

The use of gamma radiation for the elimination of salmonellae from various foods

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INTRODUCTION

A wide variety of foods have been implicated as sources of salmonellae. As pointed out by Hobbs (1962) in a paper summarizing the current position, certain imported foods are heavily contaminated. Present methods employed for the elimination of these pathogens, when the hygiene of production has failed, are based on the use of heat treatment, but gamma radiation is proposed as an alternative process offering important advantages for some products. The radiation is penetrating; it can be applied without removing the food from its container, and the temperature rise during treatment is negligible; hence its particular applicability to frozen items such as whole egg and meats.

The usefulness of gamma radiation for the treatment of frozen whole egg was recognized earlier by Brooks, Hannan & Hobbs (1959), and more detailed bacteriological studies on this product are reported in this paper. Evidence is also presented on the use of the process for the elimination of salmonellae from frozen horse-meat, desiccated coconut, and bone meal. The practical dose requirement is estimated from the results of experiments on artificially or naturally contaminated material, and the inactivation factors realized are calculated from dose/survival curves prepared for different serotypes in each product. For comparative purposes the results are also given for the radiation resistance of salmonellae in a buffer solution under various environmental conditions.

MATERIALS AND METHODS

Materials

Dose/survival curve experiments

Home-produced frozen whole egg; frozen boneless horse-meat as imported in blocks; desiccated coconut purchased locally, packaged as for retail sale. Bone meal imported for use as fertilizer.

M/15 phosphate buffer of neutral pH.

Salmonella serotypes

Five serotypes were used during the course of these studies: *Salmonella typhimurium* phage-type 2c (14), *S. senftenberg*, N.C.T.C. 9959 (775 w), *S. paratyphi* B phage-type Beccles var. 3, *S. gallinarum* and *S. meleagridis*. *S. typhimurium* was used in all the experiments because it has been isolated from each of the types of food under investigation and was detected in 64 % of food-poisoning incidents in human beings in 1961 (see Report, 1962*a*). The heat-resistant strain of *S. senftenberg*, N.C.T.C. 9959 (775 w), was chosen in order to check any possible relationship with radiation resistance.

Inoculation and irradiation

Nutrient agar slope cultures grown for 18–24 hr. were used throughout. Before inoculation each of the foods studied, with the exception of coconut, was irradiated with a dose of 1 Mrad. (in the frozen state for meat and whole egg) to remove organisms originally present, thus simplifying the recovery and counting of salmonellae. General observations on the growth characteristics of salmonellae in the irradiated food showed that such treatment did not influence the effectiveness of the food as a growth medium. Bacteriological examination of the desiccated coconut showed that it was only slightly contaminated and therefore pre-treatment by irradiation was unnecessary.

The different serotypes were studied separately in each of the following media.

(a) *Whole egg*. The slope culture was washed off with 10 ml. buffer, and 0.5 ml. of this suspension was added to 200 ml. of liquid whole egg and mixed. After incubation at 37° C. for 3 days the colony count of each serotype used was approximately 10⁸/ml. Ten ml. quantities of infected egg were placed in universal containers for irradiation in the liquid state and 30 ml. quantities were placed in aluminium cans and frozen rapidly at –17° C. for irradiation in the frozen state. The cans were maintained frozen during irradiation by surrounding them with solid CO₂ in an outer vessel. The cans were subsequently placed in water at 37° C. until thawed.

(b) *Frozen horse-meat*. The meat was thawed and cut into 20 g. samples which were placed individually in aluminium cans with screw caps. The slope culture was washed off with 5 ml. of blood exudate from the thawed meat, and 0.3 ml. of this suspension was inoculated into the centre of each meat sample. After overnight (16 hr.) incubation at 37° C. and then rapid re-freezing at –17° C., the colony counts for salmonellae were approximately 5 × 10⁸/g. The samples were maintained frozen during irradiation as described for whole egg, and thawed in a similar manner after treatment. Each sample was macerated with 100 ml. sterile distilled water in an M.S.E. homogenizer, and from this suspension 1 ml. portions were withdrawn to make suitable dilutions.

