Intestinal bacteria and cancer

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INTRODUCTION

It is generally accepted by most epidemiologists that diet plays a major role in the causation of large bowel cancer, although there are still some dissenters [1]. There is little agreement on the dietary item involved, since meat, fat, protein, fibre, alcohol, milk and vitamins A and C have all been implicated; to a large extent this is due to the shortcomings of the diet recall methods used in case control studies and it is notable that all of the studies of large numbers of populations agree on a role for meat or its component fat or animal protein. Many carcinogens or co–carcinogens can be detected in food, (such as N–nitroso compounds, aflatoxins, polycyclic aromatic hydrocarbons) but these are usually detected at maximum concentrations in the food of populations at *low* risk of large bowel cancer. Consequently we postulated [2, 3] that a carcinogen or co–carcinogen is produced by the intestinal bacterial flora from some benign substrate whose concentration is determined by the diet.

This postulate is not unreasonable. It has been demonstrated that the potent carcinogen methylazoxymethanol is released from its harmless glucoside cycasin by bacterial β- glucosidase. The intestinal mucosal β–glucosidase has a narrow substrate range and is inactive on cycasin; consequently when cycasin is fed to germ–free rats it is excreted unchanged in the faeces and the animal suffers no toxic effects. When fed to conventional animals, in contrast, the β–glucosidase of the gut bacterial flora releases the methylazoxymethanol which then gives rise to hepatotoxicity and subsequently to intestinal carcinogenesis. This has been reviewed by Laqueur and Saptz [4].

In order to test this postulate we need to determine (*a*) the likely nature of the substrate, (*b*) the chemical modification necessary to convert the benign substrate into a carcinogen or co–carcinogen, and (*c*) the intestinal organisms able to carry out the chemical modification. Clues to the nature

of the substrate come from the epidemiology described in Table 1. The best data incriminate dietary meat or its component fat or animal protein; if the proponents of the protective value of fibre are correct, then the carcinogen/co-carcinogen is still likely to be derived from meat or its components with the fibre causing a stool bulking effect and the resultant dilution of its contents. Simililarly, any protective effects of vitamins A and C are likely to be due to their inhibition of the enzymes responsible for carcinogen production. A number of possible substrates for carcinogen production and determined by the amount of dietary meat have been suggested (Table 2). They are derived both from the protein and the fat components of meat, and will be discussed in turn.

TABLE 1

The relation between diet and large bowel cancer

Diet item	Type of study	Reference
Meat	Case-control	Haenszel and Coll. [20]
	32 Populations	Armstrong & Doll [21]
	3 Populations	Hill and Coll. (in prep.) [10]
Fat	Case-control	Wynder and Coll. [22]
	37 Populations	Drasar & Irving, [23]
	32 Populations	Armstrong & Doll, [21]
	3 Populations	Hill and Coll. (in prep.) [10]
Animal protein	28 Populations	Gregor and Coll. [24]
	37 Populations	Drasar & Irving, [23]
	32 Populations	Armstrong & Doll, [21]
	3 Populations	Hill and Coll. (in prep.) [10]
Fibre (protective)	2 Populations	Walker and Coll [25]
	General	Burkitt, [26]
	2 Populations	IARC Working Party [27]
	Case-control	Modan and Coll. [28]
Fibre (causative)	Case-control	Haenszel and Coll. [29]
	3 Populations	Hill and Coll. (in prep.) [10]
Alcohol	2 Populations	IARC Working Party [27]
	47 Populations	Enstrom, [30]
Milk (protective)	2 Populations	IARC Working Party [27]
Vitamins A and C (protective)	Case-control	Bjelke [31]

TABLE 2

Possible substrates derived from meat which might undergo gut bacterial metabolism to carcinogens or co-carcinogens

Meat component	Substrate	Microbial metabolite
Protein	Tryptophan	Various metabolites
	Tyrosine	Volatile phenols (phenol and p-cresol)
	Methionine	Ethionine
	Basic amino acids	N-nitroso compounds
Fat	Lecithin	Dimethylnitrosamine
	Cholesterol	Various metabolites (protective)
	Bile acids	Unsaturated products

SUBSTRATES FOR CARCINOGEN PRODUCTION FROM MEAT

Tryptophan

The excretion of urinary metabolites of tryptophan has long been associated with cancer of the bladder [5, 6] and a range of them has been shown to carcinogenic including kynurenine, 3-hydroxykynurenine, 3-hydroxyanthranilic acid, quinaldic acid, 8-hydroxyquinaldic acid and xanthenuric acid. The urinary tryptophan metabolites are usually assumed to be the products of hepatic metabolism but it is important to remember that all of the relevant pathways were first described in bacteria. Thus, if these metabolites can act as cocarcinogens in bladder carcinogenesis and if they are produced in the colon by bacterial action, then it is possible that they might also be implicated in colon carcinogenesis.

We have no data on the relative contributions of the gut flora and the liver to the pool of urinary tryptophan metabolites and we have no data on the extent of tryptophan metabolism in the human gut. However Bone has shown that the faecal concentration of tryptophan is much higher in cases of large bowel cancer than in controls, and that in a study of six populations there was a relation between the faecal tryptophan concentration and the incidence of large bowel cancer. These were very preliminary studies but at this stage we cannot rule out a role for tryptophan metabolites

in the causation of bowel cancer. The faecal tryptophan concentration increased with increased dietary protein (Cummings and Coll. in preparation).

Tyrosine

Normal human urine contains 50–100 mg of volatile phenols per day and these are the products of tyrosine metabolism in the large intestine. The factors determining the amount of urinary volatile phenols (UVP) have been reviewed by Bone and Coll [7]. In a case–control study the amount of UVP per day in large bowel cancer cases did not differ from that in controls [7] indicating that these products of tyrosine metabolism are unlikely to be of major importance in the causation of colorectal cancer.

Methionine

Methionine can be converted *in vitro* to its S-ethyl analogue (ethionine) in the presence of sulphate ions by a number of bacteria including *Escherichia coli*. This may be important because Farber [8] showed that ethionine is carcinogenic in rats, but its production in the human gut has not been demonstrated and it has been investigated as a possible cause of bowel cancer.

Basic amino acids

The basic amino acids lysine, arginine and ornithine are metabolised by the gut flora to the cyclic secondary amines piperidine and pyrrolidine. About 1–2 mg per day of these cyclic secondary amines are absorbed from the colon and excreted in the urine and a further small amount is unabsorbed and is excreted in the faeces. Piperidine and pyrrolidine may be further metabolised by colonic bacteria in the presence of nitrate to give the highly carcinogenic N–nitroso derivatives.

