*Clin. exp. Immunol.* (1974) **16**, 643–655.

# EFFECT OF X-IRRADIATION ON VARIOUS FUNCTIONS OF MURINE MACROPHAGES

B. GEIGER AND RUTH GALLILY

*Department of Immunology, Hebrew University—Hadassah Medical School, Jerusalem, Israel*

(*Received* 25 *September* 1973)

SUMMARY

Unstimulated macrophages of X-irradiated mice have lost the capacity to induce an immune response to *Shigella*. Other studied functions of macrophages were unaffected or activated by X-irradiation. The phagocytic ability of irradiated macrophages, both *in vivo* and *in vitro*, was the same as that of normal cells. Irradiation of macrophage donors caused activation of several macrophage functions: DNA synthesis in irradiated macrophages was slightly higher, and RNA synthesis was six times higher than in non-irradiated controls. Choline uptake was significantly enhanced by irradiation. The levels of two lysosomal enzymes, acid phosphatase and cathepsin D, were elevated in macrophages taken from irradiated donors. The rate of particulate antigen (*Shigella*) degradation was slightly faster in irradiated macrophages, whereas no difference could be detected when soluble antigen ((T,G)-PRO--L) was employed.

## INTRODUCTION

The suppressive effect of X-irradiation on the immune mechanism has been known for many years. Several studies have demonstrated that the inductive phase of the immune response is especially radiosensitive (Taliaferro, Taliaferro & Janssen, 1962; Makinodan, Kastenbaum & Peterson, 1962; Taliaferro, Taliaferro & Jaroslow, 1964a) and investigations concerning the cells involved have focused extensively on both macrophages and lymphocytes. However, while the susceptibility of lymphocytes to irradiation has been well documented (Trowell, 1952; Murray, 1948; Cottier *et al.*, 1964; Taliaferro *et al.*, 1964b; Vos, 1967a and b) neither histological studies (Bloom, 1948; Brecher, Endicott, Gump & Brawner, 1948) nor tests for phagocytic capacity of macrophages (Gordon, Cooper & Miller, 1955; Bencerraf, Sebestyen & Schlossman, 1959) showed any changes following X-ray treatment. On the other hand, several macrophage functions were shown to be highly sensitive to even sublethal doses of X-ray, notably the ability to efficiently degrade and retain antigen (Donaldson *et al.*, 1956)

Correspondence: Dr R. Gallily, Department of Immunology, Hebrew University—Hadassah Medical School, Jerusalem, Israel.

and the capacity to kill bacteria (Nelson & Becker, 1959). Moreover, it was demonstrated that macrophages irradiated *in vivo* failed to induce either anti-*Shigella* (Gallily & Feldman, 1967) or anti-BSA antibodies (Mitchison, 1969) in mice or anti-BGG antibodies in rabbits (Pribnow & Silverman, 1967). Because of the limited information available, this study was undertaken to investigate several functions of macrophages following X-irradiation *in vivo*.

## MATERIALS AND METHODS

### *Mice*

Female 12–15-week-old BALB/c mice were used throughout our study.

### *Antigens*

(1) Ethanol-killed *Shigella paradysenteriae* were fixed with 0·25% glutaraldehyde.

(2) (TG)-PRO--L. A branched synthetic polypeptide poly-L (Tyr-Glu) poly-L-Pro--Poly-L-Lys, [(TG)-PRO--L], having a molecular weight of about $2 \times 10^5$ was kindly provided by E. Mozes and M. Sela (Mozes *et al.*, 1969).

### *Iodination*

Bacterial cells were iodinated ($Na^{125}I$) according to the method of Carpenter (1966) using 2 mCi for 400 mg bacteria. The intensity of the labelling of *Shigella* was $2{\cdot}5 \times 10^{-4}$ cpm/bacterium. (TG)-PRO--L was iodinated according to the procedure of Greenwood, Hunter & Glover (1963), using 2 mCi of $^{125}I$ for 200 μg of (TG)-PRO--L; about 95% of retained radioactivity could be precipitated by 10% trichloracetic acid.

### *X-irradiation*

Mice were exposed in lucite containers to total body X-irradiation using a Picker Vanguard X-ray machine (250 kV, 15 mA, target distance of 50 cm, with 1 mm Cu filter; dose rate 100 rads/min).

### *Macrophages*

Cells were harvested from normal unstimulated mice, or from mice irradiated 48 hr previously, by washing the peritoneal cavity with Hanks' balanced salt solution (HBSS). The total number of cells obtained from a normal animal was $3\text{–}5 \times 10^6$, 30–40% of which were macrophages (normal macrophages). The number of cells obtained from the peritoneal cavity of an irradiated donor was about $10^6$, 85–90% of which were macrophages (irradiated macrophages). Cells were defined as macrophages by morphological and phagocytic criteria.

