J. Hyg., Camb. (1970), **68**, 293
Printed in Great Britain

The use of gamma radiation for the elimination of *Salmonella* from frozen meat

By F. J. LEY, T. S. KENNEDY and K. KAWASHIMA*
Wantage Research Laboratory (AERE), Wantage, Berks.

and DIANE ROBERTS and BETTY C. HOBBS
Food Hygiene Laboratory, Central Public Health Laboratory, Colindale Avenue, London, N.W. 9

SUMMARY

The use of a gamma radiation process for the elimination of *Salmonella* from frozen meat is considered with particular reference to the treatment of boned-out horsemeat and kangaroo meat imported into the UK and intended for use as pet meat.

Examination of dose/survival curves produced for several serotypes of *Salmonella* in frozen meat shows that a radiation dose of 0·6 Mrad. will reduce a population by at least a factor of 10^5. The influence on the radiation resistance of salmonellas of such factors as preirradiation growth in the meat and temperature during irradiation have been examined and considered. It is also demonstrated with both preinoculated and naturally contaminated meat that postirradiation storage in the frozen state does not lead to the revival of irradiated salmonellas.

The properties of *Salmonella* survivors deliberately produced in meat using conditions of irradiation designed to simulate a commercial process are studied after six recycling treatments through the process. There were no important changes in characteristics normally used for identification of *Salmonella* but radiation resistance was lowered. Survivors grown *in situ* in meat after irradiation showed an abnormally long lag phase, and removal of competitive microflora in meat by the radiation treatment can influence the growth of salmonellas.

INTRODUCTION

Meat and meat products including poultry are perhaps the commonest known vehicles of *Salmonella* infection. Pet meat sold raw has also caused concern as a potential source of human salmonellosis, contamination spreading to human foods from knacker meat (Beasley *et al.* 1967) or from imported meats such as boneless horsemeat (Galbraith *et al.* 1962; Hobbs, 1965) or kangaroo meat (Anderson, Crowder & Woodruff, 1964) which are used in this trade. The high rate of contamination of frozen carcasses or boneless horsemeat from three countries in South America is confirmed by the investigations of van Schothorst & Kampelmacher (1967) in the Netherlands where this meat is often included in minced meat for human consumption.

* Present address: Food Research Institute, Ministry of Agriculture and Forestry, Tokyo, Japan.

https://doi.org/10.1017/S0022172400028758 Published online by Cambridge University Press

In an attempt to obviate the danger associated with the raw pet-meat trade in the UK, new regulations (The Meat (Sterilization) Regulations 1969) came into operation on 1 November 1969 superseding those made in 1960. These regulations require that knacker meat and meat imported other than for human consumption as well as butchers' meat or imported meat which in either case is unfit for human consumption, must now be sterilized before entering the chain of distribution. Sterilization is defined in terms of the processes to be applied. In relation to the pet meat trade, 'sterilized' means treated by boiling or steam under pressure until every piece of meat is cooked throughout. Where the trade is concerned, this process must present practical problems to importers of frozen meat in large blocks, in addition to eliminating an established market in raw pet-meat. Furthermore, the heat process, even if carried out effectively, must be supervised to ensure that recontamination of the cooked meat, which is a distinct possibility (Galbraith *et al.* 1962), does not occur.

In contrast to the heating process, gamma radiation will penetrate blocks of frozen meat and inactivate bacteria without changing the physical state of the meat. Frozen meat could be irradiated in its original package as imported, no handling of the meat is involved and cross-contamination between unprocessed and processed meat at a radiation plant is readily avoided. In describing the efficacy of a gamma-radiation process for the elimination of salmonellas from frozen boneless meat Ley (1962), while confident of the technical feasibility of such an operation, referred to the legal situation surrounding the use of the process as the main difficulty in implementation; since that time important steps have been taken to clarify the position.

Following recommendations published in the report from a Ministry of Health Working Party on Food Irradiation, 1964, regulations controlling the use of ionizing radiation for the treatment of food for *human* consumption were made and came into force in 1967. The regulations impose a system of prohibitive control similar to that already adopted for certain classes of food additives. Each specific process must be approved through the Ministry of Health Advisory Committee on the Irradiation of Food and a memorandum issued by the committee includes a broad description of the nature of the scientific evidence required. Microbiological evidence forms a part of this requirement and the studies presented in this paper are directed to the provision of data related to the control of salmonellas in frozen meats with particular emphasis on imported frozen boneless meat intended as pet meat. Legally, irradiation of such meat is not subject to the Food (Control of Irradiation) Regulations 1967, but such a process has many features in common with irradiation of meat for sale for human consumption. The scrutiny of scientific data related to its use is appropriate, not only with regard to the effectiveness, but also in the light of possible changes in the properties of salmonellas which might survive radiation treatment and in certain circumstances multiply.

This paper gives quantitative inactivation data following the preliminary studies reported by Ley, Freeman & Hobbs (1963). The data relate to the choice of radiation dose for the elimination of salmonellas from frozen horsemeat, kangaroo meat and veal. The influence on radiation resistance of preirradiation

https://doi.org/10.1017/S0022172400028758 Published online by Cambridge University Press

growth conditions and of temperature during irradiation was investigated as well as the possibility of postirradiation recovery during storage. The radiation resistance and biochemical and serological properties of survivors of irradiation, deliberately produced under simulated practical conditions of the proposed process, were examined and their growth rate measured and compared with that of normal salmonellas.

MATERIALS AND METHODS

Meat

Imported frozen boneless raw horsemeat, kangaroo meat and veal. For experiments requiring meat naturally contaminated with salmonellas, samples were obtained from the remainder of those blocks used for routine examination at Colindale which were shown to be positive. These samples were stored in the frozen state until required.

Salmonella serotypes

Six serotypes were used during the course of these studies—*Salmonella typhimurium* phage type 14, *S. senftenberg* 1502, *S. good*, *S. oranienburg*, *S. anatum*, *S. minnesota*. These serotypes occur frequently in imported horsemeat. *S. typhimurium* was selected for detailed study because it is the predominant serotype isolated from humans (Vernon, 1969). The particular phage type 14 was used because it is streptomycin resistant and thus marked for easy recognition, also it is comparatively radiation resistant. *S. senftenberg* 1502 was chosen because of its high resistance to freezing, also *S. seftenberg* is commonly found in foods of animal origin.

Radiation source

The Spent Fuel Element Assembly was used as a source of gamma radiation for the treatment of naturally contaminated frozen meat used in the storage experiment; the dose rate was 1·0 Mrad./hr. In the other investigations a 2000 curie Cobalt-60 'hot spot' source with a dose rate of 0·8 Mrad./hr. was used. Both sources are described by Ley & Rogers (1968).

