Journal of Applied Bacteriology 1978, **44**, 1–56

A REVIEW

Possible Sources of Ethanol Ante- and Post-mortem: its Relationship to the Biochemistry and Microbiology of Decomposition

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Received 14 March 1977 and in revised form 3 November 1977

Contents

1. Introduction

ETHANOL (ETHYL ALCOHOL) consumption is a frequent direct or indirect cause of death and injury, and a great deal of attention has been paid during the last 50–100 years to the development of methods of determining ethanol and to assessing their reliability, particularly in samples of blood and tissue taken after death. British public interest in this subject was stimulated during the inquest in April 1975 into the death of 42 passengers and the driver of an underground train after it had crashed into a dead-end tunnel at Moorgate in the City of London.

No satisfactory explanation to account for the accident has been found and, during the inquest there was some disagreement about the origin of ethanol found in the corpse of the train driver (e.g. Robinson 1975*a*,*b*; Williams 1975). Almost five days elapsed between the crash occurring and the taking of samples from the corpse.

The statement by the coroner at the same inquest that '... there is a paucity of work—almost a desert—on decomposition alcohol in a body' (Inquest Transcript, p. 535), although the coroner also claimed that examination of putrefied bodies for drugs and ethanol was common in his district, indicated that a review of literature on this subject, to draw together the considerable body of information, would be useful and might help resolve the difference of opinion.

There is no evidence that ethanol can be produced after death other than by microbial activity. However, disappearance of ethanol can occur, also, by physical (evaporative) and chemical mechanisms (Smalldon & Brown 1973). This review will concentrate on microbial aspects of ethanol production. Available literature on ethanol formation in bodies, (ante- and) post-mortem, information on the types of micro-organism normally present in the tissues after death, and their relative importance during decomposition will be summarized. Finally, an attempt will be made to suggest those organisms most likely to produce ethanol post-mortem on a basis of available information on yields of ethanol during fermentation and the substrates available post-mortem. The evidence of post-mortem ethanol levels will be discussed with respect to the Moorgate Inquest in a second publication (Corry, in preparation).

2. Methods of Ethanol Determination

Any assessment of the significance of ethanol levels should include some information on the methods of determination used. Publications on this subject are numerous, and it is not proposed to attempt any comprehensive survey of methods. Review articles by Dubowski (1956), Lundquist (1959) and Jain & Cravey (1972), among others, cover the subject adequately.

A. Chemical methods

Until the 1950s all the methods used were chemical, usually involving distillation or evaporation of the volatile substances from the sample, followed by determination of reducing substances in the distillate, usually by titration with a dichromate/sulphuric acid mixture (see Gonzales *et al.* 1954). Popular methods were those of Widmark (1918*a*,*b*, 1922) or Cavett's (1938) modification of Widmark's method, in which a known weight of sample is enclosed with a standard solution of dichromate/sulphuric acid in separate compartments of the same container, incubated to allow volatile

products to diffuse, and then back-titrated to determine the level of reducing substances. This method is quite satisfactory for examination of samples where ethanol is the only volatile reducing substance present, but it is non-specific and requires more elaborate tests to exclude the possibility of the presence of other volatile reducing compounds—e.g. acetone or methanol. For the routine examination of fresh or preserved samples of blood or urine taken from living subjects, this method is generally satisfactory—except, for instance, if the subject has inhaled or injected a mixture of solvents or is an untreated diabetic, when ketone bodies might be expected. Also, care must be taken to avoid dirty equipment and contamination with solvents, especially when using a micro-method.

B. Enzymic method

The development of an enzymic method, using purified alcohol dehydrogenase (ADH) and nicotinamide adenine dinucleotide (NAD) in the early 1950's (Bonnichsen & Theorell 1951; Bücher & Redetzki 1951) gave a more specific and sensitive system for determination of ethanol levels, which did not require distillation procedures. However, yeast ADH (normally used) reacts to a lesser extent with higher primary alcohols—particularly *n*-propanol and *n*-butanol. Reactivity decreases with increases in carbon chain length, but the reaction rate with methanol is very low (Bücher & Redetzki 1951; van Eys & Kaplan 1957; Smith & Olson 1975). The enzymic method is less sensitive to contamination than the chemical method, but samples cannot be preserved with any substance which would interfere with ADH activity—fluoride or mercury-containing preservatives (commonly added to blood and urine samples) could not be used.

C. Gas chromatographic methods

The third and most specific method, now widely used in forensic science laboratories for the routine examination of large numbers of specimens is that of gas chromatography. Many variations of the method exist (Jain & Cravey 1972) but the method used in this laboratory (a modified version of that described by Curry *et al.* (1966)), involves dilution of the specimen (blood or urine) with an internal reference compound (*n*-propanol), equilibration of the sample in a closed vessel at elevated temperature (60 °C), and sampling of the gas phase. Volatile substances are detected by flame ionization after passing through a column (in this laboratory the column contains Porapak Q, a cross-linked polystyrene resin) which gives a good separation of the volatile substances (and other volatile substances that may be encountered). This method of analysis has the advantage of being almost completely specific for ethanol (other volatiles can be detected and identified, which may also be of value) and preservatives do not cause problems.

3. Levels of 'Endogenous' Ethanol

Alcohol dehydrogenase is found at very high levels in the human liver, and at lower concentrations in other tissues such as the kidney, spleen, lung and gastric mucosa (Von Wartburg & Papenberg 1966). Naturally there has been some speculation as to the 'original' function of the enzyme—i.e. is there a source of ethanol other than food or drink? This subject was reviewed by Lester (1961), who concluded that available data indicated levels of ethanol in human blood of 2–3 mg/100 ml. However, a later experimental study (Lester 1962) indicated much lower levels—of the order of 0·15

mg/100 ml blood (determined using GLC). McManus *et al.* (1966) found levels of ethanol from 1 to 3·6 mg/100 ml in various rat tissues using an ADH method. These authors considered that the ethanol could have been formed in the tissues by the action of a 'pyruvate dehydrogenase system' requiring pyruvate dehydrogenase, thiamine pyrophosphate, pyruvate, Mg^{++} and NADH. This system is similar to that found in the Enterobacteriaceae (see Section 7*A*). A study by Krebs & Perkins (1970), using a yeast ADH detection method, indicated that ethanol was normally found in the alimentary canal of rats, the highest levels being in the stomach with an average of 3·7 mM (17 mg/100 ml). Some of this ethanol was absorbed into the blood—levels in the portal vein averaged 0·045 mM (0·21 mg/100 ml), while in the hepatic vein, inferior vena cava and aorta it was about 15 times lower. This indicated that the liver was able rapidly to remove the ethanol. In germ-free rats the levels of ethanol were much lower, both in the intestine (1/10) and in the blood (1/3), and the authors postulated that this ethanol could have arisen from acetaldehyde formed during degradation of threonine, deoxyribose phosphate and β-alanine. The stomachs of rats normally contain high numbers of yeasts and lactobacilli (Savage 1970) but human stomachs normally contain much lower numbers of micro-organisms (Savage 1970; Bauchop 1971). It would therefore seem likely that 'normal' levels of ethanol in the human stomach would be lower. There do not, however, appear to be any data on levels of ethanol in the human portal vein. Further evidence of the effect of microbial ethanol production has been obtained, where high levels of ethanol in the blood of lambs, piglets and calves fed on milk substitutes were associated with high numbers of *Torulopsis* yeasts in the stomach (White *et al.* 1972; White 1974). These authors, using GLC, demonstrated up to 500 mg/100 ml ethanol in the stomach, and levels of 300–500 mg/100 ml of ethanol in plasma from the recurrent tarsal vein or jugular vein of lambs. They state that 'Clinical signs of "drunkenness" were readily observed'. In piglets high levels were also observed in the stomach, but not in the plasma, presumably because they were better able to metabolize ethanol. Ethanol levels were found to be lower in the intestine than in the stomach. Ethanol has been demonstrated in concentrations from 11–144 mg/100 ml in jejunal aspirates from humans suffering from tropical sprue, when high levels of micro-organisms were also detected (Klipstein *et al.* 1973). Levels of blood ethanol from 0·15–4·12 mg/100 ml (mean 1·8 mg/100 ml) have been detected in 7/20 patients after jejunoileal bypass for morbid obesity (Mezey *et al.* 1975). Blood ethanol levels were negligible in the same patients before operation and in eight normal control subjects, and ethanol production was presumably associated with increased microbial activity due to the reduced intestinal absorption of nutrients, although microbiological sampling was not carried out. Similar levels of blood ethanol might be expected in other conditions besides tropical sprue, where intestinal absorption of nutrients is impaired (e.g. coeliac disease). Stomach infections with *Sarcina ventriculi*, which produces high levels of ethanol from glucose (see Section 7) are another possible source of 'endogenous' ethanol (Barnes 1854; Smit 1933; Canale-Parola 1970).

4. Evidence of Post-mortem Production of Ethanol

Numerous authors (Nicloux 1936*a*; Redetzki *et al.* 1952; Gonzales *et al.* 1954; Gormsen 1954; Schwerd 1954; Wolthers 1958; Paulus & Janitzki 1959) agree that very inaccurate results may be obtained if putrefying tissues are assayed for ethanol

using one of the traditional chemical methods such as that of Widmark (1918*a*,*b*, 1922) or Nicloux (1896, 1906). This is because many volatile reducing products other than ethanol may be produced during putrefaction. Modifications to the chemical method for assaying ethanol have been devised in order to overcome the problem (Nicloux 1936*a*; Bonnichsen *et al.* 1953; Gonzales *et al.* 1954), but the development of the more specific alcohol dehydrogenase (ADH) and gas chromatographic (GC) methods have simplified matters. Gas chromatography even enables other volatile products to be detected and identified (cf. Section 2).

Since it is very difficult to be certain that human subjects did not imbibe ethanol before death, and because storage experiments with putrefying human corpses are difficult both ethically and aesthetically, studies on laboratory animals may give more accurate information, and will be considered before evidence of ethanol production in humans.

A. Ethanol formation in dead animals

Survey of the literature has revealed only five studies on neoformation of ethanol in animal corpses after death (Nicloux 1935*b*; Nanikawa & Kotoku 1971; Davis *et al.* 1972; Iribe *et al.* 1974; Tomita 1975). All the studies were carried out on rodents. Nicloux carried out elaborate experiments with mice, strangled, enclosed in miniature coffins and left in air at various temperatures or buried. He also studied the rate of disappearance of ethanol in mice injected with ethanol immediately before death, as well as appearance of ethanol in control mice (Fig. 1). Ethanol disappeared at a rate that depended on the temperature of storage—the rate was slowest at the lowest temperature, taking more than 100 d to reach zero in mice at 3 °C, and about 25 d in mice at 20–22 °C (readings were corrected for neoformation of ethanol by use of the control mice). Concentrations of post-mortem ethanol reached a maximum at a time that again depended on the temperature and conditions of storage. Levels of ethanol as high as 100 mg/100 g were detected in mice that had taken no ethanol before death. Only one mouse/sampling time was tested, although ethanol levels in pairs of mice treated in the same way were similar (Nicloux 1936*a*). The method of analysis was, at that time, of course, chemical and involved triple distillation and acid and alkaline treatment to remove non-ethanol compounds. Added ethanol could be recovered quantitatively from similar samples (Nicloux 1936*a*). Butanol was always detected with neoformed ethanol.

Nanikawa & Kotoku (1971), using GC, found levels of ethanol up to about 100 mg/100 ml in the intraperitoneal fluid of mice incubated for 4–5 d at 30 °C in air. Yields after incubation in water were lower. Subcutaneous injection of glucose (1 ml of 50% glucose solution) 2 h before death resulted in over 200 mg/100 ml ethanol after incubating the bodies at 15 °C for 5 d, compared with about 70 mg/100 ml in control bodies. In addition to ethanol, these authors reported that *n*-propanol and acetaldehyde were also found, but in lower concentrations. They therefore suggested that *t*-butanol should be used as internal standard when assaying for ethanol by GC, rather than *n*-propanol. Comparison of levels of putrefactive ethanol in rabbit corpses after 7 d at 15 °C showed that levels were highest in pleural fluid and cardiac blood (means of 61 and 41 mg/100 ml respectively), lower in femoral vein blood and vitreous humour (means of 25 and 24 mg/100 ml respectively) and lowest in the urine (mean of 12 mg/100 ml).

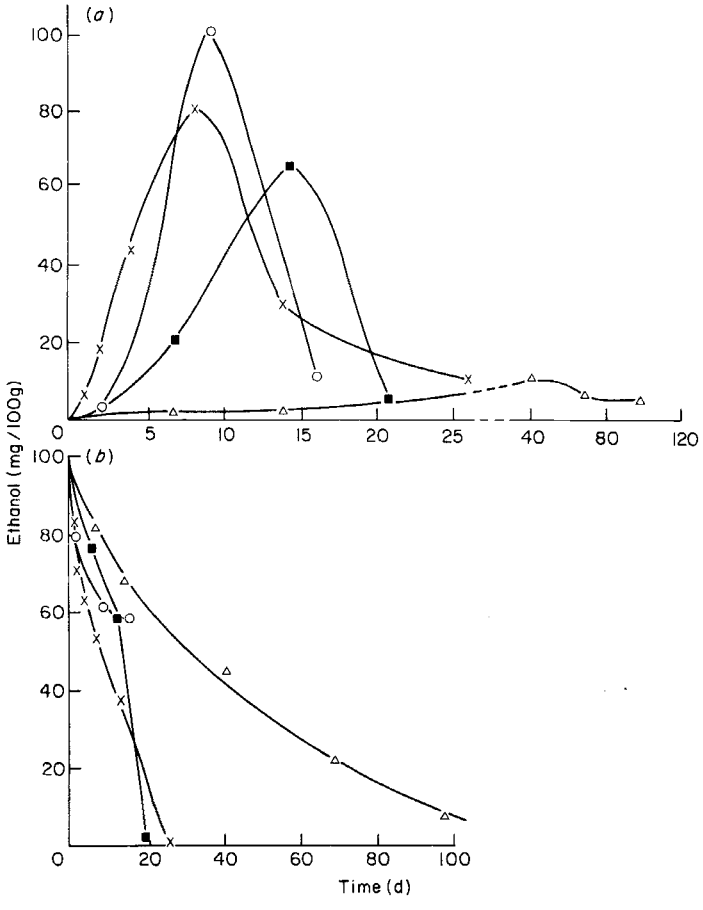


Fig. 1. (*a*) Neoformation of ethanol, (*b*) disappearance of ethanol (corrected for neoformation), of mouse corpses stored in lead-lined, screwed-down oak coffins: ×, 20–22 °C; ○, 15–18 °C; △, 3 °C; ■, buried. (From data of Nicloux 1935*b*.)

