

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO	
RAD: 11-102361- -14	FECHA: 2016-05-12 16:19:36
DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 378 FASE NACIONAL INCLUIDO CAPITULO I
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 24
ACT: 519 TRASLADOINFORME	

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
CARLOS R. OLARTE GARCIA

Asunto: Radicación: 11-102361- -14
Trámite: 11
Evento: 378
Actuación: 519
Oficio: 5612
Folios: 24

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/IT2010/020712				
Fecha de Presentación Internacional	12/01/2010				

Como resultado del examen de fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Radicado N° 11-102361-00000-0000	11/Agosto/2011
Reivindicaciones:	Radicado N° 11-102361-00000-0000	11/Agosto/2011
	Radicado N° 11-102361-00008-0000	05/Junio/2013
Prioridad:	IT MI2009A000019	12/Enero/2009

2. Análisis de la Prioridad

Se acepta la reivindicación de prioridad porque ésta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del capítulo primero del Título X de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por la solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa

pagada. En el presente caso se estudiarán las reivindicaciones 1 a 22 del capítulo reivindicatorio (Radicado N° 11-102361-00008-0000).

Título de la solicitud: “composiciones que comprenden componentes probióticos y prebióticos y sales minerales, con lactoferrina”.

El problema planteado en esta solicitud consiste en la necesidad de proporcionar composiciones que contengan los materiales probióticos que no solo sobrevivan al proceso de fabricación, sino que puedan sobrevivir en el tracto gastrointestinal liberando los materiales probióticos viables en el huésped que lo necesite.

Para resolver este problema técnico la solicitud en estudio proporciona una composición probiótica que comprende a) uno o más componentes probióticos, que comprenden *Bifidobacterium longum* R175 y al menos una especie de bacteria seleccionada del grupo de bacterias que consta de *Lactobacillus rhamnosus* R11, *Lactobacillus helveticus* R52 y *Lactobacillus plantarum* R1012; y b) una composición vehículo que comprende: 1) uno o más componentes prebióticos; 2) lactoferrina; 3) una o más sales minerales, y opcionalmente, 4) glutatión.

El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A61K 35/74, A61P 1/00, C12N 1/20.

4. De la solicitud de patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

El capítulo reivindicatorio carece de claridad y concisión por cuanto:

Se sugiere reemplazar la expresión “composición vehículo” por “vehículo”, puesto que del modo como está reclamada la reivindicación 1 se entiende como una combinación de dos composiciones.

Una composición debe reclamarse a partir de sus ingredientes (ingrediente activo y excipientes) y las proporciones de los mismos (reivindicación 1, 10 y 21).

En primer lugar, en la reivindicación independiente 1 no se han indicado las proporciones de cada uno de sus ingredientes ni tampoco se han definido claramente los excipientes puesto que se refiere de forma amplia a componentes prebióticos sin la indicación particular de los componentes que tienen sustento en la descripción. Así mismo, se refiere de forma general a sales minerales, pero no se indica específicamente cuáles. Se recuerda a la solicitante que las reivindicaciones deben estar completamente sustentadas en la descripción, y en la presente solicitud no existe sustento para cualquier componente prebiótico ni para cualquier sal mineral y por ello se sugiere limitar el alcance de la reivindicación.

Composición reclamada	Soporte de los ejemplos presentados
Reiv. 1: Composición probiótica que comprende: <i>Bifidobacterium longum</i> R175 y al menos una especie de bacteria seleccionada del grupo de bacterias que consta de; <i>Lactobacillus rhamnosus</i> R11 <i>Lactobacillus helveticus</i> R52 <i>Lactobacillus plantarum</i> R1012; Vehículo: uno o más componentes prebióticos; lactoferrina; una o más sales minerales, y opcionalmente, glutatión.	Ejemplo 1: Composición probiótica que consiste de: <i>Lactobacillus helveticus</i> R52 <i>Bifidobacterium longum</i> R175 <i>Lactobacillus plantarum</i> R1012 Vehículo: Inulina, fructosa, óxido de magnesio, gluconato de magnesio, citrato de potasio, gluconato de zinc, lactoferrina, citrato de cobre y glutatión

Reiv. 21. Composición probiótica que comprende: <i>Saccharomyces boulardii</i> <i>Bifidobacterium longum</i> R175 <i>Lactobacillus rhamnosus</i> R11 Vehículo: Fructosa, inulina, mezcla de sales minerales que constan de magnesio y zinc, y lactoferrina	Ejemplo 2: Composición probiótica que consiste de: <i>Saccharomyces boulardii</i> <i>Bifidobacterium longum</i> R175 <i>Lactobacillus rhamnosus</i> R11 Vehículo: Fructosa, inulina, gluconato de magnesio, gluconato de zinc y lactoferrina
Reiv. 10. Composición probiótica que comprende: <i>Bifidobacterium longum</i> <i>Lactobacillus helveticus</i> <i>Lactobacillus plantarum</i> vehículo: Una mezcla de prebióticos lactoferrina sales minerales seleccionados del grupo que consiste en sales de magnesio, potasio y zinc; glutatión. Reiv. 22: Composición probiótica que comprende: <i>Bifidobacterium longum</i> <i>Lactobacillus helveticus</i> <i>Lactobacillus plantarum</i> Vehículo: Inulina, fructosa, lactoferrina, óxido de magnesio, gluconato de magnesio, citrato de potasio, gluconato de zinc y glutatión	Ejemplo 3: Composición probiótica que consiste de: <i>Lactobacillus helveticus</i> R52 <i>Bifidobacterium longum</i> R175 <i>Lactobacillus plantarum</i> R1012 Portador: Inulina, fructosa, lactoferrina, óxido de magnesio, gluconato de magnesio, citrato de potasio, gluconato de zinc y glutatión

La reivindicación 10 no son claras las proporciones de los componentes del vehículo utilizados, puesto que, por ejemplo, si el vehículo está compuesto por 100% magnesio, el vehículo no podría tener siquiera 1% de los otros componentes como lactoferrina o prebióticos. Se sugiere reclamar una combinación real de ingredientes para la composición.

Se sugiere sustituir los términos “aproximadamente” y “alrededor de” por un término preciso o un rango concreto para asegurar la concisión de las reivindicaciones. Además, se sugiere reemplazar el término “sal mineral” empleado en la solicitud por “suplemento mineral”, ya que las sales minerales como zinc, magnesio y potasio se encuentran conjugadas con compuestos químicos como el ácido glucónico y por ello no existe sustento en el capítulo reivindicatorio para composiciones que comprendan sales minerales como tal.

5. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad: enero 12 de 2009, y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D2	Nicar Laboratories Inc.	“Probaclac adult & child”	18/Mayo/2008
D3	US7101565	“Probiotic/prebiotic composition and delivery method”	05/Septiembre/2006
D4	Bengmark and Gianotti 1996	“Nutritional Support to Prevent and Treat Multiple Organ Failure”	1996
D5	D'Souza et al. 2002	“Probiotics in prevention of antibiotic associated diarrhoea: meta-analysis”	08/Junio/2002

El documento D2¹ divulga una composición probiótica combinada con agentes prebióticos para tratar la diarrea causada por antibióticos y los síntomas gastrointestinales causados por estrés.

1 http://web.archive.org/web/20080512083340/http://www.nicar.ca/engprod_probaclac.html

El documento D3² divulga una composición que comprende prebióticos y probióticos que confiere un efecto benéfico al tracto intestinal (resumen).

El documento D4³ revela el concepto de nutrición ecoinmune, el cual consiste en la suplementación nutricional de agentes probióticos, prebióticos, antioxidantes, surfactantes e ingredientes que favorezcan la mucosa gastrointestinal (Resumen).

El documento D5⁴ divulga la utilidad de los probióticos para la prevención y el tratamiento de la diarrea asociada con el uso de antibióticos (Resumen).

6. Examen de Patentabilidad (Art 14 D486)

Nivel inventivo (Art 18 D486)

La reivindicación 1 consiste en: *“Una composición probiótica que comprende a) uno o más componentes probióticos, que comprenden Bifidobacterium longum R175 y al menos una especie de bacteria seleccionada del grupo de bacterias que consta de Lactobacillus rhamnosus R11, Lactobacillus helveticus R52 y Lactobacillus plantarum R1012; y b) una composición vehículo que comprende: 1) uno o más componentes prebióticos; 2) lactoferrina; 3) una o más sales minerales, y opcionalmente, 4) glutatión”.*

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D2	D3
Composición probiótica	Composición probiótica	Composición probiótica/prebiótica (resumen)
que comprende	que comprende	Que comprende
a) <i>Bifidobacterium longum</i> R175 y al menos una especie de bacteria seleccionada del grupo de bacterias que consta de <i>Lactobacillus rhamnosus</i> R11, <i>Lactobacillus helveticus</i> R52 y <i>Lactobacillus plantarum</i> R1012;	<i>L. helveticus</i> R52 <i>L. rhamnosus</i> R11, <i>B. longum</i> R175	Especies de lactobacilos y bifidobacterias como (Col. 5 ln. 5-26): <i>Bifidobacterium longum</i> <i>Lactobacillus rhamnosus</i> <i>Lactobacillus helveticus</i> <i>Lactobacillus plantarum</i>
b) un vehículo que comprende: 1) uno o más componentes prebióticos; 2) lactoferrina; 3) uno o más minerales	Prebióticos (Fructooligosacáridos FOS)	-Uno o más componentes prebióticos (Col. 6 ln. 4-11) -lactoferrina (Col. 9 ln. 2-9) -minerales (Col. 9 ln. 2-9)

El documento D2 se considera el estado de la técnica más cercano a la invención definida en la reivindicación 1 porque divulga una composición probiótica llamada “Probaclac”, la cual consiste en siete probióticos *L. helveticus* R52, *L. rhamnosus* R11, *S. thermophilus* R83, *L. lactis* ssp. *lactis* R1058, *B. bifidum* R71, *B. longum* R175, *L. delbruecki* ssp. *bulgaricus* R9001 y prebióticos FOS en combinación con hidrolizados de soya.

La diferencia entre la invención, definida en la reivindicación 1 y la composición divulgada en D2 consiste en la presencia de lactoferrina y de minerales.

En los ejemplos proporcionados por la solicitante no existe evidencia de un efecto que la adición de lactoferrina y minerales le confieran a la composición reclamada.

2 <https://www.google.com/patents/US7101565>
3 Bengmark, S. and Gianotti, L. 1996. Nutritional Support to Prevent and Treat Multiple Organ Failure. World J. Surg. 20: 474 – 481
4 D'Souza, A. et al. 2002. Probiotics in prevention of antibiotic associated diarrhoea: meta-analysis. BMJ 324:1361-4.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: cómo modificar la composición conocida en el documento D2 para conseguir una composición probiótica alternativa.

Sin embargo, la adición de compuestos como minerales, ácido glucónico y lactoferrina a composiciones de probióticos/prebióticos ya había sido divulgada en el documento D3. En consecuencia, la persona del oficio normalmente versada en la materia interesada en conseguir una composición probiótica alternativa, incluiría los minerales y la lactoferrina del documento D3 en la composición probiótica conocida en el documento D2, para llegar así al objeto de la reivindicación 1 de la solicitud.

Nótese que el efecto de supervivencia incrementada en el tracto gastrointestinal, que describe la solicitante en el capítulo descriptivo (ejemplo 4), no es atribuible a la combinación de ingredientes como lactoferrina o minerales en la composición reclamada, por el hecho de que no hay nada en los ejemplos o resultados que indique una relación causal, así como tampoco existen datos comparativos con otras composiciones del estado de la técnica que permitan evidenciar los efectos descritos. Vale la pena aclarar que para una persona medianamente versada en la materia resulta conocido que el incremento de la supervivencia de los microorganismos probióticos es logrado por la combinación de diferentes cepas probióticas⁵ y la combinación con componentes prebióticos^{6,7} como los fructooligosacáridos divulgados en los documentos D2 y D3 o como la inulina divulgada en el documento D3. Por ello, el efecto descrito por la solicitante no se considera inesperado y tampoco se considera que el efecto pueda atribuirse a las diferencias encontradas con el estado de la técnica más cercano, es decir, con el documento D2. Adicionalmente, el efecto de estabilidad de almacenamiento a temperatura ambiente también había sido descrito en la composición del documento D2. Es decir, no existe evidencia de que una composición como la divulgada en el documento D2 no tenga los mismos efectos de incremento de viabilidad en el tracto gastrointestinal, más aún cuando dicha composición resultó efectiva para la restauración de la salud intestinal bajo condiciones de estrés y para la diarrea post-antibiótico.

Por lo cual, la solución propuesta por la solicitante se considera obvia y sin altura inventiva.

Puesto que las reivindicaciones dependientes 2 – 9 no mencionan características inventivas adicionales tampoco se pueden considerar inventivas.

Las reivindicaciones 1 – 9 cumplen el requisito de novedad (Art. 16), pero no cumplen con el requisito de nivel inventivo (Art. 18).

