SALMONELLA RADICIDATION OF POULTRY CARCASSES

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ter verkrijging van de graad van
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van de Landbouwhogeschool te Wageningen

VOORWOORD

Aan het tot stand komen van dit proefschrift is, behalve door de promovendus en de promotores, door velen bijgedragen. Een aantal van hen wil ik op deze plaats graag apart noemen en hartelijk dank zeggen : Jan en Roely van Arkel, Riek Beuving, Nico Bolder, Ria van der Hulst, Ineke Mulder en Joke Werler.

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Cover page : Scanning Electron Microscopy photograph showing microorganisms on broiler skin

STELLINGEN

1. Het Salmonella-probleem bij slachtkuikens kan worden opgelost, indien koppels slachtkuikens zoveel mogelijk Salmonella-vrij worden gemest, het geslachte dier op de meest hygiënische wijze wordt behandeld en ter eliminatie van de onvermijdelijke residuale besmetting een dekontaminatie-behandeling ondergaat, bijvoorbeeld met ioniserende straling.

2. Het effekt van de toevoeging van katalase aan selektieve voedingsbodems, om ook de groei van sublethaal beschadigde cellen op deze voedingsbodems mogelijk te maken, dient nader te worden onderzocht.

3. De bewering :"pectin and pectic enzyme research constitute a vicious circle", is van toepassing voor diverse gebieden van enzyme onderzoek, bijvoorbeeld bij het cellulase onderzoek.

W. Pilnik en F.M. Rombouts (1981) in :Enzymes and Food Processing, Applied Science Publishers

4. De ironische wet van Maier :"if facts do not confirm theory, they must be disposed of", wordt in wetenschappelijk onderzoek nog te veel toegepast.

5. Wei-eiwit preparaten kunnen kip-heelei niet vervangen bij het vervaardigen van bakprodukten.

J.N. de Wit en E. Hontelez-Brackx (1981) International Dairy Federation Case Report, subject 6b, Luxembourg

6. Er is een ontoelaatbare wildgroei in gebieden van onderzoek die tot de biotechnologie worden gerekend.

7. Bij de bereiding van parenteralia wordt, in vergelijking met andere kontaminatie-bronnen, te veel aandacht besteed aan de luchtkwaliteit in de produktieruimten.

Bereiding van parenteralia in ziekenhuisapotheken (1981) Pharmaceutisch Weekblad 116, 712-736

8. Het ontwikkelen van theorieën omtrent "ideal points" c.q. "bliss points" gedurende enkele decennia, heeft (nog) niet kunnen voorkomen, dat onderzoekers pogingen ondernemen rechtlijnige verbanden aan te tonen tussen intensiteit van kwaliteits-eigenschappen en algehele sensorische waardering van voedingsmiddelen.
H.R. Moskowitz (1981) Journal of Food Science 46, 244-248

9. Alvorens vlokkingsslib als veevoeder c.q. veevoedergrondstof kan worden toegepast dient dit te worden geproduceerd op een wijze welke garandeert, dat het eindprodukt voldoet aan redelijke microbiologische eisen.

10. De versheid van het konsumptie -(tafel)- ei dient met behulp van konsumenten-panels te worden vastgesteld.

11. De selektie van de jeugd volgens het fasenplan van de KNLTB gaat uitsluitend uit van de tenniskwaliteiten van de kinderen; het verdient aanbeveling slechts dan tot selektie over te gaan, indien beide ouders tegen deze selektie lijken te zijn opgewassen.

12. Het handhaven van de koopkracht kan schadelijk zijn voor de gezondheid.

Proefschrift van R.W.A.W. Mulder
Salmonella radicidation of poultry carcasses
Wageningen, 19 mei 1982

Ter nagedachtenis aan mijn vader

Aan mijn moeder, Ineke, Nicoline en Wijnand

CONTENTS

LIST OF ABBREVIATIONS AND SYMBOLS

a_w	water activity
BG	Brilliant green phenol red agar
BGS	Brilliant green sulphadiazine agar
BGSD	Brilliant green sulphadiazine sodium deoxycholate agar
BHI	Brain heart infusion broth
BP	Buffered peptone water
BPA	Baird Parker agar
BS	Bismuth sulphite agar
cfu	colony forming unit
D_{10}-value	Decimal reduction dose. Dose (kGy) needed to reduce a certain number of microorganisms N_0 to 0.1 N_0
DC	Deoxycholate citrate agar
DHL	Deoxycholate hydrogen sulphide lactose agar
DNA	Deoxyribonucleic acid
EE	Enterobacteriaceae enrichment broth
HE	Hektoen enteric agar
ISO	International standardization organization
KAA	Kanamycine aesculin azide agar/broth
kGy	(kilo) Gray, unit of dose of irradiation (1 kGy = 100 krad) (rad, unit of dose of irradiation before the acceptance of SI (Système International d'Unités) units)
kve	kolonie-vormende eenheid
Λ	Lethality = N_0/N_f
LB	Lactose broth
LIC	Lysine iron cystine broth
MA	Minimal agar
MC	MacConkey agar no.3
MEP	Mannitol egg yolk polymyxine agar
MM	Minimal medium
$MPED_n$	Most probable effective dose of irradiation to achieve n overall decimal reductions
MPN	Most probable number
MSA	Mannitol salt agar
N_0	Number of microorganisms before lethal treatment
N_f	Number of microorganisms after lethal treatment

NA	Nutrient agar
NB	Nutrient broth
NDA	Nalidixic acid
OYDA	Oxytetracyclin yeast extract dextrose agar
PCA	Plate count agar
Pw	Peptone water
RNA	Ribonucleic acid
SBG	Sulphapyridine brilliant green agar
SCB	Selenite cystine broth
SCM	Mannitol selenite cystine broth
SEB	Selenite broth
TBB	Tetrathionate brilliant green broth
TBN	Tetrathionate novobiocin broth
TSA	Tryptone soya agar
TSB	Tryptone soya broth
TSI	Triple sugar iron agar
VRBG	Violet red bile glucose agar
XLD	Xylose lysine deoxycholate agar
XLDN	Xylose lysine deoxycholate novobiocin agar

INTRODUCTION

Foods of animal origin, such as poultry and poultry products have often been found contaminated with pathogenic microorganisms. Amongst these salmonellae, Clostridium perfringens and Staphylococcus aureus are responsible for the largest proportion of food transmitted incidents (400,401,407). Salmonellae are very important because of their predominant role in human food infections, particularly in high-risk groups of the population, such as very young children, diseased and elderly persons. Annually an average of 7500 cases of human salmonellosis are reported in The Netherlands. This number of reported cases is probably only 1-5% of the real incidence (22,100,296,403).

Contaminated raw foods introduce salmonellae into kitchens where they are spread onto initially Salmonella-free foods (89,332,361). Poultry plays an important role in this spread of salmonellae. Many investigations have shown that up to 90% of the examined broiler carcasses may be Salmonella-positive.

Among the sources of infection of broilers with salmonellae feed is very important, since it affects all parts of the poultry production chain. In The Netherlands attempts to free broiler flocks from salmonellae by decontamination of feed have failed. As long as the farm environment is contaminated with Salmonella, decontamination of feed as the only measure taken seems ineffective.

Better hygienic conditions during the slaughtering procedure have not resulted in a decreased percentage of carcasses contaminated with these bacteria either. The inefficacy of preventive measures to control salmonellae in broilers has led to a search for processes and methods to decontaminate the end product.

Heating applied as a surface pasteurization (184,185) can not be used as the product is changed too much.

Treatment of carcasses or processing water with chemicals like citric acid, glutaraldehyde, lactic acid, lysozyme, sorbic acid and succinic acid have not yet resulted in a commercially applicable process (166,292,341). The addition of chlorine to the processing water gave good results with respect to the total bacterial load of the water, but no effect was observed with respect to Salmonella (217). Moreover, this procedure is not legally permitted in several West-European countries.
Lactic acid decontamination of pork seems to be a promising process (408). Its effect in terminal decontamination of broilers remains to be substantiated.
One of the remaining but very promising possibilities for protecting the consumer against the Salmonella hazard is the use of irradiation to eliminate these microorganisms from the slaughtered poultry carcasses. In red meat processes like infrared and ultraviolet radiation gave good results in the decontamination of the meat surface (248,320). As the microbial contamination of a poultry carcass is not limited to the skin, but also occurs in the abdominal cavity these processes are less applicable because of their low penetration capacity. For the radiation treatment of poultry carcasses gamma-radiation can be used, because of its high-penetration capacity. Low doses of ionizing radiation have already been suggested many years ago to extend shelf life of broiler carcasses at refrigeration temperatures. This has not resulted in a practically applicable process, as improvements in both the cold chain and the packaging of slaughtered poultry have led to better preservation. The costs of the process are not the reason that it has not become commercial. The main reason lies in the consumer's resistance against the use of any process in which atomic energy is involved (409).
The application of low doses of ionizing radiation results in the decrease of the level of potentially harmful pathogenic microorganisms in poultry products. It is known that bacteria like Escherichia coli, Pseudomonas, Salmonella and Staphylococcus aureus are sensitive towards ionizing radiation. Therefore a treatment of slaughtered poultry carcasses with ionizing radiation would result in a virtually pathogen-free end product. The application of this radiation treatment has also some disadvantages. The use of gamma-radiation at doses over 5.00 kGy to poultry and other food products may cause undesirable side-effects, such as changes in colour, odour and flavour. These effects can be reduced by performing the process at temperatures below freezing.

Even so, the application of a radiation process is limited to a certain maximum dose.

This thesis reports investigations using gamma-radiation to decontaminate poultry carcasses. The application to foods of doses of ionizing radiation sufficient to reduce the number of viable specific non-sporeforming pathogenic microorganisms so that none is detectable in the treated food by any standard method (130,405) is termed radicidation. The doses used in this study were at such a level that no undesirable or unfavourable side-effects occurred. The effects of these doses were studied on salmonellae and other microorganisms present in, or associated with poultry carcasses and in liquid and on solid culture media as well.

Decimal reduction (D_{10}) values were estimated. These represent the dose (kGy) required to achieve a reduction in initial colony count from N_0 to 0.1 N_0. Together with the estimation of the numbers of Salmonella present per carcass the data were used to predict the effect of an ionizing radiation treatment of poultry. Data on the effect of ionizing radiation on the total microflora of poultry carcasses were also collected.

CHAPTER 1
1. LITERATURE REVIEW

1.1. The role of Salmonella in food infection.

The genus Salmonella belongs to the family of Enterobacteriaceae of which some (Arizona, Salmonella, Shigella) have an important pathogenic significance. As far as food infection is concerned, Salmonella is by far the most important agent in many countries and regions of the world. For that reason statistics are published in all parts of the world reporting Salmonella isolations from human and non-human origin. These statistics give an insight into the origin, the presence and the distribution of these bacteria.

In some countries, e.g. The Netherlands, a record of Salmonella isolations is kept. The total number of isolations per year, from human or animal origin or from different food products is recorded (17,22,23,135,402). In case of human Salmonella isolations, the number is related to the total number of cases of disease.

Salmonellae can be found in the digestive tract of healthy humans and animals and can easily be spread in and via the environment where the bacteria can survive or even multiply under favourable conditions. Once present in the digestive tract salmonellae may cause clinical symptoms of illness or may stay without causing clinical symptoms ("carrier state"). In the so-called carrier state, large numbers of salmonellae are excreted with faeces. Faeces of animals and birds may contaminate surface waters. The utilization of manure on agricultural land for fertilizer reasons is also a link in the chain of the Salmonella-cycle. The spread of the bacteria can take place for example via birds, insects or rodents.

In animals Salmonella is found in excreta and internal organs of cats, dogs, pigeons, chickens, ducks, geese, turkeys, cows, horses, sheep, rabbits, hares, rats and mice. Animal feed may be a factor in the contamination of these animals.

The environmental influence on Salmonella infections and contaminations was studied in The Netherlands.

In the project "Walcheren" a correlation was demonstrated between human salmonellosis, Salmonella infections in pigs and Salmonella contamination of feed, foods and effluents (99,100). Evidence was found for the existence of permanent Salmonella contamination cycles resulting in marked infection pressure from the environment.

Annually an average of 7500 cases of human salmonellosis are officially reported in The Netherlands (22,296). It is estimated that at least 20 million cases of human salmonellosis occur all over the world (16,78). Literature data suggest that only 1 to 5% of the real incidence of human food infection is reported to the authorities (16,83,100,105,146,333,357, 402), although salmonellosis is a notifiable disease in many countries. Inadequate isolation techniques also cause apparent low incidence rates. Investigations into the causes of Salmonella food infection are hampered by the fact that, for example in The Netherlands, in contrast to countries such as the United Kingdom, the United States and Western-Germany, the majority of cases are individual or family outbreaks. It is therefore very difficult to trace the food product or products aetiologically involved. In the USA 9 - 13% of all reported cases of food infection over the period 1963-1976 were caused by Salmonella (9,48,58). In Canada (347,348,350) these percentages were 22.8 - 25%, whereas in Australia (331) in the years 1967-1971 27% of the cases of food infection were caused by Salmonella. In England and Wales (370) this was 40% over the period 1969-1972. Over the period 1973-1975 74% of the total number of cases of food infection with identified causes were due to Salmonella (369). In those countries another high percentage (73%) of Salmonella food infections was also reported in 1968 (221).

1.2. Salmonella in man

Salmonellosis belongs to the zoonoses, diseases which can be transferred from animals to man and vice versa. The latter only applies for an indirect transmission by the environment. In view of the infection pressure indicated in Section 1.1. it is not surprising that 6-10 serotypes most frequently isolated from animals or animal products also belong to the main 10 serotypes isolated from human patients. As the same serotypes are found in humans and animals, it is not surprising that serotypes frequently isolated from animal feed, foods of animal origin and surface waters, cause clinical symptoms in man.

The severity of the disease depends on the age and the overall condition of the consumer and of course on the organism : number of cells absorbed, serotype and strain. Babies, young children, aged people and persons with a delicate health are more sensitive to salmonellae than healthy persons (16,17,22,23,296,404).

The incubation time for salmonellosis is usually 6 to 24 hours (average 12) with 3 hours as a minimum and 72 hours as a maximum. Acute symptoms of disease include nausea, stomach ache, vomiting, diarrhoea, cold chills, fever and exhaustion. The symptoms of disease normally persist for 2 to 6 days, but in exceptional cases for several weeks. These more or less serious symptoms of gastro-enteritis are caused by so-called "enteritis"-salmonellae. They differ from the very serious clinical symptoms of illness (septicaemia), which are caused by two other serotypes of the genus Salmonella, viz. paratyphi B and typhi. The mortality of the disease caused by the "enteritis"-salmonellae is below 1% (17,22,23,333). The mortality is usually expressed per 100,000 inhabitants, but the best standard for measuring the mortality of salmonellosis is the case fatality rate : the number of cases with a fatal outcome divided by the total of reported cases. In The Netherlands it is difficult to give an exact case fatality rate, because only a minority of outbreaks is reported and even fewer investigated (22). In the USA (48) the case fatality rate during the years 1962-1971 was 0.41% :in 1967 in 29 epidemics in which 5761 persons were involved, 0.22%; in 1971 0.79% in 44 epidemics in which 1391 persons were involved. In cases in which salmonellosis was fatal, the patients always showed symptoms of previous diseases.

In 1951 the morbidity in The Netherlands (333) per 100,000 inhabitants was 4.1; in 1958 : 28.6. From 1967-1972 this figure ranged from 25 tot 43. In 1974 and 1975 this figure was 44 and 47 respectively. 0.2 - 0.5% of the infected patients became carrier of Salmonella bacteria (22).

The total number of cells of salmonellae required to trigger clinical symptoms of disease vary per person and per Salmonella serotype (212,213, 214,368). In literature it is stated that 1.0×10^4, 5.0×10^5 and 5.0×10^7 cells of Salmonella typhimurium, Salmonella anatum and Salmonella meleagridis were necessary (212). 1.5×10^5 cells of Salmonella bareilly and Salmonella newport proved sufficient to cause the disease (213,214). In literature higher numbers of cells are also given for these and other Salmonella serotypes causing disease.

However, in most of the publications, the physiological condition of the persons, mostly volunteers in prison, is not mentioned. Until 1974 there was hardly any mention in the literature of clinical symptoms of salmonellosis caused by products containing less than 100 Salmonella cells per gram.

Only once (Salmonella muenchen) it was reported that Salmonella food infection had been caused by a product containing as few as 10 cells per gram (311). Recently there have been publications indicating that salmonellae present in a very low cell concentration can cause salmonellosis. The Salmonella eastbourne epidemics caused by chocolate (79), Salmonella wien via pepper, Salmonella panama via caseinate (268) and Salmonella typhimurium via apple cider (300) are examples of salmonellosis caused by a very low concentration of these bacteria. In the case of the food infection caused by caseinate growth may have taken place during the preparation of the dietary meals in a children's hospital; in the case of food infection via chocolate and via home made apple cider growth can be excluded because of the low water-activity (a_w) in case of the chocolate and because of the pH of 3.6 - 3.9 in case of the cider. At this pH, however, cells may survive. It therefore depends on the physiological condition of the consumer, whether or not absorption of low numbers of Salmonella will result in clinical symptoms of disease.

Outbreaks due to hamburgers and via pasteurized milk, both contaminated with Salmonella newport were also reported to be caused by less than 10 cells per gram or per ml (301). It is possible, however, but not yet proven, that the virulence of salmonellae depends on the composition of the food or product in which survival or growth has taken place.

The infecting dose of given salmonellae also depends greatly on the carrier in which the bacteria are contained, particularly water versus solid products. Because of the short retention time of water in the stomach no bactericidal effect of the gastric juice occurs (231). The same applies to other commodities taken on an empty stomach (231).

1.3. Salmonella and poultry

The good sanitary conditions during the hatching of eggs and the hatching process itself guarantee a very low percentage of contaminated day-old chicks. On delivery to the rearing farms these Salmonella-free day-old chicks will invariably be exposed to environments allowing Salmonella and other pathogenic microorganisms to invade the population.

As salmonellae can survive in litter, dust, water and manure for weeks or even months, on the farms conditions exist allowing the newly arrived chicks to become infected.

During the rearing period the broiler flocks can be contaminated by water, feed, dust, birds, rodents, flies and other insects.

Man, animals from the intensive animal breeding industry other than poultry and domestic animals being carriers of salmonellae are also important for the spread of these microorganisms by the environment (16,106,205).

Measures which can be taken to control Salmonella in broiler flocks are :

a. effective hygienic control in breeding and hatching centres to prevent flocks from becoming contaminated with Salmonella;
b. effective cleaning and disinfection of the broiler houses before and after the rearing of new flocks;
c. optimal conditions during the rearing of broilers;
d. effective control and monitoring of the absence of salmonellae in the water and feed administered to broilers.

Measures to be taken in a relatively small area of the intensive animal breeding industry and its environment alone will never be successful, since the total contamination of this environment is influenced by many factors to which no specific measures are applicable.

1.3.1. Incidence of Salmonella infection in broiler flocks

Over the years salmonellae have regularly been isolated from poultry and poultry products. Many data on this subject can be found in literature (21,41,136,244,247,269,270,363,391,406). These data are not consistent with respect to the percentage of broilers contaminated with Salmonella. Literature on the Salmonella contamination of broilers can be divided into two groups.

One group deals with Salmonella isolations from live, dead or sick birds, in which mainly the economic consequences of the disease are studied. Serotypes involved are bareilly, gallinarum-pullorum, infantis and typhimurium. The other group of publications deals with Salmonella isolations from eviscerated, ready-to-cook broiler carcasses, which have economic relevance too, but deal more specifically with the consequences for public health. The salmonellae which are reported by this group are mainly present in the broiler flocks without causing disease symptoms. The broilers are healthy carriers of these bacteria; over 20 serotypes were found in one flock (1).

The contamination rate within clinically healthy broiler flocks varied in a given flock from 0.5 to 14.8% (108). Some investigators could not recover any salmonellae from certain flocks, whereas other flocks proved to be very highly contaminated (90,149,242,330,371). An investigation among 46 flocks of broilers covering in all approx. 600,000 broilers showed 19 positive flocks (242). An investigation of livers and samples from the intestinal tract of 31 flocks (total 519 samples) yielded 1.16% positive samples ; the same investigations on 811 samples of 28 broiler flocks resulted in 2.59% samples positive for Salmonella (299).

In The Netherlands in 1963-1964 7.3% of 1530 samples from the caeca of broilers were found Salmonella-positive. The same investigation in 1970 showed 11% of 960 samples positive (362,363). A German investigation (39) during the years 1956-1958 found 14.8% of 317 samples positive for salmonellae. In 64.5% of the positive broilers symptoms of salmonellosis were also diagnosed.

Another German study (304) in 1960-1962 resulted in 47.2% out of 2925 broilers being Salmonella-positive. The serotype isolated from 80% of the positive broilers was Salmonella gallinarum-pullorum. All broilers infected with this serotype showed symptoms of salmonellosis. These results can not be translated into Dutch circumstances as the disease due to Salmonella gallinarum-pullorum is eradicated from the broiler flocks.

In Canada during the period 1949-1966, 5.9% of 23,000 dead or sick broilers examined were Salmonella-positive. Isolations of the serotype gallinarum-pullorum were omitted from the recordings (41). In Denmark (205) in the early sixties salmonellae were regularly isolated in 1 or 2% from ill broilers. The serotype causing the disease was Salmonella typhimurium. 20-30% of all dead animals had died from salmonellosis caused by Salmonella typhimurium (199). The mortality of salmonellosis in broilers and turkeys is indicated as 1.9-4.1% (224). This percentage found in a population of about one million broilers depends on the serotypes involved. In Finland in 1975-1976 the mortality of Salmonella infantis infections in broilers was 3-6% (406). An exception should be made for the mortality caused by infections with Salmonella gallinarum and Salmonella pullorum, which serotypes yielded a mortality of 25.4% for broilers and 62.5% for turkeys. For two reasons the data are somewhat confusing. Firstly because of the fact that during the first two weeks of the rearing period, there is always a high percentage of dead broilers and these broilers are not routinely examined for the presence of Salmonella.

This is rather strange since salmonellosis is a typical juvenile disease. Secondly data can not always be compared because of the enormous differences in microbiological culturing methods used during the last twenty years in all parts of the world.

1.3.2. Salmonella in slaughtered broilers

During the slaughtering procedure the percentage of Salmonella contaminated carcasses increases. The main cause for this phenomenon is the faecal contamination of the carcasses during the various slaughtering stages.

Table 1. Salmonella isolations from eviscerated broiler carcasses in The Netherlands

Material investigated	Number of samples	Percentage of Salmonella	Reference
skin, neck, breast	740	15	363
thaw water	95	64.2	307
skin + thaw water	75	13.3 - 14.7	131
skin, neck	250	8	244
thaw water	250	20	244
skin, cloaca	4800	0 - 90	259
skin, neck	160	10.6	21
skin, neck	200	35.5	243
thaw water	200	49.5	243

Investigations with artificially marked broilers showed that the external contamination of the feet and feathers of broilers plays a very important role in the spread of bacteria over the carcasses (245).
In literature it is stated that Salmonella does not belong to the normal microflora of the avian or animal intestinal tract. Nowadays, however, it seems that Salmonella has become a normal inhabitant of the intestinal tract of many animals (31).

In the tables 1, 2 and 3 data on Salmonella isolations from poultry carcasses reported in The Netherlands and neighbouring countries and the USA have been summarized. These data show that Salmonella is widely distributed in broilers in many countries.
In comparing the data collected in the tables notice should be taken of the fact that different isolation methods were used in the studies referred to.

1.4. Human salmonellosis associated with poultry

In The Netherlands hardly any information exists on the total number of cases of human salmonellosis caused by or associated with the consumption of poultry and poultry products. According to the yearbook of the Medical Inspection Service (22) one epidemic in 1975 was due to the consumption of meat. In future better data on this subject can be expected as the epidemiological know-how and the man-power employed has increased during the last years. To demonstrate the role of poultry in human salmonellosis we have to look for data in foreign literature. Differences in food habits, food preparation and food consumption do not allow extrapolation with regard to data in a specific country.
During the period 1934-1947 about 12,000 isolations from epidemics of Salmonella food infection in the USA were examined, from which 53.5% were originally isolated from poultry and poultry products involved (107). In 1945 (88) 20% of 232 epidemics in the USA were caused by poultry. From 1953-1958 30% of the food infection outbreaks were caused by or associated with poultry and poultry products (373). It was only once that the consumption of poultry meat was explicitly established as the cause of the clinical symptoms.
In the period 1960-1974 there were two epidemics of Salmonella food infection in the USA which had been caused by poultry (57,211).
According to American data 15 outbreaks of salmonellosis in the period 1972-1974 could be traced to poultry or poultry products (156). The period 1968-1977 showed in total 70 outbreaks : the total for meat and poultry was 157 (58).
In England and Wales (153) 10 epidemics of salmonellosis from 1959 to 1970 were caused by poultry (incl. turkeys) or poultry products. From 1964-1968 46.8% of the outbreaks were probably caused by the consumption of poultry products. In 1968 15 outbreaks in a total of 24 caused by all meat products together, were caused by poultry (368).

Table 2. *Salmonella* isolations from dressed broilers in some EEC countries

Material investigated	Number of samples	Percentage of *Salmonella*	Reference
Belgium			
thaw water	30	16.6	307
Denmark			
skin, neck	185	0.4	21
thaw water	232	5	307
France			
skin, neck	160	12.5	21
thaw water	30	16.7	307
Italy			
skin, neck	160	14.4	21
United Kingdom			
skin, neck	240	22.5	21
abdominal cavity, swab	297	10	90
skin, meat, organs	201	0 - 100	293
Western - Germany			
skin, neck	200	55	243
thaw water	200	47	243
thaw water	298	62.8	307

Table 3. *Salmonella* isolations from dressed broilers in the USA

Material investigated	Number of samples	Percentage of *Salmonella*	Reference
abdominal cavity, swab	53	2.7	124
skin, breast, swab	292	1.9	124
abdominal cavity, swab	240	0	372
skin, swab	240	0	372
skin, swab	580	4.5	56
abdominal cavity, swab	263	0	56
skin, swab	24	33	222
abdominal cavity, swab	1045	20	222
skin, swab	318	9	384
skin, swab	237	0.3	384
skin + abdominal cavity, swab	2057	50.2	387
skin, swab	525	11.2	387
skin + meat, swab	196	16.8	391
organs, swab	196	31.3	391

In another study in England it was shown (191) that from 1966-1970 36.9% of the epidemics were caused by poultry. In 1968-1970 the total of 47 epidemics can be divided into : 31 caused by broilers and 16 by turkeys. A third English study compiled all cases of salmonellosis from 1941-1972 (210). From 1955-1956, poultry was involved in 3% of all cases which could be traced to meat products; from 1956-1963 this percentage was 14%, from 1964-1968 45% and from 1969-1972 60%.

Data (369) on the period 1973-1975 show that poultry in England and Wales was involved in Salmonella food infection in 49 (64.6%) out of a total of 76 cases for all meat products together. A summary of the data from Canada, England, Wales and the United States from 1966-1976 (349) showed that in 17% out of a total of 12,315 outbreaks poultry was the identified vehicle. In Western - Germany (270) poultry was involved in 3 out of a total of 30 and 48 cases in 1973 and in 1974 respectively. In 1975 no salmonellosis associated with poultry was reported (24).

In several countries as well as in The Netherlands, the risk of becoming infected with salmonellae originating from foods of animal origin is determined by (413):

a. the total consumption per capita of foods such as poultry and red meats;
b. the way of preparation, including consumption of raw and uncooked foods and the kitchen hygiene;
c. the level of contamination with salmonellae of these foods;
d. the physiological condition of the consumer.

Since the level of contamination of pork and poultry are the highest (134,173,357) these types of meat will be the most important ones in the spread of Salmonella.

1.4.1. The role of broilers as infection source

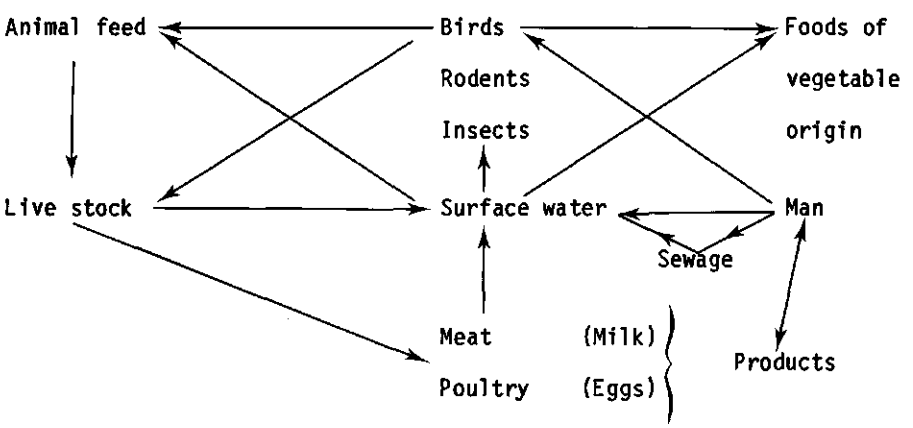
Human infection with salmonellae often occurs after the consumption of raw and insufficiently heated contaminated meat (374,379). As poultry meat is not eaten raw, but is invariably consumed in a cooked or grilled state, the role of poultry in the mechanism of dissemination of salmonellae has to be a different one. The thaw water released from the frozen carcasses during thawing could be the most important factor. During the preparation of a meal this thaw water can contaminate the cooked products, but also other foods and vegetables which are consumed without further heating (362). This has been demonstrated in a study in which artificially infected carcasses (Escherichia coli K12) were prepared (89).

However, poultry products which have not been frozen, but were kept at refrigeration temperatures, must not be excluded. These products also release "thaw"water which has been picked up during processing.
In Western - Germany in 1974 one out of the three cases of salmonellosis in which poultry was involved, was associated with poultry which had been kept at refrigeration temperatures (270). Another possibility in the spread of salmonellae would be contact infection (303). These contact infections can occur in case cooked carcasses get in contact with surfaces on which raw Salmonella contaminated carcasses have been prepared for the meal. The number of thus transmitted Salmonella-cells will be low and therefore growth must take place during storage of the contaminated product involved, before clinical syndromes will occur.

1.5. Control of salmonellae in poultry

Figure 1 illustrates the environmental cycle of Salmonella contamination. The figure shows the difficulties which can be met in controlling these bacteria.

Figure 1. Salmonella contamination cycles (396)



The poultry production chain starts with poultry breeding and ends with poultry processing. The control of Salmonella-infection in the different components of the poultry industry is therefore very complex. In primary breeding relatively small numbers of selected birds are multiplied to form the grandparent stock.

From this stock the parent stock is produced and then this stock is further multiplied to rear large numbers of broiler chickens.
Throughout the production chain there is an increase in "infection - pressure" (cf. Section 1.1.) on the live stock, being kept in increasingly larger numbers at higher stocking densities.
Among the sources of infection with Salmonella the feed is very important, since it affects all parts of the production chain. Therefore an extended review of the literature is given at this point.
The first isolations of salmonellae from animal feed were reported shortly after the second World War. Many investigations have shown that Salmonella-positive feed have led to Salmonella-positive flocks of broilers and turkeys or other animal herds (14,42,46,47,64,122,143,174,176,178,251,256, 295,316,321,337,384,389). These results can be influenced by the addition of certain antibiotics to the feed, which reduces (127,169,190,317) or favours (27) the shedding of salmonellae and thus the percentages of positive carcasses after slaughtering.
Feed components of animal and vegetable origin and mixed feed were examined for the presence of salmonellae (2,11,12,14,42,43,86,152,164,174,176,221, 223,251,273,279,295,298,319,337,373,375,378,385,386,389,393,395). All animal feed, mainly of tropical or sub-tropical origin are considered to be the source of Salmonella contamination. In The Netherlands 12.5% of all imported feed or feed components of animal origin were demonstrated to be contaminated in 1961, whereas 0.5% of the imported fish meal was Salmonella-positive (171,354). Another investigator showed (168) that 26% of the lots of imported fish meal were contaminated. According to the Animal Feed Commodity Board (18,19) 13% and 18% of the samples taken in 1973 and in 1974 from packed imported feed ingredients of animal origin were Salmonella-positive. As the role of feed in the dissemination of salmonellae is considered to be very important, many methods of feed decontamination have been suggested.The following processes of decontamination can be applied :
a. heating and pelleting;
b. treatment with chemicals;
c. treatment with ionizing radiation.
Many data can be found in literature on the decontamination effect of the heating and pelleting of feed, with respect to Enterobacteriaceae and salmonellae (e.g. 81,175,179,202,223,230,240,281,287,328,355,396).

In the pelleting process moisture is used for conditioning the meal. The temperature of the meal entering the pelleting process should be between 80 and 90 ^{o}C, for at least one minute, before passing into a cooler unit in another part of the feed mill.

The decontamination effect of the addition of chemicals like Endgerm^{R}, formic acid, propionic acid, iso-propylalcohol, phosphoric acid, lysozyme and formaldehyde has also been studied (94,287,336,381).

The effect of ionizing radiation on salmonellae and other Enterobacteriaceae in feed has in particular been studied intensively (81, 113,228,240,290,355).

In commercial practice the only acceptable process of removing salmonellae is the heating and pelleting of feed. The effect of heating and pelleting of feed in experiments with pigs has been thoroughly studied. Much less information is available on the influence of pelleted feed on salmonellae in poultry. However, it can be assumed that the results with pigs are also valid as far as rearing and growing of broilers is concerned (81,101,102, 103,104,106,172,174). For pigs it was shown very clearly that heat-treated, pelleted feed decreased the number of Salmonella contaminated carcasses without influencing feed conversion (81,102,104, 106). In a Dutch study on the dissemination of salmonellae within a closed broiler integration (371) an important role was attributed to pelleted feed in this respect. However, the data presented (7 positive feed samples in a total of 694 during a four year period) did not support this conclusion.

For broilers it was shown (72) that the pelleting of feed has a positive effect on feed conversion.

The annual report of the Animal Feed Commodity Board (19) shows that less than 50% of the feed producers use an installation for pelleting feed. This means that in practice a considerable part of the broilers are raised on feed that might be Salmonella-contaminated.

In a Salmonella-control programme it is essential that all primary breeding stocks are fed on heat treated pelleted feed. After pelleting and cooling are completed it is most important to prevent recontamination of the feed from outside foci of infection.

Day-old chicks are another Salmonella-infection source. The incidence of chicks contaminated with salmonellae in The Netherlands, over a period of 3 weeks (60% of the total Dutch production was sampled), was 2.2% (396). In total 1256 specimens were examined.

As the percentage of Salmonella - positive chicks appears to be low the production of Salmonella-free starting material will not present insuperable difficulties, provided good sanitary conditions are maintained during the production of hatching eggs and during hatching and incubation. Feed and day-old chicks are not the only sources of Salmonella-infection. A case study of the situation in a broiler house during the whole rearing period (1) showed amongst others that important sources of contamination with salmonellae were: feed, water, supplied air and dust. In the "project Walcheren" in The Netherlands evidence was found of several other factors in Salmonella-cycles in this instance in the dissemination in pigs (99). It is clear that measures aiming to prevent a Salmonella-infection should be taken at the start of the production chain. It should be remembered that only a relatively small number of carrier birds during rearing are required to result in cross-contamination during the slaughtering process. Even the most hygienic slaughtering process can not change a Salmonella - positive flock into a Salmonella-negative one.

During the slaughtering process some measures can be taken at critical points to prevent further contamination of the carcasses. During scalding, defeathering, evisceration and cooling the maintenance of hygienic and mandatory process conditions can lead to an end product, in which the percentage of carcasses contaminated with Salmonella has not been increased.

A decontamination treatment of the end product should only be promoted in case measures to be taken at the start of the production chain fail, or when these measures are not successful because of a higher infection pressure from other foci.

CHAPTER 2

2. THE EFFECT OF IONIZING RADIATION ON BACTERIA

2.1. Radiation sensitivity of bacteria in liquid and on solid culture media

When cells are exposed to stress conditions, such as heat-treatment (160, 297), freezing (204,329), freeze-drying (314,315), aerosolized conditions (377), contact with disinfectants (148) firstly sublethal and only subsequently irreversible ("lethal") lesions will occur.

Similar effects result from exposure to ionizing radiation. As a result of irradiation, molecules in bacterial cells are ionized and activated. The absorption of radiation energy and the formation of radicals are fast processes. These are followed by slower ones in which biochemical processes ultimately cause death of the cell or at least arrest of cell division. These two processes have the same effect in actual practice, because the demonstration of the presence of living microbial cells depends on the ability to divide (326).

It is assumed that damage of cells, caused by irradiation is a result of changes in DNA molecules. Experiments have shown that ionizing radiation causes changes in dissolved DNA (380); in the presence of oxygen, hydroperoxides can be formed from DNA, but also from compounds present in biological materials, like RNA, pyrimidine bases, amino acids, peptides and steroids.

The sensitivity of different bacteria (spores and vegetative cells) to irradiation differs greatly (114,342,382), while the sensitivity of a given bacterial strain also varies depending on intrinsic and extrinsic factors. As DNA is the main target in the bacterial cell, sensitivity towards ionizing radiation will be determined by attributes of DNA. Ionizing radiation causes single and double strand breaks in DNA. There is a possibility of repair, the so-called dark-repair mechanism. The differences in sensitivity to ionizing radiation among bacteria can not be explained in this way (226). In the following paragraphs the effects of some external factors influencing the sensitivity of bacteria to ionizing radiation, which are much better understood will be discussed.

Since these are expressed in the so-called survivor-curves, that will be introduced first.

The survivor curve.