Previously we have considered this to be an unlikely event in the human colon because preliminary studies indicated that all of the dietary nitrate was absorbed from the small intestine. However, Bruce and Coll. [9] have tentatively identified faecal N–nitroso compounds and if this is confirmed we will need to re–assess the pharmacology of dietary nitrate. Perhaps nitrate is secreted into the colon by the same mechanism as its secretion in saliva and gastric juice.

If the production of N–nitrosamines by bacterial action in the colon were important in large bowel carcinogenesis, then the disease should be associated epidemiologically with high levels of dietary nitrate. In fact this is not so, although dietary nitrate is associated with gastric cancer in a number of studies (Table 3). This has been reviewed by Hill [10].

TABLE 3

Studies indicating a relation between nitrate intake and gastric cancer

Country of study	Reference
Britain	Hill and Coll. [32]
Chile	Zaldivar & Robinson [33]
	Armijo & Coulson [34]
Colombia	Hawksworth and Coll. [35]
	Cuello and Coll. [36]
Japan	Haenszel and. Coll. [20]

Lecithin

Lecithin is metabolised to choline by bacterial phospholipases, and the released cholin is dealkylated to dimethylamine. This secondary amine is N–nitrosatable in the presence of nitrate, but is unlikely to be involved in the causation of large bowel cancer for the reasons described above.

Cholesterol

Cholesterol has been shown to be carcinogenic in animals by Hieger, who reviewed his work in 1958. Cholesterol is excreted in normal human faeces unchanged or as its metabolites coprostanol, coprostanone and cholestenone. Wilkins and Hackman [11] claimed two patterns of neutral steroid excretion in Americans; in 8/31 (26 %) less than 30 % of the cholesterol was converted to metabolites whilst in 23/31 more than 60 % of the cholesterol had been metabolised. They suggested that the «poor metabolisers» were the more likely to develop large bowel cancer and this would fit with the observations of Hieger [12]. However, the finding of 26 % poor metabolisers in a normal healthy western population is unique in the literature, with most workers reporting much less than 10 % of persons. In our studies of 10 populations the results, if anything, indicated an *inverse* correlation between bowel cancer incidence and the percentage of low metabolisers [13] and in our case–control study we could show no difference between the two groups in this respect.

Bile acids

The faecal bile acid concentration is related to the amount of dietary fat [14] and so the bile acids are suitable substrates for this study. There is now a large (and growing) body of evidence incriminating faecal bile acid metabolites in the causation of large bowel cancer (Table 4) from metabolic epidemiology and from animal studies.

TABLE 4

Evidence in favour of bile acid metabolites being incriminated in the causation of large bowel cancer

Type of study:

Humans:

1. Populations around the world or from around the world:
2. Populations within a country:
3. Case–control study or comparison of groups of patients with groups of controls:
4. Study of high risk groups of patients.

Animals:

1. Bile acids are co–carcinogens:
2. Bile diversion studies.

A number of bile acids have been shown to be co–carcinogens in animals [15] and in mutagenesis assay systems [16] in particular deoxycholic acid. The faecal concentration of this bile acid correlated well with the incidence of large bowel cancer in 9 populations [3] but was non better than total faecal bile acids as a discriminant in a case–control study [17]. The results of this case–control study (Table 5) are compatible with the postulate that the co–carcinogen causing large bowel cancer in an unsaturated bile acid produced by certain Clostridia (referred to as NDC) from the colonic bile acids. Such bile acids have been detected in the faeces of certain animals and we are currently attempting to identify them in human faeces.

Much of our current work is directed towards the predictive value of faecal bile acids in colorectal carcinogenesis and we are conducting a pro-

TABLE 5

The results of a case–control study to test the hypothesis that the combination of high faecal bile acid (FBA) concentration and the presence of NDC characterises large bowel cancer cases

FBA CONCENTRATION	Presence of NDC	% of patients with colorectal cancer (116) (*)	% of patients with other gastrointestinal disease (120)
High	+	71	8
Low	+	12	35
Ligh	−	12	11
Low	−	5	46

(*) Total number of patients.

spective study of 8000 persons aged 45–75 living in three areas (Glyncorrwg, a mining village in South Wales; Hay-on-Wye, a farming area in Herefordshire; West Granton, a region of Edinburgh). We should have clear results from this study within about five years.

In addition, we are attempting to improve the discriminant value of the combination of bile acid concentration and the presence of NDC by adding further factors. The production of unsaturated bile acids by NDC requires the presence of a suitable hydrogen acceptor and this is most likely to be vitamin K [18]. If this is so, then the combination of faecal bile acids, NDC and high levels of vitamin K should give an improved discriminant. Further, it has been shown that the faecal activity of bile acid 7–dehydroxylase, an enzyme produced principally by the anearobic bacterial flora [3] is higher in large bowel cancer cases than in controls [19]. This would suggest that our co–carcinogen might be a 4,6–dien–3–one and that the best discriminant should be a combination of 4 factors–faecal bile acid concentration, presence of NDC, high faecal vitamin K activity and high 7–dehydroxylase activity. This is currently being tested.

Summary. — Epidemiology indicates that diet plays an important part in the causation of colorectal cancer. We have postulated that this is because the diet determines the amount of substrate available for microbial

metabolism to carcinogenic or co–carcinogenic products. We have studied a wide range of possible substrates and the evidence to date indicates that the most likely ones are the bile acids. Much more work is necessary before we can confirm or refute this postulated association.

Riassunto. (*Batteri intestinali e cancro*) — Studi epidemiologici indicano che la dieta svolge un ruolo importante nel determinismo del cancro del colon e del retto.

Noi abbiamo ipotizzato che questo succede perché la dieta determina la produzione di un substrato utilizzabile nel metabolismo batterico per la produzione di sostanze carcinogenetiche o precarcinogenetiche. Abbiamo studiato diversi substrati ed i risultati hanno messo in evidenza che tra questi quelli che hanno maggiore importanza sono gli acidi biliari.

Tuttavia sono necessari ulteriori studi che confermino o rigettino questa ipotesi.

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Infezioni anaerobie in chirurgia

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Abbiamo assistito negli ultimi anni ad un mutamento della flora patogena nelle infezioni chirurgiche non solo da Gram–positivi verso Gram–negativi, ma, in particolare, da germi aerobi a germi anaerobi. Questo è stato in parte determinato dal fatto che l'antibioticoterapia ha sempre rivolto la sua attenzione al trattamento delle infezioni sostenute da batteri aerobi Gram–positivi e Gram–negativi e solo di alcuni particolari tipi di infezioni anaerobie come il tetano e la gangrena gassosa. A ciò si aggiunge l'affinamento delle tecniche microbiologiche per l'isolamento e la coltura dei germi anaerobi (per cui si è fatta giustizia di alcuni luoghi comuni come la definizione di « pus sterile » per materiale ascessuale da cui non si isolavano germi aerobi [1, 2]).

