INFLUENCE OF LYMPHATIC TISSUE ON THE DEVELOPMENT OF ADJUVANT-INDUCED POLYARTHRITIS IN RATS

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The experimental model of rheumatoid arthritis has not yet been established. Currently, two acceptable models of rheumatoid disease are being studied (Pearson, 1964), *i.e.* adjuvant-induced polyarthritis in rats and homologous disease (Ryżewska and Ryżewski, 1965; Ryżewski and Ryżewska, 1965).

Polyarthritis appearing in rats after a subcutaneous injection of spleen cell suspension or homogenate with Freund's adjuvant was first described by Stoerk, Bieliński, and Budzilovich (1954). These results were confirmed by Pearson (1956), who used for the immunization skeletal muscles with Freund's adjuvant or adjuvant alone. The development of adjuvant-induced polyarthritis in rats and the course of the disease have since been described in greater detail (Waksman, Pearson, and Sharp, 1960; Ward and Jones, 1962b; Pearson, 1963). It was found that a single intradermal injection of Freund's adjuvant (killed tubercle—either saprophytic bacilli or chemical fractions of it, mineral oil, and an emulsifying agent) was sufficient to produce polyarthritis. The paw is the most effective site of injection. A few hours after adjuvant administration a local inflammatory reaction develops at the injection site and persists during the whole latent period of the disease. The onset of the disease usually occurs on the 10th to the 16th day or later, being characterized by polyarthritis which often affects many of the peripheral joints but also some of the joints in the tail and at the base of the spine. The arthritis may be transitory or may become chronic.

Histologically, there is an acute and subacute synovitis with proliferation of synovial lining cells, mononuclear infiltration of the articular cartilage

and para-articular tissues, invasion of bone and articular cartilage by connective tissue pannus, and periosteal new bone formation. These destructive changes often cause fibrous or bony ankylosis (Pearson and Wood, 1962).

Adjuvant polyarthritis is accompanied by inflammatory lesions of other tissues, including acute and chronic dermatitis, inflammation of serous membranes manifested by diarrhoea and urethritis, and inflammatory changes in the eye giving rise to conjunctivitis, keratitis, and iritis. In the lymph nodes draining the adjuvant injection site, in the lungs, and occasionally in the liver, the typical "post-adjuvant miliary granulomata" can easily be shown. They consist of an oil drop in the middle with histiocytes and lymphocytes at the circumference (Pearson, Waksman, and Sharp, 1961). Jones and Ward (1963) also observed purulent infiltrations in the spleen capsule, pleura, bronchi, and heart muscles.

The pathogenesis of adjuvant-induced polyarthritis in rats has not so far been established. Three main mechanisms may be involved:

(1) A delayed hypersensitivity response resulting from the tubercle bacilli introduced and disseminated in the organism (Waksman and others, 1960; Ward and Jones, 1962a; Kalliomäki, Saarimaa, and Toivanen, 1964; Newbould, 1964b);

(2) An autoimmunization resulting from the development of autoantibodies and/or specifically sensitized lymphocytes acting against their own tissues (Pearson, 1964; Ryżewska, 1967);

(3) An infectious agent, of the nature of a latent virus activated by a hypersensitivity reaction of the organism (Jones and Ward, 1963).

Considering the role that the lymphocytes may play in the development of adjuvant-induced polyarthritis in rats, it seemed appropriate to investigate the influence of separate parts of the lymphatic system on the course of this disease.

Methods

The experiments were carried out on randomly-bred rats of Wistar strain 5 to 6 weeks old. Altogether 175 animals of both sexes were used, being divided into experimental and control groups each containing five to eight rats. In all of them polyarthritis was induced by means of Freund's adjuvant by the method of Pearson (1956) and Ward and Jones (1962b). A dose of 0.1 ml. liquid paraffin containing 3 mg. heat-killed *Mycobacterium phlei* was injected intradermally into the hind foot pad. In the experimental groups the following were removed:

- (1) The popliteal and inguinal lymph nodes draining the injection site, which can be the most active producers of immunologically competent cells and antibodies;
- (2) The spleen, which can be one of the principal organs of immunological response;
- (3) The thymus, which influences the maturation of immunologically competent cells and can act indirectly in the production of antibody.

Other experimental animals were submitted to laparotomy or had the contralateral lymph nodes removed to study the influence or surgical operation.

