A BACTERIOLOGICAL STUDY OF DRIED WHALE MEAT WITH PARTICULAR REFERENCE TO THE PRESENCE OF CLOSTRIDIA

By R. H. M. ROBINSON, A. M. PUGH and F. R. SELBIE
From the Bland-Sutton Institute of Pathology, Middlesex Hospital, London, W. 1

In February 1948 we were asked by United Whalers Ltd. to make a bacteriological investigation of dried whale meat, with particular reference to the presence of pathogenic clostridia. This was a matter of some importance since it had been suggested that the sale of dried whale meat products to the public was not without danger, there being a risk of anaerobic infections in the handlers of these products and of food poisoning from their use in made-up dishes. Since then we have examined thirty-nine samples of dried whale meat which arrived in nine batches from several factory ships in the Antarctic. In this communication we shall describe our methods and results, and discuss the significance of our findings.

METHODS

The thirty-nine samples were contained either in sealed tins or wide-mouthed glass containers with wax-sealed corks. The first batch, comprising samples 1 and 2 was from the 1946–7 whaling season, and the remaining eight were from the 1947–8 season.

When the containers were opened the superficial contents were discarded, and 2 g. were weighed out from the deeper contents. Each 2 g. sample was ground in a mortar, and 20 ml. of one-quarter strength Ringer's solution was added gradually to give a 1 in 10 weight for volume suspension. After allowing the gross particles to settle four serial 10-fold dilutions were made in one-quarter strength Ringer's solution from the supernatant fluid. Plate cultures were prepared from the initial suspension and the first two serial dilution tubes by putting 1 ml. in a Petri dish, adding 15 ml. of tryptic digest broth agar at 55° C. and thoroughly mixing by rotating and tilting the Petri dish. One set of plate cultures was incubated at 37° C. and a second at 25° C. After 48 hr. incubation an estimate of the numbers of viable aerobic organisms was made by taking an average of the counts of the two dilution plates of each series, showing a suitable number of colonies for counting. The results were expressed as the number of organisms per g. of dried whale meat, allowance being made for dilution. Estimation of the numbers of anaerobic bacteria was made from tubes of Robertson's meat broth inoculated with 1 ml. amounts of the 10-fold dilution series. After 48 hr. incubation the last tube of meat broth showing the characters of clostridial growth was regarded as containing one clostridium and from this tube the number of clostridia per g. was estimated. A series of tubes of iron litmus milk was also inoculated, and an attempt was made to estimate the number of *Cl. welchii* present by observing the formation of a stormy clot at 48 hr. Two tubes of Robertson's meat broth were inoculated with 1 ml. of the whole original suspension, including gross particles, and after incubation for 48 hr. one tube was set aside as a reference culture, and the second was examined microscopically for the types of bacteria present. The second culture was incubated for a further 5 days for pathogenicity tests. The pathogenicity of the 7 days' culture was tested by administering 0·5 ml. by oesophageal tube to three mice and by inoculating 0·5 ml. intramuscularly into three mice.

Isolation of clostridia

Isolation of the different types of clostridia present was made from the reference cultures representing thirteen samples, seven of which had proved pathogenic to mice by intramuscular inoculation of culture. A subculture from the reference culture was made in Robertson's meat broth and incubated at 37° C. for 48 hr. A tube of litmus-milk medium was then inoculated from the 48 hr. culture and incubated at 37° C. At 6 hr. the litmus-milk culture was subcultured on a blood agar plate which had been dried in the incubator at 37° C. for 2½ hr. The 48 hr. meat-broth culture was also heated at 80° C. for 10 min. and subcultured in Robertson's meat broth, which was incubated for 48 hr. and then subcultured to dry blood agar plates. The blood agar plates were incubated overnight anaerobically in a McIntosh and Fildes jar, and examined for the presence of clostridia, special attention being given to the detection of *Cl. welchii* on the plates inoculated from litmus milk. Colonies of different appearances were picked off and sub-cultured to blood agar plates, previously dried, which were then set up under anaerobic conditions as rapidly as possible.

https://doi.org/10.1017/S0022172400014546 Published online by Cambridge University Press

Subculture on blood agar of selected colonies was repeated twice, and pure cultures thus obtained were finally subcultured on Fildes's agar (5 % peptic digest of blood in tryptic digest broth agar) and in Robertson's meat broth for future reference. This procedure in most instances was sufficient to obtain pure cultures of the organisms examined, but in some cases further purification was required by selective plating and sometimes it was necessary to go back to the original culture.

