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Suppression of antibody formation by the reticuloendothelial (RES)-blockade. I. Effects of the RES-blockade with macromolecular polyvinyl pyrrolidone

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Suppression of antibody formation by the reticuloendothelial (RES)-blockade. I. Effects of the RES-blockade with macromolecular polyvinyl pyrrolidone*

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Abstract

For the purpose of revealing the role of the reticuloendothelial system (RES) for the antibody formation, the rats which received the repeated intraperitoneal and subcutaneous injections of a vast amount of PVP were challenged by bovine serum albumin (BSA) introducing through 2 routes of intramuscular and intravenous, and then antibody formation was observed. Blood cell count and clearance rate of radiogold were observed for the purpose of obtaining the information of blockade grade of the RES by PVP. Phagocytic activity of macrophages ingesting PVP against iron colloid were also observed in vitro. 1. A severe anemia was induced by the administration of a vast amount of PVP, 15 ml of 3% solution daily or every other day for 63 days. Histological picture indicated the suppressed erythropoiesis probably by iron deficiency or the lowered iron transporting activity of the RES, as the anemia recovered after intraperitoneal iron injections. 2. With the generalized and marked swelling of the RES, the cells in germinal center of spleen and lymph nodes were extremely swollen and lymphocytes disappeared completely, suggesting that the macrophages in germinal center play an important role in reproduction and differentiation of lymphocytes. 3. The phagocytic activity of the RES as understood from the clearance rate of radiogold was suppressed only slightly even by a heavy deposition of PVP after the repeated injections. The state of blockade or the suppressed phagocytic activity persisted for 48 hours or more after the several PVP injections. However, complete blockade of the RES or inactivation of the phagocytic activity by PVP injection was not attained. 4. A prolonged treatment of animals with PVP caused delay in the appearance of circulating antibody but the final titration reached the same level as that of control. The data suggest that the blockade of the RES by PVP induces the delay in the transmittance of the information for the antibody formation from the macrophages to the immunologically competent cells but no delay in the ingesting antigen by the macrophages.

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**SUPPRESSION OF ANTIBODY FORMATION BY THE
RETICULOENDOTHELIAL (RES)-BLOCKADE**

**I EFFECTS OF THE RES-BLOCKADE WITH
MACROMOLECULAR POLYVINYL
PYRROLIDONE**

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It has been well established that the phagocytic cells of the reticuloendothelial system (RES), such as Kupffer cells of the liver, sinus-lining and reticular cells of spleen and lymph nodes, and wandering macrophages in the tissue ingest antigen specifically and fix them in the localized area, as has been clarified by many workers who observed the distribution of antigen by COONS' fluorescent antibody technique (1). And yet it is true that lymphocytes and plasma cells are the antibody forming cells. Many investigators have tried to find the antigen in the antibody forming cells only to fail, excepting some cases in which such cells are occasionally situated in the area where the antigen is introduced (2, 3).

The fact suggests that some specific information comes from macrophages to immunologically competent cells such as lymphocytes and plasma cells. FISHMAN'S work (4, 5, 6, 7) indicates that this information will be a kind of RNA. *In vitro* he had succeeded in inducing *de novo* antibody formation of lymph node cells in culture after incubating them with antigen and macrophages, and he also observed antibody formation of the lymphoid cells exposed to extracts from macrophages and disappearance of the effects after treating with RNase. The antibody formation by such cells was not induced unless previously treated with macrophages or their extracts. The fact strongly suggests that RNA released from macrophages which ingest antigen will act as to induce antibody formation in immunologically competent cells.

Electronmicroscope picture suggested that RNA may be transferred by the contact or fusion of macrophages with lymphocytes (8).

From these observations it may be reasonably deduced that the loading of the RES cells with some blockading agent will result in a lowered

production of information bearing substance and the subsequent suppression of antibody formation in immunologically competent cells.

In view of this, the author aimed to observe the effects of the RES blockade on immune response to bovine serum albumin (BSA).