The survivor curve presents the relation between the radiation dose (kGy) and the numbers of surviving colony forming units (cfu). There are three different types of survivor curves (figure 2; 137,310). These are : the exponential, the sigmoid and the complex survivor curve, the latter consisting of two exponential curves. The survivor curve is called exponential or linear when a straight line is obtained in a semi-logarithmic log N vs dose-plot, used for this purpose in actual practice. Radiation sensitive microorganisms mostly show such linear curves. In that case the radiation sensitivity can be expressed in a so-called D_{10}-value. The D_{10}-value is the dose (kGy) required to achieve a reduction in initial colony count from N_0 to 0.1 N_0 .
The curves of type B in figure 2,i.e. with a shoulder at the beginning, are most common in practice. They can be described by the following equation :

surviving fraction $N/N_0 = 1 - (1 - e^{-kN})^n$

N = number of cells that survive a certain dose
N_0= the initial number of cells
k = constant, in which the death rate of the cells is incorporated
n = an extrapolation number, used to give the line a linear character

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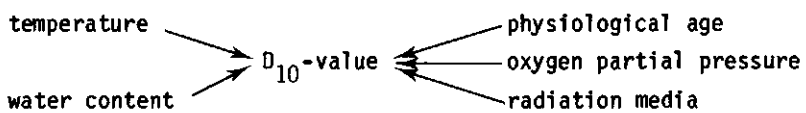


Figure 3. Factors which effect D_{10}-values.

Bacteria showing the phenomenon of a shoulder in their survivor curves, possess more than one vital target per cell, while all targets should be lethally hit to inactivate the cell.

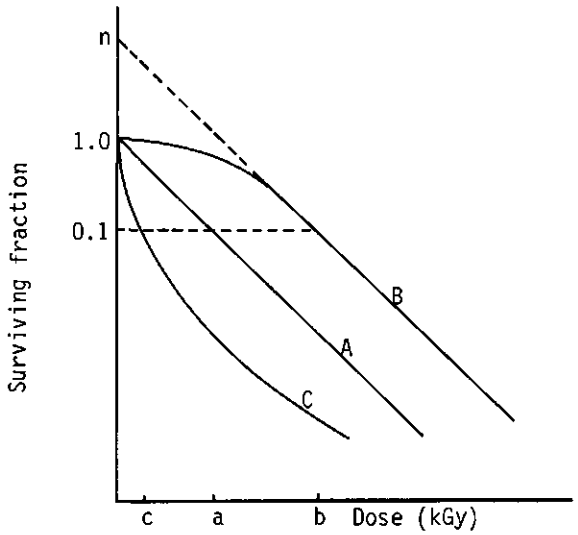


Figure 2. Types of survivor curves for radiated bacteria. The D_{10}-values of curves A, B and C are represented by doses a, b and c, respectively (310).

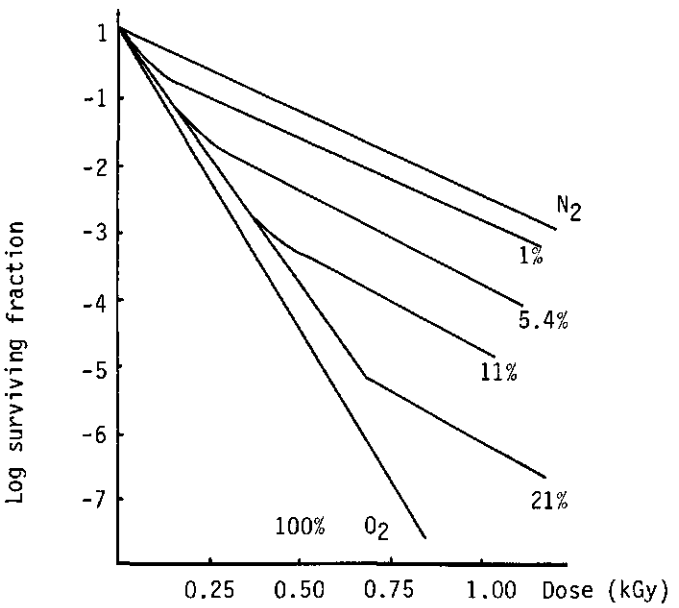


Figure 4. Survival of Escherichia coli B/r irradiated in the presence of various concentrations of oxygen (in pure N_2): high intensity electron dose delivered in single pulses of 30 nsec (112).

Sometimes a so-called tailing effect of the survivor curves is observed (figure 2-C). It is much less easy to incorporate a tail in the mathematics of survival than it is to include a shoulder.

Various factors influence the shape of survivor curves (figure 3). When Escherichia coli cfu's were irradiated in buffer solution and the surviving cfu's were enumerated on a synthetic culture medium, a linear survivor curve was found (69). In case the surviving cells were grown and enumerated in peptone broth and agar, a complex survivor curve, composed of two linear curves was obtained.

Linear curves for Escherichia coli were also reported after irradition in buffer solution and in nutrient broth to which yeast extract was added (115). However, in Hartsell's broth the survivor curve of Escherichia coli showed a shoulder (figure 2, 95).

Survivor curves for salmonellae upon radiation treatment have also been described in literature. Some serotypes show linear survivor curves in buffer, Hartsell's broth and in different foods (95,194). When salmonellae were irradiated in Brain Heart Infusion broth curves with a shoulder were obtained (277).

In complex media a tailing effect is often observed (figure 2-C). This phenomenon can be caused by all kinds of environmental factors. In each case a given higher radiation dose exerts a smaller lethal effect than a lower dose (95,353).

In summary, the shape of a survivor curve is strongly determined by the medium in which the cells were irradiated, in combination with the medium in which the cells were pre-grown and the medium in which the cells are recovered after irradiation. It may well be that all curves possess a shoulder and a tail, but that these are often too small to be observed or concealed by the use of particular techniques.

The sensitivity of bacteria to ionizing radiation is often compared on the basis of D_{10}-values. To accommodate non-linear survivor curves, the so-called most probable effective dose to achieve n log cycles reduction $(MPED)_n$ has been introduced (230). For all practical purposes the MPED is the dose of irradiation that, at a given level of initial contamination, reduces the final cfu count to a non-detectable level, i.e. no more than 10^1 - 10^2 cfu's per gram of product.

Influence of the" physiological age".

Vegetative cells differ in sensitivity depending on the phase of growth. Immediately after cell division has taken place, the cells become more sensitive to ionizing radiation than before or after completion of cell division. During the logarithmic phase of growth Escherichia coli B/r cells proved more sensitive than cells from a lag phase culture (324). Cells were most sensitive at the end of the logarithmic phase of growth, whereas, during the stationary phase of growth the sensitivity decreased, though not reaching the value of the lag-phase.

Other authors also described the fact that logarithmic phase cells showed maximal sensitivity to ionizing radiation (150,322). An explanation for increased resistance to irradiation during the lag-phase could be the following. During the lag-phase a high enzyme activity prevails (392). This causes a low oxygen concentration in the cells. As cells are more resistant under anaerobic radiation conditions (vide infra), this could explain their increased resistance (392).

The oxygen effect.

The presence of oxygen during the radiation treatment increases the lethal effect on bacteria (112,120,151,154,157,207,324,342). Under completely anaerobic conditions the D_{10}-values of some vegetative bacteria increase with a factor 2.5 to 4.7 (342), in comparison with aerobic conditions. Figure 4 shows the effect of different oxygen concentrations on the survival of Escherichia coli B/r (112). The oxygen concentration during the radiation treatment will influence the sensitivity and is therefore to be taken into account in case D_{10}-values of bacteria to ionizing radiation are compared.

The tailing effect as seen in the survivor curve type C in figure 2 could be caused by the generation of an anaerobic environment during the radiation treatment. Another explanation may be the influence exerted by the complex culture media in which the radiation treatments were performed. In literature some evidence is also given for the fact that the chemical nature of radiation damage depends on the presence or absence of oxygen. Repair from damage incurred under anaerobic conditions proceeds at a higher rate (52,226).

Influence of the medium wherein irradiation is carried out.

As indicated before, cells can be sublethally damaged by ionizing radiation (4,60,73,147,151,208). A very complex interaction between cell damage and cell repair exists and this markedly affects the results of recovery experiments (54,73). It is known that cells of certain strains of Escherichia coli can not repair double strand DNA breaks, whereas other microorganisms can (44).

It will therefore be evident that in case cells after irradiation are brought into an environment that favours repair, more surviving cells will be found than when cells are brought into a less favourable environment. The temperature during the radiation treatment and the composition of the medium in which the cells are irradiated influence the sensitivity to ionizing radiation.

The influence of the medium is connected with the composition of the medium used for pre-growing the cells. The highest sensitivity of bacteria is found when cells are suspended in a buffer solution before irradiation. The medium in which surviving cells are cultured after the treatment also influences sensitivity. In rich media cells appear less sensitive than in minimal, chemically defined media (5,92,114,151,188,195,276,346).

This effect is attributed to the presence of protective agents in the former. These agents are mainly those with a sulphydryl group (for example cysteine) (51,93) and compounds with a related chemical structure (271). The effective action of these compounds during the radiation treatment lies in scavenging free radicals produced by primary radiolysis of intracellular water, or by reacting with free radical centres on vital cell macromolecules.

Components, which sensitize bacteria to ionizing radiation are also known. These include some derivatives of naphtalene (252), N-ethylmaleimide (50), iodoacetamide (87), iodoacetic acid, phenylmercuric acetate (50), methylglyoxal and compounds with carbonyl groups (38). The effective action of these substances lies in their capacity to activate oxygen when irradiated. Cells pre-grown in a "poor" culture medium repair more easily after irradiation in a simple medium than those pre-grown in a rich culture medium. The former possess a great many different enzymes for synthetic reactions. The latter do not possess these enzymes. So, when cells after irradiation are inoculated in a poor culture medium these enzymes have first to be synthesized. This illustrates the differences between adaptive and constitutive enzymes.

The possibility for enzyme synthesis, however, is more or less impaired by radiation treatment.
Cells pre-grown in a rich culture medium therefore need for their repair a rich culture medium after irradiation (69,325).
In literature (69,325) a chemically defined culture medium is described in which repair of damaged cells was favoured by stimulating enzyme synthesis. Although the stimulation of enzyme synthesis is the condition for repair of damaged cells (325) there is also some evidence indicating that inhibition of enzyme synthesis stimulates repair (118).
By using selective media, for example the media used for the detection and enumeration of salmonellae and other Enterobacteriaceae, containing bile salts, repair of damaged cells is in general inhibited (73,199,346). The evidence for radiation induced, sublethal damage in Enterobacteriaceae is controversial. Consequently in actual practice the importance of a selective medium effect should not be overrated.

Temperature.

Salmonellae are destroyed faster when the radiation treatment is carried out at sublethal temperatures of 45-55°C (198). At reduced temperatures (particularly below freezing) bacteria are less sensitive to ionizing radiation (53,114,120,128,195,198,207). When cells are irradiated in frozen condition, there is an increase in decimal reduction (D_{10}) value, because of the protection afforded by the frozen water layer, markedly reducing the formation of free radicals (8,207,302). For the repair and growth of damaged cells a rich culture medium and a suboptimal incubation temperature have been found favourable (5,155,160,285,313,323,324,325). Other investigators (4) could not demonstrate this effect, but this may have been due to the small temperature range investigated.
Bacteria which suffer from metabolic damage caused by radiation treatment need a sub-optimal incubation temperature (3); bacteria with a more or less damaged mechanism of cell division require a poor culture medium and a normal or higher temperature during incubation (3).

Water content.

Water is the largest single constituent of bacteria and it is not surprising that it exerts a profound influence on radiation sensitivity. The effect is complex and involves interaction with oxygen.

Free radicals from intra-cellular water are mediators of radiation damage, so dehydration of the cell might be expected to increase its radiation resistance.

The radiation sensitivity of microorganisms decreases indeed at a lower water-activity (128,353). Hardly any data are available to demonstrate the effect of different a_w-values on radiation sensitivity of vegetative cells (158). Bacteria are most resistant when irradiated in dry condition (63,110).

Temperature plays an important role, too. At freezing temperatures when the amount of available water is markedly reduced the radiation resistance increases (128).

2.2. Relative radiation resistance of bacteria.

Bacterial cells of varying taxonomic position differ in their relative sensitivity to ionizing radiation (55,186,187,266,342,392). From the reported survival data it can be seen that the range of resistance is very large. The highest resistance values in vegetative microorganisms are found in cells of *Micrococcus radiodurans* (6,92,93). In general the most sensitive species are Gram-negative rods. Examples are the genera *Aeromonas*, *Bacteroides*, *Proteus*, *Pseudomonas*, *Serratia* and *Vibrio*. Higher D_{10}-values are found for the genera *Escherichia*, *Salmonella* and *Shigella*. The more resistant organisms among the Gram-negative genera are members of the psychrotrophic *Acinetobacter-Moraxella* group. The Gram-positive vegetative microorganisms represent a more diverse spectrum with respect to variations in radiation resistance. Some are relatively sensitive, others more resistant, e.g. *Micrococcus radiodurans* (84,225), *Streptococcus faecium* and *Clostridium botulinum* (table 4). Within the family of *Enterobacteriaceae* there are differences in radiation sensitivity. The differences between *Escherichia coli* and *Salmonella* are important, since the former organisms are often used as index organisms for salmonellae. Some *Salmonella* serotypes were demonstrated to be more resistant than *Escherichia coli* (114). Other investigators (95) found *Escherichia coli* more resistant than the *Salmonella* serotypes they investigated. As the reported data were not determined under essentially identical conditions, the different influence of environmental factors can explain the above mentioned discrepancies. Variation in sensitivity within the genus *Salmonella* is described in literature (199,204,276,346) (see also table 4).

However, when strains of Salmonella anatum, Salmonella heidelberg, Salmonella newport, Salmonella thompson and Salmonella typhimurium were irradiated in tryptone soya yeast extract broth and subsequently plated on non-selective agar media, no essential differences in D_{10}-values were found (199). Different strains of the same serotype may also differ in sensitivity (277,353). It is necessary to realize that the differences in sensitivity of certain serotypes can be a result of the differences in sensitivity of the used strains themselves.

D_{10}-values of microorganisms can be found in abundance in literature. It is understandable that most data are related to microorganisms which are of importance to food microbiology. So the majority of data are related to Salmonella and Clostridium botulinum. Table 4 lists D_{10}-values of some Gram-negative and Gram-positive microorganisms. The conditions of irradiation were not always identical. Nevertheless the data presented give an idea of the D_{10}-values one can encounter in actual practice.

2.3. Elimination of salmonellae from poultry carcasses by ionizing radiation

The initial contamination of broilers with salmonellae is not removed by the slaughtering procedure. On the contrary, due to cross-contamination during the slaughtering procedure carcasses can become more severely contaminated with Salmonella. The end product, kept at refrigeration or at freezing temperatures, will remain contaminated.

The major part of the Dutch broiler production is sold as frozen products. It has been shown that salmonellae can survive a storage period at low temperatures (111). Poultry products which are kept at refrigeration temperatures are also frequently found contamined with these bacteria (41,136,205,244,247,269,270,363).

By applying a radiation treatment to poultry carcasses, which are kept in refrigerated or frozen condition, a considerable reduction of the number of contaminated carcasses can be achieved. A dose of 6.00 kGy was found quite effective in this respect (237), but minor changes in odour, colour and flavour of the product were observed. These side effects could be eliminated by performing the radiation treatment when the products were frozen (49,67,140,383). Because of the absence of oxygen during radiation treatment of frozen products radical formation is inhibited or at least delayed. So no reactions causing the above mentioned unwanted changes of the product occur.

Table 4. D_{10}-values (kGy) of some bacteria in air

Bacterium	Medium	Temperature °C	D_{10}	Reference
Acinetobacter	PB	10	0.31	207
Acinetobacter	PB	-79	1.25	207
E. coli K12	PB	NS	0.13	352
E. coli	NB	room	0.23	114
E. coli	PB	10	0.21	207
E. coli	PB	-79	0.40	207
M. radiodurans	PB	room	2.10	225
Pseudomonas	PB	10	0.05	207
Pseudomonas	PB	-79	0.32	207
Pseudomonas	PB	NS	0.05	197
Salmonella	NB	room	0.39	114
S. gallinarum	PB	NS	0.13	194-195
S. gallinarum	PB	-15	0.21	194-195
S. paratyphi B	PB	NS	0.19	194-195
S. paratyphi B	PB	-15	0.49	194-195
S. senftenberg	PB	NS	0.13	194-195
S. senftenberg	PB	-15	0.30	194-195
S. senftenberg	PB	room	0.17-0.23	353
S. typhimurium	PB	NS	0.21	194-195
S. typhimurium	PB	-15	0.39	194-195
S. typhimurium	PB	0	0.12	85
Staph. aureus	NB	room	0.46	114
Str. faecalis	NB	room	0.62	114
Str. faecium	NB	room	0.90	114
Str. faecium	NB	-30	2.40	114
Str. faecium	BHI	10	0.92	207
Str. faecium	BHI	-79	3.10	207
Cl. botulinum	PB	0	1.57-2.60	7-382

PB = Phosphate Buffer; NB= Nutrient Broth; BHI= Brain Heart Infusion broth;
NS = Not Stated

However, higher radiation doses are required to achieve the same lethal effect than when the radiation treatment is performed at room temperature. Higher doses are needed because of the protection against ionizing radiation provided by the frozen water layer in the product (207). Sensitivity to irradiation of bacteria and especially of salmonellae is less when the treatment is performed under anaerobic conditions (207). In the irradiation of frozen carcasses anaerobic conditions prevail immediately after starting the treatment.

The difference in the resistance of microorganisms to ionizing radiation under anaerobic and aerobic conditions can be expressed in the ratio of the D_{10}-values under these conditions. Sensitive bacteria have a high value, for example a Pseudomonas species : 8.5; less sensitive microorganisms like Streptococcus faecalis possess a low value : 2.5. So the same lethal effect under anaerobic conditions can be achieved by increasing the dose approx. 2 to 10 times (207).

The composition of a food also influences radiation resistance. The presence of proteins and polysaccharides may provide protection (13). This may be the explanation for the higher D_{10}-values of pure cultures in foods than those obtained in buffer solution (table 4). Compared with those for egg products, there are not too many data on the influence of ionizing radiation on Salmonella in poultry products. Table 5 lists the D_{10}-values of salmonellae irradiated in poultry meat and poultry skin (161,199,200, 276). In table 6 D_{10}-values of some Salmonella serotypes in other foods are listed.

American and Canadian investigators (30,200) estimated that after irradiation with a dose of 3,00 kGy only 1 in 80,000 carcasses would be contaminated with Salmonella, when 25% of the broilers were contaminated and maximal 5 Salmonella-cells were present on a carcass. A quantitative investigation in The Netherlands showed that on an average less than 100 cells (colony forming units = cfu) per 1000 cm^2 of skin or per 500 ml of thaw water were present (246,260). Similar results were obtained later in a German study (306). From the D_{10}-values of some salmonellae and the percentage of broiler carcasses which are Salmonella-contaminated figures similar to those arrived at in the Canadian study can be derived for Dutch circumstances. Therefore, in this thesis experiments are described on the effects of ionizing radiation on salmonellae on naturally and artificially contaminated carcasses. The results of these experiments allow to estimate the radiation dose needed to guarantee Salmonella-"free" poultry products.

Table 5. D_{10}-values (kGy) of Salmonella in poultry meat or skin

Serotype	Substrate	Gaseous atmosphere	Temperature °C	D_{10}	References
anatum	meat	air	22	0.40	161
enteritidis	meat	air	22	0.37	161
give	meat	air	22	0.40	161
heidelberg	meat	air	0,22	0.40-0.69	161-199
heidelberg	skin	air	0	0.25	200
infantis	meat	air	22	0.30	161
newport	meat	air	0	0.52	199
newport	skin	air	0	0.18	200
oranienburg	meat	air	0,22	0.46-0.72	161-199
oranienburg	skin	air/anaer.	0	0.24-0.39	200
pullorum	meat	air	22	0.46	161
senftenberg	meat	air	22	0.30	161
thompson	meat	air	0	0.62	199
thompson	skin	air	0	0.25	200
typhimurium	meat	air	0,22,22,22	0.41-0.43-0.48-0.58	161-199
typhimurium	meat	air	4, 4	0.41-0.70	276
typhimurium	meat	air	-20,-20	0.73-0.99	276
typhimurium	skin	air/anaer.	0	0.20-0.31	200
worthington	meat	air	22	0.50	161

2.4. Influence of ionizing radiation on shelf life of poultry carcasses

When using radiation doses of 20 to 50 kGy it is possible to sterilize poultry carcasses (82), but this is accompanied by severe changes in quality characteristics such as odour, colour and flavour. More attention has therefore been paid to radurization (139) of carcasses by ionizing radiations. This is defined as the use of low doses of ionizing radiation with the aim to reduce the bacterial contamination in such an extent that shelf life is increased considerably, without affecting the sensoric properties of the product.

Table 6. D_{10}-values (kGy) of Salmonella in different food products

Serotype	Substrate	Temperature °C	D_{10}	Reference
bareilly	whole egg	-15	0.59	68
blockley	whole egg	-15	0.69	68
canada	whole egg	-15	0.60	68
enteritidis	whole egg	-15	0.53	68
gallinarum	whole egg	0,-15	0.43-0.57	195-196
give	whole egg	-15	0.77	68
heidelberg	whole egg	-15	0.73	68
indiana	whole egg	-15	0.68	68
infantis	whole egg	-15	0.56	68
kentucky	whole egg	-15	0.60	68
london	whole egg	-15	0.73	68
manhattan	whole egg	-15	0.76	68
meleagridis	horse meat	-15	0.93	195-196
montevideo	whole egg	-15	0.57	68
oranienburg	herring meal	-15	0.56	68
paratyphi B	crab meat	5	0.30	95
paratyphi B	horse meat	-15	1.07	195-196
pullorum	whole egg	-15	0.60	68
senftenberg	herring meal	22	1.92-2.00	353
senftenberg	whole egg	0,-15,-15	0.50-0.47-0.51	68-194-195
senftenberg	whole egg	1	0.10	227
tennessee	whole egg	-15	0.68	68
thompson	whole egg	-15	0.73	68
typhi	crab meat	5	0.48	95
typhimurium	whole egg	0,-15,-15	0.63-0.68-0.60	68-195
typhimurium	whole egg	1, 55	0.50-0.13	227
typhimurium	cangaroo meat	20,-15	0.56-0.93	196
typhimurium	horse meat	-15,-15	1.28-0.93	195-196
typhimurium	beef	20,-15	0.56-0.96	195-196
wichita	crab meat	5	0.87	95

Gaseous atmosphere: air

Refrigerated poultry products are generally stored and distributed at a temperature of +2°C to +4 °C. Depending of the mode of packaging the carcasses are packed individually or in bulk.

Shelf life of poultry products which are kept at refrigeration temperatures is determined by the initial bacterial load and by the storage temperature (109). In literature data on shelf life are given; 6 days at +4 °C, 8 days at +1°C and 10 days at -0°C, are mentioned (33,109,170). Criteria for shelf life are the development of off-odour or slime formation and discolouration. Total colony counts of the products at spoilage range from $10^{6.5}$ - 10^{8} per cm^2 (108,162).

An ionizing radiation treatment with doses up to 2.50 kGy is very suitable for application to poultry. The side-effects of this radiation treatment are low and these can be eliminated by irradiation under frozen condition. Literature on this point shows evidence for the development of a radiation odour in the raw product directly after the use of a radiation dose of 0.50 kGy. The flavour of the product was affected after the application of a dose of 2.50 kGy (291). There is enough evidence that irradiation with doses above 2.50 kGy causes changes in quality characteristics such as odour, colour and flavour (65,126,170). However, by cooking or roasting of the meat, these defects can be eliminated (65,170,291).

In general, when a dose of no more than 2.50 kGy is used, no changes can be perceived after cooking of the material involved; by doses up to 7.50 kGy the changes due to the use of ionizing radiation can also be diminished by applying drastically reduced temperatures (-18 °C) during the radiation treatment (67,140).

Microbiological shelf life of poultry products which are kept at refrigeration temperatures can be increased considerably by radiation treatment. The application of ionizing radiation to retard spoilage of poultry kept at refrigeration temperatures and thus increase shelf life, has been studied intensively. The total bacterial load is decreased to such an extent, that together with the low storage temperature an extension of shelf life is obtained.

Doses of 1.00 to 3.00 kGy appeared necessary to destroy the spoilage flora, consisting of bacteria like Acinetobacter, Flavobacterium and Pseudomonas (65,109,140,161,183,215,218,242,278,291). Sometimes higher doses are needed, depending on initial colony counts and the composition of the food or substrate from which the bacteria have to be eliminated (161,201,241, 275,342).

In table 7 data from different literature sources are summarized.

Table 7. Extension of shelf life of poultry carcasses by ionizing radiation

Dose (kGy)	Storage temperature °C	Extension of shelf life days	Reference
1.50	+ 5	6	292
1.50	+ 5	7.5	344
2.50	+ 5	15	344
2.50	+ 3	8	65
2.50	+ 1	14	65
2.80	+ 4.4	14	170
2.80	+ 1.6	21	170
4.00	+ 6	10	59
4.00	+ 4	5	215
4.00	+ 1	9	215
5.00	+ 5	14	161
5.00	+ 1.1	14	219
8.00	+ 2	40	163

This table gives only data concerning the extension of shelf life by radiation treatment and storage temperature. For the sake of completeness the use of antibiotics together with irradiation and reduced storage temperature (65,66,182,267,345) and the addition of adrenalin, mixtures of salts and polyphosphates together with radiation treatment and storage temperature should be mentioned as possible methods to extend shelf life of poultry carcasses (10).

The dose studied in this thesis to radicidate salmonellae (2.50 kGy) improves, as is shown in table 7, the shelf life of carcasses. When low doses of ionizing radiation are applied, the spoilage flora consisting of Acinetobacter, Flavobacterium, Pseudomonas and some other psychrotrophic Gram-negative and Gram-positive bacteria is reduced markedly (70,161,163, 345).

After a radiation treatment with a dose of 2.50 kGy Lactobacillus, Moraxella, Streptococcus, but also Acinetobacter were isolated (345). Radiation treatment thus causes a completely changed spoilage pattern, the spoilage association now consisting of yeasts, micrococci and other coccoid cells (59,70,71,163).

Yeasts become more important in this respect. After treatment with 5.00 kGy only isolates of yeasts and moulds were obtained (345). By applying ionizing radiation, which destroys a certain part of the natural microflora, it is possible that microorganisms will develop on the commodity, which have never been isolated from the same product before (61,70). The application of ionizing radiation might in that way present problems, because in theory, the possibility exists that, for example, pathogenic microorganisms will survive the treatment and, as a result of the absence of a competitive microflora develop freely. Examples are the isolation of a Moraxella and a Herellea vaginicola species (161), which had not previously been isolated. Although these are not pathogenic microorganisms, their occurrence indicates the potential risk of the use of ionizing radiation in this respect.

Therefore it is very important to know how certain microorganisms and especially pathogenic microorganisms, respond to treatment by ionizing radiation. Resistance to irradiation determines the surviving microflora on poultry carcasses.

CHAPTER 3
3. SCOPE OF THE INVESTIGATION

First of all it was investigated which was the best Salmonella isolation method for refrigerated, frozen and radiated poultry. Most of the experimental work was carried out on the basis of the recommendations given in the ISO document 3565/1975 on the isolation of salmonellae from meat and meat products. This method was also compared with several other Salmonella isolation methods, using broiler carcasses which had been either refrigerated, frozen and irradiated or had been treated by a combination of these processes.

In the first experiments skin samples of ducks and broilers as well as whole broiler carcasses were irradiated with a dose of 2.50 kGy. This radiation dose was chosen, because at that dose no organoleptical or toxicological changes could be detected in the products. The duck samples were examined because of the fact that, due to raising conditions for ducks, one may expect more salmonellae in these flocks than in broiler flocks. The samples were examined for the presence of salmonellae before and after treatment. Besides, the number of Enterobacteriaceae cfu was determined. The absence of these microorganisms is assumed to be an indication of good sanitary conditions. Traditionally, Escherichia coli has been the indicator microorganism for faecal contamination and therefore the index for pathogenic faecal organisms. The Enterobacteriaceae count indicates the presence of other, particularly also lactose negative pathogenic organisms and can therefore be used as group of index-organisms. Nowadays the term indicator organisms refers to those organisms demonstrating inadequate sanitary conditions during processing (397). In poultry, the ratio of the number of Enterobacteriaceae cfu to the number of cfu of salmonellae, the ε-factor (91), is estimated to be at least 1000 : 1. An Enterobacteriaceae cfu-count can therefore provide useful information in the examination of these products.

As the number of Salmonella cfu on commercially processed broiler carcasses was very low, it was obvious that for studies concerning the effects of ionizing radiation on salmonellae on broiler skin artificially contaminated carcasses had to be used. A simple and repeatable method for the artificial contamination of carcasses was hence elaborated. Three microorganisms were chosen for the experiments : an Escherichia coli strain and two Salmonella strains (serotypes niloese and panama). These strains were selected after completion of experiments in which D_{10}-values in liquid and solid culture media of a number of microorganisms associated with refrigerated or frozen broiler carcasses were determined. In these experiments the influence on the D_{10}-value of the cfu concentration and of the physiological age of the cells was also determined.

The results of experiments in which different pre-enrichment, enrichment and counting media were tested showed that the Escherichia coli strain was comparable in radiation resistance with salmonellae and therefore could be used in further experiments with artificially contaminated carcasses. D_{10}-values estimated with artificially contaminated carcasses showed that the skin afforded protection against Salmonella niloese and Salmonella panama. Higher D_{10}-values were estimated than for the Escherichia coli strain. This showed that these two microorganisms were not as similar as was assumed from the results of previous experiments with pure cultures in liquid and on solid culture media.

A study was made of the effect of a radiation dose of 2.50 kGy on the number of salmonellae on broiler carcasses. As in nearly all other experiments two radiation temperatures were used during the experiments : +5 °C and -18 °C.

As an extra dimension, a study on the influence of a combination of freezing and frozen storage was added to the experiment. In the first stage of the experiment carcasses which were kept at refrigeration temperatures were irradiated with doses of 0.50 kGy and 1.00 kGy; after this treatment the carcasses were deep-frozen at -18°C, followed by a radiation treatment of 2.00 kGy and 1.50 kGy respectively. The total dose applied was hence again 2.50 kGy. The effect was measured by examining the carcasses for the presence of salmonellae and counting the cfu of Enterobacteriaceae. In the second stage of the investigation broiler carcasses were irradiated with a dose of 2.50 kGy in refrigerated or deep-frozen condition, after which the carcasses were deep-frozen and stored at -18 °C.

During a four month period of frozen storage carcasses were examined for the presence of salmonellae and Enterobacteriaceae.
In the final part of the investigations the effect of increasing doses of ionizing radiation from 0 to 2.50 kGy with steps of 0.50 kGy on the total microflora of broiler carcasses was assessed, allowing an estimation of the shift in the composition of the microflora as caused by the radiation treatment. Counts were carried out on selective agar media and isolates from these media were biochemically characterized. In these experiments some agar media were surface treated with catalase, because there are indications that catalase favourably affects the recovery of stressed cells (206).

CHAPTER 4
4. MATERIALS AND METHODS

4.1. Radiation treatments

All treatments were carried out at the Proefbedrijf Voedselbestraling, Wageningen, The Netherlands. The source was Cobalt-60 with a flux of 1.50 kGy per hour. Doses of 0.50, 1.00, 1.50, 2.00 and 2.50 kGy were applied. The temperature during the experiments was +5 ± 1 ^{o}C and -18 $\pm 1^{o}$C.

4.2 Estimation of decimal reduction (D_{10}) values of bacteria in solid and liquid culture media

Bacteria.

Escherichia coli K12 lysogenic, and Escherichia coli K12 non-lysogenic, were kindly provided by Prof. P. Fredericq, University of Liege, Belgium. The Escherichia coli K12 resistant to 200 ppm nalidixic acid (NDA) was made available by Dr. P.A.M. Guinee, National Salmonella Centre, Bilthoven, The Netherlands.

The other strains used were from the culture collection of the microbiology laboratory of the Spelderholt Institute and had been isolated from poultry.

Method for the estimation of the D_{10}-values in solid culture media.

The bacteria were pre-grown at +30^{o}C for 20 hours in bottles containing 50 ml of Nutrient Broth (NB) or 50 ml of Minimal Medium (MM). Before starting the experiments this procedure was repeated twice.

From the 20-hours' cultures dilutions were made in peptone saline. Per dilution 1 ml was transferred to a petri dish, to which 15 ml of Nutrient Agar (NA) or 15 ml of Minimal Agar (MA) tempered at +45^{o}C (132) were added. After solidification of the agar, the dishes were packed in polystyrene boxes, transported to the radiation source and irradiated. After the radiation treatment the petri dishes were incubated at 37^{o}C and 22^{o}C (Pseudomonas testosteroni).

All tests were carried out in duplicate; the average values of the surviving colony forming units (cfu's) were used for the estimation of the D_{10}-values. The initial concentration in all experiments was approx.2.0 x 10^8 cfu per ml.

Method for the estimation of the D_{10}-values in liquid culture media.

Jars containing 50 ml NB or MM were inoculated with pure cultures. Incubation was at +30°C. Every 20 hours 1 ml of this medium was transferred to 50 ml fresh medium.

20-hours' cultures were used for the experiments, unless otherwise stated. The jars were packed in polystyrene boxes. After the radiation treatment the numbers of surviving cfu were estimated by plating in 15 ml NA and MA, after serial dilution. After 24-36 hours of incubation at +37°C and +22°C the colonies were counted. All tests were carried out in duplicate; the mean values of the number of surviving cfu were used for the estimation of D_{10}-values.

Method for estimating the effect on D_{10}-values of the medium in which the cells are grown before irradiation

This experiment was carried out with an Escherichia coli strain K12 resistant to 200 ppm NDA. Escherichia coli K12 was incubated in NB and in MM at 37°C for 20 hours during three days before the experiment. 1 ml of the 20 hours' culture was inoculated in 50 ml of fresh medium and incubated at 37°C during 1, 2, 3 and 9 hours. After incubation the cultures were packed in polystyrene boxes and transported to the radiation source. After the radiation treatment the numbers of surviving cfu were estimated by plating, after serial dilution in NA and in MA. After incubation at 37°C for 24-36 hours the colonies were counted.

Influence of the physiological age on D_{10}-values of Escherichia coli K12.

The above mentioned experiments were performed with 20-hours' cultures. According to the growth curves, which had been estimated in a preliminary experiment, the cells were then in the stationary phase of growth. In addition, experiments were carried out with 1, 2, 3 and 9 hours' cells. The cultures were then in their lag-phase and logarithmic growth phase respectively.

The influence of the physiological age on D_{10}-values was estimated from the results of the experiments with 1, 2, 3 and 9 hours' cells.

Effect of the use of a pre-enrichment medium on D_{10} -values.

After a radiation treatment in NB and in MM counts were performed:

a. after incubation at 30°C for 6 hours in lactose broth (LB)(OXOID)
b. after incubation at 30°C for 6 hours in NB (OXOID)
c. immediately in the agar media used, NA and MA

Incubation was at 37 °C during 24-36 hours.

A second experiment was carried out in the following way.

Counts were performed :

a. after incubation at 7°C for 2 and 19 hours in buffered peptone water (BP)
b. after incubation at 7°C for 2 and 19 hours in LB
c. after incubation at 7°C for 2 and 19 hours in NB
d. immediately in the agar medium used, Plate Count Agar (PCA)(OXOID)

Incubation was at 37 °C during 24-36 hours.

Influence of the initial cell concentration on D_{10}-values.

20-hours' cultures of Escherichia coli K12 were used. Before starting the experiments the bacteria were subcultured three times for 20 hours in 50 ml NB at +30°C. After serial tenfold dilution in physiological saline 1 ml of a dilution was transferred to 50 ml fresh medium (NB) three times and subsequently packed in polystyrene boxes and transported to the radiation source.

After irradiation the numbers of surviving cfu were determined in Violet Red Bile Glucose Agar (VRBG)(OXOID) to which 200 ppm nalidixic acid (NDA) (Sterling Winthrop, Haarlem, The Netherlands) was added. Incubation was at 37 °C during 24 hours. The experiment was carried out in duplicate. For enrichment of surviving Escherichia coli K12 cells 3 x 1 ml inoculated medium was transferred to 3 tubes containing 9 ml of Enterobacteriaceae Enrichment broth (EE)(OXOID) each. After incubation at 37°C for 24 hours subcultures were made on VRBG and incubated at 37°C for 24 hours. Positive or negative results were recorded.

Influence of the presence of oxygen on the D_{10}-values.
A single experiment was carried out for this purpose. Sterilized plastic bags containing cultures of Escherichia coli K12 in NB and in MM were flushed several times with nitrogen gas, sealed and irradiated. The D_{10}-values resulting from this experiment were compared with the D_{10}-values obtained with Escherichia coli cultures which were not given a nitrogen gas treatment.

Effect of transportation to the radiation source on the oxygen concentration in the liquid culture medium.
An oxygen electrode (Radiometer, Copenhagen, PH M 71 MK2) was connected with the surface of the liquid culture medium in which Escherichia coli K12 was present. Transportation to the radiation source was simulated by shaking vigorously in a water bath. The amount of oxygen dissolved in the water was measured, as was the time needed for bacteria to consume this oxygen; bacteria of a different physiological age (lag-phase, logarithmic phase and stationary phase) were used for these experiments.

Mathematical treatment of the results.
In all cases the survivor curve, the relation between the logarithm of the number of surviving cfu and the radiation dosis, was assumed to be a straight line. Regression lines were fitted. From the regression coefficients the D_{10}-values were estimated.
Equality of D_{10}-values implies that the corresponding regression coefficients are equal. Hence, in each experiment the equality of D_{10}-values is estimated by testing whether or not the regression coefficients are equal (318).

4.3. Estimation of D_{10}-values on artificially contaminated skin samples or broiler carcasses.

Skin samples, broiler carcasses.
Skin samples were dissected from the carcasses immediately after the evisceration process by means of sterile instruments. The number of samples used in each experiment is indicated in the tables.
The broiler carcasses used in these experiments were scalded at 60 ^{o}C, machine plucked and eviscerated in the usual way. The weight of the broilers was 750-1250 grams.