La reivindicación 10 consiste en “Una composición probiótica que comprende: a) una mezcla de componentes probióticos...”

Comparación de las características técnicas esenciales, con las del estado de la técnica:

5 Timmerman et al. 2004. Monostrain, multistain and multispecies probiotics—A comparison of functionality and efficacy. En línea: <http://www.sciencedirect.com/science/article/pii/S0168160504002855>

6 Bezkorovainy. 2001. Probiotics: determinants of survival and growth in the gut. En línea: <http://ajcn.nutrition.org/content/73/2/399s.full>

7 Henriksson & Mitchell. 2007. Prebiotics enhance survival and prolong the retention period of specific probiotic inocula in an in vivo murine model. En línea: <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2672.2007.03469.x/pdf>

Características esenciales	D3	D4
Composición probiótica	Composición probiótica/prebiótica (resumen)	Suplemento nutricional (resumen)
que comprende	Que comprende	
a) <i>Bifidobacterium longum</i> 50 billones de UFC/g, <i>Lactobacillus helveticus</i> 150 billones UFC/g y <i>Lactobacillus plantarum</i> 150UFC/g;	Especies de lactobacilos y bifidobacterias como (Col. 5 ln. 5-26): <i>Bifidobacterium longum</i> <i>Lactobacillus rhamnosus</i> <i>Lactobacillus helveticus</i> <i>Lactobacillus plantarum</i>	Lactobacillus (Resumen)
b) un vehículo que comprende: 1) una mezcla de prebióticos que comprende alrededor del 80% del total del vehículo; 2) lactoferrina en una cantidad aproximadamente 1 a 10% del vehículo; 3) minerales de magnesio, potasio y zinc en una cantidad aproximadante 1 a 100% cada uno del vehículo; 4) glutatión en una cantidad de 1 a 20%.	-Uno o más componentes prebióticos (Col. 6 ln. 4-11) -lactoferrina (Col. 9 ln. 2-9) -minerales (Col. 9 ln. 2-9)	Glutatión (Resumen, Pág. 477)

El documento D3 se considera el estado de la técnica más cercano a la invención definida en la reivindicación 10 porque divulga una composición probiótica/prebiótica que comprende bacterias de lactobacilos y bifidobacterias como *Bifidobacterium longum*, *Lactobacillus rhamnosus*, *Lactobacillus helveticus* y *Lactobacillus plantarum* combinadas con prebióticos como FOS e inulina, con lactoferrina y minerales.

La diferencia entre la invención, definida en la reivindicación 10 y la composición divulgada en D3 consiste en la presencia de glutatión.

En los ejemplos proporcionados por la solicitante no existe evidencia de un efecto que la adición de glutatión le confiera a la composición reclamada.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: cómo modificar la composición conocida en el documento D3 para conseguir una composición probiótica alternativa.

Sin embargo, la adición de glutatión a suplementos alimenticios ya había sido divulgada en el documento D4, el cual da a conocer la importancia de antioxidantes alimenticios como el glutatión en el tratamiento postoperatorio para aminorar la diarrea y restablecer la flora intestinal normal (resumen, Pág. 477). En consecuencia, la persona del oficio normalmente versada en la materia interesada en conseguir una composición probiótica alternativa, incluiría el glutatión del documento D4 en la composición probiótica conocida en el documento D3, para llegar así al objeto de la reivindicación 10 de la solicitud.

Por lo cual, la solución propuesta por la solicitante se considera obvia y sin altura inventiva.

Puesto que las reivindicaciones dependientes 11 – 20 no mencionan características inventivas adicionales tampoco se pueden considerar inventivas.

Las reivindicaciones 10 – 20 cumplen el requisito de novedad (Art. 16), pero no cumplen con el requisito de nivel inventivo (Art. 18).

La reivindicación 21 consiste en “Una composición probiótica que comprende: a) una

mezcla de componentes probióticos...”

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D3	D5
Composición probiótica	Composición probiótica/prebiótica (resumen)	Probióticos
que comprende	Que comprende	Que comprende
a) <i>Bifidobacterium longum</i> R175 y <i>Lactobacillus rhamnosus</i> R11	Especies de lactobacilos y bifidobacterias como (Col. 5 ln. 5-26): <i>Bifidobacterium longum</i> <i>Lactobacillus rhamnosus</i> <i>Lactobacillus helveticus</i> <i>Lactobacillus plantarum</i>	<i>Lactobacillus</i> (Pág. 1362-1363)
b) un componente probiótico que consta de inulina y fructosa	-Uno o más componentes prebióticos (Col. 6 ln. 4-11)	
c) lactoferrina	-lactoferrina (Col. 9 ln. 2-9)	
d) una mezcla de sales minerales que consta de magnesio y zinc	-minerales (Col. 9 ln. 2-9)	
e) <i>Saccharomyces boulardii</i>	No menciona	<i>Saccharomyces boulardii</i> (Pág. 1362-1363)

El documento D3 se considera el estado de la técnica más cercano a la invención definida en la reivindicación 21 porque divulga una composición probiótica/prebiótica que comprende bacterias de lactobacilos y bifidobacterias como: *Bifidobacterium longum*, *Lactobacillus rhamnosus*, *Lactobacillus helveticus* y *Lactobacillus plantarum* combinadas con prebióticos como FOS e inulina, con lactoferrina y minerales.

La diferencia entre la invención, definida en la reivindicación 21 y la composición divulgada en D2 consiste en la presencia de *Saccharomyces boulardii* y en la selección de particular de cepas de *B. longum* y *L. rhamnosus*.

En los ejemplos proporcionados por la solicitante no existe evidencia de un efecto que la adición de *Saccharomyces boulardii* y la selección de las cepas R175 y R11 le confiera a la composición reclamada.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: cómo modificar la composición conocida en el documento D2 para conseguir una composición probiótica alternativa.

Sin embargo, el empleo de *Saccharomyces boulardii* como agente probiótico para el tratamiento de la diarrea ya había sido divulgada en el documento D4. De hecho, en dicho documento se establece que *S. boulardii* y lactobacilos tienen el potencial para ser usados en el tratamiento de la diarrea asociada a antibióticos por el hecho de que ambos organismos no son patógenos, actúan contra patógenos, tienen un rápido mecanismo de acción y sobreviven en el tracto gastrointestinal (Pág. 1363 col. izquierda). En consecuencia, la persona del oficio normalmente versada en la materia interesada en conseguir una composición probiótica alternativa, incluiría a *S. boulardii* del documento D4 en la composición probiótica conocida en el documento D2, y seleccionaría cepas alternativas de *L. longum* y *L. rhamnosus* para llegar así al objeto de la reivindicación 1 de la solicitud. Más aún, cuando las cepas R175 y R11 ya eran conocidas en el campo técnico

(D2).

Por lo cual, la solución propuesta por la solicitante se considera obvia y sin altura inventiva.

La reivindicación 21 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

La reivindicación 22 consiste en “Una composición probiótica que comprende una mezcla de componentes probióticos...”

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D3	D4
Composición probiótica	Composición probiótica/prebiótica (resumen)	Suplemento nutricional (resumen)
que comprende	Que comprende	
a) <i>Bifidobacterium longum</i> 50 billones de UFC/g, <i>Lactobacillus helveticus</i> 150 billones UFC/g y <i>Lactobacillus plantarum</i> 150UFC/g;	Especies de lactobacilos y bifidobacterias como (Col. 5 ln. 5-26): <i>Bifidobacterium longum</i> <i>Lactobacillus rhamnosus</i> <i>Lactobacillus helveticus</i> <i>Lactobacillus plantarum</i>	Lactobacillus (Resumen)
b) un vehículo que comprende: una mezcla de prebióticos que comprende inulina en una cantidad de 20% del total del vehículo y fructosa en una cantidad superior al 50% del vehículo; lactoferrina en una cantidad 0.5% del vehículo; gluconato de magnesio en una cantidad entre 14 y 16% del vehículo; citrato de potasio aprox. 5%; gluconato de zinc en una cantidad alrededor de 5% y glutatión alrededor de 1 % del vehículo.	-Uno o más componentes prebióticos (Col. 6 ln. 4-11) -lactoferrina (Col. 9 ln. 2-9) -minerales (Col. 9 ln. 2-9)	Glutatión (Resumen, Pág. 477)

El documento D3 se considera el estado de la técnica más cercano a la invención definida en la reivindicación 22 porque divulga una composición probiótica/prebiótica que comprende bacterias de lactobacilos y bifidobacterias como *Bifidobacterium longum*, *Lactobacillus rhamnosus*, *Lactobacillus helveticus* y *Lactobacillus plantarum* combinadas con prebióticos como FOS e inulina, con lactoferrina y minerales.

La diferencia entre la invención, definida en la reivindicación 22 y la composición divulgada en D3 consiste en la presencia de glutatión y en la selección de suplementos minerales.

En los ejemplos proporcionados por la solicitante no existe evidencia de un efecto que la adición de glutatión y la selección particular de suplementos minerales le confiera a la composición reclamada.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: cómo modificar la composición conocida en el documento D3 para conseguir una composición probiótica alternativa.

Sin embargo, la adición de glutatión a suplementos alimenticios ya había sido divulgada en el documento D4, el cual da a conocer la importancia de antioxidantes alimenticios como el

glutación en el tratamiento postoperatorio para aminorar la diarrea y restablecer la flora intestinal normal (resumen). Además, la selección de suplementos minerales conocidos en el campo técnico⁸ para la elaboración de productos nutricionales se considera una tarea rutinaria. En consecuencia, la persona del oficio normalmente versada en la materia interesada en conseguir una composición probiótica alternativa, incluiría el glutación del documento D4 en la composición probiótica conocida en el documento D3, para llegar así al objeto de la reivindicación 22 de la solicitud.

Por lo cual, la solución propuesta por la solicitante se considera obvia y sin altura inventiva.

La reivindicación 22 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

7. Conclusión

Los requisitos de Patentabilidad de la presente solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D2	Sitio web	1 - 9	Nivel Inventivo
D3	US7101565	10 - 22	Nivel inventivo
D4	Bengmark and Gianotti 1996	10 – 20 y 22	Nivel Inventivo
D5	D'Souza et al. 2002	21	Nivel Inventivo

8 Table of Active ingredients as vitamins and minerals in food supplements. 2006. <http://goo.gl/MAS6JL>

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Si como respuesta a este requerimiento el solicitante llegara a modificar: i) el capítulo reivindicatorio, ii) la descripción de la invención a proteger, iii) o presente un nuevo capítulo reivindicatorio, y desee que se realice un nuevo examen de patentabilidad a esta modificación, no deberá pagar la tasa de modificación voluntaria pero sí la correspondiente a la tasa de examen de patentabilidad. En todo caso, el número de requerimientos por no patentabilidad estará supeditado a que el término del estudio de patentabilidad no sea superior a 18 meses, contados desde la publicación de la solicitud de patente de invención. Adicionalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones deberá pagar la tasa por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (c)

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Nutritional Support to Prevent and Treat Multiple Organ Failure

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Abstract. Enteral nutrition (EN) has several advantages over parenteral nutrition (PN) for postoperative/posttrauma patients. Modern technologies for tube-feeding have made early EN possible. Jejunal tube-feeding has advantages over gastric tube-feeding: faster metabolic recovery, less vomiting, and less risk of regurgitation and aspiration. Immediate or early EN stimulates the splanchnic and hepatic circulations, improves mucosal blood flow, prevents intramucosal acidosis and permeability disturbances, and eliminates the need for stress ulcer prophylaxis. Saliva containing important antimicrobial substances and gastric acidity are important in sepsis prevention. Chewing, saliva, and gastric acidity support gastric nitric oxide (NO) release, important for mucosal blood flow, gastrointestinal (GI) motility, mucus formation, and bacteriostasis. An oral supply of NO-donating substances and chewing of nitrate-rich food, such as lettuce or spinach, can be useful. Oral and mucosa-protective lipids are recommended. H₂ blockers and saliva-inhibiting drugs are avoided. Immediate EN should be given, starting with 25 ml/hr and increasing to 100 ml/hr over 24 to 48 hours. For the immunocompromised patient special attention should be given to the purity of water. Bottled water can contain bacteria such as *Pseudomonas*. Food antioxidants such as glutathione, vitamin E, and β -carotenes are important. Ingredients for the colonic mucosa are important. Approximately 10% of caloric need is satisfied by so-called colonic food (prebiotics), fermented at the level of the colonic mucosa to produce colonic mucosa nutrients and to prevent gut origin sepsis. More than 10 g of fiber per day is recommended. The fermenting flora (probiotic flora) is deranged owing to disease or antibiotic treatment, and resupply of flora is important. A new concept of ecoimmune nutrition is presented for enteral supply of mucosa-reconditioning ingredients: new surfactants, pseudomucus, fiber, amino acids such as arginine, and mucosa-adhering *Lactobacillus plantarum* 299.