Molti sono i dati in letteratura che evidenziano l'entità di tale problema riportando l'incidenza degli anaerobi nelle principali infezioni chirurgiche: in 67 casi di infezioni intraddominali abbiamo il 79 % di infezioni miste ed il 15 % di infezioni anaerobie pure; in 33 casi di infezioni ginecologiche l'incidenza delle colture anaerobie pure è stato del 36 %, e per l'apparato respiratorio del 52 % in 64 casi esaminati (Tab. 1).

La diagnosi di infezione anaerobia non è sempre sostenuta dal reperto batteriologico di coltura positiva; spesso infatti il solo quadro clinico suggerisce e suffraga tale diagnosi in base a particolari caratteristiche delle infezioni anaerobie (Tab. 2). Innanzitutto la sede, profondamente situata; la via di entrata del tratto gastrointestinale e peritoneale; l'odore fetido; la formazione di gas; la necrosi tissutale [3].

In generale gli anaerobi vengono dalla flora normale dell'individuo. Le mucose della bocca, dell'apparato respiratorio, del tubo digerente come anche del tratto genitale femminile sono le zone anatomiche più comunemente colpite dalla colonizzazione della flora normale. Una soluzione di continuo di tali tessuti, traumatica o chirurgica, porta alla contaminazione ed, eventualmente, alla infezione da anaerobi.

TABELLA 1

Incidenza isolamento anaerobi nelle infezioni chirurgiche

FLOGOSI	Casi n.	Misti ad aerobi %	Puri %
Endoaddominale	67	79	15
Ginecologica	33	64	36
Apparato respiratorio	64	34	52
Testa e collo	83	20	31
Vie urinarie	107	9	—
Vie biliari	—	—	—
Cute e tessuti molli	57	47	16

TABELLA 2

Caratteristiche della flogosi da anaerobi

— Sede
  - Flogosi profondamente situate nella pelvi e nella cavità peritoneale
  - Porta d'ingresso
— Odore fetido;
— Formazione di gas;
— Tendenza allo scollamento;
— Tendenza alla diffusione con necrosi tissutale;
— Negatività colturale per aerobi.

Per quello che riguarda la chirurgia addominale, branca di nostro specifico interesse, è importante sottolineare che tutto il tratto gastroenterico, e specialmente il grosso intestino, è popolato da una flora complessa, in cui il rapporto tra anaerobi ed aerobi è di 1000:1. Difficile talora valutare la reale patogenicità degli anaerobi, sia perché frequentemente associati a germi aerobi, sia perché, come taluni AA. ritengono, la loro presenza potrebbe essere espressione di una colonizzazione fortuita.

Possiamo trovarci di fronte a quattro possibilità diverse (Tab. 3): nella prima abbiamo una infezione con coltura positiva per anaerobi; questa è la condizione ottimale che ci può far affermare di trovarci di fronte ad una infezione da anaerobi; la seconda in cui vi è una infezione con coltura positiva per aerobi, ed anche in questo caso potrebbero essere in giuoco gli anaerobi se non è stata fatta una chiara ricerca degli anaerobi medesimi; la terza possibilità è la presenza di infezioni con colture di aerobi ed anaerobi; la quarta possibilità, che è la più frequente, almeno sino ad oggi, è data dalla presenza di infezioni con colture negative, determinandosi così il caso del cosiddetto « pus sterile».

TABELLA 3

Infezioni da anaerobi

1) Infezione + coltura positiva anaerobi
2) Infezione + coltura positiva aerobi
3) Infezione + coltura positiva aerobi ed anaerobi
4) Infezione + coltura negativa anaerobi (pus sterile!)

Tipicamente le infezioni da anaerobi si sviluppano nei cosiddetti spazi chiusi.

Nella peritonite sperimentale, indotta da Gorbach e Coll. (Fig. 1) in ratti Wistar sotto trattamento antibiotico, inserendo nell'addome una capsula gelatinosa contenente meteriale fecale, l'infezione ha presentato un decorso bifasico. Inizialmente si è verificata una peritonite acuta generalizzata sostenuta da batteri aerobi, associata ad un tasso di mortalità del 43 %; successivamente si è sviluppata una seconda fase caratterizzata dalla presenza di ascessi multipli, dai quali sono stati isolati anaerobi puri [4, 5].

Frequentemente nella esperienza clinica si può avere la formazione di ascessi multipli endoaddominali, senza una evidente fase precedente di peritonite diffusa, a causa delle variazioni indotte dalla antibioticoterapia principalmente attiva contro la flora aerobica [6].

Questo tipo di infezioni anaerobiche spesso è complicato da una batteriemia causata nel 97–99 % da batteri Gram–negativi (*B. fragilis*, Bacteroides) e nell'1–3 % da Gram–positivi (Clostridi, Cocci). Grave conseguenza di tali batteriemie è lo shock settico, la cui insorgenza è determinata dalle endotossine prodotte dai germi Gram–negativi aerobi ed anaerobi [7].

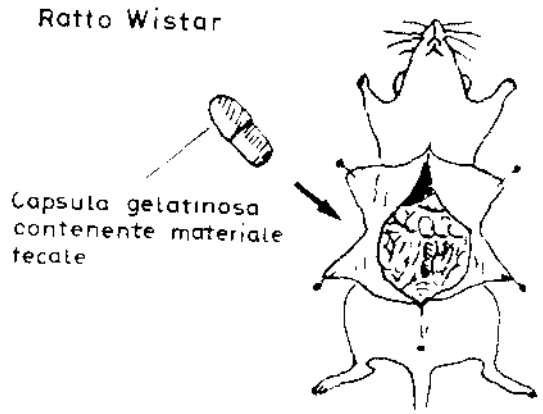

Fig. 1. — Peritonite sperimentale

1ª fase

peritonite generalizzata • coliformi
• enterococchi

mortalità 43 %

↓ 1 settimana

2ª fase

ascessi multipli • anaerobi (*B. fragilis*)

Le endotossine dei Gram–negativi, pur differenziandosi l'una dall'altra, agiscono con il medesimo meccanismo, anche se con tossicità diversa. Tale meccanismo determina una alfa o beta–stimolazione [8]. L'alfa–stimolazione provoca un effetto simpaticomimetico, con vasocostrizione dello sfintere precapillare e quindi aumento delle resistenze periferiche; si realizza così il quadro del cosiddetto shock freddo, ipodinamico, ipovolemico, che si differenzia dallo shock caldo iperdinamico, caratterizzato da basse resistenze, per il quale si è ipotizzata la teoria della beta–stimolazione [9, 12]. Questa provocherebbe l'apertura degli *shunts* periferici, con riduzione dell'afflusso di sangue in vari distretti e specie nello splancnico, con il risultato di una anossia stagnante periferica, vasoparalisi e sequestrazione del sangue.