All these procedures were carried out in different groups 5 days before and 5 days after the injection of Freund's adjuvant. Additionally, the removal of the draining or contralateral lymph nodes was performed 24 hours after the injection of adjuvant. All surgical procedures were performed under ether anaesthesia with strict asepsis.

The control animals, which were not submitted to any surgical procedures, received only adjuvant. Preliminary observations of the course of the polyarthritis were made on 36 rats. Subsequently four groups with five to eight rats in each were used as controls.

Both the experimental and control groups were examined every day. The number of joints with arthritic lesions was scored by means of one to four points for each limb separately. One point indicated inflammation of one joint, four points meant that all the small joints in the given limb were involved. The statistical evaluation of the results was carried out by means of the "t" test.

The experimental and control animals were kept under observation for 6 weeks, after which they were killed by ether anaesthesia and submitted to autopsy.

Results

A. Course of Adjuvant-induced Polyarthritis in Controls

In all control animals a local inflammatory reaction developed 2 hours after intradermal

injection of Freund's adjuvant and persisted until the onset of polyarthritis on the 8th to 27th day. The onset was characterized by erythema and oedema of peripheral joints in the adjuvant-injected limb as well as in the remote limbs and occasionally in the tail. In the skin above these joints an excessive desquamation or disruption of the epidermis with serous exudate becoming purulent was observed. After about 10 days these symptoms regressed slowly, and after 3 months thickened ankles and ankylosis were noted. At this time fresh acute arthritic lesions limited to a few joints were occasionally observed.

It was found that the arthritic inflammatory lesions occurred in the remote limbs in 27 of the 36 control rats—in one remote limb in fifteen, in two limbs in six, and in three limbs in six.

Similar results were found in the 25 control animals in the subsequent experiments (Table I), arthritic inflammatory lesions occurring in the remote limbs in 22 of them (in one remote limb in thirteen, in two remote limbs in six, and in three remote limbs in three).

TABLE I
DEGREE OF GENERALIZED ARTHRITIS IN CONTROL RATS AFTER ADJUVANT INJECTION

Control Groups*	Number of Animals		Number of Remote Limbs with Arthritis
	Total	With Generalized Arthritis	
I	7	7	11
II	8	7	11
III	5	4	6
IV	5	4	6
Total	25	22	34

*Groups I and II were controls to the experiments carried out on animals operated 5 days before injection;
Group III was control to the experiments carried out on animals operated 24 hours after injection of adjuvant;
Group IV was control to the experiments carried out on animals operated 5 days after adjuvant injection.

B. Effect of Removal of Ipsilateral Draining Lymph Nodes

In all the experimental animals in which the popliteal and inguinal draining lymph nodes were removed 5 days before and 24 hours and 5 days after adjuvant injection, a distinct depression of local inflammatory reaction was observed, and the severity of the arthritic lesions was diminished. Arthritis occurred in the remote limbs only occasionally.

Removal 5 days before adjuvant injection (Table II).—Arthritic lesions appeared in only three of eight rats, and only one remote limb was affected. The inflammation was limited to a single peripheral joint.

TABLE II
DEGREE OF GENERALIZED ARTHRITIS IN 8 RATS WITH
NODES REMOVED 5 DAYS BEFORE INJECTION OF 0.1 ml.
ADJUVANT (E) AND IN 7 RATS WITHOUT OPERATION (C)

Group	Number of Animals		Number of Remote Limbs with Arthritis
	Total	With Arthritis	
(E) Draining Lymph Nodes Removed	8	3	3
(C) No Operation	7	7	11

In all seven control animals injected at the same time, one or more (in one case all) of the peripheral joints became inflamed and polyarthritis occurred in one, two, or three limbs.

These results are shown in Fig. 1 (opposite).

The statistical analysis of the number of joints with arthritic lesions observed each day showed a significant difference on most days (Table III), the greatest difference ($P < 0.01$) being observed on the 22nd day.

Removal 24 hours after adjuvant injection (Table IV).—A slight arthritic lesion appeared in the ankle of the contralateral limb in only one of seven experimental animals. A mild polyarthritis appeared in four of the five controls. Three had

TABLE IV
DEGREE OF GENERALIZED ARTHRITIS IN 7 RATS WITH
NODES REMOVED 24 HOURS AFTER INJECTION OF 0.1 ml.
ADJUVANT (E) AND IN 5 RATS WITHOUT OPERATION (C)

Group	Number of Animals		Number of Remote Limbs with Arthritis
	Total	With Arthritis	
(E) Draining Lymph Nodes Removed	7	1	1
(C) No Operation	5	4	6

inflammation in one remote limb and one in three remote limbs.