Davis *et al.* (1972) used GLC methods to study ethanol levels during decomposition of germ-free and conventional mice. The mice were stored in sterile, humidified (to prevent desiccation and hence inhibition of microbes) aerated containers at room temperature. Individual organs were stored, as well as intact bodies. Ethanol was detected in all three tissues examined from whole bodies (brain, liver and lung). By five days the mean ethanol concentration in all organs was over 50 mg/100 g, although there was frequently a wide variation in ethanol concentration between organs from different mice and from one sample-time to another (see Fig. 2). Three mice or sets of organs were examined at each sampling time. Ethanol production in brain seemed to take a little longer than in the other two tissues. Isolated organs gave lower levels of ethanol than those left *in situ*. Germ-free mice neither putrefied nor produced ethanol during storage. In addition to ethanol, acetaldehyde and acetone were detected in the organs of both conventional and germ-free mice after 3 d (and lesser quantities in isolated organs). Other volatile products were detected after 3 d or more in organs from conventional intact mice only. Presumptive propionic acid was found repeatedly, and presumptive

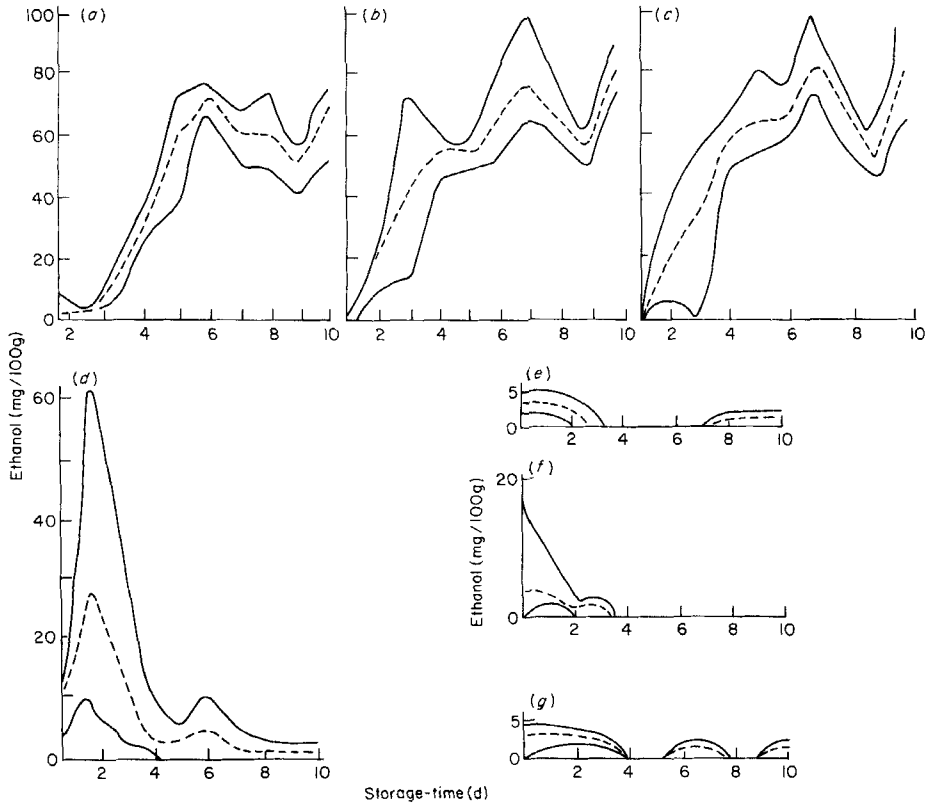


Fig. 2. Neoformation of ethanol at 22 °C in (*a*)–(*c*) organs from intact mice ((*a*) brain, (*b*) liver, (*c*) lung); (*d*)–(*g*) organs stored separately ((*d*) liver, (*e*) lung, (*f*) muscle, (*g*) brain). Areas enclosed by continuous lines, range of ethanol concentrations; broken line, mean ethanol concentration. (From Davis *et al.* 1972.)

*iso*propanol and *n*-propanol were found sporadically. Unfortunately no microbiological studies were carried out, but this work confirms the results of Nicloux (1935*b*, 1936*a*) showing neoformation of ethanol in bodies of putrefied mice, and indicates that the product is probably due to microbial action, since no ethanol was found after incubation of germ-free corpses. Presence of volatile compounds other than acetone and acetaldehyde also appears to correlate with ethanol production and the presence of micro-organisms (Yueh & Strong 1960; see Section 5*C*).

The study of Iribe *et al.* (1974) on ethanol production in rat corpses gave results similar to those of the previous two studies (Fig. 3), but maximum ethanol levels were attained later and were generally higher. Rats drowned and stored in water attained levels of 158 mg ethanol/100 ml after 8 d at 20 °C and 104 mg/100 ml after 13 d at 14 °C. In air, maximum ethanol levels of 152 mg/100 ml at 25 °C after 7 d, and 106 mg/100 ml after 8 d at 17 °C were achieved. Rats burned to death showed much lower ethanol levels after incubation, possibly because of the inhibition of microbial proliferation by desiccation, since no mention was made of humidification during storage.

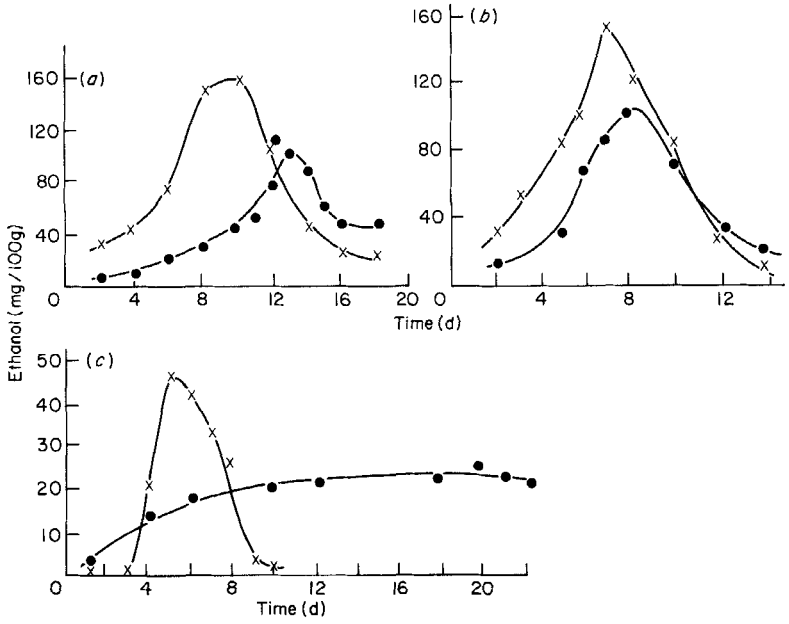


Fig. 3. Neoformation of ethanol in corpses of rats (average levels in fluid from chests of three rats at each sampling time). (*a*) Drowned and stored in water: ×, at 20 °C; ●, at 14 °C. (*b*) Strangled and stored in air: ×, at 25 °C; ●, at 17 °C. (*c*) Burnt to death and stored in air: ×, at 25 °C; ●, at 17 °C. (From Iribe *et al.* 1974.)

TABLE 1

Ethanol levels in human corpses (from Bonnichsen et al. *1953)*

Tissue	Case No. (mg ethanol/100 g)			
	1	2	3	4
Stomach contents	310	23	—	17
Pleural fluid	230	—	—	—
Blood	19	164	0	235
Liver	26	—	—	112
Brain	21	24	31	38
Urine	0	6	—	—
Kidney	—	39	79	107
Muscles	—	—	—	0
Heart	—	—	—	0
Spleen	—	—	—	111

—, Not done.

Tomita (1975) detected various alcohols during putrefaction of mouse corpses. Ethanol was found at concentrations up to *ca.* 110 mg/100 ml in corpses stored in air at 25 °C for 2–3 d. *n*-Butanol was also detected at levels up to 60 mg/100 ml. *n*-Propanol was present at lower levels (up to 25 mg/100 ml) and *iso*butanol, *iso*-propanol and *iso*pentanol were found occasionally, in addition to acetone, acetaldehyde, methylethyl ketone amines and acids (Tomita 1976; Table 7*b*). As in all the other studies, ethanol levels (and those of other alcohols) increased and subsequently

decreased during incubation. The time at which maximum ethanol levels were achieved, and the maximum level, depended on the temperature and conditions of incubation.

B. Ethanol formation in human corpses

Although Turkel & Gifford (1957) considered that microbial activity did not produce ethanol in the human body after death, this view is not a general one. The earliest report of detection of ethanol in human corpses, using a specific method to detect ethanol, appears to be that of Nicloux (1936*b*) who examined five bodies, 17 d–3½ months after death and found levels of ethanol from 40–132 mg/100 g in all tissues examined. There was no reason to believe, at least for three of the five, that they had drunk alcohol before death. The method of analysis was similar to that used during his studies on mouse corpses. Another study was published in the same year (Wagner 1936), which used Widmark's non-specific method of measuring ethanol. Wagner observed that levels of ethanol in corpses held at 6–8 °C from 8 h post-mortem fell by 5–6 mg/100 ml/d (i.e. 20–25% during the first 4 d). Results after 4 d were considered unreliable because of putrefaction. Samples from the heart and head were considered particularly unreliable and femoral vein samples were recommended.

Bonnichsen *et al.* (1953) gave details of several cases where high levels of ethanol were detected in corpses most of which had not been refrigerated for some time after death and where ingestion of alcohol prior to death was thought to be very unlikely. Ethanol levels were determined using the ADH method which agreed quite well with a specific chemical method when this was also carried out. Table 1 summarizes the results obtained, using the ADH method of ethanol analysis.

Case No. 1 showed very high levels of ethanol in the stomach and pleural fluid and low levels elsewhere. Microbiological examination of these two fluids revealed *Candida albicans*, *Saccharomyces* spp., coliform organisms, *Streptococcus faecalis* and non-haemolytic streptococci. The *Candida* and *Saccharomyces* spp. were both shown to be capable of producing ethanol in glucose broth but the other organisms were not further examined. Ethanol diffuses rapidly when it is ingested during life, and the deceased, according to police enquiries, had neither taken nor been administered any alcohol during the last 24 h of her life. These facts and the detection of micro-organisms capable of producing ethanol indicate that the source of ethanol was microbial.

Cases 2 and 3 were found dead together, again widely differing ethanol levels were found. The blood from case 2 was found to contain many 'ferment' (yeast) cells as well as a number of Gram positive and Gram negative rods, but the micro-organisms were not further identified. Some of this blood inoculated into 1% glucose in phosphate buffer yielded 80 mg ethanol/100 ml after 24 h and 260 mg/100 ml after 3 d at 25 °C. Cross-contamination during the post-mortem examination was suspected to account for the ethanol detected in other samples from cases 2 and 3.

Case 4, a three-month-old infant that had died suddenly, was found to have four strains of bacteria present in the blood—*Escherichia coli*, a *Klebsiella* sp., *Clostridium perfringens* (*welchii*) and *Streptococcus faecalis*; although none of these produced high levels of ethanol when incubated in glucose solution, it was considered that different substrates and/or conditions in the body might account for the high ethanol level.

Redetzki *et al.* (1952) observed wide variations in ethanol levels determined by the ADH method in different parts of putrefying bodies, and also that samples from the extremities showed lower levels than samples taken from deeper sites. Blood taken post-mortem and stored for 24 d showed increases of ethanol up to 35 mg/100 ml. Thoracic

TABLE 2

Ethanol levels (mg/100 g) in tissues from putrefied bodies (adapted from Gonzales et al. *1954)*

Case No.		Brain	Liver	Ratio liver/brain
Submerged cases (? specific chemical method)	1	210	430	2·05
	2	150	350	2·33
	3	80	280	3·50
	4	120	370	3·08
Non-submerged cases (using a specific chemical method)	5	16	19	1·19
	6	13	21	1·61
	7	81	107	1·32
	8	60	77	1·28
	9	102	113	1·18
	10	8	13	1·62

cavity fluid was sampled before and after storage of corpses at room temperature in sealed containers. Ethanol increased from zero initially to levels ranging from 6–136 mg/100 ml (mean 68). Gonzales *et al.* (1954) consider that ethanol production in putrified human bodies is common, and that levels 'approach the range of borderline intoxication as a limit' in bodies putrefying in air, but submerged putrefied bodies may contain higher ethanol levels—'although histories are seldom available in these cases, the consistently high alcohol findings are out of proportion to the probable percentage of intoxication in such cases' (see Table 2).

Wolthers (1958) after a comprehensive study concluded that neoformation of ethanol in human corpses was common, and that the presence of volatile products other than ethanol (indicated by higher ethanol estimations by the (chemical) Widmark method than by the ADH method) and inconsistencies in ethanol levels from one part of the corpse to another, both indicated that ethanol had not been ingested prior to death. Ethanol levels were generally highest in the deep tissues and lowest in the peripheries, related presumably to the levels of available oxygen and to slower cooling in deep tissues. He also detected the higher alcohols propanol, butanol and pentanol in some putrefied bodies (cf. Nanikawa & Kotoku 1971; and Tomita 1975.

Plueckhahn (1967) reported ethanol levels in heart blood of eight putrefied corpses ranging from 33–127 mg/100 ml, in spite of the fact that there was reliable presumptive evidence that at least four had not taken alcohol before death, and that in two cases (with levels of 89 and 127 mg/100 ml) the evidence was almost conclusive. In agreement with the observations of Wolthers (1958) levels of ethanol estimated from (peripheral) femoral blood vessels in the putrefied bodies were invariably lower than those detected in heart blood (deep tissue), and determinations carried out by the ADH method gave lower estimates for ethanol levels than using even the relatively specific chemical method of Kozelka & Hine (1941).

C. Ethanol formation in isolated tissues

Blood is the most frequently studied tissue for evidence of ethanol formation during storage. Nicloux (1935*a*) stored ox blood at various temperatures but found only low levels of ethanol after incubation (maximum of 9·1 mg/100 ml after 4 d at 20–22 °C; 5·5 mg/100 ml after 8 d at 15–18 °C and 3·7 mg/100 ml after 13 d at 3 °C). Ethanol added to the blood disappeared at a rate dependent on the temperature of

storage. Freimuth *et al.* (1951) measured levels of presumptive ethanol up to 210 mg/100 g in post-mortem blood incubated for up to 13 d at 20–26 °C, but since they used a non-specific chemical method of analysis, other reducing compounds would have increased the apparent ethanol levels. Samples of brain and liver (having, like the blood, initial ethanol levels of zero) also developed high levels of presumptive ethanol at 20–26 °C—up to 420 mg/100 g in brain and up to 210 mg/100 g in liver. Average values were generally lowest in blood and highest in brain (in contrast to the relationship observed by Gonzales *et al.* (1954) for brain and liver which had putrefied in whole bodies, Table 2). Ethanol levels in refrigerated tissue samples were much lower, generally not exceeding 20 mg/100 g although one sample of liver developed 140 mg/100 g presumptive ethanol after 13 d at 5 °C. Redetzki *et al.* (1952) using the ADH method observed increases in ethanol level up to 35 mg/100 ml in post-mortem blood stored for 2½ weeks at room temperature.

Gonzales *et al.* (1954) found only low levels of ethanol in human blood allowed to putrefy during refrigeration—4 mg/100 ml after 7 d and 40 mg/100 ml after 47 d. In agreement with the results of Nicloux (1935*a*,*b*) ethanol levels in tissues containing high levels of ethanol initially were found to decrease during storage, more rapidly at room temperature and more slowly at refrigeration temperature.