A tali meccanismi adrenergici si associano reazioni immunitarie indotte da costituenti della parete cellulare dei germi Gram–negativi, di natura lipopolisaccaridica ed aventi potere antigene.

Tale reazione immunitaria dà luogo a liberazione di sostanze vasoattive di tipo vasoparalitico, in particolare istamina e serotonina, che, associate agli alti livelli di catecolamine circolanti, sono responsabili delle gravi reazioni emodinamiche e cellulari.

A ciò si aggiunge un probabile danno cellulare diretto (cioè non mediato per via vascolare) da parte della endotossina [13].

Dal diverso rapporto tra reazioni adrenergiche e reazioni immunitarie risultano i molteplici quadri clinici dello shock tossinfettivo; tale shock conserva a tutt'oggi grande importanza clinica essendo in ordine di frequenza il terzo tipo di shock e, per la sua gravità, quello associato alla più elevata mortalità (25–30 %) [9, 14–16].

In chirurgia addominale lo shock settico si ritrova più frequentemente come complicanza di peritoniti acute generalizzate [17, 18].

Normalmente esiste a livello della sierosa peritoneale una *clearance* che assicura un drenaggio dei batteri e delle tossine presenti nel cavo, rispettivamente attraverso i vasi linfatici e venosi. Tale *clearance* fisiologica viene alterata dalla presenza nel cavo peritoneale di materiale organico, come mucina ed emoglobina.

Sleeman [10] iniettò nel peritoneo albumina marcata insieme con un certo numero di *E. coli*, poi controllò sia la concentrazione plasmatica di albumina che il numero delle colonie batteriche nella sierosa peritoneale [9]. Quando batteri sono introdotti senza altre sostanze si ha un massimo riassorbimento di albumina marcata ed un numero minimo di colonie. Quando c'è materiale organico, mucina ed emoglobina, si verifica invece un minimo riassorbimento di albumina e massima proliferazione batterica.

In caso di flogosi peritoneale, i vasi linfatici vengono compressi e funzionalmente esclusi con riduzione della *clearance* batterica, inoltre c'è un maggior assorbimento per via ematica di endotossine.

La presenza di abnormi quantità di germi nella cavità peritoneale determina una reazione flogistica con essudazione fibrinosa [4]; la fibrina determina la formazione di innumerevoli sepimenti e quindi di una miriade di piccoli ascessi annidati nelle innumerevoli concamerazioni esistenti tra ansa ed ansa, nelle pliche e nei recessi [6].

Sulla base di queste considerazioni si può comprendere l'inefficacia dell'antibioticoterapia e dell'uso dei drenaggi, in corso di peritonite, mentre molto efficace risulta essere il lavaggio peritoneale (Fig. 2) in quanto agente meccanico di rimozione dei germi e delle sostanze organiche presenti nel cavo, e pertanto capace di ripristinare la fisiologica *clearance* peritoneale [19–21].

Una corretta tattica operatoria prevede quindi l'utilizzazione di drenaggi per permettere il lavaggio peritoneale; la soluzione per tale lavaggio è costituita da una soluzione elettrolitica bilanciata con l'aggiunta di eparina e sostanze fibrinolitiche [5].

Per quanto riguarda il trattamento antibiotico delle infezioni chirurgiche, riportiamo brevemente la nostra esperienza dell'uso di un nuovo antibiotico della famiglia delle cefamicine, la cefoxitina, che ha la particolarità di essere attiva sui germi Gram-positivi e Gram-negativi.

La cefoxitina inibisce la sintesi della parete cellulare batterica e svolge effetto battericida [22–25]. La particolare struttura molecolare conferisce

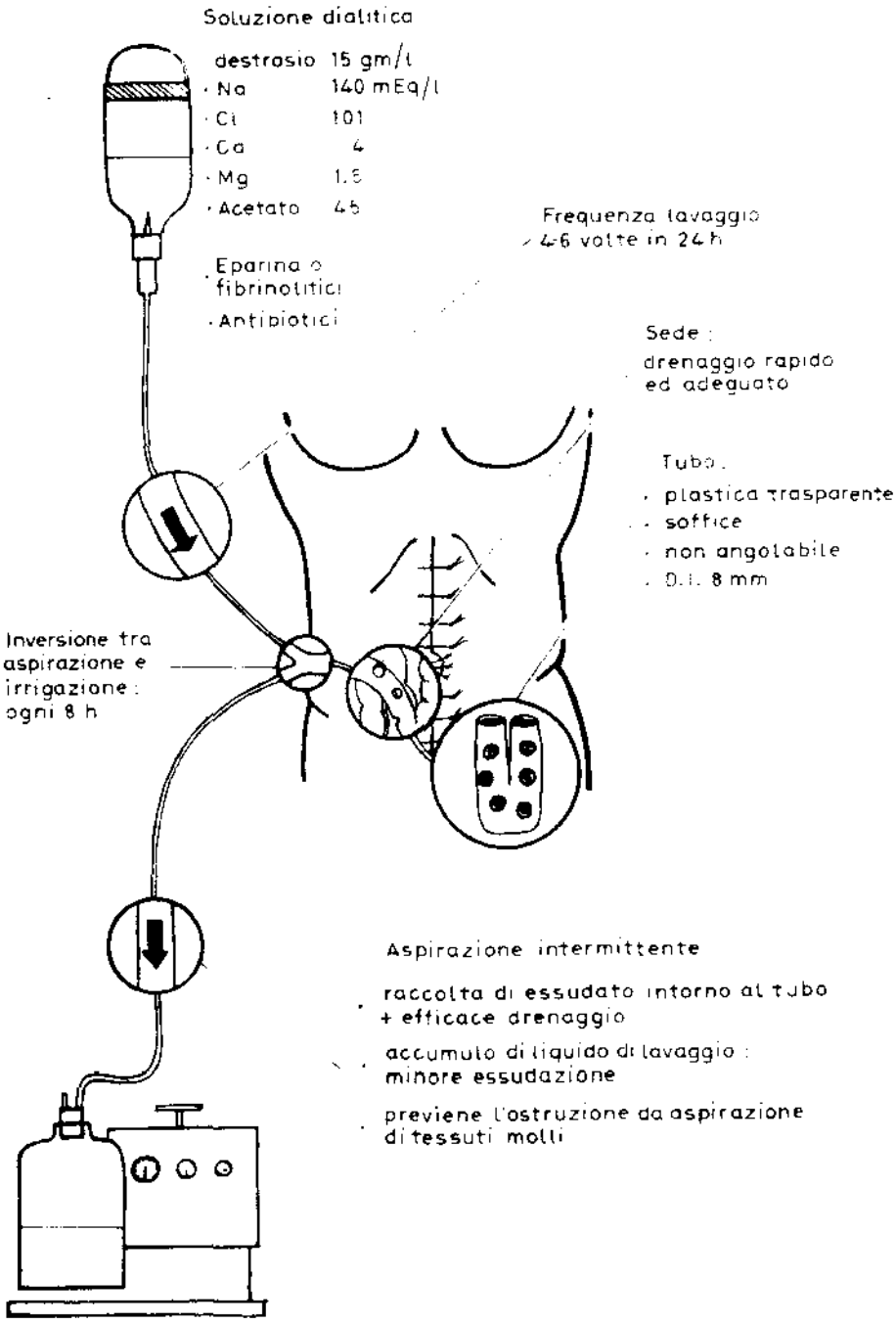

Fig. 2. — Tecnica del lavaggio peritoneale.

al farmaco un eccezionale grado di resistenza alle beta-lattamasi [25-29], le quali rappresentano uno dei principali meccanismi della resistenza batterica alle penicilline ed alle cefalosporine.