### *Macrophage cultures*

Peritoneal cells were washed in HBSS and about $3 \times 10^6$ cells from normal mice and $1\text{–}1{\cdot}5 \times 10^6$ cells from irradiated animals were cultured in $30 \times 10$ mm Petri dishes (Nunc, Denmark) in HBSS supplemented with 10% newborn calf serum (NBCS) (Microbiological Associates, Bethesda, Maryland). The cells were grown overnight at 37°C in a moist atmosphere containing 5% $CO_2$. Non-adhering cells were then removed by intensive rinsing of the plates with phosphate-buffered saline (PBS) and new medium added. All tests were performed 3 hr thereafter. The numbers of adherent cells were determined by counting

ten to fifteen microscopic fields per plate, and multiplying the arithmetic average by the ratio of plate area to field area, *viz.* $15^2/0{\cdot}1125^2 = 17778$.

*In vivo clearance of Shigella*

$^{125}$I-labelled *Shigella* was injected intravenously into normal and irradiated mice at a concentration of $3 \times 10^8$ bacteria ($8 \times 10^4$ cpm) in 0·15 ml. The animals were bled into tubes containing sodium heparin at various times thereafter and radioactivity in constant blood volumes was counted in a well type $\gamma$ scintillation counter (Elron Electronics, Haifa, Israel).

*In vitro uptake of $^{125}$I-labelled Shigella*

$^{125}$I-labelled *Shigella* was added to macrophage cultures at a concentration of $2{\cdot}5 \times 10^8$ bacteria ($6 \times 10^4$ cpm) per $8\text{–}10 \times 10^5$ macrophages. After various periods of incubation ranging from 30 to 180 min, the culture medium was removed and the macrophage monolayers were intensively rinsed with cold PBS. The macrophages were lysed with 1 ml of 1% sodium dodecylsulphate (SDS) and the cell-associated radioactivity was determined in a $\gamma$ scintillation counter.

*Degradation of $^{125}$I-labelled Shigella and $^{125}$I-labelled (TG)-PRO--L*

Macrophages ($8\text{–}10 \times 10^5$) were incubated with $^{125}$I-labelled *Shigella* ($2{\cdot}5 \times 10^8$ bacteria $6 \times 10^4$ cpm) or with $^{125}$I (TG)-PRO--L diluted with unlabelled antigen to a specific activity of $10^6$ cpm per 1 $\mu$g for 1 hr at 37°C. The cells were then thoroughly rinsed and fresh medium was added. After additional incubation for 2, 4, 10 or 24 hr, the medium was removed and the cells lysed as described above. Radioactivity in the cell lysate and in the medium was measured. In addition trichloroacetic acid (TCA) (final concentration of 10%) precipitable material in the media was counted (Gallily & Eliahu, 1973). The amount of radioactivity in the soluble fraction of medium not precipitable by 10% TCA was calculated and considered to represent the total degraded antigen.

*Enzyme assays*

Macrophage monolayers were rinsed with PBS and 1 ml of distilled water was added to each plate. The cells were scraped from the plates after freezing and thawing five times. Cell lysates were assayed for the following hydrolytic enzyme activities: acid phosphatase, $\beta$-glucuronidase and cathepsin D (Gallily & Eliahu, 1973). Acid phosphatase and $\beta$-glucuronidase activities were determined by hydrolysis of *p*-nitrophenyl phosphate and *p*-nitrophenyl-$\beta$-D-glucuronide (Sigma) respectively (Beck *et al.*, 1968). Cathepsin D activity was evaluated by hydrolysis of bovine haemoglobin (Sigma) (Barrett, 1967).

*[$^3$H]Thymidine incorporation*

Macrophage cultures were incubated for 24 hr with [$^3$H]thymidine (2 Ci/mmol, The Radiochemical Centre, Amersham) at a final concentration of 1 $\mu$Ci/ml. The cultures were then rised with PBS and lysed in 1% SDS. The TCA-insoluble fraction was collected on filters (GF/C glass filters, Whatman, England) and radioactivity counted in a Packard Tri-Carb spectrometer.

*[$^3$H]Uridine incorporation*

[$^3$H]Uridine (20 Ci/mmol, The Radiochemical Centre, Amersham) was applied to macro-

phage cultures for 1–4 hr at a final concentration of 1 $\mu$Ci/ml. Radioactivity in the TCA-precipitable fractions was determined as described for thymidine incorporation.

*[$^3H$]Choline uptake and incorporation*

Macrophages were incubated with [$^3$H]choline (17 Ci/mmol, the Radiochemical Centre, Amersham) at a final concentration of 1 $\mu$Ci/ml for 2 hr. Thereafter the cells were rinsed with PBS and lysed in 1% SDS. Both total and TCA-insoluble radioactivity were determined.