Identification of clostridia

Identification was based on morphological characters, colonial appearance, biochemical reactions and the results of pathogenicity tests.

Morphology. Morphology was determined from smears, stained by Gram's method, from Robertson's meat-broth cultures and from surface colonies on Fildes's agar. Examinations were made after 18 hr. incubation and at 5–7 days, and on each occasion the culture was examined immediately after removal from the incubator and again after 24 hr. at room temperature. Special methods were also used for demonstrating spores, such as Fleming's carbol fuschsin-nigrosin and the following modified Ziehl-Neelsen stain:

Carbol-fuchsin, 5–7 min.
Wash in water.
Decolorize with 10 % H_2SO_4 for 1–2 min.
Counterstain for 2 min. with 1 % malachite green.

Colonial appearances

Surface colonies. Colonies of pure cultures on Fildes's extract and blood agar plates were examined daily for 3 days. The points noticed were colour, translucency, shape, elevation, appearance of the periphery and haemolysis on blood agar.

Deep colonies. Deep agar cultures for the observation of deep colonies were made from the Pasteur pipette that had been used for the inoculation of the fermentation media to be described later. The bacterial suspension remaining in this pipette was discarded, and the pipette was filled once with normal saline and emptied. The residual fluid was then sprayed on the surface of 5 ml. of digest broth agar at 55° C. in a 5 in. × ½ in. plugged tube by pressure on the teat of the pipette. The pipette was again rinsed as before but successively in two tubes of fresh saline and inoculation of the spray was made in the same manner to a second tube of melted broth agar. The latter process was repeated to inoculate a third tube of broth agar. After each inoculation the inoculum was thoroughly mixed by repeated inversion of the tube. Samples of the inoculated melted agar from each tube were removed with Pasteur pipettes which were about 4 in. in length from the shoulder to the plugged end. The drawn-off ends of the pipettes were sealed in the bunsen by-pass and the sealed pipettes were held with the point downward in test-tubes during incubation at 37° C. By this method it was possible to get sufficient dilution of the original suspension to obtain well-spaced deep colonies for observation in at least one of the inoculated pipettes. As in some cases growth was very difficult to obtain with the basal medium, five drops of Fildes's extract were added to the tubes before inoculation. In a few cases 0·1 % sodium thioglycollate was added to the medium to improve anaerobic conditions.

The tubes were examined after incubation for 1, 2, 3 and 7 days. The points noted were: density, shape, colour, appearance of the periphery with special reference to the outgrowths and splitting of the medium by gas formation. These observations were also a check on the purity of the cultures, because mixed cultures were immediately detected on examination by this method.

Motility

A half-inch column of fluid was drawn up from an 18 hr. Robertson's meat-broth culture in a Pasteur pipette. The capillary of the Pasteur pipette was sealed in the bunsen by-pass above and below the column of culture. The detached part of the capillary was fixed to a glass slide by plasticine, and the organisms directly observed in the capillary under polyric oil with the $\frac{1}{12}$ in. immersion objective.

Biochemical tests

Fermentations. The medium used for fermentation tests consisted of 1 % bactopeptone, 0·5 % sodium chloride, 0·5 % agar, 0·1 % sodium thioglycollate and 1 % of the fermentable substance under test with phenol red as indicator. The medium including the test substance was made without the addition of sodium thioglycollate, sterilized by steaming for 20 min. on 3 successive days and stored in 1 oz. universal containers. Sodium thioglycollate in a concentration of 10 % was autoclaved at 10 lb. pressure for 10 min. A drop of phenol red was added to the thioglycollate solution because it tended to become acid on standing and sometimes required adjustment before use. For fermentation tests the medium was melted in the steamer, distributed in 5 ml. quantities in 5 in. × ½ in. tubes, cooled to 55° C. and maintained at the latter temperature in a water-bath. Four drops of the 10 % sodium thioglycollate solution were added to each tube to give the required concentration of 0·1 %. The inoculum for each fermentation tube consisted of two drops from a Pasteur pipette loaded with a heavy suspension of organisms procured from a 24–48-hr. Fildes's agar slope culture washed down with 2 ml. of saline. The tubes were mixed by repeated inversion and incubated at 37° C. These were observed for 3 successive days, the final

reading being made on the third day. Some organisms completely bleached the indicator and, before making the final reading, it was necessary to add a few drops of indicator, which was mixed into the culture by rapid rotation of the tube between the palms of the hands.

