THE INACTIVATION OF PYROGENS BY GAMMA RADIATION

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Gamma irradiation in a dose of $5{\cdot}0 \times 10^6$ rad reduced the pyrogenicity of London tap water but $10{\cdot}0 \times 10^6$ rad was required for it to pass the B.P. test, 20×10^6 rad reduced the pyrogenicity of a pyrogen from *S. abortus equi* but 25×10^6 rad was required before it passed the B.P. test.

The fact that micro-organisms can be inactivated by exposure to ionising radiations has been known for many years. The possibility of using such radiation for the treatment of food has been the subject of a review by Hannan[1]. Ionising radiations have also been used for the sterilisation of numerous pharmaceutical and surgical products. Their use in these fields has been summarised by Horne[2].

The late D. E. Lea's book "Actions of radiations on living cells"[3] describes radiation effects in great detail. It has been suggested that doses about 2×10^6 rad or more are required to inactivate all micro-organisms. Vegetative organisms are killed with $0{\cdot}5 \times 10^6$ rad and moulds and yeasts with $1{\cdot}0$ or $1{\cdot}5 \times 10^6$ rad. Spores are more resistant and need $2{\cdot}0 \times 10^6$ rad. Virus particles are inactivated only at still higher amounts of $2{\cdot}0$–$5{\cdot}0 \times 10^6$ rad. The inactivation amount depends upon the number of organisms and their environment. Exceptionally large amounts are needed with massive numbers or special conditions, for example in anoxia.

Davis, Dole, Izzo and Young[4] examined the haemolytic effects of radiation and Huber[5] investigated the inactivation of the virus of homologous serum jaundice.

Because of the possible use of radiation methods to sterilise plasma we have examined the effect of gamma radiation on pyrogens, since these are a possible source of contamination in plasma.

Experimental

The sources of pyrogens used were London unsterilised tap water, which has been found to be pyrogenic[6,7], and lipopolysaccharide Wander, Pyrexal, a potent pyrogen from *Salmonella abortus equi* supplied in ampoules containing 1 μg. in 2 ml.

Samples of each were irradiated with various doses of gamma radiation and were injected intravenously into the ear veins of sensitised rabbits of 2 to 2·5 kg. The doses used were 10 ml./kg. of tap water and 1 μg. per rabbit of Pyrexal. A dose of 10 ml./kg. of unsterilised tap water could be expected to give a response of about 1·5°. The dose of Pyrexal represents about 250 times the dose which could normally be expected to give a response of 0·6°. The rabbits were kept in restraining boxes during

the experiment and their temperatures were measured by means of a thermistor electrical thermometer for at least one hour before and three hours after injection. The results are shown in Tables I and II.

TABLE I

THE EFFECT OF GAMMA RADIATION ON PYROGENICITY OF LONDON TAP WATER

Irradiation dose (10^6 rad)	Number of rabbits	Mean rise ° C.	Limits
0·5	3	1·38	Limits 1·65° and 0·88°, i.e. +19·6 −36·2 per cent
1·0	2	1·36	,, 1·43° and 1·30°, ,, + 5·1 − 4·4 ,, ,,
2·0	3	1·04	,, 1·30° and 0·85°, ,, +25·0 −18·27,, ,,
3·0	2	1·23	,, 1·53° and 0·93°, ,, +24·1 −24·1 ,, ,,
5·0	12	0·59	S.D. 0·47° Coefficient of variation 80·1 per cent
5·0	7	0·78	,, 0·52° ,, ,, ,, 66·4 ,, ,,
5·0	12 + 7	0·66	,, 0·48° ,, ,, ,, 72·1 ,, ,,
7·5	12	0·85	,, 0·32° ,, ,, ,, 37·9 ,, ,,
10·0	12	0·35	,, 0·18 ,, ,, ,, 50·3 ,, ,,
20·0	3	0·28	Limits 0·45° and 0·10°, i.e. +60·71 −64·28 per cent