*Cell transfer*

Macrophages were withdrawn from the peritoneal cavities of normal mice and mice irradiated 48 hr previously. Thioglycollate-stimulated macrophages were prepared as described by Gallily & Feldman (1967). The cells were washed, counted, and suspended in HBSS. Incubation of macrophages with a final concentration of 0·01% suspension of *Shigella* was carried out for 1 hr as described by Gallily & Feldman (1967). $10^7$ macrophages were then injected intraperitoneally into mice irradiated with 450 rads 2 days prior to cell transfer and protected by intravenous injection of $5 \times 10^6$ normal bone marrow cells. Some recipients were injected intraperitoneally with $10^7$ normal lymph node (axillary, inguinal, mesenteric) cells.

*Anti-Shigella agglutinin*

Agglutinins were measured in the sera of mice 7 days after cell transfer. Antibody titre was expressed as the reciprocal of the highest dilution causing agglutination of *Shigella.*

*Statistics*

The statistical significance of the data was determined according to students *t*-test in which a *P* value of 0·05 or less was considered significant.

## RESULTS

*Antibody production following inoculation of unstimulated macrophages*

The capacity of normal and irradiated unstimulated macrophages to elicit antibody formation was studied. Macrophages withdrawn from either normal mice, or mice exposed to 550 rads 48 hr previously, were incubated for 1 hr with *Shigella*. The washed macrophages were then inoculated intraperitoneally into 450 rad-irradiated recipients at a concentration of $10^7$ cells per mouse. Table 1 shows that after 7 days normal macrophages elicited an anti-*Shigella* antibody titre of 1:20. Inoculation of recipients with both normal macrophages and lymph node lymphocytes ($10^7$ lymphocytes/recipient) yielded a titre of 1:60. Conversely, *in vivo* irradiated, unstimulated macrophages failed to trigger any anti-*Shigella* response whatsoever (group 3). A similar lack of activity had been previously demonstrated in thioglycollate-stimulated and irradiated macrophages (Table 1, group 5; Gallily & Feldman, 1967).

*Effect of irradiation on blood clearance*

Mice were irradiated with 550 rads or 800 rads total body X-irradiation. Two days later a suspension of $^{125}$I-labelled *Shigella* ($3 \times 10^8$ bacteria ($8 \times 10^4$ cpm)/mouse) was injected

TABLE 1. Effect of X-irradiation on the capacity of unstimulated macrophages to induce anti-*Shigella* antibody production

| | | Irradiated recipients injected with | | | |
|---|---|---|---|---|---|
| Group number | Number of animals | *Shigella*-incubated macrophages* (source) | Lymphocytes† | *Shigella*‡ | Mean reciprocal titre |
| 1 | 3 | Unstimulated | — | — | 20 |
| 2 | 7 | Unstimulated | + | — | 60 |
| 3 | 4 | Unstimulated and irradiated§ | + | — | 0 |
| 4 | 15 | Stimulated | + | — | 60 |
| 5 | 8 | Stimulated and irradiated | + | — | 0·4 |
| 6 | 4 | — | + | + | 0 |
| 7 | 4 | — | — | + | 0 |

* $10^7$ macrophages incubated previously with *Shigella* (0·01%) were injected intraperitoneally into 450 rad-irradiated recipient.

† $10^7$ lymph node lymphocyte/recipient.

‡ 0·1 ml of 0·1% *Shigella*/recipient.

§ Donors of macrophages were irradiated at 550 rads for 48 h previous to cell withdrawal.

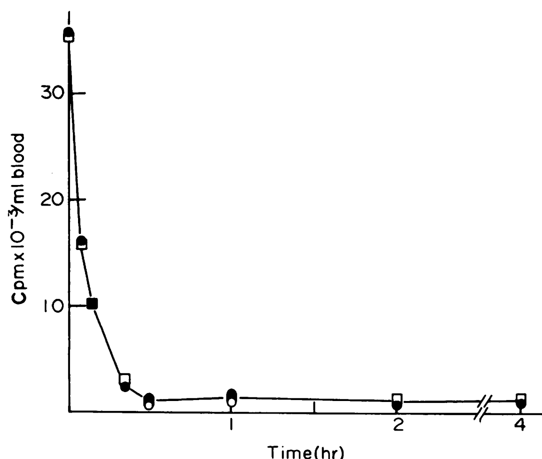


FIG. 1. Rate of blood clearance of $^{125}$I-labelled *Shigella* injected into normal and X-irradiated mice. Each point represents a mean value of twelve to fifteen measurements. (●) Control. (□) Mice irradiated with 550 rads. (○) Mice irradiated with 800 rads.

intravenously into both normal and irradiated animals. At different times (5 min–4 hr) the mice were bled and the radioactivity of the blood was measured. No differences between normal and irradiated mice could be detected in rates of clearance of radiolabelled bacteria (Fig. 1).

