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**Radiation Control
of Salmonellae
in Food
and Feed Products**



INTERNATIONAL ATOMIC ENERGY AGENCY - VIENNA, 1963

RADIATION CONTROL OF SALMONELLAE
IN FOOD AND FEED PRODUCTS

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**RADIATION CONTROL
OF SALMONELLAE IN FOOD
AND FEED PRODUCTS**

REPORT OF A PANEL ON
IRRADIATION CONTROL OF HARMFUL ORGANISMS
TRANSMITTED BY FOOD AND FEED PRODUCTS,
WITH PARTICULAR REFERENCE TO SALMONELLAE
HELD IN VIENNA,
12-14 DECEMBER 1962

INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA, 1963

RADIATION CONTROL OF SALMONELLAE
IN FOOD AND FEED PRODUCTS,
IAEA, VIENNA, 1963
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FOREWORD

A panel on radiation control of harmful organisms transmitted by food and feed products, with special reference to salmonellae, was convened by the International Atomic Energy Agency in December 1962. The meeting was motivated by a consideration of the increasing risk of transmission of pests and diseases that is a consequence of the growth in world trade with such commodities. Most food and feed products are distributed from large centralized plants, and an incidence of primary infection at such centres can lead to the spread of diseases over wide areas and among a great number of individuals. Although the problem exists in all countries, it is often more severe in tropical and subtropical regions where good sanitation is difficult to maintain.

The primary purpose of the panel was to advise the Director General of the International Atomic Energy Agency as to how the Agency can best fulfil its role in this field. The meeting was attended by twelve experts on public health problems, food hygiene, radiomicrobiology and radiation technology and by representatives of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO). In view of the seriousness of the problem of the spread of salmonellae and of the fact that radiation control seems to offer significant advantages in a number of cases, it was recommended by the panel members that the Agency should undertake to publish the papers presented. This seemed to be desirable also, because no meeting appears to have been held previously at which the usefulness of radiation for the elimination of harmful microbes was reviewed in such detail and against the background of so much information on the overall problem.

In presenting this compilation, the International Atomic Energy Agency would like to express its gratitude to all participants in the panel for their advice and guidance.

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THE EPIDEMIOLOGY OF SALMONELLOSIS IN RELATION TO ITS TRANSMISSION BY FOOD AND FEED PRODUCTS

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Abstract — Résumé — Аннотация — Resumen

THE EPIDEMIOLOGY OF SALMONELLOSIS IN RELATION TO ITS TRANSMISSION BY FOOD AND FEED PRODUCTS. The occurrence of salmonellosis in animals and man is being considered, and on the basis of an extensive literature survey attention is drawn to the frequent presence of Salmonella in animals and man. The modes of contamination now believed important, particularly the part played by food and feed products and of water, are also reviewed. A survey is made of different existing methods for disinfection of such products.

The author ends by recalling general measures of hygiene which should be observed to minimize the hazard of food poisoning by Salmonella.

ÉPIDÉMIOLOGIE DE LA SALMONELLOSE ET SA TRANSMISSION A L'HOMME ET AUX ANIMAUX PAR L'INTERMÉDIAIRE DES ALIMENTS. L'auteur étudie la salmonellose de l'homme et des animaux; se fondant sur de nombreux ouvrages, il souligne la présence fréquente de Salmonellae chez l'homme et les animaux. Il examine également les voies de contamination actuellement considérées comme importantes, en particulier le rôle joué par les aliments et l'eau. Il passe en revue les différentes méthodes actuelles de désinfection de ces produits.

L'auteur termine son exposé en rappelant les mesures générales d'hygiène à prendre pour réduire les risques de contamination des aliments par les Salmonellae.

ЭПИДЕМИОЛОГИЯ САЛМОНЕЛЛЕЗА В СВЯЗИ С ПЕРЕНОСОМ ЕГО С ПИЩЕВЫМИ ПРОДУКТАМИ И КОРМАМИ. Рассматривается распространение салмонеллеза у животных и человека, и на основе изучения обширной литературы обращается внимание на частые случаи наличия салмонеллы в организме животных и человека. Рассматриваются также считающиеся сейчас важными пути заражения, и особенно та роль, которую играют в этом пищевые продукты, корма и вода. Рассматриваются различные существующие методы дезинфекции этих продуктов.

В заключение автор напоминает об общих гигиенических мерах, которые необходимо осуществлять, чтобы свести к минимуму опасность заражения пищевых продуктов салмонеллой.

EPIDEMIOLOGÍA DE LA SALMONELOSIS: SU TRANSMISIÓN POR LOS ALIMENTOS Y LOS FORRAJES. La memoria estudia la salmonelosis en los animales y en el hombre sobre la base de una revisión muy completa de la bibliografía, destaca la frecuencia con que se encuentran Salmonellae en los animales y en el hombre. Examina las formas de contaminación que hoy se consideran importantes, en especial por conducto de los alimentos, los forrajes y el agua. Estudia los diversos métodos que se aplican para desinfectar dichos productos.

El autor termina recordando las medidas generales de higiene que se deben adoptar para reducir a un mínimo el peligro de que los alimentos queden contaminados con Salmonellae.

Salmonella infections were long considered simply as zoonoses, i. e. animal diseases that can be transmitted to man. The present trend is to consider them rather as anthroozoonoses (van der SCHAAF [200]), i. e. diseases transmitted directly or indirectly by man to animals; the role of man is not always easy to determine.

We shall not here describe Salmonella, the causal organism, but merely point out that every month new serotypes are being discovered and identified.

Although 700 to 800 are now known, Kaufmann believes there may be as many as 3000 (LANG [117]).

Since human beings are usually infected by consuming contaminated food of animal or vegetable origin, we shall first consider salmonellosis in animals. This will give us a better understanding of salmonellosis in man and its epidemiology.

SALMONELLOSIS IN ANIMALS

A distinction is usually made between acute salmonellosis (primary or secondary to an affection which, for example, has necessitated emergency slaughtering) and chronic salmonellosis (the presence of Salmonella in carriers which temporarily or permanently excrete it and in such organs as the liver and the mesenteric ganglia).

Acute salmonellosis sometimes appears as minor or even large-scale epizootic disease, manifested in livestock diarrhoea, frequently bloody, which in certain animals causes extremely serious - and sometimes fatal - disorders. One case, caused by Salmonella dublin, was reported very recently by PERDRIX et al. [154]. Of thirty cows, ten were very sick, and two died. Coccidiosis was first thought of, but then a non-gasogenic Salmonella dublin (previously isolated in Iran and France) was isolated. This organism has also been found in the cerebrospinal fluid of a child. The herd was heavily infested by flukes and strongyles.

In England MORTEN [142] reports a very serious enzootic in a farm of 128 animals (cows and calves). Salmonella typhimurium, type IA, was isolated. Besides exhibiting the customary symptoms of bloody diarrhoea and a sharp drop in the output of milk, several cows and calves died and others were slaughtered in extremis. Human cases were also reported, and the Health Officer ordered that milk should be boiled. The primary cause of the infection is unknown, but there is agreement in thinking in terms of an infection transmitted by an animal.

PÜSCHNER [163] (Munich) reports that 7 out of 10 cows which continuously eliminated Salmonella dublin had distomatosis and that, over an eight-week period, 8 pigs from a sty had to be slaughtered because of acute salmonellosis from Salmonella typhimurium.

Pigs are often chronic carriers of germs, harbouring the Salmonella in their ganglia and eliminating them with the faeces; the Salmonella are sometimes absent from the latter yet present in the former. In 1959 we published a tabulation summarizing numerous data on this subject (GRANVILLE [71]). Meanwhile, DRAGER [50] in Germany and KAMPELMACHER et al. [107] in the Netherlands have been emphasizing the growing importance of pigs as reservoirs of Salmonella.

In Germany statistics of Lerche and Barthel published in 1943 show that, of 6100 strains isolated, 55% were from calves and 7% from pigs. Today pigs account for 15% in the Federal Republic of Germany and for 49% in the German Democratic Republic (KELCH [108]).

In Belgium GRANVILLE and FIEVEZ [72] found Salmonella in 25% of the excrements of pigs.

In sheep, salmonellosis causes abortions (S. abortus ovis). The role

of rams in harbouring the organism in their testicles is significant (WOJTEK [212] and JACOB [97]).

In calves suffering from diarrhoea, Salmonella infection is quite frequent and is often serious, involving a rapid generalization. The fact that Salmonella are observed in the organs (liver, bile) and not in the muscles does not justify releasing the meat for consumption. Working with 63 calves found to be carrying Salmonella in organs but not in their meat, STRUCK [190] isolated Salmonella in the meat of 28 during a 15-d storage of the carcasses in a refrigerator. If total seizure had not been ordered, this meat from infected calves would have been consumed.

In Denmark 3.5% of calves died of salmonellosis in recent years. DYKSTRA [52] considers this percentage similar to what he observed in the Netherlands. However, in farms affected by salmonellosis, the mortality may exceed 25%. Modern therapeutic techniques have greatly decreased this figure. Dykstra believes that it is the germ-carrying adults which transmit the disease to the young animals. On affected Dutch farms he isolated 63 adult animals out of 2190 as being carriers, i. e. 2.9%. The figure for non-affected farms was 0.56%. Transmission is supposed to have occurred through the milk or by contact (faeces, shoes, clothing, equipment, dogs etc.).

ROKEY and ERLING [170] (United States) report a 33% mortality among the 85% young calves sick in an epidemic caused by S. dublin.

Bacteriological examination of the carcasses of emergency-slaughtered animals or suspects yields valuable data. WOLZ [213] (Germany) notes the leading role of Salmonella, 7674 strains of which were isolated from 1937 to 1941 (5 yr) and 17 965 from 1951 to 1956 (6 yr). Calves account for 3.5%, bovines for 1.3%, pigs for 0.8% and other animals for 1%. Considering how small the number of slaughtered calves is in relation to slaughtered bovines, it will be seen that Salmonella infection is frequent in calves slaughtered because of disease. DENNLER [42] (Munich) estimates the figure for 1960 as being 40.9% of the calves examined. S. typhimurium was isolated in 51% of cases over a 16-yr period (PÜSCHNER [162]).

In Hamburg the percentage of Salmonella detected in emergency-slaughtered animals dropped from 10.5% in 1952 to 2% in 1960. The credit goes to the Veterinary Service, which in every case investigated at the places of origin (STRUCK [189]). In 1959 and 1960 BULLING [33] found 804 strains of S. dublin for 100 strains of S. typhimurium.

In bacteriological examinations of cases of miliary necrosis of the liver of calves, STOLL [187] isolated Salmonella 41 times in 103 samples; in the others he found Coli bacteria, Micrococci, Corynebacteria and even one Brucella.

LIPPMANN [127] considers it dangerous and even uneconomical to prepare the meat from a calf that has been suffering from diarrhoea, believing it preferable to attempt to avoid the risk of infecting successive calves as well as avoiding sterilization of equipment, clothing and premises. He proposes that such calves should not be accepted for slaughter until the result of the bacteriological examination of the faeces becomes negative.

In Poland GAUGUSCH [66, 67] notes that since 1957 Salmonella infection in animals subjected to biological examination has shown a markedly downward trend. In 1961 the figure for pigs which were carriers of Salmonella

dropped to 0.02%, S. choleraesuis definitely predominating over S. typhimurium. He attributes the improvement to special ecological and nutritional conditions.

In contrast, BOEFF [26] (Bulgaria) found Salmonella in 23.3% of the pigs, 2.3% of the bovines, 2.6% of the calves and 3.4% of the goats slaughtered for meat.

ASDRUBALDI and COPPINI [16] in the Italian province of Perugia report Salmonella in 0.004% of the 11 340 bacteriological examinations (4294 autopsies, 6646 for meat) made between 1950 and 1959; S. typhimurium predominated in cattle, S. choleraesuis (Kunzendorf variety) in pigs.

KAMPELMACHER et al. [107], summarizing extensive research in the Netherlands in 1961, conclude from complete bacteriological examinations (muscles, organs, ganglia, faeces) of 600 pigs that 181, or 30.1%, were carriers of Salmonella. In similar examinations on calves, Salmonella (mainly S. typhimurium) was isolated in 6% of the cases.

In Belgium from September 1958 to April 1960 STAELENS [184] examined the mesenteric ganglia of 1007 pigs and isolated S. newington in one case and S. dublin in another.

Frequent infection in poultry by various types of Salmonella (S. galinarum, S. pullorum, S. thompson, S. bareilly etc.) was reported by MORRIS and AYRES [141].

Out of 16 655 samples of faeces from 76 duck farms, PULST [160] found 714 to be positive (i. e. showed Salmonella). Out of 2254 samples of faeces from 29 geese farms, 101 were positive.

In 17 samples of imported duck feathers, LANG [117] isolated 16 types of Salmonella. Similar findings were obtained in feathers imported from Asia (HOFMANN et al. [93]).

RASMUSSEN [166] found that 1.3% of 62 476 ducks examined had arthritis. In half the cases S. typhimurium was isolated from the lesions.

PRICE et al. [159] found 491 cases of Salmonella in 7029 ducks examined over a 10-yr period; 93% involved S. typhimurium.

According to GOETZ [70], turkeys are sometimes affected by S. typhimurium infections. In young turkeys (3 to 21 d) Paracolon arizona can lead to 90% mortality. A campaign similar to the pullorum disease campaign is needed.

Salmonellosis has also been described in a large number of other birds, animals and fish.

Van DORSEN et al. [202] reported S. typhimurium in gulls on the German and Danish coasts; NIELSEN [147] found 2% carriers of S. typhimurium; ELLEMANN [54] reported that, of 306 gulls shot down, 5.2% were infected with Salmonella: 12 cases of S. typhimurium, 2 of S. virchow and 1 of S. newport.

In sparrows DÉOM [44] identified S. californica. TIHSEN [196] found in 152 common sparrows 1 case of S. pullorum. See LUCAS et al. [129] for data on larks. On canaries, turtle doves, goldfinches, parrots and peacocks, see GRANVILLE and FIEVEZ [72].

GUERRE [78] reported that of 450 individuals 60 nutria died in 10 d from S. typhimurium. STEINIGER [185] found that S. typhimurium existed for several months in a viper. STEINIGER [185] observed S. typhimurium for 9 months in tortoises.

VINCENT *et al.* [203] reported that 96.3% of Morocco tortoises in rural areas are infected. The rate falls off during hibernation; this is quite normal, since there is no reinfestation. Tortoises in parks or with which children play are a definite hazard. On hares, guinea-pigs, minks, see LUCAS *et al.* [129].

KENDERESKI [110] noticed that, of 750 rats examined between 1956 and 1958, 6.6% were diseased.

S. choleraesuis has been isolated in hatchery-bred trout fed on scraps of pig stomach and intestine from a slaughter-house (HAMMER [79]).

SCHULTE and SCHOLZ [178] have reported that, of 707 cadavers and organs of pigeons submitted for autopsy, *Salmonella* was found in 300. Of 11 010 samples of excrement, 1545 were positive, containing mainly *S. typhimurium* (Copenhagen variety).

HAUSER [85], in a study of doves lasting over 30 months, found *Salmonella* in 15% of faeces and 37.5% of the autopsies (1126 *S. typhimurium*, 2 *S. dublin*, 4 *S. montevideo*); 17.5% of the dovescotes were infected.

BULLING [32] isolated 400 strains of *S. typhimurium* from pigeons; 85% were of the Copenhagen variety (in geese, ducks and turkeys, 15%). GRANVILLE and FIEVEZ [72] isolated 25 strains from pigeons, 23 *S. typhimurium*, 22 being the Copenhagen variety.

Hence the danger of semi-wild pigeons which live in towns and contaminate public squares, fountains, streets and window-sills with faeces. Together with ducks and swans, they represent a latent danger to human beings.

MOREL [140] is of the opinion that in rabbits salmonellosis infections are diseases of breeding, which produce sterility, abortion and stillbirth. Cases caused by *S. pullorum* have resulted in progressive posterior paraplegia. *Salmonella* infections are not as rare as supposed, *S. typhimurium* often being isolated in dead animals and believed to be caused by the mice frequently found in badly kept rabbit farms (MAYER [134]).

Among carnivora, dogs and cats are frequently infected. *Salmonella* has been found in 15-18% of dogs, especially those fed on minced horse meat (HEATHER and NOBLES [86], MESSOW and HENSEL [137], vander SCHAAF [199], SINGER and BRANDLY [182] and GALBRAITH *et al.* [65]).

Neither dogs nor cats are supposed to become permanent excretors of the organism. Except when their resistance is altered by another infection, they usually get rid of *Salmonella* within three weeks of being infected. They are sometimes infected by human beings or animals and play a significant part in infecting man, because, like small cage birds, they live close to people who seldom take even elementary hygienic precautions with them.

Salmonellosis symptoms are benign in adult horses; but, with foals, serious septicaemia appears in 90% of cases. BRYANS *et al.* [29] reports an epidemic of colitis at a United States military depot which resulted in the death of 8 foals out of 28. Some of the survivors continued to be carriers for 14 months. As *S. typhimurium* (Copenhagen) was isolated, a disease transmitted by pigeons may have been involved.

Although *Salmonella* are often found in various animals and birds, the serotypes now being isolated in both animals and man are no longer limited to species considered as common and usual. More and more frequently, investigators are finding serotypes that have been considered as

rare and peculiar to other parts of the world - especially to tropical or subtropical regions.

MODES OF CONTAMINATION NOW BELIEVED IMPORTANT IN ANIMALS

In recent years careful consideration has been given to the importance of Salmonella contamination of animal feeds - meals composed of meat, blood, fish, soybeans, peanuts, linseed, lucern etc. Many of these meals, produced by drying in the sun, came from developing countries and were often contaminated by a wide variety of Salmonella strains, the contamination coming from germs eliminated by infected humans or animals.

In Italy CASTAGNOLI et al. [34] found that 12% of the animal meals are infected. RÖHR, Germany [169], reported positive Salmonella findings in 23.6% of animal feeds and 5.4% of powdered cereals.

In 18 cows which died out of 575 on 9 Australian dairy farms, 15 different serotypes of Salmonella were isolated in the animals and feed meals (GRAY et al. [74]).

In Sweden RUTQVIST [172] found 53.1% of batches of bran, meal, cotton cake extract, soybean concentrate, peanut concentrate, sunflower seed concentrate, linseed meal and lucern meal to contain Salmonella, but in only 3.3% of the samples taken, indicating that the organisms are not very abundant. The danger is not limited to imports since Salmonella has also been found in domestic products.

Between 1 March 1958 and 31 August 1958, 195 out of 284 samples of various meals in Germany were found to be infected (POPPE and RACKOW [158]). RACKOW and NIESE [164] in 1959 found Salmonella in 8.5% of foreign blood meals and meat; in 1960, in 2%. The decrease did not result from better hygiene at the production stage but from the reduced import of highly infected meals from America (50% with Salmonella) and Argentina (43% with Salmonella). The importing countries are making a great effort to improve the bacteriological quality of the meals, providing better supervision of the actual preparation, ensuring that heating is effective and protecting the finished products from pollution by packaging in plastic or strong paper bags.

ELLINGSEN and SKOGSHOLM [56], in Norway, have isolated Salmonella (in 3 cases, S. para B) in copra from Ceylon and the Philippines.

HARVEY and PRICE [83] in 1962 in England studied shipments of crushed bones from India. Of 57 samples, 56 contained Salmonella and 8 Paracolon arizona. Of the 56 serotypes isolated, 38 had already been isolated in cases of food poisoning in persons living in the area.

NOORDAM and POSTMA [149] and KAMPELMACHER and GUINÉE [103] in the Netherlands also report heavy infestation of animal feeds; similar observations have been made in many other countries, e. g. THOMAS [195] in Belgium.

Many health workers wondered how bone meal could contain Salmonella after being heated for hours at 126° during preparation. It has been shown that the bones are infected at the outset by Salmonella (inter alia). These are killed by the heating. The bone meal is next subjected to a number of

operations before being put into bags, then transported to sheds before going on board for export. It is during these operations subsequent to heating that the bacterial reinfection takes place. Inadequate separation or none at all between the clean and the dirty areas results in the infection of clothing, workers' hands, equipment and the dust which is carried everywhere by air currents and by men, rats and dogs. Salmonella are found in the outlet spouts of the heating equipment and in the buckets for carrying the powder (influence of the external temperature and the droplets of water of condensation).

GRAY et al. [73] have shown that this reinfection could be prevented by operation in a closed circuit and heating at 150°C. Proper packing prevents the meals' being contaminated by mice and rat urine. Careful bacteriological examinations show that it is often the outside parts of the contents in bags which are infected.

Since it is definitely established that these meals are infected, the question arises whether the ingredients entering into the preparation of animal feeds, and possibly even the feeds themselves, could be sterilized.

1. Heat treatment

An important English study [14] made in 1961 provides evidence to what extent heat treatment is effective. When the heating temperature is over 60°C, the number of Salmonella is reduced by 80-98% in 6 cases out of 7, but only in 5 cases out of 9 when the temperature is less than 60°C. Preparation in pellets (lumps) reduced the rate of infection from 9% to 1.7% in the case of meals and mashes, and thereafter the Salmonella were present only in small numbers.

Heat treatment is thus partially but not totally effective.

The problem is complicated by the nature of Salmonella diagnosis in these meals. Salmonella detected in the bacteriological control examination constitute proof, but negative results do not necessarily mean that the batch from which the sample comes is itself free of infection. It is known that Salmonella are distributed very irregularly in the mass of the product and very few remain after the heat treatment. For technical reasons (number of samples, mass to be analysed), the bacteriological examination does not appear to reveal Salmonella if their number is less than 10-20 per kg of feed*.

It has been suggested that these examinations be replaced by animal tests, i. e. that suspect meals could be fed to uncontaminated animals. Such tests, with pigs and calves, are slow to provide results and involve considerable expense; Smith (van der SCHAAF et al. [201]) speaks in terms of 14 to 18 d for pigs given 200 Salmonella per day.

As a precaution against the most urgent danger, countries have adopted various types of emergency legislation: some require compulsory sterilization of all imports; others impose a bacteriological examination or intend to do so (KAMPELMACHER and GUINÉE [103], KAMPELMACHER [101]).

* For a discussion of sampling techniques, see Annex II.

2. Treatment with ethylene oxide

In 1962, ZINN [214] recommended, for a healthy product, the packing of the finished product in three layers of heavy paper, proper sealing of the package to prevent any meal's escaping and cold sterilization of bag and contents by ethylene oxide. Winkle suggested chambers taking 100 50-kg bags. A vacuum is created, and ethylene oxide is then introduced at the rate of 1 kg/m³ of chamber. The bags are left in contact with the gas for an hour, whereupon the product is sterile; for prevention of recontamination, all persons handling it should wear disinfected clothing and work in premises which are clean and regularly disinfected.

This method, also used for other foodstuffs like spices, has yet to prove its efficacy while still not altering the physicochemical properties of the product treated.

3. Treatment by ionizing radiation

The prospects for this method were discussed by the experts who attended the Panel organized by the International Atomic Energy Agency at Vienna. Reference is made in particular to the papers by Thornley and Ley.

The danger from meals which are even slightly infected is obvious (LEISTNER *et al.* [120]). Salmonellosis can be transmitted to animals by very small numbers of Salmonella (less than 10): the animals become carriers which may never show any abnormal symptoms or which become sick when their resistance drops or when suffering from great fatigue (MOREHOUSE and WEDMAN [139]).

As KNOTHE [112] has pointed out, Salmonella infection from meals is less likely to come from epidemics than from latent infections, without symptoms, in the animals which have eaten them.

It may have seemed surprising that the number of pigs which were carriers of Salmonella was much smaller on the farm than at slaughter (e.g. 3% as against 43%) (LEISTNER *et al.* [121] and LEISTNER [119]). Slightly infected feed infects some pigs while still on the farm. They are then sent to be fattened, or else, when ready for the butcher, they go in groups to markets or slaughter-houses and are kept in enclosures or stables. During the hours or days spent there, the infection is rapidly transmitted to the healthy animals by the carriers. The ground, the walls and the various pieces of equipment are often infected and still further increase the dangers of transmission. The American Meat Institute [8] found 18% of infected pigs after a short stay and 59% when they stayed longer, Salmonella then being found in the ganglia of 25% of the pigs.

Confirmation of this mode of infection comes from the large, industrial-scale pig farms now being established to reduce production costs; these farms sometimes have several hundred or several thousand pigs for fattening. It is not uncommon to find human subjects on them who are excretors of Salmonella but are only very belatedly recognized as such (THAMM [194]).

Research on the sources of contamination of animals by Salmonella has naturally led to a study being made of water. Bacteriological exami-

nations have shown that river waters often transport Salmonella. After heavy rains or floods these rivers overflow and invade low-lying areas and swamps, where the grasses and plants become contaminated by Salmonella, which then may live on for weeks or even months in the specially favourable microclimate they find on the surface of green plants. It has been demonstrated that the excrement of wild birds infected with Salmonella, when deposited amid living or dead plants, continues to be infectious for 12 yr (STEINIGER [186]). The soil protects the bacteria against the external elements and can play an important part in the propagation of infections (SLAKOV [183] and MAIR and ROSS [131]).

Much of the pollution in river and estuary waters comes from germs and parasites emptied into them in waste from sewers. Swabs placed at various points in sewage systems have been revealing. SCHMIDT and LEUK [117] report that 60 samples out of 66 contained Salmonella. These swabs have permitted the detection of high percentages of infection by various types of Salmonella, some of which (S. para B) are quite resistant and are found in 35% of the isolated samples, whereas others (e.g. the germs of the S. enteritidis group) are less resistant and are found in only 2% of the cases. Certain waters not only transport Salmonella but even help them to multiply if there is enough dissolved protein, i.e. 100 mg/l. This rarely happens in flowing waters, but it does occur in stagnant waters (SCHAAL [174]).

Left undisturbed, the water begins to putrify after 4 d and undergoes a biological purification. The germ content falls sharply since the proteins which serve as nutrients for the organisms disappear together with the oxygen (SCHAAL [174]). Hence the importance of decantation chambers in installations not connected to a public sewage system.

It has been thought by some that slaughter-houses introduce a considerable number of Salmonella into sewers and therefore constitute a special danger. In fact, however, the infection of residual waters from slaughter-houses seems no higher than that of ordinary city sewers.

VALETTE [198], in Geneva, Switzerland, found Salmonella in 56.3% of water samples from the city sewers, in 29.4% of samples from residual slaughter-house water and in 33.3% of samples from the Arve and the Rhone. Similarly, SUMMA [192] considers that slaughter-house water requires no special treatment.

However, since mechanical and biological purification of sewage water proves inadequate, chemical disinfection, by chlorine, seems desirable. SCHAAL [175] estimates that to ensure adequate disinfecting action a residual chlorine content of 5 mg/l should persist after completion of the operation. This is generally obtained by the addition of 8 to 20 mg of chlorine per liter (the amount depending on whether or not the water has been treated mechanically before chlorination).

DENNER *et al.* [43] advocate the disinfection of water from hospitals, clinics, diagnostic centres, yards where animals are cut up, places where vaccines are prepared, butchers' and pork butchers' shops, meat processing plants, slaughter-houses and markets. KOTTER [113] fully supports this view, insofar as slaughter-houses are concerned. However, these measures could well be extended, since certain English investigators [13] have isolated Salmonella in 31 out of 111 swabs placed in the drains of a bakery.

Something should also be said about human infection of animals. By way of example, we refer to two recent reports:

MESSERLI [136] states that S.typhimurium was isolated in a farm where a 4-d old calf had died. A second calf fell sick but was successfully treated with chloramphenicol. Anamnesis established that the farmer's wife had had enteritis two months previously. At the time the animals were sick, the farmer himself had fallen ill. S.typhimurium was found in four members of the family.

Secondly, FRANCONY [64] studied an enzootic of porcine salmonellosis which had resulted in the death of several large sows. The animals had been fed on non-sterilized scraps from the kitchen of the psychiatric hospital at Bassens (Savoy, France). S. para B - a pathogenic agent very rarely met with in pigs - was isolated from their blood. In the course of the investigation, cracks were found at the level of a drain passing along the wall of the pig sty. In the hospital there had been human paratyphoid B fever for several years.

SALMONELLOSIS IN MAN

Salmonella infections in man have become quite extensive in many countries during recent years.

KAMPELMACHER [101] gives a significant table for the Netherlands for the period 1951-1961 (strains isolated in man and in animals). It shows a slow increase from 1951 to 1957, then a sharp rise, with peaks in 1959 and 1961, corresponding to human epidemics which could be correlated, in part, with continuous warm, humid weather during the summer of 1959 and the autumn of 1961. The total number of Salmonella serotypes identified in 1961 was 118. In 1951 the number was only 18. Without unnecessary detail, the predominance of S.typhimurium (over 50% of the types) should be pointed out [102].

In Germany, according to BULLING [31], the number of intoxications in human beings have more than tripled by comparison with the pre-war figure (for a smaller area):

1936, 1937, 1938: yearly average: 1819 cases

1956, 1957, 1958: yearly average: 6944 cases, i. e. 3.8 times as many.

ROSS [171] reports that in 8 yr (1949-1956) the number of human intoxications in England rose from 2428 to 7713. However, the 1962 statistics shows a falling off, the figure for 1961 being 5387, i. e. 16% less than in 1960.

The significance of Salmonella in episodes of known cause is considerable (96% of cases). These episodes are particularly frequent during the summer months. S.typhimurium is the most common serotype, the peak being reached in 1959-1960 (PARRY JONES *et al.* [153]). In 1961 it was identified less frequently (14% less than in 1960). Salmonella caused death in 23 cases out of 27 in 1959 [11] and in 21 out of 25 in 1960 (COCKBURN and VERNON [35]).

EDWARDS [53] seems to attribute less importance to Salmonella in the United States than in England, because they account for only 10% of all intoxications.

In Switzerland the reporting of salmonellosis in man has been compulsory since 1952. As compared with an average of 172 for the years 1952-1953 and 1954, the number of cases was 558 in 1956, 460 in 1957, 504 in 1958 and 396 in 1959 (FEY and WIESMANN [60]).

In Belgium the Salmonella and Shigella Centre began operations on 1 April 1960. Within 19 months 811 strains had been isolated, 45% being S. typhimurium; 128 were from animal meals, 337 from human patients, 4 of various origin and 342 from the Congo and Ruanda Urundi (Report of the Director, Dr. E. van Oye).

Salmonellosis in man has a very extensive clinical picture, ranging from the classical typhic or paratyphic types to gastro-enteritis and localized conditions. Infected individuals may also be carriers without showing any clinical symptoms.

The significance of the typhic and paratyphic (A and B) forms is decreasing. DAUER [41] reports that in 1950 twice as many cases of typhoid fever were recorded in the United States as infections by other types of Salmonella, but in 1957 cases of typhoid were five times less frequent.

LEVINE et al. [125] showed that for Hawaii the number of cases of typhoid, paratyphoid A and paratyphoid B had been reduced by 95% thanks to vaccination, but there were unfortunately many other types of Salmonella infection.

In the Netherlands KAMPELMACHER et al. [105] likewise report a decrease in typhoid fever and paratyphoid fever B in man but otherwise a pronounced increase in especially S. bredeney, S. heidelberg and S. newport.

When Salmonella-induced gastro-enteritis affects large numbers (e.g. communities) or, having caused deaths, leads to a serious investigation, it does not go unnoticed by diagnosticians. This is not true, however, of isolated cases of salmonellosis in humans or small numbers of cases in which the clinical symptoms are negligible and clear up unnoticed. Sometimes Salmonella are belatedly isolated in localized purulent conditions (such as abscesses), pleurisy, nephritis, osteomyelitis, aneurysms, appendicitis, meningitis, enteritis subsequent to a gastrectomy and endocarditis (BLACK et al. [24], JUNG and NESSELER [100] and JARNIQU and MOREAU [98]).

In addition, there are all those who suffer intermittently from mild gastro-intestinal disorders and those who have no clinical symptoms but whose faeces and urine, on proper bacteriological examination, will be shown to contain Salmonella; these are carriers or excretors of germs. NOORDAM [148] estimates that in Amsterdam, out of a population of 750 000, 50 are carriers of S. typhi or S. para B, while about one out of 500 excretes one or more serotypes of Salmonella. The author believes that man continues to be a carrier of Salmonella for an average of 4 weeks, i.e. the number of persons infected annually is 10-15 times higher, i.e. 15 000 to 20 000 persons.

Taking the views of ARBUZOVA [15], HUMBERT [96] and MILLER et al. [138] that man continues to be a carrier and temporary excretor for much longer periods (2 yr and even over 6 yr), this disastrous role human carriers can play in the transmission of disease will be appreciated.

Human reactions to the same Salmonella can vary greatly. Persons who have absorbed certain drugs are found to show a pronounced change in

the compositions of their microbial flora. Various organisms which normally offer strong and vital competition to the Salmonella (e. g. coliform organisms) may no longer be present, so that a new, more serious (even grave) infection can develop after a primary infection which has required treatment (FLIPPIN and EISENBERG [61]).

MODES OF CONTAMINATION NOW BELIEVED IMPORTANT IN MAN

(a) Human infection by other humans who are carriers

We shall not consider here the epidemiology of typhoid and paratyphoid (A and B), which are described in all standard textbooks.

