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Modification of the Biological Effects of Ultraviolet Irradiation by Post-irradiation Treatment with Iodoacetate and Peptone

With an appendix on an interpretation of the effects of post-irradiation treatments based on a chemical model

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SUMMARY: A study has been made of the effects of post-irradiation treatment of ultraviolet irradiated spores of *Streptomyces* sp. strain T12 with distilled water, iodoacetate and a peptone medium.

At doses exceeding 400 ergs/mm.² (which exceed that corresponding to the peak of the dose/variant-frequency curve) treatment in distilled water results in increases in both the proportion of survivors and the frequency of variants amongst the survivors. Treatment with iodoacetate caused larger increases, whereas treatment with peptone medium suppressed the increases. A partial resolution of the lethal and mutagenic effects of the radiation was obtained at lower doses, for it was possible to modify the proportion of survivors without changing the variant frequency.

Preliminary studies (Wainwright & Mullaney, 1953) have shown that the extent of survival of ultraviolet irradiated cells of *Escherichia coli* could be increased by a brief period of post-irradiation treatment with iodoacetate, an inhibitor of carbohydrate metabolism. In further studies of these effects we have employed spores of *Streptomyces* sp. strain T12, in which the frequency of radiation-induced variants has been found to be exceptionally high (Newcombe, 1953). A partial resolution of the lethal and mutagenic actions of ultraviolet light has been effected, and it would appear that the post-irradiation metabolism of the spores may be of major importance in determining the extent of the observed effects of a given dose of irradiation.

METHODS

All experiments were performed with suspensions of spores of *Streptomyces* sp. strain T12 freshly prepared from plates inoculated with standard suspensions and incubated at 28° for 5 days. The spores were suspended in distilled water, agitated in a blender for 30 sec. to ensure complete dispersion, and freed of adhering mycelial mat by filtration through sterile Whatman no. 2 filter-paper. For experiments with washed suspensions the spores were washed once with sterile distilled water immediately before filtration.

A G.E. germicidal lamp was used for the irradiation and doses were measured with a Westinghouse dose meter. All operations subsequent to the irradiation were performed by the light of red lamps to prevent photo-reactivation.

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4 ml. Samples of irradiated suspension were diluted with 1 ml. of distilled water or reagent solution and incubated, usually for 3 hr. at 37°. The suspensions were then diluted, plated on standard medium and incubated at 28°. The maximal number of colonies could be observed after 3 days of incubation at 28°, but plates were incubated for 5–7 days to facilitate the scoring of variants.

Estimates of either the proportion of surviving spores or the proportion of induced variant colonies made from plates containing more than eighty colonies per plate were lower than estimates from less crowded plates. Therefore, wherever possible, estimates both of proportion of survivors and of induced variant frequency were made from eight to ten replicate plates each containing between ten and eighty colonies. Usually this condition could be satisfied and all estimates for a given series of treated suspensions made at the same dilutions. In experiments where estimates could not be made from the same dilutions the same qualitative effects of treatment with a given agent were shown by counts made for two dilutions of each of the suspensions.

The basal peptone medium had the composition: K_2HPO_4 0.5 g., asparagine 0.5 g., 'Bacto' peptone 0.5 g., water to 1 l. The standard medium used for plating consisted of basal peptone medium supplemented with 0.5 % (w/v) glucose and 1.5 % (w/v) agar.

Types of variant colony

The most frequently observed types of variant have been described (Newcombe, 1953) and classified according to variations in the quantity of aerial mycelium and in the colour of the mycelial mat.

In addition, we have repeatedly recovered two types of 'pink' variant which differ from both the wild type and the previously described 'red' variant in their response to the inclusion of arabinose in the standard medium in place of glucose. The latter two types give rise to colonies of an intense scarlet colour in the presence of arabinose. This scarlet coloration was not obtained if arabinose was replaced by gluconate, xylose or any other carbon source tested, nor was it obtained by variations in the pH value of the medium. The pink variants yield pink colonies with either arabinose or glucose as carbon source. Although the biochemical basis for these phenomena is not known, they serve to demonstrate that the pink variants represent a distinct class and differ from the red variants in more than a quantitative difference in the amount of red pigment produced. One type of pink variant yields colonies of an intense pink hue under all conditions of growth tested. The second type gives colonies of a light hue when grown as discrete colonies, but gives a growth indistinguishable from that of the wild type when grown under crowded conditions.