*Uptake of $^{125}$I-labelled Shigella by irradiated macrophages*

$^{125}$I-labelled *Shigella* was added to cultures of normal and irradiated macrophages and the uptake of labelled bacteria determined after various times of incubation. X-irradiation

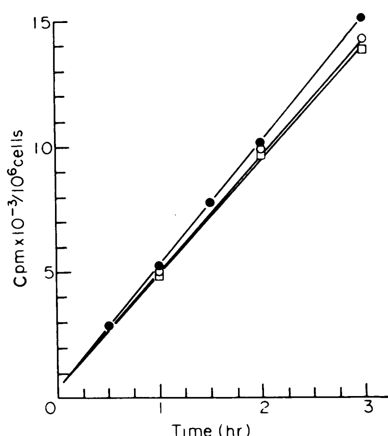


FIG. 2. Phagocytic activity of normal and irradiated macrophages tested *in vitro*. Macrophage cultures were incubated with $^{125}$I-labelled *Shigella* for various times. Each point represents a mean value of twenty measurements. Standard deviation did not exceed 5%. (●) Control. (□) Macrophages irradiated with 550 rads. (○) Macrophages irradiated with 800 rads.

of macrophage donors with 550 rads or 800 rads had no detectable effect on the phagocytic ability of the cells (Fig. 2).

*Enzyme levels*

Three hydrolytic lysosomal enzymes were assayed in normal unstimulated macrophages and in macrophages withdrawn from mice 48 hr after irradiation. The two cell populations were cultured for 24 hr in the absence or presence of 0·07% *Shigella* or polystyrene particles (latex (Difco) 0·81 μm) at a concentration of $10^8$ particles/culture. The results indicate that irradiated macrophages had significantly higher acid phosphatase and cathepsin D levels than normal cells (Tables 2 and 4), while β-glucuronidase activity was unchanged after irradiation (Table 3). Following incubation with *Shigella*, acid phosphatase levels were increased both in normal and irradiated macrophages, while some decreases were observed in β-glucuronidase and cathepsin D activities under similar conditions. The engulfment of latex particles did not affect acid phosphatase activity in normal and irradiated macrophages (Table 2), but caused decreased levels of β-glucuronidase and cathepsin D (Tables 3 and 4).

TABLE 2. Specific activity of acid phosphatase in normal and irradiated macrophages

| Incubation of macrophages | Milliunits* ± SD/$10^6$ macrophages | | |
|---|---|---|---|
| | Normal | 550 rads† | 800 rads† |
| — | 1·655±0·449 | 2·997±1·017 | 2·913±0·663 |
| *Shigella* | 3·662±0·605 | 5·193±0·576 | 5·247±0·815 |
| Latex | 1·719±0·167 | 2·733±0·452 | 2·713±0·337 |

* Enzyme activity was expressed in milliunits, one unit being that amount which released 1 μmol of nitrophenol per min. Each value represents the mean of ten to twelve determinations.

† Donors of macrophages were irradiated 48 hr prior to cell withdrawal.

TABLE 3. Specific activity of β-glucuronidase in normal and irradiated macrophages

| Incubation of macrophages | Milliunits* ±SD/$10^6$ macrophages | | |
|---|---|---|---|
| | Normal | 550 rads† | 800 rads† |
| — | 0·904±0·348 | 0·958±0·087 | 0·955±0·089 |
| *Shigella* | 0·712±0·055 | 0·778±0·100 | 0·802±0·037 |
| Latex | 0·526±0·100 | 0·572±0·074 | 0·616±0·046 |

* Enzyme activity was expressed in milliunits, one unit being that amount which released 1 μmol of nitrophenol per min. Each value represents the mean of 10–12 determinations.

† Donors of macrophages were irradiated 48 hr prior to cell withdrawal.

TABLE 4. Specific activity of cathepsin D in normal and irradiated macrophages

| Incubation of macrophages | OD 280 nm* /$10^6$ macrophages | | |
|---|---|---|---|
| | Normal | 550 rads† | 800 rads† |
| — | 0·907±0·226 | 1·364±0·135 | 1·336±0·192 |
| *Shigella* | 0·805±0·122 | 0·946±0·145 | 1·104±0·299 |
| Latex | 0·654±0·073 | 0·862±0·126 | 0·691±0·123 |

* The optical density of the TCA-soluble fraction of the reaction was read at 280 nm. Enzyme activity was expressed in terms of optical density units. Each value represents the mean of ten to twelve determinations.