Sono stati trattati 50 pazienti, compresi tra i 20 ed i 73 anni, età media 46 anni, ospedalizzati per varie patologie: infezioni intraaddominali postoperatorie (30 casi), infezioni genito-urinarie (9 casi), infezioni delle vie respiratorie (6 casi), setticemia (4 casi), e infezioni ossee (1 caso).

Di ogni paziente è stata ottenuta una emocoltura per gli aerobi e la conta batterica su urine e colture di altri liquidi di drenaggio. In alcuni casi si è provveduto a colture specifiche per anaerobi; su queste colture è stato eseguito un antibiogramma standard, saggiando anche la sensibilità alla cefoxitina. Durante il trattamento con la cefoxitina non è stato somministrato alcun altro antibiotico.

L'antibiotico è stato somministrato al dosaggio iniziale di 2 g x 3 endovena al dì, per una media di 5,57 gg., seguito da 1 g x 3 i.m. al dì, diluito con soluzione di lidocaina, per una media di 4,99 gg. [12].

Tutti i patogeni isolati si sono dimostrati sensibili alla cefoxitina mentre alcuni ceppi hanno dimostrato vario grado di resistenza ad altri antibiotici tipo penicillina, gentamicina, cloramfenicolo, ampicillina; la cefoxitina, inoltre, si è dimostrata anche più attiva di altre cefalosporine (Tab. 4) [23, 30, 31].

L'intensità della infezione è stata giudicata grave in 17 pazienti e moderata in 13. I risultati batteriologici del trattamento nei pazienti nei quali è stato possibile isolare il germe patogeno sono riportati nella Tab. 5.

I controlli batteriologici dopo il trattamento hanno confermato la validità terapeutica della cefoxitina. Gli ottimi risultati clinici conseguiti a nostro avviso debbono essere attribuiti in gran parte proprio alla efficace azione sugli anaerobi.

Non c'è dubbio che, nell'ambito della chirurgia gastroenterica, la chirurgia del grosso intestino è quella gravata da un maggior numero di complicanze infettive postoperatorie.

I motivi di ciò vanno senz'altro ricercati nel più alto contenuto batterico a livello del tratto distale dell'intestino. Il numero di batteri per grammo di contenuto del viscere aumenta dall'alto verso il basso, sino a raggiungere un massimo di 10^{11} nel sigma-retto. È pertanto necessario ridurre il contenuto intestinale dei pazienti da sottoporre ad interventi chirurgici sul grosso intestino, sia attraverso diete, purganti e lavaggio del colon, sia con l'uso di chemioterapici (metronidazolo) od antibiotici (cefoxitina, neomicina, kanamicina).

In conclusione gli anaerobi rivestono un ruolo di sempre maggiore importanza nelle infezioni chirurgiche. Dal momento che la maggior parte delle infezioni chirurgiche sono causate da una flora mista aerobia ed anaerobia,

TABELLA 4

Test di sensibilità

PATOGENI	N. isolati	Cefoxitina			Cefalotina			Cefazolina		
		S	I	R	S	I	R	S	I	R
E. coli	25	25	—	—	8	13	4	4	17	4
Proteus mirabilis	4	4	—	—	—	4	—	—	2	2
Proteus vulgaris	5	5	—	—	2	3	—	—	3	2
Staph. aureus	5	5	—	—	—	5	—	—	3	2
Strep. faecalis	5	5	—	—	—	5	—	—	3	2
Klebsiella	2	2	—	—	—	2	—	—	2	—
Streptococcus	2	2	—	—	2	—	—	2	—	—
Bacteroides fr.	2	2	—	—	—	2	—	—	2	—

S → sensibili
I → intermedi
R → resistenti

TABELLA 5

Risultati batteriologici

PATOGENI	N. TOTALE	Eradicati	Soppressi	Persistenti sensibili
E. coli	25	20	5	—
Proteus mirabilis	4	4	—	—
Proteus vulgaris	5	4	—	1
Staph. aureus	5	5	—	—
Strept. faecalis	5	3	2	—
Klebsiella	2	2	—	—
Streptococcus	2	2	—	—
Bacteroides fr.	2	2	—	—

anche in assenza di un reperto batteriologico positivo per gli anaerobi, è necessario somministrare un antibiotico od una combinazione di antibiotici attivi su entrambe le specie.

Riassunto. — L'incidenza degli anaerobi nelle infezioni chirurgiche è negli ultimi anni notevolmente aumentata in seguito ad un aumento assoluto della flora anaerobia ed al miglioramento delle tecniche di isolamento e di coltura che hanno drasticamente diminuito l'incidenza del cosiddetto « pus sterile».

L'evoluzione dello shock tossi-infettivo da germi anaerobi Gram-negativi, presenta seri problemi di ordine clinico, essendo lo shock gravato dalla più alta mortalità. Un esempio paradigmatico di shock determinato da infezioni chirurgiche è quello che complica la peritonite. Una condotta postoperatoria corretta necessita di lavaggio peritoneale attraverso i drenaggi posti *in situ* preoperatoriamente.

È riportata la personale esperienza degli AA. con 50 pazienti ospedalizzati e trattati chirurgicamente e sottoposti a terapia antibiotica con cefoxitina, nuova cefamicina resistente alla betalattamasi.

Summary (*Anaerobic infections in surgery*). — In the past few years the incidence of anaerobes in the aetiology of surgical infections has definitely increased due to a distinct increase in anaerobic flora and to the noticeable improvement in isolation and culture techniques which have drastically decreased the incidence of so-called « sterile pus».

The development of the toxi-infective shock due to Gram negative anaerobes, presents the most serious clinical problems, being the shock with the highest mortality rate. A paradigmatic example of shock due to surgical infections is the shock complicating peritonitis. A correct postoperative behaviour includes peritoneal lavage through drainages placed during surgery.

The AA. personal experience with 50 hospitalized patients treated surgically and with cefoxitina, a new cephamycin semisynthetic antibiotic resistant to beta-lactamases, is reported.