Among carriers, those working in the food industries are most to be feared. An annual bacteriological examination will at most detect only 10%. MARCUSE *et al.* [133] report that from 1955 to 1957, 28 persons out of 10 000 had been detected in Germany as carriers of Salmonella infections and that 68% of the workers in foodstuff factories excreted the germs without knowing it (123 in meat industries, 79 in bakeries and confectioners, 22 in dairies and 17 in fish-processing and food-preservation industries).

Large-scale intoxication by S. bareilly occurred in Germany in June 1953. Bonitz and von Lureck (LANG [116]) describe the intoxication of 10 000 patients (1000 in Hamburg alone) following the consumption of Camembert cheese made in a dairy in Lower Saxony; the cause was traced to a woman employee at the dairy, hired shortly before to paste labels on the tin foil in which the cheese had been packed.

ESCHMANN [59] describes an epidemic in Zug, Switzerland, in 1961. A carrier employed in a butcher's shop infected sausages which were responsible for 60 cases of intoxication. DRAGER [49] reports slaughterhouse staff found to be carriers but said it was difficult to tell whether they had infected the meat or vice versa.

Some persons who eliminate Salmonella by way of the bile occasionally have chronic cholecystitis and gallstones (HUMBERT [96]).

TSEYNKOV [197] gives the following results for the Crimea: normal persons, 0.8% carriers of Salmonella; patients with acute diarrhoea, 10.4%; children, 1.4%; hospital staff, 2.7%.

PUSCHMANN [161] reports a German nurse who was a carrier and infected 54 children and infants at the Langensalza children's hospital.

DATTA and PRIDIE [40] describe an epidemic of S. typhimurium, lysotype 27, at an English hospital which lasted 20 months, caused 102 cases of enteritis and resulted in the detection of 150 healthy carriers. The authors consider this a definite case of inter-contamination between humans since up to 1960 lysotype 27 had not been found in animals.

FLIPPIN and EISENBERG [61] report the transmission of Salmonella to newborn babies in delivery rooms as a result of inhalation of air contaminated by non-sterilized resuscitators. He also stresses the appearance of pleuropulmonary forms of salmonellosis in patients having lesions of the urinary tract. The genito-urinary tract is considered (in addition to the intestine) to be a portal of entry for Salmonella.

In autopsies on patients at Dakar who had died of various causes (chronic nephritis, syphilitic aortitis, malignant malaria), BAYLES *et al.* [30] isolated Salmonella 5 times in the mesenteric ganglia of 20 persons.

All the strains of Salmonella are potentially pathogenic in man. The cases reported in the literature are abundant, but there is no need to review them here. It is no longer possible to speak of serotypes of Salmonella peculiar to man or peculiar to animals. Even Salmonella paratyphi B has been found in pigs and in a cow (LENK *et al.* [122]). To be sure, infective doses vary. According to BERGSMAN [18], 125 000 S. bareilly or S. newport would cause oral infection (ANGELOTTI *et al.* [5]), as compared with 1.25 to 45.5 million S. anatum and 1300 to 10 000 million S. pullorum.

(b) Infection of persons by food

(1) *Home-produced meats*

Meat from animals with primary salmonellosis may contain Salmonella, which, if there are enough of them, can cause human salmonellosis. Calf meats are often responsible, but infections may also originate with other meats (beef, pig, horse, poultry, kangaroo etc.).

ACHAMEDOW [1] writes that calf meat was involved in 18.7% of the infections caused by meat in one of the Transcaucasian republics. Some patients had enteritis, but others had clinical symptoms resembling those of influenza. The author establishes a close relation between the amount of calf salmonellosis and the number of human cases.

A serious epidemic in England in 1961 is described by ANDERSON *et al.* [4]. In the most seriously ill of 90 patients, S. typhimurium (phage 20 A) was isolated - and also found in 6 diseased calves at an early stage in the human outbreak. The calves on the farm concerned came from a collection centre near Oxford which distributes calves to various slaughter-houses in the towns where human intoxication was observed. Calf meat was suspected in three-fifths of the cases studied. Moreover, type phage 20 A was found in 12.7% of the calves which remained longest at the collection centre. The meat of these calves was intended for some Jewish customers and was therefore cut into small pieces for removal of all blood vessels. This unusual type of cutting merely facilitated the infection of the meat, which, in addition, was eaten by the purchasers after only slight cooking.

PANTALEÓN [152] considers that Salmonella infection of the muscle is rare in Paris but that superficial pollution of carcasses by Salmonella of intestinal origin is much more frequent.

This is also the view of Dutch investigators after an intensive effort to find out how Salmonella could be present in such great numbers in minced meat - in other words, how secondary contamination of the meat took place (KAMPELMACHER and associates [104, 106, 107]). Salmonella are found, on the average, in 6% of minced pig meat. Analysis of the slaughtering process revealed that Salmonella were present in the scrapings of the skin of 22% of the pigs after bleeding. Scalding at 62°C did not eliminate the Salmonella, which were harboured in pieces of excrement or in the depths of the hair follicles. The depilating machines are often infected, and the way in which they are cleaned and disinfected leaves much to be desired.

Singeing in a high-temperature oven reduces Salmonella-induced infection only temporarily. The flames do not reach the anal region or the depths of the skin. During the scraping of the burned parts and the final sprinkling infection is spread again.

Many pigs have Salmonella in their lymphatic, mesenteric and hepatic ganglia. Since the germs have been found on 502 experts' knives on 25 occasions, the question arises as to whether it is advisable to cut the lymphatic and mesenteric ganglia of apparently healthy pigs.

The importance of refrigerating the meat at all times is repeatedly emphasized; it prevents multiplication of the Salmonella and has given excellent results in the United States, where the infection of pig meat is no less than in the Netherlands.

The idea is old and based on the following data: Salmonella do not grow in minced meat kept for 5 d at 7°C (SULZBACHER [191]). According to JOHNE [99], there is no multiplication at 5°C but some at 12°C. S. typhimurium begin to multiply at 9°C. The author believes multiplication of Salmonella in meat in refrigerators to be improbable.

A refrigeration cycle will come into fashion again if it becomes the practice to sell previously prepared minced meat in refrigerated or frozen form. Public health supervision will have to be strict and constant (PANTALÉON [151]).

Certain substances, e. g. antibiotics, supplied to living animals in their food can have an effect on Salmonella content. For example, the meat of animals given antibiotics in alimentary doses (i. e. 20-66 mg of aureomycin per animal per day) show Salmonella if conserved at 5°C, but the number decreases considerably if the meat is conserved at 15°C (CORETTI [37]).

Mention should also be made of ionizing radiation used in the conservation of meat. Without going into the details, we may mention, *inter alia*, the studies of LEA et al. [118], COLEBY et al. [36] and KEMPE and GRAIKOSKI [109], which indicate the radiation doses necessary and the alterations they cause. ERDMAN et al. [58] have examined the sensitivity of various strains of bacteria to ionizing radiation.

(2) Imported meats

Various meats imported frozen have been shown by HOBBS [88], HOBBS and GREENWOOD [91] and anonymous authors [12] to be infected with Salmonella. It was found in 3-4% of the frozen carcasses and in 10% of the pieces of boneless and frozen beef, mutton and veal. The probability of external contamination occurring during slaughtering, cutting and handling in the country of origin is stressed. Fatigue, hunger and thirst are definitely said to affect the young animals.

Horse meat imported for use as animal feed contained Salmonella in 100% of the cases (GREENWOOD [75], 1962).

Boneless kangaroo meat exported from Australia does not always seem to have been treated with adequate care, being very often infected by diverse types of Salmonella. Infection rates of over 50% are quoted. The consequent danger led to a prohibition of imports into Germany (BISCHOFF [21], SCHAAL [176], MAYER and HAUS [135] and BISCHOFF [22, 23]).

(3) Poultry meat

Poultry carcasses are very often infected by Salmonella. Despite the considerable progress made towards controlling pullorum disease, infections caused by S. pullorum continue to occur and to cause losses. However, in adult animals they are often localized in one organ (e.g. the ovaries). There seems more reason to fear infection by S. typhimurium, which, very pathogenic in man, are frequently found in the hepatic system and intestine, leading to contamination during dressing of the carcass. Some recent references are COSGROVE and LINDENMAIER [38], LINSERT [126] BLAXLAND et al. [25], THAL et al. [193] and WEIDLICH and NIEDEREHE [207]. Salmonella are present in 17% of the organs and 2.27% of excrements (BIGLAND [20]).

All authors agree in believing that poultry are the major non-human reservoir of Salmonella. To cope with the considerable increase in the consumption of poultry in many European and American countries, enormous breeding farms and specialized slaughter-houses have been set up; and this has, of course, greatly contributed to the spread of avian salmonellosis and the frequent infection of poultry meat. In these slaughter-houses the poultry are plucked by machinery, mass-eviscerated by the same staff and then refrigerated in tanks of iced water before sale or deep freezing.

DIXON and POOLEY [48] isolated Salmonella in 13.8% of the samples taken at a plant where 12 000 chickens are killed and packed daily. He stresses the infection which results from evisceration. To avoid this continual contamination, the refrigeration water is sometimes chlorinated, but this is often largely counteracted by the high content of organic substances in the water. In a supplementary paper, the same authors [46] suggest that fowl carcasses, to be freed of Salmonella, need to be kept for 10 min in fresh water containing 200 ppm of chlorine. Antibiotics (chlortetracycline and oxytetracycline), mixed with the iced refrigeration water, were tried but it was found (HOBBS et al. [90]) that S. typhimurium gradually developed a resistance to chlortetracycline. Of 662 strains isolated in man, animals and food, 1.6% was found to be resistant. Thus, the antibiotic treatment eliminates or considerably slows down the multiplication of putrefying organisms but accelerates the multiplication of resistant S. typhimurium. The carcasses become dangerous but are eaten because they look all right.

RAMSEY and EDWARDS [165], for the period 1958-1960, indicate that the number of tetracycline-resistant strains of S. typhimurium isolated in man rose from 0% to 13.9%; in animals, from 0% to 29%. Some strains are also resistant to chloramphenicol. Resistance on the part of other types of Salmonella is observed less frequently. Similar observations are reported by MANTEN et al. for the Netherlands [132].

WALKER [205] reports resistant strains of S. typhimurium in birds receiving chlortetracycline in their diet. The increase in resistance is nine-fold, i. e. from 25 ppm to 225 ppm by the third passage.

Poultry products proved to be the cause of Salmonella infections in persons in 16% of cases (SADLER et al. [173]), and a high incidence of a particular Salmonella serotype in fowls in a given area goes hand in hand with the same incidence of this serotype in man. Similarly, the same serotypes are isolated in fowls and in their food (BROYER et al. [28]).

Parts of poultry sold raw are important sources of infection; Salmonella have been found in one piece out of six (WILSON *et al.* [209]). They must be cold-stored by the consumer. Similar products, sold cooked, no longer contained Salmonella.

Cooked products may be contaminated by infected equipment, instruments and utensils; e.g. the investigation of the large-scale food poisoning in a prison in the United States where 200 persons became infected after eating slices of turkey showed that the cooked turkeys had been sliced on blocks that had previously been used for preparation of the raw carcasses. The slices had been stored and simply reheated before serving. The temperature was not high enough to sterilize them, nor did cleaning of the blocks with soap and water prevent the transmission of S.typhimurium (MACKEL *et al.* [130]).

Salmonella infections similar to those observed in chicken slaughter-houses have also been found in turkey slaughter-houses. DIXON and POOLEY [47] found rates of 19.3% and 7.8%; in 46 strains isolated, there were 43 anaerogenic S.typhimurium belonging to phage type 1 A, var.I. They recommend strict hygiene during the dressing; the intestines should not be torn, and the refrigeration water should be chlorinated.

(4) *Meat products*

Many public health experts consider meat products the major culprits in many types of human intoxication. English statistics shows this clearly. The following may be mentioned among the most recent cases.

S.california and S.haifa were isolated in two sausages. Almost at the same time they were found in the faeces of children between the ages of 4 months and 2 yr who had violent enteritis. A kitchen hand and a meat-market employee were found to be carriers (HULTSCH [95]).

S.infantis, transmitted by a piece of smoked ham, was responsible for poisoning in the case of 8 persons in the United States (ANGELOTTI *et al.* [7]). The patients probably ingested 1-2 million Salmonella, which is considered very close to the toxic dose. The presence at the same time of Streptococci of Group D was apparently a complicating factor.

S.blockley in Braunschweig sausage resulted in the poisoning of 561 persons and 3 deaths (HANDLOSER [80]). The sausage contained very large numbers of Salmonella. Only after 50 weeks did the last convalescent cease eliminating Salmonella via the faeces.

Meat products are rarely cooked properly. This is true of certain types of sausage, e.g. "Brühwurst" (hot sausage), in which a fatty envelope helps the Salmonella escape destruction by heating (PFAFF [155]). In poorly regulated ovens temperatures may vary between one section of a meat-pie and another, whereas only a temperature of at least 65°C held for 12 min will ensure proper protection (ANGELOTTI *et al.* [6]).

The Salmonella detected on some meat products may originate with the packing or wrapping. The casing used for sausages may be another important source of infection to them. In unsalted casings from pigs Salmonella were found in 80% of the samples (0-15 Salmonella per g). Salted pig casings did not contain Salmonella. Dry salting is supposed to be toxic for Salmonella although they are quite resistant to brine (GHYSEN [69]), but

SEIDEL and HERRLER [179] believe dry salting of the casings will not kill the Salmonella within a reasonable time. At a storage temperature of 4-7°C and in the presence of 22% salt, he found living Salmonella after 24 weeks.

In cases of intoxication reported by PAHR [150], Salmonella (S. enteritidis and S. typhimurium) were shown to be present on casings and also in the faeces of the staff of the enterprises concerned. In order to avoid as far as possible the danger of infected casings, he advocates the systematic seizure of the mesenteries and the intestines in all cases of emergency slaughter. The potential danger of transmission which these organs involve is out of all proportion to their economic value.

Contrary to what might be thought, synthetic packings can also be infected by contact with contaminated products, hands and utensils. Cellophane-wrapped sausages like "Mettwurst" and Theewurst" are handled manually. Carriers may deposit Salmonella on the surface of the products, and these can remain alive for 24 h. At the time of slicing, they can pass into the meat (GROSZKLAUS [77]). If the cellophane casings are smoked, the survival time of the Salmonella is reduced (GROSZKLAUS [76]).

The a_w (activity of water) of a product has a considerable effect on the development of bacteria. Food-poisoning germs require a minimum of 0.95. In moderately salted, vacuum-packed meat products, Salmonella have no opportunity of multiplying. In fresh, sliced meat, however, the a_w is adequate, and for counteraction of the development of bacteria (including Salmonella) the product should be kept at a low temperature (HANSEN and RIEMANN [81]).

All the points considered above reduce very often simply to questions of general hygiene as applied to staff, premises and equipment.

It has been observed that Salmonella from faeces deposited on shoes, clothing etc. can remain resistant for a long time: 10-11 months in moist faeces, 2½ months in dry faeces (DYKSTRA [52]). The aprons of slaughterhouse workers and butchers are often so filthy that the deposits removed from them may contain 1 million germs per gram. Simple contact with dry skin can result in the deposit of 90 000 germs per cm² (ALLAN [3]). One gram of faeces contains 80-100 × 10⁶ germs.

After proper cleaning of aprons, shoe soles and the treads of cart wheels, the degree of infection can be considered as having been reduced, on the average, 100 times (HÖHN [94]).

(5) Eggs and egg products

The dangers of eating insufficiently cooked duck eggs have been appreciated for a long time. The legislation of various countries is very explicit on this subject.

Chicken eggs sold in the shell are less frequently infected by Salmonella. According to W. Brederick (in WEIDLICH and NIEDEREHE [206]), the figure is between 1 per thousand and 4 per thousand. While the egg-white has well-known bactericidal properties, the alleged protection of the shell is an illusion, because it is traversed by Salmonella pullorum within 24 h and by S. typhimurium, Coli and Proteus bacteria within 48 h (vor den ESCHE [204]).

When the eggs are opened, considerable contamination takes place; and germs, including Salmonella, are found in frozen eggs (whole, yellow, white) and in powdered eggs. The infection is sometimes so extensive that egg products can be said to play an exceptional part in the transmission of Salmonella to man and animals. Recent statistics from Germany (KRESSMANN and ALBERT [114]) summarizing the results of bacteriological examinations during the last five years shows that 30.7% of the shipments imported were considered "badly processed".

As in the case of meals, each batch has to be examined, because the techniques of preparation are far from perfect and the certificates issued by many exporting countries do not afford a sufficient guarantee. ALBERT [2] has shown that 16% of pasteurized egg products from the United States were infected by Salmonella and 5-6% from China, the Netherlands and Yugoslavia, while those from France, Denmark and Norway were free.

It is not always easy to determine specifically which intoxications of man and animals are caused by eggs and egg products, and prolonged and difficult epidemiological investigations are necessary. At Cologne in 1962 8 out of 12 types of Salmonella isolated from egg products were identified in enteric intoxications in persons (ENTEL [57]). A decrease in the percentage of positive samples has been observed.

WEIDLICH and NIEDEREHE [206] report the illness in a German hospital of several patients who died within a few hours. At first this was blamed on sausages, but a few months later it was found that eggs had been responsible, i. e. S. muenchen, in eggs from poultry farms which had been supplying the hospital for a long time. This very difficult case was cleared up only by close medical and veterinary co-operation.

On a State farm in Eastern Germany, 23 cows died of S. typhimurium salmonellosis in 5 d. The same germs were found in the ducks of the farm, and it was discovered that the head of the dairy had been feeding calves which had been born weak with milk to which duck eggs had been added (KLOTZ [111]).

The cooking method used in preparing eggs is important. GERNEZ-RIEUX et al. [68] state that frying does not provide the necessary bacteriological safeguards. Nor is the type of baking used in pastry-making any more certain, and the resulting product is often responsible for poisoning in man (HARVEY et al. [84]). Salmonella are killed in the pastry cooks' establishments by heating at 62°C for 4 min. However, a strain of S. senftenberg resisted 60 min of heating at 62°C and was killed only after 2 min at 71.2°C (HELLER and SALTER [87]).

Bacteriological examination of both imported and home-prepared egg products is necessary, though even this does not give a complete guarantee. It does at least prevent unduly contaminated products from getting sold (LORENZ [128]).

Various methods are used or recommended to eliminate or reduce the sources of infection by pathogenic germs.

1. Pasteurization: KRESSMANN and ALBERT [114] and anonymous authors [10] have recommended pasteurization of whole eggs at a temperature of 64°C, the yellow at 66°C and the white of eggs at 57°C. Checking can be

made by a study of the inactivation of gamma-amylase (SHRIMPTON et al. [180]).

2. Use of ammonia: Since pasteurization has an unfavourable effect on the capacity of beaten egg whites to rise, LERCHE [123] studied the use of 0.25% ammonia. After heating for 9 h at 37°C, the germs are destroyed, and the ammonia is then eliminated by drying.

3. In the United States the shells are fumigated with formaldehyde at a concentration 3 times higher than that used in incubators or by treatment with 1% zinc sulphate to counteract infection. The efficiency is 94% (BIERER and BARNETT [19]). Experiments have been done with 0.06% and 0.07% β -propiolactone with a view to preventing the growth of Salmonella in the liquid egg as a whole or in the egg white (BRUCH and KOESTERER [27]). Germs are being sought which will be inimical to Salmonella but nevertheless ensure satisfactory fermentation of glucose in the egg whites. A non-pathogenic E. coli is reported to have given good results (FLIPPIN and MICHELSON [62]).

4. In Germany tests have been done on powdered eggs with gas (ethylene oxide and propylene). The results are reported to be encouraging, but authorizations have not yet been issued (KRESSMANN and ALBERT [114] and WINKLE and ADAM [211]).

5. In Switzerland since 1957 importers' invoices must include the following phrase: "As a protection against bacteriological hazards, foods prepared with egg products must be well heated or cooked (at least 80°C at the centre of the product) before being eaten" (FORSTER and GASSER [63]).

Eggs are used in the preparation of mayonnaise. The higher the temperature of conservation and the lower the pH, the sooner any Salmonella introduced will be killed. In the refrigerator they disappear in 10 d, according to LERCHE [124], and in 43 d, according to ROEMMELE and WALL [168]. At room temperature they resist only 24 h [124] or 46 h. [168]. It is believed that this destruction is caused by acetic and lactic acids (ROEMMELE and WALL [167]). Storage for 13 d at 15°C causes the Salmonella to disappear from mayonnaise (MOSSEL and van der MEULEN [146]).

In principle, acid fruit juices cannot be the source of Salmonella or Shigella (MOSSEL and de BRUYN [145]).

The use of ionizing radiation might prove to be advantageous, especially for pasteurization (MOSSEL [144]). See also the papers by Thornley and Ley included in this publication.

(6) *Milk and dairy products*

The infection of milk can be primary, as in the case of Salmonella-induced mastitis; but most often the infection is secondary: the Salmonella get into it by way of dust, excrement, equipment, insects etc. The clothing

of workers who are carriers is often infected. The statistics and details published in the Monthly Bulletin of the Ministry of Health and Health Labor Service (1962-21-180) are eloquent on this subject.

Milk was certainly, or at least probably, the cause of the poisoning of a group of 219 persons and a large number of families - often special-quality, tuberculin-tested milk, sold crude. S. typhimurium, S. enteritidis and S. heidelberg were involved.

HARMS [82] shows that in adult bovines which are carriers, the udder does not eliminate Salmonella but that the milk can sometimes be infected during milking if hygienic care is not rigorous.

Pasteurization destroys pathogenic germs and is to be recommended for all types of milk, including milk described as being of exceptionally high quality (POPPE [157]) and milk for cheese - it has been found in Denmark that Salmonella can persist for 6 months in cheese (anonymous authors [9]).

(7) Foods of vegetable origin

Since 1955 (WILSON and MACKENZIE [210]) it has been known that coconut shavings can be infected by Salmonella, including S. para B and, in one case, even S. typhi. Shipments of coconuts analysed in 1962 by STRAWBRIDGE [188] in England contained Salmonella in 8.9% of cases. Similar findings have been made in Germany (ELLINGSEN and SKOGSHOLM [55]). The danger is very real. Coconut shavings are used in the preparation of certain sweets, macaroons or toasted articles in which the final heating destroys the pathogenic germs; but they are also used in "cold" preparations, in which, if they are stored at too high a temperature, a dangerous multiplication of Salmonella can take place (HOCKE [92]).

Instant foods represent a further danger. There is a report on the intoxication of 110 children under 2 yr of age by a cereal preparation which, after mixture with tepid water, was ready for immediate consumption. It proved to be infected by S. muenchen, found in the barley, which had come from Africa (SILVERSTOLPE and PLAZIKOWSKI [181]). KRUGERS DAGENEUX [115] draws attention to powdered coffee, powdered puddings, powdered soups, powdered cocoa and the powders used for preparing ice cream.

It has also been shown that Salmonella and Staphylococcus aureus can develop in the presence of the normal flora present on frozen peas. Strict hygiene is therefore necessary in frozen fruit and vegetable processing plants (WHITE and WHITE [208]).

(8) Water

The infection of certain river waters has resulted in a general prohibition on public bathing in the low-lying and swampy areas of the province of Braunschweig (POPP [156]).

While drinking water has played a large part in the transmission of certain types of typhoid and paratyphoid fever, it may also be very dangerous if polluted by excretions of birds and animals which are carriers of Sal-

monella. DARASSE *et al.* [39] isolated 27 strains of Salmonella in a lizard-infected reservoir in a foreign country.

(9) *Molluscs and crustaceans*

These are a source of infection to man when the waters which harbour them are infected by Salmonella (danger of estuaries).

PROBLEMS OF THE MODERN WORLD

Mention must also be made of the new culinary habits of the modern world. The housekeeper works less and less in her kitchen because employed in a factory or office. To an increasing extent, she buys ready-made dishes in supermarkets and self-service shops. In many factories and offices the staff members eat meals prepared on a large scale. In order to feed so many people at one time, modern techniques have been developed; and there is a growing use of machinery, both in the preparation of food and in its distribution. In these circumstances it is quite obvious that the slightest infections from the food prepared, from the persons handling it or from the machines used can spread rapidly throughout the whole preparation process. As FASQUELLE (in [45]) points out, alimentary toxoinfections are a coming problem since the trends in living cannot fail to encourage them.

Overpopulation in certain areas and the prohibition of preservatives (e. g. sulphite in minced meat) has contributed to the extension of salmonellosis in man (van der SCHAAF [200]).

Nor should it be forgotten that animals which live with human beings (dogs, cats, small birds, parrots etc.) can be agents of infection.

SALMONELLA INFECTION CONTROL MEASURES RECOMMENDED

In addition to the general and special hygiene necessary in food production and utilization, the following measures can be suggested:

- (a) Compulsory declaration of cases of salmonellosis by physicians, veterinarians and diagnostic laboratories.
- (b) The attention of the general public to be drawn to the ill-defined symptoms of enteritis that often reveal bacterial activity.
- (c) A tireless campaign to control forms of salmonellosis which are not well known - in other words, study the sources of infection and the human and animal carriers; break the man-animal-man cycle; try new methods of controlling the infection of food products (e. g. ionizing radiation).
- (d) Recommend that all perishable food be refrigerated and that all suspect foods (minced meat, food products based on eggs, milk, meals etc.) be adequately cooked (MOSEL [143], KAMPELMACHER [102], FLIPPIN and EISENBERG [61] and FEY and WIESMANN [60]).
- (e) Establish effective co-operation between all public health workers with a view to carrying out the most extensive possible epidemiological in-

vestigations. Do not be satisfied with hasty results, which are often misleading. Improve diagnostic techniques as much as possible and raise the standards in specialized veterinary science (BARTELS [17]; DUTSCHKE [51]).

After so discouraging a picture of the present situation in man and animals, it might be asked why the number of Salmonella toxoinfections is not considerably higher. The reason, as Miss HOBBS [89] has said, is that most human beings are probably resistant to everything (with the exception, of course, of S. typhi) less than an extremely high dose of Salmonella.

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SALMONELLAE IN FOODS AND ANIMAL FEEDING STUFFS

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Abstract — Résumé — Аннотация — Resúmen

SALMONELLAE IN FOODS AND ANIMAL FEEDING STUFFS. After the problem of *Salmonella* infections in foods and feeding stuffs is emphasized, an account is given of the current ways of manufacturing bone meal, meat meal, blood meal, fish meal, fish flour, egg products and coconut. The effectiveness in eliminating salmonellae and the chance and possible sources of recontamination are described for each production method.

Besides heat treatment, fumigation by ethylene oxide and irradiation with gamma rays are considered. The bacteriological tests required to establish the effectiveness of treatment are also discussed, as well as the effect of the treatment on the nutritive value of the product.

LES SALMONELLAE DANS L'ALIMENTATION DE L'HOMME ET DES ANIMAUX. Après avoir souligné le problème de la contamination des aliments de l'homme et des animaux par les *Salmonellae*, l'auteur donne un aperçu des méthodes courantes de préparation de noix de coco, de poudres d'os, de viande, de sang et de poisson, de farine de poisson et de produits à base d'œufs. Pour chaque méthode de production considérée, il décrit l'efficacité de l'élimination des *Salmonellae* ainsi que les risques d'une nouvelle contamination.

Outre le traitement par la chaleur, l'auteur considère la fumigation par l'oxyde d'éthylène et l'irradiation aux rayons gamma. Il examine également les essais bactériologiques nécessaires pour déterminer l'efficacité du traitement ainsi que son influence sur la valeur nutritive du produit.

САЛМОНЕЛЛА В ПИЩЕВЫХ ПРОДУКТАХ И В КОРМАХ ДЛЯ СКОТА. Придается особое значение проблеме заражения пищевых продуктов и кормов для скота салмонеллой, приводятся сведения о методах приготовления костной, мясной, кровяной и рыбной муки и пудры, изделий из яиц и кокосов. Каждый метод сопровождается сведениями об эффективности уничтожения салмонеллы а также о вероятности и возможных источниках повторного заражения.

Помимо термообработки рассматриваются также окуривание окисью этилена и облучение гамма-лучами. Рассматриваются также бактериологические опыты, необходимые для определения эффективности обработки, а также влияние обработки на пищевые качества продукта.

SALMONELLAE EN LOS ALIMENTOS Y EN LOS FORRAJES. La memoria destaca en primer lugar el problema de la infección de alimentos y forrajes por *Salmonellae* y, a continuación, describe los métodos corrientemente utilizados para la preparación de harina de huesos, carne, sangre, pescado, productos derivados de huevos y de la nuez de coco. Para cada método de producción, describe la eficacia con que permite eliminar las bacterias y menciona las posibles fuentes de nueva contaminación, así como la probabilidad de que ésta se produzca.

Además de los tratamientos térmicos, estudia la fumigación con óxido de etileno y la exposición a los rayos gamma. Discute también los ensayos bacteriológicos requeridos para establecer la eficacia del tratamiento y el efecto de éste sobre el valor nutritivo del producto.

THE PROBLEM

Foodstuffs containing small numbers of *salmonellae* have become well established sources of human salmonellosis and of symptomless excreters amongst food handlers [1, 2, 3]. With animals and birds too, the salmonellae are retained and excreted for at least a few days and sometimes longer [4, 5, 6, 7]. Proof that salmonellosis in animals may occur after they eat

Symp...

infected feeds is difficult to find, and it is likely to depend on a number of environmental factors as well as on the physical state of the animal exposed to infection. Investigations have shown that, however small the carrier rate on the farm, there is a steady increase in symptomless excretors as the animals move or are transported from the farm through markets or other collecting centres to abattoirs or meat-packing stations [8]. Furthermore, in young animals particularly there may be a transition to a septicæmic state as the physical condition deteriorates under stress or perhaps at the moment of death [9]. Studies with pigs, calves and cattle have provided data to verify the increased rate of infection of carcass samples over that of the live animals on the farms [10].

It has been suggested that contaminated feedstuffs combined with bad hatchery practice may be responsible for the high incidence of salmonellae in some broiler factories [11, 12] and also in broken-out egg products [13, 14]. The relationship between egg products, confectionery and paratyphoid fever and salmonellosis has been described many times in the English literature [15, 16] and also in Canada [17] and the United States of America (publication by the National Office of Vital Statistics, Morbidity and Mortality Weekly Reports from 1956-1960).

Links in the chain connecting illness with meat contamination and the animal source are beginning to appear in England [18, 19] and were reported in Sweden in 1955 [20] and 1959 [21]. In the Proceedings of the 65th Annual Meeting of the United States Livestock Sanitary Association held in October 1961 [22], there were a number of papers dealing with the Salmonella contamination of feeding stuffs, animals and processing plants and with the international significance of the transportation of salmonellae from country to country by means of contaminated foods.

Sixteen years ago published data [23] revealed that spray-dried whole egg from the United States and Canada contained salmonellae of serotypes hitherto unknown in the United Kingdom but at the same time isolated from cases and outbreaks of salmonellosis. The same serotypes were demonstrated in the mesenteric glands, offal and flesh of pigs fed uncooked dried egg waste, the degree of contamination depending on the hygienic or unsanitary arrangements of the killing and processing establishments. Eleven years elapsed before another report was issued on the Salmonella contamination of egg products in general [24].

The 1961 figures for salmonellae in meat, eggs and coconut are given in Table I [25] and a summary of published data on feeding meals in Table II [26].

MANUFACTURING PRACTICES FOR MEAT AND BONE MEALS

Meat, meat-and-bone meals and bone meals are defined in the following reports: "Fertilizer and Feeding Stuff Regulations of 1960, No. 1165" and "The Diseases of Animals (Boiling of Animal Foodstuffs), Order of 1957, No. 1175, dated May 2nd, 1947."

TABLE I

CONTAMINATION OF IMPORTED FOODSTUFFS WITH SALMONELLAE
with particular reference to S. paratyphi B, S. typhi-murium and S. thompson
(1961)

Food	No. examined	Total No. positive	%	<u>S. paratyphi B</u>		<u>S. typhi-murium</u>		<u>S. thompson</u>	
				No.	%	No.	%	No.	%
Egg									
Frozen whole	1042	165	15.8	8	4.8	35	21.2	24	14.5
Dried whole	898	107	11.9	10	9.3	1	0.9	17	15.9
Dried yolk	57	3	5.3	0		0		0	
Frozen white	675	41	6.1	0		13	31.7		
Dried white									
Flake	536	68	12.7	3	4.4	22	32.3	2	2.9
Powder	214	18	8.4	0		2	11.1		
Meat (frozen and boneless)									
Horsemeat for animal feeding	769	454	57.0	5	1.1	3	0.7		
For human consumption	795	41	5.1			15	36.6		
Coconut	683	28	4.1	6	21.4	2	7.1		

TABLE II
SALMONELLAE FROM ANIMAL FEEDING STUFFS

Specimens		Salmonellae	
Description	Number	No.	%
Ingredients			
Meat-and-bone meal	32	11	34.4
Bone meal	8	1	12.5
Fish meal	13	0	-
Vegetable products	77	5	6.5
Total	130	17	13.1
Finished feeds			
Meals, mashes etc.	67	6	9.0
Pellets, foods	296	5	1.7
Total	363	11	3.0
Grand total	493	28	5.7

Methods of preparation

Those in use in the United Kingdom generally consist in dry rendering in a steam-jacketed pan, with or without pressure, and solvent extraction or a combination of these two.