Pretreatment of meat by irradiation

In all experiments involving quantitative recovery of salmonellas, with the exception of growth-rate studies, the meat was irradiated at 1·0 Mrad. in the frozen state ($-15°$ C.) as a routine procedure before use. This treatment improves recovery by removing interfering microflora. The validity of the use of irradiated meat as substrate for subsequent experiments on the radiation resistance of salmonellas was investigated in preliminary studies; the growth rate, recovery and resistance of salmonellas were compared in irradiated and unirradiated meat.

https://doi.org/10.1017/S0022172400028758 Published online by Cambridge University Press

Inoculation of meat

Stock salmonella cultures were maintained on Dorset egg medium, transferred for experimental work to nutrient agar slopes and incubated for 17 hr. at 37° C. Using M/15 phosphate buffer the growth was removed, washed twice, and then resuspended in the buffer. Appropriate volumes of buffer were mixed with meat exudate for inoculation. Horsemeat was thawed and cut into 20 g. samples which were then placed individually into sterile aluminium cans. Inoculations were made using an Agla syringe, 0·3 ml. being transferred half to the centre and half to the outside of the meat.

Preirradiation growth conditions

For those experiments in which the salmonellas were grown in the meat before irradiation, the buffer suspension was diluted with $\frac{1}{4}$ strength Ringer solution and then transferred to meat exudate. In order to obtain dose/survival curves covering more than 5 log. cycles it is necessary to have initial numbers of organisms before irradiation of at least 10^8/g. This number was obtained by inoculation of the meat samples with 10^2/g. followed by incubation for 17 hr. or 2 days at 37° C., 7 days at 22° C. or inoculation with 10^6/g. followed by 2 days incubation at 22° C. For experiments not involving preirradiation growth of salmonellas in the meat, an inoculum of *ca.* 10^8/g. was added directly to the samples.

Temperature during irradiation

Samples for irradiation at -15° C. were frozen rapidly to this temperature in the cold room (48 min.) and irradiated in a vacuum flask containing a freezing mixture of ammonium chloride and powdered ice (-15° C.). Thermocouple measurements showed a variation from the inside to the outside of the meat of ± 2° C. during the longest period of irradiation. Crushed ice was used in a similar way for meat irradiated at 0° C. Samples were also irradiated at room temperature which was *ca.* 20° C.

Influence of postirradiation storage on recovery

Recovery was examined quantitatively using artificially contaminated meat and qualitatively using naturally contaminated meat.

(*a*) *Artificially contaminated meat.* Twenty-four 20 g. meat samples were used each with counts of *ca.* 10^8/g. of *S. typhimurium*, this number was attained by inoculation with 10^2/g. followed by incubation at 37° C. for 17 hr. After freezing to -15° C., 12 samples were irradiated at 0·5 Mrad. calculated to give 10^3/g. survivors and 12 were kept as unirradiated controls. Viable counts were made at intervals over a 10 week storage period at -15° C. using solid media (agar).

(*b*) *Naturally contaminated meat.* One-hundred 50 g. samples (controls) were taken at random from various blocks of frozen meat. Each sample was placed individually in a polythene bag and heat sealed. These samples were returned to frozen store while the remainder of the meat blocks were packed lightly together in insulated containers for irradiation in the frozen state. The meat was packed

https://doi.org/10.1017/S0022172400028758 Published online by Cambridge University Press

in a manner which gave a dose distribution through the meat within the range 0·5–0·75 Mrad. After irradiation, a further 100 × 50 g. samples were taken. Half the controls and half the samples of irradiated meat were examined after 6 days' storage (−15° C.) and the remainder after 10 weeks. In addition, 12 × 10 g. samples were taken for total counts, six from the meat prior to irradiation and six immediately after irradiation.

Performance of viable counts, both total and of salmonellas, is described in a later section. The qualitative examination for salmonellas of the 50 g. samples referred to in (*b*) was carried out as follows: each sample was thawed and finely chopped and 25 g. added to 25 ml. ¼ strength Ringer solution and this mixture added to 50 ml. double strength Selenite F (Liefson, 1936), modified by replacement of lactose with mannitol and sterilization by Seitz filtration. A further 25 g. was added to tetrathionate enrichment broth (medium A of Rolfe, 1946) in a similar manner. Incubation was for 72 hr. at 37° C. Deoxycholate-citrate lactose agar (Hynes, 1942), modified by the addition of 1 % sucrose, and Oxoid bismuth sulphite agar were inoculated from the tubes of enrichment media and incubated for 48 hr. at 37° C. Characteristic colonies from the plates were examined and identified as *Salmonella* using biochemical and serological tests.

Production and isolation of radiation survivors

Meat samples were inoculated with 10^2/g. and incubated for 17 hr. at 37° C. to give a count of *ca.* 10^8/g. After freezing at −15° C., the samples were irradiated at 0·65 Mrad. to reduce the count to 10^2/g. Surviving organisms were recovered directly on Oxoid nutrient agar and also through Selenite F and tetrathionate broth on deoxycholate citrate sucrose agar; they were transferred to Dorset egg media for storage. This procedure is referred to as 1 cycle of treatment. In the case of *S. typhimurium* up to 6 cycles of treatment were used.

Dose/survival curves

The fraction of surviving organisms was calculated using the average count from two unirradiated control samples, one plated at the start and the other at the end of the longest irradiation period. Individual samples of meat were used to obtain the data for each of the 6–8 points used for the construction of each dose/survival curve.

Three dose/survival curves were prepared on separate occasions for each treatment in the studies, using *S. typhimurium*, of the influence on resistance of pre-irradiation growth conditions, of temperature during irradiation, and of different meat substrates, and in studies on the resistance of radiation survivors. A common regression line was fitted to these curves by means of the method of least squares. The regression line is in the form $y = ax + b$, where y is the logarithm of the surviving fraction, x is the dose of radiation, a is the slope of the line and b is the logarithm of the extrapolation number (Alper, Gillies & Elkind, 1960). For comparative purposes D 10 values (dose required to reduce the number of survivors to one-tenth) were calculated from the linear part of the curves and confidence limits derived following an analysis of variance. In the studies illustrating the shapes of

https://doi.org/10.1017/S0022172400028758 Published online by Cambridge University Press

curves obtained with different serotypes, one dose/survival curve was produced in each case using solid media and at least two curves using liquid media.

Biochemical and serological properties of surviving organisms

Phage typing, serological typing, fermentation and other cultural studies were carried out on the strain of *S. typhimurium* used in these studies before irradiation and after each of six cycles of radiation treatment. Serological patterns only of control cultures of *S. senftenberg*, *S. good*, *S. oranienburg*, *S. anatum* and *S. minnesota* and those of each strain after one cycle of treatment were examined.

Growth rate

The growth rate of salmonellas in meat was studied in the following situations, (i) as natural contaminants or after inoculation into untreated meat, (ii) after inoculation into irradiated meat (0·65 Mrad.), (iii) after inoculation of first cycle survivors into irradiated meat (0·65 Mrad.), (iv) survivors *in situ* in meat (previously artificially contaminated with high numbers of salmonellas) following irradiation with 0·65 Mrad.

(i) Horsemeat suspected to be naturally contaminated with salmonellas was finely chopped, well mixed and distributed in 12 g. quantities into sterile screw cap bottles. The 0 hr. sample was examined immediately and the remainder incubated at 30° C. for different periods up to 48 hr., an individual sample being used for each time interval—four growth curves were produced on separate occasions. Salmonella counts were performed using liquid media by the Most Probable Number (MPN) technique (see following section). Similarly curves were constructed showing growth in normal horsemeat following artificial inoculation with *S. typhimurium* or *S. anatum* at different concentrations; 1 ml. of an appropriate dilution of a 6 hr. broth culture was added to each 12 g. sample.