Other hitherto undescribed classes of variant included (a) 'golden' and (b) 'watery'. The former yield colonies with both aerial mycelium and mycelial mat of an intense golden hue. These colonies prove to be unstable, for they give a mixture of golden and 'white' variant colonies when subcultured. Watery colonies show a diminished quantity of aerial mycelium, are usually white or pale yellow in colour and superficially appear to be mucoid. These colonies are, however, of the firm texture typical of the *Streptomyces* mycelium.

RESULTS

When spores irradiated at a dose of 400 ergs/mm.² were incubated with 2×10^{-4} M-iodoacetate (neutralized to pH 7.2) the extent of survival was from 2 to 3 times greater than for control irradiated spores similarly treated in distilled water (Table 1). This effect of iodoacetate treatment has been repeatedly observed (e.g. Tables 2 and 3). The extent of the difference in survival varied from as little as 20 % (in one exceptional experiment) to sevenfold, but usually was in the range of two- to threefold.

Table 1. *Influence of treatment with 2×10^{-4} M-iodoacetate and with peptone + arabinose medium upon survival and frequency of variants*

Expt. no.		Control		Treated		
		Distilled water	Plated with iodoacetate	Iodoacetate	Medium* iodoacetate	Medium* iodoacetate
1	Survival $\times 10^6$	16	14	34	10	10
	Percentage variants	39.5	34.8	44.5	34.4	30.1
	Counts	71/180	43/124	274/617	57/166	50/166
2	Survival $\times 10^6$	152	140	343	78	117
	Percentage variants	53.1	48.5	58.1	42.5	47.6
	Counts	114/215	97/199	255/437	47/111	79/166
3	Survival $\times 10^6$	11	12	35	11	13
	Percentage variants	53.1	50.7	62.9	37.2	28.6
	Counts	99/186	92/182	299/479	61/164	61/213

* Basal peptone medium (see methods) plus 10^{-3} M-arabinose.

Dose: 400 ergs/mm.². Temp. 37°. Time of treatment 3 hr.

The counts are the actual numbers of colonies (variants/total) from which the percentage variants was estimated.

Results obtained with washed spore suspensions were no more marked, nor more quantitatively reproducible than with unwashed spores (Table 2). We have, therefore, assumed that the variations in the initial proportions of survivors with unincubated spores and in the extent of the effect of iodoacetate upon survival were due to uncontrollable variations in the initial physiological condition of the unirradiated spores or in the initial viable count of the unirradiated suspension (and, hence, the dose of radiation received per spore). As these quantitative variations have not masked the qualitative effects studied, no attempt has been made to standardize either of the variables at the expense of the other.

We have also consistently found that the proportion of variant colonies arising from iodoacetate-treated spores was greater than with the control irradiated spores incubated in distilled water (Tables 1 and 2). This difference in variant frequencies was highly significant. In the first eighteen of our experiments involving iodoacetate treatment the difference in variant frequency was significant at the 5 % level in fifteen, the sum of individual χ^2 was 246 (18 D.F., $P < 0.001$) and the χ^2 of the pooled data was 248 (1 D.F., $P < 0.001$).

These effects of iodoacetate upon the proportions of survivors and upon the variant frequencies could not be attributed to the small concentrations of iodoacetate carried with the spores on to the plating medium, unless firmly

Table 2. *Influence of treatment with 2×10^{-5} M-iodoacetate and with peptone medium upon survival and frequency of variants*

