

CLEARANCE OF Fe^{59} -LABELLED ERYTHROCYTES FROM NORMAL AND INFLAMED RABBIT KNEE JOINTS

I. RELATIONSHIP TO THE ANAEMIA OF RHEUMATOID ARTHRITIS

BY

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The anaemia of rheumatoid arthritis has features suggestive of iron deficiency even in the absence of occult blood loss (Jeffrey, 1965). There is some evidence that this may be due to reduced availability of iron from storage sites in the reticulo-endothelial system (Weinstein, 1959; Owen and Lawson, 1966). Extensive deposits of iron are frequently found in the synovial membrane in this disease and these have recently been considered as a further important storage site for iron (Muirden and Senator, 1968; Mowat and Hothersall, 1968). Sequestration of iron in the synovium could contribute to the hypoferraemia and anaemia of this condition.

To test this hypothesis a study was made of the clearance of Fe^{59} labelled to red blood cells from inflamed and normal knee joints of rabbits. The radiation was measured by surface counting and by liquid scintillation of ashed segments of tissue. The eventual distribution of iron in the joint was measured histochemically and by autoradiography and these results will be described in a subsequent paper.

Material and Methods

Carrageenin arthritis was induced in the right knee joints of rabbits by the method of Gardner (1960). Eight to ten injections of 0.8 ml. of a 1 per cent. solution were given over a period of 5 to 6 weeks. This is known to produce a chronic inflammation which will persist for at least 5 weeks without additional stimulus before beginning to subside. The left knee joint was given an equal quantity of normal saline at the same time.

Fe^{59} labelled red cells were prepared by intravenous injections of Fe^{59} citrate into a further rabbit. Approximately 150 $\mu c.$ were required to produce a peripheral blood radioactivity of 1 to 2 $\mu c./ml.$ after 2 to 3 weeks.

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At this stage the donor rabbit was bled and the plasma separated. The erythrocytes were washed with normal saline and then resuspended in additional saline. 1.5 ml. of this suspension was injected into the right arthritic knees and into the left control knees of recipient rabbits in the manner described by Rodnan (1960).

Immediately after the injection of the labelled cells the gamma radiation was measured by means of a Thyatron probe maintained in a fixed position immediately over the patella ligament. Each knee was measured separately by insulating the opposite knee with a lead shield. The rabbits were kept lightly anaesthetized during this procedure. Counts on subsequent days were recorded as the percentage of radioactivity remaining in the joint by comparison with the original radioactivity, corrected for background and natural radioactivity decay.

Rabbits were killed, 1, 3, 7, 10, 20, 36, and 42 days after injection, and the joints dissected and rinsed out with saline. Some of the tissue was kept for histological examination and the remainder was ashed. The samples were weighed and then digested in Pirie's reagent (3 parts nitric acid, 1 part perchloric acid, and added saturated magnesium nitrate). The solution was evaporated to dryness and after cooling, the salts were taken up in 2.0 ml. of 1N HCl. An aliquot (0.1 ml. with 2 ml. scintillation fluid) was counted in a Packard Scintillation Counter using the setting for C^{14} . The tissue examined in this way came from synovium, articular cartilage, bone marrow from the femoral shaft, and from the liver and spleen. The material from the joints was selected from equivalent anatomical areas on the two sides.

Results

The means of clearances of Fe^{59} from left and right knee joints, as measured by the probe, are shown in Table I. The means from days 1 to 10 are also compared in the Figure. There was a slow decline in radioactivity with only one-third disappearing from the left control knee within one week. The clearance from the inflamed right knee was

TABLE I
PERCENTAGE OF ORIGINAL RADIOACTIVITY IN JOINT

Day	Number	Knee				P
		Left		Right		
		Mean	S.E.	Mean	S.E.	
1	9	80.0	2.8	86.8	3.2	<0.1 N.S.
2	9	76.4	4.1	84.2	3.6	<0.1 N.S.
3	9	70.3	3.2	82.1	3.6	<0.05
7	7	60.6	5.6	71.3	3.5	<0.05
10	5	59.0	8.1	71.6	5.1	<0.1 N.S.
20	3	46.0	4.3	56.3	4.7	—
36	1	25.0	—	52.0	—	—
42	1	60.0	—	69.0	—	—

