

EXPERIMENTAL STUDIES ON HYPERERGIC CHANGES IN THE SPLEEN.

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I. INTRODUCTION.

The spleen is anatomically and physiologically a very complicated organ which has, among other things, destructive action on foreign erythrocytes when they are induced into the blood stream. Recently, much attention has been focused on the pathological condition of spleen specially related with the iron metabolism, immunity and embryonic tissue construction. Clinically, on the other hand, the spleen often shows its abnormality by means of enlargement or atrophy in various diseases such as some infectious disease, Cacheia, malaria and hemophylaxis. The genesis of this abnormality is still in confusion.

Arthus (1) in 1903 injected horse serum repeatedly into the subcutaneous tissue of a rabbit and after 4 successive injections or more at intervals of several days found the animal reacts with acute inflammation at the site of injection; the skin about the injection becomes red, edematous and occasionally necrotic. Same result was obtained in the case where the preliminary injection was performed into the peritoneum. Arthus (1) first called this phenomenon "Local Anaphylaxis". This experiment has been fully verified in human beings by Pirquet (4). He called it as of the same nature as allergy. The tuberculin test is one of the clinical applications of this phenomenon. Confronting the general anaphylaxis, the local anaphylaxis has been studied topographically in various organs.

Lucas (3) and Gay (3) observed the same local anaphylatic phenomenon on the skin of children; Asada (6) observed in both skin and mucous membrane while Migikawa (7) observed on the testicles, lungs and muscle tissue. Apparently no one studied the local anaphylatic phenomenon on the spleen.

Recently, the problem of local immunity or local production of antibody has been a target of investigation and many investigators have proved that the site which produces antibody is not confined to those blood forming organs but any other organs, specially there is much relation with the reticulo endothelial system.

There is no doubt that the spleen is one of the main organs which produce antibody. As Pfeiffer and Merx (4) already pointed out there is an abundant bacteriolysin in a rabbit's spleen in the earliest stage of immunity against Colera. If spleen plays an important part in the formation of antibody, there might be a close relationship between spleen and local anaphylaxis. Thus I have undertaken a careful observation on the local anaphylaxis of spleen by means of injecting antigen directly into the splenic artery.

II. EXPERIMENTAL PROCEDURES.

Normal rabbits weighing about 2.000 Gm. were used for this study. The antigen used in this study is made freshly by the Sanitary laboratory, Chosen Government General.

Rabbits were sensitized by the injection of horse serum 2 cc into the abdominal subcutaneous tissue and the same dose was repeated every day for 4 days in succession. On the 15th day reinjection was made by means of an operative intra-splenic arterial injection of horse serum 0.2 cc. The rabbits were divided into 4 groups and spleen was removed for examination on the 1st week, 2nd week, 3rd week and 4th week respectively. The spleen was fixed immediately in the 10% formalin solution. The stains are the hematoxylin-eosin stain, the Sudan III stain, and the Hemosidelin pigment stain.

III. RESULTS OF EXPERIMENT.

1. THE CHANGE OF SPLEEN ONE WEEK AFTER REINJECTION.

Follicles are much shrunken, and shows an irregular shape. The outline of each follicle is not quite clear, connective tissue increased markedly around the follicles which has undergone hyalin degeneration partially. In some follicles lymphocytes are proliferated and necrobiotic change can be observed. The nucleus of follicular cell shows in general pyknotic change.

Splenic subcortical tissue shows remarkable capillary congestion, the trabeculae lienis is hypertrophied and densely adherent or wound up. The endotelial cells of the venous sinus and reticular cells show practically normal appearance. In the subcortical region there is much yellow brownish pigment more abundant than in the central region. Lipoid substance around the follicles is somewhat less than in the central region. Hemosiderin pigment deeply stained as blue granules or lumps in the reticular tissue more abundantly than the central but no such pigment found in the follicles.

Table No. 1.

Rabbit No.	Sex	Body Weight	Lipoid substance	Deposit of Hemosid. Pigmt.	Degree of Follicular Atroph.
1	♀	1400 g	+	##	Slight
2	♂	1300 g	—	##	Moderate
3	♂	1420 g	—	++	//

2. THE CHANGE OF SPLEEN TWO WEEKS AFTER REINJECTION.

Follicles show in general more atrophic condition than that of the 1st week. The shape of each follicle in this stage is irregular but well scattered; some of them are broken. The boundaries between the follicular and the stroma can be scarcely distinguished. No remarkable changes in the lymphocytes except more or less atrophic, no necrotic or hyalenic changes can be observed in this stage.