(c) *Desiccated coconut*. The slope culture was washed off with 10 ml. of buffer and 0.5 ml. of the suspension (10⁹ orgs./ml.) was added drop by drop to each 3 g. sample of coconut in a sterile Petri dish. The coconut was dried at 37° C. for 1½ hr. with the lids of the dishes raised. The initial colony count was approximately

10^8 /g. The dried samples were transferred to universal containers for irradiation. Buffer solution, 20 ml., was added after irradiation and the containers were shaken vigorously for 5 min., after which 0.5 ml. portions were withdrawn for dilution and plate count.

(d) *Bone meal*. Concentrated suspensions of salmonellae were prepared by washing off cultures from four nutrient agar slopes with buffer, centrifuging and resuspending in 5 ml. of buffer. The final concentration was 10^{10} orgs./ml., and 1 ml. was added drop by drop to 5 g. samples of bone meal in Petri dishes as for the coconut. After drying at 45°C . for 1 hr., irradiation was carried out in the same way. The initial plate count was 10^7 /g. After irradiation, 10 ml. of phosphate buffer were added and the containers shaken vigorously for 5 min. before withdrawal of 0.5 ml. quantities for dilution and plating.

(e) *Buffer suspensions*. The slope was washed with 10 ml. buffer, centrifuged for 30 min. and re-suspended in 10 ml. of buffer to give a final concentration of 10^9 orgs./ml. For non-aerated conditions the suspension was irradiated in a universal container. To obtain aeration conditions, air was bubbled through the suspension in suitable glass vessels throughout the irradiation period. Anoxic conditions were obtained by bubbling 'oxygen-free' nitrogen through the suspension for 6 min. before and throughout irradiation. For irradiation in the frozen state the suspension was held for 1 hr. at -15°C . and this temperature was maintained during treatment. Samples were thawed by standing in water at 37°C . Suitable dilutions were made from all samples and used for surface plate counts.

Counting technique

Because the food media were irradiated before inoculation to remove contaminants, the requirement was merely to count salmonellae in the absence of other organisms. Suitable dilutions were made with quarter-strength Ringer's solution. The surface-plate count technique used throughout was a modified form of the method of Miles & Misra (1938) with 'Oxoid' nutrient agar as the medium. Initial runs were carried out to reveal which medium gave the best recovery for salmonellae after irradiation. Apart from 'Oxoid' nutrient agar, the media tested were deoxycholate-citrate agar, MacConkey agar and 8% horse blood agar. The poorest recovery was on the selective medium deoxycholate-citrate agar; there was no significant difference in numbers recovered on the other three media. 'Oxoid' nutrient agar did, however, produce the most reproducible results and the largest colonies, and was the simplest to prepare; it was therefore used for the dose/survival curve experiments.

Construction of survival curves

Three dose/survival curves were obtained separately for each of the systems examined. A common regression line was fitted to these by means of the method of least squares with an electronic computer. The regression line is of 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).

Radiation source

Gamma radiation from a 1000-curie Cobalt-60 source was used throughout. The source is a modified form of the 'hot spot' described by Eastwood, 1955; the dose rate was 0.25 Mrad./hr.

Large-scale experiments—artificially and naturally contaminated products

Direct evidence of the effectiveness of radiation in eliminating salmonellae from frozen horse-meat, desiccated coconut and bone meal was obtained by treating samples known to be naturally contaminated with salmonellae; this was followed by bacteriological examination. Frozen whole egg was heavily contaminated by artificial inoculation before irradiation.

(a) Whole egg

(i) During experiments described by Heller *et al.* 1962, on the pasteurization of whole egg, 1000 gal. of liquid egg held in the mixing tank at a commercial plant were inoculated with *S. gallinarum* to give a final salmonella count of approximately 500,000/ml. Samples were withdrawn, canned and frozen (-15°C .) at the plant, and used for radiation experiments. The cans were maintained frozen during both transport and irradiation with a dose of 0.5 Mrad., which was applied within 48 hr. of the inoculation. General colony, coliform and salmonella counts were made at all stages of the experiments.

In a similar experiment carried out earlier, *S. typhi-murium* phage-type 2b was used; again the final salmonella count was approximately 500,000/ml., but the dose used was only 0.35 Mrad.

