

SOME HISTOLOGICAL ASPECTS OF FORMALIN "ARTHRITIS" IN RATS.

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SELYE (1949) described a method of inducing experimental "arthritis" in rats by the injection of formaldehyde beneath the plantar aponeurosis of the hind paws. This method has been used by other authors to estimate the anti-arthritic effect of various substances. The anti-arthritic effect has been "measured" by the ability of the test substance to reduce the oedema which develops rapidly after injection of formalin. However, since oedema is only one of the manifestations of inflammation, and since very little information has been published concerning the histological changes induced in the joint and its neighbouring tissues by injection of formalin into the plantar pad, it was considered worth while making a histological examination of the effects of this procedure, not only in the injected limbs but also in the contralateral limbs. At the same time changes in the cortical lipoid of the adrenal glands were noted.

METHOD.

Sixteen rats were injected in the plantar pad of the left foot with 0.1 ml. of a 2 per cent solution of formaldehyde, and then pairs of rats were killed, and their tissues fixed in formaldehyde, at the following periods of time: 5 minutes, 1 hour, 3 hours, 8 hours, 24 hours, 3 days, 1 week, 2 weeks.

The left and right ankle-joints were fixed for 48 hours, then washed in tap water and decalcified electrolytically. Paraffin sections were stained with haematoxylin and eosin and in some cases with azan and aniline blue. The adrenals were washed for 5 hours; frozen sections were cut and stained with Sudan black to demonstrate fat and lipoid.

In addition the ankle-joints and adrenals of four rats which had been injected with formaldehyde one month previously were examined. The left ankle-joints of nine other rats injected a month previously were also examined, and so were those of three rats which had had three injections of formaldehyde into the left hind plantar pad at two-day intervals and had been killed a month following the last injection.

RESULTS.

The foot began to swell during the first hour after injection of the formalin, and this continued until eight hours and then began to decrease. This decrease continued, the ankle-joints of most animals returning to normal somewhere

between 1 and 3 weeks after the injection. Some swelling was present in the joints of some animals over a month after injection.

In the ankle-joints of animals killed five minutes after the formalin injection little change could be seen, but there seemed to be a swelling of parts of the synovial membrane in one animal.

At the end of an hour very little histological change was yet visible. There was some microscopical indication however of the oedema, which was by then visible macroscopically. Swelling of cells of the synovial membrane could be seen in the left ankle-joint of one animal; in the other a granular precipitate in some of the synovial cavities suggested an increase in the solid matter of the synovial fluid (? protein), which presumably indicated some change in the permeability of the synovial membrane at this stage.