(a) Dry-rendering, or as commonly known in the United Kingdom, iwelling

Meat and bones are heated in a steam-jacketed pan for 2½ h at 120°C with agitation. After cooking, the mass is centrifuged and pressed, and the moisture and fat are reduced, the fat to 15% oil or grease. At this stage the material is known as "greaves" and may be ground and/or kibbled and sold for poultry or dog and cat food. It is normally packed in bags, but some transportation takes place loose.

(b) Post-dry-rendering or iwelling

There is often a second process of solvent extraction with benzene or trichlorethylene for 24 h at 105°C (221°F); this meal has a 4% oil or grease level. In some plants (a) and (b) proceed as a continuous process. There are approximately twelve solvent plants in operation, producing two-thirds of the meal. After extraction, the meal is free from salmonellae, but as with any other commodity it can be recontaminated; the use of sealed paper bags instead of hessian is a favourable factor in preventing recontamination.

Other processes include the following: (1) the degelatinizing of bone, during which process the nitrogenous components are removed by steam pressure to form glue. The resulting product is sterilized feeding bone flour. (2) The treatment of degreased bone by hydrochloric acid to produce

"ossein", which is a precursor of gelatin. This product is described as dicalcium phosphate or precipitated bone phosphate, and it contains 40% phosphorous and no nitrogen. Both these products are used as mineral supplements.

~~Meat-and-bone meal~~ contains 40% to 55% protein; above 55% the product is described as a meat meal. ^{meat} The phosphoric acid varies from 20% to 10%. Sterilized feeding bone flour is sold with an analysis of 5% protein and 30% phosphorous pentoxide. Bone meal with less than 40% protein down to, say, 30% protein is not extensively used for poultry or animal feeding; the phosphorous pentoxide of this grade would be approximately 25%.

Raw materials come from butchers' shops, abattoirs, food factories, knackers' yards, hatcheries and so on. Although the importation into the United Kingdom of raw meat and raw bones for animal food for Europe is prohibited except under the Tay Landing Licence issued by the Ministry of Agriculture, Fisheries and Food, nevertheless horse meat for pet food is imported under laws relating to food for human consumption. Bones, however, can be imported from all other countries, including Turkey, Marocco, India, Pakistan and Argentina. Crushed Indian bone has been described as a prolific source of salmonellae [27]. With the increasing awareness of Salmonella contamination, improvements are being made in the industry, and this will undoubtedly continue.

In Northern Ireland the cooking time for products intended for meals is said to be 115.6°C (240°F) for 1½ h, the time depending on the fat content of the material processed. The material is shovelled from the cooker to the press, which extracts the fats. At this stage the material is too hot to touch, but it is cool by the time it passes through the press and is bagged in the form of cakes.

In the United States virtually all animal by-products intended for feeds are treated by a so-called dry-rendering process. This entails the use of large steam-jacketed vessels, in which the ground-up material is placed, approximately 60 lb steam pressure is utilized in the jacket, and the material is agitated and vented as it is being heated. The mass is heated to the boiling point and maintained at this temperature as long as moisture remains. Ultimately, the temperature rises to 112.8-115.6°C (235-240°F), which is considered to be the end point, the primary purpose being to drive off the moisture and rupture the fat cells. The rendered material is then dumped and conveyed to presses for the separation of fat and other products; there are said to be many modifications for treating the rendered material. There is a tendency, for economic reasons, toward bulk shipment of both rendered animal by-products and the mixed feeds; this procedure affords opportunities for re-contamination. The prevention of contamination in the rendering plant may be spoiled by subsequent recontamination in transit or elsewhere.

It seems that most of the present methods used for the manufacture of feeding meals include a cooking process which theoretically should kill salmonellae; but even if this were so, the opportunity for recontamination from raw to finished products frequently occurs because of thoughtlessness in the design of factory plant, movements of workers and equipment and failure to realize the weak points in the system.

Sometimes the heating is insufficient for sterilization purposes, or the penetration of heat into the centre of a mass of meat in a large cooker may be inefficient, particularly when there is a build-up of material from infrequent cleaning. Subsequent drying processes may reduce the count, but they will not eliminate all salmonellae. Furthermore, with powdered products, airborne particles containing salmonellae could be scattered all over the factory. The floor and also the materials spilled or stored on the floor are likely to be contaminated. An investigation in the United Kingdom [18] showed that 12% of samples of cooked compressed pet food were still positive for salmonellae after raw meat, including knackers' meat, abattoir scraps and imported frozen horse meat, had been cooked by boiling for about 45 min.

It seems likely, therefore, that insufficient attention has been given to ensuring (a) that manufacturing procedures adequately destroy salmonellae or (b) that recontamination of the finished product does not occur.

Pellets, cubes and cakes

Information from both Sweden and the United Kingdom indicates that the heat treatment used for producing pellets kills most if not all salmonellae in meals [26, 28]. Also, pellets are said to be nutritionally equivalent to meal and less subject to oxidative changes. The following method of manufacture is used in the United Kingdom: A uniform flow of compound feeding meal passes into the mixing chamber, where it is steam-heated, at a temperature which varies between 51.7°C and 71.1°C (125°F and 160°F) for 5 to 15 min according to the type of ration and the throughput of the machine. Specially constructed worm blades mounted on a shaft throw the material backwards and forwards and keep it suspended in the chamber. An adjustable discharge gate controls delivery from the mixer to the pelleting section. The steamed mash passes from the mixer through a spout to the centre of a revolving die, where heat is generated by extrusion of the mash through the die. A temperature of 65°C to 82.2°C (150°F to 180°F) for 3 to 4 s is attained, varying with the quality of the grist and the state of the die. The pellets are cooled in an automatic cooler which draws air through the mass of the material. The cooling time to ambient temperatures is about 10 min (Sutcliffe, personal communication). A figure of 0.3% per 1000 samples has been given for the level of Salmonella contamination.

Swedish workers (SWAHN and RUTQUIST [28]) investigated lucerne flour from which salmonellae had been isolated from 10 of 28 (36%) samples. Dry sterilization was considered too harmful for the product, but after warm-pelleting with a steam pressure of 1.8 kg and a temperature of 90°C (194°F) for 6 to 8 min plus the time taken to cool, in all 20 to 25 min, 1 only of 37 samples from the preheater was positive for Salmonella and none of 229 samples of pellets. The meal should be reinforced with vitamin A before heat treatment. Pelleting may be carried out at much lower temperatures such as 50°C to 60°C (122°F to 140°F) for a short time only to avoid the destruction of vitamin A, but it is uncertain if all the feed reaches even this temperature. The procedure is called "cold-pelleting", and it is not effective for the destruction of salmonellae, which have been isolated on several occasions from cold-pelleted samples, although it is thought possible

that the frequency of salmonellae in pellets made by the cold process is somewhat lower than in feed which has not been pelleted (Rutquist, personal communication).

The chances of recontamination after pelleting are naturally great, and much depends on the conditions under which the pelleting process and the cooling of the pellets takes place. The air of these localities may be dusty, the dust originating from contaminated raw materials such as oil, feed, bone or fish meal, so that surface infection of the pellets can easily occur. Therefore, it is imperative that processes for the elimination of salmonellae in feed should be established, in the first place, in plants producing raw material such as oil, feed, meat, bone and fish meals. RUTQUIST and WAXBERG [29] discuss basic principles to be observed by factories to prevent contamination of the finished products.

Other post-processing heat treatments

In some countries imported feeding meals are steam-treated before distribution; for example, the Danish Ministry of Agriculture requires all imported bone meal, meat meal and blood meal, as well as all other imported feeding materials containing these kinds of meal, to be sterilized before release. Steam is blown into the meal, which is kept under mechanical agitation, in a retort which may be used one-fifth full only (800 kg per 4000 l capacity). The temperature in the retort is raised to 125°C (257°F) in 15 min and maintained at this temperature for at least 45 min. MULLER (1952) [30] reported that meat and bone meal imported into Denmark with a certificate stating that it had been heated for at least 1 h at 115°C to 135°C (239°F to 275°F) (moist heat) or at least 3 h at 140°C (284°F) (dry heat) and was free from pathogenic organisms had, in fact, been found positive for Erysipelothrix and salmonellae.

Niven (personal communication) reports an investigation into the thermal tolerance of Salmonella serotypes in dried, rendered animal by-products with a view to post-processing treatment. Dried meat and bone meal were inoculated with 10 000/g of the heat-resistant strain of Salmonella senftenberg 775 W; after heating to 68.3°C (155°F) for 15 min the organism could not be detected. However, subsequent work on naturally contaminated meals was disappointing. S. bredeney and S. derby at a level of 1/g survived 76.7°C (170°F) for 15 min but could not be found after heating at 82.2°C (180°F) for 15 min. Feather meal naturally contaminated with 0.5/g of S. montevidео required heating at 82.2°C (180°F) for 15 min before the organism could no longer be detected. It was concluded that decontamination by post-processing heat treatment was economically impracticable in the United States but that the prevention of recontamination after processing was feasible and already being practised successfully in some rendering plants.

In a report, "Studies on the control of Salmonella contamination in rendered animal by-products", HANSEN et al. (1962) [31] describe visits to several plants where samples were collected for examination. Two plants were exceptionally clean with good sanitary conditions, and all samples were free from salmonellae. Samples from a third plant gave positive results from meal spilled on the floor only, indicating that the floor was contaminated. A fourth plant was old, and it gave some positive samples from two

visits and all positive samples collected on a third visit. Salmonellae were found in sweepings from floor, walls and overhead beams where dust had collected; the dust could be dislodged and mix with the finished meals. Most samples from a fifth plant were positive, particularly samples from floor spillage; 110/g salmonellae were found in one sample. Dusty meal from the floor contaminated the finished product, and meals from other plants were dumped on the floor before storage in bins. Insects and rodents as well as airborne particles played a part in spreading contamination. Prolonged storage of meals led to a higher contamination rate indicative of contamination from the environment. In the sixth plant dusty meals from overhead beams and material from the floor were positive.

The sources of the salmonellae were thought to be the raw materials, insects, birds and rodents; and, once introduced, the salmonellae could spread and probably multiply in wet patches, for example, on the floor or, if the air became humid, in moist meal on beams.

The conclusions and recommendations from these studies were as follows: "Salmonellae are present in relatively high numbers in the environment of some rendering plants. They have been found on floors, or overhead beams where spilled meal and dusty meal have been allowed to accumulate for considerable periods of time. It seems likely that these places can serve as important focal points from which salmonellae can be spread to the finished product.

"It is therefore recommended that wherever possible, the accumulated material on these focal points be eliminated. Overhead beams, floors, pieces of machinery and similar places should be swept or vacuum cleaned periodically. Every possible effort should be made to minimize the opportunity for workers or trucks to transfer material from floors or any other potentially contaminated area to the finished product.

"Finished meals should never be placed on a surface which is used also as a passage way for man or vehicles".

It was also stressed that the opportunity for salmonellae present in raw materials to reach part of the plant where finished meals were handled should be reduced, and to do this "non-handling" equipment was required in areas where both finished products and raw materials were handled unless equipment was thoroughly cleaned and sanitized after handling of the raw materials. "Workers should not be allowed to cross from one area to another without changing or cleaning their boots. Insects, birds and rodents should be eliminated." It was recognized that such measures would be costly, but it was thought that they would be less costly than procedures to reheat the finished meals.

MANUFACTURING PROCESSES FOR FISH MEAL

Fish meal is produced from herring and other oily fishes by the extraction of oil, from the remains of filleting non-oily fish (white fish meal) and from sun-dried fish (Angola). It is incorporated into compound feeds for various animals, usually in amounts from 2.5 to 10% of the total ingredients. The distribution of the quantities used in the United Kingdom is approximately 48% for poultry, 35% for cattle and 17% for pigs. Producer

samples examined in the United Kingdom in 1955 showed that the Angola dried fish meal was highly contaminated with salmonellae to the extent of more than 90% of the small number of samples examined.

The Salmonella contamination of other types of fish meal varied from 3 to 8%, and it was thought that most of this resulted from post-processing contamination. From 1957-1959, 47 of 315 (14.9%) of all samples were found to be positive for salmonellae (Report, 1959 [32]); from 1959-1961, 17 of 439 (3.9%) were reported to be positive [26]; and in 1961-1962, McCoy (personal communication) found 4 of 70 (5.7%) of positive samples.

Little or no Angola fish meal appears on the United Kingdom market at the present time although presumably it is being used in other countries and may find its way to the United Kingdom blended with other ingredients.

The bacteriological condition of fish meal imported into the United Kingdom from Peru shows a big improvement since 1957, when total bacterial counts were in the millions per gram; recent results show that 90% of samples have counts less than 100 000/g and 70% of samples less than 5000/g and salmonellae are rarely found. The reasons for the improvement are listed as follows: the use of new bags, which are closed immediately the material is out of the plant, clean water, increased temperature of processing, drying at a higher temperature of 80°C (176°F), and many smaller factories closed and the reduced numbers of owners enabled to make more profit and spend more on improvements to the factories.

News from elsewhere is also encouraging. In some South African factories (Wiechers, personal communication), whole fish, mostly mackerel, were flushed from the hold of the ship to the cooker where they were heated at 98.9°C to 100°C (210°F to 212°F) for 20 min. The cooked fish proceeded to the press, and it was held at a temperature greater than 93.3°C (200°F) for 10-15 min. The press liquor was evaporated in a 4-5 stage process, during which time a temperature greater than 200°F was reached. The pressed cake was broken up and the concentrated evaporated liquor sprayed onto it before dehydration. As a proportion of the pilchard catch was used for canning, the waste heads and tails were returned to the fish-meal factory for conversion to meal. When salmonellae were found in the early stages of production, particularly when the plant was first started up, the contaminated meal was returned to the cooker for reprocessing; as a direct result the Salmonella situation improved. The plants run continuously for a week, and of six factories two to three were investigated at a time.

The following sampling technique was suggested for the examination of batches of fish meal required free from salmonellae for export to other countries:

For quantities of 50 tons the sacks should be divided up into six batches of 300 to 400 and five sacks examined from each batch; from each of the five sacks 20 g should be taken and bulked to 100 g so that in all there should be 600 g for examination. Four lots of 150 g of meal should be distributed into two 600 ml volumes of selenite F and broth for incubation and subculture in the usual way.

When material is sampled from a continuous process, the importance of taking samples from the first, fifth and tenth bag and from another three bags spaced throughout the day was stressed.

Recommendations for obtaining Salmonella-free fish meal included

- (1) Reprocessing of the first output of meal;
- (2) Cleaning of plant;
- (3) Keeping the pressed cake hot, 80°C to 90°C, during breaking up and until dried;
- (4) Maintenance of the correct temperature control of processes, the temperatures to be watched by an independent observer;
- (5) Chlorination (2-3 ppm) of all water used for the plant; the water should be free from Escherichia coli. Sea water could be used only if chlorinated in this way.
- (6) Meal should not be allowed on the floor, and any spilled material should be reprocessed;
- (7) The factory should be cleaned preferably with a vacuum cleaner and a solution of hypochlorite used for the floors.

Information from the United States was not so reassuring [33]. Many millions of menhaden fish, which constituted the United States' largest commercial catch, were netted each year from mid-spring through late fall, along the Atlantic Coast from New England to Key Largo and in portions of the Gulf of Mexico. The total catch amounted to over 2 000 000 lb. A hundred thousand fish at a time might be pumped into the hold of a fishing vessel. At the conclusion of several "sets" yielding as many as 500 000 fish, the fish were brought to the dock-side processing plant. They were discharged into a storage bin and, throughout the night, continuously pumped into huge cookers. After cooking, the oil and solid matter were separated in a press. Dock water was used for transferring the fish to the tank which filled the cooker heating the fish at 140°C (284°F) for 3 min.

In one survey 19 of 20 samples from the cooker were positive for salmonellae, some fish being raw in the centre as they came out. Positive samples were also obtained from the cake, the press liquor, the deodorizer, the meal after steam-drying at 60°C (140°F), from the sacked meal and oil polisher to sower. Salmonellae were isolated from dock water as well as from swabs from the hold of the ship; 4 of 25 swabs were positive at the start of the journey, but 25 of 35 were positive at the end of the fishing voyage, 20 h later.

Salmonellae were found in pools of flies also. Batches of 5 to 10 flies were placed in tubes of tetrathionate broth, and of 29 pools 14 were positive for salmonellae. Four serotypes were identified, all of which appeared in the fish meal. No salmonellae were found in the freshly caught fish sampled at sea. Although sampling of the product at various stages of processing indicated that salmonellae survived even the final heat treatment of dehydration, it seemed that flies in heavy infestations would have excellent opportunities for spreading salmonellae over much of the meal which may have been negative previously. Therefore, fly control was considered to be essential in efforts to reduce the Salmonella contamination of food products, particularly in the light of the number of flies from which the organism could be isolated.

FISH FLOUR

In view of the recent interest in fish flour by the United Nations Children's Fund (UNICEF) in its plan to provide under-nourished countries

with this valuable source of protein, detailed bacteriological examinations had been carried out on ten samples by the Campbell Soups Company. The results are given in Table III. Arising out of these results, tentative specifications were proposed by the Food and Agriculture Organization of the United Nations (FAO) for fish flour types A and B: less than 10 000/g maximum plate count at 37°C and freedom from coagulase-positive staphylococci, salmonellae, shigellae, enterococci and clostridia. Later these specifications were regarded as too stringent, and the following bacteriological pattern for preliminary specifications for fish-protein concentrates classified as types A and B were suggested: total bacterial count at 37°C, 10 000/g up to 50 000/g, salmonellae absent in 50 g, coagulase-positive staphylococci less than 100/g, coliform bacilli less than 100/g; clostridia, enterococci, yeasts and moulds were not included. The pattern was considered to be adequate for preliminary work on the development of solvent-extracted fish-protein concentrates; but it was not decided whether these tentative standards could be applied also to the type C products. Coag-
solv...

The proposed standard in South Africa (Wiechers, personal communication) are as follows:

Plate count (37°C)	< 10 000/g
<u>Enterobacteriaceae</u>	< 10/g
<u>Clostridium welchii</u>	< 10/g
Coagulase-positive staphylococci	absent in 100 g
<u>Salmonella</u>	absent in 100 g
<u>Shigella</u>	absent in 100 g
Lancefield Group D streptococci	< 10/g

It was suggested that a sampling scheme for the bacteriological examination of fish protein concentrates should be set up in order to obtain a large number of results so that a practicable specification could be evolved. The sampling scheme was similar to that previously described, six samples per day, three days during the week, until a total of 500 samples was reached. Each samples would amount to 100 g, of which 60 g would be used for the actual test; the remaining 40 g could be used for repeat tests, if necessary.

If a continuous process were used, two samples should be taken in the morning, early afternoon and evening. If a batch process were used, a minimum of two samples per batch was required up to a maximum of six samples per day. The samples would be taken by bacteriologically trained personnel using sterile sampling equipment and containers, and they should reach the investigating laboratory within the shortest possible time. The scheme would be carried on for a period of time long enough, at least 6 to 7 months, to enable control of the sanitary conditions during a significant processing period. Variations in the climatic conditions in the area where the scheme was being carried out should be considered.

HEAT TREATMENT OF COCONUT

Experience with coconut has shown that steaming at 96.1°C (205°F) for 5 min followed by drying at 48.9°C (120°F) effectively eliminated salmonellae.

TABLE III
FISH FLOUR

Sample code	Total plate count	Coliform bacilli	Staphylococci	Salmonellae, shigellae	Enterococci	Anaerobic sporing bacilli	Yeasts and moulds
FF-Q-2A*	6500	2.3	45	-	-	<0.18	310
FF-Q-4A	11000	24.0	20	-	-	4.3	-
FF-Q-5A	3706	0.36	<10	-	-	4.3	-
FF-Q-6A	6100	4.3	<10	<10	<0.18	2.3	-
FF-Q-11A	1500	-	<10	-	-	2.3	-
FF-Q-3HA	16000	9.3	4.5	-	-	<0.18	90
FF-Q-7HA	3100	-	<10	-	-	24	-
FF-Q-8HA	2200	-	<10	-	-	24	-
FF-Q-9HA**	53000	-	<10	<10	<0.18	24	-
FF-Q-10HA	900	-	<10	-	-	24	-

* Q-2A produced when adjusting and starting up equipment and should not be considered as fish flour produced by UNICEF on a production basis for human consumption; diverted to animal feed.

** Hole in bag when received.

The Mitchell Drier gives a 2-h cycle consisting of 25 min heating to 100°C (212°F), maintenance at this temperature for 30 min with continuous live-steam injection, followed by cooling for 1 h under controlled humidity conditions to give a final moisture content of 2 to 3%. Coconut could be treated in this way at the rate of 330 lb/h. Other methods are described in a Technical Circular written by the Technical Sub-Committee Organization concerned with desiccated coconut [34]. Proper attention to hygiene in the country of origin has reduced the contamination rate from 9 to 10% [35] of samples to 1% or less in approximately 3 yr.

PASTEURIZATION OF EGG PRODUCTS

Another instance where application of heat treatment eliminates salmonellae from foodstuff is that of the pasteurization of liquid whole egg. Recently, it has been recommended that high-temperature, short-time treatment should be carried out at 64.4°C (148°F) for 2½ min, without homogenization of milk, ice cream and imitation cream [36, 37]. Bakery tests showed that the pasteurized material could be used satisfactorily for most articles of confectionery [37], and adjustments to recipes helped with the exceptions [37]. An enzyme test was adapted by BROOKS [38] to testify to the efficiency of heat treatment. After exposure to 148°F for 2½ min both α -amylase, the enzyme, and the most heat-resistant strain of S. senftenberg 775 W were destroyed [39].

Pasteurization has also been used for liquid egg white with variable degrees of success. In addition, dried egg albumen in the flaked form may be subjected to dry heat at 54.4°C (130°F) for 9 to 10 d, including the warming and cooling periods [40]; this treatment is usually successful, but there have been failures in the presence of large numbers of contaminating salmonellae. Dry heat for spray-dried albumen has also been described. In a limited number of trials the method has not been wholly successful in the United Kingdom.

A Canadian regulation prohibits the sale of any egg product for use as human food unless it is free from detectable numbers of salmonellae of any serotype, according to the official method of testing. Some other countries require a certificate of freedom from salmonellae to accompany imported egg products, but without pasteurization such certificates are valueless.

FUMIGATION BY ETHYLENE OXIDE

Ethylene oxide can be used to sterilize medical equipment, particularly disposable articles made of plastic [41]. It is also used for foodstuffs as a fumigant against pests in spices, cocoa beans, sugared fruit and other commodities; but its use as a bactericidal or sporicidal agent is of more recent origin. Coconut, spray-dried egg, whole egg, yolk and white, and feeding stuffs have been freed from salmonellae by treatment with ethylene oxide, but there is concern about the toxic effects of residual ethylene glycol.

Ethylene oxide experiments were carried out (Lategan, unpublished) with desiccated coconut sterilized in 40-g lots, inoculated with an equal

weight of water containing a test organism such as Bacillus subtilis, E. coli or S. senftenberg. The coconut and suspension were thoroughly mixed and dried. Quantities of 5 g of the inoculated coconut packed in glacine bags were exposed to ethylene oxide in small bottles. The concentration of ethylene oxide could be varied by repeated replacement of air quantitatively evacuated. After treatment, 25 ml of quarter-strength Ringer's solution was shaken with the coconut and allowed to stand for 15 min before Salmonella counts were made by dilutions dropped onto the surface of suitable media. The experiments were carried out at exposure temperatures of 18°C, 22°C and 45°C and at ambient humidity.

Quantities of 50 to 60 lb of coconut containing at various depths 5-g samples of inoculated coconut in glacine bags were treated in a commercial sterilizer. A gaseous mixture of 90% carbon monoxide and 10% ethylene oxide was given at 35°C and at ambient humidity. After exposure, the gas was removed by evacuation and replaced by sterile air; counts were made in the usual way. A semi-logarithmic, linear survival-curve was found in all instances, and it was thus possible to determine for a given concentration of ethylene oxide the sterilization time for a particular organism at any cell concentration. This kind of procedure could be applied to any other powdered food.

For investigation of the practicability of treating coconut in bulk, 100-lb sacks of coconut were treated with ethylene oxide in an overnight sterilization chamber, used regularly for the treatment of other material. Salmonellae were eliminated, but in three instances residual quantities of ethylene glycol were found. Objections were raised to the use of ethylene oxide if residues could be detected after treatment. Methods of storage or aeration required to disperse residual ethylene oxide have not been properly determined. Spray-dried whole egg and white have been successfully treated with ethylene oxide, but the fumigation of flaked albumen failed to kill all salmonellae, presumably because the gas could not penetrate large flakes. Information on the use of ethylene oxide in the United States is conflicting. Fifty parts per million of residual ethylene glycol was said to be permitted, but later reports suggested that the method was discouraged. On the other hand, information from Germany indicates that the treatment of certain products with ethylene oxide is used without restriction [42].

The use of propylene glycol is said to be satisfactory but less efficient.

IRRADIATION WITH GAMMA RAYS

Relatively small doses of gamma rays have been shown to destroy salmonellae in a number of products. Frozen whole egg in cans may be freed from salmonellae by treatment with 450 000 rad without any deleterious effect on the taste and odour of the cooked product or the baking quality [43, 44]. 250 000 rad destroyed salmonellae in frozen blocks of horsemeat [45], and the irradiated product was palatable to dogs.

The use of irradiation pasteurization would be practicable for all frozen blocks of boneless meat whether intended for human or animal consumption, but the present United Kingdom Meat (Staining and Sterilization) Regulations, 1960 [46], state that meat unfit for human consumption must be either stained

TABLE IV

SALMONELLAE IN FROZEN, PACKED, BONELESS MEAT
(1962-1963)

Meat	No. examined	Salmonellae	
		No. positive	Positive (%)
Horse	1 360	605	44
Veal	333	36	11
Beef	362	98	27

(knackers' yard meat only) and sold raw or sterilized by heat and sold cooked. Horsemeat, although imported as meat not intended for human consumption, may nevertheless be fit for human consumption and as such is sampled at the Port [47]. The Salmonella contamination of frozen boneless horse and other meat is given in Table IV.

Animal feeding meals have also been treated satisfactorily by irradiation to free them from salmonellae.

Coconut could be irradiated also, but the physical effects of oxidation were apparent.

See the subsequent papers in this publication, in particular the articles of Thornley and Ley.

BACTERIOLOGICAL TESTS TO ESTABLISH THE EFFECTIVENESS OF TREATMENT

For experimental evaluation of any process, an agreed number of samples is examined before and after treatment.

Where Salmonella contamination is thought to be fairly regularly distributed in the sample, the naturally contaminated material is used and a number of samples within the same batch is taken for the experiments. Where contamination is scanty, artificially inoculated material may be used.

General coliform and E. coli counts are carried out, as well as qualitative tests for salmonellae in 50-g quantities of material with 2×25-g amounts of each sample in 100 ml of selenite F and tetrathionate or nutrient broths incubated overnight for the first subculture and for 3 d at 37°C for the second subculture, when cultures from the first-day plates are negative. There is relatively little known about the effect of irradiation or other treatments on the metabolic activities of micro-organisms and therefore their ability to grow in or on selective or enrichment media. It would seem to be advisable, therefore, to use nutrient broth as the enrichment liquid. Comparative results have shown that enrichment in nutrient broth gives more

broth

satisfactory results than does selenite F for the isolation of salmonellae from dried egg products and coconut [25].

When it is desired to examine more than 50 g of each sample, the amount of liquid enrichment added should be approximately four times the sample size.

Plating media include bismuth sulphite and deoxycholate citrate agars incubated for 1 to 2 d at 37°C. Suspicious colonies are inoculated into the Gillies fermentation tubes [48, 49] and on the MacConkey agar; after overnight incubation, serological tests are carried out from growth on the sloped Gillies tube. Mac--

For routine examination of treated material a chemical test is of value, for example, the α -amylase test for pasteurized liquid whole egg [38, 39]. For irradiated meat, coconut or feeding stuffs, salmonellae should be absent from 50-g samples. Quantitative estimations may be carried out on the original material to find out the level of contamination but should not be used as a yardstick for efficiency although the dosage of irradiation or of fumigant may be judged from logarithmic survival-curves.

SAMPLING

For assessment of the bacteriological efficiency of a new process, samples should be taken at regular intervals until a significant number of results have been obtained. If it is a continuous process, it is suggested that six samples per day, covering morning, afternoon and evening production, should be taken on alternate days until approximately 500 samples have been examined. For batch processes a significant proportion of each batch should be examined; this has been considered in more detail in the section above entitled "Manufacturing processes for fish meal".

Sterile sampling equipment and containers must be used, and the samples should reach the laboratory in the shortest possible time. When Salmonella contamination is suspected, examination should include tests for this organism; but if it is desired to use a coliform test instead, then at least 50 g of material should be examined. Yet this appears to be unduly stringent because of the hazards of airborne and equipment-borne contamination; also, heat-resistant strains of E. coli may escape low-temperature pasteurization processes. equip--

The routine examination of floor, shelf and beam dust should not be neglected.

A standard cannot be set unless its practicability has been established by the examination of hundreds of samples.

THATCHER [50] describes international discussion on sampling, tolerance levels and microbiological safety limits for certain frozen foods. A comment on sampling techniques is offered in Annex I of this publication.

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BACTERIOLOGICAL STUDY OF FISH MEAL IN PERU

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Abstract — Résumé — Аннотация — Resumen

BACTERIOLOGICAL STUDY OF FISH MEAL IN PERU. The importance of fish-meal production in Peru is pointed out, and the methods of manufacture are described.

The bacteriological status at different stages of the fish-meal production process is reviewed. It is stated that the bacterial count of fish meal is related to the bacterial count of the fish pools, the environmental sanitation in ship holds and factories and the method of preserving the fish meal.

ÉTUDE BACTERIOLOGIQUE DE LA POUDRE DE POISSON PRÉPARÉE AU PÉROU. L'auteur souligne l'importance de la production de poudre de poisson au Pérou et décrit les méthodes de préparation.

Il étudie l'état bactériologique aux différents stades du processus de production. Il indique que la numération bactérienne de la poudre de poisson est fonction de la numération bactérienne des viviers, de la salubrité des cales de bateaux et des usines ainsi que de la méthode de conservation de la poudre.

БАКТЕРИОЛОГИЧЕСКОЕ ИССЛЕДОВАНИЕ РЫБНОЙ МУКИ В ПЕРУ. Подчеркивается значение производства рыбной муки в Перу и описываются методы ее изготовления. Рассматривается бактериологическое состояние на различных этапах процесса изготовления рыбной муки. Указывается, что бактериальное число в рыбной муке зависит от бактериального числа рыбных бассейнов, санитарных условий в трюмах судов и на предприятиях и от метода хранения рыбной муки.

ESTUDIO BACTERIOLÓGICO DE LA HARINA DE PESCADO EN EL PERÚ. La memoria subraya la importancia de la producción de harina de pescado en el Perú y describe los métodos de elaboración.

Examina las características bacteriológicas de la harina de pescado en las diferentes etapas del proceso de producción. Establece que la flora bacteriana de la harina de pescado guarda una relación con la que existe en los mismos pescados, con las condiciones sanitarias en las bodegas de los barcos pesqueros y en las fábricas, y con el método de conservación de la harina de pescado.

The fishing industry in Peru is the youngest of all industries, but one that has grown at an exponential rate to become in recent years the most important in Latin America and the second in the world after Japan. It is probable that it will be the world's first for the year 1962. This development has been referred to as the "anchovy fever".

The fishing industry receives a great deal of attention from private as well as governmental institutions. This was shown very clearly at a symposium held in Lima during the month of November 1962. The National Fishing Society of Peru, conscious of the potential of this industry, has started a campaign directed to inform the people about the nutritive value of fish meal fed to domestic animals. This natural resource is capable of supplying most of the food so badly needed by our population as well as by people from other countries where there is a great shortage of high quality protein. At present this industry is providing man with appreciable quantities of food and is also producing feed for livestock and poultry. Peru has

actually become the most important exporter of fish meal, as shown by statistics during the past few years.*

For all these reasons it became necessary to examine the sanitary conditions under which the fish meal is being produced. Emphasis was given to the presence of pathogenic bacteria.

For the production of fish meal in Peru, we use the anchovy (Engraulis ringens). The fish used is generally very fresh and most of the time processed within 12 h after capture. This is because of the short distances from the fishing areas to the processing plants. For this reason the fish-meal producers do not add any preservatives, as is customarily done in other countries.

Once the anchovy is in the processing plant, it is subjected to quick treatment consisting in the following steps:

- (a) Cooking by steam heat;
- (b) Pressing to eliminate water and oil;
- (c) Drying in hot air, which eliminates extra water so that the product can be preserved for longer periods of time;
- (d) Pulverizing, to facilitate mixing with other foods; and finally
- (e) Packing in paper, jute or polyethylene bags.

Very little work has been published on the bacteriology of fish meal. According to unpublished data, the fish meal manufactured today in Peru is relatively free from pathogenic bacteria. However, it should be mentioned that during its infancy this industry had considerable trouble, and the degree of bacterial contamination was high. At that time when the control measures were of low standard, Salmonella were found in fish meal that was stored in places where no rodent control was carried out. Six years ago S. typhimurium, S. cholerasuis and S. scottmuerleri were often isolated.

I shall report some of the work that was done at the Faculty of Veterinary Medicine and at the Microbiology Laboratory of the Agricultural University. The purpose of this work was to find out the bacteriological status at different stages of the fish-meal process.