During the past 30 years, a considerable number of experiments have been made on the RES blockade for the purpose of observing the suppression of phagocytic activity by intravenous injection of colloidal particles of a variety of substance (9, 10, 11, 12, 13, 14, 15, 16).

The present paper deals with antibody formation to BSA in the animals whose RES were loaded with polyvinylpyrrolidone (PVP) by the repeated injections of the PVP suspension in saline.

MATERIALS AND METHODS

Sixty Wistar strain young adult male rats, weighing 250–300g were used: Forty of these animals for assessing the RES blockade and twenty of these animals for the antigenic challenge after the blockade of the RES.

These forty animals were used for assessing the grade of the RES blockade by PVP: Twenty of them served as control and the other twenty animals received the PVP injection. They were divided further into 4 subgroups, 5 animals each. First group received intraperitoneal injections of PVP daily for a week, second group every other day for 2 weeks and third group every other day for 3 weeks. In these 3 groups clearance test was performed by injecting radiogold intravenously immediately after the last PVP injection, and in the fourth group which received the same treatment as third group, the clearance test was performed at 12, 24, 48 and 72 hours after the last injection of PVP. The 20 animals as control were also divided into 4 groups, 5 animals each, and received the injections of saline, the same amount through the same routes and same frequencies as PVP injection. In these animals clearance test was made in the same fashion as those of 4 groups which received the PVP injection.

These twenty animals to be challenged by BSA were divided into 2 groups, 10 animals each. The animals in one group were injected 3% PVP solution from subcutaneous and intraperitoneal routes, 15 ml per day every other day for 56 consecutive days and the other animals received saline injection instead of PVP. The 10 animals, 5 animals each from 2 groups were challenged by the injection of antigen starting from the 21st day of the treatment with PVP or saline, and other 10 animals, 5 animals each, served as control without receiving antigenic challenge and blood cell count, hematocrit value and hemoglobin level were observed in the selected animals.

Twenty-four hours before sacrifice all the animals received the intraperitoneal injection of an excess amount of ferric colloid (Blutal, Dainihon Pharmaceutical Inc., Osaka) for the purpose of examining the phagocytic activity of the RES in histological section.

As the blocking agent PVP of 900,000 in molecular weight (Wako Pure

Chemical Industries, Osaka) was used as 3% solution in germ-free physiological saline.

For the clearance test radioactive gold colloid, with a specific activity of more than 5 mc/mg and stabilized with 10 mg/ml sodium acetate, 5 mg/ml ascorbic acid, 3 mg/ml gelatin and 0.9 % benzyl alcohol, (Dainabott Laboratories, Tokyo) was used. Two μ c of radiogold were injected into penis vein and blood samples (0.1 ml) were taken in the heparinized hematocrit tube at successive interval from retro-orbital sinus and radioactivity was measured by the well-type scintillation counter and clearance curves were drawn.

As the antigen, Armour's crystalline BSA was used. 0.5 ml of 1% BSA in saline and 0.5 ml of Freund's complete adjuvant (Difco) conjugate was injected into foot pads, subcutaneous and intramuscular tissue twice with one week interval. Two weeks after the last injection of antigen the additional injection of 0.5 ml of 1% BSA in saline was injected intravenously as a booster. Antibody titration was evaluated by the two-fold serial dilution technique employing BOYDEN's sheep red cell hemagglutination reaction (17).

The phagocytic activity of peritoneal macrophages affected by PVP was also tested *in vitro*. The peritoneal macrophages were obtained from Ehrlich cancer cell ascites of mice by the method of YOKOMURA and others (18) and the phagocytosis of iron colloid by the macrophages loaded with PVP was observed after 10 and 20 minutes incubation.

After sacrifice liver, spleen, mesenterial lymph nodes and bone marrow were fixed in formol, dehydrated with alcohol, embedded in paraffine, sectioned and stained with hematoxylin-eosin for the routine histological section. And these series of the section were stained by PERLS-STIEDA's method for the detection of iron colloid.

