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# ON THE MODIFICATION OF THE TOXICITY OF X RAYS BY IMMUNIZATION

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(With the technical assistance of Mary Pigford)

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# ON THE MODIFICATION OF THE TOXICITY OF X RAYS BY IMMUNIZATION

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(With the technical assistance of Mary Pigford)

Hektoen suggested (1), but did not claim to prove, that immunization might increase the resistance of the dog to total-body X irradiation. In the mouse, Graham and Graham (2) found that 0.5 ml. of horse serum prevented death when injected 10 days before exposure to the ID-25%; no titer determinations were made. The effect of injecting the rat with sheep erythrocytes has been studied. In two strains, but not in a third, the injection raised the ID-50% by 50 - 75 r. The effect was not correlated with the hemolysin titer, but did specifically change one component of the biochemical reaction to total-body irradiation.

## MATERIALS AND METHODS

Adult rats, more than 90 days old, were employed throughout. The Sprague-Dawley (SD) and Holtzman (H) strains were obtained at the age of 30 days from their commercial breeders. The Osborne-Mendel (OM) strain, derived from the stock at the Duke Medical School, was bred in the laboratory.

Good hemolysin titers in the rat are readily obtained only after intravenous injection of antigen. The SD and H strains, which have visible tail veins, were given 1 ml. of a 0.5-per-cent suspension of saline-washed sheep erythrocytes by vein, plus 1 ml. of a 20-per-cent suspension subcutaneously, and peak titers were obtained six days later. As the tail vein of the OM strain could not be seen, these animals were given, during the course of three weeks, two intraperitoneal (3- and 1-per-cent) and two subcutaneous (20-per-cent) injections of 1 ml. each. The titers were determined as previously described (3), and are expressed as,

$\log_2$ (reciprocal of plasma dilution causing 50 per cent hemolysis).

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The experiments were designed to indicate whether immunization changed the LD-50%. For several reasons, this procedure is more desirable than testing for survival at a single dosage. First, the shift in LD-50%, measured in r, provides a broader scale for the comparison of various agents. Second, an erroneous impression might be gained from the results at a single dosage. Clark and Uncpaher (4) showed that the curve relating mortality to dosage is quite steep over most of its course, especially when young animals are employed, and in their work a rise of only 50 r in the LD-50% would have changed survival from 10 to 95 per cent.

In each of the present experiments, two or three groups each of immunized and nonimmunized animals were exposed to various doses of X rays straddling the region of the LD-50%. The composition of the groups within each experiment was such that they matched each other with respect to age, weight, sex, and history. The animals were irradiated as previously described (3) with 250 kv X rays (HVL 0.4 mm. Cu) one week after the last antigen injection. In each run, equal numbers of immunized and nonimmunized animals were exposed in order to insure equality of dosage in the two series. Although small numbers of animals were employed, the results at each of the dosages within a given experiment were consistent, and could be repeated. The data for each strain of rats were therefore pooled in Table I.

## RESULTS AND DISCUSSION

Tests involving 344 animals are shown in Table I. In the case of the SD and OM strains, immunization raised the LD-50% by some 50 - 75 r. In the case of the H strain, immunization decreased the LD-50% by about 25 r. There was no correlation between magnitude of hemolysin titer and survival in any one of the immunized groups. Furthermore, in the OM strain, the mean titer of 5.7, with a standard deviation of 3, was less than 1 per cent of that in the H strain, yet the former

Table I: Survival Following Immunization

Under each exposure is shown the number living over the number dead at 30 days after irradiation. Tests on different lots of animals of the same strain have been combined, since each was consistent with the pooled result. The mean and standard deviation of the hemolysin titer at the time of irradiation is given in brackets.

| Strain | No. of tests | Immunized | 725 r | 775 r | 850 r |
|---|---|---|---|---|---|
| SD | 2 ♂♂ | No | 6*/13 | | 3#/16 |
| | | Yes | 10(12.2 ± 2.7)*/10(13.1 ± 1.6) | | 8(12.3 ± 3.8)#/12(12.1 ± 2.3) |
| OM | 1 ♂ | No | 8/7 | 2/12 | 0/16 |
| | | Yes | | 7/9 | 2/10 |
| Holtzman | 3 ♂ ♀♀ | No | 30/2 | 18/18 | 1/33 |
| | | Yes | 24(13.2 ± 2.6)/8(13.1 ± 0.9) | 10(12.9 ± 2.2)/26(13.5 ± 2.1) | 3(13.8 ± 1.4)/31(13.0 ± 2.9) |

* 700 r

\# 825 r

showed increased resistance while the latter showed increased sensitivity.