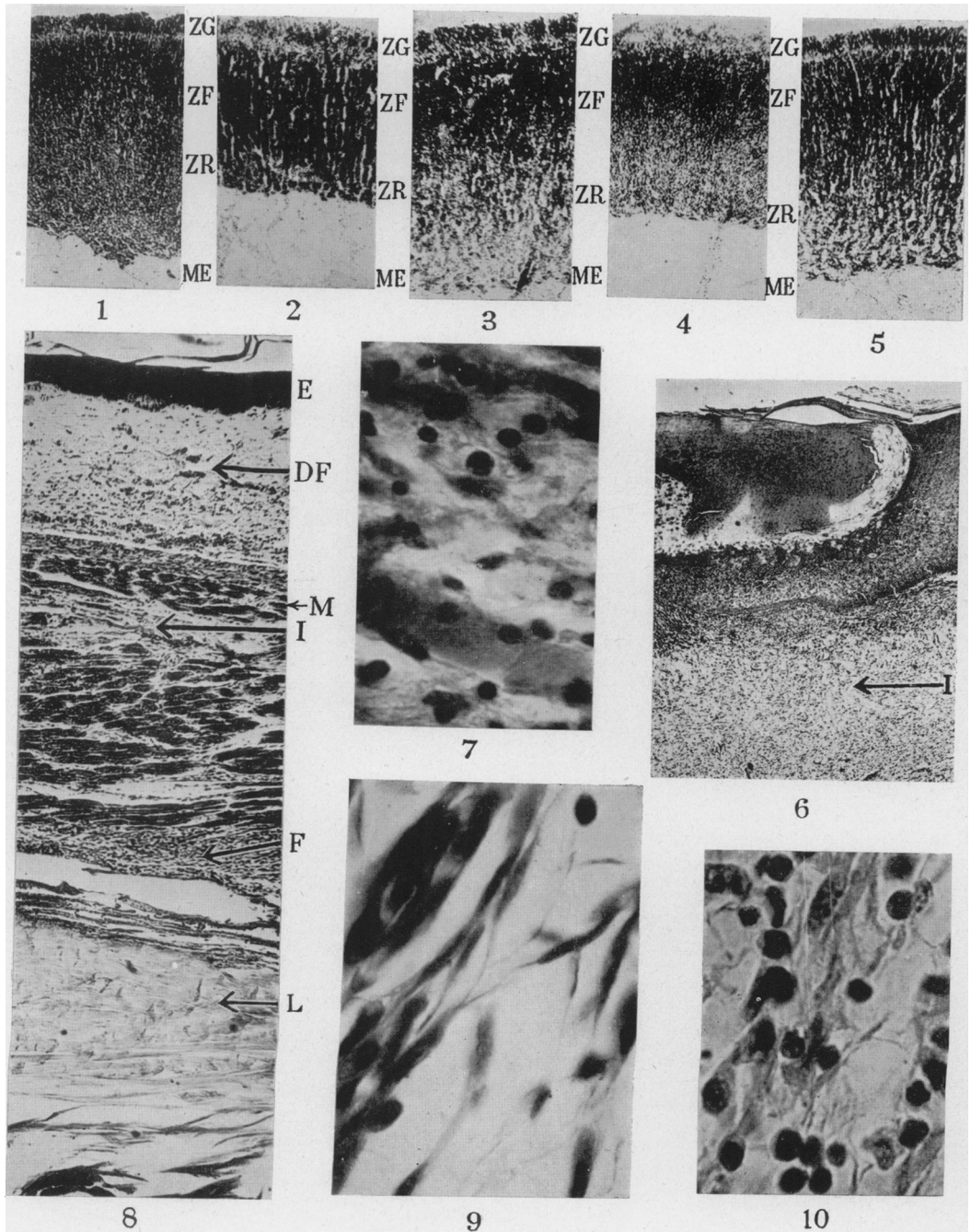
No significant changes could be seen in the right ankle-joint of either animal.

After 3 hours there was considerable increase in the granular precipitate (which stained basophilically) in the synovial cavities of the left ankle-joints of both animals. This was also found in the right ankle-joints.

Increase in the fragility of the capillary walls was also evidenced by the presence of free red cells in the dermal fascia near the site of the injection of the formalin. The first cellular evidence of inflammation occurred in one animal in the form of a small infiltration of macrophages into the dermal fascia and into some of the peri-articular tissues. Although the majority of these cells were macrophages many neutrophils were also present.

EXPLANATION OF PLATE.