RESULTS

By the repeated injections of PVP, red cell count, hemoglobin level and hematocrit value of the circulating blood fell gradually, and on day 28 when the animals were injected about 7.5 g PVP in total, a severe anemia developed; about 3 million per cu mm, 28% Sahli in hemoglobin (Hb) contents and 20% in hematocrit (Ht) value. The severity of the anemia was nearly the same in all the animals. The anemia recovered rapidly by the intraperitoneal injections of an excess amount of iron, 5mg per animals daily for 3 days; 5 million RBC per cu mm, 68% Sahli in Hb and 35% in Ht, 2 weeks after the iron introduction (Fig. 1). Granulocyte count showed no significant changes during the first 4 weeks and thereafter increased slightly toward the end of the experiment (Fig. 2). Monocytes having foamy cytoplasm increased slightly in number.

Clearance test on the animals receiving PVP injections by using radiogold revealed a marked impairment in the clearance rate in removal of

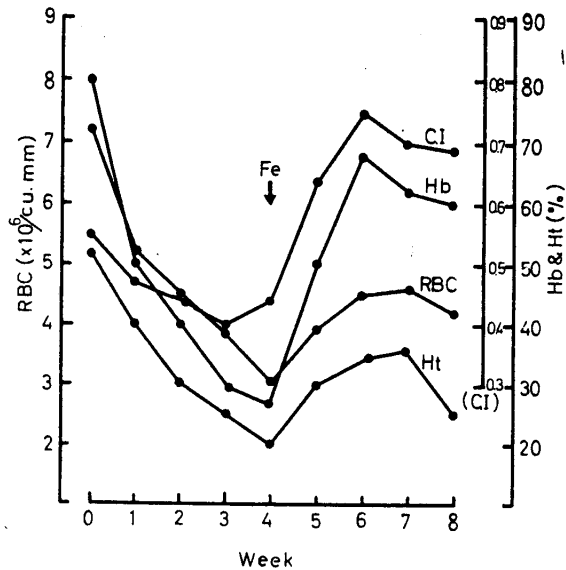


Fig. 1 Changes in RBC number, hemoglobin and hematocrit value of the rats induced by the repeated intraperitoneal and subcutaneous injections of PVP, in 15 ml every other day for 56 consecutive days, about 13.5 g in total. Each curve shows the mean value of 5 animals. RBC; red blood cell count, Hb; hemoglobin level, Ht; Hematocrit value, CI; color index.

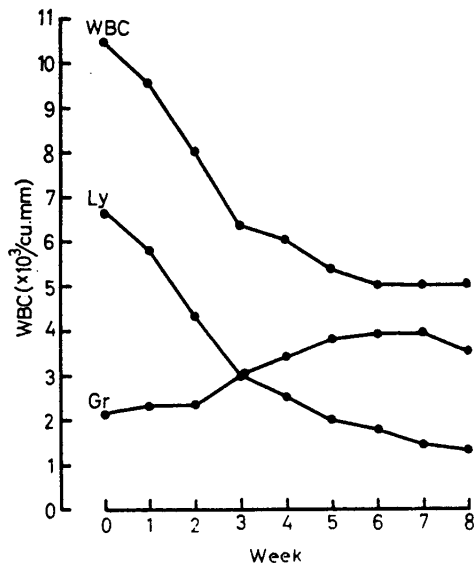


Fig. 2 Change in white blood cell count of the rats. The values are of the same animals appearing in Fig. 1 and each curve gives mean value of 5 animals. WBC; white blood cell, Ly; lymphocyte, Gr; granulocyte.