† Donors of macrophages were irradiated 48 hr prior to cell withdrawal.

*Retention and degradation of antigens*

The capacity of normal and irradiated macrophages to retain and degrade both $^{125}$I-labelled *Shigella* and $^{125}$I-labelled (TG)-PRO--L was tested. After incubation with labelled antigen for 1 hr, the amount of radioactivity retained by cells was measured over a period of 24 hr. Figs 3 and 4 show that between 20 and 31% of $^{125}$I-labelled *Shigella* and 21–28% of $^{125}$I-labelled (TG)-PRO--L were retained in both normal and irradiated macrophages. No significant differences ($P \geqslant 0{\cdot}10$) in retention capacity were detectable between the two cell populations.

The rates of degradation of both $^{125}$I-labelled *Shigella* and $^{125}$I-labelled (TG)-PRO--L were measured by determining radioactivity in TCA-soluble fractions of media at various times after antigen uptake. Irradiated macrophages degraded *Shigella* slightly faster than did normal cells (Fig. 5). However, $^{125}$I-labelled (TG)-PRO--L was broken down at the same rate by both cell types (Fig. 6). Thus irradiation with 550 rads and 800 rads did not markedly affect the capacity of macrophages to retain and degrade both *Shigella* and (TG)-PRO--L antigens.

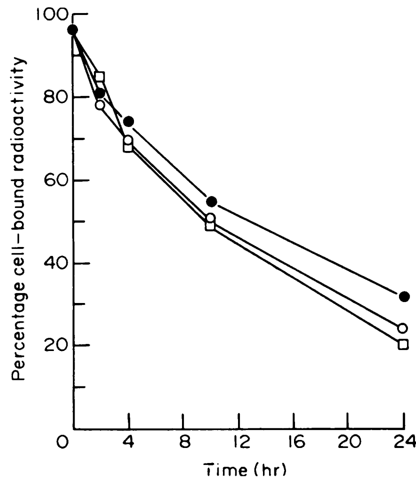


FIG. 3. Percentage of retained $^{125}$I-labelled *Shigella* in normal and irradiated macrophages Cells were cultured with $^{125}$I-labelled *Shigella* for 1 hr at 37°C The cells were then rinsed and fresh medium added. The radioactivity of the cells was measured after 2, 4, 10 and 24 hr. Each point represents a mean value of ten to twelve measurements. (●) Control. (□) Macrophages irradiated with 550 rads. (○) Macrophages irradiated with 800 rads.

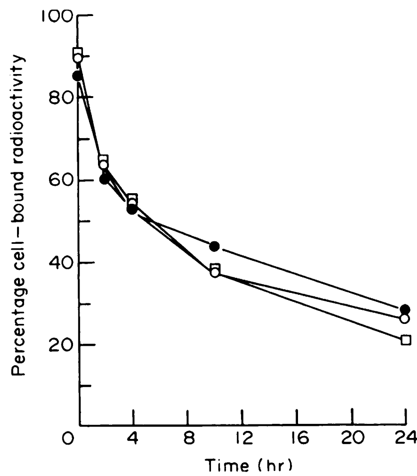


FIG. 4. Percentage of retained $^{125}$I-labelled (TG)-PRO--L in normal and irradiated macrophages. Cells were incubated with $^{125}$I-labelled (TG)-PRO--L and radioactivity of the cells was measured after 2, 4, 10 and 24 hr. Each point represents a mean value of ten to twelve measurements. (●) Control. (□) Macrophages irradiated with 550 rads. (○) Macrophages irradiated with 800 rads.

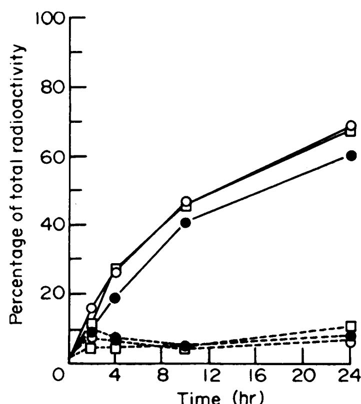


FIG. 5. Degradation of $^{125}$I-labelled *Shigella* by normal and irradiated macrophages. Cells were cultured with $^{125}$I-labelled *Shigella* for 1 hr at 37°C. The cells were then rinsed and fresh medium added. The percentage of TCA-non-precipitable radioactivity in the medium was calculated from the total radioactivity present. Each point represents a mean value of ten to twelve measurements. (●) Control. (□) Macrophages irradiated with 550 rads. (○) Macrophages irradiated with 800 rads. The dashed lines show the percentage of radioactivity in TCA-insoluble material, and the solid lines show the percentage of radioactivity in TCA-soluble material.

