Hypothermia, Radiation, and the Immune Response (34655)

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High levels of ionizing radiation (c. 1000 R) result in increased mortality of mammals following whole-body exposure. The pathology leading to death is primarily due to hematopoietic and gastrointestinal syndromes. Specific manifestations include: modified absorption and pathologic lesions of the intestinal mucosa (1), destruction of almost all medium sized lymphocytes within a few hours and a diminution in heterophils somewhat later (2). The latter stage not only leads to a direct impairment of defense mechanisms, *i.e.*, antibody formation and phagocytosis, but may also adversely affect immunity by a derangement of the normal metabolic functions of the cells involved.

One could postulate that, since early damage evoked by ionizing radiation seems to be associated with those cells having high mitotic indices (bone marrow, lymphoid follices, intestinal mucosa), a depression of metabolic rates and subsequently mitotic activity might have a radio-protective effect. Two forms of depressed metabolism which are characterized by reduced metabolic activity and reduced radiosensitivity are hibernation and hypothermia. Substantive reviews of the early literature are presented in works by Smith (3), Weiss (4) and Musacchia and Barr (5).

South and Andjus (6) showed that hamsters in deep hypothermia (0°) had a mean survival time (MST) of 49.5 days compared to 6 days following whole-body irradiation of 2000 R. Barr and Musacchia (7) noted that many hibernating ground squirrels exposed to lethal levels of radiation survived for post-arousal periods of 1 month and longer, whereas animals irradiated in the active state died much sooner. Musacchia and Barr (8) postulated that a variety of physiological features, such as reduced mitotic activity, tissue hypoxia, and increased chemical protectants, could account for the decreased radiosensitivity. Recently, Musacchia and Barr (5) reported increased radioresistance in "helium-cold" hypothermic hamsters.

In a comparison of the radio-protective effects of hibernation and hypothermia, these same workers were able to show that both states of depressed metabolism exerted some degree of radio-protection (5). The MST at 2000 R was about 19 days versus 8.5 days for ground squirrels irradiated at a body temperature of 5 and at 37° respectively. The MST at the same radiation dose was 8 days versus about 6 days for hypothermic hamsters at 5°.

In view of these findings with respect to the radio-protective effects of depressed metabolism together with the finding that circulating antibody is formed by hibernating ground squirrels (9), it became of interest to determine if there was any protective effect afforded to those cells which synthesize immunoglobulins.

Materials and Methods. Animals. Immunologically mature hamsters (*Mesocricetus auratus*), from 2 to 4 months old of random sex, were used from a colony which has been inbred since the fall of 1965. All hamsters were given a commercial lab chow (Wayne Lab Blox), a weekly fresh vegetable supplement and water *ad libitum.*

Antigen. Influenza A virus vaccine (PR8) was supplied through the courtesy of Dr. E. S. Barclay, Merck, Sharp and Dohme, West Point, Pennsylvania.

Experimental Procedures. In early experiments, antigen was given to the animals as soon as they became hypothermic in order to have the fullest possible effect of hypothermia on the immune response. However, the animals were disturbed by the injections, and it was not possible to tell if they remained

hypothermic. The experimental design ultimately used in the experiments reported here was as follows. All animals received 500 chick cell agglutinating (CCA) units/0.5 ml PR8 vaccine intraperitoneally for the primary response studies and 50 CCA units/0.5 ml for the secondary studies. Details of the immunization and serum assay procedures have been published (9). Following injection of antigen, selected animals were exposed in individual chambers to 80%:20% helium:oxygen at 0° to induce hypothermia according to the method of Fischer and Musacchia (10). After an average time of about 5 hr (range 3.5–6) required for the animals to become hypothermic (body temp. 10–8°), they were immediately removed to a refrigerator at 6–7° for 18 hr. In the refrigerator, body temperature continued to fall and paralleled the 6–7° of the refrigerator. The irradiation source consisted of approximately 150 Ci of ^{60}Co in a "normalized" configuration from a Model GR-12 gamma irradiator (U. S. Nuclear Corporation). All animals, both hypothermic and normothermic, were irradiated 5 hr after the injection of antigen. Details of the irradiation procedure have been published earlier (7, 8). All irradiated animals received 2000 R whole-body irradiation.