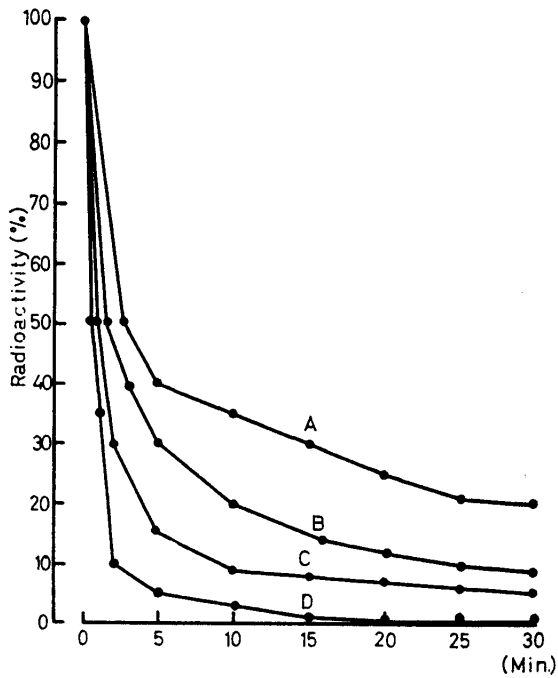


Fig. 3 Changes in clearance rate in removal of radiogold in various preliminary doses and periods of PVP injections. Each curve gives mean value of 5 animals. A; 450mg of PVP per day every other day for 3 weeks, 4.5 g in total. B; 450 mg of PVP per day every other day for 2 weeks, 3.15 g in total. C; 450 mg per day every day for a week, 3.15 g in total. D; control.

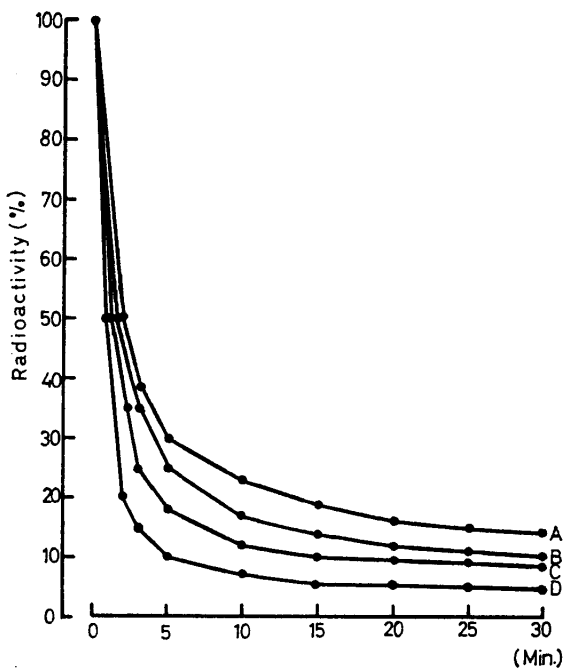


Fig. 4 Changes of clearance rate in removal of radiogold at various time intervals after the repeated injections of PVP, every other day for 3 weeks, 4.5 g in total. Each curve shows mean value of 5 animals. A; 12 hours after the last PVP injection. B; 24 hours after the last PVP injection. C; 48 hours after the last PVP injection. D; 72 hours after the last PVP injection.

radiogold from blood stream and this impaired clearance rate became severe with the increase in dose and period of administration of PVP; an extremely impaired clearance rate in the animals being injected 3.15 g in total, 450 mg per day every other day for 2 weeks.

Further injections of PVP for one additional week resulted in a significant blockade effect with prolonged half-time ranges and high level of persistent radioactivity remaining in the blood stream (Fig. 3). This state of blockade persisted for 48 hours or longer (Fig. 4).

The experiment of the antigenic challenge revealed that all of the 5 animals pretreated with PVP, were poor in antibody titer during 14 days after the initial antigenic stimulation as compared with that of control.

After the booster injection, however, the antigenic titer of these animals rose gradually and reached the level of the control 6 days after the treatment, and later it showed higher level than that of control as revealed by the test on the 8th day and 10th day of the booster injection (Fig. 5).

