

Effect of Radiation on Activity of Antibiotics

K. G. GUPTA*, K. K. VYAS*, and N. S. SEKHON†[▲]

Abstract □ Thirteen antibiotics belonging to different groups were exposed to 5, 10, and 15 megarads of neutron and γ -radiations, and their activity in comparison to nonirradiated antibiotics for *Escherichia coli* and *Staphylococcus aureus* was studied. With γ -irradiation, oxytetracycline at 15 megarads and penicillin G at 5 and 10 megarads showed considerable decrease in activity. With penicillin G, the activity was somewhat restored at the 15-megarad dose. Other antibiotics did not show a significant change in activity by this method. Neutron irradiation did not change the activity.

Keyphrases □ Antibiotics—effect of neutron and γ -radiation on activity □ Neutron radiation—effect on activity of 13 antibiotics of different groups □ γ -Radiation—effect on activity of 13 antibiotics of different groups

UV light is known to inactivate urease, papain, and trypsin (1), although the degree of inactivation may vary depending upon the type of the molecule. Similarly, amino acids have been shown to be modified when exposed to radiation (2). Iodine compounds such as potassium iodide, potassium iodate, and iodoacetic acid have been shown to possess higher toxicity to bacteria when irradiated (3). Irradiated antibiotics have also been found to have changed activity against sensitive and resistant strains (4). γ -Radiation with cobalt 60 does not bring about any chemical or biological changes in oxytetracycline and chlortetracycline in the dry state or in organic solvents, but it inactivates them significantly in aqueous solutions (5).

In the present investigation, various antibiotics were exposed to different doses of neutron and γ -radiations and the activity of such treated antibiotics against Gram-negative and Gram-positive bacteria was studied.

EXPERIMENTAL

Staphylococcus aureus (NCTC 6571)¹ and *Escherichia coli* (06)² were maintained on nutrient agar at 4°. Subculturing was done every month. Incubation temperatures for *S. aureus* and *E. coli* were $31 \pm 1^\circ$ and $36 \pm 1^\circ$, respectively.

Antibiotics—The following antibiotics were used in this study: ampicillin³, bacitracin⁴, chloramphenicol³, cloxacillin⁵, erythromycin³, kanamycin⁵, neomycin sulfate³, nisin⁶, oxytetracycline³, penicillin G³, streptomycin sulfate³, spiramycin³, and vancomycin⁷.

Standard solutions of antibiotics were made in glass double-distilled water.

Irradiation of Antibiotics—A known quantity of each antibiotic (in triplicate) was irradiated in the dry state⁸. Neutron irradiation was done with a neutron flux of 1.28×10^{12} neutrons cm.²/sec.; the duration was 12, 24, and 36 min. for 5, 10, and 15 megarads, respectively. The rate of γ -irradiation of the antibiotics was 0.356 megarad/hr.; the doses given were 5, 10, and 15 megarads.

Antibiotic Testing—*S. aureus* and *E. coli* were grown in nutrient broth. A 24-hr.-old culture containing approximately 10^8 cells/ml. was prepared. One milliliter of this suspension was mixed with every 15 ml. of nutrient agar and plated in 85–90-mm. diameter petri dishes so that the thickness of the medium in each plate was between 3.4 and 3.6 mm. The dishes were then allowed to dry in an incubator at 37° for 0.5 hr.

Disks, 6.25 mm. in diameter, were punched from Whatman No. 1 filter paper. These disks were sterilized by dry heat at 140° for 1 hr.

Table I—Effect of γ -Radiation on the Activity of Antibiotics

Antibiotic	Radiation Dose, megarads	Activity ^a (in Terms of Zone Diameter, mm.) against	
		<i>E. coli</i>	<i>S. aureus</i>
Ampicillin	— ^b	19	32
	5	18	30
	10	18	30
	15	18	30
Bacitracin	—	Nil	14
	5	Nil	14
	10	Nil	14
	15	Nil	14
Chloramphenicol	—	18	20
	5	19	21
	10	19	21
	15	19	21
Cloxacillin	—	Nil	29
	5	Nil	28
	10	Nil	28
	15	Nil	29
Erythromycin	—	Nil	13
	5	Nil	13
	10	Nil	12
	15	Nil	12
Kanamycin	—	16	23
	5	16	23
	10	15	23
	15	16	23
Neomycin	—	14	17
	5	13	16
	10	14	16
	15	14	16
Nisin	—	Nil	9
	5	Nil	9
	10	Nil	8
	15	Nil	8
Oxytetracycline	—	23	27
	5	23	26
	10	23	26
	15	Nil	16
Penicillin G	—	12	40
	5	Nil	29
	10	Nil	30
	15	9	35
Streptomycin	—	21	15
	5	21	15
	10	21	14
	15	21	15
Spiramycin	—	Nil	14
	5	Nil	13
	10	Nil	13
	15	Nil	13
Vancomycin	—	Nil	22
	5	Nil	21
	10	Nil	21
	15	Nil	20

^a Strength of the disk used = 5 mcg. ^b Nonirradiated.