The epidermis was removed from the carcasses during the slaughtering procedure. In one experiment broiler carcasses were used which were slaughtered in the pilot plant slaughter-house of the Spelderholt Institute, where a combined scalding and plucking process is in use. For each experiment 5 refrigerated or 5 deep-frozen, artificially contaminated carcasses were used per radiation dose.

Bacteria.

Experiments were carried out with a strain of Escherichia coli K12 resistant to 200 ppm NDA and with two Salmonella strains, Salmonella niloese and Salmonella panama. These strains had been isolated from poultry products.

Inoculation technique.

The bacteria used were grown at 37°C for 20 hours in a small quantity of Brain Heart Infusion broth (BHI)(OXOID), which was subsequently added to an inoculation bath containing 25 litres BHI. The bacteria used were in their stationary phase of growth. The inoculation bath was a plastic vessel which was cleaned and disinfected thoroughly before the experiments. The disinfectant used was Tego 51/15DL (Goldschmidt N.V. Amsterdam, The Netherlands).

Carcasses were immersed in the bath for 20 minutes. The temperature of the liquid was 20°C, the pH 6.5. Subsequently the carcasses were hung by their wings for a two minute drainage period. This was followed by packaging in plastic bags and deep-freezing at -40°C for 6 hours, or air-cooling (air temperature 0°C) during 2 hours. In the latter case the final temperature of the carcasses was +5 °C, in case the carcasses were deep-frozen the storage temperature was -18 °C, applied overnight before the radiation treatment. The refrigerated carcasses were transported in polystyrene boxes to the radiation source without delay. The skin samples were treated in the same way.

Irradiation of broiler carcasses under nitrogen conditions.

The packaged carcasses were evacuated and flushed with nitrogen gas, after which the plastic bags were sealed again. The carcasses were frozen at -18 °C and irradiated at the same temperature.

Microbiological examination.

For the experiments with deep-frozen broiler carcasses all carcasses were thawed at +7 °C for 24 hours under the standardized conditions routinely used at the Spelderholt Institute. The individual carcasses were placed, in opened packages, at a distance of 15 cm from each other. The air temperature during thawing was +7°C. Skin samples of about 100 cm^2 (approx. 10 grams) from the breast area of the refrigerated or thawed carcasses were used to determine the number of cfu's by a maceration method using the Colworth Stomacher. For this purpose the samples were homogenized after dilution 1:9 in peptone saline during 1 minute.

For the determination of the numbers of cfu's on the whole carcass a rinsing method was used. The numbers of cfu's in the thaw water and in the inoculation bath were estimated by conventional methods. Skin samples were also homogenized by maceration in a Stomacher.

Salmonellae were estimated using the method recommended in ISO-document 3565-1975. This method was chosen, because of experience obtained during several years in the routine examination of carcasses for salmonellae. However, as can be concluded from the data presented in Chapter 5 more positive carcasses could have been obtained with other methods.

The samples were pre-enriched in 100 ml BP at 37°C for 18 hours. 10 ml of this culture were transferred to 100 ml tetrathionate brilliant green broth according to Mueller and Kauffmann (TBB).

Incubation took place at 43°C during 24 hours. After incubation subcultures were made on brilliant green phenol red agar (BG)(OXOID) incubated at 37 °C for 24 hours. Suspect colonies were purified on MacConkey nr. 3 agar (MC)(OXOID). After testing on TSI agar (OXOID), for lysine decarboxylase and phenylalanine deaminase activity the cultures were examined with polyvalent Salmonella-serum. Final serological identification was carried out by the National Salmonella Centre at Bilthoven, The Netherlands. In addition a direct enumeration of Salmonella cfu on BG, using spread plates, was carried out.

Escherichia coli K12 counts were made in VRBG to which 200 ppm NDA was added. Incubation was at 37°C for 18-24 hours. Escherichia coli K12 was also determined with EE broth incubated at 37°C for 24 hours 100 cm^2, approx. 10 grams of sample being added to 90 ml broth, followed by streaking on MC for further examination of suspect colonies.

Mathematical treatment of results.

The regression of the logarithm of the counts with the dose was estimated for the counts on skin, in rinsing water and in thaw water after thawing. From the regression coefficients the D_{10}-values were estimated.

4.4. Estimation of the effect of a dose of 2.50 kGy on salmonellae and Enterobacteriaceae on poultry carcasses.

Estimation of the effect on duck and broiler skin samples and on whole broiler carcasses.

In a slaughtering plant for ducks 100 samples of neck skin were dissected from the carcasses before the chilling procedure and 100 from neck skin samples after immersion chilling. All samples were cut into equal parts of approx. 50 cm^2(approx. 5 grams) each and packed individually in plastic bags. One half of the total sample material was treated with 2.50 kGy and the other half was used as a control.

The samples of broiler skin were taken in 3 different slaughter-houses. In the first experiment the scalding temperature applied was 60-61°C as with the experiment with the duck skin samples.

The epidermis was removed during the plucking process. In the other experiments samples were taken from broilers which were slaughtered in a plant where the scalding temperature was 52°C and the epidermis was not removed from the whole carcass.

100 samples in each experiment were dissected from the carcass after the evisceration procedure, and were cut in two equal parts and treated as described above.

500 broiler carcasses were collected in 8 different poultry slaughtering plants. The carcasses were deep-frozen at -18°C. Half of these were treated with 2.50 kGy; the other half served as control.

Before and after the radiation treatment skin samples were examined for salmonellae. After the thawing procedure (at +7 °C for 24 hours under standardized conditions) thaw water and skin samples from the neck skin area were collected and examined for the presence of salmonellae and the number of cfu of Enterobacteriaceae.

Microbiological examinations.

Salmonellae were estimated as described in 4.3.

Counts of Enterobacteriaceae cfu were made after homogenization of 100 cm^2 (approx. 10 grams) of the skin samples diluted in peptone saline by maceration during 1 minute in a Stomacher. The thaw water obtained after thawing was examined by conventional methods. Poured plates of VRBG with a 10 ml overlayer were used. Typical colonies were counted after incubation at 37 °C for 18-24 hours.

Quantitative estimation of the effect on salmonellae on broiler carcasses.

Materials.

240 refrigerated broiler carcasses were used for these experiments. The scalding temperature was 52 °C, the carcasses were air-cooled at a temperature of 0°C during 60 minutes. In addition 140 frozen carcasses were used, which were scalded at 60°C, immersion cooled in water, packed and deep-frozen at -40 °C for 20 hours.

In the first three experiments refrigerated carcasses from three different commercial slaughter-houses were used. In each experiment 80 carcasses divided into 16 series of 5 were used. The number of cfu of salmonellae present was estimated per series before and after the radiation treatment. For this purpose skin samples were taken. From each series of 5 carcasses approx. 30 cm^2 of neck skin, 30 cm^2 of peri-cloacal skin and 30 cm^2 of lateral skin were taken (approx. 9 grams in total).

After irradiation, corresponding samples were taken from the same series of broilers. The number of cfu of salmonellae was estimated with a Most Probable Number-(MPN) technique. The skin samples were homogenized for 1 minute in BP (four times the volume/weight) using a Waring blender. Four fold portions of 50 ml, 5 ml and 0.5 ml were examined. The experiments with deep-frozen carcasses, obtained from one single processing plant, were carried out in the same way. After the radiation treatment the carcasses were thawed at +7°C for 20 hours. Skin samples were dissected from the carcasses and examined before freezing and irradiation and after irradiation and thawing. Sampling of the skin was carried out as previously described.

Salmonella isolation method.

The method for Salmonella-isolation was based on the recommendations given in ISO document 3565/1975: The isolation of salmonellae from meat and meat products.

In using this method 50 ml BP was added to 50 ml and 5 ml skin macerate; 10 ml BP was added to each of the 0.5 ml skin samples. After incubation at 37 °C for 20 hours one-tenth of each volume of culture was transferred to a tenfold volume of TBB and incubated at 43°C. After incubation for 24 and 48 hours these cultures were streaked on BG plates. The plates were incubated at 37°C for 24 hours. Suspect colonies were examined biochemically and serologically, as described earlier

Estimation of the combined effect of irradiation at +5°C, with 2.50 kGy, deep-freezing and irradiation at -18°C on salmonellae and Enterobacteriaceae on broiler carcasses (Figure 5).

Materials.

40 broiler carcasses were used for this experiment. During the radiation treatment the temperature of the carcasses was either +5°C or - 18°C, freezing was done at -40 ± 1°C for 6 hours and the carcasses were kept at -18 °C overnight. After thawing, skin and thaw water were collected for examination for salmonellae and assessment of Enterobacteriaceae cfu. Counts of Enterobacteriaceae cfu were made after homogenizing the samples for 1 minute in a Waring blender with peptone saline. The thaw water was examined by conventional methods.

Culture methods.

BP was used as a pre-enrichment medium for salmonellae: incubation was at 37 °C for 18 hours. The enrichment medium was TBB. After incubation at 37°C for 24 hours the enrichment culture was streaked onto BG. The plates were incubated at 37°C for 24 hours. Suspect colonies were cultured and examined as described previously. Enterobacteriaceae cfu's were estimated in VRBG using layered poured plates incubated at 37 °C for 18-24 hours.

Estimation of the effect of subsequent storage at -18°C on the combined effect of irradiation at +5°C, deep-freezing and irradiation at -18°C on salmonellae and Enterobacteriaceae on broiler carcasses (total dose applied 2.50 kGy)(Figure 6).

Materials.

186 broiler carcasses divided into 4 groups were used in this experiment.

Figure 5. Estimation of the combined effect of irradiation at +5°C, deep-freezing and irradation at -18°C on salmonellae and Enterobacteriaceae on broiler carcasses

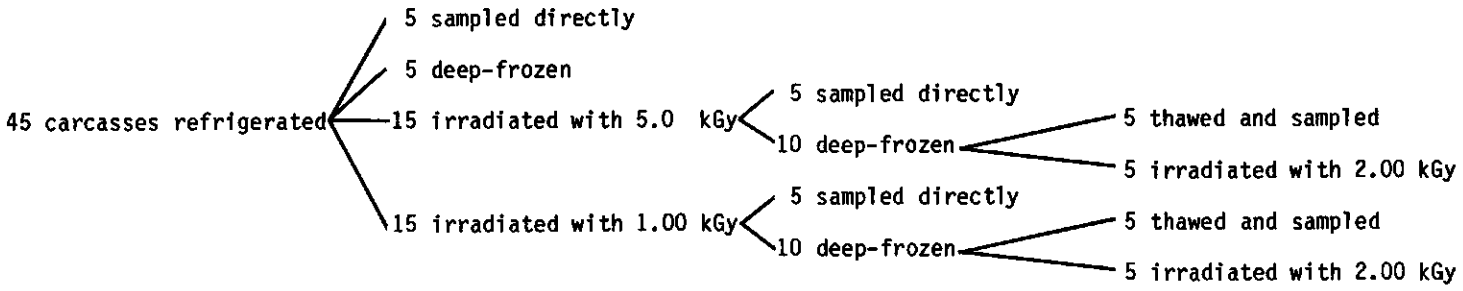
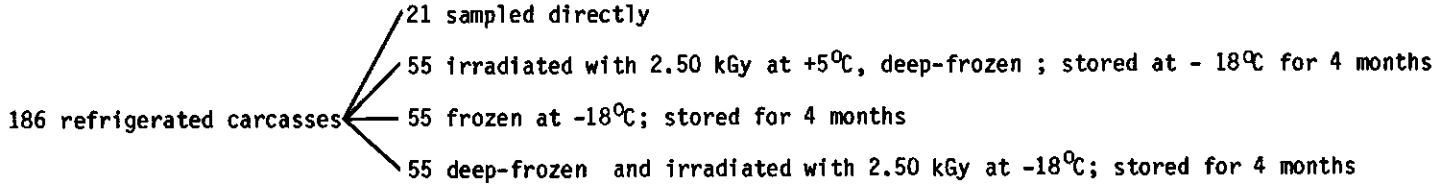


Figure 6. Estimation of the effect of additional storage at -18°C on the combined effect of irradiation at +5°C, deep-freezing and irradiation at -18°C on salmonellae and Enterobacteriaceae on broiler carcasses



Carcasses were sampled after 0, 1, 2, 3 and 4 months of storage.

a. 21 carcasses were examined after the cooling chain; the temperature of the carcasses was approx. +5 ^{o}C
b. 55 carcasses were deep-frozen and stored at -18 ^{o}C
c. 55 carcasses were irradiated with a dose of 2.50 kGy and then deep-frozen and stored at -18 ^{o}C
d. 55 carcasses were deep-frozen and then irradiated with a dose of 2.50 kGy and stored at -18 ^{o}C.

Skin samples from the neck, breast and cloaca area of 5 carcasses (approx. 100 cm^2 or 10 grams per carcass) were homogenized in a Stomacher with peptone saline (1:9) for 1 minute and 10 ml of this homogenate was used for further examination.

One carcass was examined separately, in an attempt to assess the effect of deep-freezing and irradiation and irradiation and deep-freezing by the examination of one carcass instead of the examination of 5 carcasses. The same procedure was followed in the sampling of the thaw water released after thawing of the carcasses. Mixed samples from 5 carcasses and samples from one carcass were used. Monthly 11 carcasses were examined.

Culture media. Salmonella isolation methods.

Skin and thaw water samples were resuscitated and pre-enriched (1:9) respectively in 90 ml tryptone soya broth (TSB) at 37 ^{o}C for 2 hours and in BP at 37 ^{o}C for 18-24 hours. Subsequently 10 ml of broth were transferred to 2 bottles containing 90 ml TBB and to 2 bottles containing 90 ml selenite cystine broth (SCB) according to Stokes and Osborne (327). Both enrichment media were incubated at 37 ^{o}C and 43 ^{o}C for 24 and 48 hours.

Subcultures were made on the following media:

a. BG (OXOID)
b. BG + 100 ml/l sulphadiazine + 2.5 g/l sodium deoxycholate (BGSD)
c. Sulphapyridin brilliant green agar (SBG)(Difco)
d. Xylose lysine deoxycholate agar (XLD)(OXOID)
e. Hektoen Enteric agar (HE)(OXOID)

Incubation was at 37 ^{o}C for 18-24 hours. Suspect colonies were subcultured onto MC and examined biochemically and serologically as described earlier.

Enterobacteriaceae cfu's were estimated in VRBG by using serial dilutions of the homogenate mentioned before. Layered poured plates were made and incubated at 37 ^{o}C for 18-24 hours.

4.5. Assessment of the surviving microflora on broiler carcasses after different radiation doses (0 -2.50 kGy, with steps of 0.50 kGy).

Materials.

Four experiments were carried out to determine the effect of given doses of ionizing radiation on the microflora of broiler carcasses. In the experiments no. 1 to no. 3, 60 carcasses (10 carcasses per dose), 30 carcasses (5 carcasses per dose) and 25 carcasses (10 carcasses were used as untreated reference material and 5 carcasses per radiation dose), respectively, were used in deep-frozen condition. The temperature during irradiation was -18 °C. After thawing the carcass, counts were made of Stomacher homogenates of skin samples using peptone saline as a diluent. The same procedure was followed with thaw water, if it was available, but then no initial dilution was carried out.

In the 4th experiment 25 carcasses kept at +5°C (10 carcasses were used as untreated reference material and 5 carcasses per radiation dose) were used. The radiation temperature was +5°C. Skin samples were treated as described above.

Methods of enumeration.

In experiments 1 and 2 serial tenfold dilutions of the homogenates were made and used to determine the number of cfu of specific groups of bacteria, applying the following media:

a. "total" count : poured plates, Plate Count agar (PCA)(OXOID) incubated at 30°C for 48 hours;
b. "total" psychrotrophic count : poured plates, PCA incubated at 10°C for 120 hours;
c. Enterobacteriaceae : layered poured plates, VRBG incubated at 37°C for 18-24 hours;
d. Escherichia coli : spread plates of MC incubated at 37°C for 24 hours. Typical lactose positive isolates were confirmed by studying lactose dissimilation and formation of indole at 44°C and failure to assimilate citrate.
e. Faecal streptococci : spread plates of Kanamycin Aesculin Azide agar (234,235,411)(KAA)(OXOID) incubated at 37°C for 30 hours;
f. Micrococci : spread plates of Mannitol Salt agar (MSA)(OXOID) incubated at 30°C for 48 hours;

g. Staphylococcus aureus : spread plates of Baird Parker agar (BPA)(OXOID) incubated at 37°C for 48 hours. Isolates were confirmed by DNA'se and coagulase tests

h. Bacillus cereus : spread plates of Mannitol Egg Yolk Polymyxine agar (410)(MEP)(Merck) incubated at 30°C for 36 hours;

i. Yeasts and moulds : spread plates of Oxytetracyclin Yeast Extract Dextrose agar (OYDA)(233,412) incubated at 25°C for 48 hours.

The following enrichment procedures were used :

j. Salmonella : BP at 37°C for 18-24 hours, TBB at 43°C for 24 hours, streaking on BG and incubating at 37°C for 24 hours;

k. Enterobacteriaceae : EE broth at 37°C for 24 hours, streaking on VRBG and incubating at 37°C for 24 hours;

l. Micrococci and staphylococci : Giolitti and Cantoni broth (233) incubated at 30°C for 24 hours, streaking blackened enrichment cultures on MSA and incubating at 30°C for 48 hours;

m. Faecal streptococci : KAA broth (411) at 37°C for 24 hours, streaking on KAA agar and incubating at 37°C for 30 hours.

The agar media which were used as spread plates have also been tested with the addition of catalase to the surface (117,206). The catalase (Sigma, Heidelberg, Western-Germany) concentration was approx. 780 units.

The following media were used in the experiments 3 and 4 :

1. Enterobacteriaceae :
   a. layered poured plates of VRBG incubated at 37°C for 18-24 hours;
   b. spread plates of VRBG with addition of catalase to the surface as described above;
   c. after 6 hours of resuscitation on 5 ml Trypticase Soy Agar (TSA) at 25°C ("solid medium repair") a top layer of VRBG was poured over the agar. The plates were subsequently incubated at 37°C for 18-24 hours (282).
2. Escherichia coli : the same procedure 1 a-c was followed, except that the agar medium was MC and incubation was at 44°C for 24 hours.
3. Faecal streptococci :
   a. spread plates of KAA agar were incubated at 37°C for 30 hours;
   b. spread plates of KAA agar with the addition of catalase to the surface were incubated in the same way;
   c. samples were incubated at 25°C for 2 hours in peptone water (Pw) and then plated on KAA agar and incubated at 37°C for 30 hours.

4. Staphylococcus aureus :
   a. spread plates of BPA were incubated at 37°C for 24-48 hours;
   b. the same agar medium was used after a two hour resuscitation treatment of the sample material in Pw at 25°C.
5 Salmonella : the method used was based on the recommendations in ISO document 3565/1975 : BP at 37°C for 18-24 hours, TBB at 43°C for 24 hours, streaking on BG and incubating at 37°C for 24 hours.

From enumeration plates showing 20 to 200 colonies a number almost equal to the square root of the total number of colonies were picked at random for tentative taxonomic grouping of the surviving microflora.
A rapid screening test was carried out by using Gram-negative and Gram-positive diagnostic tubes as described in the literature (236). The Gram-negative diagnostic tube consists of a butt of VRBG with a top-layer of motility test agar and hence permits direct reading of resistance to crystal violet plus bile salts, mode of attack on glucose, formation of pigments, motility and oxydase reaction. Gram-positive diagnostic tubes consist of a butt of Mannitol Purple agar with a covering layer of Tryptone Soya peptone agar and permit a direct reading of oxygen tolerance, mode of attack of mannitol and catalase reaction. Further identification was carried out using conventional biochemical tests and novel techniques, such as API and Enterotube.

4.6. Estimation of shelf life at +7°C of thawed carcasses after a radiation treatment with 2.50 kGy.

Two experiments were carried out in which a total of 100 broiler carcasses were irradiated at -18 °C with a dose of 2.50 kGy; 25 carcasses per experiment were left untreated. The carcasses were collected in a commercial slaughter-house, using a scalding temperature of 60°C for 100 seconds, water cooling and deep-freezing. After thawing the carcasses at +7 °C for 24 hours shelf life was estimated after storage at +7°C for several days. Five carcasses were sampled daily. The following microbiological tests were performed with the skin samples:

a. "total" counts in poured plates of PCA, incubated at 30°C and 10°C
b. the number of Enterobacteriaceae: cfu in VRBG
c. the number of faecal streptococci: cfu on KAA agar
d. the number of Staphylococcus aureus: cfu on BPA
e. the number of yeasts and moulds: cfu on OYDA

f. the number of Clostridium perfringens cfu was counted in sulphite iron cycloserine agar (233,414); incubation at 46°C for 24 hours.

The samples were enriched and subcultured for an estimation of Salmonella. Details of the method used were described earlier.

4.7. Assessment of required lethality.

Lethality or reduction factor refers to the number of microorganisms destroyed after a certain radiation treatment. It is defined as (413)

$$\Lambda = N_0/N_f \qquad \text{in which}$$

N_0 is number of cfu of microorganisms before irradiation and N_f is number of cfu of microorganisms after irradiation.

Lethality (Λ) of radiation treatment is expressed as $\Lambda = n.D_{10}$ or, in cases where survivor curves show a "shoulder" and/or a "tail", as $(MPED)_n$ (230).

One approach to assess the required lethality of the radiation treatment is to accept a lethality of 7, meaning a reduction of seven log cycles. This reduction factor is used, for example, in the pasteurization of eggs and milk products. The dose (kGy) required would then be $7.D_{10}$.

Another possible approach is to fix on a certain N_f in the end product, i.e. the "absence" of salmonellae, and to estimate the lethality or reduction factor using known N_0 's.

Both approaches will be dealt with.

4.7.1. Effect of different parameters on lethality of irradiation with a dose of 2.50 kGy.

Different parameters have exert a distinct effect on the lethality of irradiation, with a fixed dose of 2.50 kGy, with respect to Salmonella initially present on broiler carcasses.

Parameters in case of Salmonella radicidation of broiler carcasses are:

1. the biotype
2. the serotype
3. the number of cfu's of Salmonella initially present
4. the distribution (heterogeneous, homogeneous or in clumps) of cfu's on carcasses
5. the physiological condition of the cells
6. the attachment to, or detachment of, salmonellae from carcasses

7. the competition with other microorganisms during attachment or detachment
8. the effect of radiation media and -temperature
9. the effect of oxygen partial pressure
10. the lethal effect of irradiation as such
11. the effect of sampling techniques
12. the effect of Salmonella-isolation methods
13. the effect of MPN or colony count estimates
14. the effect of deep-frozen storage after irradiation

The effect of some of these factors was studied in more detail, as they could be expected to influence lethality more substantially than others. For instance, the effect of initially present numbers of Salmonella cfu's (N_0) on Λ was only studied grossly, as a generally valid first approximation is that Λ does not depend on N_0, but only on radiation dose under, for the rest, standardized intrinsic and extrinsic conditions.

CHAPTER 5
5. EVALUATION OF METHODOLOGY

5.1. Sampling methods for microbiological examination of broiler carcasses

Various methods are used for the determination of the microbiological condition of broiler carcasses or parts. The sampling site on the carcass is very important in attempts to assess hygiene during slaughtering, to estimate shelf life of the product and to examine the slaughtered products for the presence of pathogenic microorganisms. For this purpose broiler skin and its connective tissue have to be examined in the first place. In healthy animals the deeper muscle tissues are sterile, as shown by the observation that bacterial growth in the meat could not be detected when carcasses were stored at -10 °C to +15 °C during a longer period of time (34,35,36).

Experiments in which the attachment of bacteria to broiler skin was studied indicate (254,255) that the skin of the carcass and not the meat should be sampled. More flagellated bacteria than non-flagellated strains were found attached to the skin or in the surrounding water film. However, attachment to skin is not only due to the presence of flagella on bacteria (216).

The methods to estimate the microbiological condition of the carcasses can be divided into two groups:

a. non-destructive methods
b. destructive methods

The advantage of the use of non-destructive methods, irrespective of reliability, is that the material to be examined remains intact. Destructive methods, especially when poultry carcasses are examined, result in a downgrading of the examined product. Altogether over fifty non-destructive methods have been described in literature (32,37,263, 264,340).

The methods used for the examination of poultry carcasses vary from the sampling of a small piece of surface skin, by swabbing, rinsing or maceration, to the sampling of a considerable part or even a whole carcass by swabbing, rinsing or maceration.

A comparative study of several methods (119) resulted in the use of the alginate swab method. This method for the examination of poultry carcasses (229) was found to give counts about 10 times higher than contact plate methods (238).

Recently it has been observed (256) that no differences in counts of cfu were obtained when cotton instead of alginate swabs were used.

Other investigators completely rely on skin-maceration methods (28,29,75, 241,257,263,366). With this method counts are often at least 10 times higher than with any other method (29,119).

The most severely contaminated area of the broiler carcass appeared to be the neck skin (257,263,364). Examination of a piece of neck skin is therefore recommended. Another reason for taking neck skin is the relatively undamaged condition of the carcass after sampling.

There are also some literature references (193,312) in favour of rinsing parts of the whole carcass with a diluent. Generally, however, higher counts have been found with a destructive method, than with swab and rinsing techniques (74,75,119,312). A publication in which scanning electron microscopy photographs of broiler skin were shown (216) explains the increased recovery of microorganisms by destructive as compared with non-destructive sampling methods. The surface of the broiler skin consists of crevices and channels of capillary size and once contaminated it is extremely difficult to remove the microorganisms.

A comparison of the merits of the applied methods is difficult, because the bacterial counts are expressed as cfu per gram, per cm^2 or per ml. Conversion formulae have therefore been used, like : Surface = Weight + 500 (cm^2).

Different formulae (312, 365) for the conversion of counts expressed as cfu per gram to cfu per cm^2, by using a formula for the specific weight of a certain piece of skin (35,312,365) are in use. Even when using such conversion formulae it has not been possible to appraise the value of the results of comparative experiments with skin maceration methods, rinsing or swab techniques (241,257.364).

If one is only interested in the contamination of the slaughtered end product other methods could also be applied to examine carcasses for the

presence of pathogenic microorganisms (216,254,258,364). An examination of the thaw water obtained by hand pressing the carcass or rinsing of the whole carcass can be carried out (75,77,193,239,258). The "built up" of bacteria during the whole slaughtering process can not be measured by these sampling methods.
For practical reasons, especially when large carcasses, for example turkeys, are to be examined, a skin maceration method is to be preferred (26). The experiments to be described in this thesis were therefore performed with a skin maceration method.

5.2. Salmonella isolation methods for broiler carcasses

Several sequential steps in Salmonella analysis can be distinguished i.e. non-selective resuscitation and pre-enrichment, enrichment, plating on selective agars, biochemical and serological tests. Good review articles on this subject can be found in literature (116,144). In this paragraph only the main principles of suitable cultural methods are described.

Resuscitation and pre-enrichment methods.

Resuscitation and pre-enrichment is necessary when salmonellae have to be detected which are injured by a process such as drying, freezing or heating. When it is expected that low levels of contamination are present, for example when examining poultry products, a pre-enrichment step is becoming quite common. The best known resuscitation and pre-enrichment media are tryptone soya broth (TSB), buffered peptone water (BP), lactose broth (LB) and nutrient broth (NB). These media contain compounds like peptones and phosphate which favour recovery of damaged salmonellae (96,249,250). For Escherichia coli cells which were sublethally damaged by freezing to different degrees (274,284, 360) a minimal time of 1 to 2 hours of resuscitation (356) was needed. A two hour resuscitation at a suboptimal incubation temperature is recommended for slightly damaged cells and a 18 hour incubation period for severely damaged cells (356). In literature some authors (232,283,356) found 1 to 2 hours to suffice; others (80,313,351,358,361) proved that even 6 hours of resuscitation at room temperature were not sufficient under certain conditions.
The difference in required resuscitation time, reported by the different authors is not only caused by differences in stress but particularly also by the composition of the selective culture medium, ultimately used for enumerating bacteria (283).

It is worth noting that by pre-enrichment it is attempted to grow salmonellae or other microorganisms which are present in very low numbers (1 to 100 or 1000) compared to other naturally occurring microorganisms. Also stressed cells may repair and grow during pre-enrichment. Raw meat, poultry meat and unpasteurized egg products generally do not need a pre-enrichment phase in the Salmonella-isolation procedure (338). The contamination level is relatively high and no stressed cells are expected to occur. During preenrichment of these products growth of other micro-organisms present may interfere with isolation procedures for salmonellae. Dried, frozen or heat-treated products wherein the competitive microflora is partly destroyed and surviving cfu may be damaged, need a non-selective pre-enrichment step for the detection of Enterobacteriaceae, Escherichia coli and Salmonella (121,232,308,356,358,359,360). A certain number of competing microorganisms developing during pre-enrichment is considered necessary to eliminate some toxic substances in particular selective enrichment media (356).

Selective enrichment media.

Selective enrichment media for salmonellae should stimulate growth and multiplication of Salmonella and restrict the accompanying Gram-negative microorganisms present, like Citrobacter, Proteus and Pseudomonas. As a rule the Gram-positive microorganisms are completely inhibited by the composition of the media.

In literature the superiority of certain media compared to others is often claimed. As the effectiveness of an enrichment medium is dependent upon the substrate in which salmonellae are to be detected, the various most popular media will be reviewed.

The media most frequently described in literature are tetrathionate brilliant green broth and selenite media (177,192), from which selenite cystine broth, selenite cystine mannitol broth and selenite brilliant green broth were derived (96,138,253,261,327). In a later stage Rappaport's medium on the basis of magnesium-chloride (280), Gram-negative broth (138), strontium media (139) and media containing lysine, an iron salt and cystine (LIC)(141) were added to the list. In the latter, particularly Salmonella negative enrichments give a very specific reaction. As the selectivity of most media was often insufficient, further anti-microbial compounds were added; examples are tetrathionate broth with the addition of 40 micrograms/liter Novobiocin (45) and selenite brilliant green sulpha medium (253).

Temperature and time of incubation of selective enrichment media.

In 1953 (142) it was demonstrated that incubation at 43 °C gave better results than incubation at 37°C. However, as indicated previously not all Salmonella-serotypes grow at, or even survive exposure to, a temperature of 43°C in all selective enrichment media. Tetrathionate at 43°C proved lethal for some salmonellae, while in the same studies selenite broth also showed a lower isolation rate at 43 °C than at 37°C (209). Nonetheless, the incubation temperature of 43°C generally leads to more positive results (309). This is due to better selectivity at the increased temperature. Also the presence of a competitive microflora can decrease the toxic effect of tetrathionate broth on some salmonellae at increased temperature (356). Recommended incubation times vary from 16 to 96 hours. More salmonellae are generally isolated when the samples were incubated for 24 and 72 hours or for 24 and 48 hours (62,125). In the recommendations given in the ISO document 3565/1975 for the isolation of salmonellae from meat and meat products (20) it is suggested to incubate at 37°C and at 43°C for 24 and 48 hours. These recommendations were based on a collaborative trial using naturally contaminated minced meat as inoculum (97). The second subculture i.e. after 48 hours of incubation, was especially productive when competitive microorganisms presented problems.

Nowadays, based on comparative studies, it is accepted that a particular modification of Rappaport's medium is superior to both selenite and tetrathionate brilliant green broth (398,399,415).

Selective agar media.

In literature the agar media brilliant green and brilliant green sulphapyridine agar were found the most suitable ones for the isolation of salmonellae (123,189). To inhibit Proteus and Pseudomonas strains sulphadiazine was sometimes added to the brilliant green media (123,133,167). Other media such as bismuth sulphite agar (388), deoxycholate citrate lactose sucrose agar (159). Salmonella-Shigella agar (15) and the less selective MC (203) are also often used in the isolation of salmonellae. Some new media include HE (180) and XLD (334). Since lactose is fermented slowly by some strains of salmonellae, falsely negative reactions may occur. A theoretical disadvantage of XLD is that some strains of Salmonella enteritidis, a serotype frequently isolated from poultry, will develop atypically on that medium, since they do not ferment xylose and show only a weak lysine decarboxylase reaction (272).

Bismuth sulphite, XLD and LIC agars are unique among the selective agars because differentiation of Salmonella colonies is independent of lactose fermentation and relies mostly on a hydrogen sulphide indicator system. The BS medium gave good results with the Salmonella-serotypes, typhi, paratyphi B and enteritidis (388), but often fails in the isolation of other salmonellae.

XLD agar and HE agar recently added to the bile salt selective agars, were tested frequently in comparison with other older, selective agar media. The productivity of XLD- and HE agars was found to be very similar, both media permitting recovery of salmonellae (165). Others (272) found XLD- and HE agar superior to other agar media, yielding more Salmonella isolations and fewer false positives. Experiments with Enterobacteriaceae strains isolated from sausages (288) showed that the selectivity of XLD and HE agars could be improved by the addition of 5 micrograms/ml Novobiocin to XLD agar and 80 micrograms/ml to HE agar. A comparison between four plating media with and without Novobiocin (10 micrograms/1) (220) showed that XLD with Novobiocin gave results identical with tryptone soya brilliant green agar, modified for improved Salmonella isolation (376). Brilliant green agars gave excellent results in collaborative studies in nine European laboratories (97,98). A disadvantage of BG is the fact that depending on the foods examined often subculturing is needed.

Experiments with commercial brilliant green agars to which 18 antimicrobial agents were added showed that a combination of sulphacetamide (1 mg/ml) and mandelic acid (0.25 mg/ml), together called antibiotic enriched BG, gave maximum recovery of salmonellae with maximal suppression of competing microorganisms (376).

5.3. A comparative study of Salmonella isolation methods for broiler carcasses

As indicated in the previous section many methods have been suggested in literature for the isolation of salmonellae from foods, including poultry products. For the latter no specific method is used exclusively, but usually the recommendations given in ISO document 3565/1975 for the detection of salmonellae in meat and meat products are followed. It remained to be assessed whether this Salmonella reference method which is not yet generally accepted by many English speaking countries, also gives the highest percentage of Salmonella recovery from poultry products, treated in different ways.

Furthermore new selective agar media have been suggested since this standardized method was issued.
In this investigation broiler carcasses on which one could expect varying numbers of sublethally damaged salmonellae (refrigerated, frozen, radiated or treated with a combination of these processes) were examined.

Materials.
In stage 1 of these experiments 60 eviscerated carcasses originating from a Salmonella-positive flock were drawn from the normal production line in a broiler slaughter-house. 20 carcasses were examined before freezing at -18 °C, 20 after freezing and 20 after freezing and irradiation with 2.50 kGy. Approx. 100 cm^2 (10 grams) of skin from the neck, breast and cloaca areas were dissected from each carcass with sterile instruments. Pooled samples of 5 carcasses were examined for the presence of Salmonella.
In stage 2 of the experiments the carcasses mentioned in figure 4 were used.

Salmonella isolation methods.
In stage 1 of the experiments the 100 cm^2 skin samples were resuscitated and pre-enriched (1:9) respectively in 90 ml of:
a. TSB at 20°C for 2 hours
b. TSB at 37°C for 2 hours
c. BP at 37°C for 18-24 hours
d. LB at 37°C for 18-24 hours
10 ml of each enrichment culture were transferred to 2 bottles, each containing 90 ml of the following selective enrichment media:
A. TBB
B. tetrathionate broth + 40 micrograms/ml Novobiocin (TBN)(Upjohn, Belgium)
C. selenite broth (SEB)(Difco)
D. selenite cystine broth (SCB)(BBL)
E. mannitol selenite cystine broth (SCM)(Merck)
The enrichment media were incubated at 37°C and at 43°C for 24 and 48 hours.
After incubation subcultures were made on the following selective agar media:
1. BG (OXOID)
2. BG + 100 mg/l Sulphadiazine (BGS)(Boom, Meppel, The Netherlands)

3. BG + 100 mg/l Sulphadiazine + 2.5 g/l Sodiumdeoxycholate (BGSD) (Merck)
4. Sulphapyridine brilliant green agar (SBG)(Difco)
5. Deoxycholate hydrogen sulphide lactose agar according to Sakazaki (DHL)(Merck)
6. Xylose lysine deoxycholate agar (XLD)(OXOID)
7. XLD + 40 micrograms/ml Novobiocin (288)(XLDN)
8. Bismuth sulphite agar (BS)(OXOID)
9. Deoxycholate citrate agar (DC)(OXOID)
10. Hektoen Enteric agar (HE)(OXOID)

Incubation was at 37°C for 18-24 hours. Suspect colonies were purified on MC agar and characterized biochemically and serologically as described before.

Figure 7 presents the scheme of the experimental set up.

Stage 2 of these experiments was carried out as described in detail in Section 4.4.

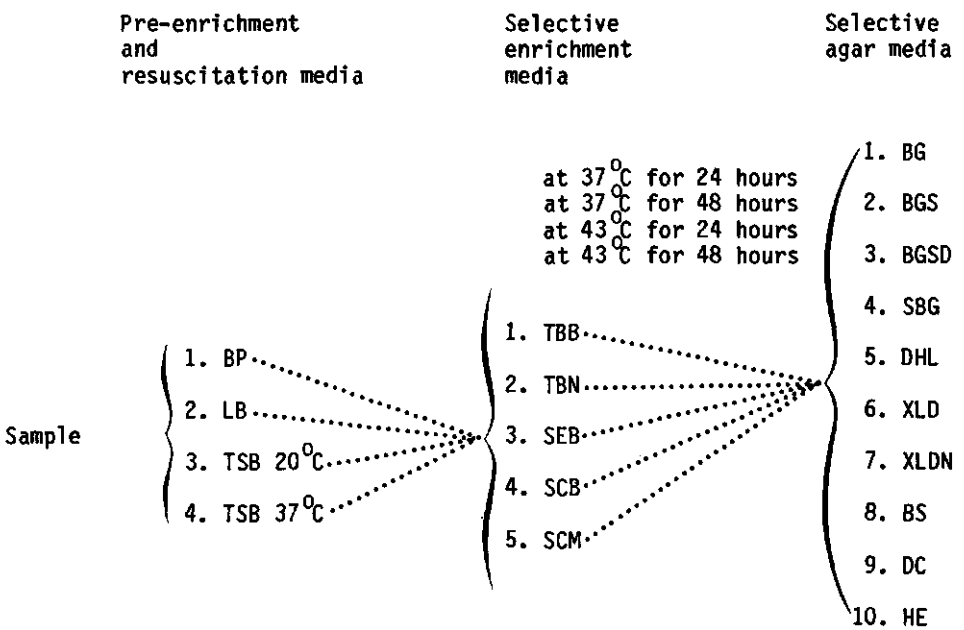


Figure 7. Scheme of the experimental set-up.