Studies of Schwerd & Garhammer (1953) and Schwerd (1954) revealed about 50 mg/100 ml ethanol after 8 d in aerated blood allowed to putrefy at room temperature and over 100 mg/100 ml ethanol in blood incubated anaerobically at room temperature. Methanol and pentanol were also detected. Similarly, Paulus & Janitzki (1959) found ethanol levels up to 29 mg/100 ml in blood from 24 of 145 'fresh' corpses with no history of alcohol consumption. Levels up to 16 mg/100 ml developed in fresh corpse blood with no ethanol initially, that was stored at room temperature for two weeks. Likewise, storage by Christopoulos *et al.* (1973) of oxalated corpse blood for 40–50 d, also at room temperature, resulted in neoformation of ethanol from zero initially to from 31 to 205 mg/100 ml. None of the above studies gave any details of initial glucose levels nor the micro-organisms present in the samples, both of which might be expected to influence the results (see Sections 5 and 7). However, these aspects have been considered in a few publications appearing within the last decade, particularly the work of Plueckhahn & Ballard (1968) and Blackmore (1968). The former authors isolated a variety of organisms from blood post-mortem (unfortunately only aerobically) and also determined glucose levels (and found a range of 19–1050 mg/100 ml). Inoculation of fresh blood plus 450 mg/100 ml glucose with a selection of the strains of micro-organism isolated (*Proteus* sp., *E. coli* and *C. albicans*) showed that up to 70 mg/100 ml ethanol (determined by a specific chemical method) could be detected in samples incubated for 2–9 d at 20–25 °C with no preservative. Incubation at 20–25 °C of 50 blood samples taken at autopsy resulted in significantly increased ethanol levels in 16 instances (see Table 3). Increases of more than 50 mg/100 ml were observed in eight instances, generally associated with high initial blood glucose levels (250–760 mg/100 ml). The greatest increases in ethanol levels were usually associated not with yeast contamination, but with Enterobacteriaceae or enterococci. Blackmore's (1968) study was on similar lines but used the specific GLC and GSC methods of ethanol analysis. Subsequent to finding raised and unevenly distributed ethanol levels in the bodies of crew members of a crashed aircraft, organisms were isolated from their bodies and reinoculated into carbohydrate and amino-acid containing media to assess their ethanol-producing capability. Table 4 summarizes the results obtained. Eleven of the 13 types of

TABLE 3

Details of the circumstances of death and collection of heart blood samples in 16 autopsies in which significant generation of ethanol occurred during storage of blood samples (adapted from Plueckhahn & Ballard 1968)

Cause of death	Age (years)	Hours after death: body refrigerated	Hours after death: blood sample taken	Organisms isolated by aerobic culture from sample at autopsy	Glucose (mg/100 ml)	Blood ethanol (mg/100 ml): at autopsy	Blood ethanol (mg/100 ml): increase after storage at 20–25 °C for 2–3 d	Blood ethanol (mg/100 ml): increase after storage at 20–25 °C for 6–10 d
Myocardial infarction	68	3	30	*Candida albicans*	258	282	43	72
Fractured skull	42	6	42	No growth	82	nil	8	10
Ruptured spleen, lacerated pericardium	19	2	34	*Klebsiella-Aerobacter* sp.	240	77	13	18
Fractured spine, drowning (sea water)	64	31	48	*Proteus* sp.	550	nil	33	53
Drowning (sea water)	26	4	20	*Staphylococcus* sp. *Bacillus* sp.	232	nil	0	18
Drowning (sea water)	37	3	41	*Staphylococcus* sp.	377	186	17	40
Fractured sternum, asphyxiation	39	1	16	*Pseudomonas* sp. *Streptococcus faecalis*	284	nil	32	52
Traumatic cerebral haemorrhage, ruptured spleen	21	8	32	*Streptococcus faecalis*	587	118	72	127
Multiple injuries, including ruptured liver and stomach	41	1	20	*Streptococcus faecalis*	193	40	8	14
Myocardial infarction	74	6	70	*Escherichia coli*	760	nil	82	102
Traumatic cerebral haemorrhage, multiple fractures	77	1	15	*Klebsiella-Aerobacter* sp. *Streptococcus faecalis*	176	nil	0	15
Myocardial infarction	74	6	70	*Pseudomonas* sp. *Paracolon* sp.	600	nil	75	75
Barbituric overdose	42	1	25	*Klebsiella-Aerobacter* sp. *Streptococcus faecalis*	153	nil	15	12
Pulping of skull, multiple fractures, including bones of chest	37	1	7	*Escherichia coli*	40	nil	0	10
Fractured thoracic spine, ruptured spleen	65	1	14	*Klebsiella-Aerobacter* sp. *Staphylococcus albus*	280	nil	21	75
Carbon monoxide poisoning	52	7	55	*Klebsiella-Aerobacter* sp.	612	194	50	67

TABLE 4

Ethanol levels in media containing peptone water + 500 mg/100 ml of various sugars or amino acids inoculated with a variety of micro-organisms and incubated overnight at 37 °C (from Blackmore 1968)

Organism	No. of strains examined	Peptone water	Glucose	Sucrose	Mannitol	Lactose	Urea	Ornithine	Lysine	Arginine
Alkaligenes faecalis	4	3	0·35	<3	<3	<3	0–6	0	0	3
Enterobacter sp.	2	8	47–54	52–72	145–148	55–56	—	5–10	6–10	5
Streptococcus faecalis	6	5–6	6–13	11	23	9–19	9–22	—	—	—
Staphylococcus pyogenes	4	8	12–25	12–21	9–26	11–12	—	—	—	—
Proteus providence	1	<3	27	11	13	8	—	—	—	—
Shigella sonnei	3	3	18–25	6	40–49	7	—	—	—	—
Pseudomonas aeruginosa	3	3	4–9	<3	3–7	<3	0	0	0	0
Proteus vulgaris	4	5	20–48	31–64	—	15	8–12	—	—	—
Proteus mirabilis	7	3	30–53	21	8–10	6	0–10	4–11	6	3
Proteus morganii	5	3	30–56	12–16	2–3	<3	0–18	—	—	—
Escherichia coli	25	8	22–92	38–107	40–120	12–65	—	—	—	—
Atypical coli	2	5	40–80	40–75	105–125	50	—	12	10	5
Klebsiella sp.	2	4	45–72	58–82	124–133	47–69	—	4	9	3
Uninoculated	—	0	0	0	0	0	0	0	0	0

organism examined produced significant levels of ethanol in carbohydrate-containing media, and for six of the nine types of organism examined, significant ethanol was produced in medium containing amino acids and only low (20 mg/100 ml) levels of sugar. (Post-mortem blood was found to contain *E. coli*, *Strep. faecalis*, *Staph. pyogenes* (*aureus*), *Staph. albus* (*epidermidis*), and *Cl. sporogenes*.) Incubation of human tissue homogenates (sterilized by autoclaving), with soil inocula gave ethanol levels up to *ca.* 70 mg/100 ml in blood, *ca.* 60 mg/100 g in brain, *ca.* 30 mg/100 g in liver and *ca.* 20 mg/100 g in kidney and bile after 48 h at 37 °C. *n*-Butyric acid and *iso*butyric acid were always found when ethanol had been produced by bacteria. Formaldehyde, acetaldehyde, acetic acid, propionic acid, methanol and propanol could only sometimes be detected.

Studies by Trojanowska (1968), Bogusz *et al.* (1970) (see also 5*C*(iii)) and Blume & Lakatua (1973) of ethanol production in human blood confirm the previous work, but unfortunately did not include any microbiological studies.

Addition of glucose to human blood by Iribe *et al.* (1974) increased ethanol levels after incubation at 27 °C for 6–10 d from about 25 mg/100 ml with no added glucose, to about 150 mg/100 ml with 1% (1000 mg/100 ml) added glucose, and 500 mg/100 ml with 5% (5000 mg/100 ml) added glucose. These authors determined lactate dehydrogenase (LDH) levels in samples from putrefying rat and human corpses, and human and rabbit blood and concluded that high levels of LDH indicated that ethanol production was due to micro-organisms—equating putrefaction of bodies with the fermentation process to yield saki. The value of LDH determination is doubtful since, although the lactic acid bacteria (e.g. *Leuconostoc mesenteroides*, *Lactobacillus* spp., *Strep. faecalis*) produce large quantities of lactic acid as well as ethanol and therefore possess high levels of LDH, many other bacteria, which are common post-mortem and are capable of producing ethanol, produce only a little lactic acid (e.g. *E. coli*, *Proteus* spp., *Cl. perfringens*); some bacteria produce large quantities of lactic acid and very little ethanol (e.g. some *Bacillus* spp. and some *Staphylococcus* strains); while yeasts produce high levels of ethanol and no lactate (Wood 1961; and Section 7). LDH determination may well be an index of putrefaction, but direct microscopic examination, or cultural methods of examination would do as well.

Blackmore (1968) found that normal urine *in vitro* was a very poor medium for microbial ethanol production, and Gormsen (1954) suggested that a positive ethanol reading in the tissues in combination with a negative reading from urine indicated that the tissue reaction was probably due to putrefaction.

D. Conclusions

There is considerable evidence to show that ethanol can frequently be found in both animal and human corpses at levels up to 150 mg/100 g after they have been stored for a few days at room temperature. Levels in isolated tissues or blood are generally lower, probably because of higher oxygen levels. The evidence suggests that this ethanol is produced by micro-organisms. Other indications (besides the presence of micro-organisms) that the ethanol has been formed after death, include the presence of other volatile compounds (particularly other alcohols) and wide differences in ethanol levels between different tissues, often with lower ethanol levels in the extremities. Ethanol is not found in all putrefied bodies; investigations of ethanol levels during putrefaction normally show an initial increase followed by a decrease (see Figs 1–3).

5. Substrates Available for Ethanol Production

Perhaps because microbial fermentation to produce alcoholic drinks always seems to require sugar (sucrose, glucose or fructose) as a substrate, it is often assumed that the most common sugar occurring in mammalian systems (glucose) must be the substrate for ethanol production during putrefaction. While glucose may indeed be one of the most readily utilized substrates, there are many other, more abundant compounds available in the body. Information on the chemical and biochemical processes and the substances present post-mortem is sparse. There is a considerable body of information available, however, on the biochemical changes that occur in skeletal muscle of animals, from the point of view of the meat industry.

A. Biochemical changes occurring in muscle prior to rigor mortis

Definition of an exact moment of death has proved impossible, but if it is correlated with cessation of brain function and heart beat, then many other tissues in the body, particularly the muscles are still 'living' and continue to be physiologically active for some time after death. Detailed accounts of biochemical events in muscle shortly after death can be found elsewhere (Bendall 1973; Lawrie 1974) and will only be summarized here.

Lack of circulating blood causes oxygen insufficiency within a short time, so that oxidative respiration ceases and tissue metabolism becomes anaerobic, with lactic acid as the end product instead of CO_2 and water. The substrate utilized in the presence or absence of oxygen is glucose-1-phosphate, obtained either by phosphorylation of glycogen reserves or from glucose. The pH level of muscle falls in proportion to the lactic acid produced—usually from about pH 7·0 to pH 5·5. The final pH attained varies from muscle to muscle and from animal to animal, depending on the limiting factor(s) that halt lactic acid production. Limitation can be caused by exhaustion of available glycogen or glucose, particularly if reserves are relatively low (e.g. in a starved animal or an animal subjected to stress before death). More commonly, glycolysis ceases due to lack of adenosine triphosphate (ATP) or adenosine monophosphate (AMP). At low pH adenosine nucleotides are rapidly deaminated, and glycolysis cannot regenerate them sufficiently quickly. Rigor mortis, a state where the muscles become rigid, occurs when ATP levels fall below a critical value. Rigor can be reversed by the addition of ATP, but the apparent disappearance of rigor observed in corpses is due to later denaturation of the tissue. At rigor the pH level is normally near its minimum and the Eh (redox potential) is also low—about zero (Ingram 1962). Eh continues to fall after rigor has set in, reaching a minimum of about −150 mV in horse muscle and about −100 mV in whale muscle (Ingram 1962). Post-mortem glycolysis occurs in other tissues besides muscles, where similar mechanisms must halt lactic acid production. Significant levels of lactate, although lower than in skeletal muscle, can be detected in other tissues post-mortem (Franks *et al.* 1974). The pH of blood, however, stays close to pH 7·4. The pH and Eh levels of tissues post-mortem undoubtedly affect the type of micro-organisms able to grow and their rate of growth (Ingram 1962; Dainty & Ingram 1971).

B. Glucose and glycogen levels in tissues

Glycogen is readily converted to glucose and this process appears to occur post-mortem, particularly in the liver. Tables 5 and 6(*a*)–(*c*) give some information on

TABLE 5

Levels of glycogen in various tissues

Tissue	Animal	Glycogen (mg/100 g) Range	Glycogen (mg/100 g) Mean	Time of sampling	Reference
Bone	Rat	16–32		Post-mortem?	Long (1961)
Brain	Dog	77–130	102	Frozen at death	Kerr (1936)
	Cat	77–101	86		
	Rabbit	70–90	82		
	Rat	74–96	85		Albaum *et al.* (1946)
Cartilage	Not stated	3–300		Not stated	Long (1961)
Retina	Ox		92	Post-mortem	Crane & Ball (1951)
Kidney	Dog		50*	Not stated	Long (1961)
Liver	Man	1430–8010	4370	Biopsy	Nilsson (1973)
	Man	950–4100	2150	Biopsy	Hildes *et al.* (1949)
	Man	2340–4860	3310	Biopsy (ordinary diet)	Martinsson *et al.* (1963)
	Man	380–2300	1350	Biopsy (24 h starvation)	
	Man	5110–10940	7880	Biopsy (extra glucose fed)	
Uterus	Man	320–1120		Not stated	Long (1961)
Muscle	Horse (heart)		276	Post-rigor mortis	Lawrie (1955)
	Horse (psoas)		606		
	Horse (diaphragm)		1109		
	Horse (l. dorsi)		1411		
	Rat (leg)		490	Not stated	Tarr (1949)
	Rabbit (psoas and l. dorsi)	0–800		Post-rigor mortis	Howard & Lawrie (1956)
	Ox (psoas and l. dorsi)	0–1500		Post-rigor mortis	
	Ox		100	Post-rigor mortis	Gardner (1965)
	Man (femoral)		1036	Biopsy (rested)	Carlson *et al.* (1971)
	Man (femoral)		340	Biopsy (post-exercise)	
	Man (gastrocnemius)	780–2190	1300	Biopsy	Hildes *et al.* (1949)
	Man (pectoralis major)	1130–3890	2200	Biopsy	
	Man (femoral)	950–2000	1390	Biopsy	Hultman (1967)
	Man (deltoid)		980	Biopsy	

* Calculated from dry weight figure, assuming 75% water.

glucose and glycogen levels in various mammalian tissues. Unfortunately most information for human tissues has been obtained from living bodies, and levels post-rigor mortis would be expected to be lower. Howard & Lawrie (1956) estimate that 1000 mg/100 g glycogen is required for the longissimus dorsi and psoas beef muscles to reach their ultimate pH of 5·44, for the same muscles in the horse the quantity of glycogen required is less (600–800 mg/100 g; Lawrie 1974) and the terminal pH is generally higher, while horse heart muscle is exceptional in that only about 300 mg/100 g glycogen are utilized, leaving about 300 mg/100 g post-rigor mortis. The rate of glycogen breakdown to glucose post-mortem also varies according to the species examined—e.g. free glucose accumulates at 0·9 mg/h/g muscle in pigs, but at only 0·04 mg/h/g in horses and oxen (Sharpe 1958). Since there appears to be no data on glycogen or glucose levels post-mortem in any human tissue except blood and liver (Table 6*b*), no comparison can be made, nor, in view of the variation between species noted above, is it possible to predict post-mortem levels with confidence. However, one might expect significant concentrations of glucose or glycogen in human muscles post rigor since *in vivo* levels are frequently in the 1000–2000 mg/100 g range (Table 5). Also, particulate glycogen has been observed in the muscles of those dying from a variety of diseases (Collins & Gilbert 1977). Levels of glycogen in human liver are frequently very high—2–10% wet wt—and after death there appears to be rapid conversion to glucose. Levels of glucose in liver post-mortem never exceed about 0·6% (Hamilton-Paterson & Johnson 1940; Table 6(*b*)) which may either be because the

TABLE 6(*a*)

Levels of glucose in various human tissues (ante-mortem) and animal tissues (post-mortem)

Animal	Tissue	Glucose level (mg/100 g)	References
Man	Cerebrospinal fluid	50–80	Long (1961) (ante-mortem)
	Vitreous humour	70	
	Gastric fluid	35–119	
	Blood (adult)	88·3 ± 6·7	
	Blood (infant)	67·1 ± 12·5	
	Blood cells	74 ± 15	
	Serum	97 ± 18	
	Pancreatic juice	8·5–18	
	Urine	4·3	
Ox	Muscle—triceps	100–200	Dainty (1975, personal communication)
	Muscle	75 (maximum)	Gardner (1965)
	Muscle—tenderloin	68	Tarr (1954)
	Muscle—T-bone steak	77 (after 2 weeks at 2 °C)	
	Muscle—round steak	55	
	Muscle—shoulder (calf)	45	
Pig	Muscle—rib	194	Tarr (1954)
Rabbit	Kidney	115	Long (1961) (immediately post-mortem)
Mouse	Brain	21–24	Medina *et al.* (1975) (immediately post-mortem)

TABLE 6(*b*)

Levels of glucose in human heart, blood and liver, post-mortem

Cause of death	Sampling time (h after death)	Blood glucose (mg/100 ml) Right atrium	Left ventricle or aorta	Liver	Reference
Cause of death not stated	5	9	9	35	Hamilton-Paterson & Johnson (1940)
	11	18	19	46	
	19	90	28	648	
	36	342	98	572	
	23	444	75	448	
	20*	390	241	640	
	24*	472	—	—	
	13*	470	410	—	
	10*	560	—	—	
Septic abortion	2·5	94	57·5	—	Hill (1941)
Reticulum cell sarcoma	4	264	0	—	
Generalized tuberculosis	8	108	2·2	—	
Subdural haemorrhage (old)	10	6·8	2·8	—	
Asphyxia choking	2·5	404	98	—	
Asphyxia hanging	6	63	51	—	
Asphyxia oedema of glottis	8	1·6	0	—	
Asphyxia hanging	10	650	44	—	
Asphyxia hanging	16	649	608	—	
Asphyxia choking	19	64	19	—	
Asphyxia hanging	20	142	0	—	
Shock	5	108	60	—	
	8	35	8	—	
	14	400	96	—	
	14	632	656	—	
	15	48·5	49	—	
	15·5	337	132	—	

* Diabetic.