These results are shown in Fig. 2 (opposite). The differences were statistically significant (Table V, opposite).

Removal 5 days after adjuvant injection (Table VI, opposite).—A slight arthritic lesion was seen in the contralateral limb in only one of seven experimental animals.

Inflammatory lesions appeared in a few peripheral joints in the remote limbs in the majority of the controls.

The results are shown in Fig. 3 (opposite).

The differences between the experimental and control animals were statistically significant in the most active period of the disease (Table VII, opposite).

C. Effect of Removal of Contralateral Lymph Nodes

The removal of popliteal and inguinal lymph nodes contralateral to the site of injection 5 days

TABLE III
STATISTICAL ANALYSIS OF RESULTS IN TABLE II

Day of Observation	Arithmetical Mean of Points scoring Number of Arthritic Joints and Standard Deviation		"t"/n-2	P
5	E C	2.25 ± 0.46 3.07 ± 0.61	2.960/13	0.02 > P > 0.01
10	E C	3.00 ± 0.93 4.00 ± 0.00	2.850/13	0.02 > P > 0.01
15	E C	4.06 ± 1.01 4.36 ± 0.31	0.749/13	P > 0.1
19	E C	3.94 ± 0.68 4.86 ± 0.90	2.254/13	0.05 > P > 0.02
22	E C	3.75 ± 0.71 4.79 ± 0.63	3.060/13	P > 0.01
24	E C	3.62 ± 0.76 4.14 ± 0.83	1.665/13	P > 0.1
28	E C	3.81 ± 0.58 4.43 ± 0.46	2.302/13	0.05 > P > 0.02
31	E C	3.63 ± 0.51 4.14 ± 0.38	2.167/13	0.05 > P > 0.02

Fig. 1.—Effect of removing draining lymph nodes 5 days before the injection of adjuvant on the course of polyarthritis in rats.

Fig. 2.—Effect of removing draining lymph nodes 24 hours after the injection of adjuvant on the course of polyarthritis in rats.

Fig. 3.—Effect of removing draining lymph nodes 5 days after the injection of adjuvant on the course of polyarthritis in rats.

The curves were determined by the aid of arithmetical means derived from the number of joints with arthritic lesions in the experimental and control groups on each day of observation. In the latent period the curves show the degree of local inflammatory reaction in the region of injection.

TABLE V
STATISTICAL ANALYSIS OF RESULTS IN TABLE IV

Day of Observation	Arithmetical Mean of Points scoring Number of Arthritic Joints and Standard Deviation		"t"/n-2	P
4	E	2.00 ± 0.00	3.412/10	P < 0.01
	C	4.00 ± 0.00		
11	E	2.00 ± 1.00	5.200/10	P < 0.01
	C	4.60 ± 0.55		
15	E	3.64 ± 0.85	2.866/10	0.02 > P > 0.01
	C	5.10 ± 0.89		
19	E	3.71 ± 0.49	3.024/10	0.02 > P > 0.01
	C	6.20 ± 2.13		
24	E	3.57 ± 0.79	4.777/10	P < 0.01
	C	6.30 ± 1.20		
30	E	3.57 ± 0.79	3.122/10	0.02 > P > 0.01
	C	5.40 ± 1.34		

TABLE VI
DEGREE OF GENERALIZED ARTHRITIS IN 7 RATS WITH NODES REMOVED 5 DAYS AFTER INJECTION OF 0.1 ml. ADJUVANT (E) AND IN 5 RATS WITHOUT OPERATION (C)

Group	Number of Animals		Number of Remote Limbs with Arthritis
	Total	With Arthritis	
(E) Draining Lymph Nodes Removed	7	1	1
(C) No Operation	5	4	6

before and 24 hours and 5 days after adjuvant injection had no distinct influence on the course of

the polyarthritis. The generalized arthritis appeared as a rule in remote limbs in the experimental animals as well as in the control animals (Table VIII, overleaf).

Differences were seen only in the latent period of the disease in those in which the contralateral nodes were removed 24 hours after injection. In the experimental rats there was a marked depression of the local inflammatory reaction and the arthritis appeared 8 days later than usual. These differences in the degree of erythema and swelling were statistically significant (Table IX, overleaf).