5.3.1. Results of a comparative study of Salmonella isolation methods when applied to broiler carcasses: stage 1

Resuscitation and pre-enrichment media.

Table 8 summarizes the results of experiments in which the performance of different media was assessed. Four samples were examined in 800 ways; if all samples had been found positive for Salmonella, the figure 3200 would have been recorded in the table.

With refrigerated carcasses no statistically significant differences were found. When frozen carcasses were examined, LB was estimated better ($P<0.01$) than the other resuscitation and pre-enrichment media. Among the other media no significant differences were determined by the statistical analysis applied. BP was more ($P<0.01$) productive than TSB with frozen + 2.50 kGy carcasses. The results did not differ significantly from those obtained with LB. In the total results no significant differences were found.

Table 8. Salmonella-positive samples of broiler carcasses found with different resuscitation and pre-enrichment media

	Refrigerated	Frozen	Frozen + 2.50 kGy	Total
Resuscitation				
TSB 20°C	45	23	35	103
TSB 37°C	40	30	38	108
Pre-enrichment				
BP	37	30	50 *	117
			n.s.	
LB	35	47*	45	127

* significant $P<0.01$; n.s. = not significant

Enrichment media.

The results are given in table 9. With all samples positive, 640 isolations would have been obtained.

Table 9. Salmonella-positive samples of broiler carcasses with different enrichment media.

Medium	Refrigerated	Frozen	Frozen + 2.50 kGy	Total
TBB	13	14 -)	37 **)n.s.	64
TBN	29	12 *	59	100
SEB	21 **	30 **	28	79 **
SCB	86	70 -)	24	180
SCM	12	4	20	36

* significant $P<0.05$ from results -)

** significant $P<0.01$

SCB showed better ($P<0.01$) results than the other media when refrigerated and frozen carcasses were examined. With refrigerated carcasses, the results of the various media did not differ significantly. When used with frozen carcasses, SCB was more ($P<0.05$) effective in isolation than all other media, except SEB. TBN proved ($P<0.01$) to be the best enrichment medium to isolate salmonellae from frozen and radiated samples. Good results were also obtained with TBB; however, it was not statistically superior to the other media. The total number of Salmonella-positive samples was better ($P<0.01$) with SCB, than with the other media, whereas SCM proved to be the medium with the lowest Salmonella recovery.

No statistically significant differences were found with respect to influence of incubation time or incubation temperature. When the results obtained with the resuscitation and pre-enrichment media were evaluated together, there was an interaction ($P<0.01$) with the results of BP and SCB. The results of the combined use of the media TSB 37 and TBB were less ($P<0.01$) successful.

Selective agar media.

The results are summarized in table 10. If all samples had been found Salmonella-positive, 320 isolations would have been obtained.

Table 10. Salmonella-positive samples on 10 different selective agar media

Medium	Refrigerated	Frozen	Frozen + 2.50 kGy	Total
BG	1) 12	1) 15	1) 16	1) 43
BGS	1) 11	1) 18	1) 16	1) 45
BGSD	* 23	1) 17	1) 16	56
SBG	17	* 30	22	* 69
DHL	15	1) 13	* 27	55
XLD	16	1) 17	1) 13	1) 46
XLDN	* 25	1)** 5	1)** 4	1)** 34
BS	1) 8	1)** 1	1)** 0	1)** 9
DC	14	1) 4	21	1) 39
HE	14	9	* 31	54

* significant $P<0.01$ from results indicated 1)
** significant $P<0.01$ from all other results

With refrigerated carcasses the results with the media BGSD and XLDN proved ($P<0.01$) better than those obtained with the media BG and BS. Among the other results, no significant differences could be detected. SBG was better ($P<0.01$) than all other media when frozen carcasses were examined for Salmonella. The recovery for Salmonella was lower ($P<0.01$) on the media XLDN, BS and DC. No difference was found among the results obtained on the other media. When frozen and radiated carcasses were examined the media DHL and HE gave higher ($P<0.01$) recoveries than the media BG, BGS, BGSD, XLD, XLDN and BS. The number of Salmonella-positive samples obtained on media XLDN and BS was lower ($P<0.01$) than the number obtained on other media. From a statistical point of view, SGB gave the best results in comparison with the media BG, BGS, XLD, XLDN, BS and DC. The lowest number of Salmonella-positive samples was obtained with BS.

Table 11. True and false positive isolations from selective agar media

Medium	True positive	False positive
BG	35	64 1)
BGS	38	78 1)
BGSD	47	47
SBG	60	39 *
DHL	47	122 1)2)
XLD	42	73 1)
XLDN	23	60 1)2)
BS	4	24 1)**
DC	35	17 *
HE	54	87 1)

* significant $P<0.01$ with respect to 1)
** significant $P<0.01$ with all other results except those indicated 2)

A second criterion to assess the effectiveness of a selective medium is the number of false positive isolations. A false positive isolation is defined as a suspect colony which, after identification, proved not to be a Salmonella.

Table 11 lists the number of false positive isolations obtained on all media. From the table it can be seen that the media SBG and DC gave less ($P<0.01$) false positive isolations than the media BG, BGS, DHL, XLD, XLDN, BS and HE. Medium BS gave fewer ($P<0.01$) false positives than the other media with the exception of the results obtained with the media DHL and XLDN.

Based on these results the following combinations of media were chosen for the second stage of the experiments : resuscitation and pre-enrichment TSB 37 and BP; selective enrichment TBB and SCB; selective agar media BG, BGSD, SBG, XLD and HE.

5.3.2. Results of stage 2 of the experiments.

Resuscitation and pre-enrichment.

Table 12 summarizes the results of the total number of Salmonella-positive samples. With all samples positive, 600 isolations would have been obtained. This applies both to skin samples and to thaw water samples.

Table 12. Salmonella-positive samples of broiler carcasses with different resuscitation and pre-enrichment media

Medium	Frozen		Frozen + 2.50 kGy		Refrigerated + 2.50 kGy		Total	
	skin	thaw water	skin	thaw water	skin	thaw water	skin	thaw water
TSB 37 °C	29 *	42 *	8	47 *	0 *	12 *	37 *	101 *
BP	71	112	0	107	18	38	89	257

* significant $P<0.01$

BP proved to be better (P<0.01) than TSB. With refrigerated carcasses the number of Salmonella-positive samples was too low to be used in a statistical analysis.

Enrichment media.

The results are summarized in table 13. The total number of Salmonella-positive samples attainable in this experiment was 600.

SCB proved (P<0.01) better than TBB. No significant differences were found between the two enrichment media, whether the samples were incubated at 37 °C or at 43 °C, for 24 or for 48 hours.

Table 13. Salmonella-positive samples of broiler carcasses with 2 different enrichment media

	Frozen		Frozen + 2.50 kGy		Refrigerated + 2.50 kGy		Total	
	skin	thaw water	skin	thaw water	skin	thaw water	skin	thaw water
Medium								
TBB	18	18	0	56	10	21	28	95
	*	*	*	*			*	*
SCB	82	136	8	98	8	29	98	263

* significant P<0.01

Isolation media.

Table 14 gives the results of the experiments in which 5 selective agar media were tested. With all samples Salmonella-positive, 120 isolations would have been obtained with all combinations.

BGSD gave better (P<0.01) results than BG when the number of Salmonella-positive samples of thaw water and skin samples were taken together. No other significant differences were found with this type of sample material.

HE proved better (P<0.01) than BSGD when frozen + 2.50 kGy samples were examined. XLD and HE were better (P<0.01) than the other media with refrigerated + 2.50 kGy carcasses, when all positive samples were taken together.

Looking for the overall score, HE proved (P<0.01) better than BG when all positive samples were taken together.

Table 14. Salmonella-positive samples of broiler carcasses with 5 different selective agar media

	Frozen		Frozen + 2.50 kGy		Refrigerated + 2.50 kGy		Total	
	skin	thaw water	skin	thaw water	skin	thaw water	skin	thaw water
Medium								
BG	13	28	2	28	0	11	15	67
BGSD	26	33	2	25	3	4	31	61
SBG	24	28	1	32	3	9	28	69
XLD	18	34	1	31	6	12	25	77
HE	19	31	2	38	6	14	27	83

1)

*

1)

*

* significant P<0.01 with respect to 1)

In assessing the effectiveness of various selective agar media, the false positive isolations were once more taken into consideration. Table 15 gives the results of the 5 media tested. HE scored more (P<0.01) false positive isolations than any other medium.

Table 15. True and false positive isolations from selective agar media

Medium	True positive	False positive
BG	82	34
BGSD	94	39
SBG	100	25
XLD	103	30
HE	111	61

*

* significant P<0.01

5.4. Discussion of both experiments.

Pre-enrichment media and conditions scored higher Salmonella recoveries than resuscitation media and conditions when samples of frozen and radiated carcasses were examined. With these two categories of samples LB gave the best overall results. In other cases no significant differences were found. No indication was found that, due to chilled storage and irradiation bacteria, were lethally injured, since the total number of Salmonella-positive samples did not differ significantly. This substantiates earlier findings (227).

When considering the overall results, SCB proved to be the best selective enrichment medium. Only when samples of frozen and radiated carcasses were examined, other media (TBB, TBN) scored higher ($P<0.01$) recoveries. To explain these observations one should consider the chemical composition of the media and the effect of individual components on the restoration of cells.

There was an interaction ($P<0.01$) between the use of BP as pre-enrichment medium and SCB as enrichment medium. Therefore, this combination of media can be recommended for this type of poultry products. It was shown that when samples had incurred more physical stress the standard deviations of the mean number of positive samples on the selective agar media increased. Four selective agar media gave the best overall results. These were BGSD, SBG and DHL in the first stage of the experiments and HE in the second stage of the experiments. From the experiments it became clear that BS can not be used for the detection of salmonellae in poultry products. When the criterion false positive isolations is taken into account the media SBG and DC gave the best results ($P<0.01$). Again medium BS gave the most false positive Salmonella-isolations ($P<0.01$). A reason for this finding may be the low number of colonies obtained in the experiments which were all marked suspect. The same can be said for the media DHL and HE with which there was no experience in routine analysis. No effects could be assessed of different incubation times and temperatures used in enrichment. Possibly the low number of Salmonella-positive samples is the reason for this finding; this observation has also been reported in several literature reviews (76,116,129,165,181,262,289,294,305,335,394,398,399,415).

A comparison with results from studies in literature is difficult, since in most of these studies other media were compared with other sample material.

Further research by which the effectiveness of new media for the isolation of salmonellae should be studied can use the results obtained here and should determine the value of the data presented.
In further experiments to be reported in this thesis, the recommendations given in ISO document 3565/1975 on the isolation of Salmonella from meat and meat products were followed. This choice was based on the experience with this method for several years and the difficulty in the recognition of suspect colonies on new and therefore not routinely-used selective agar media.

CHAPTER 6

6. D_{10}-VALUES OF MICROORGANISMS IN SOLID AND LIQUID CULTURE MEDIA

6.1. Introduction.

D_{10}-values of microorganisms are dependent on many parameters (cf. Section 4.7.), including the culture media in which the organisms were pre-grown, irradiated and counted after the radiation treatment (276). First of all it was investigated whether or not differences in radiation sensitivity in solid agar media existed between microorganisms related to or associated with poultry.

Radiation sensitivity in liquid culture media was investigated, too. Experiments were carried out with Escherichia coli 12 K and Salmonella panama to establish the influence of the physiological age, the cell concentration and of oxygen partial pressure on D_{10}-values.

Table 16. D_{10}-values (kGy) of different bacteria in solid culture media

Bacterium	Agar medium			
	NA		MA	
	D_{10}	n	D_{10}	n
Escherichia coli	0.13	5	0.09	4
Escherichia coli K12 lysogenic	0.12	4	0.14	3
Escherichia coli K12 non lysogenic	0.26	4	0.14	5
Escherichia coli K12 NDA	0.19	4	0.14	5
Escherichia coli K12 NDA	0.23	6	0.18	5
Salmonella bareilly	0.10	4	0.11	4
Salmonella panama	0.29	5	0.16	3
Salmonella panama	0.22	5	0.19	6
Salmonella senftenberg	0.13	5	0.09	3
Salmonella typhimurium	0.17	6	0.10	4
Micrococcus freudenreichii	0.20	6	Not determined	
Pseudomonas testosteroni	0.20	6	Not determined	

n = number of doses applied

The results of these experiments allowed to assess the usefulness of Escherichia coli as an index bacterium for Salmonella.

6.2. D_{10}-values on solid culture media.

All cultures were 20-hours' and the bacteria were hence in their stationary phase of growth. Table 16 gives the estimates of the D_{10}-values of some bacteria in NA or in MA.

Influence on D_{10}-values of the medium in which the cells were irradiated.

In this experiment the same media for irradiation and culturing were used. In table 16 the D_{10}-value of Escherichia coli K 12 non-lysogenic in NA (0.26 kGy) differed ($P<0.01$) from the D_{10}-value in MA (0.14 kGy). Results obtained with the other bacteria did not differ ($P>0.05$).

Influence on D_{10}-values of the physiological age of cells.

This experiment was carried out with an Escherichia coli strain K12 on NA and MA; 2 and 20 hours' cultures were compared with respect to their radiation sensitivity. D_{10}-values were estimated and are summarized in table 17.

Table 17. D_{10}-values (kGy) of Escherichia coli K12 NDA cells of a different physiological age

	Agar medium			
	NA		MA	
	D_{10}	n	D_{10}	n
2-hours' culture	0.21	6	0.18	5
20-hours' culture	0.23	6	0.18	5

n = number of doses applied

2 hours' cells were in the logarithmic phase of growth and 20 hours' cells were in the stationary growth phase. In all cases the variance of the regression was less than 2 percent of the total variance. The D_{10}-values did not differ ($P>0.05$).

6.3. D_{10}-values in liquid culture media.

Table 18 gives the results of experiments in which D_{10}-values of bacteria irradiated in NB and in MM were estimated. Table 19 gives the results of experiments in which D_{10}-values of salmonellae were estimated when irradiated at +5°C and at -18°C. This experiment was carried out with Salmonella strains isolated from poultry carcasses used in the experiments referred to in chapter 8.

Table 18. D_{10}-values (kGy) of bacteria in liquid culture media

	Agar medium			
	NA		MA	
Bacterium	D_{10}	n	D_{10}	n
Escherichia coli K12 NDA	0.43	8	0.31	8
Escherichia coli K12 NDA	0.38	5	0.40	6
Escherichia coli K12 NDA	0.37	5	0.40	6
Escherichia coli K12 NDA	0.46	6	0.48	6
Micrococcus freudenreichii	0.51	6	0.53	6
Micrococcus freudenreichii	0.54	6	0.57	6
Proteus vulgaris	0.23	5	0.23	5
Proteus vulgaris	0.24	5	0.23	5
Pseudomonas fluorescens	0.11	2	0.11	2
Pseudomonas fluorescens	0.11	3	0.11	3
Salmonella panama	0.48	8	0.35	8
Salmonella panama	0.50	6	0.43	6
Salmonella panama	0.62	6	0.57	6
Salmonella panama	0.53	6	0.49	6
Salmonella saint-paul	0.36	5	0.31	5
Salmonella saint-paul	0.34	5	0.33	5
Salmonella thompson	0.50	6	0.45	6
Staphylococcus aureus	0.29	6		
Staphylococcus aureus	0.29	6		

n = number of doses applied

Table 19. D_{10}-values (kGy) of salmonellae irradiated at different temperatures

Agar medium	NA		MA	
Serotype	D_{10}	n	D_{10}	n
Radiation temperature : +5°C				
agona	0.38	6	0.32	5
anatum	0.38	6	0.35	4
enteritidis	0.34	6	0.35	4
heidelberg	0.37	6	0.38	6
infantis	0.36	5	0.36	5
montevideo	0.32	6	0.29	5
muenchen	0.50	6	0.30	5
thompson	0.35	5	0.35	5
Radiation temperature : -18°C				
agona	0.46	6	0.46	6
anatum	0.52	6		N.D.
enteritidis	0.43	6	0.37	6
heidelberg	0.46	6	0.35	6
infantis	0.36	6	0.31	6
montevideo	0.41	6	0.39	6
muenchen	0.62	6	0.34	5
thompson	0.41	6	0.46	6

n = number of doses applied; N.D. = not determined

The results in the tables 18 and 19 give a good impression of the sensitivity of bacteria towards ionizing radiation as expressed by their D_{10}-values. The range in which these values can be found is clearly demonstrated.

The D_{10}-values did not differ significantly whether they were obtained in NA or in MA. Two exceptions to this rule were found : Escherichia coli K 12 NDA 0.43 versus 0.31 kGy and Salmonella panama 0.48 versus 0.35 kGy. In table 19 the D_{10}-values of Salmonella muenchen were found to be higher than the D_{10}-values of the other serotypes. However, a statistical superiority could not be ascertained. Irradiation at -18°C gave an increase in D_{10}-values for all serotypes tested; this finding was independent of the medium used.

Table 20. D_{10}-values (kGy) of 3-hours' cultures of Escherichia coli K12 under different culture conditions

Code media	D_{10}	n
NNN	0.66	4
MNN	0.81	4
MMN	0.25	3
MMM	0.33	3
NMN	0.28	4
NMM	0.26	4
NNM	0.74	4
MNM	0.53	4

NNN : pre-grown in NB, irradiated in NB, cultured in NB
MMM : pre-grown in MM, irradiated in MM, cultured in MM
n = number of doses applied

Effect on D_{10}-values of the pre-growing culture medium used.

The experiments were carried out with Escherichia coli K12 which bacterium showed D_{10}-values comparable with those of the Salmonella panama strain used in previous experiments. Only 3 hours' cultures were used in these experiments. The D_{10}-values obtained are presented in table 20. With 3-hours' cells there was a difference in D_{10}-values between the series of media used. Higher values were found when nutrient media were dominant in the combination of media used. One exception to this rule was found : the D_{10}-value of 0.28 kGy with the combination of media NMN. When minimal media were dominant, the D_{10}-values varied between 0.25 and 0.33 kGy. The highest D_{10}-values were found when nutrient broth was used as the medium in which the cells were irradiated (0.53 - 0.81 kGy).

Effect on D_{10}-values of the physiological condition of Escherichia coli K12 and of Salmonella panama.

In this experiment 1, 2, 3 and 9 hours' cultures of Escherichia coli K12 were used. According to their growth curve the bacteria were in the stationary phase of growth after 9 hours of incubation. This experiment was carried out to test the theory that cells at the end of their logarithmic growth phase are less resistant to ionizing radiation than early log phase cells are, but that they are more resistant than stationary growth phase cells. The results are summarized in table 21.

Table 21. Effect of the physiological age on the D_{10}-values of Escherichia coli K12 cells

Age	Code of media	D_{10}	n	Code of media	D_{10}	n
1 hour	NNN	0.31	4	NMN	0.25	4
2 hours		0.36	4		0.25	4
3 hours		0.66	4		0.28	4
9 hours		0.68	4		0.30	4
1 hour	MNN	0.27	4	NMM	0.24	3
2 hours		0.25	4		0.22	4
3 hours		0.81	4		0.26	4
9 hours		0.38	4		0.30	4
1 hour	MMN	0.24	3	NNM	0.28	4
2 hours		0.25	4		0.31	4
3 hours		0.25	3		0.74	4
9 hours		0.39	4		0.47	4
1 hour	MMM	0.22	3	NMN	0.25	4
2 hours		0.24	3		0.25	4
3 hours		0.33	3		0.28	4
9 hours		0.33	3		0.30	4

n = number of doses applied

The highest D_{10}-values were generally found with 3-hours' cultures on a combination of media in which the rich nutrient medium was dominant. In combinations of media in which the minimal medium and agar were dominant, D_{10}-values of 9-hours' cultures were maximal. In these media the bacteria have relatively small amounts of nutritive compounds available, so the cells need more time to produce radio-protective compounds; therefore the younger cultures are more sensitive towards ionizing radiation.

Table 22 gives the D_{10}-values of Escherichia coli K12 and Salmonella panama in relation to their physiological age. 1, 3.5 and 20 hours' cultures were used. Differences with the D_{10}-values recorded in the previous table are possible, since a different isolation method was used.

In MM, the D_{10}-values of Escherichia coli of an 1 hour culture in the early logarithmic phase of growth, were lower ($P<0.01$) than those of 20-hours' stationary phase cells. This D_{10}-value was as high as the D_{10}-value found with stationary phase cells.

Table 22. Effect of the physiological age on D_{10}-values (kGy) of *Escherichia coli* K12 and *Salmonella panama*

	Media			
	NB/NA		MM/MA	
	D_{10}	n	D_{10}	n
Escherichia coli K12 NDA				
1 hour	0.47	7	0.19	7
3,5 hours	0.32	7	0.23	6
20 hours	0.43	8	0.31	8
Salmonella panama				
1 hour	0.41	7	0.28	7
3,5 hours	0.31	7	0.30	7
20 hours	0.48	8	0.35	7

n = number of doses applied

Table 23. D_{10}-values (kGy) of *Escherichia coli* K12 NDA and *Salmonella panama* in dependence of their physiological age (in NB and NA)

	Escherichia coli K12 NDA		*Salmonella panama*	
Physiological age	D_{10}	n	D_{10}	n
1 hour	0.60	7	0.58	7
2 hours	0.42	7	0.29	7
3 hours	0.28	7	0.34	7
4 hours	0.28	7	0.38	7
5 hours	0.36	7	0.45	7
6 hours	0.32	7	0.50	7
7 hours	0.38	7	0.51	7
8 hours	0.39	7	0.51	7
9 hours	0.33	7	0.53	7

n = number of doses applied

The 3.5-hours' cultures consisted of logarithmic phase cells. The results obtained with *Salmonella panama* did not differ significantly. Results of experiments with cells of both strains 1 to 9-hours' are given in table 23.

The D_{10}-value of an 1 hour culture of Escherichia coli K12 NDA differed ($P<0.05$) from the values found with other cultures (3, 4 and 9 hours). The D_{10}-value of a 2 hours' culture of Salmonella panama differed ($P<0.05$) from 1, 6, 7, 8 and 9 hours' cultures. The high D_{10}-value of 1 hour cultures was striking.

Influence of a pre-enrichment medium on the recovery of Escherichia coli K12 and Salmonella panama after irradiation.

In table 24 the results are given of an experiment in which Escherichia coli K12 NDA was used. The medium in which the cells were irradiated was Brain Heart Infusion broth (BHI) or BP and the plating was done in PCA. The highest D_{10}-values were estimated from a direct plating in PCA. The different pre-enrichment media did not cause differences in the D_{10}-values of the Escherichia coli K12 NDA strain used.

A similar experiment was carried out with Salmonella panama. Table 25 gives the results. No significant differences were found between the D_{10}-values.

Table 24. D_{10}-values (kGy) of Escherichia coli K12 NDA after different treatments

	Medium	D_{10}	n
colony count	PCA	0.77	5
colony count	PCA	0.88	5
incubation time			
2 hours at +7°C	BP	0.57	5
	BP	0.65	5
	NB	0.49	5
	NB	0.57	5
	LB	0.57	5
	LB	0.74	5
19 hours at +7°C	BP	0.38	5
	BP	0.43	5
	NB	0.29	5
	NB	0.37	5
	LB	0.33	5
	LB	0.33	5

n = number of doses applied

Table 25. D_{10}-values (kGy) of Salmonella panama after different treatments

	Medium	D_{10}	n	Medium	D_{10}	n
colony count on NA	NB	0.34	5	MM	0.34	5
colony count on MA	NB	0.30	5	MM	0.31	5
count after LB on NA	NB	0.36	5	MM	0.34	5
count after NB on NA	NB	0.29	5	MM	0.36	5

n = number of doses applied

The conclusion of these experiments is, that if sublethal damage of the cells caused by irradiation occurred, it was either repaired within 2 hours or was still unrepaired after 19 hours of incubation at +7 °C.

Influence of cfu concentration on D_{10}-values.

Table 26 lists the results of these experiments which were carried out with Escherichia coli K12. D_{10}-values estimated in experiments in which the initial cfu concentration was below (log cfu) 3.00 / ml are not very accurate.

Table 26. D_{10}-values (kGy) of Escherichia coli K12 NDA based on different initial cfu concentrations

Experiment 1			Experiment 2		
Concentration (log cfu/ml)	D_{10}	n	Concentration (log cfu/ml)	D_{10}	n
5.20	0.45	5	6.92	0.46	5
4.28	0.49	5	5.83	0.49	5
3.28	0.42	4	4.65	0.49	5
2.38	-		3.77	0.51	5
1.72	-		2.75	0.47	4
0.56	-		-		

The plating medium was VRB + 200 ppm NDA
n = number of doses applied; - = not determined

Therefore a presence or absence test was carried out to estimate at which doses no Escherichia coli K12 NDA could be detected.
The results of the enrichment tests in EE broth tubes are given in table 27.

Table 27. Survival of Escherichia coli K12 NDA after different radiation doses

Dose (kGy)	0.50	1.00	1.50	2.00
Concentration (log cfu/ml)				
			+++	+++
5.20			+++	+++
			+++	+++
		+++	-++	+++
4.26		+++	+++	--+
		+++	+++	--+
3.26	+++	+++	++-	+--
	+++	+++	++-	---
	+++	+++	+--	---
2.38	+++	+++	---	---
	++-	+++	+--	---
	+++	+++	+--	---
1.73	+++	++-	---	
	+++	+--	---	
	+++	---	---	

+ = growth after enrichment in EE-broth and plating
- = no growth after enrichment in EE-broth and plating

When the initial cfu concentration was log 2.38 / ml, a dose of 2.50 kGy was sufficient to obtain negative results; only with (log) 1.73 / ml a dose of 1.50 kGy was sufficient to this respect.
This means that a 3 - 3.5 log cycle reduction in the number of cfu of Escherichia coli results from a dose of 2.00 kGy. The calculated D_{10}-values would than be 0.50-0.60 kGy, which values are comparable with data in literature, and agree pretty well with those found in this investigation.

The cfu-concentration (N_0) *had no effect on* D_{10}-*values* (*table 26*) as anticipated; cf. Section 4.7. From a methodological point of view it is better to use initial cfu concentrations of approx. (log/ml) 8.00 - 9.00. In that case the effect of the radiation treatment can be studied better, as more measuring points can be obtained before the number of cfu's becomes too low to be accurately determined.

Influence of oxygen on D_{10}-value of Escherichia coli K12 NDA.

It is well known that microorganisms are more sensitive towards ionizing radiation when the oxygen partial pressure in the surrounding medium or atmosphere is high. One experiment was carried out to investigate the effect of the presence of oxygen on D_{10}-values of the Escherichia coli K12 strain. The results are given in table 28.

Table 28. The effect of oxygen partial pressure on the D_{10}-value (kGy) of Escherichia coli K12 NDA

Medium	NA		NA	
	D_{10}	n	D_{10}	n
with oxygen	0.37	6	0.40	6
with oxygen	0.46	6	0.48	6
without oxygen	0.38	6	0.27	5
without oxygen	0.43	6	0.28	5

n = number of doses applied

The liquid medium in which the cfu had been grown was flushed with nitrogen gas replacing the oxygen present. From the results the effect of an addition of nitrogen to replace oxygen is not clear. No significant differences were found. Therefore the theory was not substantiated. It occurred to us that, because of the way of transportation to the radiation source, more oxygen than expected had been dissolved in the medium, giving lower D_{10}-values than one normally should expect under anoxic conditions.

To investigate this possibility 1, 2 and 3 hours' liquid cultures of both Escherichia coli K12 NDA and Salmonella panama were shaken in a water bath ($T = 20\ ^{o}C$) and the oxygen partial pressure measured.

It appeared that no extra oxygen was dissolved in NB or in MM under these conditions and therefore neither during transportation. The way of transportation therefore did not affect the D_{10}-value and the reason for an absence of an oxygen effect remained obscure.

6.4. Conclusions.

The experiments reported in this chapter allow the following conclusions:

1. Bacteria have no fixed D_{10}-value ; D_{10}-values can be found within a certain rather broad range
2. There is an effect on D_{10}-values of the radiation temperature
3. No effect on D_{10}-value of the initial number of cfu's could be demonstrated
4. There is an effect on D_{10}-values of different media used
5. The effect of a dose of 2.50 kGy on pure cultures can be estimated within certain limits
6. No effect of oxygen partial pressure could be substantiated.
7. D_{10}-values of Salmonella and Escherichia coli did not differ significantly. Generally, D_{10}-values of salmonellae tended to a higher value, but the statistical superiority above D_{10}-values of Escherichia coli could not be demonstrated.
8. The effect on D_{10}-values of the different media used before, during and after irradiation was equal for the strains used.

CHAPTER 7

7. D_{10}-VALUES OF MICROORGANISMS ON ARTIFICIALLY CONTAMINATED SKIN SAMPLES AND BROILER CARCASSES

7.1. Introduction.

A difficulty in assessing the effects of ionizing radiation on salmonellae on broiler carcasses is the low initial number of these bacteria per carcass. This, as explained in the previous chapter, seriously interferes with the assessment of reliable D_{10}-values. For this reason experiments were carried out with artificially contaminated skin samples and broiler carcasses. The strains used were Escherichia coli K12 NDA, Salmonella niloese and Salmonella panama. The influence of the radiation temperature on D_{10}-values of these test strains was particularly studied, in view of the impact of such effects for poultry radicidation in practice.

7.2. Method for the artificial contamination of broiler carcasses.

Tables 29, 30 and 31 give the results of the tests carried out to assess the repeatability of the contamination methods. The thaw water referred to in table 29 is the fluid, released from the carcasses immediately after artificial contamination. Counts obtained by the rinsing technique and counts obtained from skin samples were compared.

Table 29. Mean and standard deviation of counts (log cfu/ml) of Escherichia coli K12 NDA in thaw water from artificially contaminated carcasses

	Trial (5 carcasses per trial)				
	1	2	3	4	5
mean	7.90	7.77	7.57	6.55	5.62
s	0.09	0.14	0.04	0.06	0.04

Table 30. Mean and standard deviation of counts (log cfu/ml) of Escherichia coli K12 NDA and Salmonella panama in rinsing water from artificially contaminated carcasses

	Trial (5 carcasses per trial)							
	Escherichia coli K12			Salmonella panama				
	1	2	3	1	2	3	4	5
mean	6.76	7.47	4.77	5.88	5.81	5.36	4.61	3.69
s	0.10	0.09	0.07	0.03	0.21	0.06	0.12	0.13

Table 31. Mean and standard deviation of counts (log cfu/10 cm^2) of Escherichia coli K12 NDA and Salmonella panama on skin samples from artificially contaminated carcasses

	Trial (5 carcasses per trial)					
	Escherichia coli K12	Salmonella panama				
	1	1	2	3	4	5
mean	5.79	6.18	6.48	4.95	4.75	3.34
s	0.11	0.39	0.13	0.21	0.39	0.12

The differences in standard deviations between the counts in thaw water and in rinsing water were small, though in favour of the former. The standard deviations found with artificially contaminated samples were lower than those obtained with naturally contaminated samples. Counts of skin samples (Salmonella panama) gave a definitely lower repeatability in comparison with the counts in thaw and rinsing water.
In conclusion it can be said that the results obtained by the method of artificial contamination were satisfactory.

7.3. Irradiation at +5 °C and at -18 °C.

Table 32 summarizes the results of experiments in which skin samples were artificially contaminated with Escherichia coli K12 NDA and Salmonella panama. Two contamination levels were applied, viz. log cfu 3.00 and log cfu 7.00 per 10 cm^2 of skin.

For the artificially contaminated skin samples it was demonstrated that a radiation dose of 2.50 kGy applied at +5°C and at -18°C did not suffice to eliminate Salmonella panama, even when the low contamination level of (log cfu) 3.00 per 10 cm^2 of skin was used. Escherichia coli K12 NDA could, at that contamination level, not be detected after irradiation at +5°C, though positive samples were found after irradiation at -18 °C. These data substantiate our hypotheses that lower temperatures protect against radiation induced death and that Escherichia coli can not always be used as a reliable index organism for salmonellae.

Table 32. The effect of irradiation with 2.50 kGy at +5°C and at - 18°C on Escherichia coli K12 NDA and Salmonella panama on skin samples

Radiation temperature : + 5°C	Escherichia coli K12 NDA				Salmonella panama	
Contamination level (log cfu / 10 cm^2)	3.00		7.00		3.00	
	0	2.50	0	2.50	0	2.50
Number of samples	50	50	61	61	47	50
Positive samples	49	0	61	58	46	15
Radiation temperature : -18°C						
Contamination level : (log cfu / 10 cm^2)	3.00		7.00		3.00	
Number of samples	48	50	50	50	48	50
Positive samples	48	4	50	41	48	28

7.4. D_{10}-values on broiler carcasses after irradiation at +5°C and at -18°C

Table 33 gives the results of the experiments in which Escherichia coli K12 NDA, Salmonella niloese and Salmonella panama were used.

The D_{10}-values in table 33 are given in relation to the contamination level of the bacteria in the inoculation bath. The D_{10}-values of Escherichia coli K12 NDA estimated at a radiation temperature of +5°C were lower ($P<0.05$) than the other values. The mean D_{10}-values for Escherichia coli K12 NDA, Salmonella niloese and Salmonella panama at a radiation temperature of +5°C were 0.36, 0.62 and 0.67 kGy respectively.

Table 33. D_{10}-values (kGy) of Escherichia coli K12 NDA, Salmonella niloese and Salmonella panama on artificially contaminated broiler carcasses after irradiation at +5°C and -18°C.

	Contamination level (log cfu/ml)	D_{10}	n
Radiation temperature : +5°C			
Escherichia coli K12 NDA			
skin	7.00	0.44	5
	7.00	0.50	5
	6.00	0.27	3
Salmonella niloese	6.00	0.68	5
skin	6.00	0.54	7
Salmonella panama	7.00	0.67	6
skin			
Radiation temperature : -18°C			
Escherichia coli K12 NDA			
skin	7.00	0.72	3
rinsing water	7.00	0.48	4
	7.00	0.43	3
thaw water	7.00	0.38	4
	7.00	0.43	5
Salmonella niloese			
skin	6.00	0.88	3
	6.00	1.25	7
Salmonella panama			
skin	7.00	1.32	4
	7.00	1.25	3
rinsing water	7.00	0.61	4
	7.00	0.82	4
	7.00	0.83	3
thaw water	7.00	0.56	5
	7.00	0.73	4
	7.00	0.72	4

n = number of doses applied

Irradiation at -18°C gave higher ($P<0.05$) D_{10}-values for Escherichia coli K12 NDA. The temperature of -18°C during irradiation gave once more an extra protection against radiation induced death.

The D_{10}-values of Salmonella panama found in rinsing water and in thaw water were lower ($P<0.05$) than those estimated on the skin.

The experiments at -18°C showed an increase in D_{10}-value to about 2 times the value measured at a temperature of +5°C. Evidently both the skin and the low radiation temperature seemed to protect the attached salmonellae very effectivily (D_{10}= 1.29 compared to 0.67 kGy).
For skin samples the same high D_{10}-values were found for Salmonella niloese.
In table 34 the results of experiments with Escherichia coli K12 NDA using different levels of contamination in the inoculation bath are given.

Table 34. D_{10}-values (kGy) of Escherichia coli K12 NDA in relation to the level of contamination in the inoculation bath

Contamination level (log cfu/ml)	D_{10}	n
	Skin	
6.40	0.65	5
5.40	0.45	5
5.00	0.47	5
	Thaw water	
7.00	0.73	5
6.00	0.68	5
5.00	0.73	5

n = number of doses applied

The D_{10}-values are given for skin samples and for "thaw" water. The cfu concentration in the inoculation bath and therefore on the carcasses had no influence on the D_{10}-values. This was also found in the experiments reported in chapter 6.

Irradiation of broiler carcasses under nitrogen.
Table 35 gives the results of an experiment in which normal packed broilers and broilers packed under nitrogen conditions were irradiated after artificial contamination with Escherichia coli K12 NDA. The radiation temperature was -18 °C.

Table 35. D_{10}-values (kGy) of Escherichia coli K12 NDA from skin samples and thaw water of broiler carcasses treated with and without nitrogen-gas.

	D_{10}	n
Skin		
Normal packed broilers	0.58	5
Broilers under nitrogen conditions	0.59	6
Thaw water		
Normal packed broilers	0.51	5
Broilers under nitrogen conditions	0.46	6

n = number of doses applied

No significant differences in D_{10}-values were found when carcasses were irradiated under nitrogen conditions. In that particular experiment the D_{10}-values differed slightly from the previously estimated data.

7.5. Discussion.

The D_{10}-values obtained explain the findings in table 32. A dose of 2.50 kGy may achieve a 6-log cycles reduction of Escherichia coli K12 NDA; in case of the low contamination level no surviving cfu's were detected after radiation treatment at +5 °C.

A dose of 2.50 kGy provides a 4-log cycles reduction of cfu of Salmonella niloese and Salmonella panama. With D_{10}-values (measured on skin samples) of approx. 0.71 kGy for Escherichia coli K12 NDA and of approx. 1.29 kGy for Salmonella panama, after irradiation at -18 °C, it is understandable that skin samples were found positive after a radiation treatment. In comparison with the mean D_{10}-values for Escherichia coli K12 NDA in liquid media (pre-grown and irradiated in NB, the counts were estimated in MA) of 0.41 kGy, these experiments showed an increase in D_{10}-value. The high D_{10}-values obtained with skin samples after irradiation at -18°C, must be caused by this low temperature during treatment.