(ii) In an earlier experiment (Hobbs, Horne & Ingram, unpublished) carried out at a different plant, bulked liquid egg was inoculated with a mixture of *S. typhi-murium*, *S. thompson* and *S. senftenberg*, to give counts of approximately 500/ml. of each. This concentration was chosen to represent the numbers which might be encountered in practice in a badly contaminated commercial sample. The infected egg was filled into 28 lb. cans, frozen (-15°C .) and irradiated at 0.5 Mrad. From some of the cans 60 g. frozen samples were removed by the method described by Hobbs & Smith (1955), and examined for salmonellae. The tests were carried out over a period of 3 months, during which time the frozen egg was maintained at -10°C .

(b) Frozen horse-meat

Thirty-five tons of frozen horse-meat from a consignment at the Port of London, known to be contaminated with salmonellae, was transported by road in an unrefrigerated vehicle (3 hr.) to Wantage Research Laboratory, in a series of batches of 5 tons each. The material was still frozen hard on arrival. At the Port the large blocks had been sawn up into smaller blocks, each approximately 1 cu.ft., to fit the conveyor system of the Cobalt-60 Package Irradiation Plant. In order to avoid thawing of the meat and the possibility of drip during passage through the plant, the dose used was 0.25 Mrad., so that the exposure time in the irradiation

chamber was no more than a few hours. After treatment the meat, still frozen, was returned to a refrigerated store at the Port.

A large number of 200 g. samples were taken at random from each batch both before and after irradiation. The samples were sawn from different blocks, instruments being sterilized after each sample. The samples were packed in polythene bags, heat-sealed, and maintained frozen until examined for salmonellae.

(c) *Desiccated coconut*

A series of 50 g. samples of coconut was taken from a 100 lb. bag found to be positive for salmonellae on arrival at the Port of London. The samples were canned, divided into batches, and irradiated at a series of doses up to 0.55 Mrad. As well as examination for salmonellae, coliform and general colony counts were performed.

In a later experiment another contaminated bag was sampled similarly, and a number of samples were irradiated at one dose level of 0.45 Mrad. Counts and examination for salmonellae were performed in the same way.

(d) *Bone meal*

A series of 10 g. samples were taken from a sack of bone meal known to be contaminated with salmonellae, and double-packed in plastic bags. Nine samples were treated at each of a series of doses up to 0.75 Mrad. Coliform and general colony counts were carried out as well as an examination for salmonellae.

Bacteriological examination

Plate and coliform counts were carried out on most samples before and after irradiation. The surface drop technique on horse blood agar incubated at 37° C. for 24–48 hr. was used for colony counts from suitable dilutions of a suspension of 10 g. of the food in 100 ml. quarter-strength Ringer's solution. Duplicate 1/10 and 1/100 dilutions were inoculated into single strength MacConkey broth for coliform organisms at 37° C. Tubes showing acid and gas were subcultured into brilliant green bile salt broth (Mackenzie, Taylor & Gilbert, 1948), and peptone water, both at 44° C., for gas and indole production by *Escherichia coli*.

The surface drop technique on suitable media was used also for salmonella counts, and in addition a dilution method similar to that used for estimating the probable number of coliform bacilli in water (Report, 1961*a*), Selenite F or nutrient broth being substituted for MacConkey broth. In most instances at least 50 g. of each sample were examined for the presence of salmonellae. In addition, 25 g. quantities were inoculated into each of two of the liquid enrichment media, Selenite F, tetrathionate and nutrient broth. After 24 hr. and 72 hr. at 37° C. each enrichment culture was subcultured on to the two selective agar media, deoxycholate-citrate and Wilson and Blair's medium, and incubated at 37° C. for 2 days. Characteristic colonies were picked for fermentation reactions into the Gillies (1956) modification of Kohn's (1954) tubes and also on to MacConkey agar for purity and lactose fermentation. Slide agglutination was used for serological identification; obscure serotypes were sent to the Salmonella Reference Laboratory, and where

phage typing was applicable the strains were sent to the Enteric Reference Laboratory.

It was recognized that more serotypes of salmonellae may have been present in the samples, but the extra time involved in picking and identification of more colonies or in the application of a technique such as that described by Harvey & Price (1961, 1962) was considered not to be justifiable.

Treatment by irradiation may have affected surviving organisms in some way so that different methods of identification would be required, but so far there is no direct evidence to suggest that irradiation damage to micro-organisms makes different isolation techniques necessary.