During the early days of surgery, enteral nutrition (EN) produced problems such as vomiting or diarrhea. The gastrointestinal (GI) tract was regarded as paralytic and often nonfunctioning after operation or injury. Early parenteral nutrition (PN) solutions were deficient with < 10% sugar and < 500 kcal/day. Total parenteral nutrition (TPN) eliminated the problems of vomiting or diarrhea and was a major advance at the time. Now it is recognized that GI paralysis is mainly present in the stomach and colon, whereas the small intestine nearly always is functioning. Even with some ileus there is enough absorptive capacity at 25 to 60 ml/hr infused into the upper jejunum.

Early studies comparing EN and PN dealt with nutritional and

metabolic aspects, most often nitrogen metabolism [1]. As the problems of postoperative/posttrauma (POT) patients came into focus, and the etiologic relation of POTS to the gut was recognized, there was increased interest in the use of EN. Today there have been great advances in nutritional support by enteral means.

Problems of Patients with POT

Today it is accepted that many of the septic complications after injury are due to colonization of the oropharynx, the tracheobronchial tree, and the upper GI tract or to translocation of potentially pathogenic microorganisms (PPMs) in the lower GI tract. Many believe that translocation is an important etiologic factor in POT patients. Among organ donors with a median hospital stay of only 1.9 days (range 6 hours to 8 days) bacterial cultures were positive in 67%, most often in mesenteric lymph nodes, lung, liver, and spleen [2]. Endotoxin was found in abdominal fluids in approximately 50% of the cases, in peripheral blood in 20%, and in portal blood in 10% [2]—before any changes were evident in the bowel wall. Increased intestinal permeability as early as 16 to 24 hours after moderate burns was described by McDonald et al. [3]. A single dose of endotoxin given to healthy humans significantly increased permeability [4]. The first peak of endotoxin occurred in the serum of patients about 12 hours after burns [5]. It is likely that there was increased intestinal permeability at that time as well. The presence or absence of gastric intramucosal acidosis during the first 12 hours after trauma is predictive of outcome [6]; a low intramucosal pH on admission, remaining low at 12 hours, was associated with 87% mortality, compared to 36% when intramucosal pH had returned to normal after 12 hours and 27% if it was normal on admission and remained normal at 12 hours. Deranged intramucosal pH reflects poor mucosal oxygenation, and splanchnic ischemia contributes to multiple organ failure (MOF). Such tonometric measurements in the stomach may reflect the status of oxygenation of the entire GI mucosa. EN counteracts intestinal ischemia via several mechanisms and probably should be instituted in trauma cases immediately on arrival at the hospital and in postsurgical patients immediately on arrival in the recovery room to overcome the traditionally long preoperative period of intestinal starvation.

Mucosal Function during Starvation

It was shown in experimental animals by the mid-1970s that with starvation the intestinal mucosa loses a large proportion of its structure and function [7] by 7 days. There are reasons to suspect that functional down-regulation occurs within 12 to 18 hours of intestinal starvation (e.g., the usual time of no eating before elective operations). Patients may be “metabolically handicapped” and susceptible to splanchnic ischemia and translocation on arrival in the operating room. The liver at that time is devoid of glycogen. The capacity of the liver to store calories as glycogen is only 500 kcal, which is consumed before surgery even begins. The hepatocyte has no capacity to build and store glycogen during and immediately following anesthesia. Glycogen deficiency can be eliminated only by a supply of carbohydrates immediately before operation, which considerably increases insulin sensitivity. Fasting for 24 hours increases translocation after hemorrhagic stress [8], which cannot be inhibited by TPN; early EN is needed.

Four observations reported in 1980 had a great influence on the development of EN in POT patients. Roediger found that intestinal mucosa was unable to nourish itself completely from blood [9]. Half of the nutritional needs of the small intestine and > 80% of that of the large intestine must come from luminal nutrition. Mucosal atrophy develops during early intestinal starvation, even when complete TPN is given. Hoover et al. [10] described positive nitrogen balance (+ 12%) in patients given EN (by jejunostomy) compared to a negative balance (– 45%) in TPN-treated patients. Alexander and coworkers [11] found that raising the amount of protein in EN from 15% to 23% increased survival from 56% to 100% in children with severe burns. Finally, Shephard described an increase in mesenteric and hepatic circulation [12] after early institution of EN.

Role of Saliva and Gastric Acidity in Infection Prevention

Colonization of the oropharynx, tracheobronchial tree, and esophagus, often with gram-negative aerobic bacteria, occurs early in both intubated and nonintubated sick and critically ill patients [13]. It is promoted by endotracheal tubes, tubes in the GI tract and urinary bladder, and intravascular catheters. Some antibiotics, anticholinergics, other mucus-inhibiting drugs, H₂ blockers, and antacids may promote microbial translocation by facilitating intestinal colonization with PPMs. It also explains why many of these drugs, particularly H₂ blockers, produce diarrhea.

It is well known among dentists that “the saliva possesses a multiplicity of defense systems for antibacterial warfare that the Pentagon can envy” [14]. In addition to the protective role of oral bacteria such as lactobacilli, specific antimicrobial proteins such as lysozyme, lactoferrin, and lactoperoxidase are produced in saliva. Other important colonization-preventive ingredients are mucin and immunoglobulin A (IgA). Salivary mucins concentrate on mucosal surfaces and provide a barrier against environmental insults [15]. Mucus produced in the mouth follows and covers ingested food as a protective layer from the oral cavity and probably down to the colon. Extirpation of salivary glands in experimental animals reduces the content of mucus in the lower esophagus by 80%. The loss in salivary mucus can only partly be compensated for by local mucus production: A strong epidermal

growth factor stimulation increased it by 50% [16]. Mucus-producing cells, or goblet cells, increase in density with distance from the oral cavity. Dietary fiber stimulates production of intestinal mucus, thereby preventing PPMs from getting a foothold on the mucosal surfaces and initiating translocation [17]. Saliva in amounts up to 300 ml/hr is produced with stimulation, and when resting it is approximately 25 ml/hr. Drugs used in intensive care units (ICUs) inhibit saliva; among them are anticholinergics, antispasmodics, antidiarrheals, analgesics, antidepressants, antihistamines, antihypertensives, antiparkinsonian agents, antipsychotics, and diuretics. Pilocarpine best stimulates and effectively increases the salivary output [18], an important factor in patients with xerostomia and associated salivary gland dysfunction. Low pH in the stomach is one of the most important barriers against invading PPMs. Gastric pH < 3.5 is usually bactericidal for most species. This barrier may be broken by stress ulcer prophylaxis, decreased acid production, H₂ blockers, or alkalization of gastric contents by continuous gastric feeding, allowing GI overgrowth of PPMs, retrograde colonization of the pharynx, and increased risk of pneumonia and other infections [19]. Instead of H₂ blockers, mucosa-protecting drugs such as sucralfate have been recommended [19]. In experimental animals, sucralfate mixed with water, when aspirated, causes lung edema and when mixed with HCl causes severe lung edema [20]. McDonald et al., in more than 100 patients after burns [3] demonstrated that immediate (within 6 hours) nasogastric tube-feeding prevents stress ulcer bleeding and eliminates the need for prophylactic antacids for H₂ blockers. Similarly, good protection can be obtained by substances such as pectin and phospholipids [21]. If the feeding is used, it should be administered intermittently to allow the gastric pH to decrease between feedings to prevent gastric colonization [22]. This step is not necessary if enteral tube-feeding is used instead. There are several reasons to avoid H₂ blockers, especially omeprazole. Oral administration of glutathione eliminates the gastric pH-elevating effect of omeprazole [23]; most likely omeprazole also reduces glutathione, at least in the stomach. Omeprazole degrades vitamin C and eliminates the gastric production of nitric oxide (NO), which is essential for bactericidal effects in the stomach, hemodynamic normalization, and mucosal oxygenation [24].

To overcome microbial overgrowth, selective decontamination with nonabsorbable antibiotics has been used extensively during the last 10 years. A recent meta-analysis [25], based on 11 studies and about 1500 patients, failed to show any major benefits of selective decontamination. It is more likely that antimicrobial proteins alone or in combination with immediate EN can successfully prevent problems in POT patients. Such a protein could be the bactericidal/permeability-increasing protein reported to be successful in experimental animals [26]. A single bolus injection (10 mg/kg) protected 15 of 16 mice from lethal endotoxin challenge compared with none of 16 in the control group. Another interesting approach is to supply the GI mucosa with new surfactants, pseudomucus, fiber, and protective lactobacilli [21]. Whatever measure is chosen, it must be applied at an early stage. Fast progression of disease necessitates immediate actions to prevent and counteract MOF. Many adult patients have MOF on arrival to the ICU. In children, 50% of deaths occur during the first ICU day and most within 7 days [27].

Colonic Food

Food must contain nutrients for colonic mucosa that cannot be broken down and absorbed in the small intestine. Normally, about 10% of human caloric need is met by colonic feeding. Fiber and protein substances for the colon are called *prebiotics* or *colonic foods*. Sloughed endothelium, pancreatic enzymes, mucus, and so on are recycled after bacterial fermentation in the colon [28]. Sloughed epithelium in the entire GI tract measures about 287 g/day and more with some diseases [29]. Thus 80 g of protein and 12 to 30 g of lipids are recycled every day. When colonic food and other substrates reach the colon, the endogenous microbial flora (probiotic flora) metabolize it and produce nutrients for colonic mucosa. These nutrients are short-chain fatty acids (SCFAs), amino acids, peptides, and polyamines [21]. Some substrates for colonic food can be met by recycling epithelium and enzymes of 10 to 30 g/day, but fiber must be supplied. If the probiotic flora is reduced or eliminated by disease or antibiotic treatment, a resupply of human-specific probiotic bacteria is needed, especially in potential or established MOF patients, including patients with subacute or subchronic forms of MOF, such as those with human immunodeficiency virus or acquired immunodeficiency syndrome (HIV/AIDS). Colonic mucosal starvation induced by TPN or elemental/fiber-free EN leads to a deficient colonic microflora. Cosmonauts develop a deranged intestinal microflora, an increase in enteropathogens, and almost total elimination of lactobacilli [21], most likely due to emotional stress and a fiber-free diet.

Probiotic Bacteria

Probiotic bacteria have several important functions in the colon: (1) production of nutrients for the mucosa: acetate, butyrate, propionate, other SCFAs, pyruvate, lactate, and amino acids (arginine, cysteine, glutamine); (2) production of micronutrients (β group and folic acid, antioxidants, and polyamines), histamine, 5-hydroxytryptamine, piperidine, tyramine, cadaverine, pyrrolidine, agmatine, putrescine; (3) prevention of overgrowth of PPMs; (4) stimulation of the immune system, especially the gut-associated lymphoid tissue (GALT) system: NO is produced by these bacteria in the mouth and GI tract; (5) elimination of toxins from the lumen; (6) participation in intestinal regulation, mucus utilization, nutrient absorption, GI motility, and blood flow through signaling substances such as NO.

To study the effectiveness of various bacteria, a library of hundreds of lactobacilli was collected from healthy humans [30]. Studies of abilities of lactobacilli to ferment fiber in vitro, to survive the acidity of stomach and the bile acid contents of the small intestine, to adhere to the colonic mucosa, and to remain and function there after the supply stopped led us to identify a few human lactobacilli with great capability. In the past, lactobacilli for treatment were most often yogurt lactobacilli. They do not survive the acidity of the stomach; or, if so, they do not adhere and function on the mucosa. The first lactobacillus with therapeutic benefits [31] was *Lactobacillus rhamnosus* GG, isolated from human feces. In vitro it adheres to human mucosal cells (0.5–2.0 bacteria/cell). We then identified *Lactobacillus plantarum* strain 299, which has an adherence of 15 bacteria/cell, e.g. the level of *E. Coli* [I. Adleberth and A. Wold, unpublished data]; *Lactobacillus plantarum* produces lactate and acetate, deaminates serine to pyruvate and arginine to ornithine (produces NO at the mucosal surface), and produces hydrogen peroxide and probiotics called

Table 1. Nutrient content in 100 ml of nutrition solution.

