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Takenori Toyama*

*Okayama University,

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Takenori Toyama

Abstract

Macromolecular PVP was introduced intravenously into rabbits for a long period of 3 months, 10 g of PVP in total, and the observations were carried out to see disturbances in hematopoiesis, lymphopoiesis, and immune reaction with special reference to the histologic changes of marrow, liver, spleen and lymph nodes. The results were as follows: 1. A mild anemia was induced by the PVP injection. RE cells of liver and bone marrow were swollen moderately but otherwise no significant histologic changes were induced in bone marrow and liver. 2. A severe lymphocytopenia resulted: the RE cells of lymph follicles were blocked by PVP, the follicles collapsed into a homogeneous mass with fibrosis and minimized lymphopoietic tissues. These results suggest that RE cells of the so-called germinal centers are important for the reproduction or the differentiation of lymphocytes. 3. Intravenous injection of egg albumin caused the serum antibody formation with a marked proliferation of plasma cells around small vessels in lymph nodes and spleen as in the case of control animal. The data indicate that plasma cells are solely responsible for the serum-antibody formation and plasma cell may differentiate from adventitial cells of small vessels but not from lymphocyte or reticulum cell.

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STUDIES ON THE FUNCTION OF RETICULO-ENDOTHELIAL SYSTEM

II. EFFECTS OF THE R.E.S. BLOCKING WITH MACROMOLECULAR PVP ON THE LYMPHOID CELL REPRODUCTION AND THE PRODUCTION OF SERUM ANTIBODY

Takenori TŌYAMA

Department of Pathology, Okayama University Medical School, Okayama, Japan (Director: Prof. S. Seno)

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Polyvinylpyrrolidone (PVP) solution has been extensively used in clinics mainly as a plasma substitute[1-4]. It is well known that a large part of PVP of low molecules introduced into vein is excreted through kidney[5-8], but some of it is taken up by the reticulo-endothelial (RE) cells of liver, spleen and lymph nodes forming the so-called foam cells[9,10,11], swollen RE cells by taking PVP. Macromolecular PVP[11] is hardly excreted through kidney and readily deposited in the RE cells. This characteristic is advantageous for the study of reticulo-endothelial system (R. E. S.) blocking, though some ill effect may be induced by the intravenous administration of a vast amount of it at one time, because of its characteristic to remain persistently in blood, its high viscosity and erythrocyte agglutinating effect[12].

PVP is a translucent, nontoxic and chemically stable substance[5]. The histologic picture of the organs having blocked R. E. S. appears rather distinct than in the case of India ink blocking which makes it difficult to observe precise histological changes owing to the black color of carbon particles. This paper deals with the cases receiving repeated injections of PVP for a long period of time and elucidates that PVP has a specific affinity to the germinal center of lymphoid tissues resulting in a severe lymphocytopenia without any inhibitory effect on serum-antibody production.

MATERIALS AND METHODS

Ten adult male rabbits weighing 2.5 to 3.0 kg were divided into two groups, five animals each. The rabbits of the first group received the intravenous injection of 10 to 20 ml of 1 per cent PVP (mean molecular weight,

700, 000) in physiologic saline solution daily for about 3 months.

Those of the second group were the control and were given the injection of physiologic saline only.

The serum-PVP level was measured with iodine-$ZnSo_4$ solution by the method of DISCOMBE and others[13].

At certain intervals red blood cell and white blood cell counts, hematocrit value and hemoglobin level were observed by the routine method. Cell classification was made on the blood smears fixed with methanol and stained with May-Grünwald Giemsa.

After the completion of the contemplated PVP-injections, all the animals including those of the control received the intravenous injection of 2 ml of 1.5 per cent egg albumin in saline solution, twice at 48-hour interval and 3 to 20 days after the last injection, serum precipitin test was carried out by the routine method. At the termination of experiment, every animal was sacrificed by blood depletion with severance of the carotid artery. Liver, spleen, lymph node and bone marrow were fixed with neutral formol, embedded in paraffin and the sections were stained by hematoxylin-eosin. Some of the sections were stained with Congo red by the method described by FREIMAN and others[14] by which PVP in tissues is stained bright cherry red.

