crude "toxic fat" has a marked effect on testicular development without appreciably affecting the development of secondary sex characters or growth rate. At levels of 0.5 and 1.0% "toxic fat," reduced testicular development usually occurred in conjunction with hydropericardium and ascites. When the level of fat was reduced to 0.25%, hydropericardium and ascites was markedly reduced, whereas, retarded testicular development persisted. It would appear that in immature chickens testicular hypoplasia resulting from consumption of crude "toxic fat" is a more sensitive index of toxicity than is hydropericardium or ascites.

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Changes in Serum Properdin of Mice During Tumor Growth and Following Immunization to Ehrlich Ascites Carcinoma.* (27101)

M. RICHARD TROEH, CHARLES R. SLATER, WILLIAM M. LARSON, JR. AND RALPH W. MCKEE

Department of Physiological Chemistry, School of Medicine, University of California, Los Angeles

Numerous investigators have observed low levels of serum properdin in patients with advanced cancer(1,2,3,4) and leukemia(5). Ishibashi, *et al.*(6) found that patients, following surgical removal of cancer growths, have serum properdin values higher than non-operated cancer patients. Southam and Pillemer(1) and Rottino, Levy and Conte(2) showed that cancer patients with low levels of serum properdin had a low ability to reject cancer cell homografts while persons with normal serum properdin were able readily to reject the cancer homografts. Herbut and Kraemer(7, 8, 9) showed a striking drop in the serum properdin of C3H mice actively growing Gardner lymphosarcoma, 6C3HED; Bradner and Clarke(10) observed a 6-fold decrease in the serum properdin of mice growing sarcoma, S-180; and Orita(11) has determined that serum properdin of Ehrlich carcinoma bearing C6 mice and Brown-Pierce tumor bearing rabbits varied inversely with tumor growth.

This paper presents data showing: (1) diminution of serum properdin in C57/BL, A/He and C3H mice actively growing Ehrlich ascites carcinoma, L No. 2 lymphoma and Gardner 6C3HED lymphosarcoma, respectively; (2) maintenance of control serum properdin levels, following a transient fall, in C57/BL mice given injections of X-irradiated Ehrlich tumor; and (3) maintenance of control serum properdin values, following a transient drop, in C57/BL mice made resistant to Ehrlich carcinoma by injecting X-irradiated Ehrlich tumor followed by 3 to 5 challenges of completely viable Ehrlich cells.

Materials and methods. C57/BL mice were raised in our laboratory from breeding stock obtained from the Roscoe B. Jackson Memorial Labs., Bar Harbor, Me. A/He and C3H mice were obtained from the Cancer Research Genetics Lab., Univ. of California,

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Berkeley. The mice were males and females from 2 to 4 months of age at start of experiments. All animals were fed Purina Laboratory chow, *ad lib.* Ehrlich ascites carcinoma was maintained as indicated previously(12). L No. 2 lymphoma was obtained from Dr. Theodore S. Hauschka, Roswell Park Memorial Inst., Buffalo, N. Y., and Gardner 6C3HED lymphosarcoma was obtained from the Cancer Research Genetics Lab., Univ. of California, Berkeley, and maintained in A/He and C3H mice, respectively, by weekly intraperitoneal passages. C57/BL and C3H mice received, respectively, 0.1 ml of undiluted Ehrlich and Gardner tumor, while the A/He received 0.2 ml of 1:100 diluted L No. 2 lymphoma. C57/BL mice receiving X-irradiated Ehrlich tumor were given 0.05 ml and from 4 to 9 injections. The tumor was X-irradiated with 2000 r in the manner indicated previously(13). C57/BL mice given X-irradiated tumor were challenged with 0.05 ml of viable Ehrlich tumor. All tumor injections were made intraperitoneally as the ascitic forms.

Blood removed from mice under ether anaesthesia was obtained from the subclavian vein by means of a bulb pipette after cutting the vein and allowing blood to collect in the triangular pocketed area between forelimb and chest. The blood was placed in a 12 ml centrifuge tube, inclined at about a 60° angle and allowed to stand at least 1 hour. The clot was loosened from side of tube and centrifuged for 10 minutes at 2500 rpm. The serum was removed and aliquots of 0.05 and 0.10 ml placed in small glass tubes and stored at –20°C. At time of analysis a tube of serum containing an appropriate volume of serum was thawed and diluted 1:100 with Tris buffer for assay as indicated below.