1. In this study samples were taken from
 - (a) Sea water;
 - (b) Fresh anchovy;
 - (c) Water at the stores of the fishing boats;
 - (d) Anchovy that was stored for several hours at the mills or processing plants;
 - (e) Fish meal before taken to the drier;
 - (f) Fish meal after being dried; and
 - (g) Fish meal at the moment of bagging.

2. Fish-meal samples freshly prepared and stored. For this purpose 120 samples were obtained from 12 processing plants. Of the 120, 60 were at bagging time and 60 after several days of being bagged and stored. Of these last ones, 34 came from open bags and 26 from closed bags.

3. Direct samples from the environment into petri dishes containing simple agar and McConkey. These petri dishes were left open for a period of 2 h at different places.

* Information on production and international trade in fish meals can be found in Annex I of this publication.

Bacterial count for saprophytic and coliform bacteria with the usual dilution system was employed, and the media used was agar and McConkey. Adequate media for anaerobes also was used, but the investigation was mainly directed to detect Salmonella. For this purpose tetrathionate, selenite and brilliant green were used. The results are shown in Tables I, II, III and IV.

The bacteriological analyses indicate that the fish meal manufactured in Peru is a product relatively free of pathogenic bacteria. Results obtained in different laboratories are very similar.

Table I shows that the sea water does not have the same influence with regard to contamination as do the stores of the fishing boats.

TABLE I
BACTERIAL COUNT IN DIFFERENT PHASES OF
FISH-MEAL PRODUCTION

Sample	Bacterial count per gram					
	Saprophytic			Coliforms		
	1	2	3	1	2	3
Sea water	110 000*	49 000	4 900	4 000	860	200
Fresh anchovy	16 000	2 500	200	2 700	600	60
Store water	690 000	856 000	1 540 000	18 000	36 000	110 000
Anchovy at mill	17 000	26 000	55 000	3 100	4 000	15 200
Anchovy several hours at mill	52 000	726 000	1 450 000	11 000	29 000	225 000
Out of cooking and pressing	1 400	7 000	15 300	-	4	100
Drying Grinding	12	60	120	-	-	4
Packing	18	68	132	-	1	6

* The bacterial count was high because of the rough sea.

Note: Investigations for Salmonella were negative.

Table II gives a comparison of the bacterial count of the fish meal at bagging time with fish meal after several days of storage. In the first case the saprophytic count was never higher than 129 and the coliform never

TABLE II

COMPARATIVE BACTERIAL COUNT OF FRESH AND STORED FISH MEAL

Number of mills	Time	Number of samples	Saprophytic per gram			Coliform per gram			Isolated organisms
			12 to 129	750 to 100 000	100 000 to 1 290 000	negatives	1 to 6	29 to 20 000	
12	At bagging	60	60	-	-	3.5	25	-	<u>St. aureus</u> <u>B. subtilis</u> <u>E. coli</u> <u>Sarcina sp.</u>
	Several days later	60	-	37	23	-	-	60	<u>Cl. septicum</u> <u>Cl. sporogenes</u> <u>Cl. welchii</u> type A <u>Proteus vulgaris</u> <u>St. aureus</u> <u>B. subtilis</u> <u>E. coli</u> <u>Sarcina sp.</u>

TABLE III

BACTERIAL COUNT RELATED TO TIME AFTER BAGGING

Time of sampling	Condition of the bag	No. of samples studied	Bacterial count per gram								
			Saprophytic				Coliform				
			12 to 129	750-23 000	38 000-100 000	110 000-1 294 000	Negative	1 to 6	29-600	750-20 000	
Bagging time	-	60	60	-	-	-	-	35	25	-	-
4 d after	open	5	-	-	5	-	-	-	-	-	5
5 d after	open	7	-	-	1	6	-	-	-	-	7
5 d after	closed	3	-	3	-	-	-	-	-	3	-
7 d after	open	5	-	-	5	-	-	-	-	-	5
8 d after	open	7	-	-	-	7	-	-	-	-	7
14 d after	open	5	-	-	-	5	-	-	-	-	5
15 d after	closed	5	-	5	-	-	-	-	-	5	-
16 d after	open	2	-	-	-	2	-	-	-	-	2
17 d after	open	3	-	-	-	3	-	-	-	-	3
22 d after	closed	5	-	5	-	-	-	-	-	5	-
45 d after	closed	5	-	5	-	-	-	-	-	5	-
60 d after	closed	5	-	5	-	-	-	-	-	5	-

TABLE IV
COMPARATIVE BACTERIA COUNT WITHIN THE SAME MILL

Mills	Saprophytic per gram		Coliforms per gram	
	Fresh fish meal	Several days of storage	Fresh fish meal	Several days of storage
A	34	1 124 000*	0.2	18 400*
B	44	296 000*	0.6	1 010*
C	33	568 000*	0.4	812*
D	89	3 680**	2.4	170**
E	36	22 400**	1.0	448**
F	51	9 800**	0.6	420**
G	25	72 600*	0.4	6 000*
H	35	291 300*	0.6	17 600*
I	84	45 400*	2.8	7 800*
J	17	150 500*	0.2	1 700*
	17	3 766**	0.2	71**
K	35	1 440**	0.4	49**
L	25	140 600*	0.2	1 810*
	25	1 483**	0.2	183**

* open bags

** closed bags

higher than 6, while in the second case the total count was between 750 and 1 290 000 and the coliform count from 29 to 20 000/g. These results clearly show that the bacterial contamination originates mainly from the environment. This was confirmed by examination of fish meal in open and closed bags where a tremendous difference was shown (Tables III and IV). Closed bags that were kept for a period of 60 d did not show over 23 000 nor more than 600 coliform per gram, while the fish meal kept in open bags

for a period of about 4 to 17 d showed a total count from 38 000 to 1 290 000 and from 750 to 20 000 coliform bacteria per gram.

From these results we can conclude that the bacterial count in open bags is in direct proportion to the time of exposure.

Salmonella were not isolated in any of the samples, but from the surroundings S. typhimurium was isolated. This indicates the possibility that the fish meal can get contaminated with this organism.

From these studies the following conclusions were drawn:

1. The bacterial count of fish meal is related to the cleanliness of the fish pools, the method of preserving the fish meal (open or closed bags) and the environment sanitation. Fundamentally, the bacterial content has its origin in the environment.

2. The number of bacteria in the freshly prepared fish meal is from 12 to 129 saprophytic and from 0 to 6 coliform bacteria. In the stored product the number of saprophytic bacteria fluctuates from 750 to 1 290 000 and from 29 to 20 000 coliform.

3. The number of bacteria in fish meal kept for 60 d in closed bags did not surpass 28 000 saprophytic bacteria and 600 coliform per gram. Fish meal kept in open bags for periods from 4 to 17 d had 38 000 to 1 290 000 saprophytic bacteria and from 700 to 20 000 coliform bacteria per gram.

4. In the freshly prepared fish meal the following organisms were isolated: Staphylococcus aureus, Bacillus subtilis, Echerichia coli and Sarcina sp. In fish meal kept in open bags: St. aureus, B. subtilis, E. coli, Sarcina sp., Cl. septicum, Cl. sporogenes, Cl. welchii type A and Proteus vulgaris.

5. From the environment of the mills the following bacteria were isolated: St. aureus, B. subtilis, E. coli, Sarcina sp., Proteus vulgaris and Salmonella typhimurium.

6. The bacterial count of the fish-meal product is closely related to the bacterial count of the fish pool.

7. The contamination in the hold of the boat has much greater influence on the final product than does the bacteria found in sea water and fresh anchovy.

In view of these findings, the following recommendations have been offered to the fish industry:

1. Thorough hygiene should be maintained throughout the stores in the fishing boats;

2. All the equipment used in the processing should be frequently cleaned and disinfected;

3. Every effort should be made so that the anchovy arrives at the mill for processing in the shortest time;

4. As soon as the fish-meal process is finished, the product should be kept in closed bags;

5. The fish meal should be kept in clean, well ventilated and hygienic stores;

6. An effective rodent control programme should be maintained.

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MEASURES TAKEN OR RECOMMENDED
TO SAFEGUARD IMPORTING COUNTRIES AGAINST
COMMODITIES CONTAMINATED WITH SALMONELLA
AND SIMILAR ENTEROPATHOGENIC BACTERIA
IN RELATION TO THE PROSPECT
OF RADIATION CONTROL

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Abstract — Résumé — Аннотация — Resumen

MEASURES TAKEN OR RECOMMENDED TO SAFEGUARD IMPORTING COUNTRIES AGAINST COMMODITIES CONTAMINATED WITH SALMONELLA AND SIMILAR ENTEROPATHOGENIC BACTERIA IN RELATION TO THE PROSPECT OF RADIATION CONTROL. Bone meal, fish meal and vegetable products prepared in developing countries are often contaminated with Salmonella and similar enteropathogenic organisms.

For prevention of the spread of Salmonella effective measures have to be taken. An attempt is made to review the measures so far taken or suggested for this purpose and to evaluate the prospects for the use of ionizing radiation as a radiation sanitation process.

MESURES PRISES OU RECOMMANDÉES POUR PROTÉGER LES PAYS IMPORTATEURS CONTRE DES DENRÉES CONTAMINÉES PAR DES SALMONELLAE ET AUTRES BACTÉRIES ENTÉROPATHOGÈNES. EMPLOI ÉVENTUEL DES RAYONNEMENTS. Les poudres d'os et de poisson ainsi que les produits végétaux préparés dans les pays en voie de développement sont souvent contaminés par des Salmonellae et autres organismes entéro-pathogènes analogues.

Des mesures énergiques doivent être prises pour empêcher la propagation des Salmonellae. Dans ce cadre, on s'efforce d'étudier les dispositions prises ou recommandées jusqu'à présent et d'évaluer les perspectives de l'emploi des rayonnements ionisants comme moyen de décontamination.

ПРИМЕНЯЕМЫЕ ИЛИ РЕКОМЕНДУЕМЫЕ МЕРЫ ПРЕДОТВРАЩЕНИЯ ИМПОРТА ТОВАРОВ, ЗАРАЖЕННЫХ САЛМОНЕЛЛОЙ ИЛИ ПОДОБНЫМИ ЕЙ КИШЕЧНЫМИ БАКТЕРИЯМИ, В СВЯЗИ С ПЕРСПЕКТИВАМИ БОРЬБЫ С ПОМОЩЬЮ РАДИАЦИИ. Изготовленные в развивающихся странах костная и рыбная мука и изделия из овощей часто заражены сальмонеллой и подобными ей кишечными организмами.

Для предотвращения распространения сальмонеллы должны приниматься эффективные меры. Делается попытка дать обзор мер, уже применяемых или предлагаемых для этой цели, и оценить перспективы применения ионизирующих излучений в качестве процесса радиационной санации.

MEDIDAS ADOPTADAS O RECOMENDADAS PARA EVITAR LA IMPORTACIÓN DE PRODUCTOS CONTAMINADOS CON SALMONELLAE Y OTRAS BACTERIAS ENTEROPATÓGENAS SIMILARES, TENIENDO EN CUENTA LA POSIBILIDAD DE LA DESINFECCIÓN POR IRRADIACIÓN. Con frecuencia, la harina de huesos y de pescado y los productos vegetales que se elaboran en los países en vías de desarrollo están contaminados con Salmonellae y otros agentes enteropatógenos similares.

Para evitar que las Salmonellae se extiendan, es preciso recurrir a procedimientos eficaces. La memoria revisa lo más completamente posible las medidas adoptadas o propuestas hasta el presente con ese propósito, y evalúa las probabilidades de éxito del empleo de radiaciones ionizantes como medio de depuración sanitaria.

INTRODUCTION

In 1955 it was established that bone meal (BISCHOFF [1] and RASCH [2]) and fish meal (BISCHOFF [3]) prepared in developing countries are often

contaminated with Salmonella and similar enteropathogenic organisms. A few years later it was demonstrated that the same occurs in vegetable products stemming from these areas (HAUGE and BØVRE [4]).

Some of these products have been manufactured by sun-drying under rather primitive conditions and are therefore liable to be seriously contaminated (ADAM [5]). Similar products prepared in other areas, although they have been heat-processed, yet fairly often contain Enterobacteriaceae and other thermo-labile organisms. In such products the infection is often rather heterogeneously distributed, with reference both to distribution over a given batch as well as to the sequence in successive batches. This may result from two different causes: (i) the survival of Enterobacteriaceae in foci in which these bacteria are physically protected against the, in itself for asporogenous organisms lethal, heat treatment to which the bulk of the material is regularly subjected (WINKLE and ADAM [6]; van der SCHAAF [7]); (ii) post-treatment contamination which is, inter alia, most probably the route by which products of vegetable origin contract their infection with enteric organisms (WINKLE and ROHDE [8]; RÖHR [9], WINKLE, ROHDE and ADAM [10]; GRUMBLES and FLOWERS [11]; RUTQVIST [12]; van der SCHAAF [7]).

However this may be, measures have to be taken to prevent the spread of Salmonella by this route. It will be attempted in this paper to review the measures so far taken or suggested for this purpose and to evaluate the prospects for the use of ionizing radiation for this specific object.

LOGISTICS OF BACTERICIDAL TREATMENTS IN GENERAL

It has been a point of discussion whether the corrective measures necessary to decontaminate infected raw materials should be taken in the producing areas or in the region where the products are imported or incorporated in mixed feeds.

Recommendations both of the International Committee for the Prevention of Epizootics [13] and of a World Health Organization panel [14] suggested that, in principle, every area should prevent large-scale spread of food-borne disease by decontaminating its own infected commodities before shipping them elsewhere. This suggestion has certainly to be followed in the case of animal feeds which enter channels of transportation and commerce in enormous volumes and which can thereby obviously do quite some harm to ships, ports, unloading facilities, docks, store rooms, trucks, carts etc. (ZWART [15]). It seems therefore to be obvious that, as far as logistics is concerned, it should be made a principle that producing areas take care of their own contaminants by proper prevention and/or elimination techniques. However, this may for a long period of time be a very difficult task for certain countries, because they often lack the facilities to subject their products either to suitable processing or to an adequate terminal treatment - thermal or other. Certain areas will definitely need efficient help if they will ever have to be able to manufacture products of acceptable sanitary quality.

Even after such treatments have once been introduced, importers and manufacturers of feeds will still have a two-fold responsibility, *viz.* (i) to check on the efficacy of the decontaminating measures taken by their suppliers of raw materials by applying proper techniques for bacteriological examination (OLIVEIRA [16]; MOSSEL, EIJGELAAR and HENSEL [17]); (ii) in the cases where the products nevertheless appear dangerously contaminated to determine what type of processing should be applied to such lots (van der SCHAAF [7]).

PROCEDURES SO FAR REALIZED

It was often felt that the aspects dealt with in the end of the section above are of sufficient importance to public health to merit governmental control. Because contaminated raw materials and recontaminated heat-treated products have been plaguing various governments for a long time before much science could be applied to the problem in general, various countries have taken *ad hoc* measures to ban the spread of Salmonella and similar bacteria via this route.

The oldest legal measures are the Danish ones (WOLDIKE NIELSEN [18], MÜLLER [19], Einfuhrverbot [20]). Without differentiation between contaminated and sound materials, all such products are to be "resterilized" when arriving in the country. In spite of the fact that application of a suitable bactericidal heat treatment to dry goods is a far from simple procedure (WINKLE and ADAM [6]), such a system has also been advocated by some circles in The Netherlands (BERGSMA [21], CLARENBURG [22]). Such an approach has, however, for various reasons been rejected by others (ZWART [15]; van der SCHAAF, van ZIJL and HAGENS [23]). So far in that country it has only been applied to the obviously more dangerous derivatives of warm-blooded animals (Salmonellose [24]).

Germany, following BISCHOFF's suggestion [3], also issued a series of laws on the importation of animal feedstuffs (Verordnung [25]). All such produce (i) has to have been subjected to an adequate heat treatment in the country of origin; (ii) on arrival in the country of destination has to be tested for the presence of Salmonella, and only those lots found to be "free" from these organisms can be imported. Lots not satisfying these requirements have to be "resterilized" or be refused. Belgium has adopted a similar system (Arrêté Royal [26]). However, many inaccuracies are involved in applying Salmonella tests only to such goods.

First of all, Salmonella may be so unevenly distributed over a consignment that a negative outcome of one or more of the current tests lacks all significance (RÖHR [9]). Also, the detection of Salmonella may be seriously hampered by the simultaneous presence of large numbers of related enteric organisms which interfere with the enrichment and plating techniques generally used for this purpose (WINKLE, ROHDE and BISCHOFF [27]) - problems never encountered, for instance, in veterinary inspection of fresh meats where the product under examination is either infected with pathogenic Enterobacteriaceae or contains none at all. Finally, even if Salmonella are really absent but other Enterobacteriaceae present, this

still leaves the possibility open for the presence of Shigellae or enteric viruses (MOSSEL [28]). Therefore, it has been recommended that as long as an adequate preventive approach to safeguarding this type of product is not yet possible, tests applied to infected products should be such that their outcome is of potential significance too, rather than of actual significance only. A test for Enterobacteriaceae in general with the same order of sensitivity as the procedures generally used for the isolation of Salmonella from foods has been suggested for this purpose (MOSSEL, VISSER and CORNELISSEN [29]).

It goes without saying that these analytical difficulties present themselves also, albeit to a lesser extent, in the control of "resterilization" plants. This has often been overlooked by the proponents of such a system for the prevention of the spread of salmonellosis by contaminated bulk materials.

A rather satisfactory selective approach again based on the early suggestions of BISCHOFF [3] has been introduced by The Netherlands' Marketing Authority for Feeds (Health Requirements [30]). In this system the products offered for importation must again be certified as having been properly decontaminated and, in spite of this, are examined for the absence of Salmonella. If this condition appears to be repeatedly fulfilled by a given manufacturer, such products can henceforth be imported without any further obstacles, except clearly a routine check a posteriori. However, if a given production centre continues to turn out contaminated goods, every given lot will be allowed in the country only after it has appeared to be "free from" Salmonella on rather intensive a priori sampling.

PROSPECTS FOR THE USE OF RADIATION IN PRODUCING AREAS

It has become clear from American investigations on the use of ionizing radiation for the elimination of Salmonella from dehydrated egg products that a dose of the order of 7 to 10×10^5 rad will reduce the initial Salmonella contamination of this type of product with a factor of the order 10^{-7} (NICKERSON et al. [31]). Experiments carried out with fish and blood meal by van der SCHAAF and MOSSEL [32] entirely confirm these results. Hence it may be expected that such an irradiation treatment can be used in practice to decontaminate fish meal, blood meal and similar products. Such a process may be called radiation sanitation.

Gamma irradiation will be the obvious choice in this case, as this can be applied to the goods packed in finished condition in impervious bags. A pilot plant trial with a cobalt source of sufficient intensity seems therefore well indicated (INGRAM and RHODES [33]).

Proper attention should be paid in such trials to the risk of impairing the nutritive value or wholesomeness of the irradiated feed components. Our own experiments (van der SCHAAF and MOSSEL [32]) revealed no loss of available lysin in any of the proteinaceous feeds irradiated at a level ≤ 1 Mrad although a slight increase in the peroxide value of the lipids present in fish meal (KEIL [34]) was noticed.

The question of how to evaluate the bactericidal efficiency of γ -irradiation in this case again poses itself immediately here. As the various Entero-

bacteriaceae encountered in raw feeds and foods show almost the same order of resistance against various destructive agents (MOSSEL [35]), it seems preferable to use, generally, batches of naturally contaminated raw products and to estimate the reduction of total Enterobacteriaceae by a reliable technique (MOSSEL and de BRUIN [36]; MOSSEL [37]; MOSSEL, MENGERINK and SCHOLTS [38]) rather than to rely on the more or less cumbersome type of test with artificially inoculated, pre-pasteurized raw materials (van der SCHAAF, van ZIJL and HAGENS [23]). However, a few model tests with products artificially infected with relatively hyperresistant organisms might have to be carried out before the process will be acceptable to health authorities.

Next, it seems legitimate to prevent, right from the beginning, reliance, by producers who will apply terminal radiation sanitation entirely on this corrective treatment and neglect of most of the current measures of factory sanitation and prevention of microbial proliferation. As already indicated earlier, a certain degree of contamination of the raw material has to be accepted, given (i) the often rather poor hygienic state in which the animals, including fish (FLOYD and JONES [39]; GULASEKHARAM *et al.* [40]) may reach the factory; (ii) the high human carrier rate in some of the producing areas (HUGHES [41]). However, preventable contamination and subsequent microbial proliferation in the goods has to be controlled. This may be attempted by laying down a maximum tolerance for a direct microscopic count, because dangerously contaminated raw products in the past mostly showed high total counts as well (MOSSEL, EIJGELAAR and HENSEL [17]).

Finally, the question of how to realize a first pilot-plant-size experimental trial and later a full-scale application in a producing area may be briefly considered. As is generally accepted nowadays (MOSSEL [42]; LUMSDEN [43]), this will require a two-step procedure. In the initial situation a foreign scientist will most probably have to design and supervise such a pilot-plant experiment. Simultaneously, full attention should be given however, to the postgraduate or, if possible, postdoctoral training of suitable local bacteriologists. Ultimately the pilot-plant research and, as it is hoped, bacteriological supervision of the radiation treatment on a technical scale will necessarily be carried out by such local experts. However, nothing seems to speak against a more permanent "twinning" of research organizations in the producing area and a suitable country which are both interested in the subject of radiation sanitation.

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REGULATIONS ON THE SANITARY CONTROL OF IMPORTED EGG PRODUCTS AND FEEDING STUFFS IN THE FEDERAL REPUBLIC OF GERMANY

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Abstract — Résumé — Аннотация — Resumen

REGULATIONS ON THE SANITARY CONTROL OF IMPORTED EGG PRODUCTS AND FEEDING STUFFS IN THE FEDERAL REPUBLIC OF GERMANY. The federal law and the regulations of the different states of the Federal Republic of Germany concerning the sanitary control of imported egg products and feeding stuffs are reviewed.

RÈGLEMENT CONCERNANT LE CONTRÔLE DE LA SALUBRITÉ DES IMPORTATIONS D'ALIMENTS POUR ANIMAUX ET DE PRODUITS A BASE D'ŒUFS DANS LA RÉPUBLIQUE FÉDÉRALE D'ALLEMAGNE. L'auteur examine la législation fédérale et les règlements des différents Etats de la République fédérale d'Allemagne en ce qui concerne le contrôle de la salubrité des importations d'aliments pour animaux et de produits à base d'œufs.

ПРАВИЛА САНИТАРНОГО КОНТРОЛЯ ИМПОРТИРУЕМЫХ ИЗДЕЛИЙ ИЗ ЯИЦ И КОРМОВ В ФЕДЕРАТИВНОЙ РЕСПУБЛИКЕ ГЕРМАНИИ. Рассматриваются федеральный закон и правила различных земель федеративной Республики Германии, касающиеся санитарного контроля импортируемых изделий из яиц и кормов.

NORMAS QUE RIGEN EL CONTROL SANITARIO DE LOS HUEVOS Y PRODUCTOS DERIVADOS Y DE LOS FORRAJES IMPORTADOS EN LA REPÚBLICA FEDERAL DE ALEMANIA. La memoria revisa las leyes federales y los reglamentos de los diversos territorios de la República Federal de Alemania relativos al control sanitario de los huevos y productos derivados y los forrajes importados.

Since the year 1956 a series of special laws concerning the sanitary control of imported egg products and feedstuffs has been issued in the Federal Republic of Germany. The sanitary supervision of imported meat and meat products and other products of animal origin is generally regulated by the new Meat Inspection Law, 1960.

For egg products a federal law, the so-called "Regulations for Protection against Infections through Germs of the Salmonella Group in Egg Products", was issued in 1956. For imported animal feedstuffs no federal laws exists, and the single states of the Federal Republic of Germany have their own regulations. The first of these regulations was issued in the state Hamburg in 1958; the regulations of the other states are similar to this.

According to these regulations, it is generally forbidden to put on the market egg products and feedstuffs which have not been pre-treated sufficiently. "Sufficient pre-treatment" in the sense of these regulations means processes by which the bacteria of the Salmonella group and also the other bacteria of the group of Enterobacteriaceae in the products are destroyed. The imported products should be subjected to an adequate treatment in the country of origin and should be accompanied by sanitary certificates. On arrival in Germany, the products are to be tested for the presence of Sal-

monella and other Enterobacteriaceae. Only those lots found to be free from these organisms are allowed to be imported. Products which do not satisfy these requirements must be heat-treated or sterilized in other ways or have to be refused.

These regulations have been a good instrument for an effective control of imports. As a result, the level of infection, e. g. of Salmonella in egg products which are on the market, is now relatively low. Before the regulations were put into force, we found infections in about 15-20% of all samples examined. During recent years the frequency of infection has been not greater than 1-2%.

From the imported feedstuffs, especially feeding materials like fish flours, a large part must be reesterilized by heat, which naturally causes substantial losses of nutritive value.

Principally, these regulations are made with the view of import control. During recent years the situation has been changing in the case of egg products. Some years ago nearly the whole demand for egg products was imported, and only 10% of the whole consumption was produced in the country. Today the quantity of home-produced egg products has increased rapidly. It is to be expected that during the next years the home-produced supplies may be sufficient for more than half of the whole consumption. The home-products are mostly frozen whole eggs, which are made from cracked or second-grade shell eggs or from the surplus of the market in first-grade shell eggs. Sanitary supervision of home-produced egg products through normal veterinarian inspection is neither effective nor satisfactory at present. Therefore, an improvement of egg-product regulations is being discussed.

All German authorities concerned with this problem agree that many difficulties could be avoided if an effective and economic sterilization method were applied to all products which may be infected by salmonellae.

SANITARY CONTROL OF IMPORTED EGG PRODUCTS IN ITALY

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Abstract — Résumé — Аннотация — Resumen

SANITARY CONTROL OF IMPORTED EGG PRODUCTS IN ITALY. The order of the Italian Ministry of Public Health concerning sanitary control on imported egg products and the regulations governing import of feeding stuffs are described.

CONTRÔLE DE LA SALUBRITÉ DES PRODUITS A BASE D'ŒUFS IMPORTÉS EN ITALIE. L'auteur examine l'arrêté du Ministère italien de la santé publique relatif au contrôle de la salubrité des produits importés à base d'œufs et donne un aperçu des prescriptions régissant l'importation des aliments pour animaux.

САНИТАРНЫЙ КОНТРОЛЬ ИМПОРТИРУЕМЫХ ИЗДЕЛИЙ ИЗ ЯИЦ В ИТАЛИИ. Описывается распоряжение Министерства общественного здравоохранения Италии относительно санитарного контроля импортируемых изделий из яиц и правила, регулирующие импорт кормов.

CONTROL SANITARIO DE LOS HUEVOS Y PRODUCTOS DERIVADOS IMPORTADOS EN ITALIA. La memoria describe la legislación establecida por el Ministerio de Salud Pública de Italia para el control de los productos de huevo importados, así como los reglamentos que rigen la importación de forrajes.

The order of the Italian Ministry of Public Health (Department of Veterinary Service), dated 25 September 1957, concerning sanitary control of imported egg products (frozen eggs, dehydrated eggs or eggs prepared in various ways), contains the following relevant paragraphs:

ARTICLE 1

Egg products (whole egg, yolk, albumen), frozen, dehydrated or prepared in various ways and destined for human consumption, can be imported into Italian territory only with the authorization of the Italian Ministry of Public Health.

The State Veterinary Inspectors at frontiers, ports and airports verify that the hygienic characteristics of such products are those established by the above order and authorization of the Ministry of Public Health.

ARTICLE 2

To be imported into Italy the products specified above must be accompanied by sanitary certificates issued by the Government of the country of origin. These certificates must not be issued earlier than twelve months before the arrival of the products in Italy and must indicate the trade mark

of the firm from which the products originate. These certificates must also state

- (1) The species of the birds whose eggs have been used for the manufacture of the product;
- (2) the bacterial content per gram of the product and the colitre;
- (3) the possible addition to the product of sugar, salt or other substances (with percentage);
- (4) the absence of pathogenic micro-organisms in the product;
- (5) that the product has not been treated with antibiotics and others additives;
- (6) that the product is fit for all kinds of human consumption.

ARTICLE 3

Egg products which are imported for purposes other than human consumption must be treated in such way as to make them completely acceptable for human consumption. The products are inspected upon entry in Italy by the State Veterinary Inspectors.

Instruction No. 110 of the Ministry of Public Health (Department of Veterinary Services), dated 17 August 1960, states the following concerning the bacterial content of imported egg products intended for human consumption:

(1) Absence of pathogenic micro-organisms and of staphylococcal and enterotoxigenic toxins in the product

(2) Non-specific micro-organisms

in frozen eggs shall be no more than 500 000 bacteria and 100 coli per gram; in dried eggs shall be no more than 150 000 bacteria and 50 coli per gram.

Instruction No. 1 of the Ministry of Public Health (Department of Veterinary Services) of 2 January 1961 gives detailed instructions as to the bacteriological analysis of imported egg products intended for human consumption; also the method of sampling is described in detail.

Concerning sanitary control of imported feeding stuffs, it should be mentioned that the Ministry of Public Health Veterinary Regulations, approved by Decree of the President of the Republic on 8 February 1954 (No. 320), states in Article 56:

Fish meal to be imported must pass the sanitary control of the State Veterinary Inspectors at the frontier. Meat meal, bone meal and blood meal to be imported must be accompanied by sanitary certificates issued by the Government of the country of origin. These certificates must declare that the above products have been subjected to sterilization treatment.

REGULATIONS GOVERNING THE CONTROL OF SALMONELLAE IN FEED PRODUCTS IN DENMARK, AND A COMMENT ON THE USE OF RADIATION

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Abstract — Résumé — Аннотация — Resumen

REGULATIONS GOVERNING THE CONTROL OF SALMONELLAE IN FEED PRODUCTS IN DENMARK, AND A COMMENT ON THE USE OF RADIATION. The Danish Ministry of Agriculture requires reesterilization of all imported meat, bone, blood and fish meals. At present this sterilization consists of a rather severe heat treatment which leads to a considerable loss in the nutritive value of the products.

Irradiation is considered as a promising alternative. It has the advantage that the products will have a higher nutritive value than after heat treatment. This would justify a higher price for the radiation-treated commodities. However, radiation elimination of Salmonella from feeding stuffs is not likely to be more costly than is heat sterilization.

PRESCRIPTIONS RÉGISSANT LA DÉTECTION DES SALMONELLAE DANS LES ALIMENTS POUR ANIMAUX AU DANEMARK ET OBSERVATIONS SUR L'UTILISATION DES RAYONNEMENTS. Le Ministère danois de l'agriculture prescrit la re-stérilisation des poudres importées de viande, d'os, de sang et de poisson. Actuellement, cette stérilisation consiste en un traitement thermique assez rigoureux, qui entraîne une perte considérable de la valeur nutritive des produits.

On considère l'irradiation comme une méthode pleine de promesses. Son avantage est de garder aux produits alimentaires une valeur nutritive plus grande que ne le permet le traitement thermique, ce qui justifierait un prix plus élevé pour les denrées traitées par rayonnements. Cependant, le traitement par irradiation ne sera probablement pas plus coûteux que la stérilisation thermique.

ПРАВИЛА, РЕГУЛИРУЮЩИЕ КОНТРОЛЬ ЗАРАЖЕННОСТИ САЛМОНЕЛЛОЙ КОРМОВ В ДАНИИ, И ЗАМЕЧАНИЯ ПО ПОВОДУ ИСПОЛЬЗОВАНИЯ ИЗЛУЧЕНИЙ. Министерство сельского хозяйства Дании требует проведения повторной стерилизации всей импортируемой мясной, костной, кровяной и рыбной муки. В настоящее время эта стерилизация заключается в более сильной термообработке, что приводит к значительному снижению питательной ценности продуктов.

Облучение считается обещающим способом обработки. Преимущество этого метода заключается в том, что после такой обработки продукты будут иметь более высокую питательную ценность, чем после термообработки. Это должно оправдывать более высокие цены на продукты, обработанные облучением. Однако уничтожение сальмонеллы облучением в кормах, по-видимому, не будет более дорогостоящим, чем стерилизация при помощи термообработки.

NORMAS SEGUIDAS EN DINAMARCA PARA EL CONTROL DE SALMONELLAE EN LOS FORRAJES Y COMENTARIOS SOBRE EL EMPLEO DE RADIACIONES. El Ministerio de Agricultura de Dinamarca exige que se vuelva a esterilizar toda la harina de carne, de huesos, de sangre y de pescado que se importa. La esterilización consiste actualmente en un tratamiento térmico bastante intenso que disminuye considerablemente el valor nutritivo de los productos.

Se está estudiando la irradiación como medio para sustituir favorablemente este tratamiento. Tiene la ventaja de que el valor nutritivo de los productos será superior al que conservan después del tratamiento térmico. Con ello se justificaría un precio más elevado para los alimentos irradiados. Sin embargo, no es probable que la eliminación de las Salmonellae por irradiación de los forrajes resulte más onerosa que la esterilización térmica.

* Danish Meat Research Institute.