(ii) and (iii) Meat was irradiated at -15° C. with a dose of 0·65 Mrad. and after thawing inoculated with either normal (untreated) *S. typhimurium* or first cycle survivors of this serotype. Growth curves in the meat were obtained following incubation and counting as in (i).

(iv) 1 ml. of a suspension of *S. typhimurium* in meat exudate (10^{10}/ml.) was inoculated into each of 7×12 g. samples of horsemeat and a further 7 samples inoculated with 1 ml. of a 1 in 100 dilution of the suspension, thus giving samples containing two concentrations of survivors for separate growth experiments. After thawing growth curves were produced following incubation as in (i) but using a solid medium (agar). When liquid media were used in this situation as enrichment or pre-enrichment media, and counts determined by the MPN method, the phenomenon of 'skipping' was observed (North, 1961) invalidating the counting technique; this is the subject of a further investigation.

Performance of viable counts

Salmonella counts using solid media (*agar*). Each meat sample, after being thawed (when applicable) at room temperature for 30 min., was macerated for 1 min. with 100 ml. sterile distilled water in an MSE top drive macerator. 1 ml. portions were

https://doi.org/10.1017/S0022172400028758 Published online by Cambridge University Press

used for plate counts; when necessary the macerated meat suspension was diluted with ¼ strength Ringer solution. The surface plating technique used for high numbers of survivors was a modified form of the method of Miles & Misra (1938); poured plates were used when low numbers only were expected to survive.

Deoxycholate citrate sucrose agar, 'Oxoid' MacConkey agar, no. 2, 'Oxoid' Salmonella Shigella agar, 'Oxoid' triple sugar iron agar (TSI) and 'Oxoid' brilliant green agar (BGA) were tested and compared with 'Oxoid' nutrient agar (NA) for recovery of salmonellas from meat following irradiation up to 0·5 Mrad., the highest dose used; NA was found to be satisfactory by Ley *et al.* (1963) when meat used in experiments had been irradiated and the requirement was merely to count salmonellas in the absence of other organisms. BGA gave the highest viable count of the four first mentioned media and gave the same results as those obtained using NA. Both media were used in many of the experiments reported; the results for BGA only are presented in these experiments.

The characteristic pink colour of *Salmonella* colonies on BGA was not evident below the surface of the agar in poured plates. To establish whether it could be assumed that both deep and surface colonies were all *Salmonella*, colonies taken at random were transferred to TSI agar from the BGA plates. When the meat samples used had been treated by irradiation before inoculation, few organisms other than *Salmonella* were to be expected to grow and all colonies transferred to TSI agar gave H_2S production and sugar fermentation reactions for *Salmonella* sp.; the square root of the number of colonies were taken at random for slide agglutinations and all gave positive reactions against *Salmonella* O and H antisera. On the basis of these tests all colonies were assumed to be salmonellas. When the requirement was to count salmonellas in a mixed flora, for example when unirradiated meat was used for inoculation, the use of BGA medium for counting was always accompanied by the above procedure for the detection of salmonellas.

Salmonella counts using liquid media. Each meat sample was macerated as described above but with 120 ml. ¼ strength Ringer solution. The MPN technique based on the probability tables of McCrady (1918) was used for salmonella counts. For the dose/survival curve experiments in which the requirement was to count very low numbers of salmonellas, lactose broth pre-enrichment medium was used according to North (1961). Selinite F enrichment medium was used elsewhere with incubation at 37° C. for up to 48 hr. All enrichment media were inoculated on both 'Oxoid' bismuth sulphite agar and deoxycholate citrate sucrose agar for colony isolation. Identification of isolated colonies as *Salmonella* was confirmed by biochemical and serological tests.

Total viable counts and coliforms. These were performed by the technique of Miles & Misra (1938) using blood agar plates incubated aerobically and anaerobically and MacConkey agar incubated aerobically at 37° C. for 48 hr.

RESULTS

The preliminary studies showed the suitability of meat which had been previously irradiated for use in dose/survival curve experiments. *Salmonella* grew in

https://doi.org/10.1017/S0022172400028758 Published online by Cambridge University Press

the meat satisfactorily and the growth curves were of expected shape (Fig. 1). Following inoculation, more salmonellas were recovered with less variation between samples with irradiated meat ($1\cdot9 \times 10^8$/g. with S.E. $\pm 0\cdot22$) compared with unirradiated meat ($7\cdot7 \times 10^7$/g. $\pm 1\cdot95$). Furthermore, a dose/survival curve (irradiation at $-15°$ C.), prepared in previously irradiated meat using *S. typhimurium* was similar in shape to that obtained in the unirradiated meat and the D10 values

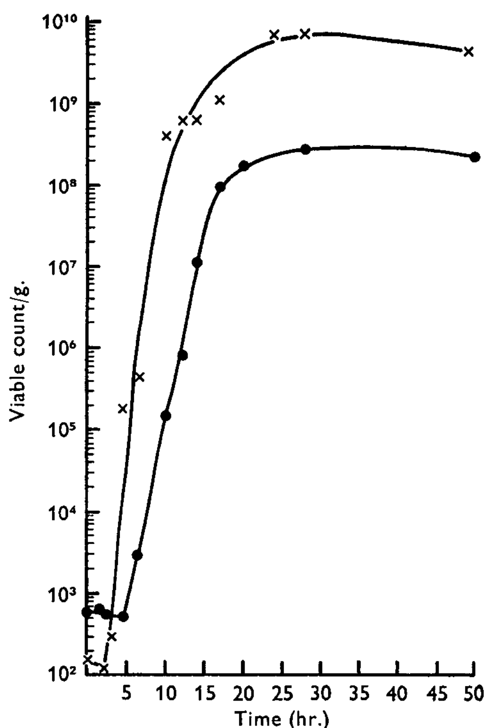

Fig. 1. Rate of growth of *S. typhimurium* at 30° C. (●) and 37° C. (×) following inoculation into irradiated horsemeat (1·0 Mrad.).

Table 1. *The influence of preirradiation growth conditions in horsemeat on the radiation resistance of* S. typhimurium, *results given as D10 values (krad.)*

Preirradiation growth conditions following inoculation	Temperature during irradiation (°C.)	
	−15	+20
No growth**	86·1 (80·7–92·4)	48·7 (45·3–52·7)
22° C. for 2 days	*	51·1 (45·1–58·9)
22° C. for 7 days	*	86·3 (76·4–99·1)
37° C. for 17 hr.	92·6 (87·9–97·7)	62·7 (54·9–73·0)

95 % Confidence limits given in parentheses.
* Not tested. ** Organisms merely added to meat.

https://doi.org/10.1017/S0022172400028758 Published online by Cambridge University Press

were also similar, i.e. 92·6 krad. (87·9–97·7) for the former and 103·7 krad. (93·3–116·8) for the latter. It can be concluded that the previous irradiation of the meat does not produce any toxic effect which influences the growth, recovery or radiation resistance of *Salmonella* subsequently inoculated into the meat.