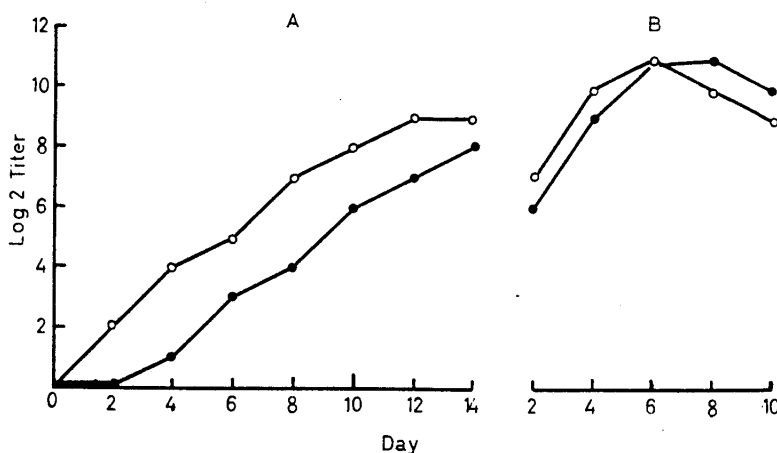


Fig. 5 Serum antibody titration against BSA, mean value of 5 animals.

○—○ : Control ●—● : PVP treated

A; initial antigenic challenge with 0.5 ml of 1 % BSA with Freund's complete adjuvant twice at one week interval.

B; booster injection of 0.5 ml of 1 % BSA intravenously 2 weeks after the last initial antigenic challenge.

The data indicates that the successive intraperitoneal administration of a vast amount of PVP results in the delay in the formation of circulating antibody, though the titer reached the level of that of the control finally.

The peritoneal macrophages taken one hour after the intraperitoneal injection of 2ml of 3% PVP to Ehrlich ascites tumor bearing mice showed

a moderate swelling of cytoplasm filled with PVP. The phagocytic activity of these swollen macrophages was tested by the incubation with iron colloid for 10 and 20 minutes at 37° C. In the cells laden with PVP 20 minutes were required for the ingestion of a moderate amount of iron colloid, while the control receiving no pretreatment of PVP could ingest a large amount of iron colloid by 10 minutes (Table 1).

Table 1 Phagocytic activity of the peritoneal macrophages obtained from Ehrlich ascites pretreated with 50 mg of PVP in incubating with iron colloid for 10 and 20 minutes.

Incubation time (min.)	10	20
Peritoneal macrophages	10	20
PVP treated peritoneal macrophages	-	+
Control	+	+

The data indicate that the blockade of the macrophages with PVP resulted only in a little delay in their phagocytosis of the metal colloid and this is consistent with the observation of the experiment *in vivo*.

Histological observation of the section of the liver, spleen and lymph nodes of the animals receiving the repeated injections of PVP revealed a marked swelling of macrophages in the RES, such as Kupffer cells of the liver, sinus-lining and reticular cells of spleen and lymph nodes. Kupffer cells of the liver were extremely swollen and the parenchymal cells appeared thinner by the pressure atrophy.

In spleen as well as in lymph nodes, lymphocytes disappeared completely with the faded lymph follicles. Foamy reticular cells of germinal centers and large basophilic cells surrounding them predominated the cellular configuration of these organs. But by the prolonged administration of PVP for 50—60 days every other day the lymph follicles turned to a homogenous mass of PVP, finally decomposing the reticular cells that had ingested PVP.

In bone marrow, the erythroblastic islets were atrophic decreasing in both islet number and cell number being surrounded by swollen reticular cells, while the granulopoiesis was not significantly affected.

The antigenic stimulation did not show the recovery of the faded structure of spleen and lymph nodes or the lymphocyte proliferation of these animals pretreated with PVP injection; lymphocytes did not reappear and foamy reticular cells and large basophilic cells surrounding them were found as in the control treated with PVP but without antigenic stimulation.

