Bactericidal Activity of Phagocytin from Rabbits Exposed to Whole Body X-Radiation. (27472)

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Hirsch(1) has extracted from rabbit heterophils a bactericidal substance called phagocytin. Experiments reported herein were undertaken to determine what effect exposure of rabbits to whole body x-radiation might have on the bactericidal activity of phagocytin since a number of investigators(2,3,4) have reported impairment of the intracellular digestive ability of peritoneal phagocytes obtained from irradiated rabbits, rats or mice.

Materials and methods. Rabbits. New Zealand albino female rabbits weighing between 1.7 and 2.8 kg were housed individually in an air conditioned, humidity controlled room with food and water available *ad libitum. Irradiation.* Rabbits were exposed to 400 or 500 r whole body x-radiation by means of a Picker x-ray machine. Radiation factors were 210 kv, 20 ma, target distance to midline of the body 96 cm and a dose rate of approximately 13.8 r per minute. No filtration was used. The animals were put into a perforated lucite box and the dose delivered half to one side of the animal and half to the other side. *Exudates.* Exudates were induced and processed according to the method described by Hirsch(1). A counting chamber differential showed the cells to be 98% polymorphonuclear. Irradiated rabbits (400-500 r) on the 1st or 5th day post-irradiation yielded cell counts ranging from 3.8×10^7 to 1.1×10^9. Thirty days after 400 r the range was 1.6×10^9 to 2.1×10^9. Unirradiated rabbits yielded cell counts ranging from 6.3×10^8 to 1.8×10^9. *Preparation of phagocytin.* Cells were extracted in a salt solution (ICS) duplicating the cationic composition of rabbit heterophil cytoplasm(1). Washed, centrifuged cells were suspended in ICS, the volume determined by the number of cells present. In this way phagocytin in each test was obtained from approximately the same number of cells. The suspension was subjected to 3 cycles of rapid freezing and thawing (37° water bath) after which it was spun 15 min. at 3000 rpm. The viscous supernate contained the bactericidal activity. *Test microorganisms.* All of the test microorganisms were gram-negative enteric bacilli. They were strains of *Escherichia coli, Salmonella enteritidis, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* Cultures used in the bactericidal tests were 18-24 hours old. *Bactericidal tests.* Serial 2-fold dilutions of cell extract were made in ICS, the final volume being 0.4 ml. Bacterial suspensions were prepared according to the technic described by Hammond, Anderle and Miller(5). Approximately 300 microorganisms in 0.1 ml of ICS were added to each tube. The tubes were incubated for 1 hour in a 37° water bath after which 0.1 ml was pipetted onto each of 3 nutrient agar plates and spread by shaking 5 sterile glass beads over the surface of the inoculated plates. After 24 hours incubation colonies were counted to determine the number of surviving bacteria. An ICS control was included in each test.

Results. Mean cell exudate counts are shown in Table I. Cell counts decreased the 1st day after the rabbits had been exposed to 400 or 500 r. Five days post-irradiation the counts were higher than on the 1st day and were, in fact, almost the same whether the rabbits had received 400 or 500 r. More cells were harvested from rabbits 30 days post-irradiation than from unirradiated rabbits.

Results of the bactericidal tests are shown in Fig. 1. The reciprocal of the highest dilution of phagocytin producing greater than 50% killing of microorganisms was taken as the endpoint. In Fig. 1 the mean titer in control rabbits is shown as 100% and the mean titers in irradiated rabbits as percent of control. It is apparent that one day after exposure to 400 r and one and 5 days after exposure to 500 r, phagocytin activity against all of the test microorganisms was depressed. The reduction in titer was observed consistently in all irradiated rabbits. Only a slight

TABLE I. Exudate Cell Counts from Peritoneal Fluid of Control and Irradiated Rabbits.

	Mean cell counts	No. rabbits
Control	1.3×10^6	5
1 day after 400 r	3.2×10^5	4
1 day after 500 r	1.9×10^5	4
5 days after 400 r	6.7×10^5	4
5 days after 500 r	6.6×10^5	5
30 days after 400 r	1.9×10^6	3

inhibition of phagocytin titer occurred 5 days after 400 r. Thirty days post-irradiation (400 r) there was a marked increase in bactericidal activity.

Discussion. Fishman and Sheckmeister(2) prepared extracts from granulocytes obtained from the peritoneal cavities of irradiated (600 r) and unirradiated rats. They found the extracts to be bactericidal for *Micrococcus aureus* on the 1st day post-irradiation but not on the 3rd day. Donaldson *et al.*(3) investigated the digestive ability of peritoneal macrophages obtained from irradiated mice. Intracellular digestion was not significantly depressed on the 1st day after exposure to 350 or 450 r but was on the 6th day. Eighteen days after exposure to 350 r it had returned to normal. It did not return to normal until 26 days after exposure to 450 r. The same workers found the digestive ability of rabbit peritoneal macrophages to be depressed 7 days after 600 r. In the present experiments phagocytin activity was lowest on the 1st day after the rabbits had been exposed to 400 or 500 r. It was almost normal on the 5th day after 400 r but had not yet returned to normal on the 5th day after 500 r. There is some indication, therefore, that the length of time phagocytin activity is depressed may be related to the dose of radiation. The increase in titer observed 30 days after 400 r is thought to be an indication of over-compensation for the original radiation insult. The increase in exudate cell yield at that time is probably a reflection of such a process.