TABLE 6(*c*)

Range and average glucose levels in samples of human blood taken in 251 consecutive autopsies (from Plueckhahn 1967)

No. of autopsies	Site of sample	Glucose (mg/100 ml) Maximum	Minimum	Average
214	Mixed heart	1050	19	186
37	Right side heart	825	29	318
	Left side heart	592	22	147
173	Femoral (leg) vessels	204	19	60
41	Cranial cavity	187	17	58

samples examined had unusually low glucose levels, or because of glucose diffusion into surrounding tissues (see below) or because not all the glycogen is hydrolysed. A number of workers have noted that post-mortem blood glucose levels are frequently higher in the right side, compared with the left side of the heart (Hamilton-Paterson & Johnson 1940; Hill 1941; Tonge & Wannan 1949; Fekete & Kerenyi 1965; Plueckhahn 1967).

This is thought to be due to diffusion of glucose from the liver via the hepatic veins and vena cava (Fallani 1961; and Section 6*G*). Post-mortem heart blood glucose levels higher than 1000 mg/100 ml have been observed at autopsy (Plueckhahn 1967; Table 6*c*).

The results of Plueckhahn (1967) show, in addition, that glucose levels in blood from the cranial cavity or femoral vessels are considerably lower than those in cardiac samples (Table 6*c*). This could indicate that glucose from the liver, or other organs influences the whole heart, or that, perhaps, glucose diffuses also from the cardiac muscle.

Raised glucose levels throughout the body can be found post-mortem, not only in diabetic subjects, but also in infants less than three-months old (Fekete & Kerenyi 1965) and in those whose death was immediately preceded by adrenalin secretion. This can occur in those dying from asphyxia, shock, acute coronary thrombosis, intracranial pressure, etc. (Hill 1941; Tonge & Wannan 1949; Fekete & Kerenyi 1965; Lawrie 1974; Table 6*b*). Extra low glucose levels are found when patients have died of debilitating diseases or suffered from liver disease (Hill 1941) or even after fasting for 12–18 h (Gibbons *et al.* 1966). Post-mortem glycolysis takes place in blood also. Average glucose loss observed by Hill (1941) was 5·98 mg/100 ml/h in blood taken from living human subjects and stored at 27·5 °C and 1·08 mg/100 ml/h at 3 °C for times up to 6 h. Presumably similar factors to those causing cessation of glycolysis in muscle operate in blood also, so that there is normally a residual glucose level of at least 17 mg/100 ml (Plueckhahn 1967; Table 6*c*). Evidence from studies of levels of glucose, glycogen, creatine phosphate and ATP in the brains of mice indicates that levels of all these substances are very low (<0·1 mmol/kg) within 20 m of death (Lowry *et al.* 1964).

Glucose and other substrate levels in normal urine are very low (Long 1961), but there appears to be a shortage of information concerning diffusion of nutrients and putrefactive products post-mortem. After death due to trauma the urine may be contaminated by blood and tissue, and is colonized by microbes even in the absence of trauma (Corry, unpublished observations). Urine from putrefying bodies (if available) frequently contains volatile products besides ethanol (Corry, unpublished observations) indicating that some compounds may indeed diffuse into the bladder.

C. Other substrates

Mammalian muscle tissue, immediately post-mortem, contains about 3·5% wet wt soluble non-protein substances, that includes all nutrients needed for microbial multiplication (see Table 7). This proportion increases with time post-mortem even in a sterile system, and autolysis occurs more rapidly in liver, spleen and kidney (Sliwinski *et al.* 1959; Sharpe 1963). There is evidence for the post-mortem formation, unassociated with any microbial action post-mortem, of hydrogen sulphide, ammonia, acetaldehyde, acetone, diacetyl and probably formic, acetic, propionic, butyric and *iso*butyric acids, and dimethyl sulphide; but not for the formation of volatile alcohols or esters (Yueh & Strong 1960; Davis *et al.* 1972).

(i) *Ribose*

Post-mortem degradation of nucleic acids, adenosine phosphates and related compounds can give rise to ribose amongst other products (inorganic phosphate,

TABLE 7

Chemical composition of typical adult mammalian muscle after rigor mortis but before degradative changes post-mortem (adapted from Lawrie 1974)

Constituent			Quantity (mg/100 g)	Quantity (% wet wt)
Water				75·5
Protein				18·0
Fat				3·0
Soluble non-protein substances:			3500	3·5
Nitrogenous		creatine	550	
		inosine monophosphate	300	
		di- and tri-phosphopyridine nucleotides	70	
		amino acids	350	
		carnosine, anserine	300	
Carbohydrate		lactic acid	900	
		glucose-6-phosphate	170	
		glycogen	100	
		glucose	10	
Inorganic		total soluble phosphorus	200	
		potassium	350	
		sodium	50	
		magnesium	20	
		calcium	7	
		zinc	5	
Glycolytic intermediates, trace metals, vitamins, etc.			*ca.* 100	

inosine, ammonia, hypoxanthine, etc.). Levels of ribose as high as 80–90 mg/100 g have been reported in some fish muscle and lower levels in beef and veal (Tarr 1953, 1954; Table 8).

(ii) *Amino acids*

Proteins in muscles (and presumably in other tissues also) are denatured at the low pH levels prevailing when glycolysis has ceased. This causes loss of water. There is also a limited amount of protein breakdown to peptides and amino acids. This (in the absence of micro-organisms) is probably caused by enzymes released from lysozomes whose membranes are weakened by the low pH; degradation of other metabolites may be caused similarly (Canonico & Bird 1970; Lawrie 1974). Table 8 shows some information on amino acid and ribose levels in various tissues. Unfortunately, almost all data are from tissues taken immediately post-mortem, although from the results of Colombo & Gervasini (1955), and Gardner (1965), levels in beef muscle increase little during subsequent storage. However, total levels of amino acids would be quite sufficient to support microbial growth.

De Duve & Beaufay (1959) reported rupture of 80% of lysozomes within 3–4 h of cutting off blood supply to dog liver tissue *in situ*. Lysozomal hydrolases, acid phosphatases, β-glucuronidase, cathepsins and acid deoxyribonuclease were detected. Fewer lysozomes occur in muscle than in liver and other organs, and it would be expected that more rapid hydrolysis would occur in the viscera than in the muscles (see 5*B*, above).

A recent study by Bonte *et al.* (1976) of protein degradation and amino acid levels in

TABLE 8

Levels of free amino acids and ribose in tissues (taken immediately post-mortem unless specified)

Compound(s)	Animal	Tissue	History of sample	Level (mg/100 g)	Reference
Alanine	Ox	Muscle	Initial	68	Colombo & Gervasini (1955)
			After 6 d refrigeration	69	
			After 12 d refrigeration	71	
Alanine	Ox	Muscle		6–43	Gardner (1965)
Alanine	Rat	Brain		3·5–7·0	Shank & Aprison (1971)
Alanine + serine	Cat	Brain		17	Tallen *et al.* (1954)
Alanine + serine	Cat	Kidney		25	
Glutamic acid	Ox	Muscle	Initial	90	
			After 6 d refrigeration	90	Colombo & Gervasini (1955)
			After 12 d refrigeration	95	
Glutamic acid	Ox	Muscle		3–24	Gardner (1965)
Glutamic acid	Rat	Brain		103–178	Shank & Aprison (1971)
Glutamic acid	Man	Brain		74–162	Perry *et al.* (1971)
Glutamic acid + glutamine	Cat	Brain		>178	Tallen *et al.* (1954)
Glutamic acid + glutamine	Cat	Kidney		>157	
Cystine	Ox	Muscle	Initial	196	
			After 6 d refrigeration	206	Colombo & Gervasini (1955)
			After 12 d refrigeration	210	
Creatine	Ox	Muscle		39–84	
Creatinine	Ox	Muscle		18–35	Gardner (1965)
γ-aminobutyric acid	Ox	Muscle		1–15·5	
γ-aminobutyric acid	Rat	Brain		15–40	Shank & Aprison (1971)
γ-aminobutyric acid	Man	Brain		14–29	Perry *et al.* (1971)
Aspartic acid	Man	Brain		24–36	Perry *et al.* (1971)
Aspartic acid + asparagine	Cat	Brain		31	Tallen *et al.* (1954)
Aspartic acid + asparagine	Cat	Kidney		10	
Threonine	Ox	Muscle		1·2–6·6	Gardner (1965)
Ribose	Pig	Muscle—'rib chop'	Market samples	<1	Tarr (1954)
	Calf	Muscle—shoulder		9	
	Ox	Muscle—tenderloin		9	
	Ox	Muscle—round steak		12·5	
	Ox	Muscle—T-bone steak	Stored 2 weeks at 2 °C	22	

TABLE 9(*a*)

Lactate levels in tissues of human air-crash victims and rats, sampled post-mortem

History of victim(s)	Lactate (mg/100 g wet wt) in: brain	heart muscle	liver	kidney	spleen	skeletal muscle	lung	thyroid	pancreas	blood	References
2 standard deviations about the mean (67%) of 60 unselected cases	125–175	400–500	250–350	150–240	325–425	500–640	200–290	100–230	150–225	240–350	Franks *et al.* (1974)
Ventricular fibrillation caused by petrol fumes	130	50–175	170–190	150	340	460	175–250	190–225	125	—	
Ventricular fibrillation of short duration	—	320–410	300	140–190	—	575–625	200	180–220	—	—	
Myocardial infarction causing ischaemia	110–170	270–350	250–260	150–200	325–335	490–550	175	100–150	225	—	
Non-stressed rats 12 h post-mortem	141	402	235	185	213	491	—	—	—	—	McBurney *et al.* (1974)

thoracic fluid of putrefying dog corpses during 46 d at 10 °C, showed progressive protein hydrolysis accompanied by small increases of amino acid levels. These rarely exceeded levels observed by other workers in unputrefied tissues, probably because of utilization by the high numbers of bacteria.

(iii) *Lactate*

There have been some studies of post-mortem changes in human bodies, but these have almost all been with a view to determining the time and/or cause of death and involved study of K^+ ion levels in vitreous humour or cerebrospinal fluid (Mant 1967) or enzyme levels in blood (Enticknap 1960). Non-protein nitrogen, urea and creatinine increase and glucose levels fall slowly (Balachowsky *et al.* 1932; Hill 1941; Jetter & McLean 1943).

Franks *et al.* (1974) examined lactate levels in human tissues post-mortem (see Table 9*a*). Levels in muscle were lower than those given by Lawrie (1974) for mammalian muscle (500–600 mg/100 g, compared with 900 mg/100 g). Levels measured by McBurney *et al.* (1974) in rat tissues were similar to those in human tissues (Table 9*a*). Levels of lactate in different organs are affected by events immediately ante-mortem, although there is disagreement about how reliable lactate measurement is as an indication of ante-mortem condition (Franks *et al.* 1974; McBurney *et al.* 1974).

D. Processes during putrefaction (adipocere and glycerol production)

A wide variety of products, besides ethanol, can be detected in corpses during putrefaction. There have been many studies on putrefactive bases, mainly carried out on viscera (liver in particular) and concerned with the problem of differentiating between drugs and putrefactive products and their derivatives (e.g. Fulton 1965; Kaempe 1969; Stevens & Evans 1973). Bonte & Bleifuss (1977) cite many of the older studies on the subject. Studies concerned with acid products and other products such as the higher alcohols are less common. Tomita (1975, 1976)) carried out an extensive GC study on a wide variety of putrefactive products, and cites a number of other publications which are, alas, in Japanese. Table 9(*b*) lists putrefactive products mentioned in a variety of publications. Marozzi & Lodi (1961) list many additional compounds (benzene and purine derivatives, besides amino acids) which were identified only by paper chromatography. A wide variety of substances can undoubtedly be detected, which is hardly surprising in such a complicated system.

The main processes during putrefaction, by analogy with other anaerobic degradation systems, such as the digestion of sewage sludge, involve hydrolytic breakdown of carbohydrates, proteins and fats, usually by bacterial action (although hydrolysis and oxidation of fats can occur in the absence of bacteria). Fatty acids, alcohols, and other products such as lactic acid, indole, acetoin, H_2 and CO_2 are produced by fermentation of amino acids and carbohydrates. Other acids and amines are produced by deamination and decarboxylation respectively of amino acids. Other degradation products include ammonia, hydrogen sulphide, methane and water. In some instances scavenging animals, particularly insect larvae, may contribute considerably to the degradation of the tissues not only by ingesting the carrion, but by secreting enzymes. They may also raise the temperature of the system (Simpson cit. Denbow & Drewett 1970; K. G. V. Smith personal communication 1977), and may contribute by facilitating access of oxygen. It might be expected, even in the absence of animals, that oxidative

TABLE 9(*b*)

*Putrefactive products detected in corpses**

Alcohols and alcohol derivatives	Acids	Amines and miscellaneous substances	Gases
methanol	acetic	ethylamine	hydrogen sulphide
ethanol	propionic	methylamine	carbon dioxide
n-propanol	*n*-butanoic	dimethylamine	ammonia
isopropanol	*iso*butanoic	trimethylamine	methane
n-butanol	*n*-valeric[4]	isobutylamine[3]	sulphur dioxide[6]
sec-butanol[1]	*iso*valeric	*n*-propylamine	phosphine[7]
*iso*butanol	*iso*caproic	*iso*amylamine	
*iso*pentanol[1]	malonic	1-phenylethylamine	
acetaldehyde	glutaric	2-phenylethylamine	
acetone[1]	succinic	tetraethylenediamine[2] (putrescine)	
methyl ethyl ketone	citric	pentamethylenediamine[2] (cadaverine)	
ethyl ether[1]	lactic	histamine	
formaldehyde	phenylacetic[2]	tryptamine	
phenylethanol	acetoacetic	tyramine	
p-hydroxyphenylethanol	pyruvic	neurine	
	2-furoic	ethanolamine	
	1-hydroxy-*n*-butyric	pyrrolidine	
	2-hydroxy-*n*-butyric	choline	
	1-hydroxy-*iso*-valeric	acetylcholine	
	1-hydroxy-*iso*-caproic	indole	
	p-hydroxyphenylacetic	indole-3-aldehyde	
	m-hydroxyphenylacetic	indole acids	
	p-hydroxybenzoic	*p*-hydroxybenzaldehyde	
	p-hydroxyphenylpropionic	tryptophol	
	2-phenyllactic	mercaptans (methyl & ethyl)	
	2-phenylpropionic[5]	pyridine	
	1-phenyllactic	agmatine	
	p-hydroxybenzoic	phosphoethanolamine[2]	
	p-hydroxybenzaldehyde		
	p-hydroxyphenylpyruvic		
	4-aminovaleric		
	2-aminoisobutyric		

* Data from Fulton (1965), Kaempe (1969), Stevens & Evans (1973), Denbow & Drewett (1970), Bhatty (1971), Tomita (1975), Oliver *et al.* (1977), Bonte & Bleifuss (1977), Holdstock & Stevens (1977, personal communication).

[1] More common in bodies in air.
[2] Produced in late stage of putrefaction.
[3] More common in bodies in water, irregular occurrence.
[4] Higher levels in human than mouse bodies.
[5] More in early stages of putrefaction.
[6] Camps and Simpson (cit. Denbow & Drewett 1970).
[7] Glaister & Rentoul (1966).

activities would become more important as decomposition progressed—after the protective barrier of the skin has been breached and fluids escape. Under optimal conditions only the bones and hair of a corpse will remain after a comparatively short time. These too disappear eventually under suitable conditions (when there is a good supply of water and oxygen).