The method of McNall(14) was employed for estimation of properdin in sera of the mice. A number of modifications suggested by McNall(15) were instituted. Tris buffer, instead of barbital buffer, was employed throughout. This buffer had the following composition: 0.85% NaCl, 0.1% Tris, 5×10^{-3} M Mg^{++} and 5×10^{-3} M Ca^{++} ($Mg\,Cl_2$ and $Ca\,Cl_2$). In the properdin-inulin complex preparation, water recrystallized inulin (Difco, control 438138) was employed. Temperature of incubation was 5-10° instead of 20°C. For preparation of R3, recrystallized inulin was used instead of zymosan, and incubation carried out at 5-10°, instead of 37°C. The final preparation was completely freed of inulin by centrifugation for 15 minutes at 5000 g and 5°C followed by filtration through an ultrafine sintered glass filter. In the preparation of RP, recrystallized inulin was employed instead of zymosan (0.25 mg inulin/ml of guinea pig serum) with incubation at 0-5°C instead of room temperature. The final preparation was centrifuged at high speed and then filtered, as was the R3. The major advantages achieved in these modifications were increased stability of the components of complement, with the yielding of R3 and RP preparations of maximum potency and stability. Furthermore, centrifugation prior to adding the activated sheep cells, was found to be unnecessary and was eliminated. The acid hematin procedure was employed for measuring concentration of activated sheep cells. The sheep cells were not used after 2 weeks of storage because they occasionally were unstable. Each new batch of hemolysin, RP and R3 was titrated and thoroughly standardized. One ml aliquots of 1:100 diluted sera were employed for assay. Duplicate analyses were made and the checks were uniformly satisfactory. Properdin units per ml of whole serum were determined by obtaining the difference in optical density between the tubes with and without inulin, dividing this difference by the optical density for 50% hemolysis, and multiplying by 100.

Results. Groups of animals, the numbers of which are indicated in the legend of the figure, were sacrificed 1, 3, 5, 7 and 9 days after receiving a single viable tumor injection. Serum properdin values for C57/BL, C3H and A/He mice growing, respectively, Ehrlich carcinoma, Gardner lymphosarcoma and L No. 2 lymphoma are shown in Fig. 1. Control mice are the zero time animals.

Properdin values for sera of control C57/BL mice ranged from 36 to 95 units, average value of 60. C57/BL mice growing Ehrlich carcinoma showed a significant decrease of properdin one day after tumor injection with

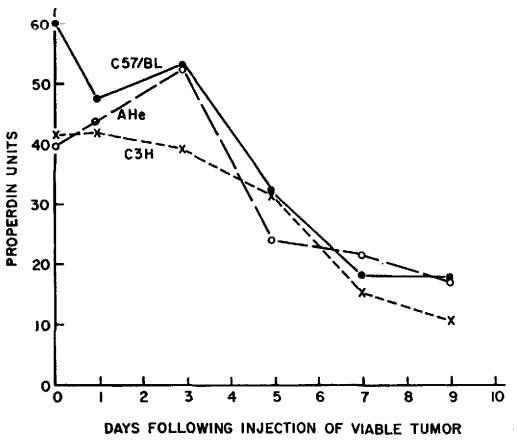

FIG. 1. Serum properdin levels at different stages of growth of Ehrlich ascites carcinoma in C57/BL mice (———); L No. 2 lymphoma in A/He mice (— — —); and Gardner lymphosarcoma in C3H mice (– – – –). No. of mice used, respectively, at 0, 1, 3, 5, 7 and 9 days were: 21, 5, 5, 4, 20 and 5 of C57/BL; 8, 5, 6, 5, 6 and 3 of A/He; and 8, 5, 5, 5, 5 and 2 of C3H.

an average value of 47 ($P = 0.03$). At 3 days there was a rebound to values not significantly different from those for control mice (average 54 units). At 5, 7 and 9 days there were highly significant decreases of serum properdin from control level ($P < 0.01$), with average values of 33, 19 and 18 units, respectively. The 7 and 9 day values were significantly lower than the 5 day values ($P = 0.02$). In contrast to this marked drop of serum properdin following a single injection of completely viable non-irradiated Ehrlich tumor is an observation with 15 mice that an injection of 2000 r X-irradiated Ehrlich carcinoma caused only a transient drop of serum properdin in the first 2 days, with a return to and maintenance at values not significantly different from control values (average 54 units).