Radiation source

The Spent Fuel Rod Assembly at A.E.R.E., Harwell, was used throughout as source of gamma radiation, except for the treatment of frozen horse-meat; the dose rate was approximately 1.5 Mrad/hr. The horse-meat was treated in the Cobalt-60 Package Irradiation Plant at Wantage, which has been described in detail by Jefferson, Rogers & Murray (1961).

RESULTS

The dose/survival curves obtained with the various media, illustrated for *S. typhi-murium* in Fig. 1, were treated as being strictly exponential, and the *D* value (dose required to reduce the number of survivors to one-tenth) for each system was calculated directly. Few of the curves extrapolated back to the ordinate to 100% survival, and therefore the dose required to reduce the population from 100 to 10% is apparently lower than the *D* value which is given in Table 1 for each of the systems examined. However, the *D* value, which is applicable in all cases below a survival level of 30%, is very useful for practical purposes; the dose needed to produce a given inactivation of salmonellae is calculated by multiplying the *D* value by the exponent of the required inactivation factor. For example, if an initial contamination of 100 orgs./g. is required to be reduced to only 1 org./1000 g., then the inactivation factor is 10^5 and the dose needed is $5 \times D$ value for the particular system. This dose would tend to be a little higher (about 8% for *S. typhi-murium* in frozen horsemeat) than that needed if adjustment is made for the initial portion of the survival curve.

The serotypes varied in their radiation resistance; for example, *S. typhi-murium* was very significantly ($P < 0.01$) more resistant than *S. senftenberg* in almost all the media. In fact it appeared to be the most radiation-resistant of the five serotypes examined, although *S. paratyphi* B was just significantly more resistant in frozen buffer and just significantly less in frozen horse-meat ($P = 0.05-0.02$). Salmonellae proved to be considerably more resistant in coconut or frozen horsemeat than in bone meal or frozen egg, and therefore no predictions regarding radiation resistance can be based on the general physical state of the food.

Though freezing had no effect on the resistance of the salmonellae in whole egg,

with the possible exception of *S. gallinarum*, it had a striking effect on the buffer suspensions, giving D.M.F.s (dose modifying factor = ratio of *D* value in the frozen state to *D* value unfrozen) of 1.6–2.8.

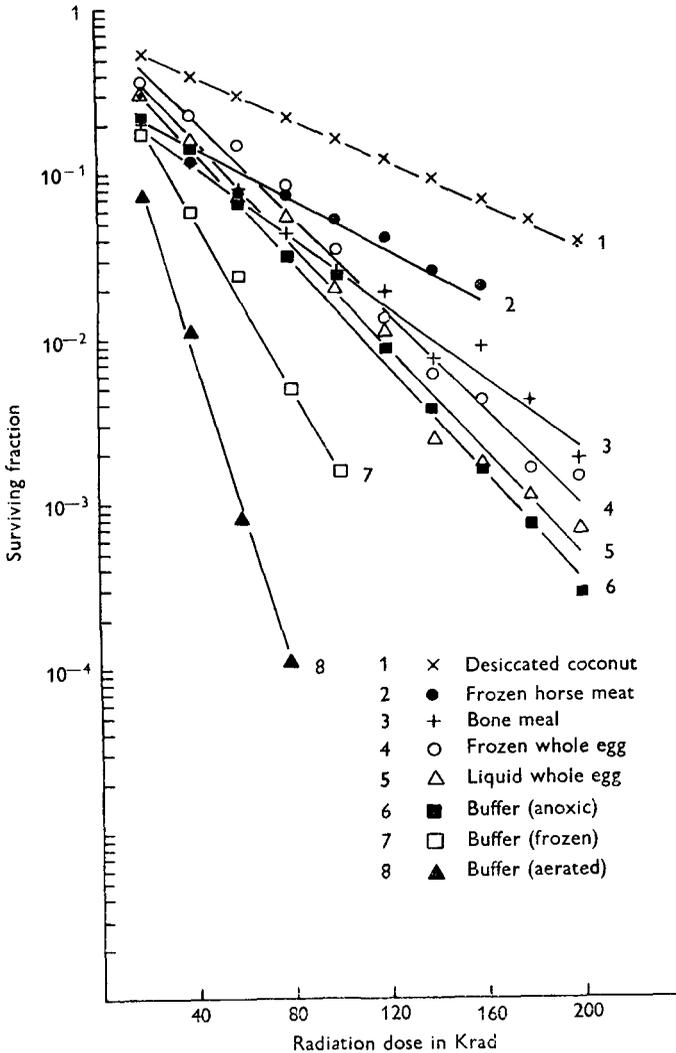


Fig. 1. Dose/survival curves for *S. typhi-murium* in various media.