Nutrient	Standard solutions with fiber (n = 8)	Immune nutrition IMPACT	Ecoimmune nutrition
Energy (KJ)	429	420	325
Protein (g)	4.0	5.6	2.8
Fat (g)	3.6	2.8	1.1
Linoleic acid (g)	1.7	0.22	0.5
Carbohydrate (g)	13.9	13.2	13.8
Fiber (g)	> 1.0		0.57
Vitamin E (mg)	2.0	6.0 IU	0.23
Thiamine (mg)	0.13	0.20	0.09
Riboflavin (mg)	0.14	0.17	0.02
Vitamin B ₆ (mg)	0.16	0.15	0.07
Vitamin B ₁₂ (g)	0.4	0.0008	< 0.1
Folic acid (μ g)	29	0.04	12
Pantothenic acid (mg)	0.7	0.67	0.09
Sodium (mg)	78.5	110	2.0
Chloride (mg)	126	130	11.3
Potassium (mg)	137	130	55
Calcium (mg)	71	80	10.7
Phosphorus (mg)	70	80	64
Magnesium (mg)	23	27	18.5
Iron (mg)	1.1	1.20	0.72
Zinc (mg)	1.1	1.50	0.62
Iodine (mg)	9.0	0.010	0.13
Copper (mg)	0.12	0.17	0.14
Manganese (mg)	0.3	0.20	0.55
Chromium (μ g)	5.0	0.001	0.37
Selenium (μ g)	5.0	0.001	0.003
Molybdenum (μ g)	10	0.002	20
Arginine (mg)	160	1358 (added)	2500 (added)
Yeast RNA (mg)	0	135	0
<i>Lactobacillus plantarum</i>			1.5×10^9
Viscosity			0.02 m PaS

plantaricins. We have used *L. plantarum* 299 to produce a new *Lactobacillus*-containing enteral feeding formula by in vitro fermentation of a fiber-rich substrate. Oats were chosen, as they are one of the most complete foods. They have a favorable amino acid pattern, are rich in glutamine/glutamic acid, and are rich in fiber, especially water-soluble β -glucans. A unique feature is its high content of membrane lipids—phospholipids—which is 100 times higher in oats than in any other known food [32]. Strong synergistic effects with arginine suggested supplementing 2.5 g of L-arginine per 100 ml (Table 1). The original formula (without arginine supplementation) was extensively studied in animals. The supplemented formula is presently undergoing clinical trials for several indications including POT nutrition. Excellent reviews have been published on the roles of arginine [33], glutamine [34], SCFAs [35], lipids such as omega-3 fats [36], and RNA [37].

Membrane Lipids

The alveolar surfactant system may be involved in the acute respiratory distress syndrome (ARDS). Similarities exist between ARDS and preterm babies with respiratory distress, where deficient surfactants are the primary cause [38]. In the traumatized patient, total bronchoalveolar phospholipid content is unchanged, but a progressive decrease in the relative amounts of phosphatidylcholine and phosphatidylglycerol parallel a progressive increase in other membrane ingredients, including surfactant proteins [39]. The extent of changes relates to the severity of ARDS.

Similar changes seem to occur in the GI mucosa. In experimental animals with subtotal liver resection, we could prevent microbial translocation by an enteral supply of phosphatidylcholine [40]. Gangliosides such as GM₁ or GM₃ may be more potent glycolipids. GM₁ has an ulcer-healing effect on gastric mucosa [41] similar to that of H₂ blockers but without inhibition of gastric acidity. Intestinal mucosa is rich in GM₃, which changes in concentration and composition with age and disease and results in low fluidity in the brush border membranes. Gangliosides are incorporated into cell membranes [42] and influence membrane function and cellular response. In rats, oral administration of GM (300 µg/day) prevents rejection after rat heart–lung transplantation [43]. Lymphocytic infiltration was not observed in the transplanted organs. GM₃-rich food may increase GM₃ content in intestinal mucosa and reduce the load on the GALT. This assumption is supported by preliminary data from our laboratory. Milk is rich in GM₃, especially colostrum.

Antioxidants

An antioxidant is any substance that “when present at low concentrations compared to those of an oxidizable substance, significantly delays or inhibits oxidation of that substrate [44]. Destruction of membrane lipids by lipid peroxidation is paralleled by destruction of deoxyribonucleic acid (DNA). Human plasma contains several water-soluble antioxidants, such as ascorbate, glutathione, urate and bilirubin, and many lipid-soluble antioxidants such as tocopherols, carotenes, lycomene, lutein, zeaxanthine, and ubiquinol-10 [45]. Tocopherols are collectively referred to as *vitamin E*, the major lipid-soluble antioxidant in plasma and red blood cells. The effect of vitamin E depends on interaction with vitamin C and β-carotenes and sulfhydryls in membrane proteins. Vitamin C is the most important antioxidant in extracellular fluids. Glutathione (GSH), a tripeptide of glutamate, cysteine, and glycine, is present in most cells and is an important antioxidant in intestinal mucosa and the liver. It is estimated that GSH in the amount of 150 mg/day is consumed with a normal diet of fresh fruits, vegetables, and meat. The intake of GSH in sick patients has been neglected. GSH deficiency contributes to organ dysfunction after hemorrhagic shock [46] and sepsis [47]. It is a strong immune modulator, required for T cell activation [48]. Oxidant-induced liver peroxidation occurs with endotoxemia and bacterial peritonitis and with severe shock and reperfusion [49]; it is preventable in part by GSA.

Pectin, present in many fruits, is a strong antioxidant against all three main types of oxidation damage: peroxide, superoxide, and hydroxyl radicals [50]. It adheres to mucosal surfaces, especially at a low pH, and serves as an artificial protection layer, or pseudo-mucus. It delays gastric emptying and intestinal transit time and thus modifies absorption of glucose, cholesterol, bile acids, metals, and vitamins [51]. It also increases intestinal barrier function and prevents microbial translocation and disruption of intestinal microflora. Pectin increases colonic production of SCFAs. Pectin has strong mucosal protection when administered alone or together with various membrane lipids in models of gastritis, colitis, GI ulceration, and translocation with sepsis. Good clinical effects have been reported in patients with HIV/AIDS [52] after administration of a pectin-rich diet called the BRAT diet (banana, rice, apple, tea/toast). This diet is effective if there is a potent colonic endogenous flora to ferment the pectin. When a large amount of pectin is supplied (36 g/day), most of it (85%) is metabolized [53].

Pectin increases the thickness of the “unstirred water layer” on the mucosal surface [54], which explains its therapeutic effects, especially when used in combination with membrane lipids. To provide the most effective mucus-adhesive gel, pectins with the lowest molecular weights should be used.

Nutrition and Immune Function

More than 35 years ago Scrimshaw et al. suggested a bidirectional interaction between nutrition, immune response, and infectious diseases [55]. Almost all nutrient deficiencies can impair the host defense [56]. Thus many nutrients also have the ability to enhance the immune defense. Patients with protein deficiency have impaired production of interleukin 1 (IL-1), which can be corrected by diet [57]. The acute-phase response seen after injection of IL-1 is impaired in protein-deficient rabbits [58]. Reduced ability to produce IL-2 or to respond to IL-2 administration is implicated in the aging process, as well as in disease. Supply of vitamin E doubles IL-2 production in aged mice [59]. MOF secondary to sepsis is associated with higher IL-8 concentrations than the MOF of noninfectious shock [60]. IL-6 in septic patients has a late peak that correlates with a fatal outcome. TNF alters surfactant composition, which may in turn contribute to the development of ARDS [61]. TNF may also affect the GI surfactants and promote increased permeability and translocation. TNF-α mediates a decrease in tissue glutathione and increases the sensitivity of pulmonary vascular endothelium cells to H₂O₂ [62], which can be prevented by supplying *N*-acetylcysteine (NAC). TNF-α induces early systemic hypotension after trauma, which is inhibited by *N*^G-monomethyl-L-arginine [63]. Inhibition of both inducible and constitutive forms of NO increases mortality in rabbit models given endotoxin, but inhibition of only the inducible form is preventive [64]. Thus NO released by inducible enzymes may exert a protective role in septic shock, favoring vasodilator tone and maintaining blood flow to visceral organs. Clinically in trauma patients and experimentally after administration of endotoxin, methylene blue is a safe option to counteract malignant NO production and to obtain benefit from the positive NO effects, including scavenging of oxygen-free radicals, and the important microbicidal effects [64, 65]. This action is nutrition-dependent, and the response to trauma and sepsis is often limited by lack of substrates for NO production; for example, vasodilation is improved by previous administration of L-arginine. The decrease in gut blood flow after burns is modified by nitroprusside infusion, which also prevents translocation [66]. In a double-blind study patients responded well to the treatment and showed an increase in gastric intramucosal pH, oxygen delivery, and cardiac index and decreased systemic vascular resistance [67]. Responders showed a higher survival (69%) than nonresponders (19%). A higher dose or repeated doses could give even better results, as observed with fulminant hepatic failure [68].

Ingested nitrate is absorbed in the GI tract, extracted from plasma, secreted by saliva, and converted to nitrite by facultative anaerobic bacteria on the tongue. After ingestion of 200 mg potassium nitrate, the nitrite level rose from 111 µM to 1030 µM [70]. Nitrite, when acidified in the stomach to approximately pH 2, produces NO at a concentration of about 600 nM. NO has a high affinity for iron/sulfur-containing respiratory enzymes, and it damages bacterial DNA [69] by a mechanism similar to that of leukocytes using NO to kill gut pathogens. It was suggested by Benjamin et al. [69] that acidified nitrite is not only effective on

Candida albicans and *Eschirechia coli*, but also on various gut pathogens, including *Helicobacter pylori*, amebae, and chronic intestinal parasites. Some food contains nitrate, especially such green vegetables as lettuce or spinach. NO also stabilizes gastric mucus by increasing its viscosity [71].

Neutrophils are involved in the induction of septic shock [72], and various neutrophilic functions are impaired in the presence of sepsis. Neutrophil respiratory burst function in critically ill patients is significantly depressed during early septic shock but not in nonseptic patients; the function recovers as the patient improves clinically [73]. It is assumed that this deficiency can be prevented by dietary manipulations.

Parenteral Nutrition

Parenteral nutrition is accompanied by decreased secretion of saliva, gastric and intestinal juices, and bile—all important for GI function and for antimicrobial defense. Bile acids are bacteriostatic, and bile contributes IgA to the intestine, an important factor in luminal control of microbial colonization. The inability of TPN to reverse catabolic states and prevent sepsis is now widely accepted. Energy requirements in critically ill and septic ICU patients are not as increased as previously thought. It should almost always be possible to meet nutritional needs by EN. A group of sick ICU patients were given aggressive TPN of 2750 kcal and 127 g amino acid intake per day. After 10 days of treatment they had lost an average of 12.5% of the body protein despite a mean gain of 2.2 kg in body fat [74]. A recent meta-analysis on perioperative TPN shows minimal effects on complication rates, varying from 12.8% better to 2.3% worse [75].

Jejunal Tube-Feeding

The development of devices for tube-feeding has made it possible to use enteral feeding before, during, and immediately after surgery. Feeding directly into the upper jejunum has the potential not only to eliminate the problem of vomiting and diarrhea but also to prevent microbial translocation and gut origin sepsis. Early jejunal EN should, if possible, be complemented with immediate, but minimal, oral/gastric feeding to increase acidity and stimulate gastric NO production. Such gastric feeding should contain NO precursors (lettuce or spinach) if possible, which should be carefully chewed.

Simultaneous intragastric pectin with phospholipids should provide gastric mucosal protection: A natural product such as the unripe banana is rich in pectin and surfactants. Pectin should never be given through a feeding tube because of the risk of clogging.

Jejunal tube-feeding should start at about 25 ml/hr and then be increased to about 10 ml/hr over the next 24 to 48 hours. Studies comparing intragastric and intrajejunal feeding have shown that metabolic recovery is considerably faster when the intrajejunal route is used [76]. The risk of aspiration is 600% greater with intragastric feeding [76]. Should one decide to use the intragastric route, it is advisable to feed intermittently [22], allowing acidity to increase between feedings to prevent microbial colonization of the stomach. Intrajejunal tube-feeding, in most cases, can be provided by preoperatively placed feeding tubes. If this maneuver is not possible, a jejunal feeding tube is placed either as a percutaneous endoscopic gastrostomy (PEG) tube or as a needle jejunostomy during surgery. Routine jejunal tube-feeding has

been hampered by poor performance of the feeding tubes presently available. The rate of spontaneous transpyloric passage of these tubes is unacceptably low, approximately 35% after 24 hours and approximately 70% [77] after 72 hours, which often necessitates additional use of endoscopy or radiography to place the tip of the tube in the upper jejunum. This fact and the high costs for extra hospital days and for the additional procedures (endoscopy or radiography) has discouraged many from routine use of tube-feeding. Hence one of us (S.B.) [78] developed a self-propelling feeding tube, which when introduced into the stomach propels itself into the upper jejunum within 4 hours. This tube has never and can never be regurgitated. It will be available in America and Europe within a year.

Content of Nutrition Formulas

Diets rich in fat, particularly omega-6-rich vegetable oils, produce diarrhea and enhance loss of fluids and electrolytes [79]. They seem also to interfere with absorption and other nutrients. Thus restriction of fats other than the omega-3 variety is recommended. High quality water is important. Immunocompromised patients should be given only boiled, filtered water. Bottled waters can contain bacteria; *Pseudomonas* organisms were identified in 6 of 8 American [80] and 39 of 87 European brands [81]. Outbreaks of infections from bottled water are rare, but the risk is important in sick patients.

Enteral Nutrition and Clinical Outcomes

A meta-analysis based on eight prospective randomized trials, published before 1991 [82] concluded that septic complications occur half as often (18%) in EN-fed patients when instituted after 6 to 72 hours than in TPN-fed patients (35%). The most significant differences were seen in trauma patients. Two randomized studies published in 1992 comparing EN and TPN in patients after abdominal trauma [83] and general trauma [84] showed as much as 65% and 76% fewer infections, respectively, in the EN-treated patients. These results were obtained with standard formulas without fiber or other specific ingredients. One could expect even better results using EN formulas to which immune-modulating ingredients, other substances, and probiotic bacteria are added.