Table 2. *The D*10 *values (krad.) for* S. typhimurium *in various meats irradiated at different temperatures following inoculation and growth at* 37° *C. for* 17 *hr.*

Temperature during irradiation (°C.)	Horsemeat	Kangaroo meat	Veal
−15	92·6 (87·9–97·7)	92·8 (86·9–99·6)	96·3 (91·2–102·0)
+20	62·7 (54·9–73·0)	55·5 (50·5–61·8)	55·8 (51·0–61·6)

95 % Confidence limits in parentheses.

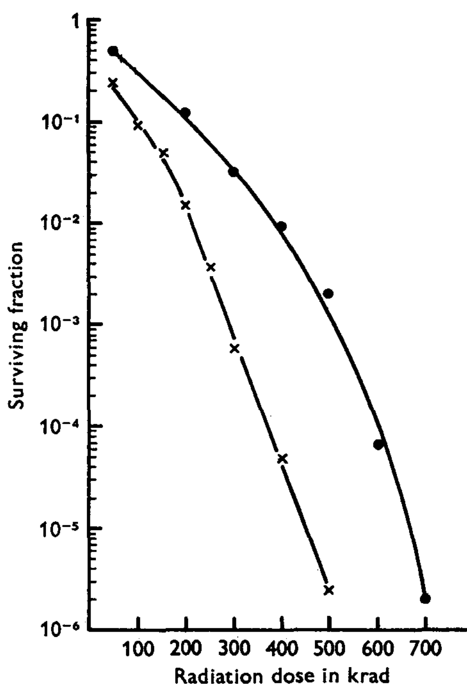

Fig. 2. Dose/survival curves for *S. typhimurium* irradiated in horsemeat at −15° C. (●) Following inoculation and growth in the meat at 37° C. for 2 days; (×) following inoculation but without preirradiation growth in the meat.

The D10 values quoted in Tables 1 and 2 were read from dose/survival curves which were linear over the dose range studied, i.e. up to 0·6 Mrad. for frozen meat and 0·4 Mrad. for unfrozen meat and covering between 5 and 6 log. cycles of inactivation in each case. The results in Table 1 show that preirradiation growth of *S. typhimurium* in meat under certain incubation conditions can have a

https://doi.org/10.1017/S0022172400028758 Published online by Cambridge University Press

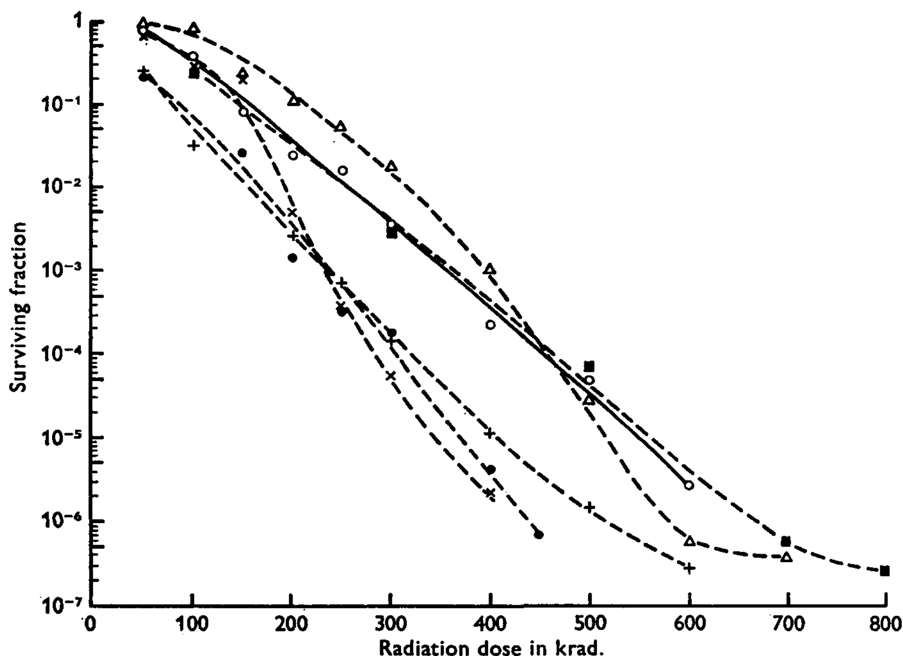

Fig. 3. Dose/survival curves for various serotypes of salmonellas in horsemeat prepared using solid recovery media. (Irradiation at $-15°$ C. following preirradiation growth in the meat at 37° C. for 17 hr.) ×, *S. minnesota*; ●, *S. good*; +, *S. oranienburg*; ○, *S. typhimurium*; △, *S. senftenberg*; ■, *S. anatum*.

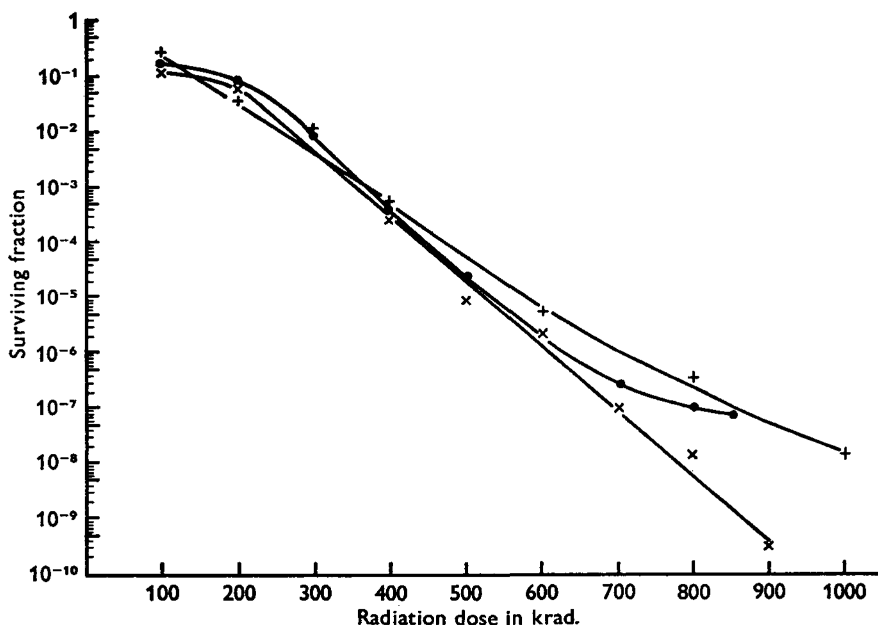

Fig. 4. Dose/survival curves for various serotypes of salmonellas in horsemeat prepared using the lactose broth pre-enrichment method (North, 1961). (Irradiation at $-15°$ C. following preirradiation growth in the meat at 37° C. for 17 hr.) ×, *S. typhimurium*; ●, *S. senftenberg*; +, *S. anatum*.

https://doi.org/10.1017/S0022172400028758 Published online by Cambridge University Press

significant effect on resistance. This is apparent with 7 days at 22° C. ($P < 0{\cdot}001$) and 17 hr. at 37° C. ($P < 0{\cdot}002$) when followed by irradiation at 20° C. compared with organisms merely added to the meat. With irradiation in the frozen state increase in resistance was also observed with 2 days at 37° C. as illustrated in Fig. 2; the non-linearity of the curve in this instance precludes the calculation of a D10 value. After perusal of all the results in the light of the commercial process envisaged for frozen meat, preirradiation growth for 17 hr. at 37° C. was chosen as a standard procedure in subsequent experiments; longer incubation in practice leads to unacceptable deterioration of meat quality. Results in Table 2 show that there is no significant difference in the radiation resistance of *S. typhimurium* when different meats are used as substrates whether irradiated frozen or unfrozen ($P > 0{\cdot}5$ throughout). The marked increase in resistance due to freezing is evident