Expt. no.	Temperature of treatment		Control		Treated		
			0 hr.*	Distilled water	Iodoacetate	Medium	Medium + iodoacetate
1	37°	Survival $\times 10^6$	—	154	214	126	120
		Percentage variants	—	34.0	44.6	24.6	21.4
		Counts	—	81/238	120/269	61/247	42/196
2	37°	Survival $\times 10^6$	—	52	122	32	35
		Percentage variants	—	36.4	49.5	23.5	29.4
		Counts	—	153/421	492/1021	60/256	83/288
3	37°	Survival $\times 10^6$	—	123	390	45	58
		Percentage variants	—	47.4	58.5	37.4	41.8
		Counts	—	68/144	267/456	197/527	286/679
4†	37°	Survival $\times 10^6$	7.4	12	21	9.5	10
		Percentage variants	23.5	32.5	41.1	24.1	24.7
		Counts	107/456	242/745	529/1292	141/584	160/647
5†	37°	Survival $\times 10^6$	36	46	77	47	40
		Percentage variants	19.6	23.8	37.4	19.6	23.5
		Counts	29/148	45/189	110/295	38/194	39/165
6	28°	Survival $\times 10^6$	15	17	54	13	20
		Percentage variants	33.6	44.0	50.2	31.9	41.1
		Counts	65/194	93/211	312/621	30/94	93/226
7	28°	Survival $\times 10^6$	15	22	56	17	18
		Percentage variants	34.7	32.2	58.9	31.6	28.7
		Counts	40/117	63/195	260/444	46/146	45/157
8†	28°	Survival $\times 10^6$	4	7	52	5	7
		Percentage variants	57.4	59.1	69.5	59.2	57.8
		Counts	102/178	174/295	162/234	128/216	191/330
9†	28°	Survival $\times 10^6$	23	27	55	26	33
		Percentage variants	21.1	28.6	44.1	22.4	24.1
		Counts	42/198	107/373	332/753	79/354	109/453

* Control suspension plated immediately after irradiation and dilution.

Dose: 400 ergs/mm.². Time of treatment 3 hr.

The counts are the actual numbers of colonies (variants/total) from which the percentage variants was estimated.

† Experiments with washed suspensions.

Table 3. *Influence of iodoacetate concentration during treatment upon survival and variant frequency*

Expt. no.		Control (distilled water)	Treated with iodoacetate at	
			2×10^{-4} M	2×10^{-5} M
1	Survival $\times 10^6$	32	95	45
	Percentage variants	44.7	64.8	53.5
	Counts	259/581	113/174	481/902
2	Survival $\times 10^6$	165	244	180
	Percentage variants	18.1	31.7	19.8
	Counts	37/205	96/302	44/222

Dose: 400 ergs/mm.². Temp. 37°. Time of treatment 3 hr.

The counts are the actual numbers of colonies (variants/total) from which the percentage variants was estimated.

bound. Plating control irradiated spores incubated in distilled water on agar containing equivalent concentrations of iodoacetate (10^{-7} to 10^{-8} M) did not result in increased survival or variant frequency (Table 1).

Two hundred and thirty-seven variant colonies were isolated from spores treated with iodoacetate, and all were found to retain their variant character upon subculture. If the increased proportion of variant colonies resulting from iodoacetate treatment had been due to the induction of temporary physiological variations of character some forty of these isolates would have been expected not to show a heritable modification of character ($P < 0.001$). Treatment with iodoacetate had not caused any significant effects upon survival or the proportion of spontaneous variants (which was of the order of 1–2 %) with unirradiated spores ($0.3 < P < 0.5$ for 66,000 colonies).

After incubation with peptone medium the extent of survival and the proportion of induced variants were lower than with irradiated control spores incubated in distilled water (Tables 1 and 2). Further, the presence of peptone medium appeared to inhibit the effects of post-irradiation treatment with iodoacetate (Tables 1 and 2). The effects of the medium were more marked in the absence of added carbohydrate. Treatment with the peptone medium had no significant effects upon survival or the proportion of spontaneous variants with unirradiated spores ($P > 0.9$ for 54,000 colonies).

The actions of iodoacetate and of peptone medium appeared to be the enhancement and the inhibition, respectively, of phenomena taking place with spores incubated in the total absence of added nutrient (Table 2).

The effects of incubation for 3 hr. with arabinose (in the absence of peptone), at a concentration of 10^{-3} M, were inconsistent.

Some effects of post-irradiation treatment with 2×10^{-4} M iodoacetate were found with a period of incubation of only 15 min. Survival was increased, from 1.1 to fourfold, in ten of twelve experiments and in two no change was observed ($P < 0.05$ by the sign test). Effects upon the proportions of variants were inconsistent and differences significant at the 5 % level were observed in only two experiments. In those cases in which there was more than a twofold increase in survival the proportion of variants was reduced, and in the remaining experiments (including those in which there was no effect upon survival) the proportion of variants was increased.