Adipocere is commonly formed during putrefaction. It is waxy, grey-white and friable and consists almost entirely of fatty acids formed by hydrolysis of body fat. Studies by Mant (1957, 1967) and Den Dooren de Jong (1961) have indicated that adipocere

TABLE 9(*c*)

Fatty acid composition (% of total fatty acids) of mouse fat and mouse adipocere compared with pig† and human‡ adipocere*

	Initial		After incubation in water at: 25°C for:		20°C for:		15°C for:		After incubation in air at 25°C for:			
	free acids	acids in fat	21 d	11 months	36 d	10 months	49 d	6 months	37 d	4 months	Pig adipocere	Human adipocere
Lower fatty acids	0·8	0·3	0·3	0·8	0·4	0·4	0·8	0·5	0·7	0·9		2·0
Myristic (C_{14})	2·3	1·2	13·1	8·1	8·8	3·7	5·2	1·7	3·4	1·6		7·0
Palmitic (C_{16})	33·0	25·3	53·0	80·0	61·0	83·1	29·2	60·4	33·0	32·2	67·0	53·0
Palmitoleic (C_{16})§	4·6	5·4	2·0	0·9	2·4	0·1	8·1	2·0	8·7	8·0		2·0
Stearic (C_{18})	17·8	11·6	2·1	4·2	5·1	5·2	3·2	2·6	9·3	12·5	3·3	9·0
Oleic (C_{18})§	25·0	31·9	11·9	2·5	4·0	3·5	25·0	26·7	41·2	43·5	5·2	22
Linoleic (C_{18})‖	14·4	22·1	3·7	0·0	0·4	0·0	11·5	2·7	2·2	0·4		
Other higher fatty acids	2·0	2·0	2·0	1·5	2·0	1·5	5·0	2·0	1·5	1·0		2·5
Hydroxystearic (C_{18})	<0·1	<0·1	12·0	2·0	16·0	2·5	12·0	1·5	0·1	<0·1	15·8	ND

* Tomita 1976.
† Ruttan & Marshall 1917.
‡ Den Dooren de Jong 1961.
§ One double bond.
‖ Two double bonds.
ND, Not done.

formation requires the presence of bacteria. Adipocere formation also seems to be favoured by anaerobic conditions and a limited supply of water. In dry situations hydrolysis of body fat would remove water from other tissues. It is not clear, however, whether this would be sufficient to inhibit further decomposition. Well-preserved bodies with adipocere have been observed after storage for over a century in sealed coffins in a dry cool vault (Mant 1957). Adipocere apparently contains no glycerol, which is a product of fat hydrolysis, is readily utilized by many bacteria and is another potential substrate for ethanol production post-mortem. Fat hydrolysis can commence within a few hours of death (Fallani & Astore 1961). It seems likely that adipocere production is common during putrefaction, but only persists under favourable circumstances—e.g. where there is little moisture or where conditions are very anaerobic. The rate of adipocere formaton appears to depend strongly on the prevailing temperature, occurring within about 3 weeks of death at 20–25 °C (Simonsen 1977).

The proportions of different fatty acids in adipocere would appear to depend on the conditions during its formation. Table 9(*c*) summarizes the proportions of various fatty acids present in fresh fat and in the free fatty acid fraction initially and after various treatments of mouse corpses (Tomita 1976). In most cases palmitic acid predominated, with lower levels of oleic and stearic acid. This agrees quite well with the proportions detected in pig adipocere by Ruttan & Marshall (1917), who found that human and pig adipocere were similar, and by Mant (1967) and Den Dooren de Jong (1961) who studied human adipocere. Mouse and human fat contain similar proportions of fatty acids (cf. Cramer & Brown 1943; Den Dooren de Jong 1961; Tomita 1976). During formation of mouse adipocere some trends were apparent (Tomita 1976; Table 9*c*): (1) the proportion of palmitic acid increased, particularly in submerged bodies; (2) proportions of linoleic, palmitoleic and oleic (unsaturated acids) and stearic acid decreased in submerged bodies, while only linoleic acid decreased in bodies stored in air; (3) the proportion of hydroxystearic acid tended to increase during putrefaction, particularly in submerged corpses, reaching a maximum in one instance. Hydrogenation of the unsaturated fatty acids seems to be a factor in the reduction of their relative levels and probably causes the waxiness of adipocere. Den Dooren de Jong (1961) considered that a major change during the formation of adipocere was the formation of palmitic acid from oleic acid by the loss of two carbon atoms and the gain of two hydrogens.

E. Conclusions

There are a wide variety of substrates available shortly after death that could give rise to ethanol by microbial action. However, although there is plenty of information on levels of substrates in tissues ante-mortem there is very little available about levels post-mortem. Glucose is frequently present in high concentrations ($\geqslant$0·5%) in the liver and in the blood on the right side of the heart, is probably present in muscles and may sometimes be elevated in the blood throughout the body. Lactic acid occurs at levels from about 150–650 mg/100 g in all tissues. Other compounds such as amino acids and ribose occur at relatively low concentration initially but as protein and fat hydrolysis proceeds available amino acids, fatty acids and glycerol must increase even though they may be rapidly utilized by bacteria and thus not detectable in high concentration.

6. 'Post-mortem' Microbiology

(Numbers and types of organisms found ante- and post-mortem, their mode of spread and their importance in decomposition)

'En ce que concerne un animal entier abandonné après la mort, soit au contact, soit à l'abri de l'air, tout la surface de son corps est couverte des poussieres que l'air charrie, c'est-à-dire de germes d'organismes inférieurs. Son canal intestinal, là surtout où se foment les matières fécales, est rempli, non plus seulement de germes, mais de vibrions tout développés que Leeuwenhoek avait déjà aperçus. Ces vibrions ont une grande avance sur les germes de la surface du corps. Ils sont a l'état d'individus-adultes, privés d'air, baignés de liquides, en voie de multiplication et de fonctionnement. C'est par eux que commencera la putréfaction du corps, qui n'a été preservé jusque-là que par la vie et la nutrition des organes.' (Pasteur 1863.)

A. Numbers

Whether or not the tissues and blood of men and other mammals contain micro-organisms immediately post-mortem has been a subject of controversy for at least the last 70 years. The problem is compounded by the difficulty of obtaining samples of tissue aseptically. However, even the most recent studies, using modern aseptic techniques agree that a significant proportion of samples of blood and tissue contains micro-organisms (Niwayama 1971; Koneman & Davis 1974; Table 10) and there is a large body of evidence to suggest that bacteria invade tissues throughout life, and may be detected frequently in blood samples and biopsies (see Table 10*a*,*b*; Reith 1926; Schweinburg & Sylvester 1953). The fact that the percentage of positive cultures does not appear to increase with time after death, at least up to 48 h post-mortem, provided the corpse is stored cold (Giordano & Barnes 1922; Burn 1934*b*; Minckler *et al.* 1966; de Jongh *et al.* 1968; Koneman 1970; Niwayama 1971), supports the idea that living bodies contain small numbers of organisms, or that the organisms are disseminated at the time of death ('agonal spread'). Experiments on animals indicate that one route of invasion during death may be from the intestine via the mesenteric lymph nodes and possibly the portal vein capillaries (Nickel & Gisske 1941; Bagadi & Sewell 1974; Dr B. Mackie, personal communication). Tanner & Ruyle (1932) were able to recover yeasts in blood samples from live rabbits, shortly after the animals had been fed with the yeast, indicating penetration of the intestinal wall (see also Desoubry & Porcher 1895; Legroux & Jeramec 1944). There is evidence of similar mechanisms in humans (Krause *et al.* 1969). A number of workers have also suggested that organisms may penetrate via the lungs (Burn 1934*a*) and another likely mechanism of entry is via skin abrasions or deeper wounds. In a healthy individual, invading micro-organisms would be rapidly eliminated by natural defences or confined to the lymph nodes, the second line of defence. The natural defences, which include not only antibodies but also non-specific substances such as lysozyme and lysins, besides phagocytes (Wilson & Miles 1975), would presumably not be completely inactivated until some time after death. Evidence for this can be seen from the results of diluting blood samples from live pigs; more positive results were obtained when blood was diluted 1/100 or 1/300 with broth than at 1/10 or 1/50 (Reith 1926). Jensen & Hess (1941) showed that fresh pigs' blood has a bactericidal effect on various bacteria, particularly *E. coli*. High numbers of

TABLE 10(*a*)

Occurrence of micro-organisms in blood

References	No. of samples	% with organisms	Source
Canavan & Southard (1915)	100	54	Human, heart blood post-mortem
Fredette (1916)	119	35	
Richey & Goehring (1918)	206	34	
	45	71	Human arm vein post-mortem
Giordano & Barnes (1921)	206	39	Human 'blood' post-mortem
Reith (1926)	6	100	Pig heart blood, live animals
	6	67	Rabbit heart blood, live animals
	6	33	Guinea pig heart blood, live animals
Epstein & Kugel (1929)	66	100	Human 'blood' post-mortem
Hunt *et al.* (1929)	567	70	
Burn (1934*b*)	134	37	Human heart blood post-mortem
Schweinburg & Sylvester (1953)	12	100	Dog heart blood post-mortem
	9	100	Rabbit heart blood post-mortem
Kurtin (1958)	50	20	Human heart blood post-mortem
Carpenter & Wilkins (1964)	2033	36	
O'Toole *et al.* (1965)	19	32	
Wood *et al.* (1965)	62	71	
De Jongh *et al.* (1968)			
(1st series)	50	72	
(2nd series)	49	51	
Niwayama (1971)	79	39	
Koneman & Davis (1974)	321	53	

bacteria (up to 10^6/ml), increasing with time post-mortem, were detected by Rose & Hockett (1971) using a syringe to obtain samples from intact human bodies, and washing the bacteria free of possibly inhibitory body fluids by the use of membrane filters. Unfortunately these authors examined their samples by aerobic techniques only and did not state what proportion of tissues examined yielded micro-organisms. The high numbers of organisms isolated may have been due to examining bodies that had been subjected to higher storage temperatures than those in other studies. Gardner (1965) observed a fall in numbers of organisms during the first 24 h storage of beef muscle at 14 °C, indicating either that some of the contaminating organisms were dying because conditions were unfavourable for growth, or that some defence mechanisms were still active during that time. Further evidence for antibacterial activity up to 24 h after death was obtained by Gill *et al.* (1976). Studies by Robinson *et al.* (1953) on numbers and types of organisms occurring in whale meat post-mortem indicated that there was little multiplication of the bacteria in the meat until rigor mortis had set in (usually after about 10 h). While there may have been a residual antimicrobial effect, there is also evidence that the high Eh (redox potential) before rigor inhibits multiplication of anaerobic organisms (Barnes & Ingram 1955, 1956).

B. Effect of mode of death

Events prior to death have been shown to be important in determining the numbers of organisms present in the carcasses of meat animals, and susceptibility to spoilage. Starvation or excessive exercise, particularly of pigs, depletes glycogen reserves so that the final pH in muscle is higher than normal (Callow 1938, 1939). Microbial

TABLE 10(*b*)

Occurrence of micro-organisms in tissues other than blood

% samples positive (no. of samples)							
Lung	Kidney	Liver	Spleen	Skeletal muscle	Brain	Animal	Reference
			40 (190)			Man	Giordano & Barnes (1922)
				100 (36)		Pig (live)	Reith (1926)
				61 (36)		Rabbit (live)	
				89 (36)		Guinea pig (live)	
87 (230)	71 (235)	57 (138)	45 (130)		26 (17)	Man	Burn (1934*a*)
80 (20)	75 (16)	90 (20)	83 (18)	76 (17)		Dog (healthy)	Schweinburg & Sylvester (1953)
45 (11)	20 (15)	46 (15)	39 (13)	23 (13)		Rabbit (healthy)	
	0 (10)	0 (10)	0 (10)	0 (10)		Hamster (healthy)	
	0 (10)	0 (10)	0 (10)	0 (10)		Guinea pig (healthy)	
	0 (15)	0 (15)		0 (10)		Rat (healthy)	
56 (213)						Man	DeVries & Pritchard (1955)
40 (50)			25 (50)			Man	Kurtin (1958)
73 (2033)						Man	Carpenter & Wilkins (1964)
50 (18)	18 (50)	24 (63)	25 (47)			Man	O'Toole *et al.* (1965)
55 (56)	24 (46)	32 (139)	37 (289)	19 (16)	31 (26)	Man	Minckler *et al.* (1966)
84 (100)						Man (1st series)	De Jongh *et al.* (1968)
53 (96)	26 (43)					Man (2nd series)	
68 (25)	50 (10)	52 (27)	46 (22)		60 (5)	Man	Dolan *et al.* (1971)
79 (343)						Man	Koneman & Davis (1974)

proliferation occurs more rapidly in meat with a high ultimate pH (Ingram 1948, 1962). Pigs show this effect more frequently than cattle, apparently because they are of a more nervous disposition—stress without starvation also raises the final pH level because of mobilization of glycogen reserves by adrenaline (Howard & Lawrie 1956, 1957; Lawrie 1974). There is evidence also that fatigue *per se* increases the number of contaminants entering the blood and lymphatic systems from the intestine (Narayan 1966; Narayan & Takacs 1966). Use of a humane killer on sheep causes shedding of the intestinal epithelium, which does not occur when sheep are anaesthetized, and may facilitate spread of bacteria from the intestine (Badawy *et al.* 1957). Similar effects have been observed in the small intestine of humans suffering from shock (Haglund *et al.* 1975).

Corpse blood has been used successfully for transfusions in both the Soviet Union and the United States (Yudin 1936, 1937; Tarasov 1960; Kevorkian & Bylsma 1961). The blood is taken from subjects who have died suddenly from various causes such as coronary thrombosis, cerebral haemorrhage or acute alcoholic poisoning(!), before 6 h post-mortem. Pruitt (1960), using dogs, reported that blood from the systemic circulation did not become contaminated with bacteria during the first 6 h post-mortem when the body was held at 24·2 °C or colder, but that 7 h samples were frequently contaminated. Blood in the portal system, however, became flooded with bacteria during the first hour following death—presumably from the intestine.

Studies by Kellerman *et al.* (1976) with isolated loops of human and canine intestine showed that at 37 °C no organisms penetrated across the gut wall until 12–15 h post-mortem. *Staphylococcus* spp. usually appeared first, succeeded by Gram positive aerobic bacilli, *Streptococci* and Enterobacteriaceae. Obligate anaerobic organisms, *Bacteroides*, *Peptococcus*, *Peptostreptococcus* and *Clostridium* spp. did not cross human intestine until about 28 h post-mortem. Comparison of these results with others mentioned previously indicates, as might be expected, that bacterial penetration across the whole of the gut wall, into the peritoneal cavity takes much longer than passage merely into the mesenteric blood and lymph system. However, the order of penetration by the different organisms is interesting, particularly the passage of fastidious anaerobic organisms other than clostridia, which have not been observed in post-mortem tissues (cf. Section 6*E*, *F*).

The use of a contaminated throat-cutting knife during slaughter of pigs has been shown to increase contamination of the tissues (Jensen & Hess 1941). Since the heart often continues to beat for several minutes after bleeding from the throat commences, there is ample opportunity for bacteria to be distributed widely throughout the body. Recent work has indicated that these organisms accumulate in the lymph nodes, liver and kidney (Labadie *et al.* 1977). A somewhat similar situation must apply when a human is subjected to a traumatic injury immediately prior to death. There is evidence that patients with intestinal lesions and neoplastic disease have a high incidence of post-mortem bacteria in the tissues (Kuklinca & Gavan 1971; Koneman & Davis 1974). The bodies of patients dying from generalized infection or gangrene tend to decompose more rapidly (Simpson 1965). However, organisms isolated post-mortem frequently show little or no relation to those causing infections before death (Koneman *et al.* 1971; Dolan *et al.* 1971; Koneman & Davis 1974).