Table 3. *The effect of storage at* −15° *C. on the surviving fraction of* S. typhimurium *in frozen horsemeat following irradiation with* 0·5 *Mrad.*

Storage (weeks)	Counts/g. Unirradiated	Counts/g. Irradiated	Surviving fraction
0	$1{\cdot}9\times10^{8}$*	$2{\cdot}7\times10^{3}$*	$1{\cdot}6\times10^{-5}$
1	$1{\cdot}6\times10^{8}$	$3{\cdot}0\times10^{3}$	$1{\cdot}8\times10^{-5}$
2	$2{\cdot}4\times10^{8}$	$3{\cdot}7\times10^{3}$	$1{\cdot}6\times10^{-5}$
3	$1{\cdot}1\times10^{8}$	$6{\cdot}2\times10^{2}$	$5{\cdot}7\times10^{-6}$
4	$1{\cdot}3\times10^{8}$	$6{\cdot}0\times10^{2}$	$4{\cdot}6\times10^{-6}$
5	$1{\cdot}2\times10^{8}$	$4{\cdot}7\times10^{2}$	$3{\cdot}8\times10^{-6}$
10	$6{\cdot}4\times10^{7}$	$1{\cdot}0\times10^{2}$	$1{\cdot}6\times10^{-6}$

* Mean of duplicate counts obtained on six individual meat samples; all other results are the means of duplicate counts on one sample.

with each type of meat; in each case the DMF (dose modifying factor = ratio of D10 value at −15° C. to D10 value at 20° C.) is *ca.* 1·7. Using horsemeat only, irradiation at 0° C. was also studied and a D10 value of 55·3 krad. (48·3–64·6) obtained which is not significantly different from that at 20° C. ($P > 0{\cdot}1$).

Curves depicted in Fig. 3 were obtained using solid media and those in Fig. 4 using liquid media. In all these experiments attempts were made to obtain curves extending as far as is practicable. The curves are of various shapes with evidence of both shoulders and tails, the latter being particularly obvious with *S. senftenberg* and *S. anatum*. Although the shapes are similar with both media, the increased sensitivity of the liquid recovery technique results in curves passing through a greater number of log. cycles of inactivation, that for *S. typhimurium* covering more than nine log. cycles. The tails to the curves appear almost one log. cycle lower with the liquid media compared with solid media implying that tailing might be associated with limitations on estimating very low numbers of survivors. It can be concluded by inspection of all the curves presented that a reduction in numbers by at least a factor of 10^5 can be achieved in an initial population of any of the serotypes examined with a dose of 0·6 Mrad.

The effect of postirradiation storage at −15° C. on the recovery of *S.*

https://doi.org/10.1017/S0022172400028758 Published online by Cambridge University Press

typhimurium in frozen horsemeat is shown in Table 3. The dose of 0·5 Mrad. was chosen to give a convenient number of survivors. The variance between counts obtained for the unirradiated organisms over the whole storage period was not statistically different ($P > 0{\cdot}1$) from that obtained for the survivors of irradiation. Although the numbers of both the unirradiated organisms and irradiation survivors fall with time, it will be seen that when the number of survivors is expressed as a fraction of the unirradiated organisms, this fraction also decreases slowly with time

Table 4*a*. *Detection of* Salmonella *in naturally contaminated meat before and after irradiation at* −15° *C.*

Dose (Mrad.)	Storage after irradiation	No. of samples examined	No. in which *Salmonella* detected	No. showing growth on selective media*		
				+ +	+	−
0	6 days	50	22	50	0	0
0·5–0·75	6 days	50	0	0	7	43
0	10 weeks	50	10	46	1	3
0·5–0·75	10 weeks	47	0	0	7	40

* On bismuth sulphite or deoxycholate citrate sucrose agar following enrichment broths. + + = Heavy; + = light; − = no growth.

Table 4*b*. *Total viable counts on meat before and after irradiation* (0·5–0·75 *Mrad.*) *at* −15° *C.*

	Sample no.	Orgs/g. Aerobic	Orgs/g. Anaerobic	Coliform bacilli
Before	1	500	500	< 500
	2	**	2×10^5	5×10^4
	3	2×10^5	3×10^4	1×10^4
	4	1×10^5	$2{\cdot}5\times10^5$	$2{\cdot}5\times10^5$
	5	2×10^5	2×10^5	2×10^5
	6	$1{\cdot}5\times10^5$	$1{\cdot}5\times10^5$	$1{\cdot}5\times10^5$
After	1–6	< 500 Throughout		Not found

** Unreadable.

Table 5. *The influence of the recycling* of survivors of irradiation* (S. typhimurium) *on their subsequent radiation resistance in horsemeat at* −15° *C.*

Treatment	D 10 value (krad.)
Uncycled (control)	92·6 (87·9–97·7)
First cycle survivors	67·5 (63·1–72·6)
Sixth cycle survivors	67·9 (60·1–77·9)
First cycle survivors (freezing only)	87·0 (80·5–94·7)

95 % Confidence limits given in parentheses.

* Each cycle is as follows: inoculation of meat with 10^2/g. incubation for 17 hr. at 37° C. to give 10^8/g., freeze to −15° C., irradiation with 0·65 Mrad., recover on nutrient agar.

https://doi.org/10.1017/S0022172400028758 Published online by Cambridge University Press

Table 6. *The growth at 30° C. of* Salmonella *in naturally contaminated horsemeat or after inoculation with* S. anatum

Time (hr.)	MPN count/g. naturally contaminated Expt. no.				Range of total plate count/g.		MPN count/g. artificially inoculated Expt. no.					Range of total plate count/g.	
	1	2	3	4	Aerobic	Anaerobic	1	2	3	4	5	Aerobic	Anaerobic
0	0·5	3·0	4·0	5·0	10^5–10^6	10^5–10^6	0·3	0·5	5	70	130*	10^3–10^6	10^3–10^4
15–17	0·1	0·1	0·6	0·2	10^8–10^9	10^8–10^9	2	20	65	$> 1·8 \times 10^6$	$> 1·8 \times 10^6$	10^8–10^{10}	10^8–10^9
24	1	4	9	14	10^8–10^{10}	10^8–10^{10}	140	60	$5·5 \times 10^2$	$> 1·8 \times 10^6$	$> 1·8 \times 10^6$	10^9–10^{10}	10^8–10^{10}
40	20	0	70	2·0	10^9–10^{10}	10^9–10^{10}	$> 1·8 \times 10^4$	50	2×10^2	2×10^5	$> 1·8 \times 10^8$	10^{10}	10^9–10^{10}
48	200	0	$2·5 \times 10^3$	130	10^{10}–10^{11}	10^9–10^{10}	$2·7 \times 10^4$	$2·5 \times 10^3$	4×10^2	$> 1·6 \times 10^8$	$> 1·8 \times 10^8$	10^{10}	10^9–10^{10}

* *S. typhimurium.*

https://doi.org/10.1017/S0022172400028758 Published online by Cambridge University Press

giving a one log. cycle fall at the tenth week. The number of samples in which salmonellas were detected in meat suspected of being naturally contaminated was reduced in storage (Table 4*a*) but the effectiveness of the radiation treatment is apparent. The effect on other bacterial species is also clear as shown by the extent of growth on selective media of mixed coliforms and by the fall in total viable count shown in Table 4*b*. Coliforms are not expected to survive the irradiation and the light growth observed in some of the irradiated samples are probably due to postirradiation contamination; a few polythene bags were found to be split.