Although the number of animals in any one group is small, the differences between immunized and nonimmunized groups in Table I appear definite because there is a consistent relationship between dosage and effect in each series. The significance of the differences is supported by the adjusted value of the chi-square test of independence (5) and the value of P (1 degree of freedom), which were calculated for each strain, using the groups specified: SD, 3 and $<.09$ (725- and 825-r groups pooled); OM, 4.6 $<.04$ (775- and 850-r groups pooled); H, 5 $<.03$ (725- and 775-r groups pooled).

Additional investigations were made of the mechanism whereby immunization affected the ID-50%. During the 10 - 14 days following total-body irradiation of the rat, the chemical composition of the plasma showed a reproducible, complex pattern of changes (6). The changes were in no sense "terminal" but represented the physiologic response of the animal to injury. Distortions of this pattern are best looked for one and four days after exposure.

In a preliminary experiment with five immunized animals, the anticipated rise in albumin-globulin ratio (A/G) after irradiation did not occur. In hundreds of nonimmunized animals previously studied, this change had occurred without exception. A second experiment on 16 animals is detailed in Table II, carried out as described previously (6). The injection of sheep cells did not change the chemical composition of the plasma. However, the injection of sheep cells one week before irradiation, prevented the usual rise in A/G on both days 1 and 4 after exposure, without influencing the changes in glucose, NPN, chloride or cholesterol.*

The rise in A/G, determined by salt precipitation or ultracentrifugation (6,7), can be abolished by preliminary extraction of the plasma with ether. Ether extraction did not affect A/G in the irradiated immunized animals. Although the nature of the factor which appears after irradiation and disturbs A/G is not

* Time did not permit checking this result with [illegible]

Table II: Plasma Composition and Immunization

SD rats, immunized as usual six days before, were irradiated on day 0. The plasma was analyzed on days 1 and 4. Each datum is the average for two rats. Different animals were used for analysis on each day. Data for the nonimmunized animals are quite typical of previous findings.

| Day | Immunized | X Ray | Total Protein | A/G | Chloride | Sugar | NPN | Cholesterol |
|---|---|---|---|---|---|---|---|---|
| 1 | | r | gm.% | | mg.% | mg.% | mg.% | mg.% |
| | No | 0 | 6.8 | 1.99 | 607 | 134 | 31 | 123 |
| | Yes | 0 | 6.7 | 2.02 | 609 | 136 | 31.9 | 117 |
| | No | 725 | 6.35 | 2.32 | 599 | 186 | 38* | 119 |
| | Yes | 725 | 6.25 | 2.08 | 600 | 187 | 37* | 123 |
| 4 | No | 0 | 6.95* | 2.0* | 600* | 134* | 31.2* | 120* |
| | Yes | 0 | 6.8 | 1.99 | 605 | 134 | 32 | 122 |
| | No | 725 | 5.64 | 2.97 | 683 | 158 | 35.2 | 192 |
| | Yes | 725 | 6.03 | 1.83 | 670 | 160 | 35.4 | 192 |

* 1 rat

understood, it is considered to be a sign of injury. That immunization can prevent its appearance is suggestive of a potential therapeutic effect.

## SUMMARY

Following injection with sheep erythrocytes, the Sprague-Dawley and Osborn-Mendel strains of rat showed a rise of about 50-75 r in the ID-50% of 250 kv X rays. The Holtzman strain showed a fall of about 25 r. The injection of sheep cells prevented the rise in albumin-globulin ratio which invariably occurs after irradiation in nonimmunized rats.

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