TABLE VII
STATISTICAL ANALYSIS OF RESULTS IN TABLE VI

Day of Observation	Arithmetical Mean of Points scoring Number of Arthritic Joints and Standard Deviation		"t"/n-2	P
7	E C	3·86±0·38 4·20±0·45	1·420/10	P>0·1
10	E C	3·14±0·69 4·60±0·55	3·910/10	P<0·01
15	E C	3·43±0·82 5·10±0·89	3·424/10	P<0·01
19	E C	3·64±0·86 6·20±2·13	2·896/10	0·02>P>0·01
24	E C	3·76±0·91 6·30±1·21	4·333/10	P<0·01
30	E C	4·00±1·00 5·40±1·34	2·079/10	0·1>P>0·05

TABLE VIII
DEGREE OF GENERALIZED ARTHRITIS IN RATS WITH CONTRALATERAL LYMPH NODES REMOVED 5 DAYS BEFORE AND 24 HOURS AND 5 DAYS AFTER INJECTION OF 0·1ml. ADJUVANT

Time of Operation	Number of Animals		Number of Remote Limbs with Arthritis
	Total	With generalized Arthritis	
5 days before injection ..	5	5	7
24 hours after injection ..	7	7	7
5 days after injection ..	6	4	4

TABLE IX
STATISTICAL ANALYSIS OF RESULTS IN TABLE VIII

Day of Observation	Arithmetical Mean of Points scoring Number of Arthritic Joints and Standard Deviation		"t"/n-2	P
4	E C	2·29±0·49 4·00±0·00	7·540/10	P<0·01
11	E C	2·13±1·46 4·60±0·55	3·556/10	P<0·01
15	E C	3·57±0·98 5·10±0·89	2·777/10	0·02>P>0·01
17	E C	3·86±0·69 5·50±0·94	3·514/10	P<0·01
19	E C	4·43±0·93 5·70±2·17	1·398/10	P>0·1
25	E C	4·86±0·69 6·00±1·94	1·456/10	P>0·1
29	E C	5·57±0·98 5·20±1·30	0·560/10	P>0·1

D. Effect of Splenectomy

Five days before injection.—A generalized arthritis including from one to three remote limbs occurred in all eight experimental animals, and also in all the controls (Table X). The difference between the groups was not statistically significant.

TABLE X

DEGREE OF GENERALIZED ARTHRITIS IN 8 RATS WITH SPLENECTOMY PERFORMED 5 DAYS BEFORE INJECTION OF 0.1 ml. ADJUVANT AND IN 7 RATS WITHOUT OPERATION

Group	Number of Animals		Number of Remote Limbs with Arthritis
	Total	With Arthritis	
Splenectomy	8	8	14
No Operation	7	7	11

Five days after injection.—Similar results were obtained: the majority of both experimental and control animals showed a generalized arthritis (Table XI) and the difference between the groups was not statistically significant.

TABLE XI

DEGREE OF GENERALIZED ARTHRITIS IN 6 RATS WITH SPLENECTOMY PERFORMED 5 DAYS AFTER INJECTION OF 0.1 ml. ADJUVANT AND IN 5 RATS WITHOUT OPERATION

Group	Number of Animals		Number of Remote Limbs with Arthritis
	Total	With Arthritis	
Splenectomy	6	4	5
No Operation	5	4	6

E. Effect of Laparotomy

Five days before injection.—A generalized arthritis occurred in all eight experimental animals, and one remote limb in two rats and three remote limbs in six rats became inflamed. Of the eight control animals only seven were affected, five having polyarthritis in one remote limb and two in two remote limbs (Table XII).

TABLE XII

DEGREE OF GENERALIZED ARTHRITIS IN 8 RATS WITH LAPAROTOMY PERFORMED 5 DAYS BEFORE INJECTION OF 0.1 ml. ADJUVANT (E) AND IN 8 RATS WITHOUT OPERATION (C)

Group	Number of Animals		Number of Remote Limbs with Arthritis
	Total	With Arthritis	
Laparotomy (E)	8	8	20
No Operation (C)	8	7	11

Statistical analysis showed that the exacerbation of polyarthritis in the experimental rats was statistically significant on the 19th and 22nd days (Table XIII).

Five days after injection.—This had a distinct influence on the course of the disease. Generalized arthritis was observed in only three of the eight experimental animals (Table XIV) and was less severe than in the controls.