As mentioned in several of these papers, we have in Denmark very strict regulations for imported feeding stuffs. The Danish Ministry of Agriculture requires a reesterilization of all imported meat meals, bone meals, blood meals and fish meals and of other imported feeding stuffs containing these meals. However, exemptions to this law have been granted for fish meal from Norway and Iceland so that fish meal from these countries can be freely imported.

The regulations prescribe exactly how to perform the reesterilization. The process is as follows:

The sterilization takes place by means of saturated steam. The steam is blown into the meal, which has to be kept under constant mechanical motion in a retort, the retort being filled only to one-fifth of its capacity. The temperature in the retort is raised to 125°C (1.37 atm overpressure) in 15 min, and maintained at this temperature for at least 45 min.

Denmark has an annual import of about 20 000 t of meat and bone meal and about 25 000 t of fish meal, the latter, however, only being imported from Norway and Iceland. The required reesterilization process means a considerable loss in the nutritive value of the products; and, considering the amount of imported meals, this means a significant economic loss. For these reasons we are interested in finding an alternative method which may give us similar assurance of the absence of *Salmonella* but at the same time less decrease in the nutritive value of the feeding stuff.

Blood meal is a feeding stuff of a high nutritive value as it is very rich in protein. In Denmark the blood from abattoirs is treated in various plants to produce either blood albumin (spray-dried blood) or blood meal. The combined annual production of these two products is on the order of 6000 t. The same heat treatment as applied during reesterilization is required for the production of blood meal, thus causing considerable losses in nutritive value. Most of the blood is at present sold in the form of albumin, which is used as an adhesive in the wood industry. However, for this purpose the albumin will soon be completely replaced by synthetic materials, and hence the whole production will be turned into blood meal. This will mean the installation of more heat sterilizers in the blood treating plants. Thus there is a two-fold incitement for the introduction of a new and more lenient sterilization process.

We think that irradiation may be the answer in the two cases mentioned above, especially in the case of imported meals; and consequently the Danish Meat Research Institute has initiated a study of the effects of irradiation on meat, bone and blood meal. The aims in our investigation are the following: (1) to compare the nutritive value of the meals after heat treatment with that after irradiation treatment; (2) to compare the prices of the two processes in order to establish the feasibility of the irradiation treatment; and (3) to establish the dose necessary to kill the *Salmonella* in the products concerned. I may add here that the work is only at a very preliminary stage and that the figures quoted in the following must be taken with reservations.

As a measure for the protein value we have chosen the amount of available lysine in the meal as determined by the method of CARPENTER [1]. It is indicated that results obtained by this chemical method correlate well with the protein value as determined in feeding experiments. We have determined available lysine in irradiated blood albumin (spray-dried blood)

and in blood meal (i. e. , heat-treated blood albumin from the same batch). The heat-treated material was found to contain 6.2 g available lysine per 16 g N whereas the albumin contained 8.3, 8.4, 8.2 and 8.3 g per 16 g N after doses of 0, 0.5, 1.0 and 5.0 Mrad respectively. Thus, no losses seemed to occur during irradiation treatment of the meal, not even at a dose as high as 5 Mrad, whereas the heat treatment caused a considerable loss in available lysine (about 25%).

The doses needed for kill of Salmonella will be established in experiments where meat and bone meal inoculated with salmonellae will be subject to various doses. We consider it most likely that the established dose will be of the order of 0.5-1.0 Mrad; consequently we have used 0.5 and 1.0 Mrad in our feasibility studies.

The prices for the reesterilization of imported meals, including re-bagging, are of the order of 1 d.(1.2 cents) per kg whereas the actual heat treatment of the blood albumin and the subsequent crushing of the blood cost 0.5-0.6 d.(1.2-1.5 cents) per kg. We want to compare this with the prices for treatment with gamma rays in a Co⁶⁰ plant and with electrons from a resonant transformer. So far we have only estimates for the electron processing. The prices, of course, vary considerably with throughput, but we estimate that the treatment of 25 000 t a year with a dose of 0.5 Mrad will amount to approximately 0.35 d.(0.4 cent) per kg (bagging is not included in this figure). Although the capital cost is high, it is indicated that the use of ionizing radiation will be able to compete economically with the conventional heat treatment, and the radiation has the advantage that the meals will have a higher nutritive value after treatment that would actually justify a higher price of the product.

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**SALMONELLA CONTAMINATION
OF FOODS AND FEEDS
AND THE POSSIBILITY OF RADIATION CONTROL
IN THAILAND**

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Abstract — Résumé — Аннотация — Resumen

SALMONELLA CONTAMINATION OF FOODS AND FEEDS AND THE POSSIBILITY OF RADIATION CONTROL IN THAILAND. The prevalence of salmonellae and other harmful micro-organisms in egg, poultry and pork is for Thailand a very severe problem, the solution of which would be of great importance to the economy of the country.

An attempt was made to use ionizing radiation for rendering chicken meat free of Salmonella. A dose of not less than 50 000 rad was required.

CONTAMINATION PAR LES SALMONELLAE DE LA NOURRITURE DE L'HOMME ET DES ANIMAUX ET POSSIBILITÉ D'UN TRAITEMENT PAR LES RAYONNEMENTS EN THAÏLANDE. La prédominance des Salmonellae et d'autres micro-organismes nuisibles dans les œufs, la volaille et le porc pose pour la Thaïlande un problème grave dont la solution serait d'une importance capitale pour l'économie du pays.

On a essayé d'utiliser des rayonnements ionisants pour éliminer les Salmonellae de la chair de poulet. A cette fin, il a fallu une dose d'au moins 50 000 rads.

ЗАРАЖЕНИЕ САЛМОНЕЛЛОЙ ПИЩЕВЫХ ПРОДУКТОВ И КОРМОВ И ВОЗМОЖНОСТЬ ИХ ОБРАБОТКИ ИЗЛУЧЕНИЯМИ В ТАИЛАНДЕ. Наличие Салмонеллы и других вредных микроорганизмов в яйцах, птице и свинине является для Таиланда очень серьезной проблемой, решение которой будет иметь огромное значение для экономики страны.

Была сделана попытка использовать ионизирующие излучения для обезвреживания курятины, зараженной салмонеллой. Для этого требовалась доза не менее 50 000 рад.

CONTAMINACIÓN DE ALIMENTOS Y FORRAJES POR SALMONELLAE Y POSIBILIDAD DE EMPLEAR EN TAILANDIA LA DESINFECCIÓN POR IRRADIACIÓN. La presencia de Salmonellae y otros microorganismos nocivos en los huevos, las aves y el cerdo constituye para Tailandia un grave problema, cuya solución será de la máxima importancia para la economía del país.

Se procuró emplear radiaciones ionizantes para liberar de Salmonellae la carne de pollo. Fue necesaria una dosis superior a 50 000 rad.

In 1961 Thailand exported nearly 800 000 eggs per day to Hong Kong. Since then, however, most of the eggs imported to Hong Kong have come from the People's Republic of China, which offers chicken eggs at a considerably lower price. Thailand's potential production capacity is actually much higher than the figure mentioned above; estimates indicate that ten times more eggs could be produced*.

It is naturally essential for the Thai economy to find another market for the eggs, and attempts have therefore been made to export them

* Note: The FAO Trade Yearbook Vol. 15, 1961, mentions the figure 16 300 t per year as Thailand's total export of hen and duck eggs during 1960.

to Europe. However, because of the fact that Thai eggs, as well as eggs from other oriental countries, are often infected with pathogenic microorganisms, particularly Salmonella, this attempt has not met with success.

Recently Thailand also started a broiler industry, using some special breeds imported from the United States and the United Kingdom. Again, Europe does not accept chicken meat from Thailand because of its content of salmonellae and other organisms.

The situation with regard to pork is similar. In 1961 Thailand exported 8000 live pigs per month to Hong Kong but has now largely lost this market owing to the competition from the People's Republic of China. In Bangkok up to 100 pig carcasses are condemned and have to be thrown away each day because of heavy infection. Thailand has a modern slaughter house with freezing chambers and could send frozen pork to Europe, provided this pork could be guaranteed free from Salmonella.

In summary, the prevalence of salmonellae and other harmful microbes in eggs, poultry and pork is for Thailand a big problem, the solution of which would be of great importance to the economy of the country.

Exact figures concerning the prevalence of food infection by Salmonella are still lacking although both Salmonella pullorum and Salmonella typhimurium are prevalent in poultry farms in Thailand. Salmonella organisms are widely distributed among chicken and ducks. The elimination of Salmonella pullorum is still a problem in the chicken-breeding farms, as both the egg and the meat of duck and chicken are common food items in the Thai diet. Slide agglutination tests with stained antigen are used for the detection of positive reactors.

Salmonella pullorum [1] was first isolated from duck in Bangkok in 1955. Salmonella typhimurium [2] was isolated in 1960 from powdered egg, produced by the Poultry Department, Kasetsart University, Bangkok. In 1962 Salmonella sp. was isolated by the author from the liver of a chicken that had died of "big liver disease".

An attempt was made to use ionizing radiation to render chicken meat free from Salmonella. Initial densities of about 10^8 /ml of Salmonella sp. were used. Irradiation was carried out with a 2000-c Co⁶⁰ source. The culture of Salmonella sp. was mixed with ground chicken meat and kept at room temperature during the treatment.

The test was made at dosages of 500 000 rad, 300 000 rad, 200 000 rad, 150 000 rad and 100 000 rad.

MATERIAL AND METHODS

120 g of the fresh ground chicken meat was mixed with 10 ml of the culture of Salmonella sp. The infected ground chicken meat was then loaded into 12 sterile test tubes with rubber caps. Each tube contained 10 g of the infected material. A tube of 5 ml of the broth culture of Salmonella sp. was used for each dosage of radiation. After the irradiation all tubes were kept in the refrigerator for bacteriological examination.

IRRADIATION

Irradiation was carried out in the Atomic Exhibition of the United States Atomic Energy Commission in Bangkok under the direction of Walter D. Tucker. The source used consisted of approximately 2000 c of cobalt-60 contained in three individual sources. The dose rates available vary with the geometrical array employed but run between 25 000 and 250 000 rad/h.

BACTERIOLOGICAL EXAMINATION OF IRRADIATED SAMPLES

Irradiated samples were examined both by enrichment media and by direct counts in solid media. The enrichment media were Difco tetrathionate broth and Difco brilliant green agar. The solid media for bacteria count was nutrient agar.

About 10 g of the irradiated material was seeded in 150 ml of tetrathionate broth, and after 24 h of incubation at 37°C the culture was streaked on the surface of brilliant green agar for the growth of Salmonella.

GENERAL EXAMINATION OF IRRADIATED SAMPLES

Normal ground chicken meat was irradiated at the dosage of 500 000 rad for evaluation of taste, odour and colour.

RESULTS

By the direct plate counts of broth cultures, the complete inactivation of Salmonella sp. required a dosage of not less than 500 000 rad (Table I).

TABLE I

RADIATION EFFECT OF SALMONELLA sp.

Dose of gamma radiation (rad)	Number of surviving cells of <u>Salmonella</u> sp. per ml (broth culture)
	8
0	10
100 000	430
150 000	300
200 000	55
300 000	30
500 000	-

General examination of the irradiated chicken meat showed that after irradiation there was irradiation odour and taste, but after the meat was kept in a refrigerator for 24 h, the odour and taste were normal. The colour of the treated sample was pale pink and looked more tempting than did the untreated chicken meat when both were kept in the refrigerator. This appearance was also observed in pork irradiated with 500 000 rad.

DISCUSSION

The bactericidal effect of gamma radiation on Salmonella sp. observed in this experiment is close to that obtained by MOSSEL [3].

The irradiation odour and taste disappeared, and the pale pink colour of the meat looked more appetizing when the treated meat was kept in the refrigerator for at least 24 h. Compared with the method of preserving chicken meat with chlortetracycline hydrochloride (10% chlortetracycline in 90% soluble substance) which results in a dirty colour of the meat, the method of preservation by radiation to free the meat from Salmonella sp. can be considered a promising process.

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MICROBIOLOGICAL ASPECTS OF THE USE OF RADIATION FOR THE ELIMINATION OF SALMONELLAE FROM FOODS AND FEEDING STUFFS

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Abstract — Résumé — Аннотация — Resumen

MICROBIOLOGICAL ASPECTS OF THE USE OF RADIATION FOR THE ELIMINATION OF SALMONELLAE FROM FOODS AND FEEDING STUFFS. Before considering the specialized application of irradiation to the elimination of salmonellae and other pathogens, the author outlines its relation to other food irradiation processes such as sterilization and pasteurization.

The radiation dose required can be estimated by determination of the D_{10} -values for the organisms, which can be calculated by means of either the "end-point" method or the "survival-curve" method. From the D_{10} -values one is able to calculate the dose which will provide any desired degree of inactivation.

The effect during irradiation of environmental factors, such as oxygen, freezing, drying and various food constituents, is discussed. The radio-resistance of different *Salmonella* serotypes can vary with the nature and physical state of the product, and the dose needed must be determined in each case.

A comparison is made between irradiation, heat treatment and gaseous sterilization. Those three procedures could be used for the elimination of salmonellae.

ASPECTS MICROBIOLOGIQUES DE L'UTILISATION DES RAYONNEMENTS POUR ÉLIMINER LES SALMONELLAE DE LA NOURRITURE DE L'HOMME ET DES ANIMAUX. Avant de considérer l'application particulière de l'irradiation à l'élimination des *Salmonellae* et autres microbes pathogènes, l'auteur souligne l'analogie de cette méthode avec d'autres procédés d'irradiation d'aliments tels que la stérilisation et la pasteurisation.

La dose d'irradiation nécessaire peut être évaluée par détermination des valeurs de la D_{10} pour les organismes, que l'on peut calculer soit par la méthode du «point terminal» soit par celle de la «courbe de survie». A l'aide des valeurs de la D_{10} , on peut calculer la dose qui assurera n'importe quel degré d'inactivation.

L'auteur étudie l'effet de certains facteurs, comme l'oxygène ambiant, la congélation, la dessiccation et les divers éléments constitutifs des aliments pendant l'irradiation. La radio-résistance de différentes catégories de *Salmonellae* peut varier suivant la nature et l'état physique du produit; aussi la dose nécessaire doit-elle être déterminée dans chaque cas.

Une comparaison est faite entre l'irradiation, le traitement thermique et la stérilisation gazeuse. Ces trois procédés pourraient être utilisés pour l'élimination des *Salmonellae*.

МИКРОБИОЛОГИЧЕСКИЕ АСПЕКТЫ ИСПОЛЬЗОВАНИЯ ИЗЛУЧЕНИЙ ДЛЯ УНИЧТОЖЕНИЯ САЛМОНЕЛЛЫ В ПИЩЕВЫХ ПРОДУКТАХ И КОРМАХ. Прежде чем рассматривать специальные виды применения излучений для уничтожения сальмонеллы и других патогенных организмов, автор описывает их связь с другими процессами обработки пищевых продуктов, такими, как стерилизация и пастеризация.

Требуемая доза облучения может быть рассчитана путем определения значений D_{10} для организмов, которые могут быть рассчитаны или с помощью метода "конечной точки" или методом "кривой выживания". Исходя из значений D_{10} , можно рассчитать дозу, которая обеспечит любую требуемую степень дезактивации.

Рассматривается влияние во время облучения факторов окружающей среды, например влияние кислорода, замораживания, высушивания и различных составных частей пищевых продуктов. Устойчивость против облучения различных серотипов сальмонеллы может меняться в зависимости от характера и физического состояния продукта, и требуемая доза должна определяться в каждом отдельном случае.

Проводится сравнение между облучением, термообработкой и газовой стерилизацией. Эти три метода можно использовать для уничтожения сальмонеллы.

D_{10} -
end -

ASPECTOS MICROBIOLÓGICOS DEL EMPLEO DE RADIACIONES PARA ELIMINAR LA SALMONELLAE DE LOS ALIMENTOS Y LOS FORRAJES. Antes de considerar la aplicación de las radiaciones con el fin concreto de eliminar la Salmonella y otros agentes patógenos, el autor indica su relación con otros procesos de tratamiento de alimentos tales como la esterilización y la pasteurización.

La dosis de radiación requerida se puede calcular determinando los valores D_{10} para los organismos, que se establecen por el método del «punto final» o de la «curva de supervivencia».

Estos valores D_{10} permiten calcular la dosis necesaria para alcanzar el grado de inactivación que se desee.

La memoria discute el efecto de los factores ambientales que prevalecen durante la irradiación, tales como el oxígeno, la congelación y el secado, así como de los diversos constituyentes de los alimentos. La radiorresistencia de diversos tipos serológicos de Salmonella puede variar con la naturaleza y el estado físico del producto, por lo que es necesario determinar la dosis requerida en cada caso.

El autor compara los modos de esterilización por irradiación o por tratamiento térmico o gaseoso. Los tres procedimientos pueden servir para eliminar las Salmonellae.

This paper concerns the microbiological aspects of the irradiation process; other changes induced by radiation in such properties as flavour and nutritive value of the food under treatment will be discussed elsewhere (see, for instance, the following paper by Ley).

A. FOOD IRRADIATION PROCESSES INVOLVING MICROBIAL INACTIVATION

Before the specialized application of irradiation for eliminating salmonellae is considered, it is useful to see how this relates to food irradiation processes with other aims, and those involving microbial inactivation are set out in Table I. Only a brief outline will be given here, full details being obtainable from HANNAN [1] and a summary of recent developments from INGRAM and RHODES [2].

(1) Sterilization

Radiation sterilization is intended to confer an indefinite storage life at room temperature on otherwise perishable products. One of the greatest hazards in non-acid foods would be survival and subsequent multiplication of Clostridium botulinum, which is highly resistant in the spore form, and therefore the inactivation requirements of this organism are the main factor in determining the dose to be used. The criterion commonly adopted in heat processing, that it should be sufficient to inactivate the C1. botulinum spores initially present by a factor of 10^{12} , has been extended to radiation work, where it results in the figure of 4.5 Mrad often quoted for the sterilizing dose. Once the requirements for inactivation of C1. botulinum are fulfilled, the main problems for this type of process lie in the organoleptic changes which tend to be developed.

(2) Pasteurization

This term is used here for the low-dose irradiation processes intended to prolong storage life. This use is more general, especially in the American literature, but the term has sometimes been applied to the killing of pathogenic bacteria.

TABLE I

FOOD IRRADIATION PROCESSES
INVOLVING MICROBIAL INACTIVATION

Process	Applied to	For destruction of	Dose range (Mrad)
1. Sterilization	Perishable non-acid foods	<u>Cl. botulinum</u> and all other bacteria	4.5 - 5.0
2. Pasteurization	Perishable foods	Spoilage micro-organisms	0.1 - 1.0
3. Elimination of <u>Salmonella</u>	Non-perishable or perishable foods	<u>Salmonella</u>	0.2 - 0.65

The lower doses employed do not sterilize the product but reduce the number of micro-organisms able to multiply and hence delay the onset of spoilage. In general, this increase in storage life is only large enough to be useful when the product is stored under refrigeration, because at higher temperatures multiplication of the surviving organisms occurs rapidly. *under*

Organoleptic changes are not so serious at the moderate doses employed, but problems arise from the possible survival of pathogenic organisms, which may be more resistant to radiation than are the spoilage flora. If the pathogens have an opportunity to multiply, the irradiated product may become more heavily infected than the control because of the lack of competition from the normal spoilage organisms. Microbiological study is particularly necessary, therefore, with radiation-pasteurized products, and each process and product has to be examined separately under the appropriate storage conditions. This consideration provides an added reason for storage under refrigeration, which prevents growth of almost all pathogens. *spoil*

(3) The elimination of salmonellae or other pathogens

The elimination of salmonellae is a very similar process to pasteurization since the doses used are in the same range, but the aim is different. It is simply to remove one group of organisms which are particularly undesirable in the product. Salmonellae are moderately sensitive vegetative bacteria (Fig. 1), and doses around 0.5 Mrad will effect a very large reduction in their numbers (by a factor of 10^7 in many products). Other pathogenic bacteria could be treated in the same way; but those forming spores, such as Clostridium welchii or Bacillus anthracis, would need much larger doses to give a comparable reduction in numbers, since spores are, in general, more radiation-resistant than are vegetative cells.

Applications so far suggested for removal of salmonellae have concerned mainly non-perishable products, such as frozen and dried egg, frozen horse meat and animal feeding stuffs. In these products there is normally no chance for multiplication of survivors, and the problems mentioned in connection with pasteurization do not arise.

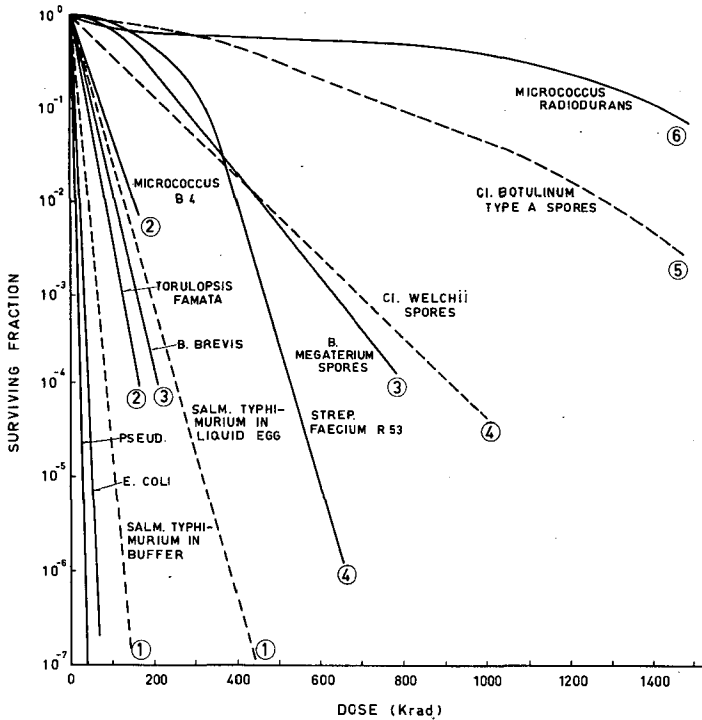


Fig. 1

Sensitivity to irradiation of some food spoilage and food poisoning micro-organisms
 Conditions of irradiation were aerobic except for Clostridium botulinum and Cl. welchii, and possibly S. typhimurium in liquid egg (see text).

----- Pathogens
 _____ Other organisms

Data from: (1) LEY et al. [3]
 (2) SCHMIDT-LORENZ and FARKAS [4]
 (3) WOESE [5]
 (4) MATSUYAMA et al. [6]
 (5) INGRAM and THORNLEY [7]
 (6) ANDERSON et al. [8]

If, however, salmonellae were to be removed from a perishable product in this way, all the considerations about survival of other pathogens would be important. For instance, the case might arise in which salmonellae were inactivated but Cl. welchii spores survived, and then storage under favourable conditions of temperature and anaerobiosis could lead to trouble. Detailed consideration of the process for control of salmonellae follows.

B. INACTIVATION OF SALMONELLAE BY IRRADIATION

(1) Estimation of dose required

Some idea of the magnitude of the dose required may be obtained by the irradiation of naturally contaminated material, but the numbers of salmonellae present are usually low, of the order of hundreds per g or less, and the information obtainable is limited. Nevertheless, this type of work is important to check that the inactivation doses calculated from artificially inoculated material have the expected effect on salmonellae in the product.

As with heat processing, it has been found most useful to inoculate the product with large numbers, about 10^7 - 10^8 /g, of strains of *Salmonella* from pure cultures and to study their inactivation. These organisms, like many other bacteria, show an exponential death rate when irradiated; that is, a given dose of radiation will inactivate a constant proportion of the viable cells previously present. This means that by determining the D_{10} values, or dose required to inactivate 90% of the bacteria, one is able to calculate the dose which will provide any required degree of inactivation.

For example, if the initial contamination is 10^2 /g and it is desired to reduce the salmonellae so that none are detected in a 100-g sample (or, for purpose of calculating, say a survival of 1 in 1000 g), then the inactivation factor required is 10^5 , and the dose necessary is $5 \times D_{10}$.

Besides the D_{10} value, one therefore has to know the maximum contamination likely to occur in the product and the level to which it must be reduced. This latter value is not so obvious as it might appear, because it depends both on the size and number of samples from which salmonellae must be absent and on the sampling plan. In the above example a level of one survivor in 1000 g has been arbitrarily chosen, and this would obviously give a low probability of a positive test for salmonellae in one 100-g sample but a much higher probability if 10 samples in the same batch were to be examined.

Some data on the sampling schemes actually used by various countries are given in Annex II.

Methods of calculating the D_{10} values from data of various kinds have been fully discussed by SCHMIDT [9] in connection with heat processing and will be mentioned only briefly here.

(a) End-point methods

In this type of method a large number of bacteria is inoculated into samples of the product, which are then irradiated at different doses. All samples are tested for presence or absence of survivors; this can sometimes be done by incubation in the product if it is suitable for growth of the organisms. The lowest dose giving no survival is regarded as the inactivation dose for the number of organisms inoculated.

Where replicate samples are used at each dose and the doses are closely spaced so that several give some positive and some negative samples, the following calculation may be applied to each dose:

$$D_{10} = D_x / (\log A - \log B),$$

where D_x is any dose giving some positive and some negative samples, A is the total number of samples treated at this dose multiplied by the number of bacteria inoculated per sample and B is the number of survivors, calculated by assuming one survivor per positive sample.

Several estimates of D_{10} are obtained, and the mean is used. This is the method of STUMBO [10], and other methods, developed from it, involve the use of the "most probable number" technique, for estimation of the survivors in the multiple samples (STUMBO et al., [11]), or a probability method (SCHMIDT [9]).

(b) Survival-curve methods

The product is inoculated with a large number of bacteria, then irradiated and sampled after various doses, and viable counts of the number of survivors in each sample are made. The \log_{10} of the number of survivors is plotted against dose, the line of best fit is drawn through the points, and this curve can then be used to obtain the D_{10} value.

Fig. 2 illustrates results of this type for Salmonella gallinarum from LEY et al. [3] and shows that the inactivation was exponential over a wide range.

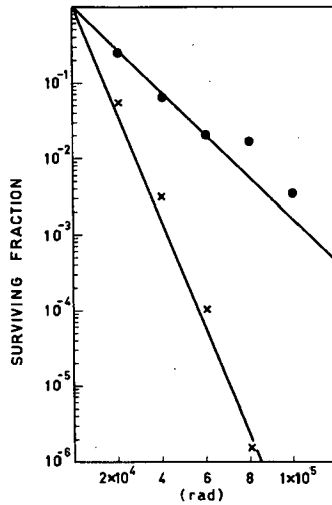


Fig. 2

Sensitivity of S. gallinarum to irradiation in buffer solution (x)
and in liquid whole egg (●), non-aerated
Data from LEY et al. [3]

(c) Relative merits of the two kinds of method

End-point methods allow the experimenter to cover a wider range of inactivation, since quantities as large as 100 g can be tested for small numbers of survivors, whereas counts cannot be made if numbers are lower

than about 10 per g. Hence the end-point procedure involves less extrapolation in the eventual calculation of the dose needed for processing.

Under certain circumstances end-point methods enable one to determine survival by incubation in the food product itself, which provides particularly relevant information (see the next section).

The survival-curve method may well be more convenient for small-scale laboratory investigation and is satisfactory provided a wide range of inactivation is covered. It is of course essential that the shape of survival curves should be checked at some stage in an investigation, since the use of D_{10} values is based on the assumption that inactivation is exponential. Ideally, methods of both types should be used and the results compared.

(d) Recovery media and conditions

Unless one can incubate in the food itself, it is necessary to transfer samples to artificial media in order to find the cells able to multiply after irradiation, whether one is using end-point or survival-curve methods. The proportion of radiation-damaged cells able to recover may be influenced by composition of the medium (STAPLETON *et al.* [12], ALPER and GILLIES [13]), presence of inhibitors (GILLIES and ALPER [14]) and atmosphere and temperature of incubation (STAPLETON *et al.* [15], PRATT *et al.* [16], ALPER and GILLIES [17], HODGKINS and ALPER [18]). These effects have all been observed with strains of *E. coli* and in one case with *Salmonella typhimurium* also. This related to greater survival of ultraviolet-irradiated cells if they were incubated anaerobically rather than aerobically (HODGKINS and ALPER [18]). Attempts to find "medium effects" with bacteria other than *E. coli* have been mainly negative (FREEMAN and BRIDGES [19], DAVYDOFF [20]); but as salmonellae are closely related to *Escherichia*, the possibility of similar effects needs to be thoroughly investigated. ultra.

MOSSEL [21] used the media listed in Table II when testing for survival of *S. typhimurium* and *S. senftenberg* strains irradiated in liquid egg and found no differences attributable to the medium. LEY *et al.* [3] compared four media (Table-II) used for surface plating by a modified Miles and Misra technique and found that desoxycholate citrate agar gave significantly lower recovery than did the other three media tested. Counts on the other media did not differ significantly, but the most reproducible results were obtained with nutrient agar. surf.

NICKERSON *et al.* [26] irradiated salmonellae in reconstituted egg white and tested for survivors by adding Difco selenite broth and incubating at 37°C for 48 - 120 h, then making various tests to confirm the presence of salmonellae. They found that increased survival was obtained if they incubated the irradiated egg white at 37°C for 48 - 120 h before adding the selenite broth. Difco

There has been evidence from work on *E. coli* strain B that a period of incubation with chloramphenicol shortly after irradiation would promote recovery (GILLIES and ALPER [14]). Mossel tried the chloramphenicol treatment in his experiments with salmonellae irradiated in egg but did not find any increase in counts (MOSSEL [21]).

TABLE II
RECOVERY MEDIA USED IN RADIATION EXPERIMENTS WITH SALMONELLAE,
AND THEIR EFFECT ON SURVIVAL

Author	Type of medium	Medium	Conclusion
MOSSEL [21]	Liquid enrichment media	(1) Brain-heart infusion broth (2) Muller's tetrathionate broth ^a (3) Osborne and Stokes' selenite bile brilliant-green sulphapyridin medium ^b	No significant effect of medium
	Solid plating media	(1) Crystal violet neutral-red bile lactose mannitol agar ^c (2) Nutrient agar (3) TDYM agar ^d	No significant effect of medium
LEY <u>et al.</u> [3]	Solid plating media	(1) Nutrient agar (2) Desoxycholate citrate agar (3) MacConkey agar (4) Blood agar	Lowest recovery on (2) The other 3 media did not differ significantly, but counts were most reproducible on (1).

^a MULLER [22]

^b OSBORNE and STOKES [23]

^c MOSSEL [24]

^d MOSSEL and KRUGERS DAGNEAUX [25]

(e) Tests on the irradiated product

It has been the usual practice in testing water supplies, and to some extent in testing foods also, to examine them for the presence of "Coliform" bacteria, the Escherichia-Aerobacter group, and, if these were present in large numbers, to assume that faecal pollution had taken place and that salmonellae might be present also. It was pointed out by ERDMAN *et al.* [27] that this method was not applicable to irradiated food, since the strains of salmonellae which they tested were substantially more resistant to radiation than were strains of Escherichia and Aerobacter. Direct tests for salmonellae would therefore be necessary on irradiated foods.

(2) Variation of sensitivity among serotypes of Salmonella

Information on only five serotypes was available at the time of the Panel meeting in December 1962, and it is shown in Table III, IV and V. In general, S. typhimurium was the most resistant strain studied, while S. paratyphi B was similar or slightly less resistant (Tables III and V) and S. meleagridis, tested only in frozen horse meat, did not differ significantly from S. paratyphi B (Table V). Both S. senftenberg and S. gallinarum were usually more sensitive than S. typhimurium, and, when tested in buffer (Table III), were similar to each other. buffer

Another factor illustrated by these tables is that the relative resistance of different strains varied with the medium in which they were irradiated. For instance, S. senftenberg appeared more sensitive than S. typhimurium in liquid whole egg and liquid and frozen egg white, while there was no significant difference between the serotypes in dried whole egg, egg yolk or egg white (Table IV). The strain of S. senftenberg used throughout this work was the particularly heat-resistant 775W, while the strain of S. typhimurium

TABLE III

EFFECT OF GASEOUS ENVIRONMENT AND TEMPERATURE
ON THE SENSITIVITY OF STRAINS OF SALMONELLA
IN PHOSPHATE BUFFER TO IRRADIATION WITH GAMMA RAYS
(LEY *et al.* [3])

Serotype	D ₁₀ value (Krad)			D ₁₀ ratio	
	Room temperature		Frozen	$\frac{D_{10} \text{ anoxic}}{D_{10} \text{ aerated}}$	$\frac{D_{10} \text{ frozen}}{D_{10} \text{ aerated}}$
	Aerated	Anoxic			
<u>S. gallinarum</u>	13.2	36.3	21.1	2.7	1.6
<u>S. senftenberg</u>	13.0	38.9	29.9	2.9	2.3
<u>S. typhimurium</u>	20.8	61.9	31.9	2.9	1.5
<u>S. paratyphi B.</u>	19.0	65.9	49.4	3.4	2.6

TABLE IV

THE EFFECT OF EGG PRODUCTS ON THE SENSITIVITY
OF STRAINS OF SALMONELLA TO IRRADIATION WITH
HIGH-VOLTAGE CATHODE RAYS

D₁₀ (Krad)* when irradiated in

Product	Serotype	State of product		
		Liquid	Frozen	Dried
Whole egg	<u>S. typhimurium</u>	40 [28]	-	55.7, 49.8 [29]
	<u>S. senftenberg</u>	17	-	60.5, 45.0
Yolk	<u>S. typhimurium</u>	-	42.7, 53.4 [29]	75.9, 66.5 [29]
	<u>S. senftenberg</u>	-	37.9, 48.6	80.6, 103
White	<u>S. typhimurium</u>	33.8, 40.3 [26]	35.6, 24.9 [26]	80.6, 86.5 [26]
	<u>S. senftenberg</u>	24.3, 30.8	16.0, 19.0	
White, sugared	Both	-	-	110, 130

* For liquid whole egg the end-point method was used. For other products two values are quoted, the first obtained by Schmidt's method and the second by the survival-curve method.