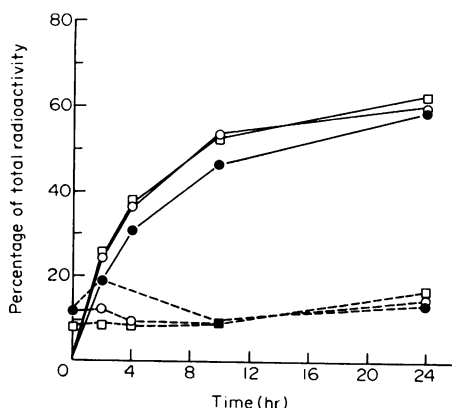


FIG. 6. Degradation of $^{125}$I-labelled (TG)-PRO--L by normal and irradiated macrophages. Macrophage cultures were maintained and the percentage of TCA-non-precipitable radioactivity in the medium was calculated from the total radioactivity present. Each point represents a mean value of ten to twelve measurements. (●) Control. (□) Macrophages irradiated with 550 rads. (○) Macrophages irradiated with 800 rads. The dashed lines show the percentage of radioactivity in TCA-insoluble material, and the solid lines show the percentage of radioactivity in TCA-soluble material.

### *Uptake and incorporation of [$^3$H]choline*

After 2 hr incubation with [$^3$H]choline, macrophages from irradiated mice took up twice as much label as non-irradiated cells (Table 5). Similarly, radioactivity determinations of TCA-insoluble fractions of these cells revealed that irradiated macrophages incorporated

TABLE 5. Choline uptake by normal and irradiated macrophages

| Macrophage source | [$^3$H]Choline uptake: Total uptake (cpm/$10^6$ cells) | TCA-insoluble fraction (cpm/$10^6$ cells) |
|---|---|---|
| Normal mice | 50,288 ± 4,520 | 519 ± 128 |
| 550 rad-irradiated mice | 96,595 ± 6,349 | 1,101 ± 333 |
| 800 rad-irradiated mice | 105,433 ± 6,940 | 1,111 ± 181 |

twice as much [$^3$H]choline into macromolecular form as did normal controls (Table 5). In all cases, TCA-precipitable radioactivity represented about 1% of the total amount of label uptake. These data suggest that levels of choline incorporation are dependent on available pool size.

*DNA synthesis*

DNA synthesis in both normal and irradiated cells was determined by incorporation of [$^3$H]thymidine into TCA-insoluble fractions during 24 hr of incubation, both in the presence and absence of *Shigella*. Although the level of thymidine incorporation detected was low as compared to other cell types (HeLa, L-929), irradiated macrophages (550 rads or 800 rads) were significantly ($P < 0{\cdot}05$) more active than normal cells (Table 6). Incubation with *Shigella*, however, did not affect [$^3$H]thymidine incorporation in the case of either cell populations.

TABLE 6. Incorporation of [$^3$H]thymidine by normal and irradiated macrophages

| Macrophage source | Number of determinations | Incorporation of [$^3$H] thymidine (cpm/$10^6$ cell/24 hr): −*Shigella* | +*Shigella* |
|---|---|---|---|
| Normal mice | 12 | 244 ± 42 | 244 ± 91 |
| 550 rad-irradiated mice | 9 | 452 ± 82 | 642 ± 191 |
| 800 rad-irradiated mice | 8 | 532 ± 53 | 480 ± 248 |

*RNA synthesis*

The incorporation of [$^3$H]uridine into 10% TCA-insoluble fractions was tested in irradiated and normal macrophages in the presence or absence of *Shigella*. A marked elevation of about six-fold in the rate of uridine incorporation was found in irradiated macrophages compared to normal ones (Fig. 7a). No parallel increase was detected in the TCA-soluble fraction of irradiated cells. Thus, the increase in isotope incorporation reflects an enhanced synthetic activity of the irradiated cells and is not merely a result of isotope accumulation. The presence of *Shigella* caused a significant increase in the rate of uridine incorporation both in normal and irradiated macrophages (Fig. 7b).

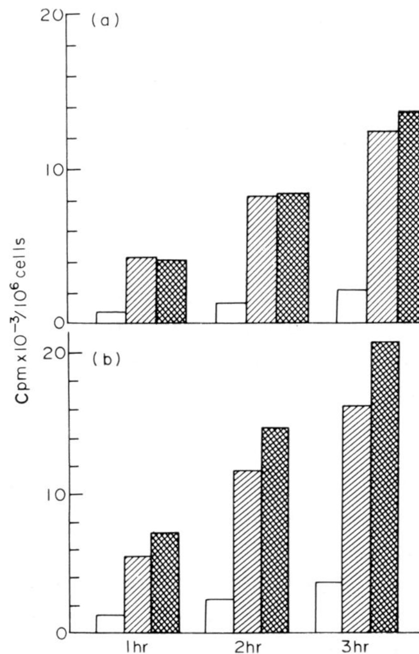


FIG. 7. [³H]Uridine incorporation into normal and irradiated macrophages both (a) in the absence and (b) in the presence of *Shigella*. Each column represents the mean value of fifteen to eighteen determinations. (□) Control. (▨) Macrophages irradiated with 550 rads. (▩) Macrophages irradiated with 800 rads.

