, and were unchanged in the remainder. In four the total protein rose, remaining unchanged in the other six. An analysis of the electrophoretic strips of the group showed no significant change. A typical example is shown in Fig. 2.

Correlation Between Clinical Effects and Laboratory Changes.—With the exception of one patient, whose joint fluid showed little change, although relief of pain and swelling occurred, clinical improvement was directly associated with the cell and protein changes described above.

Discussion

Most authors who have studied the characters of the synovial fluid are in agreement that there is a drop in the total white-cell count after treatment with hydrocortisone acetate (Duff *et al.*, 1951; Kersley and Desmarais, 1953; Duff *et al.*, 1955; Austen and Calkins, 1955). They claim that the mononuclear cells are unaltered, the fall being due to a diminution of polymorphonuclear cells. Robinson *et al.* (1955),

TABLE III.—*Electrophoretic Analysis of Synovial Fluid Protein in 40 Rheumatoid Joints*

No.	Total Protein g./100 ml.	Albumin %	α_1 -Globulin %	α_2 -Globulin %	β -Globulin %	γ -Globulin %	Duration
1	3.9	57.1	4.2	10.55	10.55	17.6	4 months
2	3.8	32.9	6.8	15.6	20.6	24.1	4 "
3	4.3	38.05	5.8	14.4	15.15	26.6	6 "
4	4.7	34.6	6.25	15.15	15.15	28.85	6 "
5	4.9	50.9	6.3	5.0	15.1	22.7	8 "
6	5.0	40.6	8.0	13.3	9.1	29.0	8 "
7	4.6	39.8	5.0	9.4	12.2	33.6	20 "
8	5.25	46.2	4.5	8.6	13.7	27.0	20 "
9	5.6	51.0	7.3	5.3	6.4	30.0	3 years
10	5.3	38.2	7.9	14.0	21.9	18.0	4 "
11	4.4	37.0	6.8	13.7	17.4	25.1	4 "
12	3.7	32.2	11.1	9.5	12.7	34.5	6 "
13	4.1	33.5	9.6	8.7	14.8	33.5	6 "
14	5.45	35.8	3.2	8.9	17.4	34.7	6 "
15	4.9	32.8	5.6	11.4	6.3	43.9	6 "
16	4.2	47.0	6.8	6.0	11.7	28.5	6 "
17	4.2	31.3	7.1	10.9	21.6	29.1	6 "
18	4.7	53.1	5.4	3.4	6.7	31.4	6 "
19	3.7	49.83	10.0	16.0	10.53	13.64	6 "
20	3.9	58.0	4.6	1.9	11.3	24.2	7 "
21	4.7	51.9	5.2	8.6	14.5	19.8	7 "
22	3.35	52.9	3.1	4.5	16.5	23.0	7 "
23	3.9	50.1	3.1	12.7	13.7	20.4	7 "
24	5.4	40.2	5.7	11.2	20.8	22.1	7 "
25	4.3	35.0	2.2	16.5	30.6	15.7	8 "
26	3.36	41.3	5.3	2.5	19.6	31.3	8 "
27	4.3	35.5	4.5	9.9	15.6	34.5	8 "
28	4.0	49.7	3.6	12.1	13.44	21.16	8 "
29	5.1	49.5	2.2	6.4	14.8	27.1	8 "
30	3.4	36.6	9.05	17.1	13.0	24.25	9 "
31	3.15	47.2	6.85	10.15	12.1	23.7	9 "
32	5.1	34.9	2.4	13.9	13.1	29.4	10 "
33	4.9	38.6	8.8	17.7	8.1	26.8	10 "
34	4.1	30.5	3.0	8.6	13.4	44.5	12 "
35	4.7	39.3	12.7	6.35	11.15	30.5	12 "
36	4.1	37.5	5.8	4.2	12.0	40.0	17 "
37	3.0	51.0	11.4	7.1	10.0	20.5	20 "
38	4.1	67.6	4.66	6.11	6.11	15.42	20 "
39	4.8	37.6	9.1	11.8	12.9	28.6	23 "
40	4.5	31.9	5.45	6.06	12.39	44.2	23 "

FIG. 2.—Changes in total protein, white-cell count, polymorph and mononuclear counts in (a) the right knee treated with prednisolone trimethylacetate, and (b) the left (control) knee given initially an equal volume of lignocaine ("xylocaine").

however, found that these changes were not consistently obtained, and lasted for only a few days. Our figures show a persistent fall in the total white-cell count after treatment with prednisolone trimethylacetate, but analysis of the absolute counts shows a real diminution in all cell types.

Those who comment on the synovial fluid protein find little or no change in the total protein concentration after treatment with hydrocortisone or hydrocortisone acetate (Dixon and Bywaters, 1953; Jessar *et al.*, 1953; Sunblad *et al.*, 1954). Our findings are in marked contrast. Whereas the total protein rose in two joints and remained unchanged in one, there was a consistent fall in the remaining 37 joints of the series. Ziff *et al.* (1953) found no change in either total protein or albumin:globulin ratio. Yielding *et al.* (1956) found that the true albumin could not be estimated in joint fluids by salting-out methods. The electrophoretic technique permits effective separation of the albumin, and, in addition, of the various globulin fractions. We have not seen reports of previous serial studies using this method. Jessar *et al.* (1953), in examining four pairs of joint fluid by the Tiselius method, found no change in the electrophoretic pattern after one injection of hydrocortisone acetate. They found in each case a fast-moving component in front of the albumin band having the same mobility as hyaluronic acid. It is of interest that this band was found in only one of our tracings. We found the albumin content to be increased in 28 of the 40 joints studied.