The salmonellae were most radiation-sensitive in buffer suspension. There was no significant difference between aerated and non-aerated suspensions, but under anoxic conditions the D.M.F. was approximately 3.

The results in Table 2 illustrate the effectiveness of a dose of 0.5 Mrad. in eliminating a high degree of contamination with *S. gallinarum* in frozen egg. In fact, based on the *D* value for this serotype, only one survivor is to be expected in 2 l. of the egg—well beyond the practical limits of bacteriological detection. In an earlier experiment, the results after the inoculation of *S. typhi-murium* at the

same concentration gave positive results in 50 ml. samples, since this serotype is very much more resistant than *S. gallinarum* and the dose used was only 0.35 Mrad. A 10^5 inactivation is to be expected as calculated from the *D* value, and

Table 1. *The D values for various serotypes of salmonella in different media*

Media	<i>D</i> value (Krad.)*				
	<i>S. gallinarum</i>	<i>S. senftenberg</i>	<i>S. typhimurium</i>	<i>S. paratyphi B</i>	<i>S. meleagridis</i>
Liquid whole egg	43.0 (39.2-47.5)	50.4 (46.6-54.8)	63.2 (58.4-68.8)	—	—
Frozen whole egg	56.9 (49.1-67.8)	46.8 (44.1-49.8)	67.9 (63.4-72.9)	—	—
Frozen horse-meat	—	—	128 (112-148)	107 (97.2-120)	93.1 (83.5-105)
Desiccated coconut	—	134 (124-145)	158 (141-177)	—	—
Bone meal	—	55.7 (52.9-58.9)	91.0 (84.0-99.4)	—	—
Phosphate buffer					
Aerated	13.2 (12.3-14.3)	13.0 (12.7-13.3)	20.8 (19.1-22.9)	19.0 (17.0-21.5)	—
Non-aerated	13.2 (11.3-15.7)	12.9 (11.6-14.4)	17.7 (15.5-20.5)	17.1 (16.4-17.8)	—
Anoxic	36.3 (34.6-38.1)	38.9 (35.5-42.8)	61.9 (58.9-65.1)	65.9 (62.6-69.5)	—
Frozen	21.1 (19.2-23.3)	29.9 (28.1-32.0)	39.1 (35.2-43.8)	49.4 (40.4-63.6)	—

95% confidence limits in brackets.

* Radiation dose required to reduce number of survivors to one-tenth

Table 2. *Bacterial examination of whole egg inoculated with Salmonella gallinarum and irradiated in the frozen state with 0.5 Mrad.*

Sample	General count/ml. at 37° C.	Coliform bacilli		<i>S. gallinarum</i> count/ml.
		0.1 ml.	0.01 ml.	
Uninoculated, liquid	55,000	*	Faecal	Nil**
Inoculated, liquid	1,000,000	*	Faecal	400,000
Inoculated, frozen (i)	680,000	Faecal	Non-faecal	380,000
Inoculated, frozen (ii)	830,000	Faecal	Faecal	530,000
Inoculated, frozen irradiated (i)	< 500	Not found	Not found	Not found in 50 ml.
Inoculated, frozen irradiated (ii)	< 500	Not found	Not found	Not found in 50 ml.

* Not tested. ** *S. pullorum* present (< 5000/ml.)

this would result in five survivors/ml. This particular experiment with *S. typhimurium* was carried out before *D* value figures were available. When the degree of contamination was only 500 orgs./ml. of each of three serotypes no salmonellae were detected in numerous samples tested after a dose of 0.5 Mrad., as the results

show in Table 3. *S. gallinarum* and *S. saint-paul*, which were not inoculated into the egg, were identified in the control samples, where they occurred as natural contaminants.