Results. The data in Table I show that serum samples taken 4, 7, and 10 days after immunization from hamsters hypothermic for 18 hr and then reanimated were lower in titer than controls. However, only the 4-day serum samples were significantly lower. All of the serum samples from the irradiated animals had titers less than 4, whether the hamsters were hypothermic or not, suggesting that the degree and duration of hypothermia which was achieved did not exert a radioprotective effect on the primary immune response.

Table II presents the data for the secondary response studies. Both hypothermia and radiation, given separately, adversely affected the secondary response, but not to the same degree as the primary response. The group with the lowest GMT was that which had been both hypothermic and irradiated, suggesting an adverse synergistic effect of the two treatments. The lack of significant difference between the irradiated group and the hypothermic–irradiated group indicates that hypothermia evidenced no protective effect on the anamestic response.

The time courses of the antibody responses are shown in composite fashion in Fig. 1. It may be noted that the primary responses were typical for mammals, and that the hypothermic–irradiated group fared much worse in the anamestic response than the other groups.

Analytical ultracentrifugal studies on the gamma globulin fraction precipitated from the different primary sera with 50% $(NH_4)_2SO_4$ revealed that both IgG and

TABLE I. Geometric Mean Titer (GMT) Hemagglutination Inhibition (HI) of Sera of *Mesocricetus auratus* to Influenza A Virus Vaccine (PR8).

Hypothermic (hr)	Bleeding day	Primary titer[a]		p
		Irradiated[b]	Nonirradiated[c]	
0	4	4 [8]	52 (8–256) [10]	0.02
18	4	4 [8]	14 (8–64) [14]	
0	7	4 [5]	119 (16–512) [16]	0.63
18	7	4 [13]	104 (16–256) [12]	
0	10	ND[d]	97 (8–1024) [17]	0.45
18	10	ND	69 (8–256) [10]	

[a] Titers are expressed as the reciprocal of the serum dilution showing complete hemagglutination inhibition.

[b] 2000 R; number of animals shown in brackets.

[c] Range of titers is given in parentheses. Number of animals shown in brackets.

[d] Not done.

TABLE II. Geometric Mean Titer (GMT) Hemagglutination Inhibition (HI) of *Mesocricetus auratus* to Influenza A Virus Vaccine (PR8)[a] (Secondary Response).

Group	Secondary titer[b] (35 day)	Hypothermic (hr)	Radiation dosage (R)	p
1	2048 (1024–4096) (5 animals)	0	0	0.001
1^1	239 (64–1024) (11 animals)	18	0	
1	2048 (1024–4096) (5 animals)	0	0	0.001
2	79 (8–256) (8 animals)	0	2000	
1	2048 (1024–4096) (5 animals)	0	0	0.001
2^1	49 (16–256) (9 animals)	18	2000	
1^1	239 (64–1024) (11 animals)	18	0	0.07
2	79 (8–256) (8 animals)	0	2000	
1^1	239 (64–1024) (11 animals)	18	0	0.005
2^1	49 (16–256) (9 animals)	18	2000	
2	79 (8–256) (8 animals)	0	2000	0.45
2^1	49 (16–256) (9 animals)	18	2000	

[a] Titers are expressed as the reciprocal of the serum dilution showing complete hemagglutinating inhibition.

[b] Range of titers is given in parentheses.

IgM were present in the sera of all groups. Treatment of the immunoglobulin fractions with 2-mercaptoethanol according to the method of Deutsch and Morton (11) indicated that IgM was the predominant species of immunoglobulin in the HI test in the sera of all groups.

Discussion. Despite the observance of several workers that depressed metabolic states (hibernation and hypothermia) have a radio-protective effect on mammals, this protection apparently does not extend to the lymphoid system in hypothermic hamsters. Indeed, there seems to be synergistic relationship between radiation and hypothermia which works to the detriment of the immune response (cf. groups 1^1 and 2^1, Table II). This result is similar to that reported by Rajevski *et al.* (12) who found that the combination of hypothermia, asphyxia, and sublethal irradiation resulted in a greater and longer inhibition of the immune response than the inhibition obtainable by sublethal irradiation at normal body temperature. The similarity is not necessarily a result of experimental induction of hypothermia, *per se*. There are several major differences between the experimental designs of Rajevski *et al.* and ours. The former group purposefully induced asphyxia by tracheal occlusion with body temperatures no lower than 15–16°. The helium–oxygen method of inducing hypothermia at 0° does not force the animals to undergo asphyxiation by creating an oxygen-