Values quoted in rep have been converted to rad: $\text{rep}_{25} \times 93/100 = \text{rad}$, $\text{rep}_{83} \times 83/100 = \text{rad}$.

used by PROCTER et al. [28] differed from that used by BROGLE et al. [29] and NICKERSON et al. [26]. Ley et al. found a similar situation, for S. gallinarum and S. senftenberg did not differ significantly when irradiated in buffer (Table III), whereas in whole egg the difference was significant (Table V). This shows that even the relative resistance of different strains cannot be assumed to be the same in different substrates and must be determined for any product to be irradiated.

Since the meeting in December some additional information has been supplied by COMER and is shown in Table VI [30]. Eighteen serotypes of Salmonella were irradiated in frozen whole egg *melange*, and survival curves, starting with an initial population of approximately 10^9 cells/ml, were obtained. The D₁₀ values calculated for each strain ranged from 51 to 77 Krad and S. typhimurium was not one of the most resistant strains, since its D₁₀ value was 60 Krad. This agrees well with the value of 68 Krad obtained by Ley et al. for the same organism in frozen whole egg; the only other serotype common to both workers is S. senftenberg, which Comer found to be the most sensitive, with a D₁₀ of 51 Krad, while Ley et al. quoted a D₁₀ of 47 Krad. At the more resistant end of Comer's range are a number of serotypes not examined by Ley et al., including S. give, manhattan, thompson, heidelberg and london, with D₁₀ values falling close together, the highest being 77 Krad for S. give.

TABLE V

THE EFFECT OF VARIOUS FOOD PRODUCTS ON THE SENSITIVITY OF STRAINS OF SALMONELLA TO IRRADIATION WITH GAMMA RAYS (LEY et al. [3])

D ₁₀ value (Krad) when irradiated in					
Serotype	Whole egg (liquid)	Whole egg (frozen)	Horse meat (frozen)	Bone meal	Desiccated coconut
<u>S. gallinarum</u>	43.0	56.9			
<u>S. senftenberg</u>	50.4	46.8		55.7	134
<u>S. typhimurium</u>	63.2	67.9	128	91.0	158
<u>S. paratyphi B</u>			107		
<u>S. meleagridis</u>			93.1		

Comer also showed that three cultures of S. pullorum, which gave identical reactions on routine examination, differed appreciably in their D_{10} values, which were 57, 66 and 70 Krad.

To sum up the results on variation between serotypes, this has now been studied quite thoroughly for irradiation in frozen egg, and the most sensitive and most resistant strains differed by a factor of only 1.5. This difference is considerably smaller than those which can be produced by variation in the environment during irradiation (see the next section). However, there is little information for products other than frozen egg, and more would be needed before processing conditions for any such products could be determined.

(3) Effect of environment during irradiation on sensitivity

Vegetative bacteria are strongly affected by conditions during irradiation; some of these factors also influence the radiosensitivity of spores, but to a much smaller extent. This brief account will deal only with main effects on vegetative cells, and fuller information can be obtained from the review by BRIDGES and HORNE [31].

(a) *The oxygen effect*

It is one of the most widespread observations in radiobiology that more damage takes place following irradiation in the presence of oxygen than in its absence. In vegetative bacteria the D_{10} values may be diminished by a factor of 2.5 - 4.7, with a factor of about 3 commonly occurring. Completely anoxic conditions are necessary to attain the higher values, for even small amounts of oxygen will markedly reduce the D_{10} value. For instance, with Shigella flexneri the concentration corresponding to a D_{10} value halfway between the anoxic and fully aerobic values was $4.0 \mu\text{M}/\text{l}$, produced by equilibration with a gas mixture containing only 0.3% oxygen (HOWARD-FLANDERS and ALPER [32]).

With salmonellae irradiated in buffer, an oxygen effect of the usual magnitude was observed by Ley *et al.* (Table III). NICKERSON *et al.* [26] and BROGLE *et al.* [29], irradiating thin layers of various egg products with cathode rays, found no overall difference in inactivation of salmonellae whether air or nitrogen was present. In their detailed results the expected differences were shown in a few instances (e.g. Brogle *et al.*, Table I, results for S. senftenberg in whole egg solids, from 10^7 -fold reduction test), and it seems possible that the system they used did not always give either fully anoxic or sufficiently aerobic conditions. If the oxygen effect was indeed lacking, this might result from protective substances in the egg.

Foods and feeding-stuffs may be in either state: for instance, the interior of frozen meat blocks would be fully anoxic, while meal in sacks would have a supply of oxygen. It is obvious that determinations of sensitivity for processing requirements should be made under the appropriate conditions for the product concerned.

(b) Freezing

Temperature changes either above or below the freezing point have little effect on the radiosensitivity of vegetative bacteria, unless temperatures lethal to the bacteria are approached. The difference between the liquid and solid state is marked, however, with much less inactivation taking place in the frozen state. Under aerobic conditions the ratios of D_{10} values for irradiation at -75°C and at room temperature ($10-15^{\circ}\text{C}$) were from 3.1 - 6.7 for strains of *Pseudomonas* and *Alcaligenes*, while with anaerobic conditions during irradiation the difference was smaller, with ratios from 1.3 to 1.9 (MATSUYAMA *et al.* [6]). The radiation sensitivity of a *Pseudomonas* suspension under the different conditions is shown in Fig. 3.

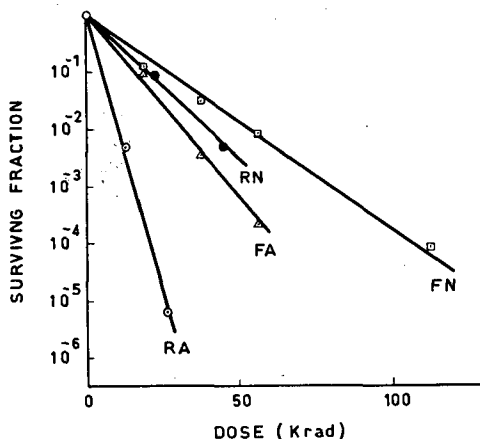


Fig. 3

Sensitivity of a *Pseudomonas* strain (MJT/FS/261) in heart infusion broth to irradiation under different environmental conditions.

- RA Temperature $10-13^{\circ}\text{C}$, air-saturated
- RN Temperature $10-13^{\circ}\text{C}$, N_2 -saturated
- ▲ FA Temperature -75°C , air-saturated
- FN Temperature -75°C , N_2 -saturated

Data from MATSUYAMA *et al.* [6]

In a buffer suspension of *E. coli*, the transition was shown to take place over a temperature range of a few degrees, just below 0°C (STAPLETON and EDINGTON [33]), while with naturally occurring bacteria in minced meat the difference was spread over a wider range, from 0°C to -20°C , possibly coinciding with the freezing of free water in the meat (COLEBY *et al.* [34]).

Salmonellae were irradiated frozen and at room temperature in air-saturated buffer by Ley *et al.* (Table III), and the ratio of D_{10} values varied from 1.5 to 2.6. In liquid and frozen whole egg (Table V) there was little difference in sensitivity, a result in agreement with that of Nickerson *et al.*

for egg white (Table IV), where, if anything, the salmonellae seemed slightly more sensitive in the frozen state.

(c) *Drying*

There is some evidence that vegetative bacteria are more resistant to radiation when dried (MOOS [35], LAWTON and BELLAMY [36]), as would be expected by comparison with the effects of freezing, since both processes remove free water from the system. Investigation is difficult, however, with vegetative cells, since a large proportion of them is killed by drying alone.

Results from the Massachusetts Institute of Technology (Table IV) illustrate effects on salmonellae in egg products. *S. typhimurium* was slightly more resistant in dried whole egg than in the liquid product, while *S. senftenberg* was very much more resistant when dried. Results for egg white showed less difference between the two serotypes, the D_{10} values for both being greater by a factor just over two in the dried product.

Information on inactivation at different levels of moisture content is lacking and would be particularly necessary for irradiation processing of dried meals.

(d) *Food constituents*

Some chemical compounds exert a "protective" effect on bacteria under irradiation, or, in other words, radiation damage is less if the compound is present during irradiation. Particularly effective compounds are cysteine and cysteamine, and many others have some protective action.

Such effects have been known for some time when bacteria were irradiated in food (see FULD *et al.* [37]) but are still little understood, since the medium is here so complex. Results for salmonellae in food will be discussed in the next section, and any protective effects observed will be mentioned.

(4) The inactivation of salmonellae in various products

Experiments in which salmonellae have been irradiated in food are shown in Tables IV, V and VI and have already been mentioned in connection with environmental effects. The inactivation doses calculated by various authors are summarized in Table VII.

(a) *Egg products*

These have received more attention than any other product because of their importance as carriers of salmonellae and because of the suitability of radiation for treating egg in the frozen state (INGRAM *et al.* [40]).

It is difficult to tell from the literature whether egg constituents exerted any protective action on salmonellae. Ley *et al.* grew the organisms for 3 d at 37°C in the liquid egg to reach a population of 10^8 /ml and used this culture for irradiation. It seems likely that all oxygen was consumed in this process and that the bacteria were irradiated under anoxic conditions.

TABLE VI

**RELATIVE SENSITIVITY OF 18 SEROTYPES OF SALMONELLA
IRRADIATED IN FROZEN WHOLE EGG
(COMER [30])**

Serotype	D ₁₀ (Krad)	Dose for 10 ⁷ -fold reduction (Krad)
<u>S. give</u>	77 ± 3	540 ± 20*
<u>S. manhattan</u>	76 ± 3	530 ± 20*
<u>S. thompson</u>	73	510*
<u>S. heidelberg</u>	73	510
<u>S. london</u>	73	510
<u>S. blockley</u>	69	480
<u>S. tennessee</u>	67	470
<u>S. indiana</u>	67	470
<u>S. kentucky</u>	60	420
<u>S. canada</u>	60	420
<u>S. typhimurium</u>	60	420
<u>S. pullorum</u>	59	410
<u>S. bareilly</u>	59	410
<u>S. montevideo</u>	57	400
<u>S. oranienburg</u>	56	390
<u>S. infantis</u>	56	390
<u>S. enteritidis</u>	53	370 **
<u>S. senftenberg</u>	51	360 **

* Mean of 6 replicates

** Mean of 2 replicates

When their results for egg (Table V) are compared with those for anoxic irradiation in buffer (Table III), the sensitivity of S. typhimurium was the same, while S. gallinarum and S. senftenberg were only slightly more resistant in egg. It seems possible, therefore, that the egg constituents only exerted a small protective effect, if any.

The D₁₀ values for salmonellae in liquid egg ranged from the unusually low value of 17 Krad for S. senftenberg (PROCTOR et al. [28]) to 63 Krad for S. typhimurium (LEY et al. [3]) (Tables IV and V). Inactivation doses have been based on the values for S. typhimurium, the most resistant strain tested in liquid whole egg, and values suggested by the two authors were 280 and

TABLE VII

IRRADIATION DOSES SUGGESTED FOR THE INACTIVATION OF SALMONELLAE IN VARIOUS PRODUCTS

Product	Author	Most resistant strain tested	Inactivation factor	Dose (Krad)
Whole egg (liquid)	PROCTOR <u>et al.</u> [28]	<u>S. typhimurium</u>	10^7	280
Whole egg (liquid)	MOSSEL [21]	<u>S. typhimurium</u>	10^6	200
Whole egg (liquid)	LEY <u>et al.</u> [3]	<u>S. typhimurium</u>	10^7	442
Whole egg (frozen)	BROOKS <u>et al.</u> [38]	Natural contamination*	About 10^3	300 - 500
Whole egg (frozen)	LEY <u>et al.</u> [3]	<u>S. typhimurium</u>	10^7	476
Whole egg (frozen)	COMER [30]	<u>S. give</u>	10^7	540
Whole egg (dried)	BROGLE <u>et al.</u> [29]	<u>S. typhimurium</u> & <u>senftenberg</u>	10^7	370
Egg yolk (frozen)	BROGLE <u>et al.</u> [29]	<u>S. typhimurium</u> & <u>senftenberg</u>	10^7	320
Egg yolk (dried)	BROGLE <u>et al.</u> [29]	<u>S. typhimurium</u> & <u>senftenberg</u>	10^7	570
Egg white (liquid)	NICKERSON <u>et al.</u> [26]	<u>S. typhimurium</u>	10^7	260
Egg white (frozen)	NICKERSON <u>et al.</u> [26]	<u>S. typhimurium</u>	10^7	212

TABLE VII (cont'd)

Product	Author	Most resistant strain tested	Inactivation factor	Dose (Krad)
Egg white (dried)	NICKERSON <u>et al.</u> [26]	<u>S. typhimurium & senftenberg</u>	10 ⁷	585
Sugared egg white (dried)	NICKERSON <u>et al.</u> [26]	<u>S. typhimurium & senftenberg</u>	10 ⁷	840
Horse meat (frozen)	LEY [39]	<u>S. typhimurium</u>	10 ⁵	640
Bone meal	LEY <u>et al.</u> [3]	<u>S. typhimurium</u>	10 ⁷	640
Desiccated coconut	LEY <u>et al.</u> [3]	<u>S. typhimurium</u>	10 ⁷	1100

* In one experiment the salmonellae present were S. paratyphi B, S. thompson and S. pullorum, while in another they were S. paratyphi B, S. newport and S. thompson.

442 Krad, both corresponding to an inactivation factor of 10^7 , while MOSSEL [21] quoted a dose of 200 Krad for inactivation by a factor of 10^6 .

Freezing made only a small difference to the radiation sensitivity of salmonellae in egg, as already mentioned. The highest D_{10} value reported for frozen egg was that of COMER [30] for S. give, which resulted in a dose of 540 Krad for inactivation by a factor of 10^7 . Somewhat lower values, relating to the resistance of S. typhimurium, were quoted by Ley *et al.* for frozen whole egg (476 Krad), NICKERSON *et al.* [26] for frozen egg white (212 Krad) and BROGLE *et al.* [29] for frozen egg yolk (320 Krad).

BROOKS *et al.* [38] irradiated frozen whole egg naturally contaminated with S. paratyphi B, S. thompson and S. pullorum and found survival after 300 Krad of cathode rays, though not after 500 Krad. Using gamma rays with a different batch of contaminated egg, they found no salmonellae after 300 Krad. The former observation means that 300 Krad inactivated the initial population by a factor of less than 10^3 , implying that the D_{10} was greater than 100 Krad. As the initial population had to be estimated by "most-probable-number" methods, which have a large range of error, too much emphasis should not be placed on this observation. However, it shows the difficulties of dealing with naturally contaminated material and the necessity for doing so to check doses calculated from inoculation experiments.

As already noted, dried egg products conferred a high degree of resistance on salmonellae irradiated in them (Tables IV and VII), and this was especially marked for egg white and sugared egg white, for which the doses for inactivation by a factor of 10^7 were 585 and 840 Krad respectively (NICKERSON *et al.* [26]).

(b) Other foods and feeds

Data on these products are scanty, and much more are needed.

The application of radiation to eliminate salmonellae in frozen horse meat intended for pet food was discussed by LEY [39], and D_{10} values were quoted for three serotypes (Table VI). The most resistant was S. typhimurium with a D_{10} of 128 Krad. Ley considered that inactivation by a factor of 10^5 would be sufficient for this product, and the dose necessary was therefore 640 Krad (Table VI). The markedly higher resistance of S. typhimurium in this product than in frozen egg is presumably attributable to some protective influence of the meat constituents.

Two dried products have also been irradiated by Ley *et al.*, using S. typhimurium and S. senftenberg: bone meal and desiccated coconut. From Table V it can be seen that the resistance of S. typhimurium in bone meal was high, as it had a D_{10} of 91 Krad, while both serotypes were extremely resistant in desiccated coconut, D_{10} values being 134 Krad for senftenberg and 158 Krad for typhimurium. These last two values are higher than have been found so far for any serotype in any other product in spite of the fact that S. senftenberg strains were among the more radiation-sensitive salmonellae in other products.

The moisture content of the bone meal and desiccated coconut was not measured, and differences in this respect may well have contributed to the differences in resistance of the salmonellae in the two products. However, the increased resistance in desiccated coconut was extremely large, and

it seems more likely that this resulted from differences in composition, particularly the high fat content of the coconut. The increased heat resistance of bacteria in the presence of fat is well known and is believed to be based on a localized reduction in moisture (SCHMIDT [9], p. 750), but whether a similar effect is found with radiation has not been investigated. Its practical importance for radiation processing is obvious.

(5) The possibility of development of mutants resistant to irradiation

This possibility must be considered, because the use of antibiotics in hospitals has shown how resistant strains may be developed on a large scale over a period of a few years. The problems involved with radiation are not quite so difficult, however, because many of the antibiotics are bacteriostatic in their action, while radiation is bactericidal.

Mutants more resistant than the parent strain have been detected among the relatively small number of survivors from large doses of radiation. This has been observed several times in E. coli (WITKIN [41, 42], LUCKIESH and KNOWLES [43], ALPER and GILLIES [17]) and in an Alcaligenes strain (THORNLEY [44]). ERDMAN et al. [45] studied some organisms of particular interest in foods and used a process of repeated irradiation, the survivors from one radiation treatment being grown in broth before the next irradiation. By this means they developed more resistant strains of E. coli, Strep. faecalis, Staph. aureus and Cl. botulinum Type A, the largest increase in resistance being by a factor of 1.9 in E. coli. A strain of Salmonella gallinarum showed no change in resistance after 14 consecutive irradiations.

In spite of this negative result with S. gallinarum, it seems that the ability to develop radiation resistant mutants is general among bacteria, and they would be expected to occur among salmonellae under suitable conditions.

This would only lead to trouble in food processing if the few mutant survivors from radiation had a chance to multiply so as to be capable of recontaminating the unirradiated product significantly. The main aim of the process, to eliminate or very greatly reduce the salmonellae, makes it essential to prevent multiplication of survivors after irradiation, either by means of the non-perishable nature of the product, or, for perishable products, by chilling or other means. Control of cross-contamination in the factory is also essential; otherwise salmonellae from the untreated product might contaminate the irradiated product. Regarding resistant mutant strains, trouble might arise from cross-contamination in the opposite direction, from the irradiated product to the unirradiated, but, as already mentioned, only if the bacteria had a chance to multiply after irradiation. One could imagine that this might happen if food were allowed to accumulate on conveyor belts leading in and out of the radiation source, but such obvious deficiencies could easily be prevented.

In fact, radiation has an advantage over some other processes as regards control of cross-infection, because it can be applied to the product while it is sealed in packages.

It is clear that careful attention to factory design and hygiene in operation are essential to any process for elimination of salmonellae, and these

precautions would also serve to prevent the increase and spread of radiation resistant mutants.

C. COMPARISON OF IRRADIATION WITH HEAT AND GASEOUS STERILIZATION

(1) Relative effect on different micro-organisms

Although information is available on the relative sensitivity of salmonellae and other micro-organisms to various agents (Table VIII), the kinds and

TABLE VIII

MICRO-ORGANISMS MORE RESISTANT THAN SALMONELLAE TO VARIOUS LETHAL AGENTS

Type of process		
Radiation	Heat	Ethylene oxide
<u>Micrococcus radiodurans</u>	<u>Clostridium</u> spores	<u>Bacillus</u> spores
<u>Clostridium</u> spores	<u>Bacillus</u> spores	<u>Clostridium</u> spores
<u>Bacillus</u> spores	<u>Microbacterium</u>	<u>Staphylococcus</u>
<u>Strep. faecium</u>	<u>Strep. faecium</u> and <u>faecalis</u>	
<u>Micrococcus</u>	<u>Micrococcus</u>	
<u>Lactobacillus</u>	<u>Staphylococcus</u>	
<u>Achromobacter/Alcaligenes</u> group	<u>Alcaligenes</u>	
Yeasts	<u>E. coli</u>	
<u>Staphylococcus</u>		

numbers surviving in any situation will also depend on the initial contamination; an organism with resistance similar to that of salmonellae would survive treatment designed to eliminate the salmonellae if it were present initially in sufficiently large numbers. Conversely, some useful reduction might be effected in an organism more resistant than salmonellae, provided that it was present initially in small numbers.

(a) Radiation

The radiation sensitivity of some strains of food spoilage and food poisoning organisms is shown in Fig. 1. These results relate to aerobic conditions, except those for the two clostridia and possibly those for S. typhimurium in liquid egg. It can be seen that a dose of 450 Krad would inactivate the initial population of S. typhimurium in egg to a level of 10^{-7}

but would cause very little reduction in numbers of Micrococcus radiodurans or Cl. botulinum spores, while spores of Cl. welchii and B. megaterium would be reduced by a factor of 10^2 , and the resistant strain of Strep. faecium shown here, by a factor of about 10^3 . These are all unusually resistant organisms, and M. radiodurans is the only vegetative bacterium known with a higher resistance than clostridial spores have. Other clostridia have also shown a high resistance (e.g. Cl. sporogenes, NIVEN [46]), whereas Bacillus spores may be similar to B. megaterium (e.g. B. stearothermophilus, NIVEN [46], B. anthracis, LEY [47]) or more sensitive, like B. brevis in Fig. 1.

Besides these unusually resistant bacteria, many others have shown a greater radiation resistance than have salmonellae, particularly strains of Micrococcus, Streptococcus, Lactobacillus, the Achromobacter-Alcaligenes group and various yeasts (SCHMIDT-LORENZ and FARKAS [4], NIVEN [46], THORNLEY [48], BRIDGES *et al.* [49]).

Staph. aureus strains were more resistant than were two serotypes of Salmonellae when irradiated in broth and were inactivated at nearly the same rate in buffer solution (ERDMAN *et al.* [27]), while with crude staphylococcal toxin, emetic activity for cats was reduced by 110 000 rep and absent after 2.2×10^6 rep.

It is clear that a wide range of organisms might be expected to survive irradiation treatment designed to eliminate salmonellae, and the observations of BROGLE *et al.* [29] are interesting in this connection. They found that the doses necessary to eliminate all micro-organisms originally present at about 10^5 /g in various egg products were two to five times higher than the doses necessary to eliminate salmonellae, previously inoculated to a level of about 10^6 /g.

(b) Heat

Heat processes in use for the destruction of salmonellae have been listed by Hobbs, and of these the pasteurization of liquid egg has been most thoroughly studied. Most strains of salmonellae in egg were inactivated by heat at about the same rate, but a few strains were very much more resistant: for instance, S. senftenberg 775W differed from the most sensitive strains by a factor of 10-20 times (OSBORNE *et al.* [50], WINTER *et al.* [51], ANELLIS *et al.* [52]). This differs from the situation observed with radiation, where resistance only varied by a factor of 1.5 between the Salmonella strains so far tested (except for the observations of PROCTOR *et al.* [28], where S. senftenberg appeared unusually sensitive), and such factors may be important in processing. For instance, the pasteurization of liquid egg must be controlled within a very narrow range of temperature and time to allow destruction of heat-resistant strains of S. senftenberg without damage to the product (MURDOCK *et al.* [53], SHRIMPTON *et al.* [54]).

Bacteria likely to survive heat pasteurization are listed in Table VIII. With the exception of the clostridia and staphylococci, these were all isolated from pasteurized milk and shown to survive heating in milk at 63°C for 30 min (THOMAS *et al.* [55], ABD-el-MALEK and GIBSON [56], CROSSLEY [57]). Some food-poisoning strains of Staphylococcus are also of a comparable heat resistance (GROSS and VINTON [58]). Besides their occurrence in

pasteurized milk, the spore formers are important in all kinds of heat-processed foods, streptococci in heat-pasteurized canned hams (INGRAM and HOBBS [59], INGRAM and BARNES [60]), and both streptococci and micrococci have shown an unusual degree of heat resistance in the presence of fat (JENSEN [61], p. 273-274, INGRAM [62]).

(c) Gaseous sterilization

This subject has been reviewed recently by BRUCH [63] and earlier by many others (PHILLIPS and KAYE [64], PHILLIPS [65], PAPPAS and HALL [66], RAUSCHER *et al.* [67], PHILLIPS and WARSHOWSKY [68]); some applications in the food industry have been described by Hobbs.

All the active compounds are alkylating agents, and ethylene oxide has been much more widely used than any other.

Bacterial spores were more resistant than were vegetative bacteria, yeasts and moulds (TOTH [69]), and among the spore-formers, strains of B. subtilis and Cl. sporogenes were particularly resistant (FRIEDL *et al.* [70], BRUCH [63]). Vegetative cells of B. globigii, Staph. aureus, Mycobacterium phlei and Gaffkya tetragena were all more resistant, by a factor of two to three times, than were Eberthella typhi (= Salmonella typhosa), Klebsiella pneumoniae and E. coli (PHILLIPS [71]). Other reports also show that staphylococci may be more resistant than are some other vegetative bacteria (e.g. RAUSCHER *et al.* [67]).

The application of ethylene oxide to remove salmonellae in egg powder has been described, but ADAM [72] mentioned possible nutritional and toxicological hazards. MAYR and KAEMMERER [73] described mixtures of ethylene oxide with either methyl bromide or ethyl formate as being particularly effective for inactivating salmonellae in egg powder.

(d) Summary

Although the mechanisms of inactivation by heat and radiation are thought to be quite different, bacteria belonging to the same groups are resistant to both; in addition to the spore-formers, this applies to micrococci, faecal streptococci, some Alcaligenes strains and staphylococci, although the last named appear to be only slightly more resistant to radiation than salmonellae are. The spore-formers and staphylococci are also resistant to ethylene oxide, which is thought to have some resemblance to radiation in its mode of action (ALEXANDER and STACEY [74]). This broad correlation between resistant genera does not, however, extend to individual species and strains, since the extremely radiation-resistant Micrococcus radiodurans is not particularly heat-resistant (ANDERSON *et al.* [8]), and, as already mentioned, the most heat-resistant strain of S. senftenberg is not especially radiation-resistant.

(2) Other considerations

As shown above, the relative resistance of different micro-organisms to the three processes is broadly similar, in that bacterial spores are much more resistant than are salmonellae and some other vegetative bacteria are

TABLE IX

FACTORS AFFECTING MICROBIAL INACTIVATION BY DIFFERENT PROCESSES

Factor	Radiation	Heat	Ethylene oxide*
Anoxia	Reduces inactivation	-	-
Drying	Reduces inactivation	Reduces inactivation	Only applicable to dried finely-divided products Good inactivation at relative humidities of 25-50%, less in very dry products or near saturation [75]
Freezing	Reduces inactivation	Not applicable	Not applicable
Temperature (above freezing point)	Little influence		Q_{10} is 2.7 between 5°C and 37°C. In practice, good inactivation between 25°C and 35°C, little below 12°C [71, 67]
pH	Little influence	Most inactivation in acid products, pH < 4.5 Salmonellae in egg more resistant at pH 5.5 than at 8.0 [52]	Not known
Food constituents	Some reduce inactivation.	Some, especially fats, reduce inactivation	Reduce inactivation if bacteria are occluded in crystals

* Other gaseous sterilants differ in moisture requirements etc. (see BRUCH [63]).
Other references are cited in the text.

also more resistant. Only in exceptional cases will this factor be important in determining the type of treatment used.

Factors such as convenience of application, possible damage to nutritional properties or production of toxic substances, and economic considerations are much more likely to be decisive. Most of these are outside the scope of this paper, but factors affecting microbial inactivation are summarized in Table IX. Most of these points have already been mentioned, but it is worth stressing that radiation is the only process which can be applied to products while they are frozen.

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TECHNOLOGICAL ASPECTS OF FOOD IRRADIATION WITH PARTICULAR REFERENCE TO SALMONELLAE ELIMINATION

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Abstract — Résumé — Аннотация — Resumen

TECHNOLOGICAL ASPECTS OF FOOD IRRADIATION WITH PARTICULAR REFERENCE TO SALMONELLAE ELIMINATION. The properties and sources of gamma rays and electrons for the treatment of food are reviewed.

The dose requirement for the adequate elimination of salmonellae varies with the particular food product in question but is on the order of 0.5 Mrad. At this dose level most foods are not appreciably changed in quality. Significant losses of vitamins do not occur, and the protein value of the product is not changed.

Radiation processing seems technologically feasible for the purpose of salmonellae elimination in food and feed products. High capital costs are involved in the establishment of a radiation plant. For instance, the total investment involved in a Co⁶⁰-plant designed to process 13 000 tons of frozen horse meat per annum at a dose of 0.65 Mrad would be approximately £250 000, most of it being the purchase of the isotope. Allowing for the replacement of the cobalt and based on a ten-year amortization for the plant itself, the cost for the treatment would be approximately 0.56d per pound.

In view of the lack of technological development in many countries producing foods and feeds, it seems likely that the first irradiation plant will be installed at an import centre.

ASPECTS TECHNOLOGIQUES DE L'IRRADIATION DES ALIMENTS, NOTAMMENT EN VUE DE L'ÉLIMINATION DES SALMONELLAE. L'auteur examine les propriétés des rayons gamma et des électrons au point de vue du traitement des aliments et les sources que l'on peut utiliser à cet effet.

La dose requise pour éliminer convenablement les *Salmonellae* varie selon le produit alimentaire considéré, tout en étant cependant de l'ordre de 0,5 Mrad, niveau auquel la qualité de la plupart des aliments n'est pas altérée de façon notable. Une telle dose n'entraîne pas d'appauvrissement important en vitamines et ne modifie pas la teneur du produit en protéines.

Pour l'élimination des *Salmonellae* se trouvant dans la nourriture de l'homme et dans celle des animaux, le traitement par irradiation semble être possible du point de vue technique. La création d'une installation d'irradiation exige des investissements importants. Par exemple une installation au cobalt-60, qui traiterait 13 000 t de viande de cheval congelée par an, à raison d'une dose de 0,65 Mrad, coûterait environ 250 000 £, dont la plus grande partie représenterait le prix de l'isotope. Compte tenu du renouvellement du cobalt et d'un amortissement de l'installation en dix ans, le traitement reviendrait approximativement à 0,56d par livre.

Comme beaucoup de pays producteurs de denrées alimentaires n'ont pas encore atteint un stade de développement technologique suffisant, on a des raisons de penser que la première installation d'irradiation sera établie dans un centre d'importation.

ТЕХНИЧЕСКИЕ АСПЕКТЫ ОБЛУЧЕНИЯ ПИЩЕВЫХ ПРОДУКТОВ С ОСОБЫМ УПОРОМ НА УНИЧТОЖЕНИЕ САЛМОНЕЛЛЫ. Рассматриваются свойства и источники гамма-лучей и электронов, используемых для обработки пищевых продуктов.

Доза, необходимая для уничтожения сальмонеллы, меняется в зависимости от конкретного пищевого продукта, но должна быть порядка 0,5 мрад. При этой дозе облучения не происходит заметного изменения качества большинства пищевых продуктов. Не происходит заметного уменьшения содержания витаминов, и белковая ценность продукта также не меняется.

Применение радиационной обработки для уничтожения сальмонеллы в пищевых продуктах и кормах представляется технически осуществимым. Создание установки для облучения связано с большими капитальными затратами. Например, общая сумма капиталовложений в установку с Co⁶⁰, рассчитан-

ную на обработку 13 000 т замороженной конины в год дозой 0,65 мград, составит приблизительно 250 000 ф.ст.; большая часть этой суммы пойдет на приобретение изотопа. Если учесть необходимость замены кобальта и десятилетний срок амортизации самой установки, то стоимость обработки одного фунта мяса составит приблизительно 0,56 пенса.

В связи с тем, что многие страны, производящие продукты питания и корма, являются технически неразвитыми, представляется целесообразным установить первую установку для облучения в импортном центре.

ASPECTOS TECNOLÓGICOS DE LA IRRADIACIÓN DE ALIMENTOS - ELIMINACIÓN DE LAS SALMONELLAE. La memoria revisa las propiedades y las fuentes de rayos gamma y de electrones para el tratamiento de alimentos.

La dosis requerida para eliminar adecuadamente las Salmonellae varía con el producto alimenticio de que se trate, pero es del orden de los 0,5 Mrad. Con esta intensidad de dosis casi ningún producto alimenticio sufre alteraciones de calidad apreciables. No se producen pérdidas importantes de vitaminas y el valor proteínico del producto permanece inalterado.

Desde el punto de vista tecnológico, parece posible emplear radiaciones para eliminar las Salmonellae en los alimentos y forrajes. La planta de irradiación requiere una inversión de capital muy elevada. Por ejemplo, la inversión total en una instalación de ^{60}Co diseñada para tratar 13 000 t de carne de caballo congelada por año con una dosis de 0,65 Mrad, sería del orden de 250 000 libras esterlinas, de las cuales la mayor parte se emplearía para la adquisición del isótopo. Considerando la renovación del cobalto y que el hecho de que la planta se deba amortizar en 10 años, el costo del tratamiento sería aproximadamente de 1 penique por kilogramo.