RESULTS

By daily intravenous injection of 1 per cent PVP solution, the PVP contents in serum increased day by day showing almost a straight line of increasing tendency (Fig. 1). The PVP level in blood was controlled to keep it lower than 3 per cent by controlling the amount of PVP to be injected, because over this shield animals hardly survived through the subsequent injections. By repeating the PVP injection the red blood cell count and hemoglobin level decreased gradually with the development of moderate anemia, around 3,700,000 per cu mm or more. No severe anemia such as in the case of India ink injection[40] was observed. Reticulocyte count did not increase throughout all the stages. Hematocrit

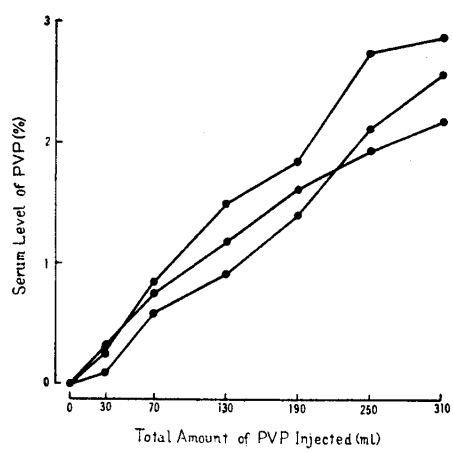


Fig. 1 Accumulation of PVP in the Serum of 3 Rabbits Received the Repeated Injections of PVP into Vein, 10~20 cc of 1% PVP Solution Daily for 15 to 20 Days
Each curve shows the changes in the value of one animal.

value decreased moderately in parallel with the decrease in the number of red cells, indicating that the PVP injection brought no concentration or dilution of the blood (Fig. 2).

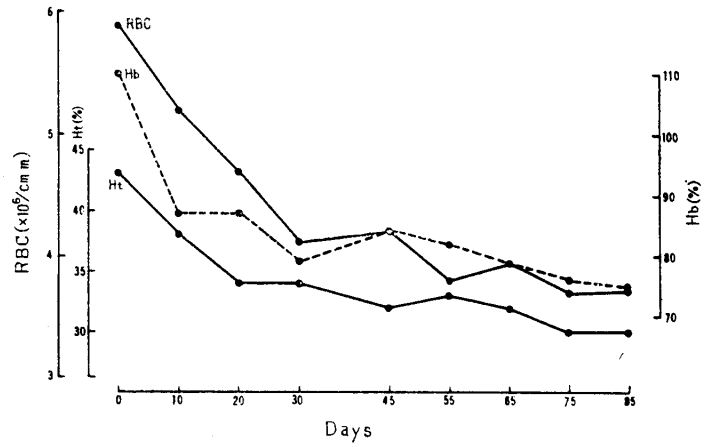


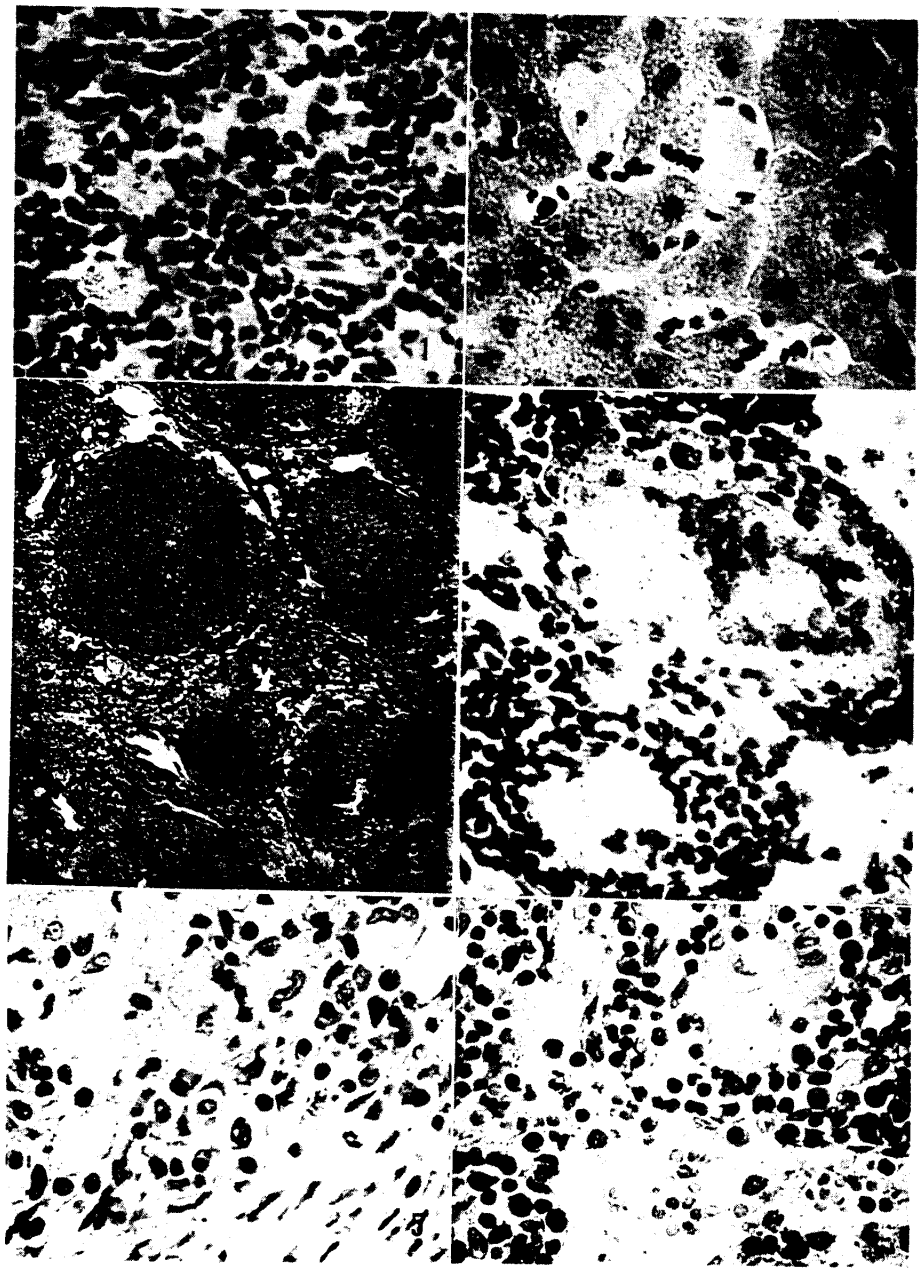
Fig. 2 Changes in RBC Number, Hemoglobin Level and Hematocrit Value of Rabbits Induced by the Repeated Intravenous Injections of PVP, in 10~20cc daily or every other day, about 10 grams of PVP in Total Each curve shows the mean value of 5 animals. RBC; Red blood cell count, Ht; Hematocrit value, Hb; Hemoglobin level.

