

Reduction and Alkylation of Immunoglobulins from Normal, Irradiated And Splenectomized Rabbits. (31941)

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Reduction and alkylation of the immunoglobulins of rabbit or of other animal species results in a mixture of their heavy (H) and light (L) chains. Starch gel electrophoresis at pH 8.2 reveals that the H chains migrate slowly toward the cathode but can be resolved into several bands(1); the L chains, under the same conditions migrate anodically in 6 to 10 separate bands (2-7). The origin of the various chains is yet unknown. Cohen and Porter (2) suggested that the different L chains may originate in different cells. It seemed conceivable that the various chains are formed in different organs or that one organ would produce predominantly one or two types of L chains. To investigate this possibility, the reduced and alkylated γ -globulins from "normal" rabbits were compared with those of splenectomized rabbits, and also with the γ -globulins of rabbits which were exposed to X-rays while their spleen was shielded.

Experimental splenectomy. New Zealand white rabbits were anesthetized with urethane and nembutal and an incision was made on the linea alba in the region of the spleen. The veins leading to the spleen were ligated and cut. After the spleen was removed, 3-4 ml of 0.85% NaCl containing 1600 units of penicillin and 4 mg streptomycin per ml were placed in the abdominal cavity. Immunization was begun one week after splenectomy.

X-Irradiation. New Zealand white rabbits and wild rabbits were exposed to X-rays (8) at a distance of 100 cm from source to subject. The G. E. Maxtron was operated at 250 kilovolts and 25 milliamps with 0.5 mm Cu and 1 mm Al filters. The animals were

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TABLE I. Conditions for Experiment with X-Irradiated Animals.

Rabbit No.	Type of rabbit	Shield	Antigen injection
Set I			
860	White	Spleen	None
861	"	"	"
870	Wild	"	Ars-R ₁ N-BSA
865	White	None	"
Set II			
862	White	Spleen	Ars-R ₁ N-BSA
863	"	"	None
864	"	"	"
867	"	None	"
868	"	"	"
871	Wild	Spleen	Ars-R ₁ N-BSA

anesthetized with approximately 700 mg urethane per kilogram body weight, followed by nembutal. They were then placed on their sides and irradiated. A shield for the spleen was prepared by placing 5 lb of lead pellets into a 6 × 24 cm cloth bag. The shield was placed on a piece of aluminum, 6 × 24 cm, which was bent to fit across the rabbit in the region of the spleen during the irradiation. In all experiments, the animals received an initial dose of 500 roentgens at the rate of 30-35 roentgens per minute.

Immunization. Ten rabbits were irradiated and immunized under various conditions (Table I). In addition to the initial dose of 500 r, the animals in set I received another dose of 200 roentgens and those in set II (Table I), 300 roentgens, every 14 days. Four of the animals were injected intramuscularly with As-R₄N-BSA, *i.e.*, bovine serum albumin coupled with equivalent amounts of diazotized arsanilic acid and p-aminophenyl-N-trimethylammonium chloride, as described earlier(7). Each rabbit received 20 mg As-R₄N-BSA with Freund's complete adjuvant, 24 hours after the initial irradiation, and a booster injection of 3 mg As-R₄N-BSA intravenously without Freund's adjuvant, 29 days after the initial injection. These 4 rab-

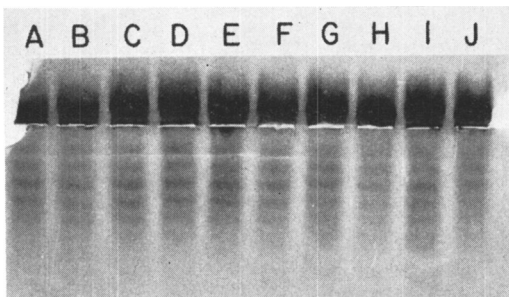


FIG. 1. Starch gel electrophoresis at pH 7.0 of rabbit γ -globulin obtained at various times after irradiation. The γ -globulin was reduced and alkylated in 6 M urea, pH 8.2. Samples A-E are from rabbit No. 860 and samples F-J are from rabbit No. 864. A and F = before first irradiation; B and G = day 6 after first irradiation; C and H = day 12 and 13; D and J = day 20; E and J = day 27.

bits (Table I) were bled 6 through 11 days after the booster injection. Only one of the 4 rabbits (No. 870) produced enough hapten-specific antibody to be isolated; this was 4 mg of R_4N -specific antibody. The remaining 6 rabbits were bled for approximately 20 ml of blood 6 days following the initial irradiation and every week following, for 7 weeks. The γ -globulin from each bleeding was obtained by Na_2SO_4 precipitation.