Litmus milk

In the preparation of litmus milk medium fresh milk was used, this being essential for anaerobic work. The cream was removed in the usual manner by steaming in a 2 l. flask for 1 hr., allowing to cool, and decanting the milk, the cream being retained in the flask. Litmus was added to the skimmed milk and the medium was sterilized by steaming for 20 min. on 3 successive days. For the test 5 ml. quantities of the litmus milk medium in 5 in. × ½ in. tubes, to give a long column of medium, were used to which were added sufficient sterile iron filings to cover the point of a penknife. The tubes were inoculated with 1–2 ml. of a 24–48 hr. culture in Robertson's meat broth as it was found that even heavy inocula from Fildes's agar slopes frequently failed to give typical reactions with known cultures. The cultures were examined after 24–48 hr. and at intervals up to 28 days. Clot, acid, gas formation, discoloration, and digestion were observed.

Tests for proteolysis

Although meat broth cultures provided good evidence of proteolytic activity the changes in coagulated serum and nutrient gelatin were also observed.

Liquefaction of coagulated serum. Tubes of a medium consisting of two parts of horse serum and three parts of digest broth were inspissated as slopes for 2 hr. at 80° C. This was a longer period of inspissation than that usually recommended for the purpose, but if only 1 hr. inspissation was used the slopes tended to fall down the tube thus making the reading of results impossible. The slopes were inoculated from a Pasteur pipette loaded with a heavy suspension from a washed down Fildes's agar slope. The point of the pipette was pushed through the surface of the slope and a few drops were forced into the depths of the medium, and the remainder allowed to flow over the surface as the pipette was withdrawn. The inoculated slopes were incubated anaerobically at 37° C. for 5 days. The results varied from almost complete liquefaction of the medium (+ + +) to an irregular erosion of the surface (+) with a deposit of liquefied medium at the bottom of the tube.

Liquefaction of gelatin. Ordinary nutrient gelatin containing 10 % of gelatin was used. The culture tubes were inoculated with a few drops of a dense suspension from a washed down Fildes's agar slope by stabbing with a Pasteur pipette and incubated anaerobically at 37° C. After 5 days' incubation the tubes were placed in a refrigerator at 4° C. for half an hour to solidify, and then kept at room temperature for 2–3 hr. when liquefaction was observed. A control tube which was not inoculated was included.

Indole formation. The presence of indole was determined in a 5-day 1 % bactopeptone water culture. Equal quantities of culture and Ehrlich's indole reagent were mixed and one drop of a saturated solution of potassium persulphate was added.

Vanillin-violet reaction (Spray 1936). Ten drops of the reagent (5 % vanillin in 95 % alcohol) were added to 1 ml. of a 5-day bactopeptone water culture, and ten drops of concentrated HCl added immediately. *Cl. sporogenes* cultures produced a brilliant violet colour which was intensified by a further addition of the reagent, whereas *Cl. bifermentans* and other indole-producing organisms gave an orange colour. Indole negative organisms other than *Cl. sporogenes* gave no colour change.

Pathogenicity tests. The method used for pathogenicity tests on the isolated clostridia was the same as that used for the original cultures of dried whale meat.

RESULTS

The estimations of the numbers of the bacteria in the samples examined are shown in Table 1. In six of the batches, comprising twenty-five samples, the average number of aerobic bacteria was about 6000 per g., a remarkably low figure, and even in batches 1, 2 and 5, with an average of about 100,000 aerobic bacteria per g., the numbers were not unduly high. Sample 16 had an exceptionally high count but, unlike all the others, was a dehydrated preparation of liver. The types of aerobic bacteria, judging from cultures and microscopical appearances, were Gram-positive rods, mainly of the spore-bearing type, found in all but one sample, Gram-positive cocci, some of the faecal type, in about one half of the samples and Gram-negative bacilli in a quarter of the samples.