TABLE II

EFFECT OF GAMMA RADIATION ON PYREXAL

Irradiation dose (10^6 rad)	Number of rabbits	Mean rise ° C.	Limits
2·0	3	1·71	Limits 1·90° and 1·50°, i.e. +11·11 −12·3 per cent
4·0	3	1·02	,, 1·50° and 0·43°, ,, +47·05 −57·8 ,, ,,
20·0	9	0·64	S.D. 0·148° Coefficient of variation 21·9 ,, ,,
22·5	12	0·27	,, 0·35° ,, ,, ,, 129·6 ,, ,,
25·0	12	0·38	,, 0·61° ,, ,, ,, 160·0 ,, ,,
45·0	12	0·15	,, 0·14° ,, ,, ,, 93·3 ,, ,,
30·0	3	0·47	Limits 0·55° and 0·30°, i.e. +17·0 −36·2 per cent
35·0	3	0·22	,, 0·43° and 0·10°, ,, +95·5 −54·5 ,, ,,
40·0	3	0·28	,, 0·63° and 0·03°, ,,+125·0 −89·3 ,, ,,

RESULTS

The irradiation of London tap water with doses of ionising radiations of from 0·5 to 3·0 × 10^6 rad, had little effect on its pyrogenicity (Table I). With a dose of 5 × 10^6 rad the results were variable. Two groups would have passed the old B.P. test and one would have passed the new test. The results of the experiments on twelve rabbits just failed to pass the new test but on the mean value it would have passed the old test by 0·01°. This appears to be a dose just below the inactivating one. Because of this doubt another seven tests were made on samples given this treatment and the mean result of these was 0·77°. Samples of London tap water treated with 10 × 10^6 rad and 20 × 10^6 rad easily passed the B.P. test.

The irradiation of Pyrexal solution (1 μg. in 2 ml.) with 2 × 10^6 rad and with 4 × 10^6 rad (Table II) did not appreciably reduce its pyrogenicity to rabbits in a dose of 0·5 μg./kg. The irradiation with 20 × 10^6 rad reduced its activity to about one third and 25 × 10^6 rad practically destroyed the activity. This is a severe test since the amount of pyrogen injected is approximately 250 times that necessary to produce a pyrogenic response.

DISCUSSION

There appear to be few references to the effects of ionising radiations on pyrogens. Bellamy and Lawton[8] report that human plasma irradiated

with $2{\cdot}0 \times 10^6$ rad or $5{\cdot}0 \times 10^6$ rad passed the standard pyrogen test in rabbits but we have now learned from them that the unirradiated plasma was not pyrogenic.

Pyrogens can be inactivated by ionising radiations but the dose is greater than that required to kill micro-organisms. The method might be of practical use in removing the pyrogenicity of intravenous preparations such as plasma or protein hydrolysates if it could be shown that the products were not damaged by radiation.

Appendix

The samples were treated with gamma radiation from a cobalt 60 source. This was in the form of a hollow cylinder and the samples were placed in the centre, thus ensuring a uniform reproducible dose.

The dose rate was measured using a standard chemical method; the oxidation of a solution of ferrous ion (ferrous ammonium sulphate). The production ferric ions during irradiation is, within limits, independant of the initial concentration of ferrous ion and is proportional to the dose. The total dose which can be measured is limited by the oxygen available in the solution. The ferric ion concentration was measured at 304 mμ and 25° as soon as possible after irradiation, with a Unicam S.P.500 spectrophotometer.

At the time of measurement the dose rate was 7200 I rad/minute. One rad denotes an energy deposition of 100 ergs per g. of material irradiated.

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DISCUSSION

The paper was presented by Mr. T. D. Whittet.

Dr. G. E. Foster (Dartford). Sterilisation by gamma radiation often caused decomposition. Had the water been tested for peroxides, for example?

Dr. J. C. Parkinson (Brighton). Was plasma fit for use after being irradiated? What was the effect of irradiation at higher than room temperature?

MR. D. H. O. GEMMELL (Glasgow). Had the authors irradiated Pyrexal as a solid or in solution?