Table 3. *Salmonellae in whole egg inoculated with Salmonella typhimurium, S. thompson and S. senftenberg (500 orgs./ml. of each) frozen and irradiated with 0.5 Mrad.*

Frozen egg sample	No. of cans examined	No. of cans in which were identified				
		<i>S. typhimurium</i>	<i>S. thompson</i>	<i>S. senftenberg</i>	<i>S. gallinarum</i>	<i>S. saint-paul</i>
Uninoculated	116	21	0	0	42	1
Inoculated	30	30	30	30	*	*
Inoculated irradiated	40	0	0	0	0	0

* Not sought.

Table 4. *Irradiation of naturally contaminated frozen horse-meat*

Radiation dose (Mrad.)	No. of 200 g. samples examined	No. in which salmonellae detected
Nil-control	72	42*
0.25	57	Nil
0.75	15	Nil

* *S. oranienburg, S. derby, S. minnesota, S. meleagridis, S. poona, S. anatum, S. saint-paul, S. newport* identified.

Table 5. *Irradiation of naturally contaminated desiccated coconut*

Expt.	Radiation dose (Mrad.)	No. of 50 g. samples examined	No. in which Salmonellae detected	General colony count per g. at 37° C.	Coliform bacilli
Expt. I	Nil-control	18	18*	7000	Present in 0.1 g.
	0.1	9	8*	< 500	Not found in 0.1 g.
	0.2	9	6*		
	0.35	18	4*		
	0.45	9	Nil	—	—
	0.55	9	Nil	—	—
Expt. II	Nil-control	3	3**	< 500	Not found in 0.1 g.
	0.45	12	Nil		

* *S. bareilly* identified. ** *S. paratyphi B* identified.

A high natural contamination rate, about 60%, was evident in the frozen horse-meat examined (Table 4), but a dose of 0.25 Mrad. was sufficient to give negative results in all the samples examined. *S. meleagridis* was identified in the controls; this has a high *D* value and only a 10² to 10³ inactivation would be expected. This indicates that the number of contaminants was small.

All the unirradiated samples of desiccated coconut examined were positive for salmonellae (Table 5), and a dose of 0.45 Mrad. was required to give negative results by normal procedures. With bone meal (Table 6) the dose was rather higher—0.75 Mrad.—which was not to be expected, comparing the influence of coconut and bone meal on the radiation resistance of the same serotypes (Table 1). However, quite a wide range of serotypes were identified in the bone meal, not all of which have been examined for resistance. Another explanation is that the numbers of salmonellae initially present might have been very high; the initial general count was high and many organisms survived at 0.75 Mrad. The initial general counts with both the coconut and whole egg (uninoculated) were comparatively low, and with the former a dose of 0.1 Mrad. reduced the numbers to < 500/g.

Table 6. *Irradiation of naturally contaminated bone meal*

Radiation dose (Mrad.)	No. of 10 g. samples examined	No. in which Salmonellae detected	General colony count per g. at 37° C.	Coliform bacilli
Nil—control	9	6*	13 million	Present in 0.01 g. (faecal)
0.1	9	6**	675,000	Present in 0.1 g. (non-faecal)
0.25	9	6***	130,000	Present in 0.1 g. (non-faecal)
0.5	9	1****	7,500	Not found in 0.1 g.
0.75	9	Nil	2,750	Not found in 0.1 g.

* *S. oranienburg*, *S. cerro*, *S. meleagridis*, *S. kentucky*, *S. typhi-murium* phage-type 2d.

** *S. kentucky*, *S. chailey*, *S. minnesota*.

*** *S. californica*, *S. oranienburg*, *S. bredeny*, *S. typhi-murium*.

**** *S. typhi-murium* phage-type 1b.

DISCUSSION

It is well known that various conditions of environment, such as temperature, media and availability of oxygen, influence the radiation sensitivity of bacteria, and this is again apparent from the results for salmonellae. The chemical composition of the media is very important; each of the foods exerts some protective effect compared with buffer and the effect can be very much greater than that produced even in an anoxic buffer suspension. No doubt a combination of protective effects contributes to the high dose requirement in certain media. It is necessary, therefore, in defining a suitable radiation process for the elimination of salmonellae, to consider each product concerned individually. This point was made by Erdman, Thatcher & MacQueen (1961) when they observed increased resistance in salmonellae and other micro-organisms of public health significance irradiated in broth compared with buffer.