Immune Nutrition and Clinical Outcomes

The first immune nutrition formula—enteral diet supplemented with arginine, omega-3 fatty acids, and RNA (IMPACT)—is regarded as a pharmaceutical agent. It is a supplement to standard nutritional formulas, containing ingredients documented to modulate the host's immune response and influence gut barrier function. It is based on many experimental studies that have shown protective effects in POT patients via components such as arginine, omega-3 fatty acids, RNA, vitamin C, and glutamine.

Gottschlich et al. [79] reported a significant reduction of nosocomial infectious complications and wound sepsis in burned patients with a diet enriched with arginine and omega-3 fatty acids. Daly and coworkers [84], randomized 85 patients to receive IMPACT or a standard formula after upper abdominal surgery for GI malignancies. The group receiving IMPACT had fewer complications (11% versus 37%), improved lymphocyte mitogenesis, and a shorter hospital stay (15.8 days versus 20.2 days). These results were confirmed in a recent report [85]. Braga and colleagues [86] showed a significant reduction of the severity (but not

the frequency) of postoperative infections and a shorter hospital stay among IMPACT-fed patients. The immune nutrients improve the phagocytic function of monocytes; the release of IL-2, IL-2 receptors, IL-1 β , interferon γ (IFN- γ), IgM, and π gG; the number of CD3⁺, CD4⁺, and β lymphocytes; the response of skin delayed hypersensitivity; and reduced the circulating levels of TNF α and IL-6 [86, 87].

Bowers et al. [88] reported a large prospective, randomized multicenter clinical trial in ICU patients. Nutrition with IMPACT significantly reduced the length of stay, but only in patients classified as septic, and decreased the rate of acquired bacteremia and urinary tract infections. These differences were most evident in the subgroup receiving an adequate amount of the experimental diet (821 ml/day). No advantages were observed in the group having the systemic inflammatory response syndrome. The results of this study were modest but better than those reported for selective decontamination and without side effects.

Ecoimmune Nutrition—A New Generation of Nutrition

Most of the ingredients provided in EN solutions (simple carbohydrates, glutamine, arginine, omega-3 fats, RNA, and amino acids) are absorbed by the small intestine, often in the jejunum; only minimal amounts, if any, reach the colon. The diet/formula should contain substances that are substrates for fermentation and production of specific substances vital for colonic mucosa. There must also be probiotic bacteria available in the colon to ferment the various substrates and to produce sufficient nutrients in quantity. In most ICU patients such bacteria are absent owing to disease or antibiotic treatment and must be supplied along with nutrition.

The need for such bacterial supplementation was described by Eisman et al. [89] and Wilmore [90], which was why we developed a new enteral feeding formula based on *in vitro* fermentation of oats and containing large quantities of live *Lactobacillus plantarum* 299. The results of treatment with this formula, without arginine supplementation, were as good as or better than those achieved by broad-spectrum antibiotics in animals with induced peritoneal sepsis [21]. It was tried, without arginine supplementation, in a small group of patients judged to be dying of MOF. In these patients, all antibiotics were discontinued, and lactobacilli and oat fiber were infused into the intestine. All five patients recovered dramatically and could leave the ICU. Their APACHE II score fell from an average of 18.4 before to 12.2 on day 5 and 8.8 on day 10. This treatment has the potential of being the next generation of immune nutrition. It is under clinical trial in the United States and Europe in posttrauma patients; after large operations; after transplantation, especially of bone marrow and liver; and in patients with acute pancreatitis, HIV/AIDS (chronic MOF), or advanced cancer, especially with heavy irradiation and cystostatic treatment. So far the results are encouraging. The product will most likely be available within 2 to 3 years.

Résumé

L'alimentation entérale présente plusieurs avantages par rapport à l'alimentation parentérale chez le patient polytraumatisé ou opéré. Les techniques modernes d'alimentation par sonde ont fait de l'alimentation entérale précoce une réalité. L'apport alimentaire dans le jéjunum a plusieurs avantages sur l'apport alimentaire dans l'estomac: meilleur rendement métabolique, moins de

problèmes de régurgitation et d'inhalation. L'alimentation entérale précoce ou immédiate est suivie d'une stimulation de la circulation splanchnique et hépatique, d'une augmentation de la vascularisation muqueuse intestinale, évite l'acidose et les perturbations de la perméabilité de la muqueuse intestinale et élimine la nécessité d'une prophylaxie antistress. La salive, contenant d'importantes substances antimicrobiennes et l'acidité gastrique sont des éléments importants dans la prévention du sepsis. Mastication, et sécrétion de salive et d'acidité gastrique, stimulent le largage d'oxyde nitreux (ON), si important pour le débit sanguin de la muqueuse, la motricité gastro-intestinale, la formation de mucus et la bactériostase. Donner oralement les substances qui augmentent la production d'ON et mâcher des aliments riches en ON tel que la laitue ou les épinards peut être utile. On recommande également les lipides pris par la bouche et ceux qui sont sensés protéger la muqueuse. Il faut éviter les anti H₂ et les médicaments qui inhibent la production du salive. L'alimentation entérale immédiate, en commençant par 25 ml/hr, doit être augmentée à 100 ml/hr en 24–48 heures. Une attention particulière doit être apportée à la pureté de l'eau chez le patient immunodéficitaire. L'eau en bouteille peut contenir des bactéries telle le *pseudomonas*. Il faut préconiser les antioxydants alimentaires comme la glutathion, la vitamine E et les β -carotènes. Les aliments destinés au côlon sont très importants. Environ 10% des besoins caloriques sont fournis par les aliments coliques dits «prébiotiques», fermentés au niveau de la lumière colique dans le but de nourrir la muqueuse colique et prévenir le sepsis d'origine intestinale. La flore de fermentation (flore prébiotique) est perturbée en raison de la maladie ou d'un traitement antibiotique: restaurer la flore est donc aussi important. On recommande des fibres en raison de 10 gr/jour. Une nouvelle conception d'«éco-immunonutrition» est présentée ici pour donner une alimentation entérale comprenant l'apport de surfactants nouveaux, de pseudomucus, de fibres, d'acides aminés comme l'arginine et du *lactobacillus plantarum* 299.

Resumen

La nutrición enteral (NE) posee diversas ventajas sobre la nutrición parenteral (NP) en pacientes postoperatorios/post-trauma (POTS). Las modernas tecnologías de alimentación por tubo han hecho posible la NE precoz. La alimentación yeyunal tiene ventajas sobre la alimentación por tubo gástrico: más rápida recuperación metabólica, menos vómito y menor riesgo de regurgitación y aspiración. La NE inmediata o precoz estimula la circulación esplácnica y hepática, mejora el flujo mucoso, previene la acidosis en la mucosa y las alteraciones de permeabilidad y eliminan la necesidad de profilaxis de ulceración de estrés. La saliva, que contiene importantes sustancias antimicrobianas, y la acidez gástrica son de importancia en la prevención de la sepsis. La masticación, la saliva y la acidez gástrica favorecen la liberación de óxido nítrico (NO) gástrico, de importancia para el flujo mucoso, la motilidad gastrointestinal, la formación de moco y la bacteriostasis. Una provisión oral de sustancias donantes de NO y la masticación de alimentos ricos en nitratos, tales como la lechuga y la espinaca, pueden ser de utilidad. Se recomiendan lípidos orales y protectores de la mucosa, y se deben evitar los bloqueadores H-2 y las drogas inhibidoras de la saliva. Se debe proveer NE inmediata, comenzando con 25 ml/hora e incrementando a 100 ml/hora en el curso de 24–48 horas. En el paciente inmunocomprometido se debe prestar especial atención a la

pureza del agua. El agua embotellada puede contener bacterias tales como pseudomonas. Antioxidantes alimentarios como la glutamina, la vitamina E y el (caroteno son importantes. Los ingredientes para la mucosa colónica son importantes. Aproximadamente el 10% del requerimiento calórico es satisfecho por los denominados alimentos colónicos (prebióticos), que se fermentan a nivel de la mucosa colónica para producir nutrientes de la mucosa colónica y para prevenir la sepsis de origen intestinal. Se recomienda fibra por encima de 10 gramos/día. La flora de fermentación (flora prebiótica) se altera debido a enfermedad o tratamiento antibiótico, por lo cual la repleción de la flora es importante. Un nuevo concepto de eco-inmunonutrición es presentado para la provisión enteral de ingredientes recondicionadores de la mucosa: nuevos surfactantes, pseudomocós, fibra, aminoácidos tales como arginina y el *Lactobacillus plantarum* 299.

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References

- Riegel, C., Koop, C.E., Drew, J., Stevens, L.W., Rhoads, J.E.: The nutritional requirements for nitrogen balance in surgical patients during the early postoperative period. *J. Clin. Invest.* 26:18, 1947
- Van Goor, H., Rosman, C., Grond, J., Kooi, K., Wübbels, G.H., Bleichrodt, R.P.: Translocation of bacteria and endotoxin in organ donors. *Arch. Surg.* 129:1063, 1994
- McDonald, W.S., Sharp, C.W., Deitch, E.A.: Immediate enteral feeding in burn patients is safe and effective. *Ann. Surg.* 213:177, 1991
- O'Dwyer, S.T., Michie, H.R., Ziegler, T.R., Revhaug, A., Smith, R.J., Wilmore, D.W.: A single dose of endotoxin increases intestinal permeability in healthy humans. *Arch. Surg.* 123:1459, 1988
- Dobke, M.K., Simoni, J., Ninnemann, T.J., Garrett, J., Hamar, T.J.: Endotoxin after burn injury: effects of early excision on circulating levels. *J. Burn Care Rehabil.* 10:107, 1989
- Doglio, G.R., Pusajo, J.F., Egurrola, M.A., et al.: Gastric mucosal pH as a prognostic index of mortality in critically ill patients. *Crit. Care Med.* 19:1037, 1990
- Levine, G.M., Deren, J.J., Steiger, E., Zinno, R.: Role of oral intake in maintenance of gut mass in disaccharide activity. *Gastroenterology* 67:975, 1974
- Bark, T., Katouli, M., Ljungquist, O., Möllby, R., Svenberg, T.: Glutamine supplementation does not prevent bacterial translocation after nonlethal hemorrhage in rats. *Eur. J. Surg.* 161:3, 1995
- Roediger, W.E.W.: Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. *Gut* 21:793, 1980
- Hoover, H.C., Ryan, J.A., Anderson, E.J., Fischer, J.E.: Nutritional benefits of immediate postoperative jejunal feeding of an elemental diet. *Am. J. Surg.* 139:153, 1980
- Alexander, J.W., MacMillan, B.G., Stinnett, D.J., et al.: Beneficial effects of aggressive protein feeding in severely burned children. *Ann. Surg.* 192:505, 1980
- Shepherd, A.P.: Intestinal blood flow autoregulation during foodstuff absorption. *Am. J. Physiol.* 239:H156, 1980
- Niederman, M.S., Mantovani, R., Schoch, P., Papas, J., Fein, A.M.: Clinical investigation in mechanically ventilated patients: the role of nutritional status in colonization of the lower airway by *Pseudomonas* species. *Chest* 95:155, 1989
- Mandel, I.D.: The function of saliva. *J. Dent. Res.* 66:623, 1987
- Starkey, R.H., Orth, D.: Radioummunoassay of human epidermal growth factor (urogastrone). *Clin. Endocrinol. Metab.* 45:1144, 1977
- Sarosiek, J., Feng, T.T., McCallum, R.W.: The interrelationship between salivary epidermal growth factor and the functional integrity of the mucosal barrier in the rat. *Am. J. Med. Sci.* 302:359, 1991
- Vahouny, G.V., Le, T., Ifrim, I., Satchithanandam, S., Cassidy, M.M.: Stimulation of intestinal cytokinetics and mucin turnover in rats fed wheat bran or cellulose. *Am. J. Clin. Nutr.* 41:895, 1985
- Fox, P.C., van der Ven, P.F., Baum, B.J., Mandel, I.D.: Pilocarpine for the treatment of xerostomia associated with salivary gland dysfunction. *Oral Surg.* 61:243, 1986
- Eddleston, J.M., Vohra, A., Scott, P., et al.: A comparison of the frequency of stress ulceration and secondary pneumonia in sucralfate- or ranitidine-treated intensive care patients. *Crit. Care Med.* 19:1491, 1991
- Toung, T.J.K., Rosenfeld, B.A., Yoshiki, A., Grayson, R.F., Traustman, R.J.: Sucralfate does not reduce the risk of acid aspiration pneumonitis. *Crit. Care Med.* 21:1359, 1993
- Bengmark, S., Jeppsson, B.: Gastrointestinal surface protection and mucosa reconditioning. *J. Parenter. Enter. Nutr.* 19:410, 1995
- Bonten, M.J.M., Gaillard, C.A., van Thiel, F.H., van der Geest, S., Stobberingh, E.E.: Continuous enteral feeding counteracts preventive measures for gastric colonization in intensive care patients. *Crit. Care Med.* 22:939, 1994
- Fujisaki, H., Oketani, K., Murakami, M., et al.: Inhibitions of acid secretion by E 3810 and omeprazole, and their reversal by glutathione. *Biochem. Pharmacol.* 4:321, 1991
- Lundberg, J.O.N., Weitzberg, E., Lundberg, J.M., Alving, K.: Intra-gastric nitric oxide production in humans: measurements in expelled air. *Gut* 35:1543, 1994
- Vandenbroucke-Grauls, C.M.J.E., Vandenbroucke, J.P.: Effect of selective decontamination of the digestive tract on respiratory tract infections and mortality in the intensive care units. *Lancet* 338:859, 1991
- Fisher, C.J., Jr., Marra, M.N., Palardy, J.E., Marchbanks, C.R., Scott, C.R., Opal, S.M.: Human neutrophil bactericidal/permeability increasing protein reduces mortality rate from endotoxin challenge: a placebo-controlled study. *Crit. Care Med.* 22:553, 1994
- Proulx, F., Gauthier, M., Nadeau, D., Lacroix, J., Farrell, C.A.: Timing and predictors of death in pediatric patients with multiple organ failure. *Crit. Care Med.* 22:1025, 1994
- Roberfroid, M.B., Bornet, F., Bouley, C., Cummings, J.H.: Colonic microflora: nutrition and health. *Nutr. Rev.* 53:127, 1995
- Croft, D.N., Cotton, P.B.: Gastrointestinal cell loss in man: its measurement and significance. *Digestion* 8:144, 1973
- Molin, G., Jeppsson, B., Johansson, M.L., Ahrné, S., Nobaek, S., Bengmark, S.: Numerical taxonomy of *Lactobacillus* spp associated to healthy and diseased mucosa of the human intestines. *J. Appl. Bacteriol.* 74:314, 1993
- Kaila, M., Isolauri, E., Soppi, E., Virtanen, E., Laine, S., Arvilommi, H.: Enhancement of circulating antibody secreting cell response in human diarrhea by a human *Lactobacillus* strain. *Pediatr. Res.* 32:141, 1992
- Jayasinghe, G., Larsson, K., Miezi, Y., Sivik, B.: Oat lipids—interaction with water and characterization of aqueous phases. *J. Disp. Sci. Technol.* 12:443, 1991
- Barbul, A.: Arginine and immune function. *Nutrition* 6:53, 1990
- Shabert, J., Ehrlich, N.: The Ultimate Nutrient Glutamine. New York, Avery Publishing Group, 1994
- Gregg Settle, R.: Invited comment: short-chain fatty acids and their potential role in nutritional support. *J.P.E.N. J. Parenter. Enter. Nutr.* 12:104S, 1988
- Nettleton, J.A.: w-3 Fatty acids: comparison of plant and seafood sources in human nutrition. *J. Am. Diet. Assoc.* 91:331, 1991
- Rudolph, F.B., Kulkarni, A.D., Fanslow, W.C., et al.: Role of RNA as dietary source of pyrimidine and purines. *Nutrition* 6:45, 1990
- Avery, M.E., Mead, J.: Surface properties in relation to atelectasis and hyaline membrane disease. *Am. J. Dis. Child.* 97:517, 1959
- Pison, U., Seeger, W., Buchhorn, R., et al.: Surfactant abnormalities in patients with respiratory failure after multiple trauma. *Am. Rev. Respir. Dis.* 140:1033, 1989
- Wang, X.D., Andersson, R., Soltesz, V., Wang, W.Q., Ar'Rajab, A., Bengmark, S.: Phospholipids prevent enteral bacterial translocation in the early stages of experimental acute liver failure in the rat. *Scand. J. Gastroenterol.* 29:1117, 1994
- Slomiany, B.L., Piotrowski, J., Samanta, A., VanHorn, L., Murty, V.L.N., Slomiany, A.: *Campylobacter pylori* colonization factor shows specificity for lactosylceramide sulfate and GM₃ ganglioside. *Biochem. Int.* 19:924, 1989
- Ostrand, G.K., Levery, S.B., Eaton, H.L., Salyan, M.E.K., Hakomori, S.I., Holmes, E.H.: Isolation and characterization of four major neutral glycosphingolipids from the liver of the English sole