- FIG. 1.—Lipoid preparation (Sudan black) of adrenal of rat injected with formalin in the plantar pad of the left hind foot 5 minutes previously. Distribution of lipoid normal.
- FIG. 2.—Lipoid preparation of adrenal of rat injected with formalin in the plantar pad of the left hind foot 3 hours previously. Considerable loss of lipoid from zona glomerulosa. Lipoid distribution of rest of cortex unaffected.
- FIG. 3.—Lipoid preparation of adrenal of rat injected with formalin in the plantar pad of the left hind foot 8 hours previously. Considerable loss of lipoid from zona reticularis as well as from zona glomerulosa.
- FIG. 4.—Lipoid preparation of adrenal of rat injected with formalin in the plantar pad of the left hind foot 24 hours previously. Considerable loss of lipoid from all zones of adrenal.
- FIG. 5.—Lipoid preparation of adrenal of rat injected with formalin in the plantar pad of the left hind foot 1 week previously. Lipoid of glomerulosa and fasciculata zones back to normal. Reticularis still shows slight loss of lipoid. Differences in width of cortex are not significant.
- FIG. 6.—Skin and surrounding tissue of left hind paw of rat in which formalin has been injected 3 days previously. Destruction of epithelium and extensive cellular infiltration of the skin and dermal fascia is apparent.
- FIG. 7.—Showing infiltrating cells in muscle near ankle-joint. Animal has been injected 1 week previously with formalin. The majority of these cells are round cells.
- FIG. 8.—Section through skin and ankle-joint of rat near site of injection of formalin 1 week previously. Infiltration of cells between muscle fibres can be seen. This infiltration passes down to but does not pass through the joint ligament seen near the bottom of the section.
- FIG. 9.—Showing well-developed peri-articular fibrous tissue in ankle-joint of rat injected 4 weeks previously with formalin.
- FIG. 10.—Showing types of invading cells in sub-dermal fascia near ankle-joint of rat injected with formalin 1 week previously. Round cells and polymorphs may be seen, also the beginnings of fibre production by fibroblasts.
- ZG. Zona glomerulosa. ZF. Zone fasciculata. ZR. Zona reticularis. ME. Medulla. E. Epithelium of skin. F. Regions of fibroblastic activity (multiplication and fibre production). M. Muscle. DF. Dermal fascia. I. Areas of cellular infiltration. L. Joint ligament.



At 8 hours, when the oedema of the ankles was at its height, the synovial fluid of the left ankle-joints of both animals showed considerable increase of solid matter. In one animal macrophages were also present in the synovial cavities and in the other some fibrin could be seen. The cells of the synovial membranes were swollen in some regions. Cellular infiltration was apparent in the peri-articular tissues of the left ankle-joints of both animals at this stage but was not yet very extensive. It was confined to the dermal fascia near the site of injection in one animal, and in the other it had spread to the joint capsule and was beginning to travel along the capsule parallel with the fibres. There was a slight fibroblastic proliferation in the dermal fascia of one animal.

The right ankle-joints of both animals at 8 hours showed some slight cellular infiltration, but the synovial cavities appeared normal.

At 24 hours there was still fibrin and increased granular deposits in the synovial fluid, and considerable increase in the infiltration of the peri-articular tissues together with a slight fibroblastic proliferation.

After 3 days the increase of solid matter in the synovial fluid was still obvious, and granular deposits were also found at the site of extra-articular accumulations of fluid.

In one animal parts of the synovial membranes had become infiltrated and fibroblastic proliferation in the dermal fascia had increased.

The right ankle-joints of the animals showed little change from normal.

By one week there was considerable cellular infiltration of the dermal fascia extending some distance from the site of the injection. At this stage too there was fibroblastic proliferation in the same site. There was also some infiltration of, and fibroblastic proliferation in, the peri-articular tissues. The synovial fluid and membranes appeared to have returned to normal.

The right ankle of one animal showed a patch of fibroblastic proliferation in the dermal fascia, while that of the other animal appeared normal.

A fortnight after injection the left ankle-joint of one animal showed extensive cellular infiltration of the dermal fascia, which, however, did not extend through to the joint. There was very little fibroblastic proliferation or activity. In the other animal the left ankle-joint showed considerable infiltration and fibroblastic activity in the same region.

In neither case had these inflammatory processes forced their way through the peri-articular tissues into the joint cavity itself. The synovial fluid and membranes and the articular surfaces in right and left ankle-joints of both animals appeared quite normal.

Of the 13 animals killed one month after injection of the formaldehyde, seven seemed quite normal and all signs of cellular infiltration and fibroblastic reaction had disappeared. Four still showed some signs of both these processes, and two had left ankle-joints which were still heavily infiltrated and in which fibroblastic activity continued.