Isolation of normal γ -globulins and antibodies. γ -Globulins were salted out with Na_2SO_4 according to the method of Kekwick (9). The final precipitation with 12% Na_2SO_4 was repeated 2 times and the γ -globulins were dialyzed exhaustively against water and lyophilized. Rabbits were injected with doubly labelled antigen, As- R_4N -BSA, as previously described (10). The hapten-specific antibodies were isolated from As- and R_4N -specific cellulose columns (10).

Starch gel electrophoresis. Following reduction and alkylation of the immunoglobulins, vertical starch gel electrophoresis was performed in 6 M urea in a manner similar to that described previously (7). The gel (15% starch) was prepared with 0.005 M phosphate, pH 7.0; the electrode vessels contained 0.067 M phosphate, of the same pH. Anodic migration was downward.

Results and discussion. Fig. 1 shows the similarity of reduced and alkylated γ -globulin taken before the first exposure to X-rays, and γ -globulin isolated at various times after the first irradiation. The L chains migrate

toward the anode and the H chains migrate toward the cathode. The electrophoretic pattern of reduced and alkylated γ -globulin from all ten rabbits (Table I) was similar. No significant difference in the number, position, or intensity of the L chain bands was found in any of the rabbits.

A comparison of reduced and alkylated γ -globulin from a splenectomized rabbit and from "normal" rabbits which were neither injected, splenectomized, nor irradiated, revealed no differences in the L chain pattern (Fig. 2). Hapten-specific antibody from the splenectomized rabbits had electrophoretic patterns similar to the hapten-specific antibody isolated from a "normal" immunized rabbit and also to γ -globulin (Fig. 2).

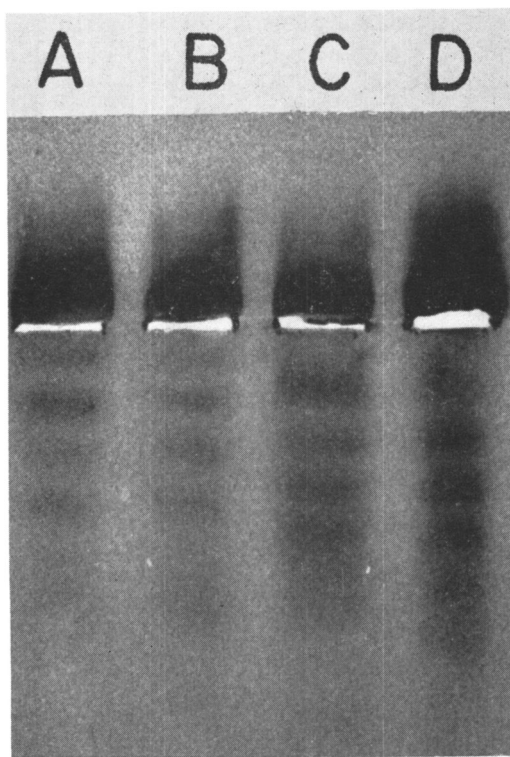


FIG. 2. Starch gel electrophoresis at pH 7.0, of reduced and alkylated hapten-specific antibody and γ -globulin from a splenectomized rabbit, No. 850. The rabbit was injected with doubly labelled antigen, As- R_4N -BSA, and the hapten-specific antibodies were isolated on hapten-specific immunosorbents. Both hapten-specific antibodies were prepared from the same serum sample of rabbit No. 850. A = Ars-specific antibody; B = R_4N -specific antibody; C = γ -globulin from splenectomized rabbit; D = γ -globulin from "normal" uninjected rabbit.

Taliaferro *et al* (11,12) have shown by serum hemolysin titers that the immune response can be completely, or almost completely, suppressed in a rabbit by one dose of 500 r of X-rays, if the antigen is injected later than 6 hours after the exposure to X-rays. Since the hemolysin titer becomes detectable again approximately 13 ± 1 days after the irradiation (13), our animals were given an additional dose of 200-300 r every 2 weeks after the initial dose of 500 r. In this manner, we expected to keep the immune mechanism suppressed (13).

The irradiated animals synthesized very little, if any, precipitating hapten-specific antibody since we were able to isolate antibody from only one rabbit, and that was only 4 mg. γ -Globulin was, apparently, synthesized, as a significant amount was obtained by Na_2SO_4 precipitation. The amount of γ -globulin obtained from any of the X-rayed animals did not differ significantly from the amount obtained before the first irradiation. Since the spleen shield was placed across the rabbits body, some of the mesenteric lymph nodes were also shielded.