TABLE XIV

DEGREE OF GENERALIZED ARTHRITIS IN 8 RATS WITH LAPAROTOMY PERFORMED 5 DAYS AFTER INJECTION OF 0.1 ml. ADJUVANT (E) AND IN 5 RATS WITHOUT OPERATION (C)

Group	Number of Animals		Number of Remote Limbs with Arthritis
	Total	With Arthritis	
Laparotomy (E)	8	3	3
No Operation (C)	5	4	6

The differences between the experimental and control animals were statistically significant from the 19th day (Table XV, overleaf).

F. Effect of Thymectomy

Five days before injection.—Generalized arthritis including remote limbs occurred in six of eight experimental animals (Table XVI, overleaf) and the results in the controls were similar. The differences were not statistically significant.

TABLE XIII
STATISTICAL ANALYSIS OF RESULTS IN TABLE XII

Day of Observation	Arithmetical Mean of Points scoring Number of Arthritic Joints and Standard Deviation		"t"/n-2	P
5, 10, 16	Differences not significant			P>0.1
19	E C	6.31±2.22 4.44±0.68	2.280/14	0.05>P>0.02
22	E C	7.12±2.90 4.25±0.76	2.813/14	0.02>P>0.01
24, 28, 32	Differences not significant			P>0.1

TABLE XV
STATISTICAL ANALYSIS OF RESULTS IN TABLE XIV

Day of Observation	Arithmetical Mean of Points scoring Number of Arthritic Joints and Standard Deviation	"t"/n-2	P
5	E C 4·00±0·00 4·20±0·45	1·321/11	P>0·1
10	E C 3·88±0·64 4·60±0·55	2·112/11	0·1>P>0·05
15	E C 4·88±0·84 5·10±0·89	0·444/11	P>0·1
19	E C 4·00±0·60 6·20±2·13	2·850/11	0·02>P>0·01
21	E C 3·88±0·44 6·00±1·77	3·781/11	P<0·01
24	E C 3·79±0·47 6·30±1·20	5·472/11	P<0·01
30	E C 3·63±0·48 5·40±1·34	3·156/11	P<0·01
40	E C 3·25±0·71 4·60±0·65	2·407/11	0·05>P>0·02

TABLE XVI
DEGREE OF GENERALIZED ARTHRITIS IN RATS WITH THYMECTOMY PERFORMED 5 DAYS BEFORE AND AFTER INJECTION OF 0·1 ml. ADJUVANT AND IN RATS WITHOUT OPERATION

Time of Operation	Group	Number of Animals		Number of Remote Limbs with Arthritis
		Total	With Arthritis	
5 days before Injection	With Thymectomy	8	6	11
	No Operation	8	7	11
5 days after Injection	With Thymectomy	8	6	11
	No Operation	5	4	6

Five days after injection.—The disease manifestations in the experimental animals were gradually exacerbated in comparison with the controls, but the differences were not statistically significant.

Discussion

The results of removing various parts of the lymphatic system on the development of polyarthritis induced by Freund's adjuvant in rats indicate that removal of popliteal and inguinal lymph nodes draining the site of injection lessens the incidence and severity of polyarthritis. Splenectomy and thymectomy appear to have no effect.

Lymph Nodes.—These results may be due either to the stimulation of the polyarthritis by lymphocytes originating from the lymph nodes, or to the mechanical occlusion of the lymph vessels preventing the spread of adjuvant.

The latter seems unlikely for three reasons:

(1) There were no symptoms of lymph stasis in the operated limb when the lymph nodes were removed 5

days before the administration of Freund's adjuvant;

(2) "Miliary granulomata"—which probably indicate the presence of adjuvant (Pearson and Wood, 1962; Waksman and others, 1960)—were found in the lungs of all animals, regardless of the time of surgical treatment;

(3) A positive result was obtained when the nodes were excised 5 days after administration of adjuvant; days are long enough for the free spread of Freund's adjuvant and according to Jones and Ward (1962), experimenting with tubercle bacilli labelled with radioisotope, Freund's adjuvant spreads in the organism within a few hours of administration.

The former theory, however, accords with the results of Waksman and others (1960), who reported that adjuvant-induced polyarthritis could be prevented or ameliorated by previous irradiation of the whole body by x rays and by a previous or simultaneous administration of corticosteroids. Ward, Cloud, Krawitt, and Jones (1964) found that the administration of methotrexate or 6-mercaptopurine as well as irradiation by x rays lessened the

incidence of polyarthritis in rats previously injected with adjuvant. Newbould (1964a) also reported that polyarthritis did not develop in rats if the more distant draining nodes besides the popliteal and inguinal nodes were removed within 5 days of injection of adjuvant.