N.S. = not significant

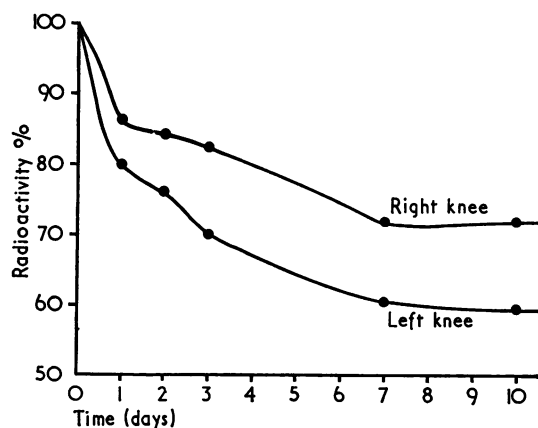


Fig. 1.—Mean clearance of Fe^{59} from right and left knees in the course of 10 days. Right knee injected with Carrageenin.

slower in all but one of nine rabbits. The difference between the two sides was marked at the 3 and 7 day stage and reached statistical significance. The trend continued at 10 days and subsequently, but the numbers were too small to show this statistically.

Radioactive counts measured by liquid scintillation appear in Table II. The results at day one are

the means for four animals and at day 7 for three, but otherwise only single animals were used. Histological studies indicate a patchy uptake of iron by the synovial cells and this could be responsible for the extreme variability of synovial counts. Differences in radioactivity of red cell samples introduced into the joints could provide another reason but the steady rise in radioactivity in bone marrow, liver and spleen suggests that this may be a minor factor. The same trend for synovial membrane counts to be higher in the right inflamed knee joint appears again, but possible tissue sampling errors tend to invalidate this comparison.

Discussion

The measurement of radioactive clearance from joints by surface counting poses certain problems of interpretation. It cannot be assumed that this technique measures radiation coming only from the synovial membrane. The probe detects counts from a wide area over the leg including the suprapatella pouch and related femur and the upper portion of the calf. In the rabbit the tendon of the extensor digitorum muscle enters the knee joint cavity and a prolongation of synovium extends along the tendon into the calf. When the joints were eventually dissected it was apparent that blood had seeped into the calf along the course of this tendon. The purpose of the experiment was to compare the inflamed and control joints, and there was no sign that inflammation in the right knee influenced this process. The other anatomical problem is that erythropoietic marrow extends into the lower third of the femoral shaft in the rabbit. Uptake of isotope by the marrow would be recorded by the surface probe and could be a factor responsible for high counts particularly after the first few days. The value of the liquid scintillation counts (Table II) was in showing that radiation from the femoral marrow was small in comparison with the synovial membrane.

TABLE II
LIQUID SCINTILLATION COUNTS*

Days after Injection of Fe^{59}		1	3	7	10	42
Synovial membrane	Right	11,875	8,285	12,250	47,193	11,320
	Left	4,920	5,811	11,915	33,333	11,720
Articular cartilage	Right	3,461	—	4,333	—	—
	Left	3,533	—	4,000	—	—
Femoral bone marrow		124	760	1,366	1,553	2,061
Liver		723	342	2,275	3,715	—
Spleen		607	—	3,615	2,137	10,666

*Recorded as total counts per 5 minutes per 1 g. wet weight of tissue

Even 42 days after injection of labelled cells, marrow counts were still less than a quarter of the radiation from the synovial membrane on a weight for weight basis. It is concluded, therefore, that the surface counts recorded came mainly from the joint tissue even 6 weeks after injection of the labelled cells.

Another possible source of error is the increased vascularity of the carrageenin joint. If the Fe^{59} had been incorporated into the haemoglobin of the recipient's red cells and these had then traversed the joint, an increase in radioactivity over the less vascular control joint could be expected. However, the vascularity of the carrageenin synovium is considerably less than the bone marrow, which provides only 12 per cent. or less of the radioactivity of the synovium over the critical 3 to 10 day stage (Table II).

Rodnan (1960) used a similar technique to study the clearance of Fe^{59} and Cr^{51} labelled red cells from rabbit knee joints. He found a more rapid clearance, in that two-thirds of the radioactivity disappeared within one week, compared with one-third in this study. The reason for this difference is not readily apparent. Rodnan also noted a slower decay from joints immobilized in plaster. The purpose of our study was to compare the inflamed joint with a control, and carrageenin, although producing a swollen joint, does not cause any loss of function. The effect of restricted activity on the human knee joint has also been studied (Nakamura, Asai, Sonozaki, and Nagano, 1967). The clearance of phenolsulphonphthalein increased in normal subjects with rest.