¹ Obtained from P.G.I., Chandigarh, India.
² Obtained from WHO International Escherichia Centre, Copenhagen, Denmark.
³ Local market.
⁴ Pfizer.
⁵ Bristol Laboratories.
⁶ Aplin and Barrett, Ltd., Yeovil, Somerset, England.
⁷ Lilly Laboratories.
⁸ At the Bhabha Atomic Research Centre, Bombay, India.

Table II—Effect of Neutron Radiation on the Activity of Antibiotics

Antibiotic	Radiation Dose, megarads	Activity ^a (in Terms of Zone Diameter, mm.)	
		—against— <i>E. coli</i>	<i>S. aureus</i>
Ampicillin	— ^b	20	30
	5	19	30
	10	19	29
	15	19	30
Bacitracin	—	Nil	12
	5	Nil	12
	10	Nil	12
	15	Nil	12
Chloramphenicol	—	19	20
	5	21	21
	10	22	22
	15	22	21
Cloxacillin	—	Nil	30
	5	Nil	30
	10	Nil	29
	15	Nil	30
Erythromycin	—	Nil	15
	5	Nil	15
	10	Nil	14
	15	Nil	14
Kanamycin	—	17	24
	5	16	24
	10	16	23
	15	16	23
Neomycin	—	13	16
	5	13	15
	10	13	15
	15	12	15
Nisin	—	Nil	10
	5	Nil	9
	10	Nil	9
	15	Nil	9
Oxytetracycline	—	21	25
	5	21	25
	10	21	25
	15	21	25
Penicillin G		Not done	
Streptomycin	—	20	13
	5	19	13
	10	19	13
	15	19	13
Spiramycin	—	Nil	12
	5	Nil	11
	10	Nil	11
	15	Nil	9
Vancomycin	—	Nil	23
	5	Nil	22
	10	Nil	22
	15	Nil	22

^a Strength of the disk used = 5 mcg. ^b Nonradiated.

in batches of 100 in screw-capped bottles. For preparing disks containing 5 mcg. of antibiotic each, 1 ml. of the solution containing 500 mcg. of the antibiotic was added to each bottle of 100 disks. Because all of this solution was absorbed, it could be assumed that each disk contained approximately 5 mcg. of the antibiotic.

Finally, four disks of each antibiotic (one nonradiated and three radiated at 5, 10, and 15 megarads) were placed on the medium, suitably spaced apart, in one petri dish and incubated overnight at 37°. For each disk the diameters of the circular areas of inhibition were measured.

The above method was adopted from Cruickshank (6). This method is semiquantitative, but it was used because the authors have observed only gross changes in the antibiotic activity. Moreover, by testing radiated and nonradiated samples of each antibiotic on the same petri dish, the difference in zone inhibition diameter due to the differences in inoculum size or thickness of the medium, etc., is overruled.

Results shown (Table I) are the mean values of three replications.

RESULTS AND DISCUSSION

The effect of various antibiotics subjected to different radiation doses upon *E. coli* and *S. aureus* was studied. Results (Table I) indicate that the activity of penicillin G and oxytetracycline was affected considerably. In the case of penicillin, the activity against *E. coli* disappeared when it was exposed to 5- and 10-megarad doses of γ -radiation but some activity reappeared when it was exposed to a dose of 15 megarads. Similarly, in the case of *S. aureus*, the activity of penicillin decreased at 5- and 10-megarad doses as compared to the control but partially reappeared at the 15-megarad dose. In the case of oxytetracycline, the activity decreased against both the organisms at 15 megarads. In all other antibiotics, the increase or decrease in the activity was not significant.

When the antibiotics were irradiated with neutrons, very little effect or no effect upon the activity of antibiotics was obtained against *E. coli* and *S. aureus* except in the case of spiramycin, which showed decreased activity against *S. aureus* when irradiated at a 15-megarad dose (Table II).

In the case of penicillin where there was an increase in the activity at the 15-megarad dose as compared to 5- and 10-megarad doses, no explanation can be made at this time for the strange behavior. The results indicate that, at least for oxytetracycline and penicillin, the activity can be affected by irradiation of antibiotics in the dry state. These results differ from earlier observations of Holland *et al.* (5); these authors reported no effect of γ -radiation on the antibiotics in the dry state up to a 10-megarad dose. Although cloxacillin, ampicillin, and penicillin are related antibiotics, only penicillin seems to be susceptible to γ -radiation. Although polypeptides are known to be more susceptible to radiation, the polypeptide antibiotics, nisin and bacitracin, did not show any change in activity whereas oxytetracycline was affected considerably.

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▲ To whom inquiries should be directed.