Table 7. *Growth at* 30° *C. of* S. typhimurium *following inoculation into irradiated* (0·65 *Mrad.*) *horsemeat*

Time (hr.)	Salmonella MPN count*/g.	Total plate count/g.		Salmonella MPN count*/g.	Total plate count/g.	
		Aerobic	Anaerobic		Aerobic	Anaerobic
0	2×10^{2}	8×10^{2}	$4{\cdot}7 \times 10^{2}$	0·2	10	5
6	$2{\cdot}6 \times 10^{3}$	$2{\cdot}4 \times 10^{3}$	$1{\cdot}6 \times 10^{3}$	13	25	45
12	$6{\cdot}6 \times 10^{6}$	$4{\cdot}4 \times 10^{7}$	$4{\cdot}3 \times 10^{7}$	$6{\cdot}0 \times 10^{4}$	$5{\cdot}9 \times 10^{4}$	$5{\cdot}9 \times 10^{4}$
18	$1{\cdot}6 \times 10^{8}$	$7{\cdot}5 \times 10^{7}$	$8{\cdot}2 \times 10^{7}$	$6{\cdot}0 \times 10^{6}$	$7{\cdot}6 \times 10^{6}$	$7{\cdot}2 \times 10^{6}$
24	$2{\cdot}2 \times 10^{9}$	$3{\cdot}8 \times 10^{9}$	$3{\cdot}3 \times 10^{9}$	$1{\cdot}7 \times 10^{8}$	$2{\cdot}8 \times 10^{8}$	$3{\cdot}1 \times 10^{8}$
36	$1{\cdot}9 \times 10^{9}$	$1{\cdot}2 \times 10^{10}$	$1{\cdot}2 \times 10^{10}$	$2{\cdot}2 \times 10^{9}$	$1{\cdot}4 \times 10^{10}$	$1{\cdot}6 \times 10^{10}$
48	$4{\cdot}2 \times 10^{8}$	$1{\cdot}7 \times 10^{10}$	$1{\cdot}7 \times 10^{10}$	$3{\cdot}5 \times 10^{9}$	$1{\cdot}3 \times 10^{10}$	$1{\cdot}5 \times 10^{10}$

* Using Selenite F.

The radiation resistance of salmonellas which have survived freezing and irradiation showed a significant decrease ($P < 0{\cdot}001$) after 1 cycle of treatment and no further decrease after 6 cycles (Table 5); this effect is not accounted for by freezing alone. The serological pattern, 1, 4, 5, 12:i:1, 2 and phage type 14, of *S. typhimurium* remained unaltered throughout the six cycles but there was a slight change in the fermentation pattern. After the first cycle inositol fermentation appeared to have been induced and, after the second cycle, rhamnose fermentation; these reactions were held throughout the subsequent cycles. There were other slight variations in the fermentation pattern, e.g. differences in the length of time required for certain fermentations to become apparent, but these were probably of minor significance. The qualities which remained constant were the cultural characteristics and the serological reactions, and acriflavine reactions which suggested that the strain had not become degraded by the radiation treatment. The serological patterns of the other serotypes examined after one cycle of treatment showed no apparent changes.

The growth rate of normal salmonellas in untreated horsemeat is influenced by the initial numbers present; low numbers had difficulty in growing, whereas an inoculum of about 10^{2}/g. grew rapidly in competition with the natural flora (Table 6). In fact, the growth rate of the heavier salmonella inoculum was comparable with that achieved in the absence of other flora as shown in the results from the preliminary work given in Fig. 1. Even a low inoculum was able to grow well in meat which had been irradiated at 0·65 Mrad. in which the natural flora was reduced considerably in number, and the growth rate of first cycle survivors

https://doi.org/10.1017/S0022172400028758 Published online by Cambridge University Press

of irradiation under the same conditions was very comparable (Tables 7, 8). In contrast, survivors of irradiation produced *in situ* in meat followed by incubation in the same meat showed a longer lag period whether the number of survivors was high or low (Table 9).

Table 8. *Growth at* 30° *C. of* S. typhimurium *survivors of first irradiation cycle following inoculation into irradiated* (0·65 *Mrad.*) *horsemeat*

Time (hr.)	Salmonella MPN count*/g.	Total plate count/g.	
		Aerobic	Anaerobic
0	3·5	10	5
6	12·0	5	15
12	$1·6 \times 10^3$	$8·8 \times 10^3$	$2·9 \times 10^3$
18	$1·3 \times 10^5$	$2·4 \times 10^5$	$2·1 \times 10^5$
24	$1·8 \times 10^8$	$2·1 \times 10^8$	$2·4 \times 10^8$
36	$3·3 \times 10^7$	$3·4 \times 10^9$	$3·9 \times 10^9$
48	$4·2 \times 10^8$	$2·6 \times 10^9$	$2·6 \times 10^9$

* Using Selenite F.

Table 9. *Growth at* 30° *C. of* S. typhimurium *survivors* in situ *in horsemeat following irradiation with* 0·65 *Mrad.*

Time (hr.)	Salmonella count*/g.	Total plate count/g.		Salmonella count*/g.	Total plate count/g.	
		Aerobic	Anaerobic		Aerobic	Anaerobic
0	$6·2 \times 10^3$	$2·7 \times 10^4$	$8·6 \times 10^3$	50	$8·4 \times 10^2$	$1·1 \times 10^2$
6	$2·0 \times 10^3$	$6·2 \times 10^3$	$4·6 \times 10^3$	15	$3·4 \times 10^2$	$3·1 \times 10^2$
12	$2·3 \times 10^3$	$5·0 \times 10^3$	$4·3 \times 10^3$	25	$2·3 \times 10^4$	$1·9 \times 10^4$
18	$5·1 \times 10^3$	$6·3 \times 10^3$	$5·5 \times 10^3$	$3·4 \times 10^4$	$6·2 \times 10^5$	$4·5 \times 10^5$
24	$3·5 \times 10^5$	$3·7 \times 10^5$	$3·8 \times 10^4$	$6·5 \times 10^6$	$1·7 \times 10^7$	u/c
36	$3·8 \times 10^6$	$6·3 \times 10^6$	$6·3 \times 10^6$	$6·9 \times 10^7$	$7·2 \times 10^7$	$6·5 \times 10^7$
48	$3·7 \times 10^7$	$6·6 \times 10^7$	$3·5 \times 10^7$	$1·1 \times 10^8$	$1·2 \times 10^8$	$1·3 \times 10^8$

* Using solid media.