C. Microflora of lymph nodes

Post-mortem examination of lymph nodes for micro-organisms should give an indication of organisms capable of initiating decomposition and the types present should

TABLE 11

*Types of micro-organism isolated from lymph nodes of healthy cattle and humans not dying of infection, immediately post-mortem**

Organisms	Lepovetsky *et al.* (1953) (23 prescapular and popliteal lymph nodes of cattle)	Nottingham (1960) (42 ischiatic lymph nodes)	Adamson (1949) (human lymph nodes)		
			88 cervical	88 mediastinal	38 abdominal
(Proportion of sterile samples)	(34%)	(11%)	(49%†	45·5%†	26%†)
Streptococcus	25·5	—	30	33	31·6
Escherichia, *Aerobacter*, *Serratia*, *Proteus* (Enterobacteriaceae)	35	12	10·2	18·2	37·0
Alcaligenes	10	—	—	—	—
Bacteroides	2	—	—	—	—
Clostridium	6	7	—	—	—
Corynebacterium	6	—	—	—	—
Flavobacterium	8	—	—	—	—
Micrococcus/Staphylococcus	4	40	5·7	2·3	2·6
Pseudomonas/Achromobacter	4	24	—	—	—
Bacillus	—	38	—	—	—

* % positive of total nodes examined (more than one type of organism sometimes detected in a node).
† Assuming one type of organism/node.

give a clue to their origin. Table 11 lists types isolated from lymph nodes during three studies, two on cattle and one on humans. Since different nodes were examined in each study, the types cannot be compared directly. However, Enterobacteriaceae were isolated frequently, especially in abdominal nodes (associated with the intestine), and other common organisms were streptococci and Micrococcaceae. *Clostridium* spp. were isolated in a small proportion of cattle nodes, but not from human nodes. Nodes from human bodies yielded a smaller range of organisms and a higher number of sterile nodes, possibly because cattle suffer more abrasions, which allow greater entry of environmental organisms (from dust, faeces, soil, etc.), and also because the nodes examined in cattle were associated mainly with muscular tissues, while those examined from humans were mainly associated with the intestine, neck and lungs. Nottingham (1960) found $\log_{10}$ numbers of viable organisms/node in the range 2·4–3·5. Higher numbers/node and high percentages of Gram positive rods (presumptive *Bacilli*) were associated with low rainfall in the preceding two months, indicating either that dust increased the contamination rate, or that dry weather reduced natural resistance, perhaps due to poorer nutrition. Log_{10} numbers of viable organisms/g of node at 37 °C in the study of Lepovetsky *et al.* (1953) ranged from 2·0–5·7 (av. 3·8). Adamson (1949) did not carry out quantitative examinations during his studies.

D. 'Bone taint'

Although most samples from the interior of fresh meat are found to be sterile, the phenomenon of 'bone taint' causes problems in the meat industry and it seems likely that organisms may be found similarly in human corpses. A small proportion of animal carcasses spoil deep down in the flesh next to the bone. A variety of organisms have been implicated (see Table 12), mostly anaerobic or facultatively anaerobic. Their origin has been suggested to be either intestinal (Callow & Ingram 1955) or from infected lymph nodes (Nottingham 1960). Growth of these organisms appears to be encouraged if the terminal pH of the meat is high (as occurs in exhausted (stressed) animals, giving poorer quality meat, see above).

TABLE 12

Organisms isolated from tainted meat

Worker	Organisms isolated	Suggested origin
Haines (1941)	Anaerobes, including *Cl. sporogenes*	Usually via synovial joint or head of femur
Callow & Ingram (1955)	*Clostridium* spp., occasionally streptococci	Intestinal
Cosnett *et al.* (1956) } Nottingham (1960) }	Clostridia, bacilli, coliforms and pseudomonads	Via lymph nodes

E. Types of organisms isolated from blood and other tissues

Types of organisms isolated from general tissues of human corpses (Table 13) are broadly similar to those isolated from tainted meat carcasses or from the lymph nodes of humans or meat animals. Enterobacteriaceae were the most commonly isolated organisms from human tissues, with lesser numbers of Gram positive cocci, coryneforms, *Clostridium perfringens* and yeasts. By contrast, the study of Vanderzant

TABLE 13

Major groups of organisms isolated from human blood and tissues post-mortem

	Burn (1934*a*)*	O'Toole *et al.* (1965)†	Minckler *et al.* (1966)†	De Jongh *et al.* (1968)†	Vanderzant & Nickelson (1969)‡	Koneman & Davis (1974)†
Enterobacteriaceae (*Escherichia coli*)	58 (51)	21 (6·25)	34·2 (15·5)	40·2 (21·4)	—	44 (18·2)
Micrococcaceae	28	21	24·4	8·2	62·2	13·4
Streptococci (excl. diplococcus)	49	16·7	15·8	16·7	—	6·6
Coryneforms	12 (aerobic)	25 (aerobic and anaerobic)	2·6	—	32·1	—
Clostridium perfringens (*welchii*)	22	—	1·6 ('Clostridium')	—	—	2·7
Yeasts	1·6	4·2	4·2	—	1·6 ('yeast and mould')	5·2
Bacillus	—	2·0	—	—	0·63	—
Bacteroides	—	2·0	0·8	—	—	—

* % positive of total number of samples tested (human).
† % positive of total number of isolates (human).
‡ % positive of total number of isolates (pigs, sheep and cattle).

& Nickelson (1969) on animals revealed no Enterobacteriaceae or *Streptococcus* spp. (see Table 12). However, streptococci and Enterobacteriaceae have been found in pork and the former in high numbers in whale meat (Ingram 1952; Robinson *et al.* 1953). Table 14 lists the most common types of organism occurring in the human large intestine and faeces. A large majority (*ca.* 90%) of organisms are strict anaerobes (*Bacteroides* spp. and Gram positive non-sporing anaerobes—bifidobacteria, eubacteria, etc.) with lower numbers of *Lactobacillus*, *Streptococcus* spp. (mostly enterococci) and Enterobacteriaceae (about 10% in all). In addition, small numbers of other groups may be detected (*Clostridium* spp. including *Cl. perfringens* (anaerobic spore-formers)), *Bacillus* spp., yeasts and, less commonly, *Staphylococcus* spp. and *Ps. aeruginosa*. Collee *et al.* (1961) found *Cl. perfringens* in all faecal samples examined while Döll & Weigand (1970) found the organism in 90%. Almost all the organisms that have been isolated from bodies post-mortem (Table 13) have also been isolated from faeces or intestines (Table 14), although not in the same proportions. In addition Micrococcaceae and coryneforms could have originated from the skin, where they are the normal predominant flora (Noble & Somerville 1974).

F. Organisms that cause decomposition (putrefaction)

Most of the textbooks of forensic medicine have little to say on the subject of the bacteria active in decomposition of human corpses. Simpson (1965) cites Burn (1934*a*), who experimented by inoculating dead animals with strains of bacteria isolated from bodies post-mortem. Burn found little evidence of active invasion and multiplication by any strains except *E. coli*, *Cl. perfringens* (*welchii*), and to a lesser extent *Staph. aureus*. Gonzales *et al.* (1954) state that the chief agent of decomposition is *Cl. perfringens* (*welchii*) which spreads through the blood vessels causing haemolysis, proteolysis and gas formation in blood and in other tissues. They quote Sydney Smith, without a reference, to say *E. coli* and *Proteus vulgaris* are found early post-mortem, in addition to *Cl. perfringens*, and that after about two days *Bacillus mesentericus* and *Micrococcus albus* are also abundant. Gradwohl (1954) states that 'a mixture of Gram positive and Gram negative cocci and bacilli is found in smears and cultures from decomposed tissues. Thick and long Gram positive rods predominate', and that 'bacteria of the coli and proteus groups migrate through the intestinal walls and into surrounding tissues, followed by overgrowth by saprophytes and welchii group' (*Cl. perfringens*). Rentoul & Smith (1973) state: 'The organisms chiefly responsible are *B.* (*sic*) *coli*, staphylococcus, non-haemolytic streptococcus, *Streptococcus viridans*, *Cl. welchii*, diphtheroid and proteus types. The most commonly found are the commensals of the respiratory and alimentary tract'. Thus, all avialable information agrees that *Clostridium perfringens* (*welchii*) is an important organism or the most important organism in decomposition presumably on account of its saccharolytic, proteolytic and lipolytic capabilities, its ability to grow at relatively high Eh and its high growth rate. *E. coli*, *P. vulgaris* and to a lesser extent *Staphylococcus* spp., *Streptococcus* spp. and *Bacillus* spp. are also said to play a part. All these organisms have been detected in the intestine, although none forms a major proportion of the intestinal flora. Since no investigation of the microbiology of human decomposition has apparently been published since Burn (1934*a*), further study would be valuable, especially to determine whether any of the more fastidious anaerobes, normally predominating in very high numbers in the intestine but whose numerical preponderance has only recently been

TABLE 14

Some bacteria of the healthy intestine (from Drasar & Hill 1974)

Families and genera represented	Prominent species	Other species isolated from the intestine
Pseudomonadaceae		*Pseudomonas aeruginosa (pyocyanea)*
Pseudomonas		*Ps. (Alkaligenes) faecalis*
Enterobacteriaceae	*Escherichia coli*	
Klebsiella		*Klebsiella (Aerobacter) pneumoniae*
Enterobacter		*Enterobacter (Aerobacter) aerogenes*
Proteus		*Proteus mirabilis*
Bacteroidaceae		*Bacteroides capillosus, B. oralis*
Bacteroides	*Bacteroides fragilis*	*B. clostridiformis, B. putredinis*
		B. coagulans, B. ruminicola
Fusobacterium		*Fusobacterium mortiferum*
		F. necrogenes, F. fusiforme
		F. girans
Neisseriaceae		*Neisseria catarrhalis*
Neisseria		*Veillonella parvula*
Veillonella		*V. alcalescens*
Micrococcaceae		*Staphylococcus albus*
Staphylococcus		*Peptococcus asaccharolyticus*
Acidaminococcus		*Sarcina ventriculi*
Sarcina		*Acidaminococcus fermentans*
Peptococcus		*Streptococcus salivarius*
Streptococcaceae		
Streptococcus	*Streptococcus faecalis*	*Strep. sangius*
		Strep. viridans (mitior)
		Strep. faecium
Lactobacillaceae		*Lactobacillus brevis*
Lactobacillus	*Lactobacillus acidophilus*	*L. casei*
		L. catenaforme, L. fermentum
		L. leichmanii, L. plantarum
Leptotrichia		*Leptotrichia buccalis*
Bifidobacterium	*Bifidobacterium adolecentis*	*Bifidobacterium (Actinomyces lactobacillus) bifidum (bifidus)*
	Bifidobacterium longum	*Bif. breve, Bif. cornutum*
		Bif. eriksonii, Bif. infantis
Ruminoccus	*Ruminococcus bromii*	*Peptostreptoccus intermedius*
Peptostreptococcus		*P. productus*
Propionobacteriaceae		
Propionobacterium		*Propionobacterium (Corynebacterium) acnes*
		Prop. granulosum
Eubacterium	*Eubacterium (Bacteroides) aerofaciens (biforme)*	*Eubacterium contortum*
		Eu. cylinderoides, Eu. lentum
		Eu. limpsum, Eu. rectale
		Eu. tortuosum, Eu. ventriosum
Corynebacteriaceae		*Corynebacterium pseudo-diphtheriticum (hofmanni)*
Corynebacterium		*C. xerosis, C. ulcerans*
Bacillaceae		*Bacillus cereus, B. subtilis*
Bacillus		*Clostridium cadaveris*
		Cl. innocuum
Clostridium	*Clostridium perfringens (welchii)*	*Cl. malenominatum, Cl. ramosum*
		Cl. sordellii
	Clostridium paraputrificum	*Cl. tertium, Cl. bifermentans*
		Cl. sporogenes, Cl. indolis
		Cl. sphenoides, Cl. felsineum
		Cl. difficile, Cl. oroticum

appreciated, can also be detected in decomposing corpses when strict anaerobic techniques for isolation are applied (cf. Kellerman *et al.* (1976); Section 6*B*). The low reported rate of occurrence of *Cl. perfringens* immediately post-mortem (Table 12) may be due to its low sporulation rate—Clostridia are frequently identified or isolated using heat-treatment to select their heat-resistant spores. Rose & Hockett (1971) might have revealed interesting results, but unfortunately they applied no anaerobic techniques at all! The study of Ingram & Hauge (1949) of the bacteria occurring in the flesh of fin whales 24 h after death is of interest in this connection, since cooling after death is very slow, even in arctic waters and after evisceration and bleeding, owing to the large size of the animal. These workers found high numbers of *Cl. perfringens*, a fastidious anaerobic streptococcus and a Gram negative facultatively fermentative rod (coliform). Dissemination of the bacteria in whales was considered to occur just before death and their origin was intestinal (Robinson *et al.* 1953). The most likely source of post-mortem bacterial contamination from the evidence above, seems to be the intestine, as predicted by Pasteur in 1863, although infected lymph nodes may also play a part, and in cases where injury has occurred near the time of death, organisms from the skin or immediate environment could also play a significant role. All the organisms at present considered important in decomposition can be found widely disseminated both inside and outside the body.

There is evidence, mostly from studies on animals and meat, that at least three factors influence the predominant organisms post-mortem:

(1) There are indications that the antimicrobial defences of the body are not completely inactivated until some time after death. (See also Section 6*A*). Recent work by Gill *et al.* (1976) with guinea-pig corpses indicated that: (a) bacteria spread from the intestines throughout the tissues immediately after death; (b) bacteria that spread in this way, that are part of the normal intestinal flora, are killed during the first 24 h post-mortem; (c) bacteria such as salmonellae can be found throughout the body when injected into the intestine after death, and are only destroyed during the first 24 h post-mortem if the animal were immunized against the organism during life. It is not clear how bacteria spread so rapidly with no circulation operative, nor is it clear how the organisms are killed (as opposed to inhibited) by the antimicrobial defences of the body unless phagocytes are active for as long as 24 h post-mortem. According to these results, tissues would remain relatively free of viable micro-organisms during the first 24 h post-mortem unless the invader was a type not previously encountered by the host. After this time colonization could occur with any suitable organism.

(2) The Eh of tissues post-mortem falls rapidly (see Section 5*A*) so that by the time antimicrobial activity has been lost, the Eh is low enough to prevent obligately aerobic organisms such as micrococci, pseudomonads and acinetobacters growing, except very close to the surface. The predominant flora in the corpse would be that capable of multiplying most rapidly—and hence *Cl. perfringens*, as mentioned previously, could fulfill this role, provided the temperature was high enough (see below). Ingram & Dainty (1971) reviewed the effect of temperature on the microbiological spoilage flora of meat. Bacteriological breakdown of meat under warm conditions (25–40 °C) is caused predominantly by *Cl. perfringens*, accompanied sometimes by other *Clostridium* spp. At intermediate temperatures (10–25 °C) there is little information on the flora, which may include the coli-aerogenes group. Clostridia other than *Cl. perfringens*, and *Pseudomonas* spp. have been isolated. At temperatures approaching freezing, *Pseudomonas*, *Acinetobacter* and *Alcaligenes* spp. predominate. These are all, or

almost all, strict aerobes, and it seems unlikely that they would be important in spoilage of intact human corpses. The universal implication of *Cl. perfringens* as the major organism in human decomposition is surprising in view of its high optimum growth temperature of about 45 °C (Buchanan & Gibbons 1974) and its minimum growth temperature of about 15 °C (Dr G. Hobbs, personal communication). It seems unlikely that this organism would be important during the decomposition of corpses in this country unless this occurred in unburied corpses during a warm summer or in a heated room, or sufficient heat was generated during putrefaction to raise the temperature of the corpse. The temperature in a vault or grave would be in the region 5–15 °C. In addition, although *Cl. perfringens* has extensive biodegradative properties, it is unable to liquefy a meat medium when grown *in vitro*, which indicates that complete disintegration of a corpse cannot be achieved by *Cl. perfringens* alone. Other *Clostridium* spp. (e.g. *Cl. putrefaciens*) can grow at lower temperatures and may have been mistakenly identified as *Cl. perfringens*. It seems probable that putrefaction is caused by a succession of organisms progressively degrading, rather than by one type.