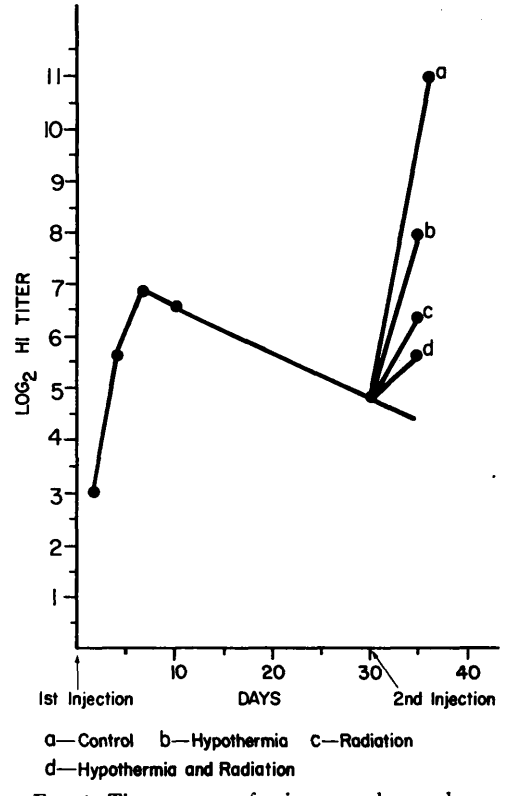

FIG. 1. Time courses of primary and secondary antibody responses in hamsters.

poor atmosphere. The animals can breathe ambient oxygen (20%), and the hypothermia is apparently a result of heat loss from the body surface and the respiratory tract together with a reduction in thermogenesis. Further, reanimation following the helium:oxygen-cold method is simply a matter of placing the animal at room temperature. Such an animal will become active in 1–2 hr without diathermy or resuscitation. The duration of hypothermia was not made clear in the report of Rajevski *et al.* (12). However, it seems likely that the animals were reanimated immediately after irradiation, and thus were hypothermic (15–16°) for only a few minutes or at best 2 or 3 hr. The preliminary studies which we report here were not designed to determine whether or not both the latent period as well as the peak titers of antibody were affected, hence further comparisons with the work of Rajevski *et al.* (12) cannot be made.

It is fully realized that the doses of ionizing radiation used in our experiments are far in excess of those normally used in studies of the immune response. However, it was decided to use radiation doses similar to those used for the studies of survival (5–7) to determine if lymphoid tissues [reported to be among the most radiosensitive of tissues (13)] would be protected by hypothermia when assayed for functional ability. Taliaferro *et al.* (2) proposed that induction of antibody synthesis occurred during the first 12 hr or less of the latent period. This interval is highly radiosensitive and it is during this time that it is determined if antibody will be formed, and approximately how much. The second part of the latent period, also radiosensitive, is primarily concerned with the development of the synthetic machinery and involves the proliferation and maturation of antibody-forming cells. The third stage in antibody formation involves protein synthesis and is the productive phase. This phase is much less radiosensitive than the others.

As mentioned earlier, antigen could not be given to the hypothermic animals after irradiation and immediately after they became hypothermic due to uncontrollable disturbance of the animals. Since it took only about 5 hr to induce hypothermia, all radiation doses were given after this time, but still within the highly radiosensitive 12 hr induction period described by others (2, 14, 15).

It is of interest to note that in recent work, Chaffee and Musacchia (16) were able to show that either heat exposure or heat acclimatization coupled with ionizing irradiation significantly reduced the survival time of hamsters. From a consideration of Arrhenius' principles regarding temperature-dependent chemical reactions, this result might be expected. Conversely, it was shown earlier (17, 18) that both heat exposure and heat acclimatization resulted in a lower metabolic rate in hamsters. Thus the protective effects of hibernation and hypothermia cannot be explained simply on the grounds of depressed metabolic rates in the animals.

While it is quite likely that extensive cellular proliferation does not occur during hibernation (19), little is known of the effects of *short-term* hypothermia on cellular proliferation. However, cell division is an essential feature of the immune response. Dutton (20) was able to inhibit antibody synthesis *in vitro* by irradiation with tritiated thymidine selectively in those lymphoid cells which were actively dividing. Resting cells were unaffected, and only those cells producing antibody were dividing. In studies of the secondary response, Nossal and Makela (21) showed that the number of plasma cells rose exponentially between the second and fourth days after antigenic stimulation, with a doubling time of about 12 hr. However, by the fifth or sixth day more than 95% of the mature plasma cells were labeled with tritiated thymidine, suggesting that they were the progeny of cells with immunologic memory. It is quite possible that the 18 hr of hypothermia sufficiently delayed plasmacytopoiesis so that the immunoglobulin titer was significantly lower in those hypothermic animals sacrificed 5 days after secondary antigenic stimulation.