In estimating the population of clostridia it was assumed at first that the total number of these organisms per g. could be directly expressed from the highest dilutions showing the formation of gas in Robertson's meat broth, a feature generally regarded as highly characteristic of the growth of clostridia. We noted, however, a few instances in which gas was formed from Gram-negative aerobic bacilli. In a more complete examination we found that this occurred in only two of twenty samples, in which instances these aerobic Gram-negative bacilli produced gas beyond the range of the anaerobic bacteria to give a 1000-fold overestimate of the numbers of anaerobic bacteria present as judged on this basis. It was noted, however, that these aerobic bacteria produced an abundance of gas without digesting the meat, giving a characteristic appearance which was

Table 1. *Dehydrated whale meat. Numbers of bacteria and pathogenicity of cultures*

Batch no.	Sample no.	Aerobic bacteria: Count in thousands per g. 37° C.	Aerobic bacteria: Count in thousands per g. 25° C.	Aerobic bacteria: Types seen: Cocci	Aerobic bacteria: Types seen: Gram-negative bacilli	Aerobic bacteria: Types seen: Gram-positive bacilli	Anaerobic bacteria: Number per g.	Anaerobic bacteria: Pathogenicity to mice of meat-broth culture at 7 days: Intramuscular	Anaerobic bacteria: Pathogenicity to mice of meat-broth culture at 7 days: Per os
1	1	80	0·1	+	+	+	10^4	−	−
	2	20	0·3	+	+	+	10^4	+	−
2	5	200	4	+	−	+	10^5	−	−
	6	14	27	−	+	+	10^3	−	−
	7	170	130	+	+	+	10^4	−	−
	8	70	65	+	+	+	10^5	+	−
	9	250	600	+	−	+	10^4	+	−
	10	200	120	+	+	+	10^2	+	−
3	14	0·4	0·5	+	−	+	10^3	−	−
	15	0·7	0·3	−	+	+	10^3	−	−
	16	3,500	2,000	+	+	+	10^3	−	−
	17	1·3	0·8	−	−	+	10^6	+	−
	18	0·5	0·3	+	−	+	10^3	−	−
	19	1·7	1·2	−	+	+	10^4	−	−
4	20	2·7	1·3	−	−	+	10^3	+	−
	21	0·7	0·4	−	−	+	10^3	+	−
	22	3·2	1·0	−	−	+	10^3	+	−
	23	2·0	1·7	−	−	+	10^4	+	−
	24	1·1	1·1	−	−	+	10^3	+	−
	25	2·8	2·8	−	−	+	10^4	+	−
5	26	77	210	+	−	+	10^1	+	−
	27	83	84	+	+	+	10^3	+	−
	28	130	140	+	−	+	10^4	−	−
	29	17	20	+	−	+	10^3	−	−
	30	160	140	+	−	+	10^3	+	−
	31	110	130	+	−	+	10^3	−	−
6	32	13	29	−	−	+	10^5	+	−
	33	19	120	+	−	+	10^4	+	−
	34	9·6	70	+	−	+	10^4	+	−
	35	5·6	9·5	−	−	+	10^3	+	−
7	36	8·5	8·3	−	−	+	10^4	+	−
	37	14	120	−	−	+	10^3	+	−
	38	3·2	64	−	−	+	10^4	+	−
8	39	5·4	4·6	−	−	+	10^3	−	−
	40	5·1	3·2	−	−	+	10^3	−	−
	41	11	8·6	+	−	−	10^2	+	−
9	42	6·0	4·8	+	−	+	10^2	−	−
	43	4·8	4·0	−	−	+	10^2	−	−
	44	2·6	3·0	−	−	+	10^4	+	−

not readily confused with the growth of proteolytic clostridia so that this infrequent error was not thought to affect the counts materially. We had considered preliminary heating as a way of getting over this difficulty, but we did not regard this method as desirable because we were concerned with the total population of anaerobic organisms present including vegetative forms, and especially *Cl. welchii* which spores rarely and is thus more susceptible to heat than other clostridia.