White blood cell count also decreased progressively. The classification study revealed that the decrease in the number of white blood cells is solely due to a marked decrease of lymphocyte count which reached less than one-third the original level at the end of experiment, while the granulocyte and the monocyte counts remained at the original level (Fig. 3).

However, no morphological abnormality of lymphocytes was observed. The percentage of medium-sized lymphocytes to the whole lymphocyte number remained in normal range, 10 to 20 per cent.

Histologic observations revealed that in spite of the administration of a vast amount of PVP, the deposition of PVP in the RE cells of bone marrow was mild and no significant change in hematopoietic tissue could be induced (Photo 1). This histologic picture agrees well with the change of hemogram, not so severe anemia or normal granulopoiesis.

In liver, PVP deposition was also mild. A few Kupffer cells of liver took up PVP, which resulted in foam cells[9.10.11.15], but on the whole, the PVP deposition of liver was surprisingly mild (Photo 2). The weight of liver increased slightly (Table 1).



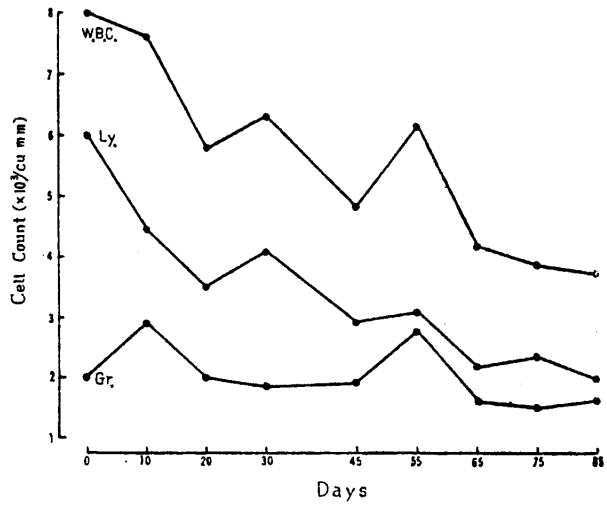


Fig. 3 Changes in White Blood Cell Count of the Rabbit
The values are of the same animals appearing in Fig. 2 and each curve gives the mean value of 5 animals. W. B. C.: White blood cell, Ly.; Lymphocyte, Gr.; Granulocyte.