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Anaerobic sepsis

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The bacterial flora of the mouth

The saliva contains aerobic bacteria and facultative bacteria in concentrations of 10^7–10^8 organisms per ml, whereas the anaerobic count is 10^7–10^9 organisms per ml [1]. Similar counts are found at tooth surfaces, but the gingival scrapings contains a much higher anaerobic count 10^{10}–10^{11} organisms per gram. The oxidation reduction potential (Eh) at the gingival crevices is —300mV. Total anaerobic counts and the relative proportion of anaerobes to aerobes increase in the presence of dental disease [2].

The commonest anaerobic bacteria of clinical significance are *Bacteroides melaninogenicus*, *B. oralis*, *Fusobacterium nucleatum*, Peptococcus and Peptostreptococcus as well as Veillonella and the anaerobic Spirochaetes.

The types of infection which are associated with oral anaerobes are predominantly peridontal and oral infections, chronic ear disease and mastoiditis, chronic sinusitis, brain abscess, pulmonary disease, in particular empyema and pulmonary abscess, bronchiectasis and aspiration pneumonia. Bacteraemia and endocarditis can occur after dental extractions and occasionally liver abscess.

Bacteriological findings of pulmonary disease

In order to study the role of anaerobes in pulmonary infections, it is necessary to use specimens such as transtracheal aspirates, empyema fluid or direct lung punctures that are devoid of contamination with the bacterial flora of the mouth where anaerobes outnumber aerobes by a factor of at least 10 to one.

On analysing the bacterial findings from 83 cases of empyema Bartlett and Coll. [3] reported that from 63 (76 %) anaerobic bacteria were recovered and anaerobic bacteria were the exclusive isolates in 29 (35 %). In most, the flora was complex, and there was an average of 2.6 different bacte-

ria per specimen. The predominant micro-organisms in order of prevalence were anaerobic and microaerophilic Streptococci, *Sthaph. aureus*, Peptococcus, *F. nucleatum*, *B. melaninogenicus*, *B. fragilis*. Clostridia, *Escherichia coli* and *Pseudomonas aeruginosa*.

The bacteriological findings from 70 patients with aspiration pneumonia studied by Bartlett and Coll. [4] using transtracheal aspirates showed that anaerobic bacteria were recovered from 61 patients (87 %) and were the exclusive organism in 32 (46 %). More than one bacterial species was recovered in 56 of 70 cases (80 %), with an average of 2.9 isolates per case. The predominant organisms were anaerobic or microaerophilic Streptococci (32 isolates), *B. melaninogenicus* (27) and *F. nucleatum* (19). In all 70 patients reported by Bartlett and Coll. [4] however, there were predisposing factors such as alcoholism or cerebrovascular disturbances.

The normal flora associated with the gastro-intestinal tract

As outlined by a previous speaker, the bacterial flora of the gastrointestinal tract consists predominantly of anaerobic bacteria. The commonest organism associated with clinical infections is *Bacteroides fragilis*.

The commonest types of infections associated with the normal flora of the intestine usually follow bowel surgery or perforation of large bowel such as peritonitis, wound infections, intra-abdominal abscess, subphrenic abscess, liver abscess and perianal infections.

The bacterial flora of the vaginal tract

Recent quantitative studies of the bacterial flora of vaginal secretions have demonstrated the predominance of anaerobic bacteria over aerobic and facultative bacteria in a ratio of at least 10 : 1. The anaerobic bacteria are present in concentrations of 10^9 organisms per gram and the aerobic flora, 10^8 organisms per gram. The commonest organisms here in order of their concentrations are the Peptococcus and then the Bacteroides sp. [5].

The infections associated with the anaerobic bacteria of the female genital tract are the non-gonococcal salpingitis, tubo-ovarian abcess, pelvic abcsess, pyometra and others. Isolation of numerous anaerobic organisms from one specimen is common in pelvic inflammatory disease.

Bacteriological analysis of 40 specimens of pus

The bacteriological findings from 40 specimens collected and processed using methods outlined below are shown in Table 1. All 40 specimens of pus derived from various abdominal, pelvic, and other sites had anaero-

bic bacteria isolated from them. From 37 out of 40 specimens, Bacteroides sp. were isolated, of which 28 (75 %) were *B. fragilis*. The next common organism was the Gram positive Peptococcus/Peptostreptococcus isolated from 15 specimens and Clostridia sp. from 9 (Table 1). From 29 out of 40 specimens, aerobic bacteria were isolated, where *E. coli* was the commonest, but other organisms were also present (Table 1).

The number of specimens with anaerobes was 40, the number with mixed aerobes and anaerobes was 29 and with anaerobes alone was 11. The isolation rate per specimen was 2.7 organisms.

The above specimens were all collected in a gassed-out transport bottle (Fig. 1). These transport bottles (jencons, Wheaton Laboratory Products)

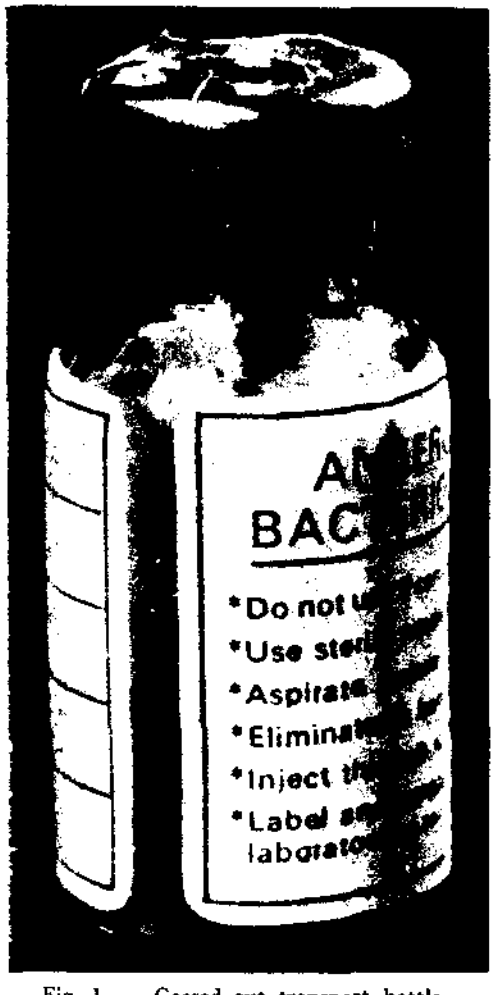

Fig. 1. — Gassed-out transport bottle.