The final estimates of the number of clostridia present in the samples of dried whale meat are shown in Table 1 from which it may be concluded, with due regard to the limitations of the methods used, that clostridia and aerobic bacteria were present in small and about equal numbers.

https://doi.org/10.1017/S0022172400014546 Published online by Cambridge University Press

Pathogenicity

As is shown in Table 1 none of the cultures had any pathogenic effect when given to mice by mouth. On the other hand, when administered by intramuscular inoculation, twenty-three out of thirty-nine samples caused the death of at least two out of three mice. All these deaths, however, occurred within 1 hr. and were preceded by characteristic symptoms. These symptoms, appearing within 5 min. after inoculation, consisted of tremor and violent tetanic spasms which were readily incited by sensory stimuli such as noise or touch, and were followed by death within an hour or extreme prostration with complete recovery.

Identification of clostridia

From the thirteen samples selected for the investigation of the different types of clostridia present a total of seventy-two strains were isolated. In Table 2 it is shown that three to nine different types of clostridia were isolated from individual samples; *Cl. sporogenes* was present in all the samples, *Cl. bifermentans* and *Cl. tetanomorphum* were present in almost all the samples, whereas the other identifiable species were much less frequent.

genicity after 7 days' incubation in Robertson's meat broth by the same method as that used for testing the primary culture of dried whale meat. As is shown in Table 3 no isolated strain was pathogenic in the sense of producing death by anaerobic infection or by clostridial toxin. Such deaths as did occur were of a similar nature to those previously described and were produced only by cultures of *Cl. sporogenes*. However, four strains of unidentifiable clostridia, all belonging to one type (no. 2), produced the characteristic symptoms without death. In further investigations we found that symptoms were not produced until the cultures were at least 4 days old, and deaths occurred only when the ammonia content of the cultures attained a level of about 700 mg./100 ml. The part played by ammonia was also confirmed by injecting mice with solutions of ammonium chloride, in which case it was found that the lethal dose was reached with an equivalent concentration of ammonia of about 800 mg./100 ml.

DISCUSSION

There are no standards in current use for the bacteriological content of meat products for human con-

Table 2. *Types of clostridia in thirteen samples of dehydrated whale meat*

Organism	Sample no. 5	6	7	8	16	18	19	20	21	26	27	32	35	Number of times isolated
Cl. sporogenes	+	+	+	+	+	+	+	+	+	+	+	+	+	13
Cl. bifermentans	+	+	+	+	+	+	+	+	+	+	+	−	+	12
Cl. tetanomorphum	+	+	+	+	+	+	+	−	−	+	+	−	+	10
Cl. welchii	−	+	+	−	−	−	−	−	+	−	−	−	+	4
Cl. tertium	+	−	+	−	−	−	−	−	−	+	+	+	−	5
Cl. butyricum	−	−	−	−	−	−	−	+	+	−	−	+	−	3
Cl. cochlearium	+	−	−	−	−	−	−	−	−	−	−	−	−	1
Unidentified types	1	2	4	3	2	0	0	2	1	1	1	1	1	19
Total number of different types isolated in each sample	6	6	9	6	5	3	3	5	5	5	5	4	5	67

In Table 3 are shown the characters by which these organisms have been identified, and also the properties of the unidentifiable strains. It will be noted that eight of the nineteen unidentified strains are closely related and have been regarded as being of one type, whilst the remainder have been differentiated into five types. It would be possible to match some of the unidentified clostridia with previously described species, but in our opinion this would serve no useful purpose. With both *Cl. welchii* and *Cl. bifermentans* we have regarded those not fermenting salicin as typical strains, and those fermenting this substance, but similar in other respects, as variants.

All the isolated strains were tested for pathosumption, but it is noteworthy that Tanner (1944) states that ground butchers' meat should contain not more than 10 million organisms per g., that is to say about 100 times more that the numbers we have found in whale-meat meal. Tanner's figure includes putrefactive anaerobic organisms, but no separate figures for these are available. The numbers of aerobic and anaerobic bacteria in the samples of whale-meat meal that we have examined would thus appear to be low for this type of meat product.