In contrast, spleen showed severe PVP deposition. RE cells in spleen were markedly swollen with giant cell formation and complete disappearance of lymph follicles being accompanied with extensive fibrotic change (Photo 3). In spite of such severe histologic changes, spleen in gross appearance showed actually no swelling, weighing only 0.76 g per kg of body weight and 1.4

Photo 1 Picture of the Bone Marrow of a Rabbit Received 80 Injections of PVP, about 10 grams of PVP in Total
The reticulum cells were loaded moderately with PVP. Hematoxylin-eosin staining, ×400

Photo 2 Picture of the Liver Tissue of the Same Animal as in Photo 1, Showing the Foam Cell Formation of Kupffer Cells. Hematoxylin-eosin staining, ×400

Photo 3 Picture of the Spleen of the Animal Treated Similarly as That in Photo 1
PVP was stained with Congo red and appearing cherry red, poststaining with light green. ×400 note the heavy deposition of PVP in lymph follicles.

Photo 4 Picture of the Limph Node of the Same Animal as in Photo 3
Note the marked swelling of reticulum cells in germinal center. Hematoxylin-eosin staining. ×400

Photo 5 Picture of the Spleen of the Animal Received 70 Injections of PVP and then Challenged with Egg Albumin Injection into Vein, 2cc of 1.5% Solution Twice at 48-Hour Interval
Picture was taken 3 days after the last injection of egg albumin and shows the plasma cell proliferation in the ruined tissues by the heavy PVP deposition. Hematoxylin-eosin staining. ×400

Photo 6 Picture of the Lymph Node of the Same Animal as in Photo. 5, also Showing a Marked Proliferation of Plasma Cells in the Area Surrounding Sinusoides.
Hematoxylin-eosin staining. ×400

times as heavy as that of control in weight (Table 1).

A striking effect of daily intravenous injection of PVP was observed in lymph nodes, whose reticulum cells in the follicles were blocked up with heavy

Table 1 The Weight Ratio of Liver and Spleen to Body Weight (×1,000) of Rabbits Treated by PVP and Physiological Saline Injection

Organ \ Animal No.	1	2	3	4	5	Mean value
Liver	21.4	23.3	26.7	27.3	32.7	26.3
Spleen	0.63	0.69	0.73	0.82	0.93	0.76

Organ \ Animal No.	6	7	8	9	10	11	12	13	Mean value
Liver	20.0	22.1	21.2	21.9	22.7	23.0	27.0	28.0	23.3
Spleen	0.26	0.52	0.45	0.50	0.62	0.59	0.72	0.73	0.55

No.1—5; Injected 1% PVP in physiological saline into vein about 10 grams of PVP in total for about 80 days.

No. 6—13; Control group injected the saline solution.

PVP deposition with poor lymphocyte production (Photo 4). These rabbits developed severe lymphocytopenia.

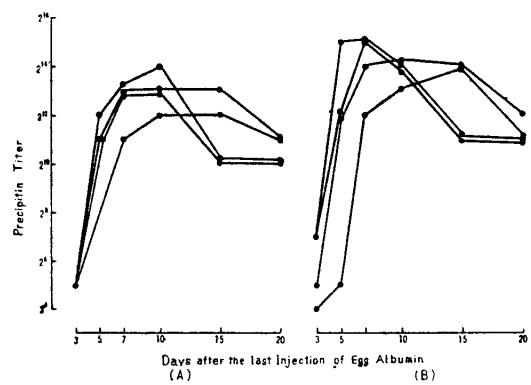


Fig. 4 Curves of the Precipitin Titer of the Animals Received the Intravenous Injections of PVP and Saline Solution

The data are of four animals of those appearing in Figs. 2 and 3. After about 70 injections of PVP solution, they were challenged with injections, 2 cc of 1.5% egg albumin in physiological saline 2 times at 48 hour interval, and the precipitin titer was estimated 3 to 20 days after the second injection. Each curve shows the value obtained on one animal. A; Control injected with physiological saline solution, B; Experimental animals received PVP injection.

The immunization of these animals with egg albumin resulted in a mild increase of serum-antibody titer. The level was almost the same or slightly higher than that of control animals (Fig. 4), indicating that the administration of PVP acts as to elevate the response to the subsequent injection of antigen.

In spleen and lymph nodes on the third day after immunization, a marked proliferation of plasma cells (Photos 5, 6), especially around small vessels, was observed, indicating that plasma cells is responsible for this serum-antibody formation and the reproduction of plasma cells has no direct relation to the R. E. S. damage or lymphocytopenia.

DISCUSSION

Intravenous administration of a vast amount of macromolecular PVP for a long period of time causes a heavy PVP deposition in RE cells in spleen and lymph nodes resulting in severe lymphocytopenia. Hematopoiesis in bone marrow is likewise affected developing a mild anemia but the damage is rather slight comparing to the cases of India ink injection.

Some authors[16-19] are of the opinion that lymphocytes originate from the germinal center. HELLMAN[20] claims that lymphocytes develop from lymphatic parenchyma. The present data may contribute to making this problem clear.