TABLE 1

Types of bacteria isolated from 40 clinical specimens of pus

ANAEROBIC ORGANISMS organism	Number	
Bacteroides sp.		37
B. fragilis	28	
B. distasonis	3	
B. vulgatus	2	
B. melaninogenicus	2	
B. corrodens	2	
Fusobacteria		2
Peptococcus/Peptostreptococcus		15
Clostridia sp.		9
Cl. welchii type A	4	
Cl. septicum	2	
Cl. glycolicum	2	
Cl. sporogenes	1	
Eubacteria		2
Actinomyces		2
TOTAL		70
Total number of specimens with anaerobic bacteria		40
AEROBIC ORGANISMS organism		
E. coli	19	
Proteus	3	
Pseudomonas	2	
Enterobacter cloacae	2	
Enterococci	2	
α haemolytic streptococci	2	
Staphylococcus aureus	1	
Staphylococcus albus	4	
Microaerophilic streptococci	3	
Candida albicans	1	
TOTAL		39
Total number of specimens with aerobic organisms		29

were prepared in the laboratory. 0.5 ml of pre-reduced Brain Heart Infusion Broth (Oxoid) and a redox indicator, resazurine (a sensitive oxidation-reduction indicator which turns pink on exposure to air) were added. The bottles were then gassed-out with oxygen-free carbon-dioxide, sealed with a rubber stopper and a metal cap and then autoclaved at 15 lbs for 25 minutes, labelled and stored at 4° C. A number of these bottles were made available in the surgical theatres and the wards.

The use of these bottles enable the clinician and surgeon to aspirate rather than swab and hence provide a better specimen for anaerobic culture. At the same time delay in the transport to the laboratory is not crucial as the specimens are under anaerobic conditions. Furthermore the use of the gassed-out transport bottles enabled us to carry out direct examination of the specimen by gas-liquid chromatography (GLC) for the presence of short chain fatty acids (SCFA).

Gas-liquid chromatography for the rapid diagnosis of anaerobic infections

Culture of anaerobic bacteria and their subsequent identification is time consuming, taking anything from one day to a week or more when mixed infections are found. Other, more rapid methods of diagnosis have been investigated and these include the use of gas-liquid chromatography (GLC). Gorbach and Coll. [6] were the first to apply the use of GLC to clinical specimens of pus in order to detect the presence of short-chain fatty acids (SCFA) and this method has been applied by others [7, 8]. Certain anaerobic bacteria produce specific patterns of SCFA from the metobolism of carbohydrate and protein, a property orginally used for taxonomic purposes. The main SCFA are the volatile fatty acids (VFA), especially acetic, propionic and butyric acids and the non-volatile fatty acids (non-VFA), in particular lactic and succinic acids. These acids are also produced *in vivo* and can be detected by direct GLC of clinical material and a result is obtained within one hour, giving presumptive diagnosis of anaerobic infections.

90 consecutive clinical specimens of pus and other body fluids (joint, pleural, peritoneal fluids and others) were examined by gas-liquid chromatography (GLC) and routine bacteriological culture methods and the results compared (Table 2). The detection of significant short chain fatty acids (SCFA) by GLC correlated well with the isolation of anerobic bacteria. In 21 of 27 specimens with positive SCFA results anaerobic organism were isolated, the remaining 6 samples with positive SCFA produced facultative organisms only (Table 3) 3 specimens with negative SCFA yielded anaerobic bacteria, one of which had anaerobic Streptococci which is known not to produce SCFA. The remaining 60 specimens with negative SCFA

TABLE 2

Summary of bacteriological findings and gas–liquid chromatography (GLC) in 90 clinical specimens

GLC	Acids (*)	Type of organisms	Specimens	
			No.	Total
Positive	A and P, B, L or S (one or more)	Anaerobic (± facultative)	21	27
Positive	A and P, B, L or S (one or more)	Facultative only	6	
Negative	No acids (or A only)	Anaerobic (± facultative)	3	63
Negative	No acids (or A only)	Facultative only	36	
Negative	No acids (or A only)	No bacteria grown	24	
				90

(*) A = acetic acid; B = butyric acid; S = succinic acid; P = propionic acid; L = lactic acid

failed to yield anaerobic organisms, 36 had facultative organisms only and there was no growth in the remaining 24 specimens (Table 2).

In general, therefore, direct GLC examination of clinical material provides a rapid, reliable and simple method of detecting anaerobic bacteria in infections and can be applied in routine bacteriological laboratories.

In our experience GLC examination of clinical specimens can be helpful even when the patients have already commenced antibiotic therapy when recovery of bacteria is difficult. Occasionally examination of blood culture broths by GLC may be rewarding.

Bacteraemia and endocarditis

Recent reports from several laboratories have indicated that there is an increase in the rate of isolation of anaerobic bacteria from blood cultures. The anaerobic bacteria appear to be responsible for 8 to 11 % of bacteraemias in a general hospital [9–11.] In Mayo-clinic series, anaerobes accounted for 11–12 % of all blood culture isolates from 1968–1970 [11], as compared to 2.2 % during 1948–1959 [12]; although this significant increase in the isolation rate of anaerobes must be attributable to improved culture techniques, also the increased number of colonic and pelvic surgery carried out at these institutions must be taken into account.

TABLE 3

Bacteriological analysis of clinical specimens with significant short-chain fatty acid production

Specimen	Source	Acids (*)	CULTURE +	
			Anaerobes	Aerobes
1	Intra-abdominal abscess	A, P, B	*Bacteroides fragilis*	Coliform sp.
2	Intra-abdominal abscess	A, P, L, S	*E. fragilis thetaiotaomicron*	Coliform sp. Enterococcus
3	Intra-abdominal abscess	A, P, B, L, S	Bacteroides *sp.*	Coliform sp. *Staph. aureus*
4	Intra-abdominal abscess	A, P, B	Bacteroides sp.	Coliform sp.
5	Sub-phrenic abscess	A, B	*Clostridium sporogen*	Staph. albus
6	Appendix abscess	A, P, B	*B. fragilis fragilis*	Coliform sp. Proteus
7	Liver abscess	A, P, B	*B. fragilis* Eubacteria	Coliform sp. *Enterobacter cloacae*
8	Bile	A, B	*Cl. septicum*	Coliform sp.
9	Abdominal wound	A, P	*B. fragilis fragilis*	Enterococcus *Staph. albus*
10	Abdominal wound	A, L, S	*B. fragilis vulgatus*	Coliform sp. Enterococcus *Staph. aureus*
11	Pelvic abscess	A, P	*B. fragilis* *Cl. welchii*	Coliform sp.
12	Pelvic abscess	A, P, iB, B iV, V	*B. gfragilis* Anaerobic strep. Actinomyces Clostridium *sp.* Fusobacteria	—
13	Perianal abscess	A, P, B,	*B. corrodens* Anaerobic strep. Eubacteria	—

(*) A = acetic acid; P = propionic acid; B = butyric acid; iB = iso-butyric acid; V = valaeric acid; iV = iso-valaeric acid; L = lactic acid; S = succinic acid.