## DISCUSSION

The present findings indicate that either lethal or sublethal X-irradiation of unstimulated mice destroyed the capacity of their macrophages to trigger an anti-*Shigella* antibody response, a finding similar to that observed in thioglycollate-stimulated mice by Gallily & Feldman (1967). When other activities of macrophages were examined for susceptibility to X-rays, the following picture was obtained. Neither the ability of the reticuloendothelial system (RES) to engulf and clear bacteria from the blood, nor the capacity of macrophages to phagocytize bacteria *in vitro* were affected by irradiation. Degradation experiments using both particulate (*Shigella*) and soluble antigen ((TG-PRO--L) failed to reveal any significant change in macrophage ability to handle antigen following X-ray treatment. However, these experiments give only quantitative information concerning the breakdown of antigen without demonstrating either the chemical character of immunogenic properties of the degradation products.

Most of the other functions tested in macrophages after *in vivo* irradiation showed that an activated state existed in irradiated cells. RNA was synthesized about six times more rapidly in irradiated cells than in normal ones, but no new RNA species could be detected in electrophoretic profiles on 3% or 10% acrylamide gels. Choline uptake was twice as fast in irradiated cells and DNA synthesis, although relatively low, was also higher in irradiated compared to normal macrophages. Two out of three lysosomal enzymes tested

(acid phosphatase and cathepsin D) were present at higher levels in irradiated cells. In addition a previous study (Geiger, Gallily & Gery, 1973) indicated that macrophages irradiated *in vivo* exhibited an enhanced capacity to release lymphocyte activating factor (LAF). It is suggested that any or all of the following explanations may account for the activities of irradiated macrophages. (1) Irradiation may select for an active subpopulation of peritoneal macrophages. It is possible that the peritoneal adherent cell population is not uniform from either the functional or morphological points of view. Furthermore, one especially immunologically active subpopulation, at least for certain antigens, may be radiosensitive and susceptible to destruction by irradiation, while the majority of peritoneal cells without immunological capacity survive or are even activated by X-ray treatment. (2) Enhanced activity of irradiated macrophages may result from active repair mechanism of the damage caused to the cells by irradiation. This assumes that the function involved in the immunogenicity of macrophages was not repaired during the period tested. (3) Debris of other cells, released following irradiation may stimulate macrophages and render them more active. It was reported (Braun & Makano, 1965; Johnson *et al.*, 1968) that nucleic acids and their derivatives contributed to activation of the immune system. It is quite possible that these compounds may help in triggering and enhancing macrophage activities.

The mechanism responsible for loss of macrophage 'immunogenicity' following irradiation is not apparent from our study. However, recent electron microscopic observations (Gallily & Ben-Ishay, 1973) revealed that irradiated macrophages interact differently with lymphocytes than do normal controls. Additional studies are presently in progress to investigate details of irradiation-induced macrophage damage.

## ACKNOWLEDGMENTS

This work was supported by research grants from the Concern Foundation of Los Angeles, the Sam Lautenberg Fellowship, and Mr and Mrs Laurence Tisch.

## REFERENCES

Barrett, A.J. (1967) Lysosomol acid proteinase of rabbit liver. *Biochem. J.* **104,** 601.

Beck, C., Mahadevan, S., Brightwell, R., Dillard, C.J. & Tappel, A.L. (1968) Chromatography of lysosomal enzymes. *Arch. Biochem. Biophys.* **128,** 369.

Benacerraf, B., Sebestyen, M.M., Schlossman, S. (1959) A quantititive study of the kinetics of blood clearance of $^{32}$P-labelled *Escherichia coli* and staphylococci by the reticuloendothelial system. *J. exp. Med.* **110,** 27.

Bloom, M.A. (1948) *Histopathology of Irradiation from Internal and External Sources.* (Ed. by W. Bloom), p. 168. McGraw-Hill Book Company, New York.

Braun, W. & Makano, M. (1965) Influence of oligodeoxyribonucleotides on early events in antibody formation. *Proc. Soc. exp. Biol.* (*N.Y.*), **119,** 701.

Brecher, G., Endicott, K.M., Gump, H. & Brawner, H.P. (1948) Effects of X-ray on lymphoid and hemopoietic tissues of albino mice. *Blood,* **3,** 1259.