- (*Parophrys vetulus*): presence of novel branched lacto-ganglio-_{iso}-globo hybrid structure. *J. Biol. Chem.* 263:18716, 1988
43. Hachida, W., Uwabe, K., Nonyama, M., et al.: The significant immunosuppressive effect of ganglioside GM₃ in heart transplantation. *Transplantation* 56:479, 1993
 44. Halliwell, B., Gutterich, J.M.C.: *Free Radicals in Biology and Medicine* (2nd ed.). Oxford, Clarendon Press, 1989
 45. Sies, H., Stahl, W., Sunquist, A.R.: Antioxidant functions of vitamins: vitamins E and C, beta-carotene, and other carotenoids. *Ann. N.Y. Acad. Sci.* 669:7, 1992
 46. Robinson, M.K., Rounds, J.D., Hong, R.W., Jacobs, D.O., Wilmore, D.W.: Glutathione deficiency increases organ dysfunction after hemorrhagic shock. *Surgery* 112:140, 1992
 47. Keller, G.A., Barke, R., Harty, J.T., Humphrey, E., Simmons, R.L.: Decreased hepatic glutathione levels in septic shock. *Arch. Surg.* 120:941, 1985
 48. Sekkat, C., Dornand, J., Gerber, M.: Oxidative phenomena are implicated in human T-cell stimulation. *Immunology* 63:431, 1988
 49. Stein, H.J., Hinder, R.A., Oosthuizen, M.M.J.: Gastric mucosal injury caused by hemorrhagic shock and reperfusion: protective role of antioxidant glutathione. *Surgery* 108:467, 1990
 50. Kohnen, R., Shadmi, V., Kakunda, A., Rubinstein, A.: Prevention of oxidative damage in the rat jejunal mucosa by pectin. *Br. J. Nutr.* 69:789, 1993
 51. Toki, A., Todani, T., Watanabe, Y., Uemura, S., Sato, Y., Morotomi, Y.: Effects of pectin and cellulose on fat absorption after massive small-bowel resection in weaning rats. *J.P.E.N. J. Parenter. Enter. Nutr.* 16:255, 1992
 52. Hickey, M.S., Weaver, K.E.: Nutritional management of patients with ARC or AIDS. *Gastroenterol. Clin. North Am.* 17:545, 1988
 53. Cummings, J.H., Hill, M.J., Bone, E.S., Branch, W.J., Jenkins, D.J.A.: The effect of meat protein and dietary fiber on colonic function and metabolism. II. Bacterial metabolites in feces and urine. *Am. J. Clin. Nutr.* 32:2094, 1978
 54. Flouire, B., Vidon, N., Florent, C., Bernier, J.J.: Effect of pectin on jejunal glucose absorption and unstirred layer thickness in normal man. *Gut* 25:936, 1984
 55. Scrimshaw, N.S., Taylor, C.E., Gordon, J.E.: Interaction of nutrition and infection. *Am. J. Med. Sci.* 237:367, 1959
 56. Chandra, R.K., Baker, M., Whang, S., Au, B.: Effect of two feeding formulas on immune responses and mortality in mice challenged with *Listeria monocytogenes*. *Immunol. Lett.* 27:45, 1991
 57. Hoffman-Goetz, L., McFarlane, D., Bistran, B.R., Blackburn, G.I.: Febrile and plasma iron responses of rabbits injected with endogenous pyrogen from malnourished patients. *Am. J. Clin. Nutr.* 34:1109, 1981
 58. Bell, R.C., Golembowski, K.A., Youngman, L.D., et al.: NK cell activity and protein intake in an aflatoxin B₁ (AF)-induced tumor model [abstract]. *FASEB J.* 4:A1043, 1990
 59. Meydani, S.N.: Dietary modulation of cytokine production and biologic function. *Nutr. Rev.* 48:361, 1990
 60. Marty, C., Misset, B., Tamion, F., Fitting, C., Carlet, J., Cavailjon, J.M.: Circulating interleukin-8 concentrations in patients with multiple organ failure of septic and nonseptic origin. *Crit. Care Med.* 22:673, 1994
 61. Hertzog, J.H., Godinez, M.H., Godinez, R.: Tumor necrosis factor- δ alters phospholipid content in the bronchoalveolar lavage-accessible space of isolated perfused rat lungs. *Crit. Care Med.* 22:1969, 1994
 62. Ishii, Y., Partridge, C.A., Del Vecchio, P.J., Malik, A.B.: Tumor necrosis factor- δ -mediated decrease in glutathione increases the sensitivity of pulmonary vascular endothelial cell to H₂O₂. *J. Clin. Invest.* 89:794, 1992
 63. Kilbourn, R.G., Gross, S.S., Jubran, A., et al.: N^G-Methyl-L-arginine inhibits tumor necrosis factor-induced hypotension: implications for the involvement of nitric oxide. *Proc. Natl. Acad. Sci. U.S.A.* 87:3629, 1990
 64. Wright, C.E., Rees, D.D., Moncada, S.: Protective and pathological roles of nitric oxide in endotoxin shock. *Cardiovasc. Res.* 26:48, 1992
 65. Preiser, J.C., Lejeune, P., Roman, A., et al.: Methylene blue administration in septic shock: a clinical trial. *Crit. Care Med.* 23:259, 1995
 66. Herndon, D.N., Ziegler, S.T.: Bacterial translocation after thermal injury. *Crit. Care Med.* 21:S50, 1993
 67. Spies, C.D., Reinhart, K., Witt, I., et al.: Influence of N-acetylcysteine on indirect indicators of tissue oxygenation in septic shock patients: results from a prospective, randomized, double-blind study. *Crit. Care Med.* 22:1738, 1994
 68. Harrison, P.M., Wendon, J.A., Gimson, A.E.S., Alexander, G.J.M., Williams, R.: Improvement by acetylcysteine of hemodynamics and oxygen transport in fulminant hepatic failure. *N. Engl. J. Med.* 324:1852, 1991
 69. Benjamin, N., O'Driscoll, F., Dougall, H., Duncan, C., Smith, S., Golden, M.: Stomach NO synthesis. *Nature* 368:502, 1994
 70. Palmer, R.M.J., Ferrige, A.G., Moncada, S.: Nitric oxide release accounts for biologic activity of endothelium-derived relaxing factor. *Nature* 327:524, 1987
 71. Brown, J.F., Hanson, P.J., Whittle, B.J.R.: Nitric oxide donors increase mucus gel thickness in rat stomach. *Eur. J. Pharmacol.* 23:103, 1992
 72. Ringer, T.V., Zimmerman, J.J.: Inflammatory host responses in sepsis. *Crit. Care Clin.* 8:163, 1992
 73. Vespasiano, M.C., Lewandoski, J.R., Zimmerman, J.J.: Longitudinal analysis of neutrophil superoxide anion generation in patients with septic shock. *Crit. Care Med.* 21:666, 1993
 74. Streat, S.J., Beddoe, A.H., Hill, G.L.: Aggressive nutritional support does not prevent protein loss despite fat gain in septic intensive care patients. *J. Trauma* 27:262, 1987
 75. Detsky, A.S., Baker, J.P., O'Rourke, K., Goel, V.: Perioperative parenteral nutrition: a meta-analysis. *Ann. Intern. Med.* 107:195, 1987
 76. Montecalvo, M.A., Steger, K.A., Farber, H.W., et al.: Nutritional outcome and pneumonia in critical care patients randomized to gastric versus jejunal tube feedings. *Crit. Care Med.* 20:1377, 1992
 77. Levenson, R., Turner, W.W., Jr., Dyson, A., Zike, L., Reisch, J.: Do weighted nasoenteric feeding tubes facilitate duodenal intubations? *Parenter. Enterol. Nutr.* 12:135, 1988
 78. Jeppsson, B., Tranberg, K.G., Bengmark, S.: Technical developments: a new self-propelling nasoenteric feeding tube. *Clin. Nutr.* 11:373, 1992
 79. Gottschlich, M.M., Jenkins, M., Warden, G.D., et al.: Differential effects of three enteral dietary regimens on selected outcome variables in burn patients. *J.P.E.N. J. Parenter. Enterol. Nutr.* 14:225, 1990
 80. Hernandez-Duquino, H., Rosenberg, F.A.: Antibiotic resistant *Pseudomonas* in bottled drinking water. *Can. J. Microbiol.* 33:286, 1987
 81. Rosenberg, S.A., Hernandez-Duquino, H.: Antibiotic resistance of *Pseudomonas* from German mineral waters. *Toxicity Assess.* 4:281, 1989
 82. Moore, F.A., Feliciano, D.F., Andrassy, R.J., et al.: Early enteral feeding compared with parenteral, reduces postoperative septic complications: the result of a meta-analysis. *Ann. Surg.* 216:172, 1992
 83. Kudsk, K.A.: Gut mucosal nutrition support: enteral nutrition as primary therapy after multiple system trauma. *Gut* 35:S52, 1994
 84. Daly, J.M., Lieberman, M.D., Goldfine, J., et al.: Enteral nutrition with supplemental arginine, RNA, and omega-3 fatty acids in patients after operation: immunologic, metabolic, and clinical outcome. *Surgery* 112:56, 1992
 85. Daly, J.M., Weintraub, F.N., Shou, J., Rosato, E.F., Lucia, M.: Enteral nutrition during multimodality therapy in upper gastrointestinal cancer patients. *Ann. Surg.* 221:327, 1995
 86. Braga, M.N., Vignali, A., Gianotti, L.K., et al.: Immune and nutritional effects of early enteral nutrition after major abdominal operations. *Eur. Surg.* 221:327, 1995
 87. Kemen, M., Senkal, M., Homann, H.H., et al.: Early postoperative enteral nutrition with arginine, omega-3 fatty acids and ribonucleic acid-supplemented diet versus placebo in cancer patients: an immunological evaluation of IMPACT[™]. *Crit. Care Med.* 23:652, 1995
 88. Bowers, R.B., Cerra, F.B., Bershadsky, B., et al.: Early enteral administration of a formula (IMPACT) supplemented with arginine, nucleotides, and fish oil in intensive care unit patients: results of a multicenter, prospective, randomized, clinical trial. *Crit. Care Med.* 23:436, 1995
 89. Eiseman, B., Silen, W., Bascom, G.S., Kauvar, A.J.: Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. *Surgery* 44:854, 1958
 90. Wilmore, D.W.: The surgeon and intestinal bacteria: reconsideration of our relationship. Presidential Address, Surgical Infection Society (Not published)