Effect of temperature of incubation

Results obtained after treatment at 28° were essentially similar to those observed after post-irradiation treatment at 37° . With the exception of one experiment, the effects observed after 3 hr. incubation were of the same magnitude at both temperatures (Table 2). Treatment with iodoacetate at 28° for 15 min. did not increase the survival above that observed with control spores similarly treated in distilled water in six of eight experiments, and only caused a 25 % increase in survival in the remaining two. On the other hand, iodoacetate raised the variant frequency above that of the corresponding controls in all but one of the experiments, and the increase was significant at the 5 % level in three ($P < 0.001$ for pooled results).

Effect of iodoacetate concentration

The effects of two concentrations of iodoacetate upon spores irradiated at a dose of 400 ergs/mm.² and incubated at 37° are illustrated in Table 3. Iodoacetate was found to have some activity at a concentration of 2×10^{-5} M, but less than at 2×10^{-4} M. Essentially similar results were also obtained for spores irradiated at a dose of 200 ergs/mm.², and for spores irradiated at either dose and incubated for 15 min. Iodoacetate was lethal to unirradiated control spores at a concentration of 2×10^{-3} M, causing extensive killing in 15 min. at 37°.

Effect of dose of irradiation

A dose of 400 ergs/mm.² exceeds that corresponding to the peak of the 'dose variant-frequency' curve (Wainwright & Nevill, 1955). Hence, an increased survival associated with an increased variant frequency as a result of post-irradiation treatment could be the consequence of a reduction in the 'effective' dose of radiation received by the spores. Therefore, the effects of post-irradiation treatments upon spores irradiated at other doses were studied.

Similar results to those obtained with spores irradiated at 400 ergs/mm.² were also obtained with spores irradiated at 600 ergs/mm.² Results obtained with spores irradiated at 200 and at 100 ergs/mm.² are given in Table 4. The effects upon survival were all qualitatively the same as with spores irradiated at the higher doses, except that treatment of spores irradiated at 100 ergs/mm.² with peptone medium did not cause any reduction below the level obtained with controls incubated in distilled water. The effects of incubation in distilled water or with peptone medium upon the proportion of induced variants with spores irradiated at 200 ergs/mm.² were qualitatively the same as with spores irradiated at the higher doses, but were quantitatively smaller. The variant frequency increased after incubation in distilled water (Table 4, columns 4 and 5, χ^2 for pooled data 11.53, $P < 0.001$) and this increase was inhibited in the presence of peptone medium (Table 4, columns 5 and 7, χ^2 for pooled data 7.39, $P < 0.007$). Treatment in either distilled water or peptone medium did not cause any change in the variant frequency in the case of spores irradiated at 100 ergs/mm.². Iodoacetate treatment had no effect upon the proportion of induced variants with spores irradiated at either 200 or 100 ergs/mm.². The effects of the various treatments upon spores irradiated at different doses are summarized in table 5.

DISCUSSION

It is improbable that the effects of the various post-irradiation treatments studied can be attributed to increases in cell numbers due to growth of the spores occurring during the period of treatment and subsequent fragmentation of the mycelium during plating. We have not detected any significant increases in the viable counts of unirradiated control suspensions. Indeed, cytological examination has shown that development of the germination tube is barely initiated after 3 hr. incubation in nutrient medium at 37°. Further, less than 2% of unirradiated control spores germinated in the absence of added

nutrient during the course of incubation for 96 hr. (Wainwright & Nevill, 1955).