G. Mode of spread of bacteria in human corpses

Although information obtained about the occurrence of bacteria in tissues immediately after death of meat animals is useful in assessing the initial situation in human corpses, the treatment of meat animal corpses and human corpses differs in several important respects. Meat animals are bled (exsanguinated) during slaughter, and the viscera (and skin, except pigs') removed very shortly after death. Removal of the intestine eliminates a very important source of bacterial contamination, removal of the skin and exposure of unprotected tissues allows access of micro-organisms both from the animal and from the environment, and removal of most of the blood must also deprive contaminating micro-organisms of a ready source of nutrients (see Section 5) and a convenient pathway to spread round the body.

Sterile tissues in bodies up to 35 d post-mortem have been reported (Nehring *et al.* 1971), presumably (although not stated) after prompt cooling and subsequent storage at low temperatures. Little or no decomposition would be expected since the organisms present would be unable to grow at refrigeration temperatures. Under cool conditions the first sign of decomposition is stated to be the appearance of a greenish tinge to the abdomen (Gonzales *et al.* 1954) due to the formation of sulphaemoglobin from hydrogen sulphide-forming organisms in the intestine. A similar effect has been observed in uneviscerated game birds, associated with large numbers of H_2S-producing coli-aerogenes bacteria and clostridia in the intestine, although the muscular tissue, unlike that of mammals, appeared to be sterile post-mortem (Barnes 1968; Mead *et al.* 1973). Text-books of forensic medicine (Gonzales *et al.* 1954; Gradwohl 1954; Simpson 1965; Rentoul & Smith 1973) give many details of the progression of decomposition and of the effect of various intrinsic and extrinsic factors on the rate of decomposition and the final products. Bacteria are stated to invade from the intestine, through the intestinal wall and into the blood vessels. They spread through the blood vessels where haemolysis causes the normally invisible vessels to become visible on the surface of the skin.

A most interesting and apparently little-known study by Fallani (1961) of the post-mortem 'circulation' of blood merits particular attention. This author carried out a detailed study of the movement of blood by injecting blood vessels of corpses with radio-opaque or coloured dye. He also measured intravascular, intra-abdominal and

intra-pleural pressures, as well as muscle tension during periods from 10 to 72 h post-mortem. The results can be summarized as follows: (a) If the blood remains coagulated no movement occurs. (Blood may become liquid within a few hours of death, particularly if death was sudden, but in some cases may remain clotted until it is liquefied by putrefactive action. This occurs most often in the corpses of those who died from debilitating diseases or prolonged shock (Rentoul & Smith 1973).) Fallani observed that when the blood did liquefy before putrefaction, liquefaction occurred first in the peripheries, then in the large blood vessels and finally in the heart. (b) The two factors that influence post-mortem circulation most profoundly (if the blood is liquid) are whether or not the muscles are in a state of rigor, and the abdominal pressure, which increases post-mortem due to the gases produced by intestinal and putrefactive organisms. The time of onset and the duration of rigor mortis can vary widely (see Section 5*A*; Rentoul & Smith 1973), but on average rigor commences about 10–13 h after death, and starts to resolve within 36 h.

Fallani observed a steady increase in pressure in the body cavities post-mortem, with higher levels in the abdominal than in the pleural cavities. After 30 h the mean abdominal pressure was about 20 mm Hg and the mean pleural about 5 mm Hg. By 70 h post-mortem the pressures had risen to about 30 and 20 mm Hg respectively. Muscular tension (rigor) was maximum at about 30 h post-mortem and by about 50 h was again at its 10 h level.

Movement of blood

Very little movement was observed during the first 24 h post-mortem, except for slight reflux of heart blood from the right side of the heart to the veins of the neck, probably caused by the onset of rigor in the cardiac muscle before it occurred in the skeletal muscle. During the second phase, which lasted until rigor was resolved, abdominal pressure increased and forced blood from the abdominal to the thoracic aorta and from the left chambers of the heart into the pulmonary veins. Blood from the inferior vena cava moved via the right atrium to the superior vena cava and into the neck veins. When rigor was resolved, blood from the left side of the heart, instead of passing into the pulmonary veins, flowed mainly into the aorta and from there into the arteries of the limbs, head and neck. Very little movement of blood in the veins of the limbs occurred because of the resistance offered by their valves, but in the head and neck, where fewer valves occur, there was significant blood movement.

Fallani postulated a fourth stage which occurs when putrefaction is well-advanced, when pressures in the abdomen and thorax are very high and cause a continuous centrifugal movement of liquids to the surface, giving rise to large epidermal blisters and loss of fluid from the external orifices. By this stage the veins would no longer resist liquid reflux.

Fallani's results indicate that blood samples from corpses (particularly if taken more than 24 h after death) may well have originated from sites different from those from which they were taken. For instance, high levels of glucose, originating from the liver, (Hill 1941), may spread by post-mortem circulation from the liver via the hepatic vein, inferior vena cava, right side of the heart and the superior vena cava to the veins of the head and neck. Similarly, glucose in the hepatic artery may be distributed to the femoral arteries. Consequently, the best site for sampling blood from a corpse more than 24 h old, with the least numbers of micro-organisms and with the minimum of post-mortem changes due to circulation, would be a vein from the lower portion of a limb.

Neither the post-mortem blood circulation observed by Fallani nor the spread of motile bacteria can explain the almost instantaneous distribution of bacteria observed by Gill *et al.* (1976; see Section 6*F*) following post-mortem intestinal injection in corpses less than 24 h old, unless this caused a very high peritoneal pressure.

As putrefaction proceeds proteins and fats are degraded and large quantities of gas are formed which distend the tissue. Finally, if conditions are suitable, putrefaction proceeds to completion and the tissues liquefy and disintegrate, leaving only the bones, teeth and hair. Insect larvae and scavenging animals may hasten the process. Under optimal conditions (a naked body in warm damp air) nothing but the bones, teeth and hair remain after a few months. The parenchymatous organs decompose more quickly than the muscular organs—with the exception of the intestines and stomach, because of their contents. The brain, lining of trachea and larynx, stomach and intestines, spleen, liver and uterus in pregnant or puerperal state, decompose early while the oesophagus, diaphragm, heart, lungs, kidneys, urinary bladder and skeletal muscle decompose more slowly. The uterus and prostate gland are the most resistant of the soft tissues.

H. Conclusions

Micro-organisms can be isolated in low numbers from blood and tissue samples taken during life and immediately after death. They gain access from skin abrasions and from the respiratory and intestinal tract (the latter especially during ingestion of food, and during violent death or when under stress). During life invading organisms are eliminated by the natural defence systems of the body, or confined to lymph nodes. Immediately after death many of these systems continue to function for a short time. Subsequently, provided the temperature of the body exceeds about 5 °C, surviving bacteria begin to multiply and other invaders penetrate, mainly intestinal organisms via the portal vein and mesenteric lymph system. Although a wide variety of species exist in the intestine, relatively few are stated to predominate within a short time in the decomposing corpse, these include *Cl. perfringens*, Enterobacteriaceae, streptococci, Micrococcaceae and occasionally bacilli. The low Eh of tissues post-mortem must prevent the growth of obligately aerobic types of micro-organism. The effect of the temperature during decomposition on the predominating types of micro-organism has not been studied, but, by analogy with studies of other systems, temperature would be expected to have a very important effect.

7. Micro-organisms Capable of Producing Ethanol

This Section consists of a brief review of present knowledge of the mechanisms of ethanol production and the yield, with particular reference to the available substrates (glucose, amino acids, glycerol and lactic acid) and the organisms most frequently encountered in bodies post-mortem (Enterobacteriaceae, streptococci, Micrococcaceae and clostridia).

A. Ethanol from glucose

Tables 15(*a*)–(*c*) summarize qualitative and where possible, quantative data available on ethanol production from glucose by micro-organisms. The ability to produce ethanol from glucose is widespread amongst micro-organisms and most information that is available concerns this substrate. These include not only yeasts but many types of bacteria, some giving as high a yield. Many of the bacteria (Table 15*c*) are found in

the human intestine or on the skin. Some species of moulds (certain members of the Mucorales, some *Fusarium* spp. and a few strains of *Aspergillus* and *Penicillium*) can also produce as high a yield as yeast, even under well-aerated conditions (see Table 15(*a*); Foster 1949). Moulds do not normally proliferate in the intestine but are common aerial contaminants and frequently colonize the surface of corpses, particularly if partial dehydration has occurred. Very few moulds or yeasts are capable of growing in completely anaerobic conditions although they can ferment. There is surprisingly little data on ethanol production in yeasts, except for *Saccharomyces cerevisiae* and a few other species. Table 15(*b*) lists data available for *Saccharomyces cerevisiae* (found occasionally on humans—e.g. see Hurley *et al.* (1975)) and one or

TABLE 15(*a*)

Ethanol production by yeasts

Organisms	Natural habitat	Ethanol yield: mmol/100 mmol glucose	Ethanol yield: mg/100 mg glucose	References
Saccharomyces cerevisiae	Widespread	198	50·5	d
		166	42 (pH 3)	a
		171	43 (pH 6)	a
Torulopsis glabrata	Skin	+		g
Candida albicans	Mainly mouth and vagina	+		h
C. parapsilosis	Skin	f		
C. guilliermondii	Skin	f		
(*Debaromyces hansenii*)	Skin	f/o		
C. pseudotropicalis	Skin and mouth	f		
C. tropicalis	Skin and mouth	f		
C. zeylanoides	Skin	?		
D. kloeckeri	Skin	?		
Torulopsis famata	Skin	?		
Rhodotorula spp.	Skin	o		
Trichosporon cutaneum	Skin	o		
Cryptococcus spp.	Skin and mouth	o		
Saccharomyces spp.	Skin	f		
Pityrosporum ovale	Skin (scalp)	o		
P. orbiculare	Skin	o		

f, ferments glucose (presumably producing ethanol); o, oxidizes glucose (presumably not producing ethanol); f/o, reaction varies depending on strain; ?, reaction not known (Smith 1969; Barnett & Pankhurst 1974); +, produces ethanol; d, Oginskey & Umbreit (1959); a, Wood (1961); g, White *et al.* (1972); h, Plueckhahn & Ballard (1968).

two other yeasts, and also lists other species that have been isolated (mostly from the skin (Marples 1974; Noble & Somerville 1974)). Ethanol has not actually been identified as a metabolic product of many yeasts but if it is assumed that all species capable of fermenting glucose produce ethanol then the majority of yeasts associated with humans have this capability. Some moulds produce high acetaldehyde levels when oxygen levels are relatively high (Foster 1949).

Dawes (1963) reviewed mechanisms of microbial ethanol production. The maximum theoretical yield is two molecules of ethanol for each molecule of glucose fermented (51 mg ethanol/100 mg glucose). This yield is approached during fermentation by yeasts and moulds that use the Embden–Meyerhof glycolytic pathway (Rose 1976) in

combination with pyruvate decarboxylase and alcohol dehydrogenase. The overall reaction can be expressed:

$$\text{glucose} \rightarrow 2\ \text{ethanol} + 2CO_2.$$

A few bacteria (e.g. *Sarcina* (*Zymosarcina*) *ventriculi* found particularly in the intestine of vegetarians (Crowther 1971) and in stomach infections (see Section 2), and *Erwinia amylovora* (Haq & Dawes 1971)) also produce ethanol by this pathway.

TABLE 15(*b*)

Ethanol production by moulds (most occur widely, especially in soil and decaying matter)

Organisms	Ethanol yield: mmol/100 mmol glucose	Ethanol yield: mg/100 mg glucose	Reference
Alternaria (3 sp.)	11–31	3–8	j
Aspergillus clavatus (2 strains)	103–114	26–29	j
A. flavus (aerated)	97	24·8	i
A. flavus (2 strains)	89–136	23–35	j
A. luchuensis (3 strains)	75–92	19–23·5	j
A. nidulans (type A, 3 strains)	53–65	14–17	j
A. niger (4 strains)	41–86	10–22	j
Clasterosporium sp.	43	11	j
Eidamia viridescens	70	18	j
Epicoccum sp.	58	15	j
Fumago sp.	28	7	j
Fusarium avenaceum	159	41	j
F. lini	76	19	j
F. oxysporum (aerated)	110	28	i
F. tuberculariodes	147	38	j
Helminthosporium geniculatum	52	13	j
Penicillium dahleae	113	29	j
P. digitatum	59–87	15–22	j
P. spiculisporum	153	39	j
P. terrestre (1 strain)	6	1·5	j
P. terrestre (7 strains)	70–110	18–28	j
Rhizopus oryzae (aerated)	20	5·1	i
Sordaria sp.	95	24	j
Trichoderma sp.	61	16	j

i, Foster (1949); j, Raistrick *et al.* (1931). Ethanol levels calculated from 'carbon in volatile neutral compounds' which may include aldehydes, ketones, etc. Cultures grown in restricted aeration in Czapek-Dox synthetic medium with glucose as sole carbon source.

In addition to the above pathway, *Sarcina ventriculi* also possesses enzymes which reduce pyruvate to acetyl phosphate and formic acid (or $H_2 + CO_2$). The acetyl phosphate is further reduced, perhaps via acetyl co-enzyme A to acetaldehyde and finally to ethanol. The Enterobacteriaceae (e.g. *Proteus vulgaris*, *Escherichia coli*, *Aerobacter aerogenes*) and some *Clostridia* spp. produce ethanol by a similar pathway, with a number of other products. For every four molecules of pyruvate formed (from two molecules of glucose), *E. coli* reduces two to lactate and oxidizes two to acetate, and one molecule of acetate is then reduced to ethanol (this is known as the 'mixed acid fermentation'). Thus, the theoretical maximum yield of ethanol from glucose by

fermentation of *E. coli* is one molecule of ethanol from every two molecules of glucose (12·8 mg ethanol/100 mg glucose, one quarter the maximum yield for yeasts). Other members of the Enterobacteriaceae (*Enterobacter* (*Aerobacter*) *aerogenes*, *Erwinia caratovora*, *Serratia* spp.) and *Bacillus* spp. produce butanediol, diacetyl and acetyl-methylcarbinol (acetoin) (Wood 1961; Rose 1976), but the yield of ethanol appears to be similar to that of *E. coli* (Wood 1961; Table 15*c*). Ørskov (in *Bergey's Manual of Determinative Bacteriology* (Buchanan & Gibbons 1974)) states that most *Klebsiella* strains produce 2,3-butanediol as a major product of glucose fermentation, with lactic, acetic and formic acids in smaller amounts and ethanol in larger amounts than in a mixed acid fermentation. *Enterobacter* spp. appear to have a fermentation similar to *Klebsiella* spp. and most *Proteus* spp. possess a mixed acid fermentation like *E. coli*. Clostridia (and some bacilli) ferment glucose via acetate by a similar pathway to that of the enterobacteria, but differ from the enterobacteria and from each other in their final products. These include butyric acid, butanol, acetone, and isopropanol, (besides ethanol, acetic acid, lactic acid, CO_2, H_2 and acetoin, also found after fermentation by enterobacteria). Many clostridia give higher yields of ethanol from glucose than enterobacteria. (see Table 15*c*). *Zymomonas mobilis* and *Z. anaerobia* produce similar yields of ethanol to that produced by yeasts (see Table 15*c*). They produce pyruvate via the Entner-Doudoroff pathway (Rose 1976) and not the glycolytic pathway, but, like yeasts, possess pyruvate decarboxylase and alcohol dehydrogenase.