Of three rats which had received three injections of formaldehyde into the same foot and were killed one month after the last injection, two showed more or less normal ankle-joints and the third still showed considerable cellular infiltration of, and considerable fibroblastic activity in, the dermal fascial and the peri-articular tissues. There was also some infiltration of the synovium, and granular deposits (from the synovial fluid) were present in the joint cavities. The articular cartilage was unaffected.

THE ADRENALS.

The changes in the adrenals can be summarized as follows :

5 minutes	.	2 animals	.	Cortex has full load of lipoids in all zones.
1 hour	.	„	.	Slight loss of lipoid from zona glomerulosa.
3 hours	.	„	.	50 per cent loss of lipoid from zona glomerulosa and slight loss from zona reticularis.
8 „	.	„	.	Considerable reduction of lipoid in all zones.
24 „	.	„	.	Lipoids considerably depleted in all zones.
3 days	.	„	.	Ditto.
1 week	.	„	.	1 animal shows lipoids in all zones nearly normal. 1 animal shows zona glomerulosa normal, but reticularis and fasciculata zones not back to normal.
2 weeks	.	„	.	All zones appear normal.
1 month	.	4 animals	.	2 animals have all zones with full load of lipoid. 2 animals show considerable depletion of lipoids particularly in zona glomerulosa and zona reticularis.

The changes in the last group can not be correlated with changes in the joints.

DISCUSSION.

It is noteworthy that apart from the initial changes in permeability of the synovial membrane as evidenced by the increased solid matter in the synovial fluid the inflammatory processes do not extend into the joint itself. Only in one or two animals was there even cellular infiltration into the synovial membranes. In fact the condition produced by formalin injection is not an arthritis but a peri-articular inflammation. It is obvious that treatment which rapidly reduces the oedema associated with such a procedure may not necessarily affect the processes of cellular infiltration and fibroblastic proliferation which follow it.

Selye found that several formaldehyde injections at intervals of a few days produced a "self-maintaining chronic inflammatory process," which continued to progress after formalin injections had been discontinued. Joints affected in this way were said by Selye to resemble the rheumatoid type of arthritis, and the acute oedematous joints to resemble the rheumatic type. Coutu, Selye and Gareau (1951) have described the histological changes which follow the injection of mustard powder into the plantar pads of rats. The peri-articular changes they describe resemble those described in the present paper but in addition they found that granulation tissue penetrated into the joint cavity and became progressively organized. They also found degeneration of the cells and erosion of the articular cartilage. Nothing of this sort was found in the present work.

The difference in the two results may be explained by the more prolonged irritation produced by the mustard powder.

The right ankles show changes in the synovial fluid (increased solid matter) at an early stage, but this in most cases passes off rapidly. Right ankles of some animals have been examined from time to time, however, a month after injection and found to show appreciable cellular infiltration around the joint.

The changes in the adrenal lipoids show that there is no effect from the formalin injections in the first five minutes, but that discharge of these lipoids is beginning by the first hour. It is of interest that the first signs of loss of lipoid show in the zona glomerulosa, a zone in which, Deane and Greep (1946) have brought forward evidence to show, the salt and water controlling hormones (desoxycorticosterone or related substances) are produced. Loss of lipoid is next shown by the zona reticularis, and there is considerable loss from both these zones before there is any obvious loss of lipoid from the zona fasciculata, where, according to Deane and Greep, the carbohydrate hormones are localized. There may be some significance in the fact that extensive discharge of lipoid from all zones which has occurred by 8 hours coincides with the beginning of cellular infiltration and fibroblast proliferation round the ankle-joints. The adrenals have regained most of their lipoid by the end of one week and appear quite normal again after a fortnight.

SUMMARY.

Injection of formaldehyde into the plantar pads of the hind feet of rats causes a peri-articular inflammation characterized by oedema of the peri-articular tissues, increase of solid matter in the synovial fluid, cellular infiltration of the dermal fascia and the peri-articular tissues and increased fibroblastic activity. By one month most of the joints have returned to normal, but some still show considerable cellular infiltration and fibroblastic activity. There appears to be some correlation between lipoid changes in the adrenal and the onset of cellular inflammatory changes in the joints.

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