MR. J. W. HADGRAFT (London). After autoclaving London tap water the author had found it to comply with the B.P. test for absence of pyrogens. The water had been used without filtration, and it might well be that the pyrogens had been adsorbed on to the finely precipitated salts. There was an alternative explanation that the water was not pyrogenic in the first place. The paper seemed to indicate that the substance present in London tap water which caused an increase in body temperature was more sensitive to gamma radiation than known pyrogens or *Salmonella abortus equi*.

MR. K. L. SMITH (Nottingham). Irradiation caused heparin to lose potency: heparin was a sulphonated polysaccharide and it had been suggested the pyrogens were polysaccharides. Was the reason that London tap water when autoclaved was rendered non-pyrogenic because it was heavily chlorinated?

DR. H. DAVIS (London). At University College Hospital some years ago a famous surgeon insisted that every injection of 5 per cent dextrose should be made with London tap water which was filtered and then boiled. In ten years hundreds of those injections were given to patients, without a rigor occurring. He was not convinced that the evidence which had been put forward as to the pyrogenicity of London tap water was sufficient. Had correlation of the rise in temperature of the rabbit and the production of rigor in a human been attempted?

MR. G. SYKES (Nottingham). One assumed that the mechanism of sterilisation by irradiation was by denaturation of proteins in the organisms. Presumably the proteins in plasma were closely allied to those in bacteria, and it was difficult to see why irradiation should attack organism protein and not plasma protein.

DR. L. SAUNDERS (London). Was the method feasible for treating water continuously?

PROFESSOR J. P. TODD (Glasgow). There was a general assumption that all substances producing a rise in temperature were polysaccharides. The production of a rise in temperature was only one of many phenomena associated with physiological stress. A great many substances could do that, not necessarily a bacterial polysaccharide, which was reasonably thermostable. Possibly there was another type of substance in London tap water, protein-like in nature.

DR. T. WALLIS (London) understood that if a precipitate were produced in the liquid the pyrogen would be adsorbed on the precipitate. Had the authors determined the hardness of the London water, because if London water were heated a precipitate was obtained which might be unobserved by anybody who had either boiled or autoclaved the water, because it might adhere to the side of the vessel in which the heating was done.

DR. J. G. DARE (Leeds) commented that there was evidence, for certain specified pyrogens, correlating the effects of injection into rabbits and man.

DR. J. W. FAIRBAIRN (London) was surprised at the very high coefficients of variation in Tables I and II.

MR. WHITTET replied. Radiations damaged a number of compounds, and some peroxide was formed. The dose necessary to destroy pyrogens would render plasma unfit for use. Pyrexal solution containing 1 μg./2 ml. ready for injection was irradiated. Thames water was strongly pyrogenic, and on autoclaving its pyrogenicity was not destroyed. Primary filtered London tap water was strongly pyrogenic after autoclaving, but Thames mains water after secondary filtration was not pyrogenic after autoclaving. The response from London tap water gave the same shape of curve as pure pyrogens. Primary filtered London tap water was pyrogenic both before and after autoclaving, but if it were left standing it lost its pyrogenicity after a month or two. A large number of samples of different origins had been examined at all seasons of the year, with both mains water and water from cisterns, and no differences had been found in any of the samples. Glass ampoules became brown when subjected to irradiation, and there was slight effervescence when an ampoule was opened which had been subjected to a high dosage. There was no apparent effect on the rabbits at all. He did not consider chlorination was the reason for the pyrogenic activity, as water before chlorination behaved in the same manner as it did after chlorination. He doubted whether it would be a feasible method for continuous water treatment. What convinced him that pyrogens were the cause was the fact that raw and primary filtered water behaved exactly as bacterial pyrogens. The material was extremely thermostable, but in secondary filtered water there was thermal instability. With unsterilised water the time: temperature curve was that of a typical bacterial pyrogen. There was no appreciable precipitate visible after autoclaving, but it was possible that one had formed and adhered to the glass. The autoclaved samples were injected unfiltered. The results shown in the Tables were not intended to give a quantitative measure of pyrogenicity.