— Signifies no bacterial growth.

Follows: TABLE 3

Bacteriological analysis of clinical specimens with significant short-chain fatty acid production

Specimen	Source	Acids (*)	CULTURE +	
			Anaerobes	Aerobes
14	Perianal wound	A, B	*B. fragilis vulgatus* Anaerobic strep.	Coliform sp.
15	Thigh abscess	A, P, IB. B, iV	*B. fragilis*	Coliform sp. Haemolytic strep.
16	Thigh abscess	A, P, B	Bacteroides sp. Clostridium sp.	Coliform sp. *Staph. aureus*
17	Thigh abscess	A, B	Bacteroides sp.	—
18	Empyema	A, P, iB, B	*Cl. glycolinum* Anaerobic strep.	Coliform sp. Non-haemolytic strep.
19	Empyema	A, P, B	Bacteroides sp. (microaerophillic strep.)	—
20	Subdural abscess	A, P, B	*B. fragilis fragilis*	—
21	Face abscess	A, P	*B. corrodens* Anaerobic strep.	—
22	Intra-abdominal abscess	A, P, B	—	Coliform sp. β-haemolytic strep.
23	Retroperitoneal	A, P, B	—	Proteus/Enterococcus Coliform sp.
24	Abdominal wound	A, P	—	Klebsiella sp.
25	Groin abscess	A, P, B	—	Coliform sp.
26	Neck wound	A, P, B	—	Coliform sp. Diptheroid *Staph. aureus*
27	Foot abscess	A, P, B	—	Enterococcus

(*) A = acetic acid; P = propionic acid; B = butyric acid; iB = iso-butyric acid; V = valaeric acid; iV = iso-valaeric acid; L = lactic acid; S = succinic acid

— Signifies no bacterial growth.

The predominant anaerobic isolates are the Gram-negative bacilli [11, 13]. Of those *Bacteroides fragilis* is by far the commonest, (58–90 %) of these organisms, followed by Fusobacteria (4–20 %) [9–11, 13, 14]. Although anaerobic species have been associated with endocarditis, the number of cases are relatively low. In reviews of 1498 cases of infective endocarditis anaerobes were recovered from 55 patients (3.8 %) and these were predominantly Peptostreptococci and microaerophilic Streptococci [10, 15]. Other anaerobic bacteria are occasionally responsible for endocarditis. Nastro and Finegold [16] reviewed thirtyseven cases involving anaerobic Gram negative bacilli and the isolates were *B. fragilis* (13), *B. oralis* (1), *B. melaninogenicus* (one), *F. necrophorum* (7), *F. nucleatum* (3) and *Dialister granuliformans* (1). The mortality rate among reported cases was 38 %.

Pathogenicity of anaerobic bacteria

Oxygen tolerance of anaerobes may be important in the pathogenicity of certain anaerobic bacteria. In studying the oxygen tolerance of 57 fresh anaerobic clinical isolates from infected sites, Tally and Coll. [17] found that all organisms survived at least eight hours and 44 of the 57 tolerated 48–72 hours of oxygen exposure. Furthermore no extremely oxygen sensitive (E.O.S.) organisms were encountered in these specimens.

The presence of the enzyme, superoxide dismutase (S.O.D.), may form part of the biochemical basis of this tolerance. Superoxide dismutase, an enzyme first described by McCord and Fridovich [18], catalyses the conversion of toxic superoxide radicals to H_2O_2. These radicals are generated in the reduction of molecular oxygen and are toxic to anaerobes. S.O.D. is the enzyme which protects cells by conversion of O_2 to $H_2O_2+O_2$. In examining the distribution of S.O.D. in the clinical isolates Tally and Coll. [19] found that aerotolerant and moderate organisms posses S.O.D., whilst strict anaerobes have very low or undetectable enzyme levels.

Although the presence of this enzyme may contribute to the pathogenicity of the organisms other factors are also involved. One such factor in *Bacteroides fragilis* is the presence of the capsular polysaccharide.

Although *Bacteroides fragilis* subsp. *fragilis* is the least common in the faecal flora accounting for only 0.04 % [20] it represents 65–85 % of clinical isolates as compared to the other Bacteroides species [21, 22]. Kasper [23] demonstrated by electron microscopy studies that *B. fragilis* was encapsulated whereas other species were not. Furthermore, it was shown in experimental animal models that the capsular polysaccharide was the virulence factor causing the formation of intra-abdominal abscess formation [24].

This capsular polysaccharide is unique to *B. fragilis* and antisera raised against the purified polysaccharide reacted with almost all strains of

B. fragilis but not with other subspecies [21]. This antisera can be used to identify *Bacteroides fragilis* strains and has been used in the rapid diagnosis of Bacteroides infections by the use of indirect immunofluorescent studies of clinical material [25].

Finally Ingham and Coll. [26] reported that anaerobic bacteria may interfere with the process of phagocytosis of aerobic bacteria by normal leucocytes. This may contribute to the pathogenesis of anaerobic infections.

Conclusions

The normal flora of the mouth, gastrointestinal tract, vaginal tract and skin consists predominantly of anaerobic bacteria. These bacteria are involved in infections of the oropharynx, the chest, the central nervous system, the abdomen and the female pelvis as well as the skin. Bacteraemia and endocarditis may also be caused by anaerobes.

A variety of anaerobic bacteria are encountered in clinical sepcimens but *Bacteroides fragilis* is by far the commonest. This may be due to virulence factors present in this species which have not been demonstrated in others. Some of the pathogenic mechanisms of anaerobic bacteria are outlined.

Summary. — Anaerobic bacteria are the major residents of the skin and mucous-membrane surfaces, it is reasonable to suspect therefore, that these organisms play a part in the pathological processes associated with disturbances of these sites. The improvements in laboratory methods and the clarification of taxonomy have resulted in an increased awareness of the prevalence and virulence of the non-sporing anaerobic organisms in clinical infections.

In this paper, only a brief outline of the types of infections associated with the normal flora and the types of bacteria isolated from these infections are given. Finally some of the pathogenetic mechanisms of anaerobic infections are discussed.

Riassunto (*Sepsi da anaerobi*). — I batteri anaerobi sono i principali colonizzatori della pelle e delle mucose, è perciò ragionevole sospettare che questi microrganismi svolgano un ruolo nei processi patologici che interessano queste aree. Le migliorate metodologie di laboratorio e la chiarificazione della tassonomia hanno dimostrato la prevalenza e la virulenza degli organismi anaerobi non sporigeni nelle infezioni cliniche.

In questo lavoro sono brevemente indicati i tipi di infezione associata con la flora normale e i tipi di batteri isolati da queste infezioni.

Sono infine discussi alcuni meccanismi patogenetici delle infezioni anaerobiche.

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