On the other hand, in spite of the depletion of lymphocytes with complete disappearance of lymph follicles and collapse of germinal centers, a marked proliferation of plasma cells in medullary sinusoid of lymph nodes and in the red pulp of spleen were observed. They seemed to originate from the adventitial cells of small vessels as AMANO had previously pointed out.

Histochemical observation of iron uptake by the RES of liver, spleen and lymph nodes of the animals, which received a vast amount of PVP and followed by an intravenous injection of iron colloid, revealed that the extremely swollen RES cells by PVP uptake still had a marked phagocytic activity showing no depressed state of trapping iron colloid.

DISCUSSION

As has been just demonstrated in this experiment the repeated intraperitoneal administration of a vast amount of PVP for a long period of time caused heavy PVP deposition in the RES cells of spleen and lymph nodes, resulting in complete collapse of germinal center and lymph follicles being accompanied by severe lymphocytopenia in circulating blood. Erythropoiesis in bone marrow was also affected by PVP developing severe anemia. These facts suggested that the well retained function of the RES cells are closely correlated to the specialization of lympho- and erythroblasts.

KOMIYA and his associates (19) succeeded in inducing an experimental aplastic anemia in rabbit by the blockade of the RES with repeated intravenous injections of silver colloid. They are of the opinion that anemia is induced by the disturbances of iron metabolism due to the blockade of the RES.

SENO and AWAI (20) reported that the function of the RES to metabolize iron for hemoglobin synthesis is affected by heavy blockade but the cells generally retained marked phagocytic activity, i. e. the function of the RES for iron metabolism and phagocytosis can be affected independently.

TOYAMA (21) and SENO (22) observed the development of the severe anemia by the blockade of the RES with the repeated injections of carbon particle suspension daily for about 2 months and in their cases they found that the anemia is solely due to the arrested erythroid cell multiplication but not to the hemoglobin synthesis.

The results indicate that the cell specialization process is arrested independent of the iron metabolism. The rats receiving the repeated injections of PVP also developed anemia of slight hypochrome which turned to hyperchromic one by treating the animals with iron. Histological observa-

tion revealed the poorly developed islet with swollen reticular cells in the center. The granulopoiesis remained normal. The findings indicate the suppressed erythroid cell specialization, but no significant change in the granulocyte specialization.

Similar changes as in erythroid cells have been observed in the lymphoid cells, indicating that the specialization of lymphocyte is also closely correlated to the well-retained function of the RES, probably the function to give the information for cell specialization to lymphocyte.

Histological observation showed the accumulation of undifferentiated large basophilic cells in the area surrounding the cells in germinal center which were swollen by engulfing PVP. Antigenic stimulation of these animals treated with PVP seemed to accentuate the swelling of the RES cells never to induce lymphocyte proliferation. The findings suggested that through the pretreatment of PVP the production of cellular antibody will largely be suppressed by the loss of lymphocytes, though in this experiment, special examination was not made to assess the cellular antibody.

NOSSAL *et al.* (23) have called attention to the dendritic macrophage which appeared to store the antigen for prolonged period and which, because of its intimate contact with antibody forming cells, is thought to play an important role in antibody response and concluded that in the process of antibody formation large primitive cells in the rabbit spleen become basophilic and are transformed into immature plasma cells, many of them in turn differentiate into mature plasma cells (24).

After antigenic stimulation in the present experiment the level of serum antibody rose, which was considered to be produced by plasma cells, though with some delay comparing to that of control.

These indicate two important facts; one is that the specialization of plasma cells will not be correlated to the function of the RES cells, and the other is that the information for the synthesis of specific antibody from antigen containing cell (23, 25) is not severely affected by the blockade, though some reduced activity of the RES in sending information may result as understood from the delay in response.

Conflicting opinions may be expected concerning the mechanism of inhibited cell specialization of lymphocyte and erythroblast by the blockade of the RES, i. e. injury of the stem cell by PVP instead of the suppressed information from the damaged macrophage. But the erythrocyte and lymphocyte which contain foreign body are hardly encountered. Therefore there is little possibility that the stem cell becomes impotent by the injection of PVP.