The heterolactic acid bacteria, which include some *Lactobacillus* spp. and all *Leuconostoc* spp., produce ethanol from glucose via the hexose monophosphate pathway (Rose 1976). Xylulose-5-phosphate is cleaved to give glyceraldehyde-3-phosphate and acetyl phosphate which are further reduced to ethanol, lactate and carbon dioxide. Ethanol yields can exceed those from Enterobacteriaceae (see Table 15*c*). Some lactobacilli ferment about 30% of glucose to glycerol, acetic acid and CO_2 (Wood 1961). Homolactic acid bacteria (*Streptococci*, e.g. *Streptococcus faecalis* and some *Lactobacillus* spp.) ferment glucose almost quantitatively via the Embden–Meyerhof pathway to lactic acid (analogous to the glycolytic pathway in muscle). However, traces of acetic acid and formic acid (and/or CO_2) and ethanol are still formed by homolactic streptococci, and at high pH may account for 25–40% of the glucose utilized (Gunsalus & Niven 1942; see Table 15*c*).

Qualitative data of Holdeman & Moore (1972) indicate that many anaerobic bacteria produce ethanol, but there is little quantitative information except for some strains of *Clostridium* (see Table 15*c*). A non-sporing anaerobic rod, isolated by Barnes & Impey (1968) from a chicken caecum was shown to produce ethanol as a major metabolic product (150 mmol/100 mmol glucose, Dr J. Peel, personal communication), a level approaching that for yeast or zymomonas. All organisms so far examined which produce ethanol have been found to possess the enzyme alcohol dehydrogenase.

All studies of glucose fermentation by staphylococci (reviewed by Blumenthal 1972) have suggested that lactate is the major end-product with small quantities of acetate and traces of pyruvate. Acetyl-methylcarbinol may sometimes accumulate also. Almost all glucose is fermented via the Embden–Meyerhof pathway. Glucose in the presence of air tends to inhibit acetate oxidation via the tricarboxylic acid cycle. However, Blackmore (1968) reported that four strains of *Staphylococcus aureus* (*pyogenes*) produced ethanol at levels between 9 and 25 mg/100 ml after incubation overnight at 37 °C with 0·5% (w/w) glucose, sucrose, mannitol or lactose in peptone water, determined using gas chromatography. Assuming all the sugar was utilized, this represents yields from 1·8–5

TABLE 15(*c*)

Ethanol production by bacteria

Organism	Natural habitat	Ethanol yield: mmol/100 mmol glucose	Ethanol yield: mg/100 mg glucose	References
Aerobacter indologenes	?	70·5	18	a
Aerobacter aerogenes	Intestine	51·5	13	a
Aeromonas hydrophila	Pathogen	52·0	13·3	a
A. punctata (formerly *Pseudomonas formicans*)	Sewage and water	64·0	16·3	a
'Anaerobe' 59/96	Chicken caecum	150	38·25	e
Bacillus subtilis	Widespread	7·65	1·94	a
B. macerans	Widespread	+		c
Bacteriodes biacutus	Pathogen	+		b
Bact. capillosis	Intestine	+		b
Bifidobacterium adolescentis	Intestine, vagina and mouth	+		b
Clostridium perfringens (*welchii*)	Intestine	trace–47 26	trace–12·0 6·6	m (6 strains) a
Cl. acetobutylicum	Soil	7·2	1·8	a
Cl. aminovalericum	Sewage	+		b
Cl. bifermentans	Intestine, soil, water	82	21	m
Cl. cadaveris	Pathogen, intestine	+		c
Cl. difficile	Intestine	51	13	m
Cl. ghoni	Soil, water	+		b
Cl. glycolicum	Pathogen	+		b
Cl. hastiforme	Pathogen	+		b
Cl. indolis	Pathogen	196	50	m
Cl. litus-eburense	Soil, water	25	6·5	m
Cl. novyi type B	Pathogen	+		c
Cl. oroticum	Pathogen, mud	137	35	m
Cl. putrificum	Intestine, soil	+		c
Cl. ramnosum	Intestine	43	11	m
Cl. septicum	Intestine, soil	45	11·5	m
Cl. sphenoides	Pathogen	180	46	m*
Cl. sordellii	Pathogen	74–176	19–45	m (3 strains)
Cl. sporogenes	Intestine	113–415	29–106	m (8 strains)*
Cl. subterminale	Pathogen, soil	+		b
Escherichia coli	Intestine	50·5 77·0	12·9 (pH 7·8) 19·6 (resting)	a
Erwinia carotovora	Rotting plants	66·2	17·0	a
Erwinia amylovora	Plant pathogen	124·0	31·7	k
Eubacterium saburreum	Mouth	+		b
E. combesii	Soil, water	+		b
Fusobacterium symbiosum	Intestine	+		b
F. russii	Intestine	+		b
Lactobacillus brevis	Widespread, mouth, intestine	75·5	19·3	d
L. mannitopoeus (*buchneri*)	Similar to *brevis*	66·6	17·0	a
L. pentoaceticum		61·2	15·6	a
L. lycopersici		59·4	15·2	a
L. fermentum		+		b
Leuconostoc mesenteroides	Fruit, vegetables and dairy products	112·0	28·55	a
Leuc. dextranicum		81·2	20·7	a
Peptococcus saccharolyticus	Skin	+		b
P. variabilis	Pathogen	+		b

TABLE 15(*c*)—*continued*

Organism	Natural habitat	Ethanol yield: mmol/100 mmol glucose	Ethanol yield: mg/100 mg glucose	References
Peptostreptoccus anaerobius	Mouth, urogenital	+		b
Photobacterium phosphoreum	Sea water and fish	80·7	20·6	a
Ruminococcus bromii	Intestine	+		b
R. albus	Rumen	+		b
Sarcina ventriculi	Intestine	100	25·5	c
Serratia marcescens	Widespread, sputum	46 (anaerobic) 29·6 (aerobic)	18·0 7·55	a
S. kielensis	Buchanan & Gibbons (1974) identifies as *S. marcescens*	46·2	11·8	a
S. plymuthicum		50·5	12·9	a
Streptococcus faecalis	Intestine	7·0 14·6 22·4	1·8 (pH 5) 3·7 (pH 7) 5·7 (pH 9)	a
Strep. liquefaciens	Intestine	2–5·5	0·5–1·4	a
Zymomomonas mobilis	Breweries and fermented drinks	130–170	33–43	c
Z. anaerobia		180	46	c

a, Wood (1961); b, Holdeman & Moore (1972); c, Buchanan & Gibbons (1974); d, Oginskey & Umbreit (1959); e, Barnes & Impey (1968), Dr J. L. Peel (1975, personal communication); k, Sutton & Starr (1959); +, produces ethanol, but yield has not been measured; m, Dr G. Hobbs (1977, personal communication) (yields measured after 48 h incubation at 37 °C in TPYG (trypticase peptone yeast extract glucose) broth; about 25% higher yield would be expected after a further 24 h incubation). * These strains produced significant yields of ethanol in TPY without glucose, so the ethanol yields observed when glucose was present must include some ethanol produced from amino acids.

mg ethanol/100 mg sugar. Unpublished observations in this laboratory have confirmed these results with glucose in approximately half of 30 strains of *Staphylococcus*, isolated from blood samples. These gave yields in the region 2·4–9·6 mg ethanol/100 mg glucose, after incubation in brain heart infusion broth containing 0·7% (w/v) glucose for 3 d at 30 °C.

B. Ethanol from Glycerol

In general, less reduced substrates such as glutarate or 2-ketoglutarate give reduced yields of ethanol compared with glucose, while more reduced substrates (such as glycerol or mannitol) give higher yields of ethanol (Wood 1961; Dawes 1963). Harden (1901) compared yields of ethanol from fermentation by *E. coli* and obtained the following data (mg ethanol/100 mg): glucose or fructose, *ca.* 10; mannitol, 28–34; glycerol, *ca.* 46. Studies by Magasanik *et al.* (1953) showed a yield of 0·86 mol ethanol/mol glycerol (43 mg/100 mg) for *Enterobacter aerogenes*. Stanier & Adams (1944) found a yield of 100·6 mmol ethanol/100 mmol mannitol (25·4 mg/100 mg) for the same organism. Other enterobacteria (e.g. *Citrobacter freundii* and some strains of *E. aerogenes*) produce high levels of trimethylene glycol or acrolein from glycerol and lower yields of ethanol (Mickelson & Werkman 1940*a*,*b*). *Cl. perfringens* also produces acrolein from glycerol (Humphreys 1924) but the ethanol yield was not reported. The cultures were self-sterilizing, which may explain why *C. perfringens* died rapidly when cultured on human fat (Mant 1957).

C. Ethanol from amino acids

There is little information on this subject. Some clostridia and other anaerobes have been shown to produce ethanol from amino acids (*Cl. tetani* produces 30 mmol/100 mmol (10·4 mg/100 mg) from aspartate and 24 mmol/100 mmol (10·5 mg/100 mg) from serine; *Cl. botulinum* produces 57 mmol/100 mmol (25 mg/100 mg) from serine; *Fusobacterium nucleatum* produces 19 mmol/100 mmol (8·3 mg/100 mg) from serine (Barker 1961)). Deamination of amino acids can yield intermediates in ethanol production, and hence, presumably, ethanol e.g. serine is deaminated to pyruvate; leucine via α-oxoisocaproate to acetoacetate and acetylcoenzyme A; tryptophan to indole and pyruvate in *E. coli* and *P. vulgaris* (Rose 1976); cysteine to H_2S and pyruvate in *E. coli*, *P. vulgaris* and other bacteria (Barker 1961); glutamate to acetate and butyrate by *Cl. tetanomorphum* (Barker 1961) and glycine to acetic acid by various anaerobic bacteria (Barker 1961). Threonine, which constitutes 4–6% of most animal proteins is degraded to ethanol via acetaldehyde by *E. coli* (Lenti & Grillo 1953), *Cl. pasteurianum* (Dainty 1967) and group N streptococci (Lees & Jago 1976). That many organisms can produce low levels of ethanol from amino acids is evident from the results of Blackmore (1968) who showed levels from less than 3 mg to 8 mg/100 ml after growth in peptone water with no added carbon source. However, Bogusz *et al.* (1972) considered conversion of alanine, serine and cysteine to pyruvate or ethanol to be almost impossible in putrefying human blood incubated anaerobically, due to lack of 1-oxoglutarate.

D. Ethanol from Fatty acids

Oxidation of fatty acids, which are already highly reduced, to acetyl-coenzyme A would need a hydrogen-accepting system and it appears unlikely that this would occur to a significant extent under anaerobic conditions.

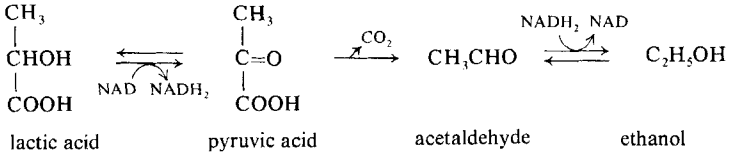
E. Ethanol from Ribose

Pentoses appear to be fermented via the hexose monophosphate pathway (Rose 1976) to glyceraldehyde-3-phosphate, which is then metabolized via the Embden–Meyerhof pathway as for glucose.

A yield of 59·6 mmol ethanol/100 mmol ribose (18 mg/100 mg) has been found for *Enterobacter aerogenes* (Wood 1961), a yield similar to that for glucose.

F. Ethanol from Lactate

Studies of Bogusz *et al.* (1970) have shown that lactate can be a major source of ethanol in putrefying blood, and that pyruvate is an intermediate. This transformation presumably relies on the reactions catalysed by lactate dehydrogenase (LDH), pyruvate decarboxylase and alcohol dehydrogenase:



Since all these enzymes are commonly found in bacteria and LDH also in human tissues, and lactate is found at relatively high concentration in all tissues examined, lactate may well be an important source of ethanol post-mortem.

G. Ethanol utilization

Ethanol is oxidized by a wide variety of micro-organisms and may be used also as a sole carbon source. Some clostridia utilize ethanol in an ethanol/fatty acid fermentation (Wood 1961) while *E. coli*, which produces ethanol in nutrient broth, subsequently utilizes it anaerobically (Dawes & Foster 1956).

H. Conclusions

Although many people concerned with forensic science still appear to believe that only yeasts produce ethanol, various micro-organisms which have been detected in bodies post-mortem, besides yeasts, are capable of producing significant yields of ethanol from glucose and ribose. Lesser quantities of ethanol can also be formed from amino-acids, and some organisms (particularly Enterobacteriaceae) give much higher yields of ethanol from glycerol than from glucose. Conversion of lactate to ethanol via pyruvate is also possible and there is evidence that it does occur, at least in blood. Because micro-organisms are also capable of utilizing ethanol, and because constitutive chemical (Smalldon & Brown 1973) and biochemical mechanisms for ethanol oxidation exist in corpses, a complicated interaction between various bacteria producing and utilizing ethanol and constitutive systems most probably occurs. The net result being that when high ethanol concentrations exist ante-mortem there is a subsequent fall post-mortem, and when levels are low ante-mortem they can be expected to increase post-mortem.

8. Summary, Discussion and Conclusions

Although ethanol can on rare occasions be detected in blood from living subjects who have not ingested alcohol, these levels never exceed 5 mg/100 ml. On the other hand, levels up to 150 mg/100 g have been detected in blood and tissues of putrefied human or rodent corpses. Ingestion of ethanol ante-mortem in these cases is known not to have taken place (in the case of the rodents), or is most unlikely to have taken place (in the case of humans). Production of ethanol has occurred, not only in tissues that have obviously putrefied, but within a relatively short time (Bonnichsen *et al.* 1953) if temperatures are elevated (i.e. above about 15 °C). Experience with decomposition of meat shows that high numbers of bacteria can be present without showing obvious signs of putrefaction.

The limited evidence available suggests that ethanol is not formed post-mortem except by microbial action, and that ethanol is both produced and utilized, so that bodies with high initial levels will show a decrease, and bodies with low initial levels will show an increase.

The method by which bacteria invade dead bodies is not entirely clear. However, the source appears to be mainly intestinal, although injury resulting in skin breakage immediately before death may introduce exogenous micro-organisms into the blood stream and thoughout the body. There is evidence that bacteria may penetrate the intestinal walls during death and be distributed throughout the tissues in the blood stream, this may also occur during food absorption and from skin abrasions, etc. throughout life. Even after clinical death has occurred these organisms may be prevented from multiplying or actually killed, by the residual antimicrobial defences of the body, and the anaerobic organisms will be inhibited initially by the high Eh, but within a few hours, provided the temperature exceeds about 5 °C, they will start to multiply. This primary invasion is probably reinforced by a secondary invasion of

intestinal organisms, starting via the hepatic portal vein and the intestinal lymph system, and spreading round the body via the vascular system.

Although the intestine harbours a wide variety of organisms, the majority obligate and fastidious anaerobes, only relatively few groups have been implicated as major colonizers of corpses during putrefaction; these include, in order of importance, *Cl. perfringens* (a vigorous saccharolytic, lipolytic and proteolytic organism) and other *Clostridium* spp., enterobacteria (frequently, *E. coli* and *Proteus* spp.), Micrococcaeae (frequently *Staph. aureus*), streptococci and *Bacillus* spp. All of these are capable of producing ethanol from glucose and other substrates. In addition, a wider variety of organisms may be detected in the early stages of putrefaction, and these include yeasts, which may produce very high ethanol levels if present in sufficiently high numbers.

Information on levels of substrates present shortly after death is sparse and further studies on this subject would be of interest. Glucose may be present in high levels in the liver and nearby blood and tissues, levels in the blood generally may be raised. Other possibly important sources are amino-acids (especially once proteolysis has commenced), glycerol (formed during fat hydrolysis), and lactate which occurs widely and at levels over 100 mg/100 g in all tissues. There is evidence that all these compounds can serve as substrates for ethanol production by bacteria commonly found in corpses.

Forensic scientists must, therefore, always bear in mind that specimens of human tissue containing micro-organisms, particularly specimens taken from corpses, may contain ethanol produced by microbial fermentation, and that extreme caution should be exercised when assessing the significance of post-mortem ethanol.

I am grateful in particular to the late Professor M. Ingram for his keen interest in this review and for helpful discussions, and suggestions and information during its preparation. I should like to thank also Professor D. A. A. Mossel, Dr B. Mackie, Dr H. M. Stevens, Mr P. Morrison, Professor K. Simpson and many others for their help. I should like to thank Dr R. L. Williams for encouragement and for the use of the facilities of the Metropolitan Police Laboratory.

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