Bacterial multiplication will not occur in a dehydrated product if the moisture content is kept below 30 %, which is well above that of the product we have been examining. From this it is evident that the bacterial population present in dried whale meat

https://doi.org/10.1017/S0022172400014546 Published online by Cambridge University Press

Table 3. *Characters on which identification of clostridia was based*

Type	No. of strains	Spores	Surface colony	Deep colony	Cooked meat	Litmus milk	Fermentations: Gluc.	Fermentations: Sac.	Fermentations: Lact.	Fermentations: Sal.	Gelatin	Coagulated serum	Motility	Indole	Vanillin violet	Pathogenicity
Cl. sporogenes	13	Subterminal, oval	Clear, rhizoid edge	Woolly ball	Digestion, foul odour, blackening	Clot, digestion, blackening	A	–	–	–	+	+++	+++	–	+	CL
Cl. bifermentans	7	Central or subterminal, oval	Large, opaque, irregular	Opaque, flaky	Gas, odour, slight blackening	Digestion, blackening	A	–	–	–	+	++	+	+++	–	–
Cl. bifermentans (var.)	9	Central or subterminal, oval	Large, opaque, irregular	Opaque, flaky	Gas, odour, slight blackening	Digestion, blackening	A	–	–	A	+	++	+	+++	–	–
Cl. tetanomorphum	10	Terminal, spherical	Small, clear, irregular	Small, irregular	Gas	No change	–	–	–	–	–	–	++	+	–	–
Cl. welchii	3	None seen	Circular, raised, opaque	Lenticular	Pink, gas	Stormy clot	A	A	A	–	+	–	–	–	–	–
Cl. welchii (var.)	2	None seen	Circular, raised, opaque	Lenticular	Pink, gas	Stormy clot	A	A	A	A	+	–	–	–	–	–
Cl. tertium	5	Terminal, oval	Small, round, central papilla	Lenticular	Gas	Late clot	A	A	A	A	–	–	+	–	–	–
Cl. butyricum	3	Subterminal, oval	Round, central papilla, opaque	Lenticular	Gas	Stormy clot	A	A	A	A	–	–	+	–	–	–
Cl. cochlearium	1	Terminal, oval	Small, irregular	Small, irregular	Gas, slight odour	—	–	–	–	–	–	–	+	–	–	–
Unidentified (1)	8	Subterminal, large, oval	Round, opaque, irregular, cent. papilla	Dark centre, fluffy edge	Gas, sour	—	A	–	–	–	–	–	+	–	–	–
Unidentified (2)	4	Scanty, subterminal, oval	Rhizoid	Dark centre, fluffy edge	Gas, and later rancid, blackening, crystals	Discoloration	–	–	–	–	–	–	+	–	–	C
Unidentified (3)	2	Subterminal, oval	Clear, rhizoid	Irregular	Gas	—	–	–	–	–	–	–	+	–	–	–
Unidentified (4)	3	None seen	Clear, irregular	Round, irregular	Gas	—	–	–	–	–	–	–	+	–	–	–
Unidentified (5)	1	None seen	Opaque, irregular	Irregular	Gas, faint odour	Clot, digestion	–	–	–	–	+	+++	+	–	–	–
Unidentified (6)	1	None seen	Flat, spreading	Small, irregular	Gas	—	–	–	–	–	–	–	+	+	–	–

A = acid. CL = convulsant and quick lethal factor. C = convulsant factor.

must have been already present as such in the original material from which the dried preparation was made or has been the result of multiplication of bacteria during the process of manufacture. We are not in a position to give an opinion on this question which can only be settled by investigation at the site of operation.

The majority of clostridia found were spore-forming types, as would be expected because of the heat applied during manufacture. This may also account for the small number of samples from which we isolated *Cl. welchii*, an organism which rarely forms spores. The types of anaerobes we have isolated from these dried products, their distribution, and the proportion of unidentified types do not appear to differ from those found in surveys made on human material (Smith & George, 1946; MacLennan, 1943). It would thus appear that the clostridial flora of the dried whale meat is what would be expected from any mammalian source.

In the examination of the isolated clostridia for pathogenicity we found that deaths occurred only with cultures of *Cl. sporogenes* with a high content of ammonia. This phenomenon is similar to that described by Barger & Dale (1915), who observed that some old cultures of *Cl. sporogenes* gave rise to toxic manifestations in the guinea-pig and regarded toxicity as being due to volatile ammonium base. It has also been observed by Meyer & Dubovsky (1922), and by Haines (1942), who points out the difference between this phenomenon and the effects of true botulinum toxin which causes characteristic symptoms only after 3–6 hr. but always with fatal results. Like Barger & Dale we have been able to correlate the toxicity of our *Cl. sporogenes* cultures with ammonium content, and have found that when this rises above 500 mg./100 ml., symptoms result in mice after injection of 0·5 ml. and in concentrations of over 700 mg./100 ml. death occurs. That ammonia itself can produce this effect has been confirmed by the injection of ammonium chloride, which causes similar effects at these equivalent concentrations of ammonia.