The role of humoral autoantibody production in the pathogenesis of the tissue lesions should also be considered. Anti-connective tissue antibodies in the serum of affected animals and passive transfer of the disease by the serum has not yet been proved (Waksman and others, 1960), but the titre of the autoantibodies may have been too low in the experiments so far conducted.

Splenectomy diminishes the organism's power to produce antibodies. Taliaferro and Taliaferro (1951a, b) found that splenectomy performed in rabbits 0-4 days after the one injection of sheep erythrocytes caused an 80 per cent. decrease in antibodies, whereas if performed after 6 days it had no effect. This seems to show that the antibodies are produced in the spleen soon after immunization and later in other tissues. Rowley (1950) stated that splenectomy in rats caused a decrease in antibody production after intravenous injection of a small quantity of antigen but not after subcutaneous or intraperitoneal injection. A similar diminished capacity of precipitin production after splenectomy was observed in hens by Wolfe, Norton, Springer, Goodman, and Herrick (1950). Selectively shielding the spleen during x-irradiation prevented the immunological reaction of the organism after the intravenous administration of antigen (Jacobson, Marks, Gaston, Robson, and Zirkle, 1949; Jacobson, Robson, and Marks, 1950; Wissler, Robson, Fitch, Nelson, and Jacobson, 1953).

In the present experiments splenectomy performed 5 days before and after the injection of adjuvant had no effect on adjuvant-induced polyarthritis. This was probably due to a lack of humoral autoantibodies acting against connective tissue antigens.

Laparotomy performed 5 days before the injection of adjuvant caused a marked exacerbation of the disease but when it was performed 5 days after injection the symptoms were milder. Perhaps the surgical procedure reacts on the autonomic nervous and hormonal systems which take part in the immunological process in rats (Ryżewski, 1966; Ryżewski and Kossakowska, 1967).

Thymectomy.—The role of the thymus in the antibody production is not yet explained. Bjørneboe, Gormsen, and Lundquist (1947) and

Harris, Rhoads, and Stokes (1947) found an almost complete lack of antibodies in the thymus after the immunization of experimental animals, and Fichtelius, Laurell, and Philipsson (1961) observed that thymectomy caused a distinct decrease in serum antibody titre. Fichtelius (1960) proposed that in the process of antibody formation the lymphocytes migrated from the thymus into the spleen where they were transformed into plasma cells. This hypothesis has been partially confirmed by Diderholm (1961), who injected thymocytes labelled with H^3 -thymidine or S^{35} -methionine into the autologous guinea-pig and found by microautoradiography that the cells were mainly localized in spleen and liver. However he did not observe any transformation of thymocytes into plasma cells.

On the other hand the role of the thymus in the maturation of immunologically competent cells should be noted. Thymectomy performed in newborn mice inhibits the development of lymphatic tissue: the lymph nodes and spleen are smaller than in normal animals and show histologically a lymphoid involution with a lack of germinal centres and a deficiency of plasma cells (Miller, 1961). In thymectomized animals the immunological response against skin or neoplasm homografts does not develop as well as the humoral antibody against exogenous antigens (Miller, 1964). Thymus grafts transplanted into animals thymectomized at birth, however, restore immunological competence (Miller and Davies, 1964).

The thymus may also take part in the maturation of the immunological apparatus in later life. Good, Dalmaso, Martinez, Archer, Pierce, and Papermaster (1962) found that thymectomy performed in mice as much as 30 to 40 days old checked or diminished the development of immunological competence.

It has recently been found in our laboratory (Ryżewska and Dąbrowski, 1967) that thymectomy performed in newborn rats prevented them from developing adjuvant polyarthritis in adult life. In the present series of experiments, thymectomy in rats 5 weeks old had no effect on adjuvant-induced polyarthritis; this is probably because the lymphocytes had already acquired an immunological competence.