The L chain patterns of the reduced and alkylated γ -globulins from X-rayed, splenectomized and normal rabbits seemed to be identical. If large amounts of certain types of L chains were formed in the normal spleen, the animals irradiated with shielded spleens should have yielded an electrophoretic pattern in which the bands corresponding to these chains would be more intense than the other bands. For the same reason, the splenectomized animals should have produced patterns in which the same L chain bands would be less intense. In the normal, untreated rabbits, both splenic and extrasplenic γ -globulins would be produced. Since no differences in the L-chain patterns of reduced and alkylated γ -globulins were detected in the irradiated, the splenectomized and the normal rabbits, none of the observed L chain bands seems to be synthesized predominantly in the spleen.

Peptide maps (7) of tryptic digests of γ -globulin preparations of the splenectomized rabbits and the X-rayed rabbits with the spleen shielded were identical to "normal" γ -globulin. Moreover, the ratio of the amounts

of Porter's fragment I to those of fragment II (15,16) in the irradiated animals did not change during the experiment, and was similar to that in "normal" γ -globulin, and in the γ -globulin of the splenectomized rabbits. The results obtained indicate that the L chains of γ -globulins formed in the spleen do not differ in their multiple electrophoretic bands from the γ -globulins formed in other organs of the lymphoid system.

Summary. The L chain patterns of reduced and alkylated γ -globulin preparations from splenectomized, irradiated and normal rabbits were compared by starch gel electrophoresis. Animals irradiated with their spleen shielded produced γ -globulin with L chains similar to those of splenectomized and normal rabbits. Thus, the L chains formed in the spleen apparently do not differ electrophoretically from the L chains of γ -globulin formed in other organs.

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Human Leukemic Polyribosomes.* (31942)

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The important discovery that polyribosomes (PRS) are connected by strands of messenger RNA led to the finding that synthesis of proteins occurs on the polyribosome complex(1,2,3). Our knowledge of mammalian cells is largely based on studies with reticulocytes, rat liver and HeLa cells, in which PRS appear to be held together by a relatively stable messenger RNA(1). Aging of reticulocytes results in a decreased capacity to synthesize protein and a progressive decrease in PRS(4).

There are no reports on PRS profiles of lymphocytes or granulocytes and the number of tissues examined is still insufficient for an adequate comparison of normal with malignant cells. The purpose of this paper is to report the analysis of PRS in normal granulocytes and in a variety of human leukemic cell types. The PRS patterns of these human leukocytes differ from most other mammalian cells which have been studied.

Materials and methods. PRS preparations were made from human leukemic leukocytes (1 normal, 3 acute granulocyte leukemia [AGL], 4 chronic granulocytic leukemia [CGL], and 3 chronic lymphocytic leukemia [CLL]). Heparinized whole blood was allowed to sediment for 30-45 minutes at room temperature and the leukocyte-rich plasma layer was then removed by aspiration. Labeled PRS were prepared after incubating leukocytes for 30 minutes at 37°C with 2 μ C/ml uridine-H³ (Sp. Act. 3.62/c/mM New England Nuclear Corp.). A minimum of 9.0×10^8 cells were used per preparation. Incubation was omitted whenever non-labeled preparations were made.

Polyribosomes were prepared from leuko-

cytes by two different methods. Method I was reported by Penman *et al*(5) for the preparation of HeLa cell PRS. This procedure utilized sodium deoxycholate (DOC) treated postmitochondrial supernatants layered over linear sucrose gradients of 5-20% (w/w). Method II, a technique originally used by Wettstein *et al*(3), was modified and adapted for this work as follows: after incubation, the cells were chilled to 4°C then washed twice with normal saline. The cells were suspended in RSB buffer (RSB buffer = 10^{-2} M Tris pH 7.4; 10^{-2} M KCL; and 1.5×10^{-3} M Mg Cl₂) 1:10 (v/v) for approximately 10 minutes and subsequently homogenized in a T-Dounce homogenizer. During homogenization, the cells were examined for breakage by light microscopy and homogenization was stopped when most of the cells were broken. The homogenate was then centrifuged at $27,000 \times g$ for 30 minutes, and the microsomal supernatant thus obtained diluted with medium A without sucrose (1:1 v/v). DOC was added to a final concentration of 0.005 mg/ml. The mixture was allowed to stand for 10 minutes, and then layered above a discontinuous sucrose gradient(3). Centrifugation was carried out at $105,000 \times g$ for 2½ hours in a Spinco model L ultracentrifuge. After centrifugation, the material found in the bottom of the 2 M sucrose layer, plus any other pelleted material found at the bottom of the tubes, was suspended in medium A without sucrose and concentrated by recentrifugation. Ribosomes thus obtained were dissolved in a minimum volume of medium A and 10-20 OD units (260 m μ) layered over 7-34% (w/w) exponential sucrose gradients. The gradients were centrifuged at 25,000 RPM for 2½ hours on an SW 25.1 rotor. Gradient analysis was performed by punc-

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