It would seem equally improbable that the effects upon the proportions of variant colonies can be attributed to effects upon the germination and growth

Table 4. *Influence of treatment with 2×10^{-4} M-iodoacetate and with peptone medium upon survival and frequency of variants with spores irradiated at doses of 200 and 100 ergs/mm.²*

Expt. no.	Ultraviolet dose (ergs/mm. ²)		Control		Treated		
			0 hr.*	Distilled water	Iodoacetate	Medium	Medium + iodoacetate
1	200	Survival $\times 10^2$	1.6	3.7	8.2	1.3	1.5
		Percentage variants	37.6	44.6	47.5	36.6	44.8
		Counts	114/303	306/688	72/152	86/238	114/255
2	200	Survival $\times 10^2$	0.8	1.9	3.7	0.7	1.0
		Percentage variants	26.4	45.7	37.2	31.8	44.9
		Counts	27/102	131/286	237/637	44/138	85/190
3	200	Survival $\times 10^2$	3.2	5.9	12.2	2.7	3.1
		Percentage variants	32.2	33.3	39.3	32.5	30.9
		Counts	149/462	287/862	70/179	115/354	138/447
4	200	Survival $\times 10^2$	1.7	4.2	5.6	1.3	1.6
		Percentage variants	37.2	40.0	39.4	40.3	38.9
		Counts	277/745	74/185	58/147	231/573	280/720
5	200	Survival $\times 10^2$	2.4	4.4	9.6	—	—
		Percentage variants	34.4	35.9	40.2	—	—
		Counts	71/207	123/342	327/813	—	—
6	200	Survival $\times 10^2$	2.6	5.7	12.0	—	—
		Percentage variants	34.0	38.5	36.4	—	—
		Counts	164/481	357/932	79/218	—	—
7	200	Survival $\times 10^2$	1.8	3.8	—	2.5	—
		Percentage variants	38.0	43.4	—	33.4	—
		Counts	95/250	237/546	—	117/351	—
8	100	Survival $\times 10^2$	43.5	59.2	65.8	55.3	53.0
		Percentage variants	17.1	16.9	17.2	20.2	20.0
		Counts	159/928	214/1272	242/1405	214/1059	227/1133
9	100	Survival $\times 10^2$	39.6	35.4	59.3	40.8	52.6
		Percentage variants	25.7	25.4	24.1	25.7	24.4
		Counts	109/423	95/375	152/631	116/433	136/558
10	100	Survival $\times 10^2$	14.5	46.9	47.2	47.9	45.8
		Percentage variants	23.4	23.3	22.4	17.1	21.0
		Counts	34/145	110/469	93/415	82/479	85/405
11	100	Survival $\times 10^2$	24.8	38.2	52.0	39.1	27.6
		Percentage variants	21.9	25.1	27.1	26.8	27.6
		Counts	70/318	100/398	165/610	134/498	72/260

* Control suspension plated immediately after irradiation and dilution.

Temp. 37°. Time of treatment 3 hr.

The counts are the actual numbers of colonies (variant/total) from which the percentage variants was estimated.

upon the plates (due to traces of reagent carried over during plating) resulting in selection favouring either variant or non-variant types of mycelial growth. The concentrations of iodoacetate present in the plating medium were inadequate to account for the effects observed (Table 1), unless these resulted

from iodoacetate firmly bound to the spores. Moreover, results obtained with irradiated spores treated with iodoacetate and subsequently washed indicate that this reagent is not firmly bound to the spores (Wainwright & Nevill, 1955). Further, the magnitude of the effects of post-irradiation treatment with iodoacetate are dependent upon the duration of the treatment for periods up to at least 96 hr. (Wainwright & Nevill, 1955), whereas the binding of a firmly adsorbed chemical would be expected to be complete well within 3 hr. The amounts of peptone medium carried over to the plates are negligible in comparison with that already present in the plating medium.

Table 5. *Summary of effects of incubation with iodoacetate and peptone medium*

Dose (ergs/mm. ²)	Water*	Iodoacetate†	Medium†
(a) On survival			
400 }	Increased	Increased	Reduced
600 }			
200	Increased	Increased	Reduced
100	Increased	Increased	Unchanged
(b) On frequency of variants			
400 }	Increased	Increased	Reduced
600 }			
200	Increased	Unchanged	Reduced
100	Unchanged	Unchanged	Unchanged

* Effects relative to control spores plated immediately after irradiation and dilution.

† Effects relative to control spores incubated with water.