In the splenic stroma small blood vessels are congested, trabeculae lienis are thin and curved. The yellow brownish pigments are also found in this stage but less than it was in the 1st week. The endothelial cells of the venous sinus and reticular cells show very little change. Lipoid pigment around the follicles shows very scanty amount; much less than the normal central. Hemosiderin stain revealed blue granules or mass in the reticular cells of splenic stroma but not in the follicles. The evidence is not so abundant as it was in the 1st week.

Table No. 2.

Rabbit No.	Sex	Body Weight	Lipoid substance	Deposit of Hemosid. Pigmt.	Degree of Follicular Atroph.
4	♀	1300 g	—	++	Severe
5	♂	1450 g	+	++	//
6	♀	1350 g	—	+	//

3. THE CHANGE OF SPLEEN THREE WEEKS AFTER REINJECTION.

Follicles in this stage show more or less hyperplastic, the small follicles are numerous found, the old follicles show somewhat atrophic and

irregular in shape but not so remarkable as seen in the 1st and 2nd week. Capillary proliferation is prominent in this stage and vascular wall thickened obviously, blood vessels are generally congested. Lymphocytes show nuclear pyknosis, (No. 10).

In the splenic stroma, the small blood vessels are congested trabeculae lienis hypertrophied similar to the state of 2nd week. The yellow brownish pigment in the reticular tissue of splenic stroma appeared in slight degree, no necrotic change found. Lipoid substance found very little in this stage. The hemosiderin stain shows about the same in its nature but smaller amount even than it was in the 2nd week.

Table No. 3.

Rabbit No.	Sex	Body Weight	Lipoid substance	Deposit of Hemosid. Pigmt.	Degree of Pollicular Atroph.
7	♂	2110 g	—	+	Slight
8	♂	2000 g	+	+	//
9	♂	2100 g	—	—	//

4. THE CHANGE OF SPLEEN FOUR WEEKS AFTER REINJECTION.

Follicles in this stage show more like normal appearance though some of them are atrophied. The connective tissue between the follicles hypertrophied, many lymphocytes are found. Vascular wall thickening and vascular congestion are found. In the splenic stroma no change in the blood vessels trabeculae lienis slightly hypertrophied. The reticular cells in the stroma contains yellow brownish pigment. Similar degree to the normal control, nearly no lipoid substance found in this stage which means much diminution of lipoid substance compared with the normal control, a maximal deposit of Hemosiderin stains show hemosiderin pigment in this study but even in this stage no hemosiderin pigment in the follicles.

Table No. 4.

Rabbit No.	Sex	Body Weight	Lipoid substance	Deposit of Hemosid. Pigmt.	Degree of Pollicular Atroph.
10	♀	1600 g	—	++	Slight
11	♀	2000 g	—	++	//
12	♂	1500 g	—	+	//

IV. SUMMARY.

In this study, the spleen removed one week after the reinjection by means of serum injection into the splenic artery in the sensitized rabbits, shows histologically moderate atrophic condition of follicles, hypertrophy of connective tissue around the follicles. In some, necrobiotic change can be observed. In the splenic stroma vascular congestion and dense hyper-

atrophied troberculæ lienis with yellow brownish pigment can be found. In the 2nd week, after the reinjection, the spleen shows more atrophied follicles and many follicles have lost their original shape and nature. Some of them are destroyed. Lymphocytes are numerous found and many of them are atrophied. In the stroma the blood vessels are congested and the trabeculae lienis moderately thickened. The yellow brownish pigment in this stage is much diminished. In the 3rd week, the follicles are atrophied slightest degree and many newly formed follicles are found with the hyperplasty of new capillaries. In the splenic stroma the changes are similar to the state of the 2nd week but the yellow brownish pigments are much diminished in this week.

In the 4th week, the histological picture shows very little change from this normal picture. The yellow brownish pigment are still present in this stage.