The mean D_{10}-values of Salmonella panama in liquid media (pre-grown and irradiated in NB) was found to be 0.52 kGy. The experiments at a radiation temperature of +5 °C gave a D_{10}-value of 0.56 kGy. The high D_{10}-values obtained with skin samples must be due to protection against irradiation provided by the broiler skin, together with the influence of the low radiation temperature.

The results of the experiments described here and those in chapter 6 differ in several points. The D_{10}-values of the enteric bacteria in liquid and solid culture media were of the same magnitude; with artificially contaminated carcasses differences in D_{10}-values of Salmonella strains and Escherichia coli were observed. In both investigations the same influence of the radiation temperature on D_{10}-values could be assessed. No differences were found when D_{10}-values were estimated in dependence of the initial cell concentration used. An influence of oxygen partial pressure on D_{10}-values could not be ascertained.

For public health reasons the presence of microorganisms in thaw water obtained upon thawing of the carcasses is important, because of the possibility of transfer of pathogens to other food products and kitchen utensils. The D_{10}-values of the bacteria tested in thaw water were 0.70 kGy for Escherichia coli and 0.65 kGy for Salmonella panama. Under the assumption of the 7 D_{10}- concept (Section 4.7.) the required lethality of the radiation treatment can be calculated at a dose of 4.90 to 4.55 kGy. If it is assumed that on an average (log cfu) 3.00 Escherichia coli and 2.00 salmonellae per ml (260) are present, the 7 D_{10}- concept would lead to 1 positive carcass in 10,000 and 100,000 respectively.

The other approach to assess the required lethality of the radiation treatment is to set the number of microorganisms tolerated after the treatment; cf. Section 4.7. In case of radicidation this assumption is the absence of one cfu of Salmonella in the end product. The initial number of cfu's of salmonellae varies between 1 and 1,000 per ml of thaw water. The required dose would then be 3 D_{10}= 1.95 kGy.

For Salmonella-bacteria attached to the skin the 7 D_{10}- concept leads to a dose of approx. 9.00 kGy, when Salmonella panama was irradiated at -18^{o}C and to a dose of 4.50 kGy when the radiation treatment was performed at +5 ^{o}C.

In the literature such high doses are also occasionally reported for the elimination of salmonellae (201,230,343).

Salmonella niloese and Salmonella panama behaved similarly with respect to D_{10}-values on artificially contaminated carcasses. Perhaps in case of artificially contaminated carcasses, salmonellae meet less competition from the naturally present microflora than Escherichia coli K12 NDA, resulting in a stronger attachment of the salmonellae to the skin.

From the experiments it is evident that the representatives of the family of Enterobacteriaceae tested, Escherichia coli K12 NDA, Salmonella niloese and Salmonella panama showed markedly different resistance to ionizing radiation. It is therefore questionable whether Escherichia coli can be used as an index organism for salmonellae. The suitability of the entire group of Enterobacteriaceae will be discussed below.

The data obtained from the artificially contaminated carcasses and from the experiments in liquid culture media indicate that D_{10}-values for Salmonella vary between D_{10}= 0.30 and D_{10}= 1.30 kGy. Even duplicate tests showed substantial differences.

In table 36 a calculation is made of the proportion of the total broiler production (3.0×10^7 kilograms per year) remaining Salmonella-positive despite having been subjected to a radiation treatment with 2.50 kGy. The following assumptions were made : the contamination rate with Salmonella varies from 5 to 50 percent and the mean number of salmonellae per carcass varies from 5 to 100 cfu.

Table 36. Calculation of proportion of contaminated carcasses remaining after irradiation with 2.50 kGy

Total broiler production : 3.0×10^7 kilograms; D_{10}-value = 0.80 kGy

Mean of salmonellae (cfu)	Percentage of broilers initially contaminated	Positive carcasses after the treatment
5	5	1 : 5.340
	10	1 : 2.670
	25	1 : 1.070
	50	1 : 534
10	5	1 : 2.680
	10	1 : 1.340
	25	1 : 535
	50	1 : 208
100	5	1 : 277
	10	1 : 138
	25	1 : 55
	50	1 : 28

If a D_{10}-value of 0.80 kGy and a contamination rate of 25 % and 100 cfu's of Salmonella are assumed, after the treatment of the whole broiler production with a dose of 2.50 kGy, one in 55 carcasses is still contaminated with Salmonella.

The radiation treatment therefore causes a decrease in Salmonella contaminated carcasses of approx. 14 times.
If D_{10}= 0.30 kGy had been chosen the reduction in Salmonella contaminated carcasses would have been 220,000 times; with D_{10}= 1.30 kGy this reduction would only have been approx. 3 times.
In our further experiments the dose of 2.50 kGy was chosen as a realistic one.

CHAPTER 8

8. EFFECT OF A DOSE UP TO 2.50 KGY ON SALMONELLAE AND ENTEROBACTERIACEAE ON BROILER CARCASSES

8.1. Introduction.

In this chapter results of experiments with a dose of 2.50 kGy, measuring the effect on salmonellae and other microorganisms naturally present on carcasses, will be reported. Quantitative and qualitative determinations were carried out. The quantitative assessments were carried out by the Most Probable Number (MPN) technique.

The effect of the application of low doses of ionizing radiation followed by a freezing process and a second radiation treatment was also determined. The total dose applied never exceeded 2.50 kGy (respectively 0.50 and 2.00 kGy; 1.00 and 1.50 kGy).

These experiments have been repeated using carcasses that have been stored at $-18^{o}C$ for 4 months and after being irradiated with a total dose of 2.50 kGy before and after freezing. This experiment allowed to answer the question whether the effect of a radiation treatment, either at $+5^{o}C$ or at $-18^{o}C$, could advantageously be combined with a storage period at $-18\ ^{o}C$.

8.2. Estimation of the effect of 2.50 kGy on salmonellae and Enterobacteriaceae on samples of ducks and broilers.

Duck- and broiler skin samples.

Table 37 gives the results of the experiments with neck skin samples of ducks. The radiation temperature was $+5\ ^{o}C$. The number of *Salmonella*-contaminated skin samples was decreased 3 times. At the same radiation temperature three experiments were carried out with 100 samples of broiler neck skin each, in order to determine the effect of an ionizing radiation dose of 2.50 kGy. In two experiments all samples were found negative before and after the radiation treatment. In the third experiment 1 sample was found positive before irradiation. The serotype was infantis. After the radiation treatment all samples were found negative.

Table 37. The effect of a radiation dose of 2.50 kGy on salmonellae on duck skin.

Number of samples	Dose (kGy)	Positive samples	Serotypes isolated
200	0	30	agona, albany, bredeney, enteritidis, montevideo, saint-paul, senftenberg
200	2.50	10	montevideo, senftenberg

Broiler carcasses.

Table 38 gives the results with respect to the number of deep-frozen broiler carcasses contaminated with salmonellae and the effect of a radiation treatment applied at -18°C on the number of salmonellae on these carcasses. The radiation dose was 2.50 kGy.

Table 38. Salmonella contamination of deep-frozen broiler carcasses before and after a radiation treatment with 2.50 kGy.

Slaughter-house	Number of samples	Number of positive samples			
		Skin		Thaw water	
		Dose (kGy)			
		0	2.50	0	2.50
1	30-30	0	0	3	2
2	30-30	4	0	19	0
3	30-30	2	0	4	0
4	30-30	1	0	0	0
5	20-20	0	0	0	0
6	30-30	7	0	18	2
7	30-30	6	1	8	0
8	50-50	0	1	2	1
Total	250-250	20	2	54	5

The following serotypes were isolated : before irradiation : agona (15x), heidelberg (25x), infantis (8x), montevideo (1x), newport (1x) and thompson (1x). After irradiation the following serotypes were isolated : agona (1x), heidelberg (2x), newport (2x) and thompson (2x).
The effect of a radiation dose of 2.50 kGy on the mean and the standard deviation of the logarithm of counts of the entire group of Enterobacteriaceae is given in table 39.

Table 39. Mean and standard deviation of counts of Enterobacteriaceae (log cfu per 10 cm^2 or per ml) on frozen carcasses before irradiation with a dose of 2.50 kGy

Slaughter-house	Number of samples	Skin		Thaw water	
		mean	s	mean	s
1	30-30	3.34	0.42	2.85	0.32
2	30-30	3.02	0.38	2.70	0.58
3	30-30	2.92	0.39	2.94	0.46
4	30-30	3.46	0.36	3.24	0.48
5	20-20	3.66	0.66	2.70	0.60
6	30-30	3.22	0.52	3.40	0.72
7	30-30	3.89	0.34	3.98	0.50
8	50-50	3.64	0.55	3.74	0.62

After the radiation treatment no survivors of Enterobacteriaceae were found, i.e. the cfu counts were below (log) 1.00 per 10 cm^2 of skin or per ml of thaw water. The findings indicate that with a dose of 2.50 kGy one log cycle reduction can be obtained in the number of Salmonella contaminated broiler carcasses, whereas the number of cfu of Enterobacteriaceae is decreased by 2 to 3 log cycles. The latter results were obtained for both types of samples : skin and thaw water specimens.

8.3. Determination of salmonellae on refrigerated and deep-frozen broiler carcasses before and after irradiation with 2.50 kGy.

Tables 40 and 41 show the MPN counts of salmonellae on 1,000 cm^2 skin of refrigerated carcasses, before and after a radiation treatment with 2.50 kGy. The temperature of the carcasses during the experiments was either +5 or -18 ^{o}C (± 1 ^{o}C).

Table 40. MPN counts (cfu/1,000 cm^2) of salmonellae of skin of refrigerated broiler carcasses before irradiation with 2.50 kGy obtained by two laboratories.

	Laboratory 1		Laboratory 2	
Batch	Count	Serotype	Count	Serotype
1 to 7	<2		<2	
8	<2		<2 *	
9	240	agona eimsbuettel	13	agona heidelberg
10	220	agona eimsbuettel	43	agona
11	62	agona	<2	
12	>1400	agona eimsbuettel heidelberg	2 **	agona
13	110	agona eimsbuettel	6	agona
14	16	agona eimsbuettel heidelberg	5	eimsbuettel
15	160	eimsbuettel heidelberg	<2	
16	390	agona	8	agona
17	23	montevideo	3	montevideo
18	5	montevideo	3	montevideo
19	8	montevideo	13	montevideo
20	6	montevideo	3	montevideo
21	6	montevideo Salmonella sp.	3	montevideo
22	9	montevideo	6	heidelberg montevideo
23	6	montevideo	3	montevideo
24	6	montevideo	<2	

After irradiation all samples yielded <2 salmonellae per 1,000 cm^2 of skin, except for those marked * and **

Table 41. MPN counts (cfu/1,000 cm^2) of salmonellae on samples of skin from deep-frozen (-18°C) broiler carcasses before irradiation with 2.50 kGy

Batch	Count	Serotype	Batch	Count	Serotype
1	9	infantis	14	23	agona
2	11	agona			infantis
		eimsbuettel	15	6	agona
3	160	agona			infantis
4	23	agona	16	94	agona
		eimsbuettel			infantis
5	62	agona	17	23	agona
		infantis			oranienburg
6	<2		18	<2	
7	23	agona	19	<2	
		infantis	20	23	agona
8	16	agona	21	2	infantis
		infantis	22	<2	
9	110	agona	23	5	infantis
10	9	agona	24	3	infantis
11	16	agona	25	3	agona
		infantis	26	2	agona
12	36	agona	27	2	agona
		infantis	28	34	agona
13	11	agona			
		infantis			

After irradiation with 2.50 kGy at +5 °C two samples were Salmonella-positive. The serotypes isolated were agona and infantis. No Salmonella-positive samples were found on the skin after a radiation treatment at -18 °C. Table 42 gives the approximate 95% two-side confidence intervals of the MPN counts in table 40.

Table 42. Approximate 95% two-side confidence interval of MPN counts of salmonellae on skin samples of refrigerated broiler carcasses before irradiation with 2.50 kGy

Laboratory 1 Batch	Number of salmonellae / 1000 cm^2		
	lower bound	estimate	upper bound
9	75	240	765
10	69	220	701
11	19	62	198
12	439	>1400	>4463
13	35	110	351
14	5	16	51
15	50	160	510
16	122	390	1243

Similar results of MPN's of Salmonella in broilers were recently reported in literature (306).

8.4. Estimation of the effect of irradiation at +5 °C, deep-freezing and irradiation at -18 °C with a total dose of 2.50 kGy on Enterobacteriaceae and salmonellae.

The results of these experiments are presented in tables 43 and 44. On the broiler carcasses which were irradiated with 0.50 kGy and 1.00 kGy at a temperature of +5 °C, deep-frozen at -18 °C and irradiated again at -18 °C with 2.00 kGy and 1.50 kGy, respectively, no surviving Enterobacteriaceae were detected. The Salmonella isolations are summarized in table 44. As could be expected salmonellae were isolated from carcasses which had been treated with a maximum dose of 1.00 kGy.

Table 43. The effect of irradiation, deep-freezing and irradiation on the Enterobacteriaceae (log cfu/ml or 10 cm^2) on broiler carcasses (total dose 2.50 kGy)

Dose (kGy)	Skin		Thaw water	
	refrigerated after thawing		refrigerated after thawing	
0	3.56	3.36	3.54	3.07
0.50	1.23 (+)	1.52 (+)	- (+)	- (+)
1.00	- (-)	1.83 (-)	- (-)	- (-)

- = below 1.00; (+) = positive after enrichment; (-) = negative after enrichment

Table 44. Salmonella isolations from broiler carcasses after different treatments

Total dose applied 2.50 kGy

Dose (kGy)	Skin	Thaw water
0	serotype : thompson	serotype : thompson
0.50	: thompson	: saint-paul
		: thompson
1.00	: thompson	: thompson

Table 45. Means and standard deviations of Enterobacteriaceae counts (log cfu/ 10 cm^2 or ml) after different treatments and storage at -18°C

Storage (month)	Refrigerated carcasses		Frozen carcasses				Frozen + irradiated, irradiated + frozen, dose 2.50 kGy
	skin	s	skin	s	thaw water	s	
0	5.43	0.52	6.37	0.26	5.96	0.43	
1			5.69	0.77	4.27	0.35	No Enterobacteriaceae
2			5.69	0.64	5.52	0.22	were detected
3			5.45	0.72	3.85	0.32	
4			5.02	1.11	3.73	0.58	

a. the samples may not have been representative for the whole poultry production
b. no data on the initial Salmonella contamination level of the sampled carcasses are available.

The results of slaughter-house 8, where a Salmonella-positive skin sample was found after the radiation treatment, despite a negative outcome of the examination of the non-radiated specimens, may have been caused by chance. The probability of this particular result could not be assessed, though a general model using the parameters given in 4.7.1. to assess probabilities of the lethality of a radiation treatment with a dose of 2.50 kGy is presented in 8.6.

After irradiation salmonellae were isolated from two out of a total of 48 pooled samples of refrigerated carcasses. These salmonellae were isolated from samples with a low degree of contamination before irradiation. This finding is not so surprising as :

1. the carcasses are not uniformly contaminated
2. the radiation dose applied allows survivors as could be anticipated from previous experiments.

The findings in table 40 for the untreated samples of skin from batches 9-24 show that laboratory 1 yielded systematically higher counts than laboratory 2. As the sign-test for the differences of laboratory counts was highly significant ($P<3 \times 10^{-4}$), it was concluded that the differences found are not due to random variation. As far as could be ascertained no systematical difference existed between the various procedures followed. The results of laboratory 2 for the batches 8 and 12, before and after irradiation, show that after the radiation treatment with 2.50 kGy Salmonella-positive samples can be found. It can be excluded that the reason for these findings lies in a laboratory contamination as suggested in literature (145).

Results of previously described experiments indicate an effect on D_{10}-values of both Salmonella panama and Escherichia coli of:

1. the culture media used before and after irradiation;
2. the radiation temperature;
3. the substrate in which irradiation took place.

However, no effect on D_{10}-values could be ascertained for cell concentration and oxygen partial pressure.

D_{10}-values of test strains were found to comprise a certain range; duplicate tests often gave substantially varying D_{10}-values.
In case of artificially Salmonella panama-contaminated carcasses, a protective action against ionizing radiation provided by broiler skin was demonstrated. This was not found in experiments in which Escherichia coli was used.
Applying the 7 D_{10}- concept here would lead to a required dose of approx. 9.10 kGy. On an average 100 cfu of Salmonella were present per pooled sample of 5 half carcasses. The approach to fix N_f, i.e. the absence of Salmonella in the specimens after irradiation at -18°C would result in a radiation dose of approx. 2.50 kGy. The finding of two Salmonella-positive samples after irradiation with 2.50 kGy indicates that other factors interfere. In the first place there is a variance in the number of cfu's of Salmonella: local N_0 values higher than 1,000 are possible. Secondly the probability of finding Salmonella-positive samples after irradiation can not be exactly calculated as the distribution of the number of salmonellae in skin samples of 5 half broiler carcasses and the distribution of the salmonellae on the skin of a group of carcasses are not known. In 8.6. a model is presented, by which this probability is assessed taking into account several assumptions regarding the parameters which influence the lethality of a radiation treatment.
It is questionable whether the MPN method as applied could have influenced the findings. Almost 75 percent of the primary macerate was used for the dilution series. It may therefore be that the usual assumption of the MPN method, i.e. that the number of salmonellae in test tubes are independently (Poisson) distributed, no longer holds. In case of dilution series, the number of salmonellae in the various test tubes and the remaining part of the primary macerate is assumed to follow a multinomial distribution. A simulation study (367) was performed to find out whether the MPN method could give usefull results under a multinomial model.
For fixed concentrations of salmonellae in the primary macerate the dilution series used were simulated under a Poisson model as well as a multinomial model for the numbers of salmonellae in the test tubes. The simulation study did not show differences between both situations; therefore the MPN method as applied gave valid results for the dilution series used.

The fact that no Salmonella survivors were found in irradiated deep-frozen carcasses seems to contradict reports in literature (207), where it was shown that a radiation treatment at freezing temperatures was less effective than the same dosage at refrigeration temperatures (see also chapters 6 and 7).
Possibly the lethal effect of frozen storage induces a stronger lethality of later irradiation on non-sporing microorganisms.
From these experiments it is evident that the use of a radiation treatment with a dose of 2.50 kGy (the maximum dose approved by the Dutch Health Council for refrigerated poultry carcasses is 3.00 kGy; nowadays 10.00 kGy is accepted by the World Health Organization) considerably contributes to the elimination of salmonellae from broiler carcasses, but that no 100% Salmonella-free product can be guaranteed. A reduction in total Enterobacteriaceae counts of three log cycles can be obtained. As the Salmonella contamination of the broiler carcasses was low, it can be expected that by a treatment with 2.50 kGy, the Salmonella hazard at the slaughtered product is decreased considerably.

Estimation of the effect of irradiation at +5°C, deep-freezing and storage at -18°C irradiation and storage at -18°C with a dose of 2.50 kGy.
The effect of irradiation on refrigerated and deep-frozen carcasses with respect to the numbers of Enterobacteriaceae is evident. Also after storage at -18 °C for 4 months no cfu of Enterobacteriaceae was detected. In this respect there was a decrease in cfu found on the skin and also in the thaw water during the frozen storage period.
During the 4 months' storage period the group of deep-frozen carcasses proved Salmonella-positive every month, whereas the irradiated carcasses, irradiated in the refrigerated or frozen condition, were negative after 3 months (table 46).
No salmonellae could be found with any of the isolation methods described before. Among refrigerated carcasses a relatively low percentage positives was estimated. It is evident that more salmonellae are isolated from thaw water than from skin samples. This can be caused by the fact that the thaw water samples were examined undiluted whereas the skin samples had to be diluted 1:5 in peptone saline solution. A reason may also be the attachment of bacteria to the skin, not detaching during enrichment of the samples, although this is not likely because of the bile salts present and the high temperature (43 °C) applied during enrichment.

A factor may also be the fact that the presence of a competitive microflora consisting of Escherichia coli, Citrobacter, Proteus and Pseudomonas species interferes with the detection of Salmonella on refrigerated carcasses.

The irradiation of carcasses under refrigerated conditions seems to give higher lethality than applying the same process under frozen conditions. This is generally reported in literature, when the influence of temperature on radiation sensitivity is studied (207). However, the differences found in these experiments (irradiation at +5°C versus -18°C) had no statistical significance.

Irradiation and subsequent storage of the carcasses at -18°C leads to a Salmonella-free product within 3 months. Therefore this combined process is a very promising method in attempts to reduce the dissemination of Salmonella.

8.6. A model aiming at the assessment of the lethal effect of irradiation on salmonellae in broiler carcasses.

Several parameters exert a distinct effect on the lethality of a radiation treatment of Salmonella in broiler carcasses. To estimate the probability [P(0)] of finding a Salmonella-positive carcass before and after irradiation with a dose of 2.50 kGy it was tried to appraise the numerical value of the parameters given in 4.7.1. In the model, assumptions were made regarding the following parameters:

1. The average number of cfu's of Salmonella in carcasses.
   Results of experiments reported in tables 40, 41 and 42 were used.
   Average cfu's of Salmonella of 2, 10, 60 and 1,000 per carcass were assumed.
2. The lethality of irradiation.
   D_{10}-values given in chapters 6 and 7 were used; they ranged from 0.30 to 1.30 kGy; the lethal effect of a dose of 2.50 kGy therefore being approx. 8 to 2 log cycles. In the model lethalities of 1, 2 and 3 were used.
3. The Salmonella-detection level achieved by the Salmonella-isolation method employed.
   As no data were available it was postulated that if Salmonella is present, 1 or more cfu's, 2 or more cfu's and 4 or more cfu's may be detected.

The computations were made with the following assumptions:

1. The average numbers of cfu's of Salmonella per carcass (μ) are Poisson distributed.
2. The lethality is named Λ. The probability of survival is $1 - \Lambda$. It is assumed that ionizing radiation hits every bacterial cell in the same way.
3. Assuming k cfu's of Salmonella in the carcass, the survivors 1 are binomial distributed, with parameters k and Λ.
4. The skin or thaw water sample examined contain all salmonellae present.

With these assumptions it is possible to assess the probability [P(0)] of finding a Salmonella-positive carcass in an a-selectly chosen number of carcasses.

$$P(0)(\text{before irradiation}) = \frac{e^{-\mu}\,\mu^{1}}{1!} \qquad ; 1 = 0,1,2,\ldots$$

$$P(0)(\text{after irradiation}) = \frac{e^{-\mu(1-\Lambda)}((1-\Lambda)\mu)^{1}}{1!} \qquad ; 1 = 0,1,2,\ldots$$

From the latter formula it can be concluded that the number of surviving cfu's of Salmonella is Poisson distributed with an average $(1-\mu)$.

If it is assumed that the Salmonella-isolation methods needs 2 or more cfu's, 3 or more cfu's and 4 or more cfu's, the formula for P(0) is :

$$P(0)(\text{before irradiation}) = 1 - \sum_{0}^{4} \frac{e^{-\mu}\,\mu^{1}}{1!}$$

$$P(0)(\text{after irradiation}) = 1 - \sum_{0}^{4} \frac{e^{-\mu(1-\Lambda)}((1-\Lambda)\mu)^{1}}{1!}$$

Table 47 gives the probabilities [P(0)] of finding a Salmonella-positive sample following the model assumptions.

Table 47. Probabilities [P(0)] of finding a Salmonella-positive sample following the model assumptions

Average cfu of Salmonella (μ)		1 - Λ		
		0.1	0.01	0.001
Salmonella-isolation method detects 1 or more cfu's				
2	before irradiation	0.86	0.86	0.86
	after irradiation	0.18	0.02	0.00
10	before	1.00	1.00	1.00
	after	0.63	0.10	0.00
60	before	1.00	1.00	1.00
	after	1.00	0.45	0.06
1,000	before	1.00	1.00	1.00
	after	1.00	1.00	0.63
Salmonella-isolation method detects only 2 or more cfu's				
2	before	0.32	0.32	0.32
	after	0.00	0.00	0.00
10	before	1.00	1.00	1.00
	after	0.08	0.00	0.00
60	before	1.00	1.00	1.00
	after	0.94	0.02	0.00
1,000	before	1.00	1.00	1.00
	after	1.00	1.00	0.08
Salmonella-isolation method detects only 4 or more cfu's				
2	before	0.05	0.05	0.05
	after	0.00	0.00	0.00
10	before	0.97	0.97	0.97
	after	0.00	0.00	0.00
60	before	1.00	1.00	1.00
	after	0.71	0.00	0.00
1,000	before	1.00	1.00	1.00
	after	1.00	0.93	0.00

The data in table 47 demonstrate very clearly that Salmonella-isolation methods exert a profound effect on the probability [P(0)] of finding a Salmonella positive sample. If it is assumed that the average cfu's of Salmonella per carcass is 60, and 1 - Λ = 0.001 (a 3 D_{10}- concept, corresponding with approx. D_{10} = 0.80 kGy at a dose of 2.50 kGy), 6 out of 100 carcasses will be found Salmonella-positive in case a Salmonella-isolation method is used capable of detecting 1 or more cfu's.

With a Salmonella-detection level of only 4 or more cfu's no Salmonella-positive samples will be found after irradiation with a dose of 2.50 kGy. Another situation exists assuming μ = 1,000 cfu's of Salmonella. P(0) after irradiation in case of a Salmonella-detection level of 1 or more cfu's = 0.63; a Salmonella-detection level of 4 or more cfu's would give 0.00.

Following the model assumptions it seems that the variance in average numbers (μ) of Salmonella cfu's per carcass and the variance in D_{10}-values as estimated in chapters 6 and 7, are less important than the Salmonella-detection level attained by the Salmonella-isolation method employed. This will, of course, vary from procedure to procedure. It may also fluctuate within a given technique and even within a given method applied in the same laboratory. It depends strongly on the equilibrium, during resuscitation and enrichment, between salmonellae and competing bacteria. The model presented here may be a start to appraise the numerical value of several parameters influencing the lethality of a radicidation treatment. From those values the lethality Λ of any radicidation process can be computed, and hence the degree of consumer protection attained. This important grossly disturbing effect of the fluctuating sensitivity of Salmonella-isolation methods on lethality can be eliminated by relying on Enterobacteriaceae-cfu-counts (413). Residual Salmonella-survival probabilities can then be calculated from Λterms established and from experimentally assessed ε-values; cf. Section 3 (91).

CHAPTER 9

9. EFFECT OF IRRADIATION ON THE MICROBIAL ASSOCIATION OF DEEP-FROZEN AND REFRIGERATED BROILER CARCASSES

9.1. Introduction.

The treatment of broiler carcasses with different doses of ionizing radiation results in the survival of a varying part of the microflora originally present. This depends on the dose applied and the temperature during treatment; as a result microbial selection occurs (70,71), as it is also observed secondary to a treatment with antibiotics (339).

The previous chapters described results of investigations wherein the effects of ionizing radiation were studied almost exclusively on salmonellae and other Enterobacteriaceae. The use of ionizing radiation can result in an almost Salmonella-free product, but on such products the microflora can be changed in such a way that the final product becomes more dangerous to human health. Gram-negative rods are readily destroyed by radiation treatments; bacterial spores and many types of non-sporing Gram-positive rods will survive. The possibility exists that the natural presence of certain bacteria limits the development of potentially pathogenic or toxinogenic bacteria, so that when these bacteria are eliminated the growth of pathogens is encouraged (161).

In the investigation to be described, the microflora of deep-frozen and refrigerated broiler carcasses which had been treated with different doses of ionizing radiation was examined. Counts of cfu's of various groups of microorganisms on the appropriate selective and non-selective media were determined before and after irradiation.

In literature it is described (117,206) that catalase, and also pyruvate, when added to the surface of selective agar media stimulates the repair of sublethally damaged cells, so that these overcome the difficulties in terms of colony forming ability when plated on selective agar media (393).

Catalase is said to promote the repair of these damaged cells by the degradation of hydrogen peroxide formed by the cells (206) or otherwise present in the media. In this investigation the selective agar media therefore were treated with catalase to evaluate the literature findings with this particular sample material. Also some other methods for resuscitation, so-called "solid-repair" methods (282), to detect possibly stressed microorganisms, were tested.

9.2. Results.

Table 48 presents the various groups of microorganisms constituting the initial microbial community structure of the broiler carcasses used in the experiments.

Table 48. Means and standard deviations of the initial contamination of broiler carcasses. (log cfu/10 cm^2 or/ml).

	Experiment 1 (10 carcasses)				Experiment 2 (5 carcasses)			
Test	skin	s	thaw water	s	skin	s	thaw water	s
Total count 30°C	5.4	0.7	6.2	0.3	4.4	0.4	4.8	0.2
Total count 10°C	3.6	1.1	Not determined		3.4	0.6	3.6	1.0
Enterobacteriaceae	4.8	0.8	5.5	0.5	3.2	0.5	3.3	0.2
Escherichia coli	3.4	0.5	3.9	0.6	2.8	0.6	3.3	0.6
Micrococci	3.0	0.7	2.8	0.5	3.4	0.4	3.8	0.4
Staphylococcus aureus	2.0	0.1	3.0	0.4	2.0	0.1	2.8	0.4
Faecal streptococci	3.0	0.4	2.4	0.4	2.7	0.5	3.1	0.8
Yeasts and moulds	3.3	0.4	3.4	0.4	2.6	0.5	3.2	0.6
Bacillus cereus	Not determined				3.1	0.6	3.5	0.2

These carcasses were treated with doses of ionizing radiation ranging from 0 to 2.50 kGy, with steps of 0.50 kGy. Tables 49 and 50 give the results of experiments in which counts of different groups of microorganisms on selective agar media were compared with counts on the same agar media to which catalase had been added to the surface.

The results indicate no increase in recovery of Escherichia coli and micrococci compared with non-treated media. Striking, however, are the facts that 1. yeasts and moulds were indeed recovered in higher numbers on the medium with catalase and 2. Bacillus cereus was completely inhibited on the medium to which catalase had been added. This points to the presence of inhibitory substances in the catalase preparation used; it is well known that Bacillus-spores are very sensitive to inhibitors of microbial growth.

Table 49. Means and logarithmic counts (log cfu/10cm^2) of microorganisms on broiler samples after irradiation at -18°C. (5 carcasses per dose).

Microorganism	Medium	Doses applied (kGy) 0	0.50	1.00	1.50	2.00	2.50
Escherichia coli	MC	2.8	-	-	-	-	-
	MC + cat	3.1	-	-	-	-	-
Faecal streptococci	KAA	-	-	-	-	-	-
	KAA + cat	-	-	-	-	-	-
Staphylococcus aureus	BPA	-	-	-	-	-	-
	BPA + cat	-	-	-	-	-	-
Micrococci	MSA	3.4	-	-	-	-	-
	MSA + cat	4.1	-	-	-	-	-
Bacillus cereus	MEP	3.7	-	-	-	-	-
	MEP + cat	-	-	-	-	-	-
Yeasts and moulds	OYDA	-	-	-	-	-	-
	OYDA + cat	5.2	4.7	4.8	-	-	-

- indicates < 2.0

Table 50. Means and logarithmic counts (log cfu/ml) of microorganisms in thaw water of broiler carcasses after irradiation at -18°C. (5 carcasses per dose).

Microorganism	Medium	Doses applied (kGy) 0	0.50	1.00	1.50	2.00	2.50
Escherichia coli	MC	3.3	-	-	-	-	-
	MC + cat	3.2	-	-	-	-	-
Faecal streptococci	KAA	3.9	-	-	-	-	-
	KAA + cat	3.2	-	-	-	-	-
Staphylococcus aureus	BPA	-	-	-	-	-	-
	BPA + cat	-	-	-	-	-	-
Micrococci	MSA	3.8	3.1	-	-	-	-
	MSA + cat	3.9	-	-	-	-	-
Bacillus cereus	MEP	3.5	-	-	-	-	-
	MEP + cat	-	-	-	-	-	-
Yeasts and moulds	OYDA	3.2	-	-	-	-	-
	OYDA + cat	5.2	4.9	4.5	-	-	-

- indicates < 2.0

Table 51. D_{10}-values (kGy) of microorganisms on broiler skin. (5 carcasses per dose).

Microorganism	Medium	Irradiation at -18°C D_{10}	n	Irradiation at +5°C D_{10}	n
Enterobacteriacea	VRBG	-		0.33	3
	TSA + VRBG	0.54	3	0.35	3
	VRBG + cat	-		0.34	3
Escherichia coli	MC	-		0.32	3
	TSA + MC	0.55	3	0.34	3
	MC + cat	-		0.34	3
Faecal streptococci	KAA	1.22	4	0.69	3
	Pw + KAA	1.27	4	0.56	3
	KAA + cat	1.31	4	0.60	3
Staphylococcus aureus	BPA	0.53	3	0.33	3
	Pw + BPA	0.54	3	0.31	3

- = D_{10}-value could not be calculated; n = number of doses applied

Table 51 gives the results of experiments in which skin samples were examined for the presence of Enterobacteriaceae, Escherichia coli, faecal streptococci and Staphylococcus aureus. Also the effect of ionizing radiation was studied. The microbiological techniques used in this experiment were in agreement with the latest ideas on the detection of possibly stressed microorganisms (282).

No significant differences were obtained between the three different detection methods used. Irradiation at -18°C resulted in higher D_{10}-values than those obtained when the radiation treatment was performed at +5°C. In these experiments no evidence was obtained suggesting that other detection methods are needed for the enumeration of stressed microorganisms in radiated samples of broiler skin and thaw water.

Table 52 gives the results divided into subgroups of strains customarily used for further identification. As expected the percentage of Gram + cocci and Gram - rods increases with increasing radiation doses.

Table 52. Isolates from broiler carcasses used for further identification

Dose (kGy)		Skin		Thaw water	
		Number	Percentage	Number	Percentage
0	Gram + cocci	108	61.7	114	67.0
	Gram + rods	4	2.3	16	9.4
	Gram - rods	57	32.6	32	18.8
	Gram - cocci				
	Yeasts & moulds	6	3.4	8	4.8
	Total	175		170	
0.50	Gram + cocci	114	70.0	91	74.5
	Gram + rods	8	4.9	8	6.6
	Gram - rods	35	21.4	16	13.3
	Gram - cocci	1	0.6		
	Yeasts & moulds	5	3.1	7	5.8
	Total	163		122	
1.00	Gram + cocci	63	71.6	78	80.4
	Gram + rods	14	16.0	2	2.1
	Gram - rods	8	9.1	12	12.4
	Gram - cocci			1	1.0
	Yeasts & moulds	3	3.3	4	4.1
	Total	88		97	
1.50	Gram + cocci	50	76.8	45	69.2
	Gram + rods	7	10.7	7	10.7
	Gram - rods	3	4.4	9	13.6
	Gram - cocci				
	Yeasts & moulds	5	8.1	4	6.5
	Total	65		65	
2.00	Gram + cocci	30	73.2	68	84.0
	Gram + rods	5	12.1	2	2.4
	Gram - rods	2	4.9	5	6.2
	Gram - cocci				
	Yeasts & moulds	4	9.8	6	7.4
	Total	41		81	
2.50	Gram + cocci	22	75.9	37	80.4
	Gram + rods	2	6.9	4	8.7
	Gram - rods			3	6.6
	Gram - cocci				
	Yeasts & moulds	5	16.2	2	4.3
	Total	29		46	

Table 53. Microflora of broiler carcasses after irradiation

Radiation dose (kGy)	Skin	Thaw water
0	Alcaligenes viscolactis	Bacillus sp.
	Alcaligenes sp.	Escherichia coli
	Bacillus coagulans	Micrococcus sp.
	Bacillus sp.	Proteus mirabilis
	Enterobacter cloaca	Salmonella albany
	Enterobacter liquefaciens	Streptococcus sp.
	Enterobacter sp.	
	Escherichia coli	
	Lactobacillus plantarum	
	Lactobacillus sp.	
	Micrococcus sp.	
	Pseudomonas fluorescens	
	Pseudomonas sp.	
	Streptococcus faecalis	
0.50	Alcaligenes faecalis	Bacillus sp.
	Alcaligenes sp.	Escherichia coli
	Arthrobacter citreus	Micrococcus sp.
	Bacillus coagulans	Salmonella newport
	Enterobacter sp.	Streptococcus sp.
	Escherichia coli	
	Lactobacillus sp.	
	Micrococcus sp.	
	Pseudomonas fluorescens	
	Pseudomonas sp.	
	Salmonella albany	
	Salmonella infantis	
	Salmonella newport	
	Salmonella sp.	
	Streptococcus faecalis	
1.00	Bacillus sp.	Bacillus sp.
	Escherichia coli	Escherichia coli
	Micrococcus sp.	Micrococcus sp.
	Streptococcus sp.	Streptococcus sp.
1.50	Alcaligenes sp.	Bacillus sp.
	Bacillus sp.	Micrococcus sp.
	Escherichia coli	Pseudomonas vesicularis
	Micrococcus sp.	Pseudomonas sp.
	Streptococcus faecalis	Streptococcus sp.
	Streptococcus sp.	
2.00	Alcaligenes sp.	Bacillus sp.
	Bacillus sp.	Micrococcus sp.
	Micrococcus sp.	Pseudomonas vesicularis
	Streptococcus sp.	Streptococcus sp.
2.50	Bacillus sp.	Bacillus sp.
	Micrococcus sp.	Micrococcus sp.
	Streptococcus sp.	Streptococcus sp.

At the same time Gram - rods decrease and are only sporadically found after higher radiation doses. This applies both to skin and to thaw water samples.

Table 53 gives more detailed taxonomic information on the microflora detected on the carcasses after irradiation with a certain dose. Striking in view of their D_{10}-values is the presence of Escherichia coli in skin samples after a radiation dose of 1.50 kGy and of Escherichia coli and Pseudomonas species in thaw water samples after a radiation treatment with 2.00 kGy and 1.50 kGy respectively.

For carcasses which had been treated with a radiation dose of 2.50 kGy the microflora of the skin samples and of the thaw water samples consisted of bacilli, micrococci, streptococci and yeasts and moulds. The yeasts and moulds were not further identified.