The radiation dose to be recommended for the adequate elimination of salmonellae from the different products will depend on what degree of inactivation is considered safe on health grounds. This is extremely difficult to define; even a small number of salmonellae widespread throughout a commodity might lead to contamination of second vehicles such as cream cakes, trifles or cooked meat products, which are completed foods not requiring further heat treatment before consumption.

There is little information on the numbers of salmonellae in frozen or dried foods such as meat, egg products and coconut. However, the dilution technique performed from time to time has revealed figures of approximately 0.12–18/ml. for frozen whole egg, and < 0.03–0.072/g. for coconut; the highest count recorded for steamed bone flour was 120/g. (Report, 1959), but counts for meat and bone meal were usually < 0.3/g.; recorded figures for various animal feeds and ingredients were 0.02–0.08/g. (Report, 1961*b*). McCullough & Eisele (1951) in volunteer experiments gave 130,000 to 150,000 organisms as the lowest dose of salmonellae which could initiate illness. It is evident, therefore, that most of the incidents of salmonella food poisoning arising from the products considered in this paper are due to cross-contamination of other foods in which the salmonellae multiply, although cases of paratyphoid fever have been suspected to arise from the direct consumption of desiccated coconut (Anderson, 1960; Semple, Parry & Graham, 1961). The method of cross-contamination has been described for egg products (reviewed by Hobbs, 1963) and coconut (Wilson & Mackenzie, 1955), and must also occur from raw meat or poultry to cooked meat (Anderson, Galbraith & Taylor, 1961; Galbraith, Mawson, Maton & Stone 1962).

There is no doubt that a process which will considerably reduce the original number of salmonella contaminants will effectively contribute to the lessening of health hazards from this source. At a recent International Atomic Energy Agency (Vienna) panel meeting on the control of salmonellae using radiation (Report, 1963) it was recommended that the estimation of the effectiveness of radiation treatment should be based on the absence of salmonellae in, for example, 100 g. of product. This can easily be attained by a comparatively low dose of radiation when the numbers of initial contaminants are small; however, in order to deal with the possibility of large numbers being occasionally present, the dose should be as high as will allow economic operation of the process and yet avoid damage to the quality of the products.

Particular attention has previously been given to the use of radiation for the elimination of salmonellae from egg products, and a difference was noted in the radiation resistance of different serotypes. In studies on liquid whole egg (Proctor, Joslyn, Nickerson & Lockhart, 1953; Mossel, 1960) and on liquid and frozen egg white (Nickerson *et al.* 1957), *S. typhi-murium* was found to be more resistant than *S. senftenberg*, and the results presented in this paper show this to be so in a variety of media; in fact, *S. typhi-murium* was the most consistently resistant serotype examined. Brogle *et al.* (1957) found no such difference in resistance for the two serotypes referred to in whole egg solids, egg yolk solids or frozen egg yolk. A recent paper by Comer, Anderson & Garrard (1963) provides information on the radiation resistance of 18 serotypes of salmonella in frozen whole egg. Several of these were shown to be more resistant than *S. typhi-murium*, *S. give* being the most resistant. The culture of *S. typhi-murium* used was, however, less resistant than our own. For the purposes of this paper an estimate of the practical dose requirement for the treatment of the products currently reported is based on the resistance of *S. typhi-murium* phage type 2c (14).

Considering data for *S. typhi-murium* in frozen whole egg, a suitable practical

dose would be 0.5 Mrad., which gives a 10^7 inactivation as shown by *D* value determination, and should ensure the destruction of a variety of serotypes of salmonellae after inoculation into egg. This dose is somewhat higher than that quoted by other workers previously referred to for a similar egg product, and only 0.04 Mrad. less than that recommended by Comer *et al.* (1963). Treatment of frozen egg at 0.5 Mrad. has a negligible effect on the egg quality (Ley, Glew & Cornford, 1962), and the cost of the process is low (Ley & Rogers, 1962). Recently, however, a successful heat pasteurization process has been developed (Heller *et al.* 1962), and legislation is now nearing completion for the compulsory pasteurization of bulked whole egg. Heat and radiation processing of whole egg have been compared by Ingram, Rhodes & Ley (1961); radiation has an obvious advantage for the treatment of imported canned frozen egg as opposed to home-produced liquid egg, which can be heat-treated as such at 'breaking-out' stations before canning and freezing. Based on *D* value data only, the dose for egg in the liquid state would also be 0.5 Mrad. but this dose would be expected to have a detrimental effect on quality, such effects being observed at much lower doses by Mossel (1960).