En vista del escaso desarrollo técnico en muchos de los países que producen alimentos y forrajes, es de suponer que la primera planta de irradiación se instalará en un centro de importación.

The ionizing radiations most appropriate for the treatment of food are gamma rays, emitted by naturally occurring and artificial radioactive elements, and electrons generated in the form of a beam by electrical machines. Both these types of radiation are lethal to micro-organisms, parasites and insect pests; they can be applied without causing an appreciable rise in temperature; and they are penetrating, so that products can be pre-packed before treatment. The choice of the types of radiation suitable for food processing is, of course, limited by the fact that no radioactivity must be induced by the treatment and the radiation must be available in large amounts and at economic cost to be of commercial value.

PROPERTIES AND PROPOSED SOURCES OF GAMMA RAYS AND ELECTRONS

(a) Gamma rays

Gamma rays are electromagnetic radiation. They can energize individual atoms and molecules in the medium through which they pass, their mode of energy transfer depending on the energy level of the gamma photons involved. The result, however, is that the energy of the electromagnetic radiation is converted largely into energy of internally generated electrons. These electrons dissipate their energy in the medium along well defined tracks, leaving molecules in their vicinity in various states of disturbance so that they become chemically active and take part in certain reactions, depending on the nature of the medium. Whilst the total amount of chemical change induced in food treated at the dose levels required for various pro-

posed processes is very small, in living cells vital changes occur which lead to the death of the cell.

The sources of gamma rays which have been seriously considered for the treatment of food are cobalt-60, caesium-137 and spent fuel rods. Cobalt-60 has been chosen as the most useful for radiation processing since it can be produced in large amounts by bombardment of cobalt-59 with neutrons in a nuclear reactor. It has a half-life of 5 yr (time taken to decay to half its original activity), and it emits radiation which is a mixture of two photon energies, 1.17 and 1.33 MeV. A low-energy beta radiation (electrons from the nuclei of radioactive elements) is also emitted, but this is largely self-absorbed in the sources and can be ignored. The gamma rays are very penetrating; it takes about 12 in (30 cm) of water to reduce the absorbed dose to 50% of that at the surface. In the construction of radiation plant the cobalt-60 source is housed in a concrete chamber with walls about 5 ft (1.5 m) thick so that the dose at the outer surface is less than the limit specified as acceptable for non-radiation workers. Regulations governing the safe operation of radiation sources already exist in most countries.

Caesium-137 is an isotope with a half-life of 33 yr. It emits gamma rays of 0.66 MeV, and it also has a small beta component. It occurs in reasonable amount in the fission products from nuclear reactors; and if it could be extracted and purified, it would make a useful source for radiation applications. However, a high capital investment in plant suitable for its large-scale production would be required, and this is not at present warranted, particularly in view of the availability of cobalt-60.

The spent fuel rods from nuclear reactors have been used experimentally as sources of gamma radiation for the treatment of food. They are used during the "cooling" period between removal from a reactor and processing for recovery of useful fuel material. The rods contain a variety of isotopes emitting gamma rays of a wide spectrum of energy levels. Many of the isotopes are of short half-life so that the initial high dose rate falls rapidly, and it would be necessary to introduce fresh rods at frequent intervals if production of material irradiated in this way were to be maintained. A radiation plant designed to use spent fuel rods would have to be constructed close to an existing nuclear reactor. This gamma-ray source is not likely to be used, therefore, in commercial radiation plant.

It should be mentioned in connection with the use of spent fuel rods that a small neutron flux exists, since some of the gamma rays emitted have photon energies greater than 2.28 MeV, the threshold energy for the gamma-neutron reaction on deuterium, which is present in all natural hydrogenous materials (e.g. water). The neutron flux is, however, quite small, and the amount of radioactivity that would be produced in food is less than that from natural radioactivity so that no health hazard exists in the use of a spent fuel rod source for experimental purposes [1].

(b) Electron beams

Electron beams (or cathode rays) are emitted from a cathode and are accelerated by electrostatic forces along an evacuated tube, reaching velocities approaching that of light. They emerge in the form of a narrow beam

through a very thin metal window. The electrons acquire kinetic energy which is usually specified in terms of the electron volt (eV). 1 eV is the energy gained by an electron in accelerating through a potential difference of 1 V and is equivalent to 1.602×10^{-12} ergs; the common practical unit of energy is a million electron volts (MeV).

Electrons entering a medium behave in the same manner as those internally generated by gamma rays, and therefore chemical reactions initiated by both types of radiation would be expected to be similar. Since electrons are charged particles, they do not penetrate into material to the same extent as gamma rays do, although this depends on the energy level employed and the density of the material. 1-MeV electrons give a reasonably uniform dose to only about 1/8-in (0.3-cm) thickness of material of unit density, but it is possible to double this figure by irradiation from both sides. Electron beams can be deflected or focussed by magnetic means and can be used to scan large areas of material. The dose rate achieved by machine sources is very high; rates of millions of rad per second compare with rates of several hundred thousand rad per hour from gamma-ray sources. In spite of the low penetration of electrons, heavy concrete shielding has to be used to house the machines, since scattered electrons produce penetrating X-rays (bremsstrahlung) when they strike objects, particularly dense metals, in the vicinity.

Various types of electron machine have been developed, considerable progress having been made in their design and construction within the last few years.

1. Van de Graaff accelerator

A moving belt carries electrostatic charge from a low-voltage source to an insulated high-voltage terminal. This voltage is used to accelerate electrons down a long evacuated tube, the beam emerging through a thin window of metal foil.

2. Linear accelerator

Electrons are injected into an evacuated wave guide energized by a high-power magnetron. The electrons ride on the travelling wave propagated by the guide and extract energy from it; 1 m of wave guide can accelerate electrons up to 4-MeV energy.

3. Resonant transformer

The voltage derived from a resonant transformer is used to accelerate electrons down a tube. Electrons flow only during one half-cycle of the anode voltage, resulting in a range of electron energies.

4. Capacitron

This consists of a number of capacitors which are charged in parallel from a DC generator and then connected in series to provide a high-voltage

pulse (about $1 \mu\text{s}$). The high voltage accelerates electrons in an accelerating tube.

5. Cockcroft-Walton generator

This generator supplies a continuous beam of electrons of uniform energy, the high voltage being produced by means of a network of rectifiers and condensers. A variant of this type of generator is known as the "Dynamitron", which is capable of several kW output at 1-4 MeV.

6. Insulated core transformer

A transformer which has a segmented magnetic core with insulation between successive segments is used to generate high voltages. The potential of the core segments can be graded to match that of the local secondary windings, thus reducing the stress on the insulation between winding and core. A continuous beam of electrons is provided, and power outputs of several kilowatts can be obtained.

RADIATION UNITS

The unit of dose of radiation is the rad, and this is defined as the quantity of ionizing radiation which results in an energy deposition of 100 erg/g of treated material. In the field of food irradiation it is useful to refer to multiples or fractions of 1 000 000 rad (1 Mrad).

Isotope source strength is measured in curies, 1 c being defined as the quantity of any radioactive material giving 3.7×10^{10} disintegrations per second. The radioactivity of 1 g of radium is approximately 1 c.

POTENTIAL FOOD APPLICATIONS

During the last ten years a considerable research effort has been devoted to the investigation of the use of ionizing radiation for the treatment of food. The potential application of radiation processing in this field is very wide indeed, as illustrated in Table I; but, as with any new process, certain scientific and technological problems must be solved before applications become commercial. The problems involved were reviewed in detail by HANNAN in 1955, in a publication which is still extremely useful [2], and accounts of recent progress have been made [3, 4].

With those applications involving the use of high doses ($> 1 \text{ Mrad}$), certain chemical changes, particularly in raw meats and fish, give rise to off-odours and flavours which diminish the quality of the product; ways and means of eliminating these changes are being sought. Another problem arises where radiation treatment is aimed at preserving raw foods at room temperature; enzyme activity is not arrested even by a sterilizing dose, and autolysis can proceed. Processes involving the use of lower doses have been more successful, particularly those where no microbiological hazards (referred to in the paper by Thornley) are involved. The inhibition of

TABLE I

MAIN FOOD APPLICATIONS UNDER INVESTIGATION
AND DOSE LEVELS REQUIRED

Application	Cause of spoilage	Dose (Mrad)
Sterilization of meats (room-temperature storage)	Bacteria (particular ref. <u>Cl. botulinum</u>)	4 - 5
Sterilization of special food ingredients, e. g. spices	Bacteria (vegetative and spore-forming)	1 - 3
Extended refrigeration life of meats, fish etc.	Bacteria (vegetative)	0.05 - 1
Fruit storage	Moulds	0.1 - 0.5
Elimination of food-poisoning organisms, e. g. from frozen egg, meat, coconut	Salmonellae	0.5 - 1.0
Disinfestation of grain	Insects	0.02
Storage of root crops, e. g. potatoes, onions	Sprouting	0.01
Elimination of parasites in meats	<u>Trichinella spiralis</u> <u>Cysticercus bovis</u>	0.01
Aging of alcoholic beverages	-	1 - 2
Treatment of dehydrated vegetables (shortening rehydration time)	-	0.25 - 2.5

sprouting in stored potatoes has aroused particular interest in some countries; in Canada a mobile irradiator designed for this purpose has been constructed and used to demonstrate the feasibility of the process on a large scale [5].

APPLICATION TO THE SALMONELLAE PROBLEM

Effect on food quality

The dose requirement for the adequate elimination of salmonellae varies somewhat with the particular food in question, but it is on the order of 0.5 Mrad. At this dose level most of the foods tested are not appreciably changed in quality. For example, irradiated frozen whole egg has been found acceptable [6], as have various dried egg preparations [7], and no

obvious changes have been noted in irradiated frozen horse meat intended as pet food [8]. Animal feeding stuffs appear to be quite unchanged on visual inspection. On the other hand, we observed in this laboratory that desiccated coconut treated at 0.7 Mrad to eliminate salmonellae was unacceptable: a rancid flavour develops, and the colour darkens.

Frozen products such as whole egg and meat have a particular advantage as far as effect on quality is concerned, since freezing has been demonstrated to protect against radiation-induced off-flavour and odour development [9]. Whole egg irradiated in the liquid state is reported to be very susceptible to undesirable quality changes [10]. In general, based on experience so far gained with several foods, it can be anticipated that no serious difficulties will be experienced with regard to detrimental changes in the quality of food treated at doses aimed at salmonellae elimination.

Elimination of salmonellae

The microbiological aspects are dealt with in detail in the preceding paper by Thornley.

Effect on food wholesomeness

Wholesomeness here concerns the nutritive value and possible toxicity (including carcinogenicity) of irradiated food, and the wholesomeness question concerns each application listed in Table I and any others which arise. The subject was discussed in detail at a meeting held in Brussels in 1961 by the Food and Agriculture Organization of the United Nations, the World Health Organization and the International Atomic Energy Agency (FAO, WHO and IAEA) [11]. The results of extensive animal feeding studies were reviewed; and while further research was recommended, it was generally concluded that sufficient data were available on several food items to allow expert bodies to reach a definite conclusion. Chronic toxicity studies on many foods, using several species of animals, have been completed, yielding negative results; and it is reasonable to anticipate that the wholesomeness question will not prevent the introduction of radiation into food processing. Indeed, in Canada irradiated potatoes have already been approved for human consumption; in the United States precooked irradiated bacon treated at 4.5 Mrad has recently been approved by the Food and Drug Administration for human consumption.

Certainly radiation, like heat, can destroy vitamins, some being more radiation-sensitive than others; but the level of destruction depends on the dose applied and the nature of the food being treated and its environment. Excessive losses are not to be expected, particularly at the doses required for salmonellae control. The nutritive value of protein in food is little changed, even at sterilizing doses; our own current work on the protein values of various animal feeds (meat and fish meals) and on the protein in egg indicates no change at all at 0.5 Mrad. Where animal feeding stuffs are concerned, this is particularly relevant since the financial value of these commodities is measured in terms of protein value. Irradiation has an advantage in this respect over its competitive process of heat treatment.

The question of induced radioactivity was also analysed at the Brussels meeting [11], and it was concluded that no hazard exists with the use of gamma rays from cobalt-60 or caesium-137 nor with electron machines, provided that the energy level used is restricted to less than 10 MeV.

TECHNICAL FEASIBILITY

Gamma rays with their property of penetration are ideal for the treatment of many of the food items listed in this report as carriers of salmonellae. Foods could be irradiated in the containers in which they are handled; for example, frozen whole egg can be irradiated in the metal cans without thawing and blocks of frozen horse-meat treated without removal of the hessian wrapping and again without thawing. Animal feeding stuffs could be irradiated in bags; but if it is practical to handle a commodity such as this in bulk, an electron machine could be used to irradiate a thin layer. The use of an electron machine in this manner has been considered for the treatment of grain handled in bulk for the purpose of disinfestation [12]. It is proposed, however, in view of the fact that irradiation has already been considered for the elimination of salmonellae with reference to foods requiring gamma-ray penetration, to illustrate technical feasibility with reference to a cobalt-60 plant.

CONSTRUCTION OF PLANT

The principles involved in the design of cobalt-60 plant for food processing and the method for calculation of source strength required have been illustrated by LEY and ROGERS [13] with reference to a plant envisaged for the treatment of canned frozen whole egg. However, a study of a plant of very similar design, previously described by LEY [8] will perhaps best serve to demonstrate feasibility. The application is the elimination of salmonellae from blocks of frozen horse meat intended as pet food in the United Kingdom.

The outline of a suitable design is shown in Fig. 1. The blocks are transported round a cobalt-60 source in metal cages suspended from a mono-rail. Each cage could hold two blocks of dimensions $36 \times 18 \times 8$ in, as illustrated, or three blocks $24 \times 18 \times 8$ or $24 \times 18 \times 6$ in, these being the approximate dimensions of the meat as handled at present at the Port of London. The cages approach the source in three layers and move away from it in a similar manner. This arrangement ensures an efficient absorption of the penetrating radiation, the dose rate increasing as the cages approach the source; and the blocks receive equal treatment from both sides. The cages enter and leave the thick concrete chamber through a labyrinth for avoidance of radiation hazard.

The cobalt-60 is contained in a number of cylindrical tubes mounted in a frame about $9 \times 2\frac{1}{2}$ ft, a size which allows for efficient absorption of the radiation emitted in all directions. Meat at the centre of the cages would, however, receive more radiation than that at the top or bottom would, so that it is necessary to pass each block twice through the circuit, with its

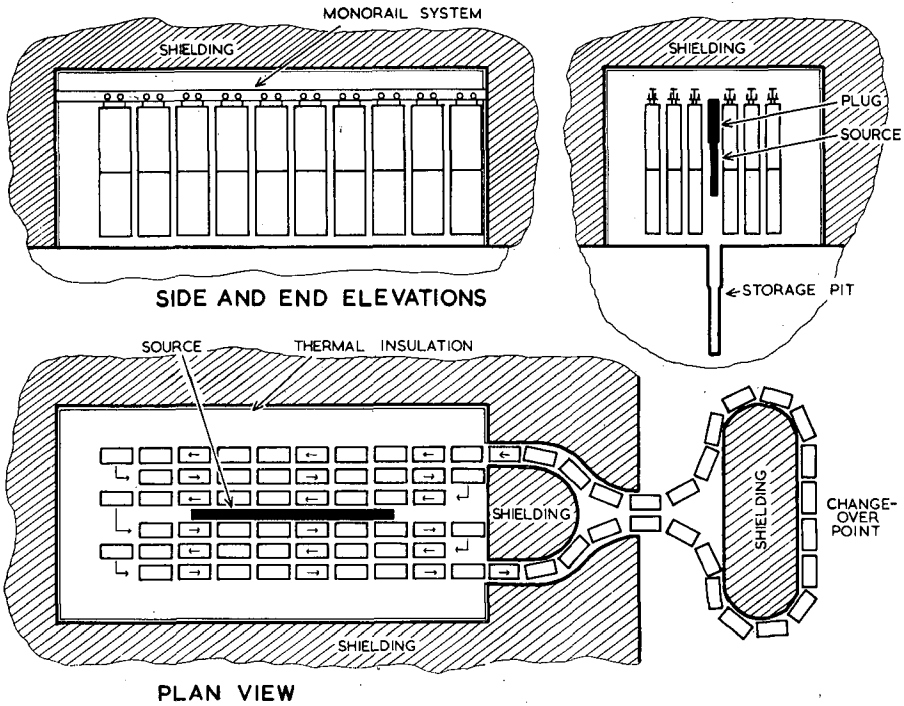


Fig. 1

Proposed design of cobalt-60 irradiation plant suitable for the irradiation of blocks of frozen horse meat.

position exchanged in the cage after one circuit in which it received half the required dose [14].

Based on throughput of 250 t of horse meat per week and a dose requirement of 0.65 Mrad, the size of source required would be approximately 700 000 c, the plant attaining about 30% efficiency and being used continuously for 6 d per week. The total holding time on the conveyor is estimated at about 5 h, but the capacity of the radiation chamber is such that blocks would be delivered off the plant after full treatment at about 2-min intervals.

COSTS

An estimate of the capital and running costs of such a plant is given in Table II. The capital investment involved is high owing to the cost of the cobalt-60 source, quoted here at 5s. 6d (77 cents) per curie; but the cost per pound for the treatment is quite low, provided that the plant is kept in almost continuous operation throughout the year. Fig. 2 illustrates the influence of source size on the final cost of treatment, the cost of plant installation remaining fairly constant. It can be seen that the cost levels off when the source is increased beyond several hundred thousand curies. Inquiries in the United Kingdom revealed that a cost of 0.56 d/lb (1.3 cents/kg) is very ac-

TABLE II

ESTIMATE OF THE CAPITAL AND RUNNING COSTS OF A RADIATION PLANT DESIGNED FOR THE TREATMENT OF FROZEN HORSE MEAT AT 0.65 Mrad [8]

Capital costs			
	£		£
700 000 c Co ⁶⁰	192 500	25% on Co ⁶⁰	48 125
Building, machinery and land	55 000	(15% replacement) (5% amortization) (5% interest on capital)	
		Amortization on machinery etc. over 10 years	5 500
		Interest at 5%	2 750
		Labour 4 shifts/week 2 men	8 000
		Overheads	<u>4 000</u>
	<u>£ 247 500</u>		<u>£ 68 375</u>

Processing, 13 000 t per annum - cost per lb = 0.56 d

Note: One Pound Sterling is approximately equal to US \$2.80.

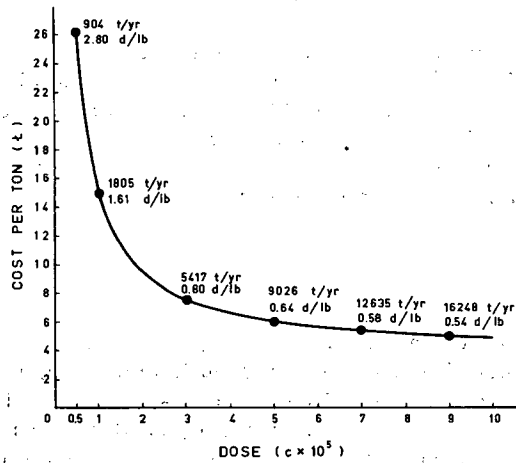


Fig. 2

Variation of cost with source size in a plant for the treatment of frozen horse meat at 0.65 Mrad

Excluding Co⁶⁰ { Capital cost £ 55 000.
Running cost £ 20 250

ceptable to the trade, whose alternative method of salmonellae elimination involves cooking the meat throughout; this process leads to high capital loss owing to the water loss from the meat. A similar cost is also acceptable for the treatment of whole egg, as revealed in a feasibility study [15].

SITUATION OF PLANT *

The question arises as to whether such a plant should be sited at the point of export of the foods demonstrated as likely to be contaminated with salmonellae or at the site of import of such foods. The former has the advantage of controlling salmonellae distribution over a wide area, since the exporter no doubt trades with many countries, and, moreover, the throughput of a particular commodity is likely to be very large and continuous, keeping such a plant in full operation. This would allow the design of a plant for a single commodity, such as that illustrated, thus leading to high efficiency and low costs. The installation of a plant at a main port of import would allow control of the same foodstuff arriving from different parts of the world. It is also feasible to design a versatile plant to treat various commodities.

Many of the foods in which salmonellae are detected originate from less developed areas of the world, but it is too early to contemplate installation of radiation plant in most of these areas. It is likely that the first plant will be at a point of import situated within the dock area. Where frozen foods are to be treated, it would be adjacent to a refrigerated store which would act as a buffer to the irregular arrival of consignments, thus ensuring continuous operation of the plant. Irradiated food would either be despatched directly from the plant to the retailer or returned to store, being kept quite separate from the food awaiting treatment.

CONTROL OF THE PROCESS AND ITS IMPACT ON INSPECTION FOR SALMONELLAE

It must be admitted that there is no method yet available which would allow authorities to detect whether foods had been irradiated or not. This is mainly because the amount of chemical change in the irradiated food is so small, and it is unlikely that a compound or a particular reaction unique to irradiated food could be readily detected. The absence of such a control device should not detract from the usefulness of the process. By the nature of the process and costs involved, irradiation treatment could only be carried out at an established centre which could be placed under the appropriate authority. Individual units of material allocated for treatment could be marked with an indicator label impregnated with a dye which undergoes a colour change specific to radiation [16]. Such labels in official seals would facilitate adequate inspection.

If radiation processing does become a commercial reality and its effectiveness is proved for salmonellae elimination, then it is quite con-

* See also the paper by Mossel.

ceivable that proof of radiation treatment at the required dose would be sufficient to eliminate bacteriological inspection for salmonellae. This would be a very great advantage because of the practical difficulties of bacteriological examination, outlined in other papers in this report*, and it would avoid the high cost which inspection involves and the inevitable delay while awaiting results. The approach to the problem is applicable to those food commodities where salmonellae contamination has been demonstrated to be high and regular.

It should be stressed that, when radiation is applied for the control of salmonellae, although the dose used will reduce considerably the total bacteriological count - will reduce, for example, the danger of anthrax and eliminate parasites and insect pests - foods will still need to be inspected for purposes other than salmonellae elimination. The use of radiation treatment should not influence in any way the normal precautions in handling and storage and should not be regarded as a preservation process.

RECENT CONSTRUCTION OF RADIATION PLANT FOR OTHER PURPOSES

Experience has already been gained in the United Kingdom of the construction and commercial operation of cobalt-60 radiation plant so that technical feasibility and costs can be estimated with reasonable accuracy. In 1962 a plant designed for the radiation sterilization of medical equipment (dose, 2.5 Mrad) was brought into operation at the factory of Johnson's Ethical Plastics Ltd., Slough [17]. It had an initial source strength of 50 000 c and is now 75 000 c; and this may be increased to 500 000 c. The conveyor system is of the monorail type, as can be seen in the photograph (Fig. 3). This year a plant was opened in Scotland for the sterilization of catgut sutures; loaded at present with 40 000 c, it has the capacity to house 150 000 c [18]. Apart from these plants, the Package Irradiation Plant at Wantage Research Laboratory, now loaded with 350 000 c, continues to operate to demonstrate the feasibility of medical sterilization and to treat large amounts of materials for research purposes, e.g. food for animal feeding studies. It was used also to treat a total amount of 35 t of frozen horse meat to demonstrate salmonellae elimination on a large scale [8].

In Canada a mobile potato irradiator has been operating successfully [5], and in the United States a large facility for food irradiation was formally opened this year. This houses 1 300 000 c of cobalt-60 and a linear accelerator; it is intended primarily for research and development. A cobalt-60 plant of 500 000 c has been in operation in Australia for several years; it is used primarily for the elimination of anthrax (*B. anthracis*) from bales of goat hair used in carpet manufacture [19]. Machine sources are also in commercial operation; Ethicon Inc. in the United States has used one for many years for suture sterilization.

Radiation processing is a commercial reality; and if it is found appropriate for the treatment of foods, there would be little delay in commencement of industrial operation.

* See the papers by Hobbs and Thornley as well as Annex II.

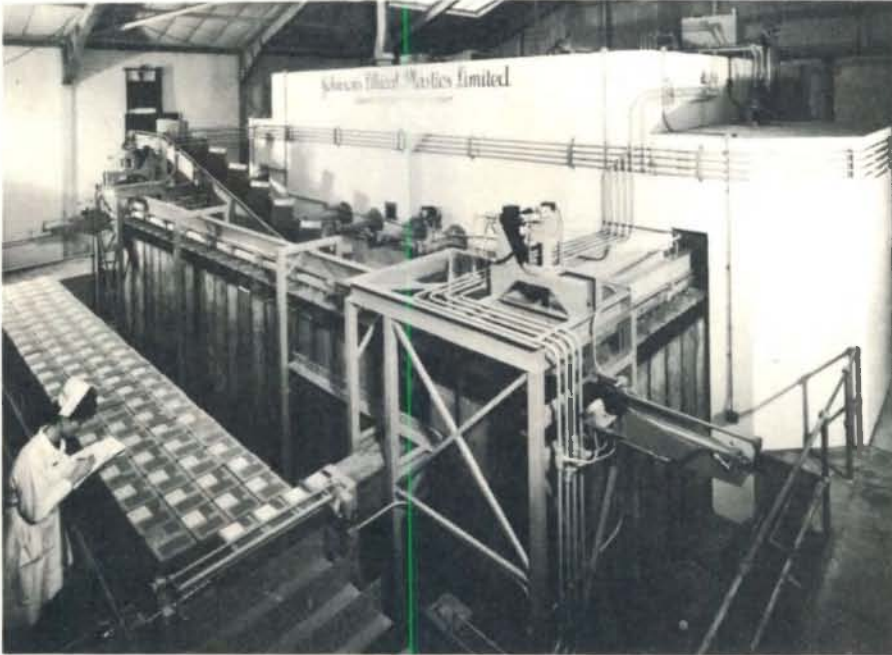


Fig. 3

Cobalt-60 radiation plant now in operation in the United Kingdom for the sterilization of medical equipment
Photograph, Johnson's Ethical Plastics Limited

LEGAL SITUATION

The use of radiation for the treatment of food for human consumption is not specifically prohibited in most countries but is controlled under general food regulations which prohibit any process which might render food injurious to health. Any individual proposed process would be examined by the appropriate authorities, who would make a decision in the light of experimental evidence available proving non-toxicity and freedom from any other hazards, as well as taking into account methods for its control. As a result of the Brussels meeting in 1961 [11], FAO, WHO and IAEA have undertaken to establish a Joint Expert Committee to advise on the special requirements for the testing of irradiated food, and their findings will no doubt assist in the final conclusion as to the wholesomeness problem. In the meantime, several countries have already given consideration to particular processes, as in Canada and the United States (see above). Animal feeding stuffs do not generally come under the specific regulations governing foods for human consumption; and in the light of the vast quantity of evidence available indicating the harmlessness of the process, it is conceivable that approval for the radiation treatment of feeds might be readily obtained.

Apart from the legal requirements regarding distribution of irradiated food, regulations exist in many countries controlling the treatment and dis-

tribution of foods found unfit for human consumption. Salmonellae are very often the cause of unfitness, and regulations would have to be amended to incorporate the use of ionizing radiation.

CONCLUSION

Radiation processing seems ideally suited for the purpose of salmonellae elimination from a variety of foods. The process appears technologically and economically feasible, but each situation must be individually examined. The wholesomeness problem may well delay some applications, but immediate consideration should be given to the treatment of certain animal foods, particularly those contributing most to a salmonellae hazard.

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ANNEX I

PRODUCTION AND INTERNATIONAL TRADE IN FISH MEALS *

Data concerning the production, export and international trade in fish meals are collected yearly by the Fish Statistics Division of the Food and Agriculture Organization of the United Nations. A summary of the relevant data for the years 1956 to 1961 (the last year for which data are available) is given in Tables I-III. These give (Table I) the total world production of white fish meal and the total exports of the five main exporting countries. Table II details the quantities exported by the five main producers to the principal importing countries. The total world production of fish meal from oily fish, with the main producing countries and their more important customers (importers), is presented in Table III.

From these statistics it will be seen that the most significant feature over the past 5 yr (1956 to 1961) has been the increasing production of fish meal from oily fish, more particularly in the South American countries (Chile and Peru) and in South Africa. Thus, in Chile production has increased from 3750 t in 1956 to 49 100 t in 1961; while in Peru and South Africa the corresponding figures are 31 000 and 71 700 t (1956) to 839 800 and 178 900 t in 1961. The corresponding export figures in metric tons are as follows: Chile, no figures available (1956), 6 300 (1961); Peru, 27 800 (1956), 708 400 (1961); South Africa, 54 400 (1956); 172 000 (1961).

It will also be noted that the main importers of South African meals are the United Kingdom, the Federal Republic of Germany and the United States of America while the Peruvian meals have been going in increasing

TABLE I

TOTAL WORLD PRODUCTION OF FISH MEAL FROM WHITE FISH AND EXPORT FROM THE MAIN EXPORTING COUNTRIES (in thousands of metric tons)

Year	1956	1957	1958	1959	1960	1961
World total production	321.0	315.0	368.0	415.0	468.0	509.0
Total exports of the five main exporting countries	72.0		89.0		66.0	
Canada	16.2		12.9		9.6	
Chile	4.1		9.4		18.4	
Japan	15.8		23.7		6.3	
Iceland	20.3		26.5		19.2	
Norway	16.0		16.5		12.5	

* Prepared by J.M. Shewan, Torry Research Station, Abbey Road, Aberdeen, Scotland, and N.E. Holmes, Nutrition Division, Food and Agriculture Organization of the United Nations, Rome, Italy.