Other isotopes have been used to study clearance rates from rabbit and human joints. The half-life of heavy water in human knees is approximately 30 minutes (Scholer, Lee, and Polley, 1959) and that of radioactive sodium 40 minutes (Jacox, Johnson, and Koontz, 1952). Both I^{131} labelled albumin and I^{131} gamma globulin have half-lives of about 30 hours (Rodnan and McLachlan, 1960). The turnover of I^{131} albumin in rheumatoid joints was observed by Ahlström, Gedda, and Hedberg (1956) to be more rapid, with a half-life of 8 to 12 hours. Clearance of radiosodium and xenon have been compared in rheumatoid and normal subjects (Harris, Millard, and Banerjee, 1958; St. Onge, Dick, Bell, and Boyle, 1968). The isotopes disappeared more rapidly from actively inflamed joints, the local state of the joint being more important than the general level of disease activity. This difference between active and inactive rheumatoid joints has also been shown with I^{131} albumin (Ahlström and others, 1956) and with PSP (Nakamura and others,

1967), and it was concluded that this was due to changes in bloody supply. None of these studies have shown a slower clearance from arthritic joints compared with controls. There is no inherent contradiction, however, between these observations and the delayed clearance of labelled red cells from the inflamed joints. The escape of molecules up to the size of gamma globulin from joints is unlikely to involve uptake by synovial cells, whereas Fe^{59} is incorporated in the haemoglobin molecule within red cells which are known to be taken up by synovial macrophages (Key, 1929).

Carrageenin arthritis has a very similar histological picture to the synovitis of rheumatoid arthritis (Gardner, 1960; Muirden and Peace, 1969). Synovial cell proliferation, inflammatory cell infiltration, prominence of blood vessels, erosive bone, and cartilage damage are common to both. Findings in the subsequent paper indicate that the injected red cells are broken down within the synovial cavity and membrane and extensive deposits of haemosiderin then appear in synovial macrophages. The similar distribution of haemosiderin in rheumatoid arthritis and the probability that this iron arises in part at least from repeated small extravasations of red cells over long periods (Muirden, 1966) suggests that the slow turnover of Fe^{59} from carrageenin arthritis is applicable to rheumatoid arthritis.

A fault in the release of iron from storage sites and in particular the synovium has been discussed as an important contributing factor to the hypoferraemia and anaemia of rheumatoid arthritis (Muirden and Senator, 1968; Mowat and Hothersall, 1968). The delay in the release of Fe^{59} injected in the form of labelled red cells from the inflamed rabbit joint supports this suggestion.

Summary

The clearance of Fe^{59} labelled erythrocytes injected into the knee joints of rabbits has been studied by surface counting and by liquid scintillation of ashed segments of tissue. Only one-third of the radioactivity disappeared from the control joints within a week and there was a significantly slower decay from joints inflamed by previous treatment with carrageenin.

A fault in the release of iron from the extensive synovial deposits has been considered as an important contributing factor to the hypoferraemia and anaemia of rheumatoid arthritis. The delay in the clearance of Fe^{59} from the inflamed knee in this experimental model supports this suggestion.

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La libération du Fer-59 des érythrocytes des articulations du genou normal ou enflammé du lapin. I. Sa relation à l'anémie de l'arthrite rhumatoïde

RÉSUMÉ

La libération du Fer-59 des érythrocytes injectés dans les articulations du genou du lapin a été étudiée par la numération à la surface et par la scintillation liquide des segments de tissu réduits en cendres. Seulement un tiers de la radio-activité avait disparu des articulations témoins avant la fin de la semaine et il y avait une dégénérescence plus lente dans les articulations enflammées par une injection antérieure de carrageen.

Une erreur dans la libération du fer des dépôts considérables de la synoviale a été considérée comme un important facteur contribuant à l'hypoferrémie et à l'anémie de l'arthrite rhumatoïde. Le retard dans la libération du Fer-59 du genou enflammé dans ce modèle expérimental corrobore cette suggestion.

Eliminación de eritrocitos identificados con hierro 59, de articulaciones de la rodilla de conejo, normales e inflamadas. I. Relación con la anemia de poliartritis reumatoide

SUMARIO

La eliminación de eritrocitos identificados con Fe^{59} inyectados en las articulaciones de la rodilla de conejos, ha sido estudiada mediante cuenta de superficie y por escintilación líquida de cenizas de segmentos de tejido. Solamente un tercio de la radioactividad desapareció de las articulaciones testigo en el término de una semana, y hubo una declinación significativamente más lenta de articulaciones inflamadas por tratamiento previo con carrageenina (hongo de Irlanda).

Una falla en la liberación de hierro de los vastos depósitos sinoviales ha sido considerada como un importante factor contribuyente a la hipoferrémia y la anemia de la poliartritis reumatoide. La demora en la eliminación de Fe^{59} de la rodilla inflamada en este modelo experimental apoya esta indicación.