The results obtained in this study (Table 5) do not correspond to a simple modification by post-irradiation treatment of the 'effective' dose of radiation received by the spores. With spores irradiated at doses exceeding that corresponding to the peak of the 'dose variant-frequency' curve (400 ergs/mm.²), there is indeed a close parallel between the effects of incubation in distilled water or with iodoacetate and of a decrease in radiation dose. Conversely, the effects of incubation in peptone medium correspond to those produced by an increase in the dose of radiation employed (Wainwright & Nevill, 1955). However, with spores irradiated at lower doses some portion of the lethal effect of the radiation can be reversed without any accompanying change in the frequency of induced heritable variants amongst the survivors. Thus, our studies demonstrate a partial resolution of the lethal and mutagenic effects of ultraviolet radiation.

Various possible mechanisms can be proposed to account for the effects of post-irradiation treatments with chemical agents in modifying the biological responses of irradiated cells. At one end of the range are simple chemical mechanisms in which the added chemical can modify a reaction between a hypothetical radiation-produced poison and a sensitive cell component as a result of combining with either of them. At the other end of the range of mechanisms are those which postulate that the added chemical changes the course of the abnormal cell metabolism resulting from the radiation because

of some mechanism known to affect normal cell metabolism (i.e. the added chemical is a metabolic inhibitor, a cell nutrient, the prosthetic group of a known enzyme). Even these extremes do not represent mutually exclusive alternatives, for it is possible to postulate that the poison of the former type of mechanism is an inhibitor of cell metabolism or a degraded substrate or enzyme. There is no limit to the number of hypothetical radiation-produced poisons which may be postulated. Further, there is no limit to the properties which may be ascribed to any one such poison in order to account for the fact that with two reagents of closely similar chemical structure one is active in modifying the effects of the radiation and the other is not. In addition, the only evidence that any applied chemical has actually entered the treated spores is the observation of a definite effect, for it is not generally possible to determine chemically which of the individual spores contain the chemical and in what concentration.

For these reasons it is not possible to provide critical evidence which would eliminate any of the various hypotheses which may be advanced. Rather, we must inquire whether any observed effect of post-irradiation treatment with a chemical reagent is due primarily to the known biochemical properties of the reagent, or to some relatively simple chemical reaction(s) involving a specific type of radiation-produced poison. This means that we must decide: (a) whether the effects of a particular post-irradiation treatment can be explained on the basis of the known biochemistry of the organism and the reagent used, and (b) how complex must be the postulates required to account for the effects by a predominantly chemical model. We may also note that with our questions posed at this level it is immaterial whether any observed effect upon the induced variant frequency is due to a preferential action of the agent leading to greater survival of potentially-variant than of non-variant spores (or vice versa), for such preferential effects would in themselves be due either to an effect upon cell metabolism or to some relatively simple chemical reaction.

The increased survival resulting from incubation in distilled water can be attributed to the replacement of material inactivated during the irradiation through the metabolism of the endogenous reserves of the spores. Any disturbance of the balance of that metabolism, either by the addition of a nutrient (i.e. peptone medium) or a metabolic inhibitor (i.e. iodoacetate), or by dilution of the suspension and plating on solid medium, would be expected to result in a modification of the observed effects of the irradiation. Further, unless the relative extents of inactivation of all the cell constituents were the same at all doses of irradiation, qualitative variations in the effects of treatment with a given agent would be expected at differing doses. Similarly, the effects of the post-irradiation treatments used in modifying the frequency of induced variant colonies can be explained on the basis of effects of the added chemicals upon normal cell metabolism.

Any model required to explain the results obtained in this study (Table 5) by a simple set of chemical reactions has to attribute very complex properties to the hypothetical radiation-produced poisons. We have indicated in the Appendix the postulates which we believe to be the minimum requirements.

Further studies of: (a) the response of ultraviolet irradiated spores given post-irradiation treatment in distilled water or iodoacetate for varying periods of time (Wainwright & Nevill, 1955), (b) of the responses to post-irradiation treatments with other agents of known significance to the cell economy (in preparation), and (c) of the influences of post-irradiation treatments upon the phenomenon of photoreactivation (in preparation), all indicate much more complex minimum sets of postulates.