Table 53. Development of microorganisms (mean of counts log cfu/10 cm^2) on thawed radiated (2.50 kGy) carcasses during storage at +7°C

Experiment 1	Skin samples (5 carcasses per sampling)			
Storage (days)	PCA 30	PCA 10	VRBG	OYDA
1	4.4	2.2	1.0	1.0
2	3.2	2.8	2.0	2.0
3	4.8	3.9	2.0	2.8
4	4.9	4.2	2.2	3.3
5	6.2	5.1	1.8	3.4
6	6.0	5.9	2.6	4.5
7	5.6	5.1	2.6	4.6
Experiment 2	Skin samples (5 carcasses per sampling)			
5	5.7	5.2	1.7	4.4
6	6.3	5.2	2.8	4.8
7	6.9	6.5	4.1	5.4
8	6.4	7.4	5.7	6.4
10	8.6	8.4	4.1	7.3

Carcasses were negative for Salmonella, Staphylococcus aureus and Clostridium perfringens; faecal streptococci were found after 7 days of storage in experiment 2.

Table 54 shows the development of cfu's of microorganisms on thawed radiated (2.50 kGy) carcasses during storage at +7 °C.

The increase in bacterial counts during storage at +7°C is comparable to that observed on untreated carcasses. There were no isolations of Salmonella, Staphylococcus aureus and Clostridium perfringens and only sporadically Streptococcus species were found on the carcasses.

9.3. Discussion.

There is a considerable lethal effect of ionizing radiation on the total microflora present on skin or in thaw water when applied to the carcasses. The total counts on the products treated with a dose of 2.50 kGy were between 100 and 1,000 cfu per 10 cm^2 or per ml. Mainly micrococci and yeasts and moulds were recovered after treatment with this dose. In some cases faecal streptococci were found. The number of these bacteria present on the carcasses was below 100 cfu per 10 cm^2 or per ml.

After a radiation treatment with 0.50 to 2.50 kGy no surviving cfu could be counted. Therefore no D_{10}-values were calculated. From the experiments it became evident that total aerobic counts could not be used to describe the lethality of the radiation process. A reason may be that the effect of ionizing radiation on mixed populations, as with the total of aerobic microorganisms present on the carcasses, results in irregular destruction curves. With every dose applied the total count depends on the fraction of relatively more resistant microorganisms.

With increasing radiation doses, sometimes more surviving cfu were counted. One of the reasons for this phenomenon may be the fact that for every radiation dose 5 different carcasses were used. This finding may therefore be caused by the variation in the microbiological counts on carcasses.

No stimulating effect as indicated in literature was observed in the enumeration of Staphylococcus aureus and Salmonella in case catalase was added to the surface of the selective agar media. The mechanism of the beneficial effect of catalase and the practical consequences for the enumeration of yeasts and moulds in foods should be studied in more detail in future.

This also applies to Bacillus cereus cfu's which were completely inhibited on the catalase treated media. The presence of inhibitory substances in the catalase solution may have caused this effect.

Solid medium repair methods (282), which were tested here to detect stressed microorganisms, did not result in significantly different D_{10}-values. As expected the use of ionizing radiation resulted in a microbial selection on the broiler carcasses.

However, no microorganisms were isolated which had not yet been found on the carcasses before the treatment. From this point of view no objection against the use of ionizing radiation of carcasses can be levelled. The surviving microflora of the carcasses consisted of bacilli, micrococci, streptococci and yeasts and moulds. Striking was the fact that Escherichia coli and a Pseudomonas species were present on the skin or in the thaw water after the application of high (1.50 to 2.50 kGy) doses of irradiation. According to their D_{10}-values these bacteria should already have been destroyed completely after a lower dose.

Discussions in previous chapters have indicated the various factors involved.

The final counts on the radiation treated carcasses are so low that no dangerous effect can be expected. Also no dangerous effect can be expected in case radiation treated frozen carcasses are thawed and stored at refrigeration temperatures. The development of the different groups of microorganisms during storage of the carcasses was comparable to the spoilage pattern of non-treated broiler carcasses.

CHAPTER 10

10. SUMMARY OF RESULTS, CONCLUSIONS AND PRACTICAL CONSEQUENCES

Validity of methods

Experiments were carried out in which it was assessed which Salmonella isolation method is the most productive one in the examination of broiler carcasses.

Refrigerated, refrigerated and radiated (2.50 kGy), frozen and frozen and radiated (2.50 kGy) samples of broilers were examined. After evaluation of all results it was concluded that the following method was the most productive one:

1. pre-enrichment in buffered peptone water at 37°C for 20 hours
2. enrichment in selenite cystine broth at 37°C and at 43°C for 24 hours
3. selective plating on brilliant green sulphapyridine agar and XLD-agar at 37°C for 24 hours

From this study it could not be concluded that other methods were not sufficiently reliable to be applied in other situations.

However, it became clear that when sublethally damaged salmonellae are expected to be present, and when, as a result of the treatment, a different competitive microflora is present, adapted isolation methods should be used.

The method chosen for Salmonella-isolation in the experiments described in this thesis was the ISO 3565/1975 reference method for isolation of salmonellae from meat and meat products. This method was ultimately preferred because of the experience obtained for many years in routine analyses.

In two instances other conditions than prescribed by the ISO-method were used; i.e. the samples were incubated at 37 °C or 43°C for 24 hours. The ISO-method as well as its slight modification proved to be reliable methods in the experiments reported.

Escherichia coli versus Salmonella.

The experiments have shown that the Escherichia coli K12 NDA strain tested can not be used as index microorganism for Salmonella : in this case Salmonella niloese and Salmonella panama on broilers, though in liquid and solid culture media the D_{10}-values of these three microorganisms were similar.

The experiments with pure cultures and with artificially contaminated carcasses showed that the following factors influenced D_{10}-values :

1. the culture media used before, during and after irradiation;
2. the temperature during irradiation;
3. the physiological age of the bacteria.

No influence on D_{10}-values could be observed from :

1. the initial cell concentration;
2. the oxygen partial pressure.

The results of the experiments showed that after a radiation treatment with 2.50 kGy Salmonella-positive samples were found; in those cases the numbers of cfu's of Enterobacteriaceae were below the detection limit. This indicates that no other microorganism or groups of microorganisms can consistently serve as the sole index microorganism(s) for Salmonella.

Effect of 2.50 kGy on salmonellae and Enterobacteriaceae.

Poultry carcasses were found frequently contaminated with salmonellae. Per carcass a maximum number of 1,400 cfu per 1,000 cm^2 skin were estimated by a MPN method. However, 90% of the total of the examined carcasses in this specific experiment contained less than 100 cfu's per 1,000 cm^2. Similar data are given in literature (161,200,260,306).

A radicidation treatment with a dose of 2.50 kGy markedly reduced the total number of Salmonella-positive carcasses, as well as the number of salmonellae per carcass.

This dose, however, could not guarantee a Salmonella-"free" product. The number of Salmonella contaminated carcasses was reduced tenfold (table 38). After irradiation at $+5^{o}C$ with a dose of 2.50 kGy two series of pooled samples of broiler skin were found Salmonella-positive by a MPN method. The serotypes isolated were agona and infantis.

Before the radiation treatment these samples proved to be very sparsely contaminated with salmonellae (numbers of 2 cfu per 1,000 cm^2).

It was shown in previous experiments that the D_{10}-values of these two serotypes were comparable to other serotypes, so the two did not belong to a radio-resistant group.

Salmonella-positive samples which were observed in experiments in which they were expected to be absent, according to their estimated D_{10}-values, were also found in :

1. experiments in which the resulting microflora after certain radiation treatments was identified;
2. experiments in which the effect on Salmonella of freezing and storage of radiated carcasses was studied.

In some experiments artificially contaminated carcasses were used. In the case of Salmonella niloese and Salmonella panama a protective action was afforded by the carcass skin. This resulted in D_{10}-values of 1.07 and 1.29 kGy respectively, when irradiation was carried out at -18 °C and in D_{10}-values of 0.62 and 0.67 kGy at +5 °C.

The D_{10}-values of Salmonella panama estimated in skin samples were found higher ($P<0.01$) than the values which were estimated under optimal conditions in liquid culture media, when the D_{10}-value was 0.52 kGy. Different factors as discussed earlier exert an influence on D_{10}-values and therefore on the lethality of the radiation treatment. Only speculations can be made on the mechanism of the protective action of skin. Perhaps salmonellae meet less competition from the naturally present microflora than Escherichia coli, resulting in a stronger attachment to the skin. It is possible that this protection is provided by skin components or by extracellular polysaccharides which are produced by attached bacteria (14,216).

A model was designed to assess the probability of survival after irradiation. Following the model assumptions it was shown that the detection level of the Salmonella-isolation methods employed was very important in the finding of Salmonella-positive samples after irradiation, and hence the estimated lethality of a given radiation treatment. Several bacteria, such as species of Alcaligenes, Escherichia, Pseudomonas and Salmonella were found in radiation treated products in which they were not expected according to their D_{10}-values.

The application of low doses of ionizing radiation followed by freezing and storage of the products during a certain period had more success in reaching the final goal : a Salmonella-"free" end product.

To demonstrate a correlation between the number of Enterobacteriaceae and the number of salmonellae present per carcass, also Enterobacteriaceae cfu counts were assessed.

Surprising was the presence of Salmonella cfu in samples of broiler skin on which the number of Enterobacteriaceae cfu's were below 10 per 10 cm^2 of skin. This effect was not found in samples of thaw water.

A difference in lethal effect on salmonellae was not found between irradiation at +5 °C and at -18 °C. In literature (207) proof of differences in lethal effects of irradiation carried out at different temperatures have been reported.

The results obtained with these experiments point to a preference for the application of the radiation treatment at +5 °C, but the statistical superiority over the other results was not proven.

By means of estimation of the number of cfu's of Enterobacteriaceae no answer to this question could be obtained. All counts of cfu's proved less than 10 per 10 cm^2 or per ml. After a three months' storage period at -18°C both groups of radiation treated carcasses were Salmonella-negative. This was not the case with untreated carcasses, from which Salmonella could be isolated during the whole storage period.

In summary, it can be said that irradiation of broiler carcasses with a dose of 2.50 kGy results in a decrease of the total number of Salmonella contaminated carcasses as well as of the number of salmonellae per carcass. As the experiments showed very promising results with these combined treatments, further investigations should be made in the field of the application of the combined use of freezing and irradiation.

Surviving microflora.

The microflora surviving after irradiation with 2.50 kGy will not cause unexpected problems as demonstrated by the nature and the composition of the microflora. The residual "total" count of 100 - 1000 cells per 10 cm^2 or per ml consists of bacilli, micrococci, streptococci, yeasts and moulds. Sporadically an Enterobacteriaceae was also isolated.

No stimulating effect as suggested in literature was observed in the enumeration of various microorganisms, especially Staphylococcus aureus and Salmonella, when catalase was added to the surface of the selective agar media. The mechanism of the beneficial effect of catalase on the enumeration of yeasts and moulds and of the inhibitive effect on cfu's of Bacillus cereus should be studied in more detail in future.

Solid medium repair methods used in the enumeration of stressed microorganisms did not result in better recoveries of the microorganisms investigated.

The presence of Salmonella in the end product after irradiation with 2.50 kGy shows that this dose does not guarantee a Salmonella-"free" end product. The question arose which dose is needed to guarantee this. The broiler skin and irradiation in the frozen condition provided a protection of Salmonella against irradiation. The highest D_{10}-value found was approx. 1.29 kGy. If the 7 D_{10}- concept is applied a dose of approx. 9.00 kGy at -18 °C is needed for thaw water.

This dose is rather high, but nowadays doses up to 10.00 kGy are accepted by international organizations. In case of poultry products it is expected that at this dose quality defects will occur.

If "absence" of Salmonella in radiated skin samples is required a dose of 3.90 kGy is needed with irradiation at -18°C and a dose of 1.95 kGy at +5°C. The data presented, however, show that such high doses are not necessary. Experiments have shown that the number of salmonellae per carcass will be lower than 100 cfu. An additional storage period for 1 to 3 months at -18 °C leads to a decrease in the number of salmonellae per carcass. In literature (259) it was shown that freezing resulted in an 0.5 log cycle reduction of the number of salmonellae cfu. The application of a radiation dose of 2.50 kGy does not guarantee a Salmonella-"free" end product. The microbiological quality, with respect to total counts and to the presence of potentially pathogenic microorganisms, is improved in comparison with the untreated slaughtered product.

Practical consequences.

The application of a radiation dose of 2.50 kGy was not sufficient to destroy all salmonellae present per carcass.

The surviving microflora of the carcasses consisted of bacilli, micrococci, streptococci, yeasts and moulds. The final counts on the radiation treated carcasses were so low that no dangerous effect can be expected.

Salmonella-contaminated broiler carcasses contained on an average 100 cfu's per carcass (minimum : 2, maximum : 1,400).

After irradiation only 2 series of pooled skin samples contained 2 cfu. D_{10}-values for Salmonella panama after irradiation at +5°C and at -18 °C were 0.67 kGy and 1.29 kGy respectively.

As can be seen from these D_{10}-values and from the number of Salmonella cfu's per carcass, generally, the carcasses will be Salmonella-negative after irradiation with 2.50 kGy. An extra effect on the number of Salmonella-positive carcasses as well as on the number of salmonellae per carcass can be obtained by freezing and additional storage for several months' at -18 °C.

An extrapolation to the whole Dutch broiler production of the lethal effect of the radiation treatment as measured in the experiments described may not be made, because of uncertainty with respect to the variance in average numbers of Salmonella cfu's per carcass, the variance in D_{10}-values and the Salmonella detection level obtained by the Salmonella-isolation method used.

Nevertheless the results presented here indicate that in case the whole Dutch broiler production (approx. $3.0 \cdot 10^7$ carcasses) is treated with a dose of 2.50 kGy and assuming a D_{10}-value of 0.80 kGy, the maximal result would be 1 positive carcass in 55 carcasses, which means that the number of Salmonella contaminated carcasses is decreased 14 times.

Additional storage for several months' at -18°C affords an extra beneficial effect to this respect.

A combined treatment of freezing and irradiation can be recommended to reduce the dissemination of Salmonella.

CHAPTER 11
HOOFDSTUK 11

11. SAMENVATTING VAN DE RESULTATEN, KONKLUSIES EN PRAKTISCHE KONSEKWENTIES

11. SAMENVATTING VAN DE RESULTATEN, KONKLUSIES EN PRAKTISCHE KONSEKWENTIES.

Methodologie.

Proeven werden uitgevoerd om vast te stellen welke methode voor de isolatie van Salmonella bacterien het best toepasbaar is voor de bepaling van kolonie vormende eenheden (kve's) van deze bacterien op het geslachte eindprodukt. Hiertoe werden gekoelde en diep-bevroren slachtkuikens al of niet bestraald met een dosis van 2.50 kGy onderzocht. Na verwerking van de resultaten werd gekozen voor de volgende methoden :

1. voorophoping in gebufferd pepton water gedurende 24 uur bij 37°C;
2. ophoping in seleniet cystine bouillon gedurende 24 uur bij 37°C en bij 43°C;
3. bepaling van kve's op briljantgroen fenolrood agar en op XLD-agar, inkubatie gedurende 24 uur bij 37°C.

Uit deze resultaten kan niet de konklusie worden getrokken, dat de andere methoden onder andere onderzoeksomstandigheden niet betrouwbaar zouden zijn. De konklusie, die wel uit deze studie kan worden getrokken is, dat indien sublethaal beschadigde kve's van Salmonella bacterien worden verwacht en indien er ten gevolge van een bepaalde behandeling een anders samengestelde konkurrerende microflora aanwezig is, aangepaste isolatie methoden kunnen worden toegepast.

In alle overige proeven, die in dit proefschrift zijn beschreven, werd voor de isolatie van kve's van Salmonella bacterien gebruik gemaakt van de methode omschreven in het ISO 3565/1975 voorschrift. Deze methode werd toegepast vanwege de praktische ervaring, die hiermede gedurende vele jaren bij routinematig en toegepast onderzoek werd verkregen. In enkele gevallen werden de monsters slechts 24 uur in het ophopingsmedium bij 37°C of 43°C bebroed.

De ISO 3565/1975 methode bleek een betrouwbare methode te zijn tijdens de proeven.

Escherichia coli versus Salmonella.

De proeven hebben aangetoond dat Escherichia coli K12 NDA onder proef-omstandigheden niet kan worden gebruikt als index microorganisme voor Salmonella : in dit onderzoek voor Salmonella niloese en Salmonella panama. Bij proeven, waarbij de decimale reduktie waarde (D_{10}) in vloeibare en vaste media werd bepaald, bleken de D_{10}-waarden van genoemde microorganismen goed met elkaar overeen te stemmen.

De resultaten van de proeven met reinkultures en met kunstmatig besmette karkassen toonden aan, dat de volgende faktoren de D_{10}-waarden beinvloeden:

1. de kultuurmedia waarin de bacterien voor, tijdens en na de bestralingsbehandeling werden gekweekt;
2. de temperatuur tijdens de bestralingsbehandeling;
3. de fysiologische leeftijd van de bacterien.

Er kon geen invloed worden vastgesteld van :

1. de koncentratie van de aanwezige bacterien;
2. de aanwezigheid van zuurstof in het medium.

Salmonella positieve monsters werden bepaald in huidmonsters, die waren behandeld met een stralingsdosis van 2.50 kGy. In die gevallen bleek het niet mogelijk kve's van de Enterobacteriaceae aan te tonen.

De Escherichia coli stam vertoonde een duidelijk ander gedrag ten opzichte van ioniserende straling op kunstmatig besmette kuikens dan de twee Salmonella stammen. Aanwijzingen werden verkregen, dat er geen microorganisme of groepen van microorganismen zijn gevonden, die als index organisme(n) voor salmonellae kunnen dienen.

Het effekt van een behandeling met 2.50 kGy op salmonellae en Enterobacteriaceae.

Het geslachte eindprodukt bleek besmet met salmonellae.

Per karkas werden met behulp van een Most Probable Number (MPN) methode tussen de 2 en 1400 kve's per 1000 cm^2 huid aangetoond. 90% van de onderzochte karkassen bevatte echter minder dan 100 kve's per 1000 cm^2huid. Ook in de literatuur worden dergelijke aantallen gegeven (161,200,260,306). Een pasteurisatie behandeling met ioniserende straling met een dosis van 2.50 kGy gaf een aanzienlijke reduktie van het aantal met Salmonella besmette karkassen, terwijl tevens het aantal salmonellae per karkas afnam. Deze dosis bleek echter niet afdoende om een Salmonella-vrij produkt te verkrijgen. Het aantal met Salmonella besmette karkassen nam door de bestralingsbehandeling met een faktor 10 af (tabel 38).

Na de bestraling met 2.50 kGy bij +5°C bleken twee series huid-mengmonsters Salmonella positief. De bepaling hiervan werd met een MPN methode uitgevoerd. De serotypen waren agona en infantis. Voor de bestralingsbehandeling bleken deze monsters met zeer geringe (2 per 1000 cm^2) aantallen kve's van Salmonella besmet. Uit voorgaande proeven, waarbij D_{10}-waarden werden bepaald, was gebleken dat de resistentie van genoemde serotypen tegen ioniserende straling niet afwijkend was van de overige onderzochte serotypen, zodat niet kan worden gesproken van twee vertegenwoordigers van een stralingsresistente groep Salmonella bacterien. Salmonella positieve monsters, waar deze, gezien de D_{10}-waarden, niet verwacht werden, werden aangetoond bij :

1. proeven, waarbij de overlevende microflora na een bepaalde bestralingsbehandeling werd bepaald;
2. proeven, waarbij de invloed van bevriezing en bewaring van bestraalde karkassen op de aanwezigheid van Salmonella werd onderzocht.

Bij enkele proeven werd gebruik gemaakt van kunstmatig besmette karkassen. Bij proeven met Salmonella niloese en Salmonella panama kon worden aangetoond, dat de slachtkuikenhuid een duidelijke bescherming tegen ioniserende straling gaf. De gevonden D_{10}-waarden voor beide serotypen waren 1.07 en 1.29 kGy, indien de bestraling bij -18°C werd uitgevoerd en 0.62 en 0.67 kGy indien de bestraling bij +5 °C werd uitgevoerd. De D_{10}-waarden voor Salmonella panama bepaald aan kuikenhuid bleken hoger ($P<0.01$) dan de D_{10}-waarden, die met reinkultures onder optimale kondities in vloeibare kultuurmedia werden bepaald (0.52 kGy). Verschillende faktoren, die van invloed zijn op de lethaliteit van de bestralingsbehandeling, werden besproken.

Over het mechanisme van de bescherming van de slachtkuikenhuid tegen ioniserende straling kan slechts worden gespeculeerd. Een mogelijke verklaring kan zijn, dat Salmonella bacterien minder worden beinvloed door de op de huid van slachtkuikens aanwezige microflora, dan de Escherichia coli. Het resultaat kan dan een sterkere hechting aan de kuikenhuid zijn. Het is mogelijk dat huid-eiwitten daarvoor verantwoordelijk zijn; het kan eveneens zijn dat bacterien extracellulaire polysacchariden produceren, die voor de bescherming zorg dragen (14,216).

Er werd een model opgesteld om de kans te kunnen berekenen, dat na een bepaalde bestralingsbehandeling nog een Salmonella positief monster zou worden aangetoond, waarbij tevens de lethaliteit van de bestralingsbehandeling kan worden vastgesteld. De resultaten van de berekeningen toonden aan, dat onder de gemaakte aannames, de gevoeligheid van de gebruikte Salmonella isolatiemethoden hierbij erg belangrijk was. Diverse bacterien, zoals stammen van Alcaligenes, Escherichia, Pseudomonas en Salmonella werden bepaald in bestraalde produkten, waarin deze bacterien gezien hun D_{10}-waarden nauwelijks konden worden verwacht.
Een gekombineerde behandeling van de karkassen met ioniserende straling met daarvoor of daaropvolgend een bewaarperiode gedurende 4 maanden bij -18°C, gaf veelbelovende resultaten op weg naar het uiteindelijke doel : een Salmonella-vrij eindprodukt.
Om een verband te leggen tussen het aantal aanwezige kve's van Enterobacteriaceae en het aantal kve's van salmonellae werd eveneens een telling van kve's van de gehele groep der Enterobacteriaceae uitgevoerd. Opvallend was de aanwezigheid van kve's van salmonellae in monsters slachtkuikenhuid, waarvan het aantal kve's van Enterobacteriaceae kleiner was dan (log) 1.00.
Bij het onderzoek van dripwater werden deze resultaten niet bevestigd. Er werd geen verschil in lethaal effect op Salmonella gekonstateerd, indien de bestraling bij -18°C of bij +5°C werd uitgevoerd. In de literatuur (207) werd op een verschil in lethaal effect geduid. Op grond van de resultaten zou een behandeling bij +5°C de voorkeur verdienen; de verschillen bleken echter niet signifikant te zijn.
Ook de bepaling van het aantal Enterobacteriaceae gaf geen antwoord op de vraag of er een verschil in lethaal effekt kan worden verkregen door een bestralingsbehandeling bij +5°C of bij -18°C uit te voeren. Alle tellingen van kve's bleken beneden de bepalingsgrens te liggen.
Het is duidelijk dat na een 3 maanden bewaarperiode bij -18 °C geen Salmonella werd geisoleerd uit met 2.50 kGy bestraalde karkassen. Onbehandelde diep-bevroren karkassen bleken gedurende de gehele bewaarperiode Salmonella positief.
Samenvattend kan worden gesteld, dat na een bestralingsbehandeling met 2.50 kGy een afname van het aantal met Salmonella besmette karkassen als ook van het aantal salmonellae per karkas werd gevonden.

De veelbelovende resultaten, verkregen met een gekombineerde behandeling van bestraling, bevriezing en bewaring van kuikenkarkassen, rechtvaardigen verder onderzoek naar het effekt van deze behandelingen op salmonellae en Enterobacteriaceae.

De microflora van het geslachte eindprodukt na een bestralingsbehandeling.
De samenstelling van de microflora van het geslachte eindprodukt na een bestralingsbehandeling gaf kwalitatief gezien, in vergelijking met onbehandelde karkassen, een normaal beeld. Het totaal kiemgetal was gereduceerd tot 100 - 1000 kve's per 10 cm^2 huid en bestond voornamelijk uit bacillen, micrococcen, streptococcen, gisten en schimmels. Sporadisch werden kve's van Enterobacteriaceae geisoleerd.
De telling van kve's van Staphylococcus aureus en van Salmonella werd niet verbeterd door aan het oppervlak van de selektive agar media katalase toe te voegen.
Het mechanisme van de groeibevordering van gisten en schimmels door de toevoeging van katalase aan selektieve agar media dient in de nabije toekomst te worden bestudeerd. Dit geldt eveneens voor de telling van kve's van Bacillus cereus, die door de toevoeging van katalase aan het oppervlak van het selektieve agar medium niet konden worden gekweekt.
De z.g. "solid medium repair" methoden, die voor de telling van mogelijk sublethaal beschadigde kve's werden gebruikt, gaven geen hogere tellingen dan die verkregen met meer konventionele methoden.
De aanwezigheid van Salmonella in een met 2.50 kGy bestraald produkt toont aan dat met deze behandeling niet zonder meer een Salmonella-vrij produkt kan worden verkregen. De vraag kwam naar boven welke dosis dan wel voor dit doel nodig is.
Zoals bekend gaven de kuikenhuid en de lage temperatuur tijdens de bestraling (-18 °C) een bescherming tegen de ioniserende straling.
De hoogste D_{10}-waarde voor Salmonella panama was 1.29 kGy.
Als voor een 7 D_{10}- koncept wordt gekozen, dan is een dosis van circa 9.00 kGy noodzakelijk, indien dripwater bij -18 °C zou worden bestraald. Deze dosis is hoog, hoewel men het er internationaal over eens is dat een dosis van 10.00 kGy kan worden toegepast. Voor pluimveeprodukten is deze dosis te hoog vanwege de optredende geur-, kleur en smaakafwijkingen.
Als alleen de afwezigheid van kve's van Salmonella in het eindprodukt is vereist, dan is bij een bestralingsbehandeling bij -18 °C een dosis van 3.90 kGy en bij +5 °C een dosis van 1.95 kGy nodig.

De resultaten van de proeven geven aan dat de hoge doses niet per se noodzakelijk zijn. Immers het aantal kve's van Salmonella per karkas lag gemiddeld gezien onder de gestelde 100 kve's. Door een aanvullende bewaarperiode bij -18°C kan een aanzienlijke extra reduktie van het aantal Salmonella worden verkregen. Uit literatuur gegevens (259) is gebleken dat door bevriezing van de karkassen een halve log-eenheid reduktie van het aantal kve's van salmonellae kon worden verkregen. De toegepaste dosis van 2.50 kGy garandeert geen Salmonella-vrij eindprodukt. De microbiologische kwaliteit van het eindprodukt is, met het oog op het totaal kiemgetal als ook op de aanwezigheid van mogelijk pathogene microorganismen, duidelijk verbeterd in vergelijking met niet behandelde eindprodukten.

Praktische konsekwenties.

De behandeling van slachtkuiken-produkten met een dosis ioniserende straling van 2.50 kGy levert geen Salmonella-vrij eindprodukt. Het totaal kiemgetal was gereduceerd tot 100 - 1000 kve's per 10 cm^2 huid en bestond voornamelijk uit bacillen, micrococcen, streptococcen, gisten en schimmels. Met Salmonella besmette karkassen bleken gemiddeld 100 kve's te bevatten (minimum : 2, maximum : 1400). Na de bestraling bleken slechts twee series van de onderzochte huid-mengmonsters Salmonella positief.

De D_{10}-waarden voor Salmonella panama na een bestralingsbehandeling bij +5 °C en bij -18 °C waren respektievelijk 0.67 kGy en 1.29 kGy. Aan de hand van deze D_{10}-waarden en van de aantallen kve's van Salmonella per karkas, kunnen over het algemeen Salmonella negatieve eindprodukten worden verwacht. Een extra effekt op het aantal Salmonella positieve monsters en op het aantal kve's van salmonellae per karkas kan worden verkregen door de karkassen gedurende een aantal maanden bij -18°C te bewaren.

Een extrapolatie naar het lethale effekt van een bestralingsbehandeling van 2.50 kGy op de gehele Nederlandse slachtkuikenproduktie mag niet worden gemaakt, vanwege de onzekerheid omtrent de variatie in het gemiddelde aantal salmonellae per karkas, de variatie in D_{10}-waarden en het Salmonella detectie niveau behorend bij de gebruikte Salmonella isolatie methode. Niettemin geven de resultaten aan dat indien de gehele slachtkuiken-produktie ($3.0 \cdot 10^7$ karkassen) zou zijn behandeld met 2.50 kGy en een D_{10}-waarden van 0.80 kGy wordt aangenomen, het maximale resultaat zou zijn dat na bestraling nog 1 op 55 karkassen Salmonella positief is; dit betekent een verbetering met een faktor 14.

Een aanvullende bewaarperiode bij -18 °C kan dit resultaat positief beinvloeden.

De gekombineerde behandeling van bevriezing en bestraling is een veelbelovende methode voor de Salmonella-decontaminatie van slachtkuikens.

CHAPTER 12

12. REFERENCES

1. AARSSE, L.W. (1976) De verspreiding van Salmonella in en via een slachtkuikenhok. IPS report 3176.
2. ADAM, W. (1957) Ueber die Herstellung und Pasteurisierung von Fischmehl im Hinblick auf dessen Gehalt an Salmonellabakterien. Berliner und Muenchener tieraerztliche Wochschrift 70, 49-51.
3. ADLER, H.I. & HARDIGREE, A.A. (1965) Postirradiation growth, division, and recovery in bacteria. Radiation Research 25, 92-102.
4. ALPER, T. & GILLIES, N.E. (1958) "Restoration" of Escherichia coli strain B after irradiation: its dependence on suboptimal growth conditions. Journal of general Microbiology 18, 461-472.
5. ANDERSON, E.H. & BILLEN, D. (1955) The effect of temperature on X- ray induced mutability in Escherichia coli. Journal of Bacteriology 70, 3543.
6. ANDERSON, A.W., NORDAN, H.C., CAIN, R.F., PARRISH, G. & DUGGAN, D. (1956) Studies on a radio-resistant Micrococcus. 1. Isolation, morphology, cultural characteristics, and resistance to gamma radiation. Food Technology 10, 575-578.
7. ANELLIS, A. & WERKOWSKI, S. (1968) Estimation of radiation resistance values of microorganisms in food products. Applied Microbiology 16, 1300-1308.
8. ANELLIS, A., BERKOWITZ, D. & KEMPER, D. (1973) Comparative resistance of nonsporogenic bacteria to low-temperature gamma irradiation. Applied Microbiology 25, 517-523.
9. ANGELOTTI, R. (1974) Recommendation to the commissioner for the control of foodborne human salmonellosis. F.D.A. Washington, D.C.
10. ANISIMOW, B.N., BUSHKANETS, T.S., GEL'FAND, S.Yu., ZAL'TSMAN, Yu. A. & SILAEV, M.P. (1972) Irradiation of chilled poultry meat to prolong its keeping quality. Trudy. Vsesoyuznie Nauchno-issledovatel'skii Institut Konservnoi i Ovoshchesushil'noi Promyshlennosti 16, 48-55.
11. ANON. (1959) Salmonella organisms in animal feeding stuffs. Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service 18, 26-35.
12. ANON. (1961) Salmonella organisms in animal feeding stuffs. Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service 20, 73-85.
13. ANON. (1965) Radiation preservation of foods. Public no., 1273 NAS/NRC Washington, D.C.
14. ANON. (1965) Salmonellae in cattle and their feedingstuffs, and the relation of human infection. Journal of Hygiene, Cambridge 63, 223-241.
15. ANON. (1968) 5th Edition BBL manual of products and laboratory procedures. BBL Division of Becton, Dickinson and Company, Cockeysville, Maryland.
16. ANON. (1969) An evaluation of the Salmonella problem. National Academy of Sciences, Washington, D.C.

17. ANON. (1971) World Health Statistics Annual 1968. Volume 1. WHO Geneve p. 174, 557, 561, 613, 636.
18. ANON. (1973) Produktschap voor Veevoeder. Jaarverslag 1973.
19. ANON. (1974) Produktschap voor Veevoeder. Jaarverslag 1974.
20. ANON. (1975) ISO 3565. Meat and meat products. Detection of Salmonellae (Reference Method). Ref. no. ISO-3565-1975 (E).
21. ANON. (1976) Evaluation of the hygienic problems related to the chilling of poultry carcasses. 1976. Series: Information on Agriculture no. 22. E.E.C., Brussel.
22. ANON. (1976) Jaarverslag 1975 van de geneeskundige hoofd-inspecteur. Staatstoezicht op de Volksgezondheid 46/1976 Den Haag, Ministerie van Volksgezondheid en Milieuhygiene.
23. ANON. (1976) World Health Statistics Annual 1973-1976. Volume II. Infectious Diseases: Cases, Deaths and Vaccinations. WHO Geneve.
24. ANON. (1977) Salmonellose ueberwachung beim Menschen in der BRD einschl. Berlin (West) Jahresbericht 1975. 2. Epidemiologische Analyse Bundesgesundheidsblatt 20, 133-148.
25. ANON. (1978) Het Salmonella-vraagstuk. Advies van de Gezondheidsraad no. 60. Staatsuitgeverij, Den Haag.
26. ANON. (1979) Microbiological methods for control of Poultry Meat, Study P. 203 E.E.C.
27. ASERKOFF, B. & BENNETT, J.V. (1969) Effect of antibiotic therapy in acute Salmonellosis on the fecal excretion of Salmonellae. New England Journal of Medicin 281, 636-640.
28. AVENS, J.S. & MILLER, B.F. (1970a) Optimum skin blending method for quantifying poultry carcass bacteria. Applied Microbiology 20, 129-132.
29. AVENS, J.S. & MILLER, B.F. (1970b) Quantifying bacteria on poultry carcass skin. Poultry Science 49, 1309-1315.
30. AYRES, J.C., WALKER, H.W., FANELLI, M.J., KING, A.W. & THOMAS, F. (1956) Use of antibiotics in prolonging storage life of dressed chicken. Food Technology 10, 563-568.
31. BARNES, E.M. (1972) Food poisoning and spoilage bacteria in poultry processing. Veterinary Record 89, 720-722.
32. BARNES, E.M. (1975) The microbiological problems of sampling a poultry carcass. Proceedings 2nd European Symposium on Poultry Meat, B. Erdtsieck (ed), Oosterbeek, The Netherlands, May 12-16, page 23 (1-8).
33. BARNES, E.M. (1976) Microbiological problems of poultry at refrigerator temperatures. A review. Journal of the Science of Food and Agriculture 27, 777-782.
34. BARNES, E.M. & SHRIMPTON, D.H. (1957) Causes of greening of uneviscerated poultry carcasses during storage. Journal of applied Bacteriology 20, 273-285.
35. BARNES, E.M. & SHRIMPTON, D.H. (1958) The effect of tetracycline compounds on the storage life and microbiology of chilled eviscerated poultry. Journal of applied Bacteriology 21, 213.
36. BARNES, E.M. & IMPEY, C.S. (1975) The shelf-life of uneviscerated and eviscerated chicken carcasses stored at 10 C and 4 C. British Poultry Science 16, 319-326.
37. BARNES, E.M., IMPEY, C.S. & PARRY, R.T. (1973) The sampling of chickens, turkeys, ducks and game birds. In: Sampling-Microbiological Monitoring of Environments. R.G. Board and D.W. Lovelock (eds), London, New York, Academic Press, p. 63-75.

38. BARNES, J., ASHWOOD-SMITH, M.J. and BRIDGES, B.A. (1969) Radiosensitization of bacterial cells by carbonyl compounds. International Journal of Radiation Biology 15, 285-288.
39. BECKER, W. (1960) Beitrag zur Gefluegelsalmonellose. Zentralblatt Veterinaer Medizin 7, 226-229.
40. BICKFORD, A.A. & ROSENWALD, A.S. (1975) Staphylococcal infections in chickens. Poultry Digest, July 1975, 285-287.
41. BIGLAND, C.H. (1962) Salmonella reservoirs in Alberta. Canadian Journal of Public Health 53, 97-104.
42. BISCHOFF, H. (1956) Ein Beitrag zur Klaerung der Frage nach der Herkunft seltener Salmonella-Typen. Berliner und Muenchener tieraerztliche Wochschrift 68, 306-307.
43. BISCHOFF, J. & ROHDE, R. (1956) Salmonellen in Fisch- und Fleischmehlen auslaendische Herkunft. Berliner und Muenchener tieraerztliche Wochschrift 68, 307-309.
44. BLOK, J. & LOMAN, H. (1973) The effect of gamma irradiation in DNA. Current topics in Radiation Research. M. Ebert & A. Howard (eds.), North Holland, Amsterdam 9, 165-245.
45. BOOTHROYD, M. & BAIRD-PARKER, A.C. (1973) The use of Enrichment Serology for Salmonella detection in human foods and animal feeds. Journal of applied Bacteriology 36, 165-172.
46. BOYER, C.I. jr., BRUNER, D.W. & BROWN, J.A. (1958) Salmonella organisms isolated from poultry feed. Avian Diseases 2, 396-401.
47. BOYER, C.I. jr., NAROBLEY, S., BRUNER, D.W. & BROWN, J.A. (1962) Salmonellosis in turkeys and chickens associated with contaminated feed. Avian Diseases 6, 43-50.
48. BRACHMAN, P.S., TAYLOR, A., GANGAROSA, E.J., MERSON, M.H. & BARKER, W.H. (1973) Food poisoning in the USA in: The Microbiological safety of food. B.C. Hobbs & J.H.B. Christian (eds), London, Academic Press p.153-163.
49. BRASH, A. & HUBER, W. (1948) Reduction of undesirable by-effects in products treated by radiation. Science 108, 536-537.
50. BRIDGES, B.A. (1962) The chemical sensitization of Pseudomonas species to ionizing radiation. Radiation Research 16, 232-242.
51. BRIDGES, B.A. (1963) Effect of chemical modifiers on inactivation and mutation induction by gamma radiation in Escherichia coli. Journal of general Microbiology 31, 405-412.
52. BRIDGES, B.A. (1976) Survival of bacteria following exposure to ultraviolet and ionizing radiations in: The survival of vegetative microbes by T.R.G. Gray & J.R. Postgate (eds). Cambridge University Press, Cambridge p. 183-208.
53. BRIDGES, B.A. & HORNE, T. (1959) The influence of environmental factors on the microbicidal effect of ionizing radiations. Journal of applied Bacteriology 22, 96-115.
54. BRIDGES, B.A. & MUNSON, R.J. (1968) Genetic radiation damage and its repair in Escherichia coli. In: Current Topics in Radiation Research, M. Ebert & A. Howard (eds), North Holland, Amsterdam 4, 95-188.
55. BRIDGES, A.E., OLIVO, J.P. & CHANDLER, V.E. (1956) Relative resistances of microorganisms to cathode rays. II. Yeasts and molds. Applied Microbiology 4, 147-149.
56. BROBST, D., GREENBERG, J. & GEZON, H.M. (1958) Salmonellosis in poultry and poultry processing plants in Western Pennsylvania. Journal of the American Veterinary Medical Association 133, 435-437.