Probiotics In Prevention Of Antibiotic Associated Diarrhoea: Meta-Analysis

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- 17 Shann F, Hart K, Thomas D. Acute lower respiratory tract infections in children: possible criteria for selection of patients for antibiotic therapy and hospital admission. *Bull World Health Organ* 1984;62:749-53.
- 18 Campbell H, Byass P, Lamont AC, Forgie IM, O'Neill KP, Lloyd-Evans N, et al. Assessment of clinical criteria for identification of severe acute lower respiratory tract infections in children. *Lancet* 1989;i:297-9.
- 19 Rahman MM, Vermund SH, Wahed MA, Fuchs GJ, Baqui AH, Alvarez JO. Simultaneous zinc and vitamin A supplementation in Bangladeshi children: randomised double blind controlled trial. *BMJ* 2001;323:314-8.
- 20 Sazawal S, Jalla S, Mazumder S, Sinha A, Black RE, Bhan MK. Effect of zinc supplementation on cell-mediated immunity and lymphocyte subsets in preschool children. *Indian Pediatr* 1997;34:589-97.
- 21 Shankar AH, Prasad AS. Zinc and immune function: the biological basis of altered resistance to infection. *Am J Clin Nutr* 1998;68 (suppl 2): S447-63.
- 22 Sempertegui F, Estrella B, Correa E, Aguirre L, Saa B, Torres M, et al. Effects of short term zinc supplementation on cellular immunity, respiratory symptoms, and growth of malnourished Ecuadorian children. *Eur J Clin Nutr* 1996;50:42-6.

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Probiotics in prevention of antibiotic associated diarrhoea: meta-analysis

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Abstract

Objective To evaluate efficacy of probiotics in prevention and treatment of diarrhoea associated with the use of antibiotics.

Design Meta-analysis; outcome data (proportion of patients not getting diarrhoea) were analysed, pooled, and compared to determine odds ratios in treated and control groups.

Identification Studies identified by searching Medline between 1966 and 2000 and the Cochrane Library.

Studies reviewed Nine randomised, double blind, placebo controlled trials of probiotics.

Results Two of the nine studies investigated the effects of probiotics in children. Four trials used a yeast (*Saccharomyces boulardii*), four used lactobacilli, and one used a strain of enterococcus that produced lactic acid. Three trials used a combination of probiotic strains of bacteria. In all nine trials, the probiotics were given in combination with antibiotics and the control groups received placebo and antibiotics. The odds ratio in favour of active treatment over placebo in preventing diarrhoea associated with antibiotics was 0.39 (95% confidence interval 0.25 to 0.62; $P < 0.001$) for the yeast and 0.34 (0.19 to 0.61; $P < 0.01$) for lactobacilli. The combined odds ratio was 0.37 (0.26 to 0.53; $P < 0.001$) in favour of active treatment over placebo.

Conclusions The meta-analysis suggests that probiotics can be used to prevent antibiotic associated diarrhoea and that *S. boulardii* and lactobacilli have the potential to be used in this situation. The efficacy of probiotics in treating antibiotic associated diarrhoea remains to be proved. A further large trial in which probiotics are used as preventive agents should look at the costs of and need for routine use of these agents.

Introduction

The term "probiotic" was first used to describe "a live microbial supplement, which beneficially affects the host by improving its microbial balance."¹ Since then, research has looked at possible clinical uses for these agents. In 1995, when a greater understanding of their properties had developed, the term "biotherapeutic agents" was proposed to describe micro-organisms with specific therapeutic properties that also inhibit the growth of pathogenic bacteria.²

Probiotics and their uses

- Probiotics are live organisms that improve the microbial balance of the host
- Probiotics have special properties that make them useful in fighting infections of mucosal surfaces such as the gut and vagina
- Different species of lactobacilli and the yeast *Saccharomyces boulardii* have the potential for use in clinical practice
- Probiotics are becoming increasingly available as capsules and dairy based food supplements sold in health food stores and some supermarkets
- The relative lack of side effects makes probiotics a possible way of preventing antibiotic associated diarrhoea

A number of agents have been isolated and studied with a view to clinical use. *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, commonly used in the dairy food industry, were among the first to be studied. Other strains that have been used are *Bifidobacterium bifidum*, *B. longum*, *Enterococcus faecium*, *Saccharomyces boulardii*, *L. acidophilus*, *L. casei*, and *Lactobacillus GG*. However, doctors are still reluctant to use these agents in clinical practice.

In this paper, we review the results from various trials carried out to study their benefits. We also look at the properties of biotherapeutic agents and options for further research.

Materials and methods

Literature search

We searched Medline between 1966 to 2000 with the terms "probiotics," "biotherapeutic agents," "lactobacilli," "antibiotic associated diarrhoea," and "*Clostridium difficile*." We also searched the Cochrane Controlled Trials Register and the Cochrane Database of Systematic Reviews. We included all randomised double blind trials that compared the effects of probiotic therapy and placebo (both given in combination with antibiotics).

Ten double blind placebo controlled trials were relevant to our area of interest (nine published in English and one in French).³⁻¹² Our meta-analysis included nine that looked at prevention of diarrhoea. We excluded the other trial, which looked at treatment of diarrhoea.¹²

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Table 1 Probiotics studied in trials and patients with absence of diarrhoea at end of trial

Trial	Probiotic	Dose	Duration of treatment	Antibiotic studied	% of patients without diarrhoea	
					Active group	Placebo group
Adam et al ³	<i>S. boulardii</i>	4 capsules/day	Variable	Mixture	96	83
Gotz et al ⁴	<i>L. acidophilus</i> and <i>L. bulgaricus</i>	1 sachet Lactinex four times a day	5 days	Ampicillin	100	86
Surawicz et al ⁵	<i>S. boulardii</i>	1 g/day	Variable	Mixture	91	78
Wunderlich et al ⁶	<i>E. faecium</i> SF68	1 capsule twice a day	7 days	Mixture	91	73
Tankanow et al ⁷	<i>L. acidophilus</i> and <i>L. bulgaricus</i>	1 g Lactinex four times a day	10 days	Amoxicillin	34	31
Orrhage et al ⁸	<i>L. acidophilus</i> and <i>Bifidobacterium longum</i>	Fermented milk with cultures 250 ml twice a day	21 days	Clindamycin	80	30
McFarland et al ⁹	<i>S. boulardii</i>	1 g/day	49 days	Mixture	93	85
Lewis et al ¹⁰	<i>S. boulardii</i>	113 mg twice a day	14 days	Mixture	79	83
Vanderhoof et al ¹¹	<i>Lactobacillus</i> GG	1-2 capsules a day (10 ¹⁰ colonies per capsule)	10 days	Mixture	93	74

Meta-analysis and data abstraction

The meta-analysis was carried out according to the recommendations of the QUOROM statement.¹³ The key outcome data taken for analysis included the sample size, treatment regimens, and numbers of patients in both arms of the study who had an absence of diarrhoea (table 1).

Results

Nine trials were included in the final analysis (fig 1). The study regimens used probiotics combined with one antibiotic or a variety of antibiotics (table 1). All trials studied the efficacy of a probiotic in the prevention of antibiotic associated diarrhoea. The numbers of patients and the duration of follow up varied greatly from study to study, but the patients' characteristics were similar for the active treatment and placebo groups within each study.

We calculated the odds ratio on the basis of the proportion of patients free of diarrhoea on treatment compared with that in control groups. After tests of

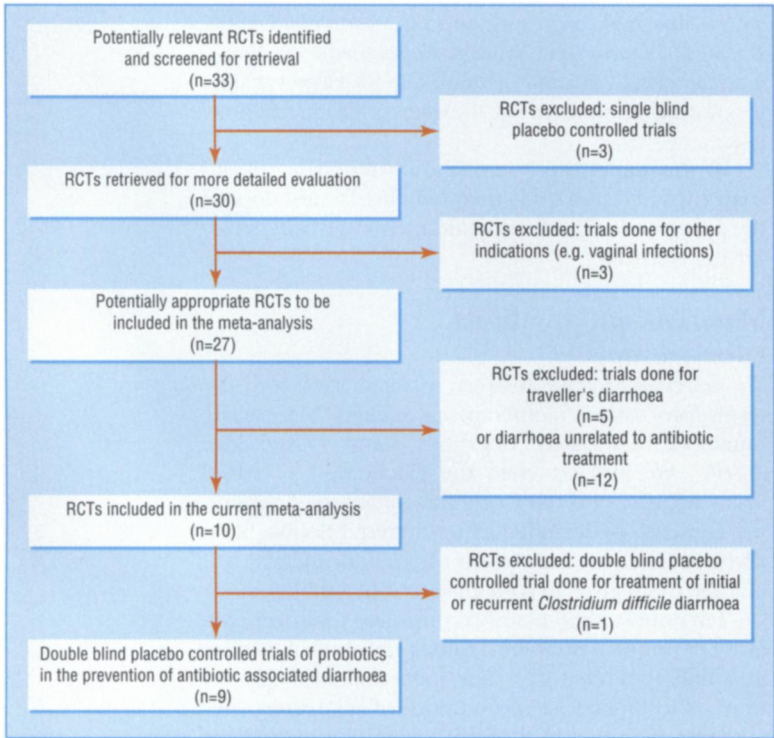


Fig 1 Meta-analysis profile summarising trial flow. RCT=randomised controlled trial

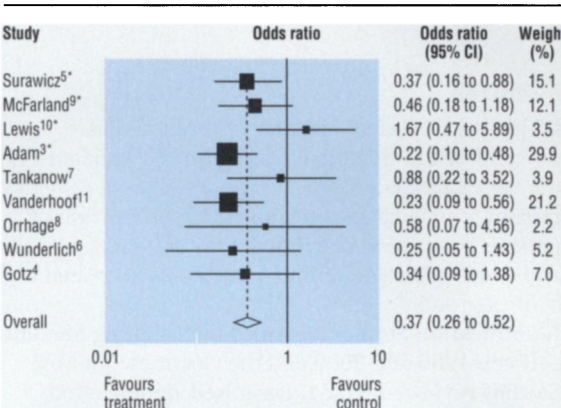


Fig 2 Plot of the log of odds ratios for the proportion of patients free of diarrhoea in treatment groups compared with control groups

homogeneity, summary odds ratios and 95% confidence interval limits were provided for the combined data of the four trials that used *S. boulardii* (yeast trials), the five non-yeast trials, and all nine trials together. The combined odds ratios for the four yeast trials and for the five non-yeast trials were similar (0.39 (95% confidence interval 0.25 to 0.62) and 0.34 (0.19 to 0.61), respectively); both favoured active treatment over placebo in the prevention of antibiotic associated diarrhoea. The odds ratio for pooled data from all nine trials was in favour of active treatment over placebo in the prevention of antibiotic associated diarrhoea (0.37; 0.26 to 0.53). Six studies showed a significant benefit of probiotic treatment compared with placebo ($P < 0.05$) (fig 2).^{3 5 6 8 9 11} One study showed benefit for only a subgroup of patients who did not receive non-antibiotic drugs likely to induce diarrhoea, such as magnesium hydroxide (for constipation), lactulose, and bisacodyl (for hepatic encephalopathy).⁴

Discussion

Our meta-analysis of trials that used live organisms to prevent diarrhoea associated with antibiotics shows that probiotics may be effective in preventing antibiotic associated diarrhoea. We had only a small number of trials in our meta-analysis, and it should be noted that the different antibiotics used in the trials may have altered the risk of patients getting diarrhoea and their response to the probiotics. Although probiotics have been used to prevent or treat diarrhoea of other

causes—namely traveller's diarrhoea and infantile infectious diarrhoea—we did not include trials that investigated probiotics in these indications; however, most of these studies showed positive results, and some reviews have been encouraging.¹⁴

The way in which probiotics affect the gut has drawn much interest. To combat the problems of gastrointestinal infection, probiotics must be non-pathogenic and must act against pathogens by different mechanisms from antibiotics—for example, by competition. More importantly, they should have a fairly rapid onset of action and survive the challenges of gastric acid, bile, or concurrent antibiotics. It is also desirable that they modify immune processes to destroy the invading organism. *Saccharomyces boulardii* and lactobacilli display these common properties.