In spleen and lymph nodes of the rabbits administered PVP for a long period of time, the RE cells of lymph follicles were blocked up with PVP, no longer mitosis of RE cells could be observed, and finally the follicles themselves collapsed into a large homogeneous mass. In proportion to these changes lymphocyte layers around follicle became atrophic and marked lymphocytopenia ensued.

Lymphocytes themselves, however, never took up PVP and showed no significant morphological change so far as the observation on the smeared cells was concerned. These facts prove that RE cells of the so-called germinal centers play an important role in the reproduction of lymphocytes. Recent morphological studies[21,22] revealed a close relationship between RE cells and lymphocytes, just as that of erythrocytes and RE cells in bone marrow.

AMANO[23] is of the opinion that lymphogonia has a dualistic potentiality which can differentiate to lymphocyte as well as to reticulum cell. However, his lymphogonia is not the macrophage and probably is not damaged by PVP.

A possible and reasonable deduction from the present experimental results is that lymphocytes will be formed from RE cells in the germinal center or that the stem cells receive some information for differentiation from RE cells.

As there is no morphologic evidence indicating the transformation of reticulum cell to lymphocyte, the latter supposition will be more acceptable at present.

Reticulum cell may give the information for the antibody formation as well as for the differentiation to lymphocyte.

It is known that the lymphocytes are responsible for tissue immunity or cellular antibody formation.[24,25,26] The author has not observed the tissue immune reaction in this experiment, but clarified that the serum-antibody formation is not affected by the damaged lymphopoiesis, proving that serum antibody is formed independently of lymphocyte.

Histological observations of the animals revealed a marked proliferation of plasma cells.

NOSSAL's experiment[27,28] and fluorescent antibody techniques[29,30,31] have already demonstrated that serum antibody is produced by plasma cells. Consequently the well retained ability of serum-antibody production can be explained by the plasma cell proliferation. Besides this, the present experiment may give a light on the cytogenesis of plasma cells.

Regarding the cytogenesis of plasma cells, YOFFEY[33,33] and GOWANS[34,35] consider a lymphocyte as a stem cell of plasma cells, FAGREUS[36] and DAMESHEK[37], that of a reticulum cell, and AMANO[38,39], the adventitial cells of vessels as the mother cell of plasma cell.

The present data indicate that plasma cells may not come from lymphocytes or reticulum cells, because plasma cell proliferation in spleen and lymph nodes can be successfully induced in the tissues where the reticulum cells and lymphocytes are extremely rare. These plasma cells proliferate markedly in the area surrounding small vessels or small arteries. Such a picture supports the opinion of AMANO[38] strongly that plasma cells originate from the adventitial cells of small vessels.

Another important fact is that PVP deposition is in a moderate extent in the reticulum cells of bone marrow, though a slight anemia is induced. Kupffer cells of liver also take up PVP but the tissue itself shows no significant damage.

It is not clear why the Kupffer cells or the RE cells of bone marrow take up PVP not so actively but probably this is the reason why PVP can be removed from the blood very slowly.

On the other hand, spleen and lymph nodes are severely affected. These changes are in contrast to the changes induced by the India ink injection, where the RE cells of bone marrow, liver and sinusoid of spleen are heavily laden with soot particles, resulting in a severe anemia, while lymph nodes are relatively free from injury.

It is not definitely clear with what mechanism these differences of phagocytosis occur, but these findings so far described offer an important suggestion that there seems to be some functional difference between the erythropoietic reticulum and the lymphopoietic reticulum.

SUMMARY

Macromolecular PVP was introduced intravenously into rabbits for a long period of 3 months, 10 g of PVP in total, and the observations were carried out to see disturbances in hematopoiesis, lymphopoiesis, and immune reaction with special reference to the histologic changes of marrow, liver, spleen and lymph nodes. The results were as follows:

1. A mild anemia was induced by the PVP injection. RE cells of liver and bone marrow were swollen moderately but otherwise no significant histologic changes were induced in bone marrow and liver.

2. A severe lymphocytopenia resulted: the RE cells of lymph follicles were blocked by PVP, the follicles collapsed into a homogeneous mass with fibrosis and minimized lymphopoietic tissues. These results suggest that RE cells of the so-called germinal centers are important for the reproduction or the differentiation of lymphocytes.

3. Intravenous injection of egg albumin caused the serum antibody formation with a marked proliferation of plasma cells around small vessels in lymph nodes and spleen as in the case of control animal. The data indicate that plasma cells are solely responsible for the serum-antibody formation and plasma cell may differentiate from adventitial cells of small vessels but not from lymphocyte or reticulum cell.

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