57. BRYAN, F.L. (1974) Microbiological food hazards today-based on epidemiological information. Food Technology 28, 52-66,84.
58. BRYAN, F.L. (1980) Foodborne diseases in the United States associated with meat and poultry. Journal of Food Protection 43, 140-150.
59. BUECHLI, K. (1969) Radurisatie van vers pluimvee. Veeteelt en Zuivelberichten 12, 165-170.
60. CERUTTI, P.A. (1974) Excision repair of DNA base damage. Life Sciences 15, 1567-1575.
61. CHARM, S. & RONSIVALLI, L. (1967) Effects of processing on the microbial flora. Food Technology 21, 744-748.
62. CHATTOPADHYAY, B. & PILFOLD, J.N. (1976) The effect of prolonged incubation of Selenite F broth on the rate of isolation of Salmonella from faeces. Med. Lab. Sciences 33, 191-194.
63. CHRISTENSEN, E.A. (1964) Radiation resistance of enterococci dried in air. Acta Pathologica et Microbiologica Scandinavia 61, 483-492.
64. CLISE, I.D. & SWECHER, E.E. (1975) Salmonella from animal by-products. Public Health Report 80, 899-905.
65. COLEBY, B., INGRAM, M. & SHEPHERD, H.J. (1960) Treatment of meats with ionising radiations. III. Radiation pasteurisation of whole eviscerated chicken carcasses. Journal of the Science of Food and Agriculture 11, 61-71.
66. COLEBY, B., INGRAM, M., SHEPHERD, H.J. & THORNLEY, M.J. (1960) Treatment of meats with ionising radiations IV.Comparison of the deterioration in quality during storage of eviscerated chicken carcasses treated with chlortetracycline or radiation. Journal of the Science of Food and Agriculture 11, 678-684.
67. COLEBY, B., INGRAM, M., SHEPHERD, H.J., THORNLEY, M.J. & WILSON, G.M. (1961) Treatment of meats with ionising radiations. VII. Effect of low temperatures during irradiation. Journal of the Science of Food and Agriculture 12, 483-490.
68. COMER, A.G., ANDERSON, G.W. & GARRARD, E.H. (1963) Gamma irradiation of Salmonella species in frozen whole egg. Canadian Journal of Microbiology 9, 321-327.
69. COOK, A.M. & WIDDOWSON, J.P. (1967) Factors affecting the recovery of gamma irradiated Escherichia coli. Journal of applied Bacteriology 30, 206-218.
70. CORLETT, D.A. (1967) Microbial selection due to ionizing radiation. Food Technology 21, 69-74.
71. CORLETT, D.A., LEE, J.S. & SINNHUBER, R.O. (1965) Application of replica plating and computer analysis for rapid identification of bacteria in some foods. II. Analysis of microbial flora in irradiated Dover sole.(Microstomus pacificus). Applied Microbiology 13, 818-822.
72. CORNELISSEN, J. (1967) Korrel of meel voor slachtkuikens. Nederlandse Pluimveehouderij 1, 53-55.
73. CORRY, J.E.L., KITCHELL, A.G. & ROBERTS, T.A. (1969) Interactions in the recovery of Salmonella typhimurium damaged by heat or gamma radiation. Journal of applied Bacteriology 32, 415-428.
74. COX, N.A. & BLANKENSHIP, L.C. (1975) Comparison of rinse sampling methods for detection of Salmonellae on eviscerated broiler carcasses. Journal of Food Science 40, 1333-1334.

75. COX, N.A., MERCURI, A.J., THOMSON, J.E. & CHEW, V. (1976) Swab and excised tissue sampling for total and Enterobacteriaceae counts of fresh and surface-frozen broiler skin. Poultry Science 55, 2405-2408.
76. COX, N.A., DAVIS, B.H., KENDALL, J.H., WATTS, A.B. & COLMER, A.R. (1972) Salmonella in the laying hen. 3. A comparison of various enrichment broths and plating media for the isolation of Salmonella from poultry feces and poultry food products. Poultry Science 51, 1312-1316.
77. COX, N.A., MERCURI, A.J., TANNER, D.A. CARSON, M.O., THOMSON, J.E. & BAILEY, J.S. (1978) Effectiveness of sampling methods for Salmonella detection on processed broilers. Journal of Food Protection 41, 341-343.
78. GRAIG, F.R. (1975) Controlling Salmonella in poultry products. Proc. 10th National Meeting Poultry Health and Condemnation. Oct. 1975 p. 110-115.
79. CRAVEN, P.C., MACKEL, D.C., BAINE, W.B., BARKER, W.H., GANGAROSA, E.J., GOLDFIELD, M., ROSENFELD, J., ALTMAN, R., LACHAPALLE, G., DAVIES, J.W. & SWANSON, R.C. (1975) International outbreak of Salmonella eastborne infection traced to contaminated chocolate. Lancet 1, 788-793.
80. DABBAH, R., MOATS, W.A. & MATTICK, J.F. (1969) Factors affecting resistance to heat and recovery of heat-injured bacteria. Journal of Dairy Science 52, 608-614.
81. DAMMERS, J., KAMPELMACHER, E.H., EDEL, W. & VAN SCHOTHORST, M. (1966) Invloed van thermisch en met gamma-stralen gedecontamineerde meelmengsels op groei en voederverbruik bij mestvarkens. Landbouwkundig Tijdschrift 79, 106-109.
82. DARMADY, E.M., HUGHES, K.E.A., BURT, M.M., FREEMAN, B.M. & POWELL, D.B. (1961) Radiation sterilization. Journal of clinical Pathology 14, 55-58.
83. DAUER, C.C. (1960) Summary of disease outbreaks and a 10 years resume. U.S. Public Health Service Report 76, 915-921.
84. DAVIES, R. (1976) The inactivation of vegetative bacterial cells by ionizing radiation in: Inhibition and inactivation of vegetative microbes by F.A. Skinner & W.B. Hugo (eds). Academic Press, London, New York, San Francisco p. 239-255.
85. DAVIES, R. & SINSKEY, A.J. (1973) Radiation-resistant mutants of Salmonella typhimurium LT2 development and characterisation. Journal of Bacteriology 113, 133-144.
86. DAWKINS, H.C. & ROBERTSON, L. (1967) Salmonellas in animal feeding stuffs. Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service 26, 215-221.
87. DEAN , C.J. & ALEXANDER, P. (1962) Sensitization of radioresistant bacteria to X-rays by iodoacetamide. Nature, London 196, 1324-1326.
88. DE CAMP, D. (1958) Publication by Inspection Branche, Poultry Division, Agr. Marketing Service.
89. DE WIT, J.C., BROEKHUIZEN, G. & KAMPELMACHER, E.H. (1979) Cross contamination during the preparation of frozen chickens in the kitchen. Journal of Hygiene, Cambridge 83, 27-32.
90. DIXON, J.M.S. & POOLEY, F.E. (1961) Salmonellae in a poultry-processing plant. Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service 20, 30-33

91. DRION, E.F. & MOSSEL, D.A.A. (1977) The reliability of the examination of foods, processed for safety, for enteric pathogens and Enterobacteriaceae: a mathematical and ecological study. Journal of Hygiene, Cambridge 78, 301-324.
92. DUGGAN, D.E., ANDERSON, A.W. & ELLIKER, P.R. (1963a) Inactivation of the radiation-resistant spoilage bacterium Micrococcus radiodurans. I. Radiation inactivation rates in three meat substrates and in buffer. Applied Microbiology 11, 398-403.
93. DUGGAN, D.E., ANDERSON, A.W. & ELLIKER, P.R. (1963b) Inactivation of the radiation-resistant spoilage bacterium Micrococcus radiodurans. II. Radiation inactivation rates as influenced by menstruum temperature, preirradiation heat treatment, and certain reducing agents. Applied Microbiology 11, 413-417.
94. DUNCAN, M.S. & ADAMS, A.W. (1972) Effects of a chemical additive and of formaldehyde-gas fumigation on Salmonella in poultry feeds. Poultry Science 51, 797-802.
95. DYER, J.K., ANDERSON, A.W. & DUTIYABODHI, P. (1966) Radiation survival of food pathogens in complex media. Applied Microbiology 14, 92-97.
96. EDEL, W. (1974) Comparative studies on Salmonella isolation Thesis, University of Utrecht.
97. EDEL, W. & KAMPELMACHER, E.H. (1969) Salmonella isolation in nine European laboratories using a standardized technique. Bulletin World Health Organisation 41, 297-306.
98. EDEL, W. & KAMPELMACHER, E.H. (1973) Comparative studies on the isolation of "sublethally injured" Salmonellae in nine European laboratories. Bulletin World Health Organisation, WHO Geneve 48, 167-174.
99. EDEL, W., VAN SCHOTHORST, M. & KAMPELMACHER, E.H. (1975) Epidemiologisch Salmonella-onderzoek in een bepaald gebied. 1. Project Walcheren. Tijdschrift voor Diergeneeskunde 100, 1304-1311.
100. EDEL, W., VAN SCHOTHORST, M. & KAMPELMACHER, E.H. (1977) Salmonella and Salmonellosis. The present situation. In: Proceedings of the International Symposium on Salmonella and Prospects for Control. June 8-11, 1977, Guelph, Canada.
101. EDEL, W., GUINEE, P.A.M., VAN SCHOTHORST, M. & KAMPELMACHER, E.H. (1966) Studies about the occurrence of Salmonella in pigs fattened with pellets and non-pelleted meal. Tijdschrift voor Diergeneeskunde 91, 962-966.
102. EDEL, W., GUINEE, P.A.M., VAN SCHOTHORST, M. & KAMPELMACHER, E.H. (1967) Salmonella infections in pigs, fed normal and pelleted meal. Tijdschrift voor Diergeneeskunde 92, 1006-1016.
103. EDEL, W., VAN SCHOTHORST, M., GUINEE, P.A.M. & KAMPELMACHER, E.H. (1970) Effect of feeding pellets on the prevention and sanitation of Salmonella infections in fattening pigs. Zentralblatt Veterinaer Medizin B 17, 730-738.
104. EDEL, W., VAN SCHOTHORST, M., GUINEE, P.A.M. & KAMPELMACHER, E.H. (1973a) Salmonella in pigs on farms on which pellets and on farms on which meal is fed. Tijdschrift voor Diergeneeskunde 98, 1157-1165.
105. EDEL, W., VAN SCHOTHORST, M., GUINEE, P.A.M. & KAMPELMACHER, E.H. (1973b) Mechanism and prevention of Salmonella infections in animals in: The microbiological safety of food. B.C. Hobbs &J.H.B. Christian (eds) London, Academic Press p. 247-255.

106. EDEL, W., VAN SCHOTHORST, M., GUINEE, P.A.M. & KAMPELMACHER, E.H. (1974) Preventive measures to obtain Salmonella-free slaugher pigs. Tijdschrift voor Diergeneeskunde 99, 249-257.
107. EDWARDS, P.R., BRUNER, D.W. & MORAN, A.B. (1948) Further studies on the occurrence and distribution of Salmonella types in the United States. Journal of Infectious Diseases 83, 220-231.
108. ELLIOTT, R.P. & MICHENER, H.D. (1961) Microbiological standards and handling codes for chilled and frozen foods. A Review. Applied Microbiology 9, 452-468.
109. ELLIOTT, R.P. & MICHENER, H.D. (1965) Factors affecting the growth of psychrophilic microorganisms in foods. A Review. ARS, USDA. Technical Bulletin no. 1320, Washington, D.C.
110. EMBORG, C. (1972) The influence of preparation technique, humidity and irradiation conditions on the radiation inactivation of Streptococcus faecium, strain A 1. Acta Pathologica et Microbiologica Scandinavia 80, 367-372.
111. ENKIRI, N.K. & ALFORD, J.A. (1971) Relationship on the frequency of isolation of Salmonellae to their resistance to drying and freezing. Applied Microbiology 21, 381-382.
112. EPP, E.R., WEISS, H. & SANTOMASSO, A. (1968) The oxygen effect in bacterial cells irradiated with high-intensity pulsed electrons. Radiation Research 84, 320-325.
113. EPPS, N.A. & IDZIAK, E.S. (1972) Poultry feed radicidation. 1. Microbiological aspects of poultry feed irradiation. Poultry Science 51, 277-282.
114. ERDMAN, I.E., THATCHER, F.S. & MACQUEEN, K.F. (1961a) Studies on the irradiation of microorganisms in relation to food preservation. 1. The comparative sensitivities of specific bacteria of public health significance. Canadian Journal of Microbiology 7, 199-205.
115. ERDMAN, I.E., THATCHER, F.S. & MACQUEEN, K.F. (1961b) Studies on the irradiation of microorganisms in relation to food preservation. II. Irradiation resistant mutants. Canadian Journal of Microbiology 7, 207-215.
116. FAGERBERG, D.J. & AVENS, J.S. (1976) Enrichment and plating methodology for Salmonella detection in food. A Review. Journal of Milk and Food Technology 39, 628-646.
117. FLOWERS, R.S., MARTIN, S.E., BREWER, D.G. & ORDAL, Z.J. (1977) Catalase and enumeration of stressed Staphylococcus aureus cells. Applied and Environmental Microbiology 33, 1112-1117.
118. FORAGE, A.J. & GILLIES, N.E. (1964) Restoration of Escherichia coli strain B after gamma-irradiation. Journal of general Microbiology 37, 33-39.
119. FROMM, D. (1959) An evaluation of techniques commonly used to quantitatively determine the bacterial population on chicken carcasses. Poultry Science 38, 887-893.
120. FULD, G.J., PROCTOR, B.E. & GOLDBLITH, S.A. (1957) Some microbiological aspects of radiation sterilization. International Journal of Applied Radiation and Isotopes 2, 35-43.
121. GABIS, D.A. & SILLIKER, J.H. (1974) ICMSF methods studies. II. Comparison of analytical schemes for detection of Salmonella in high-moisture foods. Canadian Journal of Microbiology 20, 663-669.

122. GALBRAITH, N.S., MAWSON, K.N., MATON, G.E. & STONE, D.M. (1962) An outbreak of human salmonellosis due to Salmonella saintpaul associated with infection in poultry. Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service 21, 209-216.

123. GALTON, M.M., LOWERY, W.D. & HARDY, A.V. (1954) Salmonella in fresh and smoked pork sausage. Journal of Infectious Diseases 95, 232-235.

124. GALTON, M.M., MACKEL, D.C., LEWIS, A.L., HAIRE, W.C. & HARDY, A.V. (1955) Salmonellosis in poultry and poultry processing plants in Florida. Journal of American Veterinary Research 16, 132-137.

125. GALTON, M.M., MORRIS, G.K. & MARTIN, W.T. (1968) Salmonellae in foods and feeds. Review of isolation methods and recommended procedures. U.S. Department of Health, Education and Welfare/Public Health Service C.D.C. Atlanta, Georgia.

126. GERRITS, A.R., GERMS, A.C., MULDER, R.W.A.W. & FRIJTERS, J.E.R. (1973) Irradiation of fresh tray-packed parts of softscald dry-chilled broilers. 1st Poultry Meat Symposium Roskilde, Denmark paper B2.

127. GIRARD, A.E., ENGLISH, A.R., EVANGELISTI, D.G., LYNCH, J.E. & SOLOMONS, I.A. (1976) Influence of subtherapeutic levels of a combination of Neomycin and Oxytetracycline on Salmonella typhimurium in swine, calves and chickens. Antimicrobial Agents and Chemotherapy 10, 89-95.

128. GOLDBLITH , S.A. (1971) The inhibition and destruction of the microbial cell by radiations. In: Inhibition and destruction of the microbial cell. W.B. Hugo (ed), Academic Press,London, New York p.285-305.

129. GOO, V.Y.L., CHING, G.Q.L. & GOOCH, J.M. (1973) Comparison of Brilliant Green Agar and Hektoen Enteric Agar media in the isolation of Salmonellae from food products. Applied Microbiology 26, 288-292.

130. GORESLINE, M.E., INGRAM, M., MACUCH, P., MOCQUOT, G., MOSSEL, D.A.A., NIVEN, C.F. & THATCHER, F.S. (1964) Tentative classification of food irradiation processes with microbiological objectives. Nature 204, 237-238.

131. GOETZE, U. (1974) Untersuchungen ueber die hygienische Beschaffenheit von gefroren im handel angebotenem in- und auslaendischen Gefluegel (Brathaehnchen, Enten und Puten). Fleischwirtschaft 54, 1347-1360.

132. GRAAF, F.K. DE & STOUTHAMER, A.H. (1971) Interactions of various bacteriocins with Klebsiella edwardsii var. edwardsii. Antonie van Leeuwenhoek 37, 1-14.

133. GRAU, F.H. & SMITH, M.G. (1974) Salmonella contamination of sheep and mutton carcasses related to pre-slaughter holding conditions. Journal of applied Bacteriology 37, 111-116.

134. GUINEE, P.A.M., KAMPELMACHER, E.H., HOFSTRA, K.& VAN KEULEN, A. (1965) Salmonella bij jonge biggen in Nederland. Tijdschrift voor Diergeneeskunde 90, 787-795.

135. GUINEE, P.A.M. & VALKENBURG, J. (1975) Salmonella isolations in The Netherlands 1966-1973. Zentralblatt Bakteriologie und Hygiene. I. Abteilung. Originale: A 231, 97-107.

136. GUNNARSON, A., HURVELL, B., NORDBLOM, B., RUTQVIST, L. & THAL, E. (1974) Salmonella isolated from animals and feedstuffs in Sweden in the period 1968-1972. Nordisk Veterinary Medicin 26, 499-517.

137. GUNTER, S.E. & KOHN, H.I. (1956) The effect of X-rays on the survival of bacteria and yeast. I. A comparative study of the dose-survival curves of Azotobacter agile, Escherichia coli, Pseudomonas fluorescens, Rhodopseudomonas spheroides and Saccharomyces cerevisiae irradiated in the resting state. Journal of Bacteriology 71, 571-581.

138. HAJNA, A.A. (1955) A new enrichment broth medium for Gramnegative organisms of the intestinal group. Public Health Laboratory 13, 83-90.

139. HAJNA, A.A. & DAMON, S.R. (1956) New enrichment and plating media for the isolation of Salmonella and Shigella organisms. Applied Microbiology 4, 341-345.

140. HANNAN, R.S. & SHEPHERD, H.J. (1959) Treatment of meats with ionising radiations. I. Changes in odour, flavour, and appearance of chicken meat. Journal of the Science of Food and Agriculture 10, 286-295.

141. HARGROVE, R.E., McDONOUGH, F.E. & REAMER, R.H. (1971) A selective medium and presumptive procedure for detection of Salmonella in dairy products. Journal of Milk and Food Technology 34, 6-12.

142. HARVEY, R.W.S. & THOMSON, S. (1953) Optimum temperature of incubation for isolation of salmonellae. Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service 12, 149-150.

143. HARVEY, R.W.S. & PRICE, T.H. (1970) Salmonellosis in South Wales. Public Health, London 84, 183-190.

144. HARVEY, R.W.S. & PRICE, T.H. (1979) A review. Principles of Salmonella isolation. Journal of applied Bacteriology 46, 2756.

145. HARVEY, R.W.S., PRICE, T.H. & JOYNSON, D.H.M. (1976) Observations on environmental contamination in a microbiological laboratory. Journal of Hygiene, Cambridge 76, 91-96.

146. HAUSCHILD, A.H.W. & BRYAN, F.L. (1980) Estimate of cases of food- and waterborne illness in Canada and the United States. Journal of Food Protection 43, 435-440.

147. HEINMETS, F. (1953) Reactivation of ultraviolet inactivated E. coli by pyruvate. Journal of Bacteriology 66, 455-457.

148. HEINMETS, F., TAYLOR, W.W. & LEHMAN, J.J. (1954) The use of metabolites in the restoration of the viability of heat and chemically inactivated Escherichia coli. Journal of Bacteriology 67, 5-12.

149. HERMANS, K.H. (1968) De produktie van Salmonella-vrije slachtkuikens experimenteel benaderd. Thesis, R.U., Utrecht.

150. HERNADI, F., CSOBAN, Gy. & NAGY, Zs. (1964) Die Strahlenempfindlichkeit von Escherichia coli B-Kulturen. I. Physiologische Faktoren von Einfluss auf die Strahlenempfindlichkeit. Acta Microbiologica Academiae Scientiarum Hungaricae 11, 99-103.

151. HERNADI, F., NAGY, Zs., KOVACS, P & MUCSI, O. (1966) Die Strahlenempfindlichkeit von Escherichia coli B-Kulturen. II. Die Wirkung der Behandlung vor, waehrend und nach der Bestrahlung auf die Strahlenempfindlichkeit der Zellen. Acta Microbiologica Academiae Scientiarum Hungaricae 13, 1-11.

152. HIRSCH, W. & SPIRO-HIRSCH, R. (1958) The role of certain animal feeding stuffs especially bone meal in the epidemiology of Salmonellosis. Harefuah 54, 57-59.
153. HOBBS, B.C. (1971) Food poisoning from poultry in: Poultry Disease and World Economy by R.F. Gordon & B.M. Freeman (eds). British Poultry Science Ltd., Edinburgh p. 65-80.
154. HOLLAENDER, H., STAPLETON, G.E. & MARTIN, F.L. (1951) X-ray sensitivity of E. coli as modified by oxygen tension. Nature 167, 103-104.
155. HOLLAENDER, H., STAPLETON, G.E. & BILLEN, D. (1953) The reduction of radiation damage to living cells by treatment after exposure. Science 117, 468.
156. HORWITZ, M.A. & GANGAROSA, E.J. (1976) Foodborne disease outbreaks traced to poultry. United States 1966-1974. Journal of Milk and Food Technology 39, 859-863.
157. HOWARD-FLANDERS, P. & ALPER, T. (1957) The sensitivity of micro-organisms to irradiation under controlled gas conditions. Radiation Research 7, 518-540.
158. HUGO, W.B. (1976) The inactivation of vegetative bacteria by chemicals in: Inhibition and inactivation of vegetative microbes. F.A. Skinner & W.B. Hugo (eds), Academic Press, London, New York, San Francisco p. 1-11.
159. HYNES, M. (1942) The isolation of intestinal pathogens by selective media. Journal of Pathology and Bacteriology 54, 193-207.
160. IANDOLO, J.J. & ORDAL, Z.J. (1966) Repair of thermal injury of Staphylococcus aureus. Journal of Bacteriology 91, 134-142.
161. IDZIAK, E.S. & INCZE, K. (1968) Radiation treatment of foods. I. Radurization of fresh eviscerated poultry. Applied Microbiology 16, 1061-1066.
162. INGRAM, M. & DAINTY, R.H. (1971) Changes caused by microbes in spoilage of meats. Journal of Bacteriology 34, 21-39.
163. INGRAM, M. & THORNLEY, M.J. (1959) Changes in spoilage pattern of chicken meat as a result of radiation. International Journal of Applied Radiation and Isotopes 6, 122-128.
164. ISA, J.M., BOYCOTT, B.R. & BROUGHTON, E. (1963) A survey of Salmonella contamination in animal feeds and feed constituents. Canadian Veterinary Journal 4, 41-43.
165. ISENBERG, H.D., KOMINOS, S. & SIEGEL, M. (1969) Isolation of Salmonellae and Shigellae from an artificial mixture of fecal bacteria. Applied Microbiology 18, 656-659.
166. ISLAM, M.N., GRAY, R.J.H. & GEISER, J.N. (1978) Development of antimicrobial agents for the extension of poultry shelf-life. Poultry Science 57, 1266-1271.
167. IVESON, J.B. & MACKAY-SCOLLAY, E.M. (1969) Strontium chloride and strontium selenite enrichment media in the isolation of Salmonella. Journal of Hygiene, Cambridge 67, 457-464.
168. JACOBS, J., GUINEE, P.A.M., KAMPELMACHER, E.H. & VAN KEULEN, A. (1963) Studies on the incidence of Salmonellae in imported fishmeal. Tijdschrift voor Diergeneeskunde 88, 1404-1414.
169. JAROLMEN, H., SAIRK, R.J. & LANGWORTH, B.F. (1976) Effect of chlortetracycline feeding on the Salmonella reservoir in chicken. Journal of applied Bacteriology 40, 153-161.

170. KAHAN, R.S. & HOWKER, J.J. (1970) Retention of fresh chicken quality in refrigerated poultry using radiation and other techniques. Proceedings 3rd International Congress Food Science and Technology. Washington, D.C. August 9-14.
171. KAMPELMACHER, E.H. (1970) Incidence of Salmonella with special reference to Europe. Proceedings 3rd International Congress Food Science and Technology. Washington, D.C. August 9-14.
172. KAMPELMACHER, E.H., GUINEE, P.A.M. & VAN KEULEN, A. (1965) Studies about the incidence of Salmonella in pigs fattened with decontaminated and normal meal. Tijdschrift voor Diergeneeskunde 90, 1270-1281.
173. KAMPELMACHER, E.H., GUINEE, P.A.M., HOFSTRA, K. & VAN KEULEN, A. (1962a) Studies on Salmonella in slaughterhouses. Tijdschrift voor Diergeneeskunde 87, 77-94.
174. KAMPELMACHER, E.H., GUINEE, P.A.M., HOFSTRA, K. & VAN KEULEN, A. (1962b) Further studies on Salmonella in slaughter-houses and normal slaughter pigs. Tijdschrift voor Diergeneeskunde 87, 1486-1492.
175. KAMPELMACHER, E.H., GUINEE, P.A.M., VAN SCHOTHORST, M. & WILLEMS, H.M.C.C. (1965) Experimentele onderzoekingen ter bepaling van de bij decontaminatie van voedermelen benodigde temperatuur en verhittingstijd. Tijdschrift voor Diergeneeskunde 90, 373-378.
176. KARLSSON, K-A., RUTQVIST, L. & THAL, E. (1963) Salmonella isolated from animals and animal feeds in Sweden during 19581962. Nordisk Veterinary Medicin 15, 833-850.
177. KAUFFMAN, F. (1930) Die Technik der Typhenbestimmung in der Typhus-Paratyphus-gruppe. Zentralblatt Bakteriologie Parasitenkunde Infektionskrankheiten und Hygiene Abteilung Originale 119, 152-160.
178. KAUFMAN, A.F. & FEELEY, J.C. (1968) Culture survey of Salmonella at a broiler raising plant. Public Health Reports 83, 417-422.
179. KIELSTEIN, P., BATHKE, W. & SCHIMMEL, D. (1971) Der Einfluss der Pelletierung auf den Salmonellengehalt in Futter. Monatsheft Veterinaer Medizin 26, 12-17.
180. KING, S. & METZGER, W.I. (1968a) A new plating medium for the isolation of enteric pathogens. I. Hektoen Enteric Agar. Applied Microbiology 16, 577-578.
181. KING, S. & METZGER, W.I. (1968b) A new plating medium for the isolation of enteric pathogens. II. Comparison of Hektoen Enteric Agar with S.S. and E.M.B- Agar. Applied Microbiology 16, 579-581.
182. KISS, I. & FARKAS, J. (1967) Influence du rayonnement-gamma au stockage de viande de volaille traites par l'acide tetracycline 1-dodecile-4-benzene-monosulfonique. International Journal of Applied Radiation and Isotopes 18, 65-69.
183. KISS, I. & FARKAS, J. (1972) Radurization of whole eviscerated chicken carcase. Acta Alimentaria 1, 73-86.
184. KLOSE, A.A. & BAYNE, H.G. (1970) Experimental approaches to poultry meat surface pasteurization by condensing vapors. Poultry Science 49, 504-511.
185. KLOSE, A.A., KAUFMANN, V.F., BAYNE, H.G. & POOL, M.F. (1971) Pasteurization of poultry meat by steam under reduced pressure. Poultry Science 50, 1156-1160.
186. KOH, W.Y., MOREHOUSE, C.T. & CHANDLER, V.L. (1956a) Relative resistances of microorganisms to cathode rays. I. Nonsporeforming bacteria. Applied Microbiology 4, 143-146.

187. KOH, W.Y., MOREHOUSE, C.T. & CHANDLER, V.L. (1956b) Incidence and characteristics of beta radiation survivors. (Escherichia coli) Applied Microbiology 4, 153-155.
188. KRABBENHOFT, K.L., ANDERSON, A.W. & ELLIKER, P.R. (1967) Influence of culture media on the radiation resistance of Micrococcus radiodurans. Applied Microbiology 15, 178-185.
189. KRISTENSEN, M., LESTER, V. & JUERGENS, A. (1925) Use of trypsinized casein, bromthymol-blue, brom-cresol-purple, phenol-red and brilliant green for bacteriological nutrient media. British Journal of Experimental Pathology 6, 291-299.
190. LAYTON, H.W., LANGWORTH, B.F., JAROLMEN, H. & SIMKENS, K.L. (1975) Influence of chlortetracycline and chlortetracycline + sulfamethazine supplemented feed on the incidence, persistence and antibacterial susceptibility of Salmonella typhimurium in experimentally inoculated calves. Zentralblatt Veterinaer Medizin, B 22, 461-472.
191. LEE, J.A. (1973) Salmonellae in poultry in Great Britain in: The microbiological safety of food. B.C.Hobbs & J.H.B. Christian (eds) Academic Press, London, New York p. 197-207.
192. LEIFSON, E. (1936) New selenite enrichment media for the isolation of typhoid and paratyphoid (Salmonella) bacilli. American Journal of Hygiene 24, 423-432.
193. LEISTNER, L. & SZENTKUTI, L. (1970) Zwei Methoden zur bakteriologischen Untersuchung von Schlachtgefluegel. Fleischwirtschaft 50, 81.
194. LEY, F.J. (1966) Application of radiation for the control of Salmonellae in various foods. Karlsruhe Symposium. IAEA Publication no. 127 SM-73/35, p. 349-363.
195. 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, Cambridge 61, 515-529.
196. LEY, F.J., KENNEDY, T.S., KAWASHIMA, K., ROBERTS, D. & HOBBS, B.C. (1970) The use of gamma radiation for the elimination of Salmonellae from frozen meat. AERE - R6314.
197. LEWIS, N.R., ALUR, M.D. & KUMTA, U.S. (1971) Radiation sensitivity of fish micro-flora. Indian Journal of Experimental Biology 9, 4557.
198 LICCIARDELLO, J.J. (1964) Effect of temperature on radiosensitivity of Salmonella typhimurium. Journal of Food Science 29, 469-474.
199. LICCIARDELLO, J.J., NICKERSON, J.T.R. & GOLDBLITH, S.A. (1970a) Recovery of Salmonellae from irradiated and unirradiated foods. Journal of Food Science 35, 620-624.
200. LICCIARDELLO, J.J., NICKERSON, J.T.R. & GOLDBLITH, S.A. (1970b) Inactivation of Salmonella in poultry with gamma radiation. Poultry Science 49, 663-675.
201. LINEWEAVER, H. (1966) Sensitivity of Salmonellae to beta and gamma energy. In: The destruction of Salmonellae. ARS. 74-37. Western Experiment Station. USDA, Albany, California p. 47-50.
202. LIU, T.S., SNOEYENBOS, G.H. & CARLSON, V.L. (1969) Thermal resistance of Salmonella senftenberg 775W in dry animal feeds. Avian Diseases 13, 611-613.
203. MAC CONKEY, A. (1905) Lactose fermenting bacteria in faeces. Journal of Hygiene 5, 333-379.

204. MACLEOD, R.A., SMITH, L.D.H. & GELINAS, R. (1966) Metabolic injury to bacteria. I. Effect to freezing and storage on the requirements of Aerobacter aerogenes and Escherichia coli for growth. Canadian Journal of Microbiology. 12, 61-72.

205. MARTHEDAL, H.E. (1973) The occurrence of Salmonellosis in poultry in Denmark 1935- 1971 and the eradication programme established in: The Microbiological safety of Food. B.C. Hobbs & J.H.B. Christian.(eds). Academic Press, London, New York p. 211-226.

206. MARTIN, S.E., FLOWERS, R.S. & ORDAL, Z.J. (1976) Catalase: its effect on microbial enumeration. Applied and Environmental Microbiology 32, 731-734.

207. MATSUYAMA, A., THORNLEY, M.J. & INGRAM, M. (1964) The effect of freezing on the radiation sensitivity of vegetative bacteria. Journal of applied Bacteriology 27, 110-124.

208. MAXCY, R.B. (1970) Non-lethal injury and limitations of recovery of coliform organisms on selective media. Journal of Milk and Food Technology 33, 445-448.

209. McCOY, J.H. (1962) The isolation of Salmonellae. Journal of applied Bacteriology 25, 213-224.

210. McCOY, J.H. (1975) Trends in salmonella food poisoning in England and Wales 1941-1972. Journal of Hygiene, Cambridge 74, 271-282.

211. McCROAN, J.E., McKINLEY, T.W., BRIM, A. & HUEY RAMSEY, C. (1963) Five Salmonellosis outbreaks related to poultry products. Public Health Reports 78, 1073-1080.

212. McCULLOUGH, N.B & EISELE, C.W. (1951a) Experimental human Salmonellosis I. Pathogenicity of strains of Salmonella meleagridis and Salmonella anatum obtained from spray-dried whole egg. Journal of Infectious Diseases 88, 278-289. 213.

213. McCULLOUGH, N.B. & EISELE, C.W. (1951b) Experimental human Salmonellosis III. Pathogenicity of strains of Salmonella newport, Salmonella derby and Salmonella bareilly obtained from spray-dried whole egg. Journal of Infectious Diseases 89, 209-213.

214. McCULLOUGH, N.B. & EISELE, C.W. (1951c) Experimental human Salmonellosis IV. Pathogenicity of strains of Salmonella pullorum obtained from spray-dried whole egg. Journal of Infectious Diseases 89, 259-265.

215. McGILL, J.N., NELSON, A.I., STEINBERG, M.P. & KEMPE, L.L. (1959) Gamma-ray pasteurization of whole eviscerated chickens. Food Technology 13, 75-80.

216. McMEEKIN, T.A., THOMAS, C.J. & McCALL, D. (1979) Scanning electron microscopy of microorganisms on chicken skin. Journal of applied Bacteriology 46, 195-200.

217. MEAD, G.C., ADAMS, B.W. & PARRY, R.T. (1975) The effectiveness of inplant chlorination in poultry processing. British Poultry Science 16, 517-526.

218. MERCURI, A.J., KOTULA, A.W. & SANDERS, D.H. (1966) Low dose ionizing irradiation of tray-packed cut-up fryer chickens. Poultry Science 45, 1105.

219. MERCURI, A.J., KOTULA, A.W. & SANDERS, D.H. (1967) Low dose ionizing irradiation of tray-pack cut-up fryer chickens. Food Technology 21, 95-118.

220. MOATS, W.A. (1978) Comparison of four agar plating media with and without added Novobiocin for isolation of Salmonellae from beef and deboned poultry meat. Applied and Environmental Microbiology 26, 747-751.

221. MOREHOUSE, L.G. & WEDMAN, E.E. (1961) Salmonella and other disease-producing organisms in animal by-products. A survey. Journal of the American Veterinary Medical Association 139, 989-995.
222. MORRIS, T.G. & AYRES, J.C. (1960) Incidence of Salmonellae on commercially processed poultry. Poultry Science 39, 1131-1135.
223. MORRIS, G.K., MARTIN, W.T., SHELTON, W.H., WELLS, J.G. & BRACHMAN, P.S. (1970) Salmonellae in fish meal plants: relative amounts of contamination at various stages of processing and a method of control. Applied Microbiology 19, 401-408.
224. MORSE, E.V. & DUNCAN, M.A. (1974) Salmonellosis: An environmental health problem. Journal of the American Veterinary Medical Association 165, 1015-1019.
225. MOSELEY, B.E.B. (1967) The isolation and some properties of radiation-sensitive mutants of Micrococcus radiodurans. Journal of general Microbiology 49, 293-300.
226. MOSELEY, B.E.B. (1968) The repair of damaged DNA in irradiated bacteria. Advances in Microbial Physiology 2, 173195.
227. MOSSEL, D.A.A. (1960) The destruction of Salmonella bacteria in refrigerated liquid whole egg with gamma radiation. International Journal of Applied Radiation and Isotopes 9, 109-112.
228. MOSSEL, D.A.A. (1966) Perspectives for the use of ionizing radiation in the decontamination (Salmonella radicidation) of some frozen proteinaceous foods and dry mixed feed ingredients. Food Irradiation SM 73/19 p. 365-380.
229. MOSSEL, D.A.A. & BUECHLI, K. (1964) The total object swab ("TOS") technique. A reliable and convenient method for the examination of some proteinaceous staple foods for various types of enterobacteriaceae. Laboratory Practice, dec. 1964, 1184-1187.
230. MOSSEL, D.A.A. & DE GROOT, A.P. (1965) The use of pasteurizing doses of gamma radiation for the destruction of Salmonellae and other Enterobacteriaceae in some foods of low water activity in: Radiation preservation of foods. Public. no. 1273. NAS/NRS Washington, D.C. p. 233-264.
231. MOSSEL, D.A.A. & OEI, H.Y. (1975) Person-to-person transmission of enteric bacterial infection. Lancet 29, 751.
232. MOSSEL, D.A.A. & RATTO, M.A. (1970) Rapid detection of sublethally impaired cells of Enterobacteriaceae in dried foods. Applied Microbiology 20, 273-275.
233. MOSSEL, D.A.A. & TAMMINGA, S.K. (1980) Methoden voor het microbiologisch onderzoek van levensmiddelen. P.C. Noordervliet, Zeist.
234. MOSSEL, D.A.A., EELDERING-DE VOR, I.H. & KEIZER, E.D. (1976) Use of agar immersion, plating and contact (AIPC) slides for the bacteriological monitoring of foods, meals and food environment. Laboratory Practice 25, 393-395.
235. MOSSEL, D.A.A., HARREWIJN, G.A. & ELZEBROEK, B.J.M. (1973) Recommended routine monitoring procedures for the microbiological examination of (infant) foods and drinking water. UNICEF Geneva.
236. MOSSEL, D.A.A., HARREWIJN, G.A. & NESSELROOY-VAN ZADELHOFF, C.F.M. (1974) Standardization of the selective inhibitory effect of surface active compounds used in the media for the detection of Enterobacteriaceae in foods and water processed for safety. Health Laboratory Science 11, 260-267.