Regarding the local immunity of spleen, there are many reporters such as Okasaki (8) but very little has been done on the splenic local anaphylaxis. Heineke, Ziegler (9) Sibudani (10) and Ono (11) observed the changes in the spleen histologically after X-ray stimulation on the spleen. Soper (12) and Kitaoka (9) by the vital staining found spleen showed the early destruction of lymphocytes. In the stroma the venous sinus dilated, vascular congestion, and proliferation of wandering cells. Tokumitsu (15) observed the changes of liver and spleen after the operation morphological by which the various parts of vena cava were communicated with the portal vein. 150 days after the operation the splenic follicles showed decrease in number and much atrophied, the troberculæ lienis and the splenic membrane are thickened. Some of them showed hyalin degeneration. In my study, the histological changes mainly the follicular atrophy, vascular congestion and degenerative changes of lymphocytes are possibly due to the fact that the anaphylatic toxin are more abundantly found in the antibody forming organs such as spleen.

The lipid substance in the 1st week after the reinjection shows a slight diminution from the normal deposit, on the 2nd week the diminution is more prominent and on the 3rd and 4th week it was nearly negative. In my result thus far in the splenic local anaphylaxis the animal shows gradual diminution of lipid substance in the spleen absolutely not related with the general nutritional condition.

The Hemosiderin pigment in the 1st week more abundant than the normal spleen. In the 2nd and 3rd week it shows somewhat less in amount but still more than normal. In the 4th week it is still present and slightly more than normal. It is well known fact that spleen contains iron element. Lubarsch (9) insisted the content of iron element in

the spleen has no relation with the iron content in the diet. Miwa (14) attributes this evidence to the fact of erythrocytic destruction in the spleen. Tokumitsu (15) observed same increase of hemosiderin in his experiments above mentioned and Sibudani (10) and Kitaoka (9) found an abundant hemosiderin pigment to the spleen after the X-ray stimulation, they attribute this evidence to the fact that X-ray provokes the process of destruction of erythrocytes in the spleen.

In the process of splenic local anaphylaxis it is quite possible that the anaphylatic toxin produced in the spleen might stimulate the physiological erythrocyte destructive action of spleen.

V. CONCLUSION.

1. By means of same serum reinjection into the splenic artery in the rabbits already strongly sensitized, produce an acute inflammatory reaction on the spleen which is called Hyperergic change, (local anaphylaxis).
2. In the splenic local anaphylaxis the spleen shows congestion, follicular atrophy up to necrobiotic change. The degree varies sharply; in the 1st and 2nd week those histological changes show a maximum and in the 3rd and 4th week it shows a tendency to restore.
3. In the process of splenic anaphylaxis the lipid substance decreases and runs parallel with the histological picture.
4. In the splenic anaphylaxis, the hemosiderin pigments are obviously increased.

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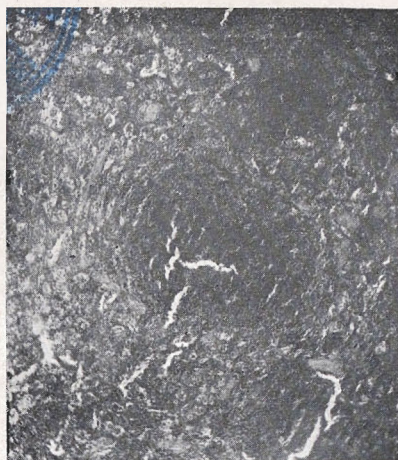


Fig. 1. The normal spleen 45 \times Leitz.

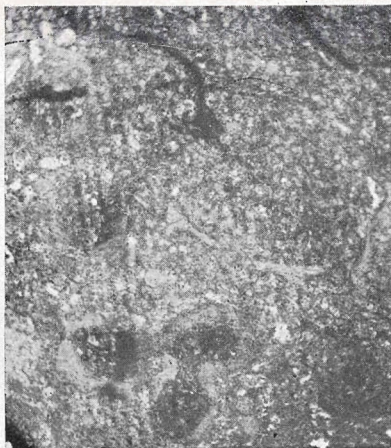


Fig. 2. One week after reinjection 45 \times Leitz.

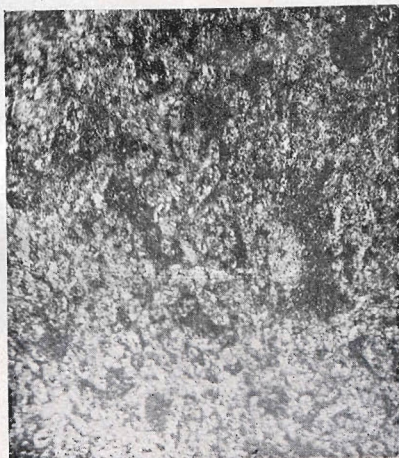


Fig. 3. Two weeks after reinjection 45 \times Leitz.