We, therefore, prefer to interpret our results as the consequences of disturbances of post-irradiation cell metabolism by the added reagents. Thus, they strongly indicate that greater attention should be given to the possible importance of the post-irradiation cell metabolism than has hitherto been accorded to it. The studies of Lwoff and co-workers (see Lwoff, 1952, 1953; Jacob, Siminovitch & Wollman, 1952) have clearly demonstrated the significance of post-irradiation cell metabolism in the phenomena of radiation-induced lysogenesis and colicinogenesis. In the study of other aspects of the action of irradiations, however, critical evidence is not yet available. Nevertheless, reports have been made of phenomena which can be at least as readily interpreted as due to effects upon the post-irradiation metabolism of irradiated cells as by any other mechanism. For example, it has been found that variations of the temperature of post-irradiation incubation cause significant changes in both the observed extent of survival and the frequency of induction of mutations with irradiated cells of *Escherichia coli* (Stapleton, Billen & Hollaender, 1952; Witkin, 1953). Similarly, it has been found that incubation of irradiated cells of this organism with yeast or meat extracts causes increased survival (Hollaender, Stapleton & Billen, 1953). Further, it may be noted that an increased frequency of mutants has been observed with spores of *Trichophyton mentagrophytes* irradiated at a high dose of ultraviolet and incubated in saline for prolonged periods (Hollaender & Emmons, 1941).

If the post-irradiation cell metabolism is of importance in determining the observed effects of the irradiation it might markedly affect the form of the observed 'dose-survival' and 'dose-mutation' curves. Indeed, it is not possible to obtain such curves without permitting considerable metabolism and cell division to occur. Thus, it would appear that conclusions concerning the mechanism of action of irradiations based upon dose-survival (or dose-mutation) curves obtained after treatment of irradiated cells with agents of significance to the cell economy, or after modifications of the environmental conditions known to affect markedly the course of cell metabolism, should be accepted with reserve.

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APPENDIX

*Interpretation of the effects of post-irradiation treatments
based on a chemical model*

Incubation in peptone medium prevented the increase in the proportion of survivors which occurred when spores irradiated at doses exceeding 200 ergs/mm.² were incubated in distilled water. However, treatment with peptone medium did not prevent the increase in proportion of survivors which occurred when spores irradiated at a dose of 100 ergs/mm.² were incubated in distilled water. Thus, we may, for example, conclude that the concentration of lethal 'poison' produced at a dose of 100 ergs/mm.² is inadequate for appreciable reaction with the peptone to occur (i.e. the concentration is less than the required 'threshold concentration'). Alternatively, we may conclude that peptone medium modifies a chemical reaction involving a lethal poison which is only produced when the dose of radiation exceeds 100 ergs/mm.². Similarly, we may conclude either that the various reagents can only react with a mutagenic poison when the concentration exceeds a certain threshold, or that one mutagenic poison is not formed at doses less than 100 ergs/mm.² and a second mutagenic poison is not formed at doses less than 200 ergs/mm.².

As incubation in distilled water of spores irradiated at doses of 100 ergs/mm.² results in an increased proportion of survivors without any change in the frequency of induced variant colonies we must postulate a minimum of two poisons. If we also postulate that both poisons are lethal and mutagenic, and that interaction with the added reagents only occurs when the concentration of poison exceeds a different threshold concentration for each added reagent,

it is only necessary to postulate this minimum number of two poisons. On the other hand, if we postulate that interaction with the added reagents occurs at all concentrations of the various poisons we must postulate a minimum of two lethal and three mutagenic 'poisons'. The lethal and mutagenic effects of doses of radiation exceeding 200 ergs/mm.² which can be modified by post-irradiation treatments could be those of a single lethal and mutagenic poison, but the results obtained with spores irradiated at doses of 200 and 100 ergs/mm.² (Table 5) require that we postulate a minimum of two distinct mutagenic poisons but only one lethal poison.

We, therefore, believe the minimum postulates required for the interpretation of the effects of the post-irradiation treatments summarized in Table 5 by a relatively simple chemical mechanism to be either: (i) that the irradiation causes the production of two lethal and/or mutagenic poisons, with differing threshold concentrations required for interaction with endogenous cell constituents, peptone and iodoacetate, respectively: or (ii) that four different poisons are produced at different doses, and that there are no threshold concentrations required for interactions with added chemicals. These poisons would be, respectively, one lethal and mutagenic poison, one other lethal poison, and two other mutagenic poisons not identical with either lethal poison.

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