Serum properdin values of control A/He mice range from 31 to 51 units, average value of 40. A/He mice growing L No. 2 lymphoma showed a non-significant change of serum properdin at one day (average 44 units); at 3 days there was a significant rise ($P = 0.01$), average of 52 units; and at 5, 7 and 9 days there was a highly significant drop from the control values ($P < 0.01$), an average of 25, 21 and 18 units, respectively.

Properdin values for the sera of control C3H mice ranged from 25 to 57 units, average value of 42. C3H mice growing Gardner lymphosarcoma showed non-significant changes from normal at 1, 3 and 5 days, average values of 42, 39 and 33 units, respectively. However, at 7 and 9 days there were highly significant decreases from the controls ($P < 0.01$), average values of 14 and 12 units.

Studies, not shown in Fig. 1, were made determining serum properdin changes in C57/BL mice following multiple (4 to 9) injections of 2000 r X-irradiated Ehrlich carcinoma. One and 2 days following the injections there was a significant drop ($P < 0.01$) of properdin, average value for 5 mice was 44 units. However, this was followed by a significant rise back to control values, average of 61 units for 13 animals. These control values were maintained, for as long as 104 days.

Additional studies, not shown in Fig. 1, were made determining serum properdin in C57/BL mice following 8 injections of 2000 r X-irradiated Ehrlich tumor and variable numbers of challenges with completely viable non-X-irradiated tumor. The 31 animals receiving the series of X-irradiated tumor plus 3 to 5 challenges had a non-significant change of serum properdin compared with the controls ($P = 0.1$), average value of 67 units. However, 20 mice receiving the series of X-irradiated tumor plus 11 to 16 challenges had a significantly lower serum properdin ($P < 0.01$), average value of 47 units.

Discussion. At present the role of properdin in the blood of animals and the relationships of this protein to cancer, or to any disease, are unknown(16). Unpublished data from our laboratory indicated that C57/BL mice immunized against Ehrlich carcinoma cells by multiple injection of 2000 r X-irradiated Ehrlich tumor showed the presence of increased quantities of serum α-2 and γ globulins. Also, the complement-fixation titre rose remarkably. This rise took place even during the first 2 days when the properdin was dropping, suggesting the possibility that properdin may be a precursor or a stimulator for production of specific antibodies.

Further studies will be necessary to establish the relationship.

Summary. Employing a modified McNall procedure for determining serum properdin it was shown that in C57/BL mice growing Ehrlich ascites carcinoma there was a progressive drop of serum properdin after 3 days. Similar decreases of serum properdin values in C3H and A/He mice occurred when they were growing Gardner lymphosarcoma and L No. 2 lymphoma, respectively. On the contrary, when C57/BL mice were given either repeated injections of 2000 r X-irradiated Ehrlich carcinoma, or repeated injections of X-irradiated tumor and 3 to 5 challenges of viable tumor, there was a transient drop with a return to a normal blood level of properdin. However, numerous challenges(11-16) of mice that received repeated injections of X-irradiated tumor resulted in a significant lowering of the serum properdin, but not to levels produced with actively growing tumor. There appeared to be a correlation of serum properdin with cancer growth. During cancer growth there was a fall in serum properdin, while regression was accompanied by a rise back to control levels of serum properdin.

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Detection of Antibody to Urease by Hemagglutination.* (27102)

W. J. VISEK, M. E. IWERT AND W. BURROWS

Departments of Pharmacology and Microbiology, University of Chicago, Chicago, Ill.

Since the demonstration by Kirk and Sumner(1) that rabbit homologous antisera will precipitate jackbean urease, the antigenicity of enzymes has become well established(2). The serologic assay apparently depends upon the antigenicity of the protein moiety. It is independent of the prosthetic group of the enzyme(3) and therefore may be considered to be indirect. The *in vitro* antigen-antibody reaction is demonstrable as specific precipitation of the enzyme by its homologous antibody in the equivalence zone or in the region of slight antigen excess. In the case of urease-antiurease, it has been quantitated by Marucci and Mayer(4) to give precipitin units as antibody nitrogen. The precipitin test is not suitable, however, for quantitative assay of small amounts of antibody. Assay of antibody by the agglutination of inert particles coated with soluble antigen has proved to be a more sensitive method than the precipitin test. Of such particles the red blood cell has been the most widely used(5). Both protein and polysaccharide antigens are readily and firmly adsorbed by tannic acid treated fresh cells which are agglutinated, often in

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