DISCUSSION

The immediate penetrating property of gamma radiation combined with the precision with which required doses can be given, allows quantitative microbiological inactivation data to be produced in the laboratory under conditions very similar to those expected in a commercial operation. This is important since many factors of environment before, during and after irradiation can influence radiation resistance (reviewed by Bridges, 1964). The importance of using the particular food itself as substrate in the measurement of the radiation resistance of *Salmonella* was illustrated by Ley *et al.* (1963) who observed considerable differences in different foods which in turn all showed protective effects when compared with buffer as suspending medium. In view of the possibility that salmonellas in meat might have the opportunity to grow during slaughterhouse handling, meat was inoculated with a small number of organisms and given a period of growth before

https://doi.org/10.1017/S0022172400028758 Published online by Cambridge University Press

freezing to the temperature (−15° C.) at which the meat is normally shipped. Preirradiation growth conditions in food are known to influence radiation resistance as observed in our investigations on salmonellas in corned beef (Ley, 1966). High resistance in vegetative bacteria is expected in the stationary phase of growth (Stapleton, 1955) and this was confirmed for salmonellas grown in nutrient broth by Licciardello, Nickerson & Goldblith (1968) who also showed that preirradiation growth temperature can influence resistance; the highest resistance was obtained at 37° C. This was the temperature used in our main experiments and held for 17 hr. after which time the organisms were at the beginning of the stationary phase of growth.

The D10 value obtained for *S. typhimurium* in frozen horsemeat is somewhat lower than that reported by Ley *et al.* (1963) but in this earlier work the meat was irradiated in solid CO_2. However, the striking increase in radiation resistance in meat in the frozen state compared with the unfrozen state confirms their findings with buffer suspensions. Since the commercial radiation process is expected to be applied at import to incoming frozen meat which, after treatment, is distributed in the frozen state, it would be unpractical to irradiate in the thawed state to take advantage of the lower dose requirement; besides, such a proposal would present an opportunity for mishandling with the possibility of microbial growth. Whilst freezing has a protective effect on salmonellas and other vegetative bacteria (Matsuyama, Thornley & Ingram, 1964*a*), it seems to have little effect on the resistance of bacterial spores (Matsuyama, Thornley & Ingram, 1964*b*). However, the data presented by Grecz (1965) indicate some increased sensitivity for *Clostridium botulinum* spores irradiated in buffer at −20° C. compared with ambient temperatures when the dose given was 0·7 Mrad.; such conditions are similar to those expected in the commercial process described in this paper. As regards the effect of irradiation on the quality of the meat itself, there is no disadvantage in the use of the higher dose in the frozen state since freezing protects against damage (Coleby *et al.* 1961; Harlan & Kauffman, 1965). The nature and reactivity of free radicals formed during irradiation is thought to account largely for this difference between the frozen and unfrozen state.

The dose/survival curves presented for different serotypes, though complex in shape, extend far enough to read off directly the dose of radiation required to reduce a population by a factor of at least 10^5. A dose of 0·6 Mrad. is suggested as the minimum to use in practice in a commercial process. In the irradiation of blocks of frozen meat in cartons of dimensions 20 × 20 × 10 in. (50 × 50 × 25 cm.) which are ideal for handling and conveying, attenuation of the dose is expected. If the minimum dose at the centre is 0·6 Mrad., then a dose of 0·85 Mrad. is received at the outside of the block resulting in inactivation factors for *Salmonella* varying between 10^5 and 10^8. This level of inactivation seems adequate considering that low numbers of salmonellas are expected in frozen meat; 5 viable organisms/gram was the highest number recorded during these studies. Mossel, van Schothorst & Kampelmacher (1968) showed that 0·6 Mrad. was adequate for the treatment of frozen poultry when the absence of Enterobacteriaceae flora in drip fluid was used as a measure of effectiveness. The same authors also reported the efficacy of this

https://doi.org/10.1017/S0022172400028758 Published online by Cambridge University Press

dose for frozen red meats using the same criteria. Our own experiments comparing horsemeat, kangaroo meat and veal indicate no differences in radiation resistance for salmonellas between these meats as substrates, although salmonellas in crabmeat, studied by Dyer, Anderson & Dutiyabodhi (1966), are more radiation resistant.

It is only a remote possibility that salmonellas surviving irradiation, expected to be $< 1/10^5$ g., would have the opportunity to grow and recontaminate meat earmarked for radiation treatment, particularly if precautions are taken at a radiation plant to separate incoming from outgoing material, and to keep the conveyor system clean. However, it is encouraging to note that *S. typhimurium* recycled through the process shows reduced radiation resistance. Other work has shown, on the one hand, no change in resistance with *S. gallinarum* after 14 consecutive cycles of radiation treatment (Erdman, Thatcher & MacQueen, 1961*a*), and on the other, increased resistance with the several serotypes examined after at least eight cycles, though not with *S. typhimurium* unless followed by storage (Idziak & Incze, 1968) and after at least six cycles (Licciardello *et al.* 1969). However, the authors of the first two papers, referring to the characteristics of recycled *Salmonella* species, note no change in the reactions normally used in identification. Apart from two minor changes in sugar fermentation, our own results support the same conclusion and it is unlikely therefore that any practical problems would arise in this connexion.

Repair mechanisms have been shown to operate in bacteria following ultraviolet irradiation (reviewed by Witkin, 1969) but the cells of *Salmonella* inactivated in meat by gamma irradiation do not regain viability during frozen storage; it appears rather that the surviving organisms are reduced in number. However, survivors could have the opportunity to grow when the meat is thawed before use or allowed to thaw at some stage in distribution through mishandling. It is an advantage that survivors in the meat exhibit a longer lag phase than unirradiated salmonellas but it is important to note that irradiation used as envisaged in this application, which is similar in aim to a heat pasteurization process, causes a considerable reduction in the total microflora thus providing a medium subsequent to radiation treatment, which would be less inhibitory to the growth of salmonellas than unirradiated meat. Our results showing the influence of competing flora on the growth of *Salmonella* support the findings of Matches & Liston (1968) in investigations on irradiated fish.

The studies as a whole confirm the adequacy of a minimum dose of gamma radiation of 0·6 Mrad. for elimination of *Salmonella* from frozen meat and reveal no new microbiological hazards which would make the commercial use of the process unsafe. The recommended dose would also result in a considerable reduction in numbers of other pathogens of public health significance (Erdman, Thatcher & MacQueen, 1961*b*; Dyer *et al.* 1966). Sufficient data are now available for scrutiny by the appropriate authorities concerned with the safety of the process including those from toxicological studies on irradiated frozen meat (Hickman, Law & Ley, 1969) and investigation of methods for detecting whether meat has been irradiated or not (Hills & Smith, 1967). If approved, the process should be

https://doi.org/10.1017/S0022172400028758 Published online by Cambridge University Press

considered as an alternative to the 'cooking process' described in The Meat (Sterilization) Regulations 1969.

We are grateful to Dr E. S. Anderson of the Enteric Reference Laboratory for phage typing and for the provision of a strain of *S. typhimurium* for experiments. Also, we thank Dr Joan Taylor of the Salmonella Reference Laboratory for the serological typing of salmonellas and examination of cultural characteristics.

REFERENCES

ALPER, T., GILLIES, N. E. & ELKIND, M. M. (1960). The sigmoid survival curve in radiobiology. *Nature, London* **186**, 1062.