237. 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. In: Elimination of harmful organisms from food and feed by irradiation. Vienna. International Agency for Atomic Energy.
238. MOSSEL, D.A.A, KAMPELMACHER, E.H. & VAN NOORLE JANSEN, L.M. (1966) Verification of adequate sanitation of wooden surfaces used in meat and poultry processing. Zentralblatt Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene abt. I Originale 201, 91-104.
239. MOSSEL, D.A.A., KROL, B. & MOERMAN, P.C. (1972) Bacteriological and quality perspectives of Salmonella radicidation of frozen boneless meats. Alimenta 11, 51-60.
240. MOSSEL, D.A.A., VAN SCHOTHORST, M. & KAMPELMACHER, E.H. (1967) Comparative study on decontamination of mixed feeds by radicidation and by pelletisation. Journal of the Science of Food and Agriculture 18, 362-367.
241. MULDER, R.W.A.W. (1974) Vergelijking huidvermalingsmethode en de dompelmethode voor het microbiologisch onderzoek van slachtkuikens. IPS report 3474.
242. MULDER, R.W.A.W. (1976a) Kruiscontaminatie tussen koppels slachtkuikens tijdens het slachtproces. IPS report 4476.
243. MULDER, R.W.A.W. (1976b) Salmonella in slachtkuikens. IPS report 5976.
244. MULDER, R.W.A.W. (1976c) Radiation inactivation of Salmonella panama and Escherichia coli K12 present on deep-frozen broiler carcasses. European Journal of Applied Microbiology 3, 63-69.
245. MULDER, R.W.A.W., DORRESTEIJN, L.W.J. & VAN DER BROEK, J. (1977) Kruisbesmetting tijdens het broeien en plukken van slachtkuikens. Tijdschrift voor Diergeneeskunde 102, 619-629. 246.
246. MULDER, R.W.A.W., NOTERMANS, S. & KAMPELMACHER, E.H. (1977) Inactivation of Salmonellae on chilled and deep frozen broiler carcasses by irradiation. Journal of applied Bacteriology 42, 179-185.
247. MULDER, R.W.A.W., DORRESTEIJN, L.W.J., HOFMANS, G.J.P. & VEERKAMP, C.H. (1976) Experiments with continuous immersion chilling of broiler carcasses according to the code of practice. Journal of Food Science 41, 438-442.
248. MULDER, S.J. (1978) Ultraviolet-bestraling van rundvlees. Vleesdistributie en Vleestechnologie 13, (no. 3) 10-13.
249. NELSON, F.E. (1943) Further studies on the effect of the medium on the apparent survival of heat-treated bacteria. Journal of Bacteriology 46, 486.
250. NELSON, F.E. (1944) Factors which influence the growth of heat treated bacteria II. Further studies on media. Journal of Bacteriology 48, 473-477.
251. NEWELL, K.W., McCLARIN, R., MURDOCK, C.R., MacDONALD, W.N. &HUTCHINSON, H.L. (1959) Salmonellosis in Northern Ireland, with special reference to pigs and Salmonella-contaminated pig-meal. Journal of Hygiene, Cambridge 57, 92-105.
252. NOAMAN, M.A., SILVERMAN, G.J., DAVIS, N.S. & GOLDBLITH, S.A. (1964) Radiosensitization of Streptococcus faecalis and Escherichia coli. Journal of Food Science 29, 80-86.
253. NORTH, W.R. & BARTRAM, M.T. (1953) The efficiency of selenite broth of different compositions in the isolation of Salmonella. Applied Microbiology 1, 130-134.

254. NOTERMANS, S. & KAMPELMACHER, E.H. (1974) Attachment of some bacterial strains to the skin of broiler chickens. British Poultry Science 15, 573-585.
255. NOTERMANS, S. & KAMPELMACHER, E.H. (1975) Further studies on the attachment of bacteria to skin. British Poultry Science 16, 487-496.
256. NOTERMANS, S., HINDLE, V. & KAMPELMACHER, E.H. (1976) Comparison of cotton swab versus alginate swab sampling method in the bacteriological examination of broiler chickens. Journal of Hygiene, Cambridge 77, 205-210.
257. NOTERMANS, S., KAMPELMACHER, E.H. & VAN SCHOTHORST, M. (1975a) Studies on sampling methods used in the control of hygiene in poultry processing. Journal of applied Bacteriology 39, 55-61.
258. NOTERMANS, S., KAMPELMACHER, E.H. & VAN SCHOTHORST, M. (1975b) Studies on different sampling methods for the determination of bacterial counts from frozen broilers. Journal of applied Bacteriology 39, 125-131.
259. NOTERMANS, S., VAN LEUSDEN, F.M., VAN SCHOTHORST, M. & KAMPELMACHER, E.H. (1975) Salmonella-contaminatie van slachtkuikens tijdens het slachtproces in enkele pluimveeslachterijen. Tijdschrift voor Diergeneeskunde 100, 259-264.
260. NOTERMANS, S., VAN SCHOTHORST, M., VAN LEUSDEN, F.M. & KAMPELMACHER, E.H. (1975) Onderzoekingen over het kwantitatief voorkomen van Salmonellae bij diepvrieskuikens. Tijdschrift voor Diergeneeskunde 100, 648-653.
261. OSBORNE, W.W & STOKES, J.L. (1955) A modified selenite brilliant-green medium for the isolation of salmonella from egg products. Applied Microbiology, 3, 295-299.
262. OTTAVIANI, F., ALIANI, A. & BENETTI, L. (1976) I. brodi di arrichimento e di isolamento delle salmonelle. Annali Sclavo 18, 481-484.
263. PATTERSON, J.T. (1972) Microbiological sampling of poultry carcasses. Journal of applied Bacteriology 35, 569-575.
264. PATTERSON, J.T. & GIBBS, P.A. (1975) Sampling methods for poultry meat. In: Proceedings 2nd European Symposium on Poultry Meat Quality, B. Erdtsieck (ed), Oosterbeek, May 12-16 p. 22 (1) - 22 (10).
265. PAYNE, D.J.H. (1969) Salmonellosis and intensive farming. Public Health London 84, 5-16.
266. PEPPER, R.E., BUFFA, N.T. & CHANDLER, V.L. (1956) Relative resistances of microorganisms to cathode rays. III. Bacterial spores. Applied Microbiology 4, 149-152.
267. PHILLIPS, A.W., NEWCOMB, H.R. & BACH, F.C. (1960) Experimental preservation of poultry by chlortetracycline and radiation. Food Technology 14, 521-522.
268. PIETZSCH, O. (1977) Zur Epidemiologie von Salmonella-Infektionen durch Heil- und Kindernahrung. Vortrag 14 Juni 1977, West-Berlin.
269. PIETZSCH, O. & BULLING, E. (1974) Salmonella-Isolierungen aus Tieren sowie Lebens- und Futtermitteln in der Bundesrepublik Deutschland 1961 bis 1972. Bundesgesundheidsblatt 17, 194.
270. PIETZSCH, O. & BULLING, E. (1976) Verbreitung der Salmonella-Infektionen bei Tieren, tierischen Lebens- und Futtermitteln in der Bundesrepublik Deutschland eischl. Berlin (West). Bundesgesundheidsblatt 19, 97-115.

271. PITILLO, R.F. (1966) A new radioprotector in Escherichia coli. Radiation Research 28, 811-821.
272. POLLOCK, H.M. & DAHLGREN, B.J. (1974) Clinical evaluation of enteric media in the primary isolation of Salmonella and Shigella. Applied Microbiology 27, 197-201.
273. POMEROY,B.S. & GRADY, M.K. (1961) Salmonella organisms isolated from feed ingredients. Proceedings 65th Annual Meeting US Livestock San. A (oct. 1961) 449-452.
274. POSTGATE, J.R. & HUNTER, J.R. (1963) Metabolic injury in frozen bacteria. Journal of applied Bacteriology 26, 405-414.
275. PREVITE, J.J. (1967) Salmonellosis the problem and potential remedy. Activities Report 19, 64.
276. PREVITE, J.J., CHANG, Y & EL-BISI. H.M. (1970) Effects of radiation pasteurization on Salmonella. I. Parameters affecting survival and recovery from chicken. Canadian Journal of Microbiology 16, 465-471.
277. PREVITE, J.J., CHANG, Y. & EL-BISI. H.M. (1971) Effects of radiation pasteurization on Salmonella. III. Radiation lethality and the frequency to antibiotic resistance. Canadian Journal of Microbiology 17, 385-390.
278. PROCTOR, B.E., NICKERSON, J.T.R. & LICCIARDELLO, J.J. (1956) Cathode ray irradiation of chicken meat for the extension of shelf life. Food Technology 10, 11-20.
279. RACKOW, H.G. & WIESE, H. (1958) Ergebnisse und Erfahrungen 3 jaehriger bakteriologischen Einfuehrungsuntersuchungen von Futtermitteln tierischer Herkunft auf gesetzlicher Grundlage. Berliner und Muenchener tieraerztliche Wochschrift 71, 340.
280. RAPPAPORT, F., KONFORTI, N. & NAVON, B. (1956) A new enrichment medium for certain Salmonellae. Journal of Clinical Pathology 9, 261-266.
281. RASMUSSEN, O.G., HANSEN, R., JACOBS, N.J. & WILDER, O.H.M. (1964) Dry heat resistance of Salmonellae in rendered animal by-products. Poultry Science 43, 1151-1157.
282. RAY, B. (1979) Methods to detect stressed microorganisms. Journal of Food Protection 42, 346-366.
283. RAY, B. & SPECK, M.L. (1973a) Discrepancies in the enumeration of Escherichia coli. Applied Microbiology 25, 494-498.
284. RAY, B. & SPECK, M.L. (1973b) Enumeration of Escherichia coli in frozen samples after recovery from injury. Applied Microbiology 25, 499-503.
285. RAY, B., JEZESKI, J.J. & BUSTA, F.F. (1971) Effect of rehydration on recovery, repair, and growth of injured freeze-dried Salmonella anatum. Applied Microbiology 22, 184189.
286. READ, R.B. jr & REYES, A.L. (1968) Variation in plating efficiency of Salmonellae on eight lots of brilliant green agar. Applied Microbiology 16, 746-748.
287. REINDERS, M.E. (1974) Bestrijding van schimmels en bacterien in veevoeder. De Molenaar 77,p. 22,50,74.
288. RESTAINO, L., GRAUMAN, C.S., McCALL, W.A. & HILL, W.M. (1977) Effects of varying concentrations of novobiocin incorporated into two Salmonella plating media on the recovery of four Enterobacteriaceae. Applied and Environmental Microbiology 33, 585-589.
289. REUSSE, U., MEYER, A. & TILLACK, J. (1976) Zur Methodik des Salmonellen-Nachweises aus gefrorenem Gefluegel. Archiv fuer Lebensmittelhygiene 27, 98-100.

290. REUSSE, U., BISCHOFF, J., FLEISCHHAUER, G. & GEISTER, R. (1976) Pasteurisierung von Fischmehl durch Bestrahlung. I. Mitteilung: Feststellung der rationellsten Strahlendosis durch die im einem kontaminierten Fischmehl Salmonellen abgetoetet werden. Zentralblatt Veterinaer Medizin B 23, 158170.
291. RHODES, D.N. (1965) The radiation pasteurization of broiler chicken carcasses. British Poultry Science 6, 265-271.
292. ROBACH, M.C. & IVEY, F.J. (1978) Antimicrobial efficacy of a potassium sorbate dip on freshly processed poultry. Journal of Food Protection 41, 284-288.
293. ROBERTS, D. (1972) Observations on procedures for thawing and spit-roasting frozen dressed chickens and post-cooking care and storage with particular reference to food poisoning bacteria. Journal of Hygiene, Cambridge 70, 565-588.
294. ROBERTS, D., BOAG, K., HALL, M.L.M. & SHIPP, C.R. (1975) The isolation of salmonellas from British pork sausages and sausage meat. Journal of Hygiene, Cambridge 75, 173-184.
295. ROHDE, R. & BISCHOFF, J. (1956) Die epidemiologische Bedeutung salmonella-infizierter Tierfuttermittel (insbesondere Knochenschrot und Fischmehl) als Quelle verschiedener Lebensmittelvergiftungen. Zentralblatt Bakteriologie I Ref. 159, 145-164.
296. ROMBOUTS, F.M. & KAMPELMACHER, E.H. (1973) Voedselvergiftigingen. Natuur en Techniek 41, 2-16.
297. RUSSEL, A.D. & HARRIES, D. (1967) Some aspects of thermal injury in Escherichia coli. Applied Microbiology 15, 407-410.
298. RUTQVIST, L. (1961) Vorkommen von Salmonella in Futtermitteln vegetabilischen Ursprunges. Zentralblatt Veterinaer Medizin 8, 1016-1024.
299. SADLER, W.W., YAMAMOTO, R., ADLER, H.E. & STEWART, G.F. (1961) Survey of market poultry for Salmonella infection. Applied Microbiology 9, 72-76.
300. Salmonella typhimurium outbreak traced to a commercial cider (1975) Morbidity and Mortality Weekly Report 24, 87-88.
301. Salmonella newport contamination of hamburger (1975) Morbidity and Mortality Weekly Report 24, 438-443.
302. SANNER, T. & PIHL, A. (1969) Significance and mechanism of the indirect effect in bacterial cells. The relative protective effect of added compounds in Escherichia coli B irradiated in liquid and frozen suspension. Radiation Research 37, 216-227.
303. SCHOENMAKERS, M.J.G. (1973) Onderzoek naar het voorkomen van Salmonella in een slagerij met verkoop van verse onverpakte slachtkuikens en delen daarvan. Tijdschrift voor Diergeneeskunde 98, 503-505.
304. SCHOLZ, H.D. (1964) Beobachtungen zur Salmonellose des Gefluegels. Deutsche Tieraerztliche Wochenschrift 71, 98-102.
305. SIEMS, H. (1978) Experience in the isolation of Salmonellae in foods of different risk categories. Zentralblatt Bakteriologie und Hygiene. I. Abteilung. Originale: B 167, 120-128.
306. SIEMS, H., HILDEBRANDT, G., ARNDT, G. & WEISS, H. (1981) Use of Most Probable Number Technique for quantitative detection of salmonellae. Fleischwirtschaft 61, 1740-1745.
307. SIEMS, H., HILDEBRANDT, G., INAL, T. & SINELL, H.J. (1975) Official inquiry into the presence of salmonella in frozen slaughtered poultry on the german market. Zentralblatt Bakteriologie und Hygiene. I. Abteilung. Originale: B 160, 84-97.

308. SILLIKER, J.H. & GABIS, D.A. (1973) ICMSF methods studies. I. Comparison of analytical schemes for detection of Salmonella in dried foods. Canadian Journal of Microbiology 19, 475-479.
309. SILLIKER, J.H. & GABIS, D.A. (1974) ICMSF methods studies. V. The influence of selective enrichment media and incubation temperatures on the detection of salmonellae in raw frozen meats. Canadian Journal of Microbiology 20, 813-816.
310. SILVERMAN, G.J. & SINSKEY, T.J. (1968) The destruction of microorganisms by ionizing irradiation. In: Disinfection, Sterilization and Preservation. C.A. Lawrence & S.S. Block (eds), Lea & Febiger. Philadelphia p. 741.
311. SILVERSTOLPE, L., PLAZIKOWSKI, U., KJELLANDER, J. & VAHLNE, G. (1961) An epidemic among infants caused by Salmonella muenchen. Journal of applied Bacteriology 24, 134-142.
312. SIMONSEN, B. (1971) Methods for determining the microbial counts of ready-to-cook poultry. Worlds Poultry Science Journal 27, 368.
313. SINSKEY, T.J. & SILVERMAN, G.J. (1970) Characterization of injury incurred by Escherichia coli upon freeze-drying. Journal of Bacteriology 101, 429-437.
314. SINSKEY, T.J., SILVERMAN, G.J. & GOLDBLITH, S.A. (1967) Influence of platen temperatures and relative humidity during storage on the survival of freeze-dried Salmonella typhimurium. Applied Microbiology 15, 22-30.
315. SINSKEY, T.J., McINTOSH, A.H., PABLO, I.S., SILVERMAN, G.J. & GOLDBLITH, S.A. (1964) Considerations in the recovery of microorganisms from freeze-dried foods. Health Laboratory Science 1, 297-306.
316. SMITH, H.W. (1960) The effect of feeding pigs on food naturally contaminated with salmonella. Journal of Hygiene, Cambridge 58, 381-389.
317. SMITH, H.W. & TUCKER, J.F. (1975) The effect of antibiotic therapy on the faecal excretion of Salmonella typhimurium by experimentally infected chickens. Journal of Hygiene, Cambridge 75, 275-292.
318. SNEDECOR, G.W. & COCHRAN, W.G. (1971) Statistical methods. Iowa State University Press, Ames, Iowa, USA.
319. SNOEYENBOS, G.H., CARLSON, V.L., McKIE, B.C. & SMYSER, C.F. (1967) An epidemiological study of Salmonellosis of chickens. Avian Diseases 11, 653-667.
320. SNIJDERS, J.M.A. (1976) Hygiene bij het slachten van varkens. Thesis University of Utrecht.
321. SPENCER, J.V., STREETER, E.M. & GEORGE, M.H. (1968) Effect of an iodophor on shelf life of poultry. Poultry Science 47, 1721.
322. SPOERL, E., LOVELESS, L.E., WEISMAN, T.H. & BALSKE, R.J. (1954) Studies on cell division. II. X-radiation as a division inhibitory agent. Journal of Bacteriology 67, 394-401.
323. STAPLETON, G.E. (1955) Variations in the sensitivity of Escherichia coli to ionizing radiations during the growth cycle. Journal of Bacteriology 70, 357-362.
324. STAPLETON, G.E., BILLEN, D. & HOLLAENDER, A. (1953) Recovery of X-irradiated bacteria at suboptimal incubation temperatures. Journal of Cellular and Comparative Physiology 41, 345-357.
325. STAPLETON, G.E., SBARRA, A.J. & HOLLAENDER, A. (1955) Some nutritional aspects of bacterial recovery from ionising radiations. Journal of Bacteriology 70, 7-14.

326. STELLMANN, C. (1964) Radiosensibite des microorganismes: consequences pratiques. Revue de Pathology Comparee 1, 20-27.
327. STOKES, J.L. & OSBORNE, W.E. (1955) A selenite brilliant green medium for the isolation of Salmonella. Applied Microbiology 3, 217-220.
328. STOTT, J.A., HODGSON, J.E. & CHANEY, J.C. (1975) Incidence of Salmonellae in animal feed and the effect of pelleting on content of Enterobacteriaceae. Journal of applied Bacteriology 39, 41-46.
329. STRAKA, R.P. & STOKES, J.L. (1959) Metabolic injury to bacteria at low temperatures. Journal of Bacteriology 78, 181-185.
330. SURKIEWICZ,B.F., JOHNSTON, R.W., MORAN, A.B. & KRUMM, G.W. (1969) A bacteriological survey of chicken eviscerating plants. Food Technology 23, 80-83.
331. SUTTON, R.G.A. (1973) Food poisoning and Salmonella infections in Australia in: The microbiological safety of food, B.C.Hobbs & J.H.B. Christian (eds). Academic Press, London-New York p. 153-163.
332. SZANTON, V.L. (1957) A 30-month study of 80 cases of Salmonella oranienburg infection. Pediatrics nov. 1957, 794-808.
333. TACONIS, W.K. & VAN FURTH, R.(1972) Kliniek en epidemiologie van de andere Salmonellosen. Nederlands Tijdschrift voor Geneeskunde 116, 2260-2270.
334. TAYLOR, W.I. (1956) Isolation of Shigella. I. Xylose lysine agars: new media for isolation of enteric pathogens. American Journal of Clinical Pathology 48, 356-362.
335. TAYLOR, W.I. & SCHELHART, D. (1971) Isolation of shigellae. VIII. Comparison of Xylose Lysine Deoxycholate Agar, Hektoen Enteric Agar, Salmonella Shigella Agar and Eosine Methylene Blue Agar with stool specimens. Applied Microbiology 21, 32-37.
336. TEOTIA, J.S. (1973) Chemical pasteurization of poultry meat. Thesis Colorado State University, Deparment of animals science. Fort Collins, Colorado 80521.
337. THAL, E., RUTQVIST, L. & HOLMQVIST, H. (1957) Salmonella isolated from animals in Sweden during the years 1949 to 1956. Nordisk Veterinary Medicin 9, 822-830.
338. THATCHER, F.S. & CLARK, D.S. (eds) (1968) Microorganisms in foods: their significance and methods of enumeration. University of Toronto, Toronto, Ontario.
339. THATCHER, F.S. & LOIT, A. (1961) Comparative microflora of chlortetracycline-treated and nontreated poultry with special reference to public health aspects. Applied Microbiology 9, 39-45.
340. THOMSON, J.E. & BAILEY, J.S. (1978) Innovations in sampling and culturing methods for bacteriological examination of broiler carcasses. Journal of Food Science 43, 1301-1302.
341. THOMSON, J.E., COX, N.A. & BAILEY, J.S. (1977) Control of Salmonella and extention of shelf-life of broiler carcasses with a glutaraldehyde product. Journal of Food Science 42, 1353-1355.
342. THORNLEY, M.J. (1963a) Radiation resistance among bacteria. Journal of applied Bacteriology 26, 334-345.
343. THORNLEY, M.J. (1963b) Microbiological aspects of the use of radiation for the elimination of Salmonellae from foods and feeding stuffs. International Atomic Energy Agency. Technical Report Series 22, 81-106.
344. THORNLEY, M.J. (1966) Irradiation of poultry and egg products. Food Irradiation, Symposium Karlsruhe, SM73/63 p. 427-436.

345. THORNLEY, M.J., INGRAM, M. & BARNES, E.M. (1960) The effects of antibiotics and irradiation on the Pseudomonas-Achromobacter flora of chilled poultry. Journal of applied Bacteriology 23, 487-498.
346. TIWARI, N.P. & MAXCY, R.B. (1972) Post-irradiation evaluation of pathogens and indicator bacteria. Journal of Food Science 37, 485-488.
347. TODD, E.C.D. (1976) The first annual summary of food-borne disease in Canada. Journal of Milk and Food Technology 39, 426-431.
348. TODD, E.C.D. (1977) Foodborne disease in Canada-1974. Annual Summary. Journal of Food Protection 40, 493-498.
349. TODD, E.C.D. (1980) Poultry-associated foodborne disease. Its occurrence, cost, sources and prevention. Journal of Food Protection 43, 129-139.
350. TODD, E.C.D. & PIVNICK, H. (1974) The significance of food poisoning in Canada. Canadian Journal of Public Health 65, 89-91.
351. TOMLINS, R.I., PIERSON, M.D. & ORDAL, Z.J. (1971) Effect of thermal injury on the TCA cycle enzymes of Staphylococcus aureus MT 31 and Salmonella typhimurium 7136. Canadian Journal of Microbiology 17, 759-765.
352. TYRRELL, R.M. (1974) The interaction of near UV and X-radiations on wild-type and repair deficient strains of Escherichia coli K12: physical and biological measurements. International Journal of Radiation Biology 25, 373-390.
353. UNDERDAL, B. & ROSSEBO, L. (1972) Inactivation of strains of Salmonella senftenberg by gamma irradiation. Journal of applied Bacteriology 35, 371-377.
354. VAN DER SCHAAF, A. (1962) Salmonellosis as a scheduled disease. Tijdschrift voor Diergeneeskunde 87, 976-980.
355. VAN DER SCHAAF, A. & MOSSEL, D.A.A. (1963) Gamma radiation sanitation of fish and blood meals. International Journal of Applied Radiation and Isotopes 14, 557-562.
356. VAN SCHOTHORST, M. (1976) Resuscitation of injured bacteria in foods. In: Inhibition and inactivation of vegetative microbes. F.A. Skinner & W.B. Hugo (eds), Academic Press, London, New York, San Francisco p. 317-328.
357. VAN SCHOTHORST, M. & KAMPELMACHER, E.H. (1971) Overzicht van infectie, besmetting en verontreiniging door organismen. Voedingsmiddelentechnologie 2, 18-25.
358. VAN SCHOTHORST, M. & VAN LEUSDEN, F.M. (1972a) Studies on the isolation of injured Salmonellae from foods. Zentralblatt Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. Abt. I. Originale A 221, 19-29.
359. VAN SCHOTHORST, M. & VAN LEUSDEN, F.M. (1972b) Wateractiviteit en resuscitatie van Enterobacteriaceae: Isolatie uit voedermiddelen. Voedingsmiddelentechnologie 3, 164-165.
360. VAN SCHOTHORST, M. & VAN LEUSDEN, F.M. (1975a) Comparison of several methods for the isolation of salmonellae from egg products. Canadian Journal of Microbiology 21, 1041-1045.
361. VAN SCHOTHORST, M. & VAN LEUSDEN, F.M. (1975b) Further studies on the isolation of injured salmonellae from foods. Zentralblatt Bakteriologie und Hygiene. Abt. Originale A 230, 186-191.
362. VAN SCHOTHORST, M., GUINEE, P.A.M., KAMPELMACHER, E.H. & VAN KEULEN, A. (1965) Onderzoek naar het voorkomen van Salmonella-kiemen bij slachtkippen en -kuikens in Nederland. Tijdschrift voor Diergeneeskunde 90, 1523-1531.

363. VAN SCHOTHORST, M., VAN LEUSDEN, F.M., EDEL, W. & KAMPELMACHER, E.H. (1974) Verdere onderzoekingen over het voorkomen van Salmonella bij slachtkuikens en -kippen in Nederland. Tijdschrift voor Diergeneeskunde 99, 815-820.
364. VAN SCHOTHORST, M., NORTHOLT, M., KAMPELMACHER, E.H. & NOTERMANS, S. (1976) Studies on the estimation of the hygienic condition of frozen broilers. Journal of Hygiene, Cambridge 76, 57-63.
365. VEERKAMP, C.H. (1973) Oppervlakte en specifiek oppervlak van slachtkuikens. IPS report 7273.
366. VEERKAMP, C.H., MULDER, R.W.A.W. & GERRITS, A.R. (1972) Reinigung und Kuehlung von Schlachtgefluegel. Fleischwirtschaft 52, 612-621.
367. VEREIJKEN, P.F.G. (1981) Gebruik van een verdunningsreekstechniek in een speciaal geval. IWIS-TNO Wageningen, C81ST 5332.
368. VERNON, E. (1970) Food poisoning and Salmonella infections in England and Wales, 1968. Public Health, London 84, 239-260.
369. VERNON, E. (1977) Food poisoning and Salmonella infections in England and Wales, 1973-1975. Public Health, London 91, 225-235.
370. VERNON, E. & TILLETT, H.E. (1974) Food poisoning and Salmonella infections in England and Wales, 1969-1972. Public Health, London 88, 225-235.
371. VOETEN, A.C., BRUS, D.H.J. & JAARTSVELD, F.H.J. (1974) Distribution of Salmonella within a closed broiler chicken integration. Tijdschrift voor Diergeneeskunde 99, 1093-1109.
372. WALKER, H.W. & AYRES, J.C. (1956) Incidence and kinds of microorganisms associated with commercially dressed poultry. Applied Microbiology 4, 345-350.
373. WALKER, J.H.C. (1957) Organic fertilizers as a source of Salmonella infection. Lancet 2, 283-284.
374. WALKER, J.H.C. (1960) The broiler industry-Transmission of Salmonella infection. Journal of the Royal Sanitation Institute 80, 142-145.
375. WATKINS, J.R., FLOWERS, A.J. & GRUMBLES, L.C. (1959) Salmonella organisms in animal products used in poultry feeds. Avian Diseases 2, 290-301.
376. WATSON, D.C. & WALKER, A.P. (1978) A modification of brilliant green agar for improved isolation of Salmonella. Journal of applied Bacteriology 45, 195-204.
377. WEBB, S.J. (1963) The effect of relative humidity and light on air-dried organisms. Journal of applied Bacteriology 26, 307-313.
378. WEDMAN, E.E (1961) Findings and recommendations of the USDA task force on Salmonella in animal by-products and feeds. Proceedings 65th Annual Meeting US Livestock San. A 458-463.
379. WEIDLICH,N. & NIEDEREHE, H. (1961) Uber eine mit Erkrankungen beim Menschen zusammenhaengende latente Salmonellen Infektion beim Gefluegel. Archiv fuer Lebensmittelhygiene 12, 121-124.
380. WEISS, J. (1959) Effects of radiations on acqueous systems under aerobic and anaerobic conditions. International Journal of Applied Radiation and Isotopes 6,52-58.
381. WESTERFELD, B.L., ADAMS, A.W., ERWIN, L.E. & DEYOE, C.W. (1970) Effect of chemical additive on Salmonella in poultry feed and host birds. Poultry Science 49, 1319-1323.
382. WHEATON, E. & PRATT, G.B. (1962) Radiation survival curves of Clostridium botulinum spores. Journal of Food Science 27. 327-334.

383. WIERBICKI, E., ANELLIS, A., KILLORAN, J.J., JOHNSON, E.L., THOMAS, M.H. & JOSEPHSON, E.S. (1970) High dose radiation processing of meat, poultry and seafood products. 3rd International Congress Food Science and Technology, Washington, August 9-14.

384. WILDER, A.N. & MACCREADY, R.A. (1966) Isolation of Salmonella from poultry. Poultry products and poultry processing plants in Massachusetts. New England Journal of Medicin 274, 1453-1460.

385. WILLIAMS, L.P. & NEWELL, K.W. (1968) Sources of Salmonellas in market swine. Journal of Hygiene, Cambridge 66, 281-293.

386. WILLIAMS, L.P., VAUGHN, J.B., SCOTT, A & BLANTON, V. (1969) A ten-month study of Salmonella contamination in animal protein meals. Journal of the American Veterinary Medical Association 155, 167-174.

387. WILSON, E., PAFFENBARGER, R.S., FOTER, M.J. & LEWIS, K.H. (1961) Prevalence of Salmonellae in meat and poultry products. Journal of Infectious Diseases 109, 166-171.

388. WILSON, W.J. & BLAIR, E.M.McV. (1927) Use of a glucose bismuth sulfite iron medium for the isolation of B. typhosis and B. proteus. Journal of Hygiene 26, 374-391.

389. WINKLE, S. & ROHDE, R. (1957) Ueber die Gefahr des bakteriologisch unkontrollierten Importes auslaendischer Tierfuttermittel mit besonderer Beruecksichtigung der Schweinezucht. Berliner und Muenchener tieraerztliche Wochschrift 70, 443-444.

390. WITTER, L.D. & ORDAL Z.J. (1977) Stress effects and food microbiology. In: Antibiotics and antibiosis in agriculture. M.Woodbine (ed), Butterworths Inc., Reading Mass.

391. WOODBURNE, M. (1964) Incidence of Salmonellae in dressed broiler-fryer chickens. Applied Microbiology 12, 492-495.

392. WOODSIDE, E.E. (1965) Differentiation between radiation-sensitive and -resistant mutants of Escherichia coli , strain B. Canadian Journal of Microbiology 11, 243-257.

393. WRIGHT, M.L., ANDERSON, G.W. & EPPS, N.A. (1962) Salmonella isolations from feed additives of animal origin. Canadian Journal of Public Health 53, 36.

394. ZAJC-SATLER, J. & GRAGAS, A.Z. (1977) Xylose Lysine Deoxycholate Agar for the isolation of Salmonella and Shigella from clinical specimen. Zentralblatt Bakteriologie und Hygiene. I. Abteilung. Originale. A. 237, 196-200.

395. ZINDEL, H.C. & BENETTE, M.V. (1968) Salmonellae in poultry feeds. Poultry Science 47, 1925-1928.

ADDENDUM

396. BOLDER, N.M., VAN DER HULST, M.C. & MULDER, R.W.A.W. (1980) Salmonellae in broiler chicks. Proceedings 6th European Poultry Conference, Hamburg, 6-12 Sept. 1980, Volume II, 271-279.

397. MOSSEL, D.A.A. (1978) Index and indicator organisms - a current assessment of their usefulness and significance. Food Technology in Australia 30, 212-219.

398. VASSILIADIS, P., TRICHOPOULOS. D., PATERAKI, E. & PAPAICONOMOU, N. (1978) Isolation of Salmonella from minced meat by the use of a new procedure of enrichment. Zentralblatt fuer Bakteriologie. Originale B. Hygiene, Praeventive Medizin 166, 81-86.

399. VASSILIADIS, P., TRICHOPOULOS, D., PAPOUTSAKIS, G. & PALLANDIOU, E. (1979) A note on the comparison of two modifications of Rappaport's Medium with Selenite Broth in the isolation of Salmonellas. Journal of applied Bacteriology 46, 567-569.
400. BECKERS, H.J. (1981) Voedselinfecties en -vergiftigingen. Nederlands Tijdschrift voor Geneeskunde 125, 2167-2170.
401. RIEMANN, H & BRYAN, F.L. (eds) (1979) Food-Borne infection and intoxications, Academic Press, New York, San Francisco, London.
402. BOUWER-HERZBERGER, S.A. & MOSSEL, D.A.A. (1980) Onderzoek naar de morbiditeit en etiologie van door voedsel overgebrachte ziekten in de regio Haarlem. Nederlands Tijdschrift voor Geneeskunde 124, 1460-1463.
403. BRYAN, F.L., FANELLI, M.J. & RIEMANN, H. (1979) Salmonella infections in : Food-Borne infection and intoxications by H. Riemann & F.L. Bryan (eds), Academic Press, New York, San Francisco, London. 73-130.
404. VAN SCHOTHORST, M., HUISMAN, J. & VAN OS, M. (1978) Salmonella onderzoek in huishoudens met salmonellose bij zuigelingen. Nederlands Tijdschrift voor Geneeskunde 122, 1121-1125.
405. INGRAM, M. & ROBERTS, T.A. (1980) Ionizing radiation in : Microbial ecology of foods. Volume I. Factors affecting life and death of microorganisms by : ICMSF, Academic Press, New York, London, Toronto, Sydney, San Francisco. 46-49.
406. RAEVUORI, M., SEUNA, E & NURMI, E. (1978) An epidemic of Salmonella infantis infection in Finnish broiler chickens in 1975-1976. Acta veterinaria Scandinavia 19, 317-330.
407. HEPNER, E. (1980) Food poisoning and Salmonella infection in England and Wales, 1976-1978. An analysis of reports to the Communicable Disease Surveillance Centre of the Public Health Laboratory Service. Public Health 94, 337-349.
408. VAN NETTEN, P. & MOSSEL, D.A.A. (1980) The ecological consequences of decontaminating raw meat surfaces with lactic acid. Archiv fuer Lebensmittelhygiene 31, 190-191.
409. KAMPELMACHER, E.H. (ed)(1982) Proceedings Symposium Food irradiation, Ede 20th october 1981. Wageningen, Pudoc. In press.
410. MOSSEL, D.A.A., KOOPMAN, M.J. & JONGERIUS, E. (1967) Enumeration of Bacillus cereus in foods. Applied Microbiology 15, 650-653.
411. MOSSEL, D.A.A., BIJKER, P.G.H. & EELDERINK, I. (1978) Streptokokken der Lancefield-Gruppe D in Lebensmitteln und Trinkwasser - Ihre Bedeutung, Erfassung und Bekaempfung. Archiv fuer Lebensmittelhygiene 29, 121-127.
412. MOSSEL, D.A.A., VISSER, M. & MENGERINK, W.H.I. (1962) A comparison of media for the enumeration of moulds and yeasts in foods and beverages. Laboratory Practice 11, 109-112.
413. MOSSEL, D.A.A. & DRION, E.F. (1979) Risk analysis. Its application to the protection of the consumer against food-transmitted diseases of microbial aetiology. Antonie van Leeuwenhoek 45, 321-323.
414. MOSSEL, D.A.A. & POUW, H. (1973) Studies on the suitability of sulphite cycloserine agar for the enumeration of Clostridium perfringens in food and water. Zentralblatt Bakteriologie, Originale A, 223, 559-565.
415. HARVEY, R.W.S. & PRICE, T.H. (1980) Salmonella isolation with Rappaport's medium after pre-enrichment in buffered peptone water using a series of inoculum ratio's. Journal of Hygiene, Cambridge 80, 125-128.

CURRICULUM VITAE

Roelof Wijnandus Antoon Wea Mulder werd op 30 maart 1946 geboren te Loppersum. Na het behalen van het HBS-B diploma in 1963 aan het Christelijk Lyceum te Stadskanaal, begon hij zijn studie in de scheikundige technologie aan de Technische Hogeschool te Delft. Deze studie werd in 1969 afgesloten met het doctoraal examen. Het afstudeeronderzoek werd onder leiding van Prof. dr. T.O. Wiken in het Laboratorium voor algemene en toegepaste microbiologie van de TH te Delft uitgevoerd.
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