At 0.64 Mrad. there is a 10^5 inactivation for frozen horse-meat, and this appears to be adequate in view of the absence of salmonellae from the naturally contaminated meat tested after treatment with as low a dose as 0.25 Mrad., when a 10^2 inactivation was obtained, although no *S. typhi-murium* were identified in the particular batches examined before irradiation. The application of 0.64 Mrad. for the treatment of horse-meat intended as pet food in the U.K. would be an economical and practical proposition (Ley, 1962). Experience in the operation of such a process would show whether the dose could safely be reduced below this level.

The microbiological data for desiccated coconut showed that 0.45 Mrad. reduced the number of salmonellae to a level which could not be detected; treatment with this dose gives an inactivation factor of 10^3 . However, taste panel studies revealed that, even at this low dose level, change in flavour was detected as well as a slight darkening in colour, and the product was unacceptable to the trade (Glew & Ley, unpublished). Improved conditions of hygiene have considerably reduced the numbers of salmonellae in coconut and therefore the need for a large-scale process is not so pressing as hitherto.

For bone meal the effective dose was between 0.5 and 0.75 Mrad. Results indicate that natural contamination in this product is often heavy, and the practical dose will therefore be higher than for the other products investigated. A dose of 0.75 Mrad. is unlikely to affect the bone meal in any way, but the practicability of a radiation process for such a product remains to be determined. Animal feedingstuffs such as meat and fish meals might also be treated by radiation; it is quite feasible for a radiation plant to be designed to treat a variety of bagged feeds and fertilizers.

Though radiation is effective against salmonellae, the doses recommended for the treatment of individual products will also have a lethal effect on the other microorganisms present, as revealed by the fall in the general count after treatment. The clostridia, however, are particularly radiation-resistant; it should therefore

be stressed that a radiation process aimed at salmonella elimination does not allow a change in the normal methods of storing and distributing the foods, e.g. at room temperature instead of frozen. The radiation resistance of various strains of staphylococcus in broth media, as shown by Erdman *et al.* (1961), is equal to or less than the resistance of the serotypes of salmonella which we have examined in foods; the numbers of staphylococci would therefore be very considerably reduced. Staphylococcal toxin is also affected; emetic activity for cats was reduced with a dose just greater than 0.1 Mrad. The same authors noted the high radiation sensitivity of coliforms, and our results, well illustrated with desiccated coconut, show that they are absent after low doses of radiation although salmonellae survived; this confirms that coliform destruction cannot be used as an indication of effective radiation treatment, although it is sometimes used as a measure of effective heat pasteurization.

Before any radiation process can be applied, evidence for the safety of irradiated food for consumption is required. A vast quantity of such evidence is now available (Report, 1962*b*), and it is not expected that the problem of wholesomeness will limit the use of the radiation process discussed in this paper.

SUMMARY

Studies on the use of gamma radiation for the elimination of salmonellae from whole egg, frozen horse-meat, desiccated coconut and bone meal show the extent to which the nature of the medium influences the resistance of these organisms to gamma radiation. There is also a variation in radiation resistance between different serotypes; *S. typhi-murium* was consistently the most resistant of those examined.

Based on experiments with artificially inoculated or naturally contaminated products, and also on dose/survival curve data, the dose requirement for the elimination of salmonellae from frozen whole egg is estimated at 0.5 Mrad., which gives a 10^7 reduction in numbers of *S. typhi-murium*; for frozen horse-meat 0.65 Mrad., giving a 10^5 reduction; and for bone meal between 0.5 and 0.75 Mrad., giving between 10^5 and 10^8 reduction. A dose of 0.45 Mrad. appears effective for desiccated coconut, with a reduction of 10^3 , but such a radiation dose affects the quality of this product.

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