In keeping with the findings of other authors (Dixon and Bywaters, 1953; Kersley and Desmarais, 1953; Ziff *et al.*, 1953; Duff *et al.*, 1955; Robinson *et al.*,

1955; Yielding *et al.*, 1956) there was an increase in viscosity with treatment in the majority of cases.

On examination of the mucin clot, though the method used was unsatisfactory, we appeared to find a decrease in clot density in response to treatment. However, we found significant protein changes during therapy, and it may be that changes in the mucin clot were partly related to these. We have this aspect under investigation.

Prednisolone trimethylacetate produced greater change in the cell content and pattern of the synovial fluid than has been reported for other steroids. In addition, it is the only compound so far noted to produce significant changes in total synovial protein and electrophoretic pattern. These findings suggest that it has a local action superior to the other steroid compounds used.

Summary

A detailed analysis is made of the serial changes in total and differential cell counts, total protein and electrophoretic pattern, viscosity, and mucin content of the synovial fluid of 40 joints (30 rheumatoid arthritis, 6 osteoarthritis, and 4 hydrarthrosis) treated with prednisolone trimethylacetate, a new compound for intra-articular injection.

The findings suggest a local action superior to that of the other steroid compounds used.

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RAPID DESTRUCTION OF APPARENTLY COMPATIBLE RED CELLS

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As serological tests have improved, it has become progressively easier to avoid the transfusion of incompatible blood. When, owing to weak reactions of the blood-group antibody *in vitro*, incompatibility of a sample of red cells is overlooked, transfusion of the cells is usually followed only by a mild haemolytic reaction.

The case reported here is remarkable in that transfusions of red cells which seemed to be perfectly compatible, judged by tests *in vitro*, were repeatedly followed by severe reactions, with haemoglobinuria.

Case Report

A married woman aged 46 was found in 1955 to have reticulum-cell sarcoma. In 1956 she received deep x-ray therapy. During the course of treatment her haemoglobin concentration dropped from 84% to 44%. She was given several blood transfusions, but the notes state that these were "not tolerated." According to the patient the first transfusion took place in February, 1956; she received 2 pints (1 litre) and developed a slight fever, but no other symptoms. The next blood transfusion was given three or four weeks later, and was followed by shivering and high temperature; the next day she was jaundiced.

Later in 1956 she was found to have enlarged glands in both groins. Further deep x-ray therapy was given. In May, 1957, she received another course of x-ray therapy, and a transfusion of 2 pints (1 litre) of blood. The transfusion produced only a transient increase in haemoglobin concentration. In June she was severely anaemic; the results of a blood examination were as follows: haemoglobin, 4.9 g./100 ml.; red cell count,

1,400,000/c.mm.; P.C.V., 15%; reticulocytes, 10%; W.B.C., 1,700/c.mm. (lymphocytes 17%, neutrophils 70%, monocytes 11%, metamyelocytes 2%); 4 nucleated red cells/100 W.B.C.; platelets 51,000/c.mm. The patient had petechial haemorrhages and was bleeding from the gums.

A transfusion with blood which was compatible by the indirect Coombs test and the albumin technique was given, but had to be abandoned after approximately 350 ml. had been transfused because of fever and haemoglobinuria. Treatment with prednisone, 5 mg. four times a day, was then begun. Five days later (June 7), a transfusion of red cells, labelled with ^{51}Cr and washed three times in saline, was given. After the patient had received about half of this over the course of several hours, she felt ill and the transfusion had to be stopped. The urine passed shortly after the end of this transfusion contained haemoglobin; a blood sample showed that most of the injected cells had already been eliminated. The patient's clinical condition improved slightly, but she still suffered from petechial haemorrhages, and her haemoglobin concentration was only 4.5 g./100 ml., with a white cell count of 3,100/c.mm. and a platelet count of 18,000/c.mm. On June 14 the prednisone was increased to 25 mg. four times a day. On July 5 the haemoglobin concentration was 4.8 g./100 ml., and the patient now improved rapidly. By July 25 the haemoglobin concentration had risen to 9.3 g./100 ml., with a platelet count of 187,000/c.mm. The patient was discharged from hospital on a reduced dosage of prednisone (15 mg. three times a day).

When seen in the out-patient department in September her haemoglobin concentration had risen to 12.2 g./100 ml. and her white cell count to 7,800/c.mm. She died suddenly at home in December, 1957.

Red Cell Survival Tests

As mentioned above, a test with ^{51}Cr -labelled red cells was made on June 7, and this showed clearly that the haemolytic reactions were due to destruction of the donor's red cells rather than those of the recipient. On June 13 an attempt was made to measure more precisely the mode of elimination of the injected red cells. Accordingly, a sample of blood was taken from a compatible donor, and 1 ml. of cells was labelled with radioactive chromium and injected. As a control, a sample of the patient's own red cells was labelled with ^{32}P and injected. From the ^{32}P counts in the patient's blood after the transfusion the number of ^{51}Cr

Survival in the patient's circulation of her own red cells contrasted with the survival of red cells from a donor whose red cells were "compatible," judged by tests *in vitro*. The results were obtained by labelling the cells with ^{51}Cr , and the estimates shown in the figure have been corrected for elution of Cr.