The present study emphasizes the role of the lymphocytes of the draining lymph nodes and indicates that they undoubtedly play a role in the pathogenesis of adjuvant-induced polyarthritis. Numerous authors (Waksman and others, 1960; Ward and Jones, 1962b; Kalliomäki and others, 1964; Newbould, 1964b) consider that the disease

process is a delayed hypersensitivity response to a disseminated exogenous antigen probably derived from the injected tubercle bacilli. Pearson (1964), however, obtained a passive transfer of polyarthritis to healthy rats by lymphocytes derived from the draining lymph nodes or from the spleens of affected animals. The evidence that polyarthritis may be an auto-immune disease has been confirmed in our laboratory (Ryżewska, 1967). Lymphoid cells derived from lymph nodes draining the arthritic lesions in rats with adjuvant-induced polyarthritis provoked a characteristic delayed hypersensitivity skin reaction when injected intradermally into autologous normal rats. It thus seems very probable that a delayed hypersensitivity reaction acts in parallel with the development of immunologically competent cells acting against their own connective tissue antigens in the pathogenesis of adjuvant-induced polyarthritis.

Summary

The influence was investigated of separate parts of the lymphatic system on the course of adjuvant-

induced polyarthritis. Experimental polyarthritis was induced in randomly-bred rats of Wistar strain 5 to 6 weeks old by intradermal injection of Freund's adjuvant. The degree of inflammation of joints in every limb was noted daily. From a statistical analysis of the results, the following conclusions were drawn:

(1) Removal of popliteal and inguinal lymph nodes draining the site of injection diminished the incidence and severity of polyarthritis in comparison with controls which were injected with adjuvant only or which received adjuvant and also had their contralateral lymph nodes removed. This diminishing effect occurred when the nodes were removed 5 days before and 24 hours or 5 days after the injection.

(2) Splenectomy and thymectomy had no significant effect.

(3) Laparotomy performed 5 days before the administration of adjuvant increased the severity of the disease, but when it was performed 5 days after injection the disease ran a milder course.

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L'influence du tissu lymphatique sur le développement de la polyarthrite provoquée par l'adjuvant chez le rat**RÉSUMÉ**

On étudia l'influence de différentes parties du système lymphatique sur l'évolution de la polyarthrite provoquée par l'adjuvant. On produisit une polyarthrite expérimentale chez des rats de la souche Wistar, élevés au hasard et âgés de 5 à 6 semaines, par injection intradermique de l'adjuvant de Freund. On nota tous les jours l'intensité de l'inflammation articulaire dans toutes les pattes. L'analyse statistique des résultats permet de tirer les conclusions suivantes :

(1) L'ablation des ganglions lymphatiques poplités et inguinaux du côté de l'injection diminuait la fréquence et la sévérité de la polyarthrite, en comparaison de témoins qui ne recevaient que des injections d'adjuvant ou qui recevaient l'adjuvant et à qui enlevait les ganglions du côté opposé. Cet effet réducteur d'inflammation survenait lorsqu'on enlevait les ganglions 5 jour avant et 24 heures ou 5 jours après l'injection.

(2) La splenectomie et la thymectomie n'exercèrent pas d'effet significatif.

(3) Une laparotomie effectuée 5 jours avant l'administration de l'adjuvant augmentait la sévérité de la maladie mais lorsqu'on l'effectuait 5 jours après l'injection, l'évolution de la maladie était plus bénigne.

La influencia de tejido linfático sobre el desarrollo de la poliartritis inducida por un adyuvante en la rata**SUMARIO**

Se estudió la influencia de diferentes partes del sistema linfático sobre el desarrollo de la poliartritis producida por un adyuvante. Una poliartritis experimental fué inducida por inyección intradérmica del adyuvante de Freund en ratas de la cepa Wistar, criadas sin selección, de 5 a 6 semanas de edad. Se tomó nota diaria de la intensidad de la inflamación articular en las cuatro extremidades. El análisis estadístico de los resultados nos lleva a las conclusiones siguientes :

(1) La extirpación de los ganglios linfáticos poplíteos e inguinales que sirven el sitio de la inyección hizo bajar la incidencia y la severidad de la poliartritis, en comparación con ratas que sólo recibieron inyecciones de adyuvante o con las que recibieron inyecciones de adyuvante y fueron sometidas a la extirpación de los ganglios linfáticos del lado opuesto. Este efecto reductor de inflamación se producía con la ablación de los ganglios 5 días antes y 24 horas o 5 días después de la inyección.

(2) La esplenectomía y la timectomía no tuvieron efecto significativo.

(3) Una laparotomía realizada 5 días antes de la administración del adyuvante aumentaba la severidad de la enfermedad pero la misma operación realizada 5 días después de la inyección era seguida de una evolución morbosa más benigna.