Recent observation by GOWAN (26, 27, 28) and SIMIC (24) revealed

that during the interaction with foreign antigen, some of the small lymphocytes are apparently transformed into large basophilic cells capable of division.

In the present experiment it is quite probable that the large basophilic cells found in the outer skirt of the germinal center will be the dedifferentiated cells which can specialize if the information comes from the macrophages.

AMANO (30, 31) is of the opinion that there are large primitive cells having the potency to differentiate to the reticular cells or macrophages on one hand and the lymphocytes on the other.

This large primitive cell will probably correspond to the large basophilic cell just described.

Concerning the antibody formation of plasma cell, the information may arise from the large dendritic cell in the germinal center.

All these past observations and the data of the present experiment support the view that the information for the specialization of lymphocytes and erythroid cells will be given by the RES cells, and the function of these cells will severely be arrested by the blockade with PVP. The information for the antibody formation from macrophage to plasma cells is hardly intercepted by the blockade of the RES, though some suppression or delay in the transmittance may be the result.

SUMMARY

For the purpose of revealing the role of the reticuloendothelial system (RES) for the antibody formation, the rats which received the repeated intraperitoneal and subcutaneous injections of a vast amount of PVP were challenged by bovine serum albumin (BSA) introducing through 2 routes of intramuscular and intravenous, and then antibody formation was observed.

Blood cell count and clearance rate of radiogold were observed for the purpose of obtaining the information of blockade grade of the RES by PVP.

Phagocytic activity of macrophages ingesting PVP against iron colloid were also observed *in vitro*.

1. A severe anemia was induced by the administration of a vast amount of PVP, 15 ml of 3% solution daily or every other day for 63 days.

Histological picture indicated the suppressed erythropoiesis probably by iron deficiency or the lowered iron transporting activity of the RES, as the anemia recovered after intraperitoneal iron injections.

2. With the generalized and marked swelling of the RES, the cells in germinal center of spleen and lymph nodes were extremely swollen and lymphocytes disappeared completely, suggesting that the macrophages in germinal center play an important role in reproduction and differentiation of lymphocytes.

3. The phagocytic activity of the RES as understood from the clearance rate of radiogold was suppressed only slightly even by a heavy deposition of PVP after the repeated injections. The state of blockade or the suppressed phagocytic activity persisted for 48 hours or more after the several PVP injections. However, complete blockade of the RES or inactivation of the phagocytic activity by PVP injection was not attained.

4. A prolonged treatment of animals with PVP caused delay in the appearance of circulating antibody but the final titration reached the same level as that of control.

The data suggest that the blockade of the RES by PVP induces the delay in the transmittance of the information for the antibody formation from the macrophages to the immunologically competent cells but no delay in the ingesting antigen by the macrophages.

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- Photo. 1 Picture of the spleen of the rat receiving a vast amount of PVP: A marked deposition of PVP and faded structure of lymph follicle.
 - Photo. 2 Picture of the liver of the same animal as Photo. 1: Extremely swollen Kupffer cells of the liver loaded with PVP and pressure atrophy of parenchymal cells.
 - Photo. 3 Picture of the spleen of the animal receiving a vast amount of PVP and then challenged with BSA: Decomposed reticular cells by PVP ingestion in germinal center and cord-like proliferation of plasma cells in medullary sinusoid.
 - Photo. 4 Picture of the spleen of the same animal as Photo 3: Large basophilic cells in outer skirt of affected germinal center and plasma cell proliferation in medullary sinusoid.
 - Photo. 5 Picture of mesenterial lymph node of same animal as Photo. 3, 4. Extremely swollen reticular cells in germinal center and large basophilic cells surrounding them.
 - Photo. 6 Picture of mesenterial lymph node of the same animal as Photo. 6. A marked proliferation of plasma cells in medullary cord.