Carpenter, R.R. (1966) Phagocytosis by guinea-pig splenic cells of *Escherichia coli* and protein-coated bentonite particles labeled with iodine$^{125}$. *J. Immunol.* **96,** 992

Cottier, H., Cronkite, E.P., Jansen, C.R., Rai, K.R., Singer, S. & Sipe, C.R. (1964) Studies on lymphocytes. III. Effects of extracorporeal irradiation of the circulating blood upon the lymphoreticular organs in the calf. *Blood,* **24,** 241.

Donaldson, D.M., Marcus, S., Gyi, K.K. & Perkins, E.H. (1956) The influence of immunization and total body X-irradiation on intracellular digestion by peritoneal phagocytes. *J. Immunol.* **76,** 192.

Gallily, R. & Ben-Ishay. (1974) Interaction between normal or irradiated macrophages and lymphocytes in mice. (In preparation.)

GALLILY, R. & ELIAHU, H. (1974) Uptake and degradation of a polypeptide antigen by stimulated and unstimulated macrophages in responder and non responder mice. *Immunology*, **26,** 603.

GALLILY, R. & FELDMAN, M. (1967) The role of macrophages in the induction of antibody in X-irradiated animals. *Immunology*, **12,** 197.

GEIGER, B., GALLILY, R. & GERY, I. (1973) The effect of irradiation on the release of lymphocyte-activating factor (LAF). *Cell. Immunol.* **7,** 177.

GORDON, L.E., COOPER, D.B., & MILLER, C.P. (1955) Clearance of bacteria from the blood of irradiated rabbits. *Proc. Soc. exp. Biol.* (*N.Y.*), **89,** 577.

GREENWOOD, F.C., HUNTER, W.H., & GLOVER, J.S. (1963) The preparation of $^{131}$I-labelled human growth hormone of high specific radioactivity. *Biochem. J.* **89,** 114.

JOHNSON, A.G., SCHMIDTKE, J., MERRIT, K. & HAN, I. (1968). Enhancement of Ab formation by nucleic acids and their derivatives. *Nucleic Acids in Immunology* (Ed. by O. J. Plescia, and W. Braun), p. 379. Springer Verlag, Stuttgart.

MAKINODAN, T., KASTENBAUM, M.A. & PETERSON, W.J. (1962). Radiosensitivity of spleen cells from normal and pre-immunized mice and its significance to intact animals. *J. Immunol.* **88,** 31.

MITCHISON, N.A. (1969) The immunogenic capacity of antigen taken up by peritoneal exudate cells. *Immunology*, **16,** 1.

MOZES, E., MCDEVITT, H.O., JATON, J.C. & SELA, M. (1969) The nature of the antigenic determinant in a genetic control of the antibody response. *J. exp. Med.* **130,** 493.

MURRAY, R.G. (1948) *Histopathology of Irradiation from External and Internal sources.* (Ed. by W. Bloom), p. 243. McGraw Hill Book Company, New York.

NELSON, E.L. & BECKER, J.R. (1959). The effect of whole-body X-irradiation on the bactericidal activity of phagocytic cells. *J. infect. Dis.* **104,** 13.

PRIBNOW, J.F. & SILVERMAN, M.S. (1967) Studies on the radiosensitive phase of the primary antibody response in rabbits. I. The role of macrophage. *J. Immunol.* **98,** 225.

TALIAFERRO, W.H., TALIAFERRO, L.G. & JANSSEN, E.F. (1952) The localization of X-ray injury to the initial phases of antibody response. *J. infect. Dis.* **91,** 105.

TALIAFERRO, W.H., TALIAFERRO, L.G. & JAROSLOW, B.N. (1964a) *Radiation and Immune Mechanisms.* p. 33. Academic Press, New York and London.

TALIAFERRO, W.H., TALIAFERRO, L.G. & JAROSLOW, B.N. (1964b) *Radiation and Immune Mechanisms.* p. 88. Academic Press, New York and London.

TROWELL, O.A. (1952) The sensitivity of lymphocytes to ionising radiation. *J. Path. Bact.* **64,** 687.

UNANUE, E.R., CEROTTINI, J.C. & BEDFORD, M. (1969) Persistence of antigen on the surface of macrophages. *Nature* (*Lond.*), **222,** 1193.

VOS, O. (1967a) Survival of lymphatic cells after X-irradiation in mice. *Effects of Ionizing Irradiation on the Haematopoietic Tissue.* p. 134. Vienna, I.A.E.A.

VOS, O. (1967b) Radiation sensitivity and post-irradiation repair of mouse lymphatic cells *in vivo* and *in vitro.* A study with the parent to $F_1$ hybrid graft-versus-host reaction. *Int. J. radiat. Biol.* **13,** 317.