TABLE II
 EXPORT OF WHITE FISH MEAL FROM MAIN EXPORTING COUNTRIES TO PRINCIPAL IMPORTING
 COUNTRIES
 (in thousands of metric tons)

Year	1956	1957	1958	1959	1960	1961
Canada - Total produced	29.8	-	-	-	-	-
Total exported	16.2	9.7	12.9	11.5	9.6	7.1
to						
United Kingdom	3.2	5.8	7.7	9.6	8.7	6.0
United States of America	12.5	3.9	5.1	1.8	0.9	1.1
Chile - Total produced	9.3	10.3	9.0	10.1	10.0?	8.9?
Total exported	4.1	4.5	9.4	11.7	18.4	34.7
to						
Federal Republic of Germany	1.1	2.0	1.2	3.1	0.9	3.2
Netherlands	1.9	0.9	0.4	3.2	1.8	13.9
United States of America	1.1	1.1	6.7	3.5	12.0	9.8

TABLE II (cont'd)

Year	1956	1957	1958	1959	1960	1961
Japan - Total produced	-	-	-	-	-	-
Total exported	15.8	2.8	23.7	24.0	6.3	4.9
to						
China (Taiwan)	-	-	-	0.1	1.2	0.7
Federal Republic of Germany	14.0	1.0	19.0	17.8	-	-
Hong Kong	0.3	0.6	0.2	0.9	1.1	1.1
Philippines	0.3	0.8	3.3	4.0	0.5	-
Singapore	-	0.2	0.1	0.4	2.7	2.8
United States of America	-	-	0.1	0.2	-	-
Iceland - Total produced	21.6	21.1	21.8	25.3	22.9	19.0
Total exported	20.3	24.3	36.5	24.2	19.2	28.7
to						
Czechoslovakia	1.6	0.5	0.2	2.2	0.7	0.6
Denmark	1.1	0.9	2.5	0.6	0.9	1.5
Finland	1.0	0.5	-	-	0.1	-
Federal Republic of Germany	6.2	8.6	11.2	2.8	5.1	12.1
Ireland	2.0	0.5	0.6	0.1	0.1	0.1

TABLE II (cont'd)

Year	1956	1957	1958	1959	1960	1961
Netherlands	0.2	0.5	0.6	0.1	0.1	0.1
Poland	1.4	1.2	0.3	0.5	-	0.4
Sweden	0.5	4.0	2.7	10.7	5.2	3.7
United Kingdom	5.3	6.1	4.3	3.0	5.5	8.7
Norway - Total produced	16.6	15.2	15.2?	17.4	15.7	13.9?
Total exported	16.0	14.2	16.5	17.0	12.5	17.3
to						
Austria	2.5	0.9	2.0	1.1	0.7	0.8
Belgium and Luxembourg	0.8	0.5	0.6	0.7	0.3	-
Denmark	3.4	3.3	3.9	3.9	0.7	3.6
German Democratic Republic	0.7	0.9	0.4	0.4	-	-
Federal Republic of Germany	1.9	2.1	1.3	-	0.8	1.4
Italy	0.3	0.2	0.7	0.4	0.1	0.2
Poland	-	-	-	-	2.6	2.2
Switzerland	6.4	6.1	7.5	7.4	5.9	6.7

TABLE III

WORLD PRODUCTION OF FISH MEALS FROM OILY FISH AND DETAILS OF EXPORT
AND IMPORT

(in thousands of metric tons)

Year	1956	1957	1958	1959	1960	1961
World total production	409.0	447.0	490.0	641.0	887.0	1181.0
<u>AFRICA</u>						
Angola - Total produced	77.7	85.2	74.8	46.2	44.8	55.3
Total exported	53.6	94.1	81.2	51.3	45.0	50.3
to						
Austria	1.3	3.3	4.3	4.5	3.2	4.6
Belgium and Luxembourg	3.1	8.1	9.7	0.8	1.0	-
Denmark	2.1	0.6	-	-	-	-
Federal Republic of Germany	27.6	30.5	4.8	3.9	5.8	13.7
Italy	1.3	6.1	2.5	14.5	9.8	16.5
Netherlands	11.9	23.9	23.9	7.2	10.2	2.1
Portugal	0.2	0.9	5.1	1.7	8.9	3.9
Spain	1.0	4.0	-	2.0	0.5	-

TABLE III (cont'd)

Year	1956	1957	1958	1959	1960	1961
United Kingdom	-	3.1	1.2	0.7	0.1	-
United States of America	3.5	11.3	27.0	10.5	0.4	-
Morocco - Total produced	5.9	12.6	20.7	15.1	8.2	not available
Total exported	6.2	12.7	16.6	14.5	13.9	not available
to						
France	1.2	2.9	12.4	8.1	5.3	not available
Federal Republic of Germany	1.2	2.7	1.9	2.8	3.1	not available
Netherlands	0.6	1.1	-	0.3	0.9	not available
United States of America	3.0	3.5	0.8	0.2	-	not available
South Africa - Total produced and Southwest Africa	71.7	81.8	90.7	120.1	136.4	178.9
Total exported (includes solubles)	54.4	57.0	89.4	99.9	126.7	172.0
to						
Australia	-	-	0.2	0.2	2.6	5.7
Federation of Malaya	1.8	3.3	3.0	4.3	4.2	3.7
German Democratic Republic	-	-	-	-	5.3	28.9

TABLE III (cont'd)

Year	1956	1957	1958	1959	1960	1961
Federal Republic of Germany	3.2	3.4	4.3	14.8	15.2	17.1
Israel	6.2	4.2	13.9	13.4	10.6	11.3
Italy	0.2	no data	0.4	0.8	1.4	0.7
Netherlands	7.4	6.4	6.6	3.8	13.1	6.3
Rhodesia and Nyasaland	4.3	5.0	4.8	5.0	4.0	4.2
United Kingdom	26.9	28.2	40.2	36.1	47.3	71.9
United States of America	3.4	5.2	6.9	11.0	6.5	13.8
<u>SOUTH AMERICA</u>						
Chile - Total produced	3.7	6.3	9.3	19.9	32.3	49.1
Total exported	no data	no data	13.8	2.0	5.7	6.3
to						
Belgium and Luxembourg	no data	no data	0.2	no data	0.3	0.9
United States of America	no data	no data	0.4	no data	4.9	2.7

TABLE III (cont'd)

Year	1956	1957	1958	1959	1960	1961
Peru - Total produced	31.0	64.5	126.9	332.4	558.3	839.8 (mainly anchovy)
Total exported	27.8	61.7	105.8	277.6	507.0	708.4 (mainly anchovy)
to						
Belgium and Luxembourg	1.7	2.3	5.2	21.2	48.2	30.7
Brazil	-	-	-	-	2.4	3.3
Canada	-	-	-	-	0.8	5.1
France	-	-	-	2.8	11.8	23.0
Federal Republic of Germany	2.5	11.5	26.7	73.2	107.3	150.5
Italy	-	-	-	0.1	8.8	15.1
Japan	-	-	-	-	17.0	22.7
Mexico	0.7	2.6	1.9	4.8	9.9	11.4
Netherlands	16.2	27.1	32.9	100.4	160.0	204.0
Spain	-	-	-	-	8.8	15.3
Sweden	-	-	-	-	0.3	4.2
United Kingdom	0.2	1.3	10.7	32.9	55.7	76.5
United States of America	6.4	16.4	27.8	40.2	60.3	118.6
Venezuela	-	0.3	0.6	0.4	4.5	6.2
Yugoslavia	-	-	-	-	3.5	3.6

TABLE III (cont'd)

Year	1956	1957	1958	1959	1960	1961
<u>EUROPE</u>						
Denmark - Total produced	46.9	54.9	65.6	70.8	50.6	57.9
Total exported	40.0	41.7	53.6	58.8	31.8	39.5
to						
Czechoslovakia	0.9	0.2	1.2	0.7	0.2	0.7
Finland	-	-	-	1.8	0.7	2.8
France	0.4	-	0.4	0.5	0.3	-
Federal Republic of Germany	4.3	2.8	5.5	3.6	2.9	6.5
Netherlands	19.0	12.8	16.6	13.6	2.5	-
Sweden	0.5	0.6	0.5	1.9	1.7	0.6
Switzerland	0.7	0.2	0.5	2.0	3.0	1.1
United Kingdom	11.6	24.5	26.0	30.9	14.5	22.5
United States of America	0.4	-	1.0	-	-	-
Iceland - Total produced	17.9	23.5	22.2	44.1	32.9	51.7
Total exported	7.8	13.0	27.7	19.3	35.2	40.6
to						
Czechoslovakia	-	0.5	1.3	0.9	1.4	1.2
Denmark	3.9	1.2	5.6	8.6	12.4	11.8

TABLE III (cont'd)

Year	1956	1957	1958	1959	1960	1961
Finland	1.0	0.7	1.2	0.6	5.8	4.4
Federal Republic of Germany	0.9	3.4	7.5	2.3	4.4	6.4
Israel	0.5	0.3	-	0.2	-	-
Netherlands	0.1	3.2	4.0	-	3.0	0.7
Poland	0.1	0.3	1.2	1.9	0.6	1.3
Sweden	-	-	0.2	0.5	1.3	1.0
United Kingdom	0.6	1.8	3.1	3.2	3.0	13.3
Netherlands - Total produced	7.5	9.1	9.6	15.7	7.7	8.0
Total exported	5.3	5.5	6.1	8.4	5.6	4.8
to						
Austria	0.7	0.6	0.3	0.2	0.1	-
Belgium and Luxembourg	0.2	0.2	0.4	0.4	0.1	0.1
Federal Republic of Germany	3.1	4.3	4.6	7.6	4.4	4.3

TABLE III (cont'd)

Year	1956	1957	1958	1959	1960	1961
Norway - Total produced	247.6	176.1	102.7	116.5	140.8	132.3 (mainly herring meal)
Total exported	174.0	120.5	89.4	71.1	88.7	109.9
to						
Austria	2.1	1.9	1.7	1.7	2.7	2.7
Belgium and Luxembourg	10.4	9.2	9.0	6.1	3.6	4.1
Denmark	2.3	0.9	-	0.2	3.9	11.6
France	18.2	15.0	15.7	12.6	14.1	19.6
German Democratic Republic	1.2	1.6	2.5	2.4	5.9	4.8
Federal Republic of Germany	18.0	18.7	12.7	5.1	9.6	4.8
Italy	6.4	2.3	2.6	2.4	1.8	0.9
Netherlands	25.9	17.3	11.3	7.0	2.7	4.1
Sweden	8.6	9.0	7.8	4.6	10.3	9.3
Switzerland	1.8	1.8	2.3	1.1	2.5	2.8
United Kingdom	65.0	32.1	18.3	25.4	28.9	41.5
United States of America	9.6	3.6	0.1	-	-	-

amounts since 1956 to the Netherlands, the Federal Republic of Germany, the United States of America United Kingdom, Belgium and Luxembourg.

It is perhaps significant that the Angola fish meals, which were first suspected in Germany of being contaminated with salmonellae, have, in general, been imported into Western European countries in decreasing amounts since 1956 (Table III).

ANNEX II

A COMMENT ON SAMPLING TECHNIQUES FOR MICROBIOLOGICAL DETECTION OF SALMONELLAE IN CONSIGNMENTS OF FOODS AND FEEDS *

SAMPLING OF FISH AND FISHERY PRODUCTS FOR BACTERIAL INFECTION

Although most people are aware that the adequate sampling of a consignment of fish or fishery products such as fish meal for infection by, say, food poisoning pathogens involves statistical considerations, little which gives the investigator any guidance on this matter seems to have been published.

The problem in the sampling of an infected consignment is to reduce the risk that samples will turn out all negative so that the infected material passes undetected. The difficulties in getting a positive sample when the level of infection is low, or when infection is scattered very unevenly over the consignment, can to some extent be lessened by methods such as increase of the number of samples, increase of the volume of material in each sample, use of bulked samples from several units, and use of more than one enrichment or plating medium. The choice of methods will depend partly on the resources available — staff to collect and prepare more samples, incubator space to hold more or larger vessels — and partly on the level and distribution

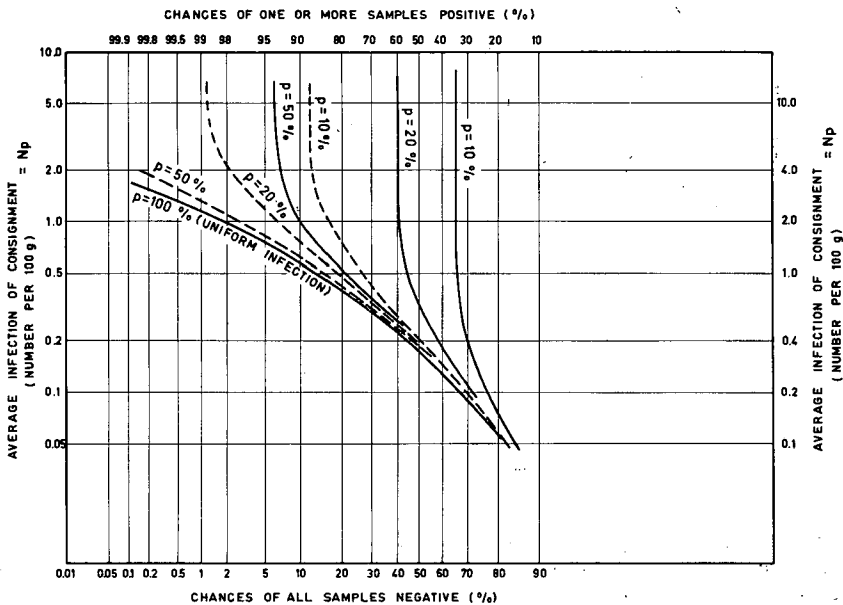


Fig. 1

Efficiency of sampling methods under various conditions

Number of samples (100 g) = 4

Infection of N per 100 g assumed to be present in p% of all units of consignment

Use left-hand scale for 100-g samples.

Use right-hand scale for 50-g samples.

————— Each sample from single unit

----- Each sample bulked from 5 units

* Prepared by J. M. Shewan and C. R. Baines, Torry Research Station, Abbey Road, Aberdeen, Scotland.

of infection that are considered the most likely to occur. Some typical situations, with appropriate sampling methods, are mentioned briefly below.

The efficiency of such a sampling scheme can be assessed by calculation of the chances of getting at least one positive sample in a consignment with a given level of infection. This is shown in Figs. 1-3 for various conditions and can be used as a rough guide for the decision as to how many samples of a given size are needed and how these samples should be taken.

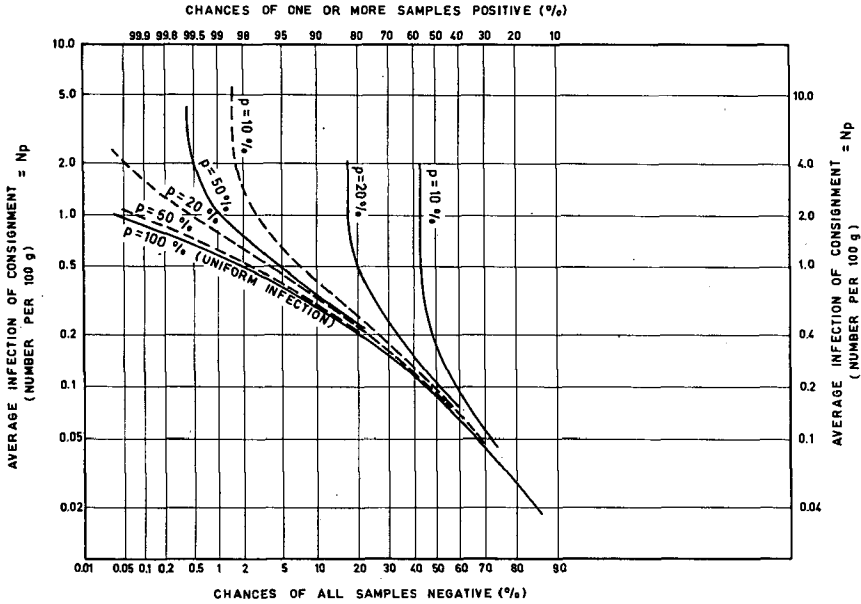


Fig. 2

Efficiency of sampling methods under various conditions

Number of samples = 8

Use left-hand scale for 100-g samples.

Use right-hand scale for 50-g samples.

———— Unbulked samples

----- Bulked samples

For the present purpose we propose to confine our remarks to the sampling of batches of fish, e. g. meal, at, say, a port of entry, for the detection of salmonellae alone. We assume that the bacteriological techniques for detecting and/or enumerating the salmonellae in fish meal have already been shown to be adequate. The number and size of the samples actually examined per day will depend to some extent on the staff and facilities available.

It is known that it is the practice of many sampling agencies to sample an amount equal to the square root of the total number of units in the batch. Thus, if there are 2500 sacks of fish meal in a consignment, one would sample 50 sacks. The question remains how to take these 50 samples.

The choice of sampling method depends on at least three considerations:

- (1) Infection may or may not be uniform throughout the batch;
- (2) The level of infection may be high or low; and
- (3) Different serotypes, including paratyphoid bacilli and therefore of varying importance from the public-health point of view, may be present.

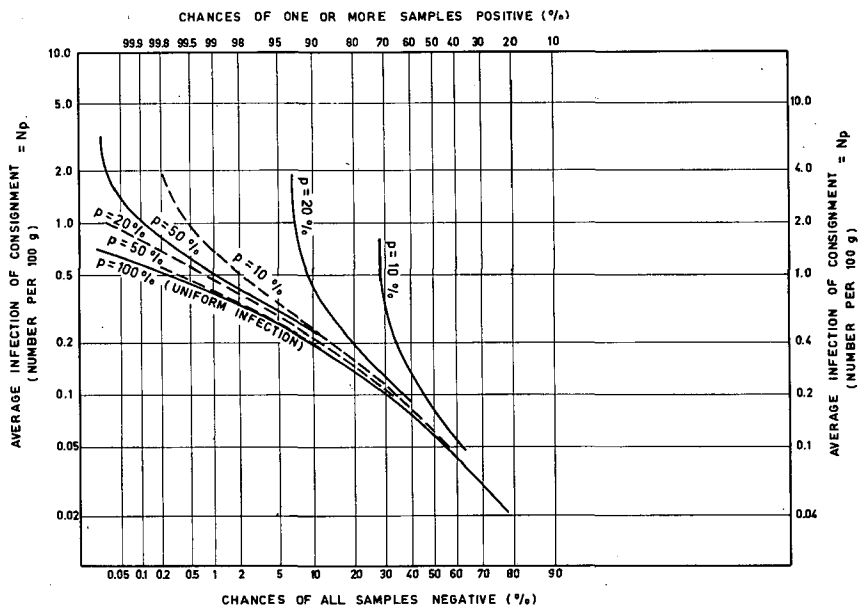


Fig. 3

Efficiency of sampling methods under various conditions

Number of samples = 12

Use left-hand scale for 100-g samples.

Use right-hand scale for 50-g samples.

———— Unbulked samples

----- Bulked samples

One can therefore envisage the following situations:

A. Only one serotype is present.

- (1) If infection is evenly distributed throughout the batch, there is little to choose between different methods. The simplest method would be to sample every 50th sack or package. If the bacterial numbers are very low, one can improve the reliability of detection by incubation of samples of larger volume, 100 g, say, instead of 25 g.
- (2) If infection is very unevenly distributed, it is advisable to use bulked samples, that is, to take 50 samples each made up of samples from five sacks or packages, so that a total of 250 sacks or packages is sampled. In this case the chances of getting all negative samples (and so failing to detect infection) are less than when the 50 samples are each taken from a single sack or package (as in (1) above). The improvement from bulking of the samples is greater when the level of infection present is high than when it is low.

B. When two or more different serotypes may be present, then, even though a knowledge of what these serotypes are may not be important, it may be thought necessary to employ two different kinds of liquid enrichment medium. Here it is assumed that during enrichment one serotype more than another may be selected by the different medium.

- (1) If the serotypes are present in low concentrations, distributed either evenly or unevenly, the best procedure would appear to be to sample every 50th sack or package (as in A (1)) and divide the samples into two for inoculation into two liquid enrichment media.

- (2) If any of the serotypes is distributed unevenly and the concentration is high, the best procedure is that suggested under A(2) but again to divide them into two and use two liquid enrichment media for each sample.
- (3) Two kinds of plating media for sub-cultures will also increase the number of positives.

SUMMARY AND CONCLUSIONS

The information made available to the Panel through the papers presented and the subsequent discussions is summarized below, together with the main conclusions drawn from this material. The summary consists of four sections. In the first section, headed GENERAL, the products and commodities causing the transmittance of salmonellae and other harmful organisms are listed, and the possible ways of eliminating the contamination, with particular reference to radiation control, are briefly discussed. The second section deals specifically with the microbiological problems associated with irradiation treatment, and the third deals with wholesomeness problems. The fourth section summarizes some technological and economical aspects of the application of ionizing radiation for the elimination of salmonellae in food and feed products.

I. GENERAL

A. There is a large number of products and commodities which are regarded as hazards to both human and animal health, because they are potential sources of salmonellae and sometimes other pathogenic organisms. The edible products can be grouped under the categories: 1. Animal feeds, and 2. Foods and food ingredients for human consumption. Also a number of inedible products are known to spread harmful micro-organisms, and these are listed at the end of this section.

1. *Animal feeds*

(a) Dry products of animal origin, e.g. meat meal, bone meal, blood meal, hoof and horn meal, whale meal, feather meal, fish meal, crushed bones.

(b) Moist products of animal origin, e.g. horse meat and other pet foods.

(c) Water for drinking and cleaning.

(d) Dry products of vegetable origin.

Attention should here be drawn to the fact that packaging materials for animal feed stuffs, e.g. bags of jute and similar materials, can also be contaminated with salmonellae and other harmful micro-organisms.

2. *Foods and food ingredients for human consumption*

(a) Boneless frozen meat, e.g. veal, beef, mutton, poultry, kangaroo.

(b) Other meats including offals, tripe and casings.

(c) Shell eggs.

(d) Egg products, e.g. frozen whole, white or yolk; dried whole, white or yolk.

(e) Milk and other dairy products.

(f) High protein concentrate meals, e. g. meat meals, meal of blood and blood albumin, fish flour.

(g) Desiccated coconut.

There are other foods, e. g. certain spices and condiments, which are known to carry pathogenic organisms, including salmonellae. Also other food stuffs not yet tested for the presence of these harmful organisms are suspected carriers.

B. In some instances the method of preparation of the products listed above will not lead to a pathogen-free produce. In other instances the method of manufacture provides a satisfactory heat treatment, but the finished product may be recontaminated either by other materials or by lack of sufficient environmental sanitation.

In some instances chemical treatments are applied to the finished product. These may or may not be successful, and there may be toxicological or nutritional objections to their use.

Therefore, exposure to ionizing radiations will in the following be considered as an alternate or complementary control measure.

1. *Animal feeds*

Meat meal, blood meal, bone meal and whale meal are all well known sources of salmonellae. At present, methods to reduce the salmonellae content include steam treatment or formation of pellets, cubes and cakes and, secondly, dry or moist heat treatment of the finished product. It would seem difficult to eliminate salmonellae in the finished product entirely by improving the hygiene of the manufacturing process, because the basic raw materials and the animals themselves are reservoirs of these organisms. The use of ionizing radiation should therefore seriously be considered for elimination of harmful organisms from these products.

Fish meal. There are countries where fish meal is still produced under primitive conditions and therefore heavily contaminated with salmonellae. Although gross contamination of fish meal thus occurs, there is good evidence that, by careful industrial practices, fish meal can be produced almost free from salmonellae.

Thermal processes are available for the elimination of salmonellae from contaminated fish meal; however, heat treatment often causes an appreciable loss in protein value. Since international trade with fish meal produced under unsatisfactory hygienic conditions still continues, further investigations are required to ascertain the usefulness of radiation treatment for the elimination of salmonellae from this product. The same appears to apply to meals of vegetable origin.

Feather, hoof and horn meal and crushed bones. There is evidence that the terminal heat treatment is not always feasible for destruction of salmonellae. It is also considered that progress in hygienic methods of manufacture will be very slow to come in this particular industry. Therefore, radiation may be applicable.

Pet foods, e. g. horse meat or open-packed, cooked, compressed meat. It is well known that frozen, boneless horse meat from many countries is very often contaminated with salmonellae. Efforts made during the last

years to produce horse meat with lower salmonellae contamination have failed. Furthermore, the final (heat-treated, cooked, compressed) product is still often contaminated. It is important that the raw frozen horse meat should be treated to render this product free of salmonellae. This can be done by irradiation.

Water. The problem of making water for various purposes microbiologically acceptable can probably be solved by the application of conventional methods, such as improved sanitation and chlorination.

2. *Foods and food ingredients for human consumption*

Frozen boneless meat. Experience has shown that it is extremely difficult to render these foods free from salmonellae by ordinary methods of hygiene in abattoirs and meat-packing stations because of a number of complex factors.

The primary reservoir is the animal. During handling and cutting up or other treatments of carcasses there is considerable spread of micro-organisms and also favourable conditions for their growth. This occurs before packing and freezing.

The use of irradiation for ridding the frozen material of salmonellae is the only feasible solution to the problem.

Poultry meat is known to be a vehicle of salmonellae to a greater or lesser extent, according to the cleanliness or sanitary practices of the processing plant. The treatment of the killed or frozen bird with ionizing radiation would eliminate these organisms.

Egg products. Heat treatment is effective for liquid whole egg intended for freezing or drying. The heat treatment of egg white is not yet fully satisfactory. Dry heat treatment for flaked albumin is applicable. It is impossible to render egg products free from salmonellae by improved hygiene alone, because poultry themselves are known to be one of the biggest reservoirs of salmonellae. Most countries exporting egg products have their own heat-treatment facilities. Radiation can be considered as an alternative method of treatment. Shell eggs cannot be irradiated.

Milk and other dairy products. Milk and most other dairy products are extremely sensitive to ionizing radiation, and even low doses often cause undesirable changes in the product. It is therefore doubtful if radiation control of harmful organisms is applicable.

Desiccated coconut. Measures of hygiene in the production of the desiccated material have already led to a marked decrease in salmonellae contamination. In addition, steam treatment followed by drying can be used as a supplementary method. So far irradiation treatment has proved unsatisfactory, because the dose required induces rancidity.

C. As indicated in the introductory remarks, there is a substantial number of inedible products that are known to transmit harmful micro-organisms, possibly including salmonellae. These can be grouped as follows:

Raw materials for manufacturing purposes. e.g. goats' hair, skins and hides, pig bristle, wool, hoof, horn and tusks, feathers.

Fertilizers, e.g. guano, compost, sludge, feather meal, bone, blood, hoof and horn.

Effluents from communities, slaughter houses and other industries.

Some of the products listed above have already been commented upon. A few remarks about the others may be appropriate.

Guano. As far as the Panel members know, there are no published results on the occurrence of salmonellae in this product, but birds are known to produce contaminated excreta. There would be no method suitable for decontamination except possibly irradiation.

Compost. Van der Schaaf (1962) provided evidence that this product is often contaminated with salmonellae. The natural heat of production, or heat applied during production, may destroy salmonellae. Where this is not the case, radiation could be applied to this product.

Sewage: effluents and sludge. It is well recognized that effluent from human and animal sources contains salmonellae and that its use as a fertilizer constitutes a hazard via crops for human and animal feeding and also for grazing animals.

Sludge and sludge cakes may be contaminated with salmonellae; and when they are, irradiation may be applicable.

CONCLUSIONS

There is evidence that many dried or frozen products of animal origin intended for animal feeding and also dried or frozen products mostly of animal origin used for human food are contaminated with salmonellae. Many of the serotypes found in these products occur frequently in human salmonellosis.

Dried animal products used as fertilizers and the sacks used for packaging fertilizer and feeding stuff may serve to maintain the spread of these organisms.

There is no information on the contamination with salmonellae of certain inedible products of animal origin used for manufacturing purposes, but it is possible that skins, pig bristles and feathers are contaminated.

In some instances, e. g. certain egg products, heat treatment provides a satisfactory method for the elimination of salmonellae. In others neither chemical nor heat treatment is practicable, and efforts to reduce the hazard by good sanitation have continually failed. For these, e. g. animal meals for feeds and fertilizers, frozen boneless horse meat and other open-pack pet foods, radiation treatment is recommended for immediate use. The application of radiation treatment for certain foods intended for human feeding, e. g. frozen boneless veal and other frozen boneless meats, is strongly recommended for consideration.

II. MICROBIOLOGY

1. Radiation elimination of salmonellae is particularly applicable to non-perishable products, such as those in the frozen or dried state, where subsequent multiplication of surviving micro-organisms is not a problem. When foods in a perishable state are to be irradiated, the possibility of the survival of pathogens must be investigated, and storage conditions must be controlled.
2. The radiation process required can be estimated by determination of

the D value (decimal reduction dose) for the most resistant serotype likely to be present in the product to be irradiated. Then the degree of inactivation required is determined by the level of initial contamination, on the assumption that the requirement for the irradiated product is absence of salmonellae in 100 g or other chosen level (e. g. initial number of salmonellae $10^x/g$, to be reduced to 1 in 1000 g; inactivation factor required, 10^{x+3} ; dose needed, $D(x+3)$).

Radiation doses calculated in this way should be checked by tests on the naturally contaminated product.

Of the five serotypes studied, none has shown higher resistance than has S. typhi-murium. A wider range of serotypes needs to be studied.

The relative resistance of different serotypes can vary with the nature and physical state of the product in which they are irradiated and must be determined in each case. Also, for any strain, D values show a wide variation, depending mainly on the presence of oxygen and the water content of the product.

3. Survival of irradiated bacteria has been shown to vary with the recovery medium and temperature, and more information is needed on the optimal recovery conditions for irradiated salmonellae.

As strains of E. coli have proved more radiation-sensitive than have some strains of Salmonella, E. coli would not be suitable as an indicator of faecal contamination after irradiation. Direct tests for salmonellae should be used on the irradiated product.

4. Compared with Salmonella strains, Clostridium and Bacillus spores are much more radiation-resistant, while yeasts and some other vegetative bacteria, such as streptococci, micrococci etc., are somewhat more resistant. If present in sufficient numbers, these would survive doses used to eliminate salmonellae. Some reduction in numbers would take place, however, even with resistant bacteria. This might prove useful for pathogens other than salmonellae (e. g. Bacillus anthracis in materials which contain it).

5. Radiation, heat or ethylene oxide processes could all be used for elimination of salmonellae and other harmful microbes, even if there is some variation in resistance of different strains (especially for heat), and would all leave resistant survivors, of which bacterial spores are the most important. The choice of process would therefore be determined by other factors. Radiation is the only process applicable to products in the frozen state.

6. Because of the formation of radiation-resistant mutants an increase in resistance by a factor of about two has been shown in a small proportion (e. g. 10^{-7}) of bacteria surviving radiation in laboratory experiments. This should not prove a hazard in radiation processing; the nature of most of the products is such as to prevent bacterial multiplication, and, where it is not, factory hygiene should be able to avoid any opportunity for the accumulation of resistant mutants.

III. WHOLESOMENESS

1. The types of radiation (gamma rays from cobalt-60 and other radioactive isotopes, and electrons from machine sources of less than 10 MeV)

being considered for this application do not give rise to any problems of induced radioactivity, but a wholesomeness problem concerning the effect on the nutritive value of food constituents and the possible formation of toxic compounds must be considered.

2. In the study of treatments of animal feeds for the elimination of salmonellae the effects of such treatments on the nutritive value of the protein must receive particular attention.

3. Evidence is available which indicates that no loss of protein value in foods and feeds would be expected at the radiation doses which would be employed in eliminating salmonellae. Preliminary experiments on some animal feeds (blood meal, fish and meat meal) have shown that no loss of "available lysine" occurs even at doses above 1 Mrad and no change in "Relative Protein Value" at 0.5 Mrad, which was the only dose tested.

4. The absence of change in nutritive value of protein resulting from irradiation contrasts with the substantial losses which occur with the conventional heat treatment and losses caused by the use of ethylene oxide.

5. Vitamin losses caused by radiation are in general comparable with those caused by heat treatment and are not likely to be significant at doses below 1 Mrad, particularly in dry food. Moreover, in individual animal feeds, the effect on vitamins is not generally of practical importance because the feeds eventually become part of a composite ration.

6. The conclusions of the FAO/WHO/IAEA 1961 Brussels Conference on the possible toxicity of irradiated food must be accepted in a consideration of irradiation application to the Salmonella problem. However, some distinction should be made between products irradiated for animal consumption and those intended for human consumption. In view of the large-scale testing programme of the possible toxicity of irradiated human foods already carried out, which has so far produced no evidence of any toxic factors, irradiated animal feeds should be seriously considered for distribution. This would be a good way of introducing radiation into food technology on a large scale.

7. Toxic effects from the use of ethylene oxide for the treatment of foods have already been observed, and its use is not permitted in many countries. Irradiation is the only method of cold treatment not involving the use of chemicals at present available and therefore warrants serious consideration for trial.

IV. TECHNOLOGY

1. It is apparently technologically feasible to process food and animal feeds by irradiation with either cobalt-60 plants or electron machines. Irradiation plants are already in operation for sterilization of medical supplies, for the elimination of B. anthracis from goats' hair and (on a pilot-plant scale) for sprout inhibition in potatoes.

2. Electron machines might be used for the treatment of loose material placed in thin layers on a conveyor belt. Gamma rays would be used for packaged foods, e.g. animal feeds in bags or frozen products such as whole egg in tins and horse meat.

3. High capital cost is involved in the establishment of a radiation plant.

For instance, the total investment involved in a cobalt-60 plant designed to process 13 000 tons of frozen horse meat per annum at a dose of 0.65 Mrad would be approximately £250 000 (\$700 000); most of this cost is the purchase of the isotope itself. Based on a 10-yr amortization, the cost for the treatment would be approximately 0.56 d/lb (US\$0.65/lb). Machine sources, where applicable, might be somewhat cheaper. This cost of treatment is comparable with, or lower than, heat processing.

4. In the case of protein concentrates, such as animal feeds, the financial value of the irradiation-treated product would be higher than that of the heat-treated product, because the protein value is not changed by irradiation.

5. A continuous and large throughput of material is essential for economic radiation processing; the plant must be used at full capacity, especially if isotopes are used as radiation source. A plant could be designed for treatment of a variety of different products, but this would result in reduced radiation efficiency.

6. Radiation treatment of a food product cannot be detected as yet by examination of the food itself, because the chemical changes are extremely small. Indicator labels specific to radiation, which can be used to distinguish between irradiated and unirradiated products, have been developed. Treated and non-treated commodities must be kept separated at the site of the plant.

7. In principle the place of an irradiation plant to eliminate salmonellae and other harmful microbes from a commodity should be at the site of production. The throughput of material for treatment would also be better assured there. However, in view of the lack of technological development in many countries producing foods and feeds, it might be practical at the present time to install a plant in a country of import. Treatment at a free port facility might be envisaged for products from certain areas which show consistent contamination.

8. Radiation treatment should also be considered for certain foods produced and consumed within a country, e.g. frozen whole egg. The feasibility of the use of radiation processing will depend on local conditions and existence of competitive methods. Such situations must be individually examined.

1. In microbiology

- (a) Study of radiation resistance of additional serotypes of Salmonella;
- (b) Study of the effects of water activity and of the lipid content of the irradiated products on radiation resistance of Salmonella;
- (c) Effect of media and temperature on recovery of irradiated salmonellae with special reference to the use of Salmonella selective media.

This is an initial programme for basic information. When a particular product is being studied, it would be desirable to investigate also radiation inactivation of other pathogens, e.g. Bacillus anthracis.

2. In wholesomeness

- (a) More direct experiments should be made on the effect of radiation on the nutritive value of protein with particular reference to animal feeds. Animal tests as well as chemical and microbiological estimates of protein value should be made.
- (b) More attention should be given to the effect of the dose levels being considered here (0.5 - 1 Mrad) on vitamin destruction in the individual foods where vitamin content might be important.

The Panel wishes to draw the attention of the "Expert Committee on Wholesomeness of Irradiated Food" being set up by FAO/WHO/IAEA to the problem of the possible toxicity of irradiated animal feeds and to its suggestion that the use of such feeds should be permitted.

3. In technology

- (a) When dose requirements for particular products have been fixed, detailed cost studies should be made in terms of a particular situation so that the best way of treatment can be established.
- (b) A detailed, on-the-spot analysis should always be made in each situation before deciding on location of an irradiation plant at import or export.

F. The Panel also recommended that IAEA support requests for the training of scientists from countries where radiation control of salmonellae and other pathogens might be considered. Long-term training as well as shorter study periods in laboratories where active research in this field is under way is highly desirable. It is essential that such training should be linked with the possibility for the trained scientist to perform research work on return to his home country.

G. The Panel finally recommended that the lectures presented at the meeting as well as an edited version of the discussions should be published by IAEA in its Technical Bulletin Series. The publication should be given as wide a distribution as possible in order to draw international attention to the problem of transmittance of pests and diseases by food and feed products and to the potential use of atomic energy for its control.

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