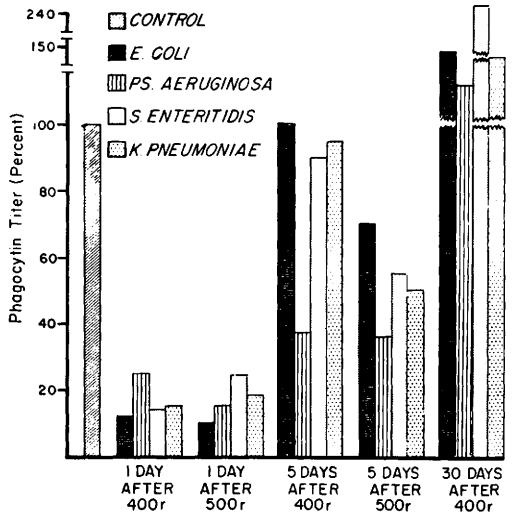

FIG. 1. Mean phagocytin titers as percent of control.

Following completion of the present studies Cohn and Hirsch(6) and Hirsch and Cohn (7) reported phagocytin and various enzymes to be contained in the granules of rabbit heterophils. These substances were released from the granules after the phagocyte ingested foreign material. There was a striking reduction in phagocytin obtained from heterophils following the uptake of live or heat killed bacteria. As phagocytin activity decreased a gross degranulation of cells was observed. Since in the present experiments heterophils were observed in the hemacytometer but not in stained smears, the condition of the granules cannot be described with any accuracy. Jacobson(8) found giant heterophils, metamyelocytes, myeloctes and degenerating granulocytes present in peripheral blood of irradiated (>300 r) rabbits during the first 4-5 days post-irradiation. It is conceivable that the reduction in phagocytin activity observed 1 and 5 days post-irradiation was due to the presence of cells containing faulty or immature granules whose phagocytin activity was impaired. Whatever the explanation, it is apparent that under the conditions of the experimental methods employed, phagocytin activity was decreased 1 and 5 days after 500 r and 1 day after 400 r.

Hirsch(9) has recently reported that administration of 25 mg per kg of cortisone for 10 days did not alter phagocytin activity of rabbit heterophils. Cortisone and x-radiation produce some similar physiological, histological and immunological changes among which is an increased susceptibility to infection. The mechanisms by which susceptibility to

infection is increased after x-radiation and after cortisone administration are, however, quite different. When an animal is exposed to moderate doses of x-radiation severe injury to the bone marrow and lymphoid tissue occurs. All of the formed blood elements are markedly depressed for 2-3 weeks. The functional capacity of the reticuloendothelial system is disturbed and capillary permeability is increased. The inflammatory response is suppressed due to scarcity of cells. Although cortisone treatment does induce atrophy of lymphoid tissue, it does not impair the function of either bone marrow or reticuloendothelial system. In fact, the number of neutrophils is usually increased during cortisone therapy. The inflammatory response is suppressed largely because of decreased capillary permeability. The increased susceptibility to infection following cortisone therapy appears to be almost secondary to the suppression of the protective inflammatory response(10,11, 12).

Summary. Peritoneal exudates were induced in female rabbits and collected either 1, 5 or 30 days after total body exposure to 400 r and 1 or 5 days after 500 r. Exudate cells from unirradiated rabbits served as controls. Phagocytin was extracted from the exudate cells (heterophils) and tested for bactericidal activity against *Escherichia coli, Salmonella enteritidis, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* Phagocytin from cells collected 1 day after exposure to 400 or 500 r and 5 days after exposure to 500 r showed a decreased activity against all the test microorganisms. Phagocytin from cells collected 5 days after exposure to 400 r showed only a slight change in activity from controls. A marked increase in phagocytin activity was observed in extracts of cells collected 30 days after exposure to 400 r.

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1. Hirsch, J. G., *J. Exp. Med.,* 1956, v103, 589.
2. Fishman, M., Sheckmeister, I. L., *J. Exp. Med.,* 1955, v101, 275.
3. Donaldson, D. M., Marcus, S., Gyi, K. K., Perkins, E. H., *J. Immunol.,* 1956, v76, 192.
4. Nelson, E. L., Becker, J. B., *J. Inf. Dis.,* 1959, v104, 13.
5. Hammond, C. W., Anderle, S. K., Miller, C. P., PROC. SOC. EXP. BIOL. AND MED., 1961, v108, 183.
6. Cohn, Z. A., Hirsch, J. G., *J. Exp. Med.,* 1960, v112, 983.
7. Hirsch, J. G., Cohn, Z. A., *ibid.,* 1960, v112, 1005.
8. Jacobson, L. O., Marks, E. K., Simmons, E. L., Hagen, C. W., Jr., Zirkle, R. E., in *Biological Effects of External X and Gamma Radiation,* edit. by Raymond E. Zirkle, McGraw-Hill Book Co., Inc., New York-Toronto-London, 1954, p283.
9. Hirsch, J. G., Church, A. B., *Bact. Proc.,* 1961, p135.
10. Gell, P. H., Hinde, I. T., *Brit. J. Exp. Path.,* 1953, v34, 273.
11. Germuth, F. G., *Pharm. Rev.,* 1956, v8, 1.
12. Thomas, L., *Ann. N. Y. Acad. Sci.,* 1953, v56, 799.

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Effect of Postganglionic Mesenteric Sympathectomy in Irreversible Hemorrhagic Shock.* (27473)

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Recent investigation has implicated the gastro-intestinal tract as one of the prime organs responsible for irreversibility of experimental shock. The most striking autopsy finding of irreversible endotoxic or hemorrhagic shock is the diffuse hemorrhagic necrosis of the bowel. Irreversibility and bowel necrosis can be prevented by pretreatment of animals

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