ANDERSON, K., CROWDER, E. F. & WOODRUFF, P. (1964). The isolation of salmonellae from kangaroo meat sold as pet food. *Medical Journal of Australia* ii, 668.

BEASLEY, J., HOPKINS, G. B., MCNAB, D. J. N., RICHARDS, A. G. & KING, G. J. G. (1967). Pet meat as a potential source of human salmonellosis. *Lancet* i, 560.

BRIDGES, B. A. (1964). Microbiological aspects of radiation sterilisation. *Progress in Industrial Microbiology* **5**, 285.

COLEBY, B., INGRAM, M., SHEPHERD, H. J., THORNLEY, M. J. & WILSON, G. M. (1961). Treatment of meats with ionizing radiation. vii. Effect of low temperatures during irradiation. *Journal of the Science of Food and Agriculture* **12**, 483.

DYER, J. K., ANDERSON, A. W. & DUTIYABODHI, P. (1966). Radiation survival of food pathogens in complex media. *Applied Microbiology* **14**, 92.

ERDMAN, I. E., THATCHER, F. S. & MACQUEEN, K. F. (1961*a*). Studies on the irradiation of micro-organisms in relation to food preservation. II. Irradiation resistant mutants. *Canadian Journal of Microbiology* **7**, 207.

ERDMAN, I. E., THATCHER, F. S. & MACQUEEN, K. F. (1961*b*). Studies on the irradiation of micro-organisms in relation to food preservation. I. The comparative sensitivities of specific bacteria of public health significance. *Canadian Journal of Microbiology* **7**, 199.

GALBRAITH, N. S., TAYLOR, C. E. D., CAVANAGH, P., HAGAN, J. C. & PATON, J. L. (1962). Pet foods and garden fertilizers as sources of human salmonellosis. *Lancet* i, 372.

GRECZ, N. (1965). Biophysical aspects of Clostridia. *Journal of Applied Bacteriology* **28**, 17.

HARLAN, J. W. & KAUFFMAN, F. L. (1965). Effect of irradiation temperature and processing conditions on organoleptic properties of beef and chemical yields in model systems. Radiation Preservation of Foods. *Advances in Chemistry Series* 65, *American Chemical Society, Washington, D.C.* 1967.

HICKMAN, J. R., LAW, A. W. & LEY, F. J. (1969). Studies on the wholesomeness of irradiated meat. *Harwell Rep. no. A.E.R.E.—R-6028 (unclassified)*.

HILLS, P. R. & SMITH, A. H. (1967). An evaluation of several gas-chromatographic and colorimetric methods for the detection of irradiated horsemeat. *A.E.R.E. Rep. no. R-5461*, H.M.S.O.

HOBBS, B. C. (1965). Contamination of meat supplies. *Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service* **24**, 123.

HYNES, M. (1942). The isolation of intestinal pathogens by selective media. *Journal of Pathology and Bacteriology* **54**, 193.

IDZIAK, E. S. & INCZE, K. (1968). Radiation treatment of foods. I. Radurization of fresh eviscerated poultry. *Applied Microbiology* **16**, 1061.

LEIFSON, E. (1936). New selenite enrichment media for the isolation of typhoid and paratyphoid (Salmonella) Bacilli. *American Journal of Hygiene* **24**, 423.

LEY, F. J. (1962). The use of ionizing radiation for the treatment of raw meat. *Sanatarian, London* **71**, 185.

LEY, F. J. (1966). Application of radiation for the control of salmonellae in various foods. Food Irradiation. *Proceedings of FAO/IAEA meeting, Karlsruhe* 1966. *IAEA Pub. no.* 127, H.M.S.O.

LEY, F. J., FREEMAN, B. M. & HOBBS, B. C. (1963). The use of gamma radiation for the elimination of salmonellae from various foods. *Journal of Hygiene* **61**, 515.

https://doi.org/10.1017/S0022172400028758 Published online by Cambridge University Press

LEY, F. J. & ROGERS, F. (1968). Processing with ionizing radiations. *Biochemical and Biological Engineering Science*, vol. 2, chap. 17 (Ed. by N. Blakeborough). Academic Press.

LICCIARDELLO, J. J., NICKERSON, J. T. R. & GOLDBLITH, S. A. (1968). Elimination of salmonella in poultry. Proceedings of an FAO/IAEA Panel on elimination of harmful organisms from food and feed by irradiation. *IAEA Pub. no. STI/PUB/200.* H.M.S.O.

LICCIARDELLO, J. J., NICKERSON, J. T. R., GOLDBLITH, S. A., SHANNON, C. A. & BISHOP, W. W. (1969). Development of radiation resistance in Salmonella cultures. *Applied Microbiology* **18**, 24.

McCRADY, M. H. (1918). Tables for rapid interpretation of fermentation tube results. *Canadian Public Health Journal* **9**, 201.

MATCHES, J. R. & LISTON, J. (1968). Growth of salmonellae on irradiated and non-irradiated seafoods. *Journal of Food Science* **33**, 406.

MATSUYAMA, A., THORNLEY, M. J. & INGRAM, M. (1964*a*). The effect of freezing on the radiation sensitivity of vegetative bacteria. *Journal of Applied Bacteriology* **27**, 110.

MATSUYAMA, A., THORNLEY, M. J. & INGRAM, M. (1964*b*). The effect of freezing on the radiation sensitivity of bacterial spores. *Journal of Applied Bacteriology* **27**, 125.

MILES, A. A. & MISRA, S. S. (1938). The estimation of the bactericidal power of blood. *Journal of Hygiene* **38**, 732.

MOSSEL, D. A. A., VAN SCHOTHORST, M. & KAMPELMACHER, E. H. (1968). Prospects for the salmonella radicidation of some foods and feeds with particular reference to the estimation of the dose required. Proceedings of an FAO/IAEA Panel on elimination of harmful organisms from food and feed by irradiation. *IAEA Pub. STI/PUB/200.* H.M.S.O.

NORTH, W. R. (1961). Lactose pre-enrichment method for isolation of salmonellae from dried egg albumin. *Applied Microbiology* **9**, 188–95.

REPORT (1964). *Ministry of Health Report of working party on irradiation of food.* H.M.S.O.

ROLFE, V. (1946). A note on the preparation of tetrathionate broth. *Monthly Bulletin of the Ministry of Health and the Emergency Public Health Laboratory Service* **5**. 158.

STAPLETON, G. E. (1955). Variations in the sensitivity of *E. coli* to ionizing relations during the growth cycle. *Journal of Bacteriology* **70**, 357.

STATUTORY INSTRUMENTS (1967). *The Food (Control of Irradiation) Regulations* 1967. H.M.S.O.

STATUTORY INSTRUMENTS (1969). *The Meat (Sterilization) Regulations* 1969. H.M.S.O.

VAN SCHOTHORST, M. & KAMPELMACHER, E. H. (1967). Salmonella in meat imported from South American countries. *Journal of Hygiene* **65**, 321.

VERNON, E. (1969). Food poisoning and Salmonella infections in England and Wales, 1967. *Public Health, London* **83**, 205.

WITKIN, E. M. (1969). Ultraviolet-induced mutation and DNA repair. *Annual Review of Microbiology* **23**, 487.

https://doi.org/10.1017/S0022172400028758 Published online by Cambridge University Press