The significantly lower titers in the sera of hypothermic animals bled 4 days after primary antigenic stimulation might also be explained on the same basis. However, the effect of hypothermia on the primary re-

sponse was not as dramatic as that on the secondary response. The titers of the 7- and 10-day sera of hypothermic animals was essentially the same as the controls, indicating a recovery from the earlier suggested inhibition of cellular proliferation.

In agreement with the findings of Fugmann and Sigel (22), we found both IgG and IgM in the sera of active as well as hypothermic hamsters following primary immunization. However, our findings indicated that, following reduction and alkylation, IgM was the dominant immunoglobulin in the HI test. It is quite possible that immunoglobulin samples following treatment were too dilute to detect the small amounts of IgG present.

Summary. An adverse effect of hypothermia for 18 hr to the immune response was observed in hamster sera up to 10 days after primary immunization with Influenza A virus vaccine. Hypothermia caused less inhibition to the secondary response than did radiation, while 2000 R completely destroyed the primary response of both active and hypothermic hamsters. Hypothermia under the conditions described did not afford any protection to the radiosensitivity of either the primary or the secondary immune response. The combination of hypothermia and radiation seemed to act synergistically in lowering the immune response. The presence of both IgG and IgM was shown in the sera of all the groups used in the primary response studies. IgM seemed to be the predominant immunoglobulin in the HI test.

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1. Bond, V. P., Amer. J. Clin. Nutr. **12**, 194 (1963).

2. Taliaferro, W. H., Taliaferro, L. G., and Jaroslow, B. N., "Radiation and the Immune Response." Academic Press, New York/London (1964).

3. Smith, D. E., Bull. Mus. Comp. Zool., Harvard Univ. **124**, 493 (1960).

4. Weiss, L., Brit. Med. Bull. **17**, 70 (1961).

5. Musacchia, X. J., and Barr, R. E., *in* "Depressed Metabolism" (X. J. Musacchia and J. F. Saunders, eds.), p. 569. Amer. Elsevier, New York (1969).

6. South, F. E., and Andjus, R. K., Cryobiology **3**, 364 (1967).

7. Barr, R. E., and Musacchia, X. J., Proc. Soc. Exp. Biol. Med. **124**, 1204 (1967).

8. Musacchia, X. J., and Barr, R. E., Radiat. Res. **33**, 348 (1968).

9. McKenna, J. M. and Musacchia, X. J., Proc. Soc. Exp. Biol. Med. **129**, 720 (1968).

10. Fischer, B. A., and Musacchia, X. J., J. Physiol. (London) **215**, 1130 (1968).

11. Deutsch, H. F., and Morton, J. I., Science **125**, 600 (1967).

12. Rajevski, O., Rajevski, V., and Andjus, R. K., Int. Astronaut. Cong., 18th, Belgrade, Yugoslavia, **1967**, 285.

13. Bloom, W., "Histopathology of Irradiation from External and Internal Sources" McGraw-Hill, New York (1948).

14. Dixon, F. J., Talmage, D. E., and Maurer, P. H., J. Immunol. **68**, 693 (1952).

15. Taliaferro, W. H., Taliaferro, L. G., and Janssen, E. F., J. Infec. Dis. **91**, 105 (1952).

16. Chaffee, R. R. J., and Musacchia, X. J., Proc. Soc. Exp. Biol. Med. **129**, 718 (1968).

17. Cassuto, Y., and Chaffee, R. R. J., Amer. J. Physiol. **210**, 423 (1966).

18. Casuto, Y., Amer. J. Physiol. **214**, 1147 (1968).

19. Thompson, J. E., Straube, R. L., and Smith, D. E., Comp. Biochem. Physiol. **5**, 297 (1962).

20. Dutton, R. W., Nature (London) **192**, 462 (1961).

21. Nossal, G. J. V., and Makela, O., J. Exp. Med. **115**, 209 (1962).

22. Fugmann, R. A., and Sigel, M. M., J. Immunol. **100**, 1101 (1968).

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