In our pathogenicity tests we have not encountered any deaths in mice that can be attributed to clostridial infection or the effects of clostridial toxin. The only organisms of possible pathogenic significance that we have isolated are five strains of *Cl. welchii*, none of which proved pathogenic to mice in our tests. We have, however, isolated a strain of *Cl. septicum* from a sample of frozen whale meat which was pathogenic to mice and could be neutralized specifically by anti-septicum antitoxin. The finding of pathogenic clostridia in whale meat was to be expected from our knowledge of the distribution of anaerobes in similar products from other sources. Since these clostridia are commonly found in mammalian faeces, and in soil, and are therefore ubiquitous, their presence in whale meat should not be regarded as a special risk to handlers of these products.

The main purpose of this investigation was to determine whether dried whale-meat products might be responsible for food poisoning when used for human consumption. The conditions under which poisoning is likely to arise would be in made-up dishes or in open packs. We therefore gave full opportunity for the development of bacterial toxins or other toxic products by testing meat-broth cultures which had been incubated at 37° C. for 1 week. As can be seen in Table 1 no such poisonous substance was detected when large quantities were given directly into the stomachs of mice. In fact, the only evidence we have obtained of the production of toxic substances is in the poisoning effect of ammonium compounds which produced death only by inoculation and had no effect whatever when given by mouth.

There have been reports in which food poisoning has been attributed to *Cl. welchii* and *Cl. bifermentans*, but these cases have always been mild and there has been no question of danger to life. There is also the possibility that some of these cases may have been due to poisoning by staphylococcal enterotoxin which had not been fully inactivated by heat, whereas the staphylococci had been killed leaving only the anaerobic bacteria for identification by bacteriological investigation. The only clostridial toxin which has been proved to be pathogenic to man by mouth is that of *Cl. botulinum*, an organism that has been shown by Haines (1942) to be common in the soil of England. *Cl. botulinum* is a very rare cause of food poisoning and, so far as we are aware, has never been isolated from fresh or dehydrated meat. By methods similar to those described by Haines we have been unable to detect any evidence of botulinum toxin or to isolate the organism from any sample derived from whale meat. In any case *Cl. botulinum* comes from the soil and is not likely to be found in whale meat unless vegetable products are added in the making up of the product for human consumption.

SUMMARY

The numbers and types of bacteria in thirty-nine samples of dried whale meat of the 1946–7 and 1947–8 Antarctic whaling seasons have been investigated.

The total number of bacteria was low for this type of product and there were about equal numbers of clostridia and aerobic bacteria.

A detailed examination of the clostridia present showed that they were of types commonly found in material from mammalian sources.

No *Clostridium* pathogenic to the mouse was found, although some cultures on prolonged incubation

killed mice by ammonia poisoning. Ammonia poisoning was produced by injection of cultures and never by oral administration.

We wish to acknowledge our debt to the late Prof. James McIntosh, M.D., LL.D., who taught us the methods used in this investigation and our thanks to the Director General, Army Medical Services, for permission for one of us (A.M.P.) to take part in this work.

REFERENCES

BARGER, G. & DALE, H. H. (1915). *Brit. Med. J.* **2**, 808.
HAINES, R. B. (1942). *J. Hyg., Camb.*, **42**, 323.
MACLENNAN, J. D. (1943). *Lancet*, ii, 94.
MEYER, K. F. & DUBOVSKY, B. H. (1922). *J. Infect. Dis.* **31**, 501.
SMITH, L. DE S. & GEORGE, R. L. (1946). *J. Bact.* **51**, 271.
SPRAY, R. S. (1936). *J. Bact.* **32**, 135.
TANNER, F. W. (1944). *Microbiology of Foods*, p. 855, Champaigne, Illinois: Garrard Press.

(*MS. received for publication* 15. vii. 49.—Ed.)