A few live organisms have been used in many trials. *S. boulardii*, a non-pathogenic yeast, is one such organism. It has a growth temperature of 37°C, rapidly colonises the bowel, does not alter the normal gut flora, and is cleared from the colon after treatment is discontinued.¹⁵ Of the four yeast trials, three studies individually showed significant benefit,^{3 5 9} but one did not¹⁰; differences in the dose and duration of treatment with *S. boulardii* and variations in the period of follow up may explain this disparity. Interestingly, *S. boulardii* can also destroy the receptor site for *C. difficile* toxin A and B by producing a protease¹⁶; this could explain how *S. boulardii* was noted to reduce the frequency of toxin B positivity.¹²

The other probiotic agent used widely in clinical trials is the *Lactobacillus* species. The mechanism of action of lactobacilli may be through multiple means: *Lactobacillus* GG has shown beneficial effects on intestinal immunity; it increases the numbers of cells that secrete immunoglobulin A and other immunoglobulins in the intestinal mucosa, and it stimulates the local release of interferon.¹⁷ It also facilitates antigen transport to underlying lymphoid cells, and shows increased uptake in Peyer's patches.¹⁷ *Lactobacillus* GG has also been shown to produce an antimicrobial substance that inhibits the growth of *Escherichia coli*, streptococci, *Clostridium difficile*, *Bacteroides fragilis*, and *Salmonella*.¹⁸ *L. casei shirata* also showed good survival in the gut in separate studies, and mucosal antibody titres (specific to lactobacilli) were increased in the presence of this agent.^{19 20} Although there was no discernible change to the numbers of clostridia or enterococci, there was an increase in the numbers of excreted bifidobacteria—a normal bowel anaerobe.^{19 20} It is possible that this increase in bifidobacteria interferes with the pathogenic potential of *C. difficile*.

Probiotics are a possible solution in the prevention of antibiotic-associated diarrhoea. *Clostridium difficile* infection is increasingly prevalent in today's hospital setting, particularly in elderly patients, in whom 10–20% of such cases occur.²¹ The incidence of antibiotic associated diarrhoea depends on the antibiotic used and each individual patient's risk factors. The standard regimens to treat colitis associated with *Clostridium difficile* are metronidazole and vancomycin; although these drugs are successful in 80% of cases, about 20% of patients suffer from recurrence.²² In light of the need to control costs in these days of managed health care, we must re-examine the benefits of using live organisms.

What is already known on this topic

Probiotics are well known for their microbiological properties and have been used to treat gastrointestinal and vaginal mucosal infections

Conflicting results have prevented probiotics from being accepted as viable alternatives to conventional treatments for antibiotic associated diarrhoea

The commercial availability of probiotics is increasing

What this study adds

Probiotics may prevent antibiotic associated diarrhoea

The potential of specific probiotics to prevent *Clostridium difficile* infection secondary to the use of antibiotics should be re-examined

A large trial looking at the efficacy of probiotics in preventing antibiotic associated diarrhoea, particularly in elderly patients, with an emphasis on the optimal dose and cost benefits is needed

Whether the use of probiotics can actually reduce the length of hospital stay by reducing the incidence of infection with *C. difficile* and the need to use antibiotics such as metronidazole and vancomycin are issues that need to be addressed in a clinical trial.

Conclusion

Our meta-analysis of nine trials shows that biotherapeutic agents may be useful in preventing antibiotic associated diarrhoea.

The increasing availability, lower costs, and relative lack of side effects of probiotics contrast with the problems associated with current antibiotic regimens. Commercially available strains are being marketed in capsules and yoghurt based drinks, but their potential benefit needs further investigation. Data from trials have provided us with clear evidence on the efficacy of some strains in the gut, but we still need to see confirmation of their clinical benefit.

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- Fuller RB. Probiotics in human medicine. *Gut* 1991;32:439–42.
- McFarland LV, Elmer GW. Biotherapeutic agents: past, present and future. *Microcol Ther* 1995;23:46–73.
- Adam J, Barret A, Barret-Bellet C. Essais cliniques contrôlés en double insu de l'ultra-levure lyophilisée: étude multicentrique par 25 médecins de 388 cas. *Gaz Med Fr* 1977;84:2072–8.
- Gotz V, Romankiewicz JA, Moss J, Murray HW. Prophylaxis against ampicillin associated diarrhoea with *Lactobacillus* preparation. *Am J Hosp Pharm* 1979;36:754–7.
- Surawicz CM, Elmer GW, Speelman P, McFarland LV, Chinn J, Van Belle G. Prevention of antibiotic associated diarrhoea by *Saccharomyces boulardii*. *Gastroenterology* 1989;96:981–8.
- Wunderlich PF, Braun L, Fumagalli I, D'Apuzzo V, Heim F, Karly M, et al. Double-blind report on the efficacy of lactic acid-producing *Enterococcus* SF68 in the prevention of antibiotic-associated diarrhoea and in the treatment of acute diarrhoea. *J Int Med Res* 1989;17:333–8.
- Tankanow RM, Ross MB, Ertel JJ, Dickinson DG, McCormick LS, Garfinkel JF. Double blind, placebo-controlled study of the efficacy of Lactinex in the prophylaxis of amoxicillin-induced diarrhoea. *DICP, Ann Pharm* 1990;24:382–4.
- Orrhage K, Brismar B, Nord CE. Effects of supplements of *Bifidobacterium longum* and *Lactobacillus acidophilus* on intestinal microbiota during administration of clindamycin. *Microb Ecol Health Dis* 1994;7:17–25.
- McFarland LV, Surawicz CM, Greenberg RN, Elmer GW, Moyer KA, Melcher SA, et al. Prevention of β -lactam-associated diarrhoea by *Saccharomyces boulardii* compared with placebo. *Am J Gastroenterol* 1995;90:439–48.

10 Lewis SJ, Potts LF, Barry RE. The lack of therapeutic effect of *S. boulardii* in the prevention of antibiotic related diarrhoea in elderly patients. *J Infect* 1998;36:171-4.

11 Vanderhoof JA, Whitney DB, Antonson DL, Hanner TL, Lupo JV, Young RJ. *Lactobacillus GG* in the prevention of antibiotic-associated diarrhoea in children. *J Pediatrics* 1999;135:564-8.

12 McFarland LV, Surawicz CM, Greenberg RN, Fekety R, Elmer GW, Moyer KA, et al. A randomized placebo-controlled trial of *S. boulardii* in combination with standard antibiotics for *Clostridium difficile* disease. *JAMA* 1994;271:1913-8.

13 Moher D, Cook DJ, Eastwood S, Olkin I, Rennie D, Stroup DF, for the QUOROM Group. Improving the quality of reports of meta-analyses of randomised controlled trials: the QUOROM statement. *Lancet* 1999;354:1896-900.

14 Elmer GW, Surawicz CM, McFarland LV. Biotherapeutic agents: a neglected modality for treatment and prevention of selected intestinal and vaginal infections. *JAMA* 1996;275:870-6.

15 Blehaut H, Massot J, Elmer GW, Levy RH. Disposition kinetics of *S. boulardii* in man and rat. *Biopharm Drug Dispos* 1989;10:353-64.

16 Castagliuolo I, Riegler MF, Valenick L, LaMont JT, Pothoulakis C. *S. boulardii* protease inhibits effects of *C. difficile* toxins A and B in human colonic mucosa. *Infect Immun* 1999;67:302-7.

17 Benno Y, He F, Hosada M, Hashimoto H, Kojima T, Yamazaki K, et al. Effects of *Lactobacillus GG* yoghurt on human intestinal micro-ecology in Japanese subjects. *Nutr Today* 1996;31(6 suppl):9-11S.

18 Gorbach SL. The discovery of *Lactobacillus GG*. *Nutr Today* 1996;31(6 suppl):2-4S.

19 Spanhaak S, Havenaar R, Schaafsma G. Effect of consumption of milk fermented by *Lactobacillus casei shirota* on intestinal microflora and immune parameters in humans. *Eur J Clin Nutr* 1998;52:899-907.

20 Yuki N, Watanabe K, Mike A, Tagami Y, Tanaka R, Ohwaki M, et al. Survival of probiotic *Lactobacillus casei shirota* in the GI tract-selective isolation using monoclonal antibodies. *Int J Food Microbiol* 1999;48:51-7.

21 Kelly CP, Pothoulakis C, Lamont JT. *Clostridium difficile* colitis. *N Engl J Med* 1994;330:257-62.

22 Walters BAJ, Roberts R, Stafford R, Seneviratne E. Recurrence of antibiotic-associated colitis: endogenous persistence of *C. difficile* during vancomycin therapy. *Gut* 1983;24:206-12.

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Science
commentary

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Science commentary: Probiotics

Probiotics are microbes that protect their host, and in some cases they can prevent disease. They are immunomodulating bacteria that have very low virulence compared with the more pathogenic gut flora such as *Escherichia coli* and clostridia. *Lactobacilli* and *bifidobacteria* are examples of probiotics found in the large intestine.

Lactobacillus GG can prevent diarrhoea and atopy in children.^{1 2} In the gut, probiotic bacteria are thought to occupy binding sites on the gut mucosa, preventing pathogenic bacteria from adhering to the mucosa. *Lactobacilli* also produce proteinaceous compounds—bacteriocins—that act as local antibiotics against more pathogenic organisms. But what is known about what happens in vitro cannot necessarily be extrapolated to the complexity of the ecosystem of the human gut.

Diarrhoea associated with antibiotics is presumed to result from the antibiotics disrupting the normal flora in the gut of a healthy person. Such disruptions cause dysfunction of the gut's ecosystem, and they may allow pathogenic bacteria to colonise the gut and gain access to the mucosa. Whether probiotic supplements stop this process by reducing the disruption or by acting as substitutes for the healthy flora is unclear. Probiotics may compete with pathogens for the nutrients the pathogens need to grow, or they may modify toxins produced by pathogens or toxin receptors

found in the gut wall, or they may stimulate immune responses to pathogens.

The exact mechanisms by which probiotics prevent atopy are also under debate.³ One suggestion is that the establishment and maintenance of innate immune tolerance is mediated by T helper 1 cells and linked in some way to the faecal flora. If the Th1 response is particularly robust, the allergic response mediated by T helper 2 cells tends not to be so strong. Probiotics may prevent atopy by supporting the faecal flora, strengthening the Th1 response, and reducing the allergic response.

In the countries of continental Europe, probiotics are regarded as medicines, and they are prescribed alongside antibiotics. In other countries, probiotics are marketed as supplements and are sold over the counter—although preparations such as “bioyoghurts” do not always contain probiotic strains proved to be clinically useful.

1 Szajewska H, Mrukowicz JZ. Probiotics in the treatment and prevention of acute infectious diarrhoea in infants and children: a systematic review of published randomised, double blind, placebo-controlled trials. *J Pediatr Gastroenterol Nutr* 2001;33 (suppl):S17-25.

2 Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 2001;357:1076-9.

3 Murch S. Toll of allergy reduced by probiotics. *Lancet* 2001;357:1057-9.

A memorable patient
Can you hear me?

The young woman, brought by the police, looked past me, perplexed, eyes darting around the room. Some of my questions obtained brief answers, some none. Not all the answers were for me; “But he can’t be dead” seemed a response to another voice than mine.

As I was about to launch into further questions to establish a diagnosis, I was caught by a recollection of an earlier voice. It was that of the ward sister on the busy medical ward where, as a psychiatric registrar, I would assess people who had taken overdoses. I had noticed the young woman lying unconscious for over a week and had wondered, as I passed, whether she would ever recover. Approaching the next bed I heard the voice of the ward sister—a woman I regarded as efficient and a little

intimidating. I paused and listened (psychiatrists are generally inquisitive). Her voice was soft and barely audible above the bustle and beeping monitors, as she gently reminded the young woman where she was, what was happening, and who had visited, even adding snippets of ward gossip.

The woman in front of me now seemed lost in a world of voices, confusion, and fears. I realised that, while I needed to elicit symptoms to clarify diagnosis and establish a rational treatment plan, I must also try to explain what was and was not happening and, as far as possible, help her contact some reality through the fears provoked by psychotic symptoms.

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INFORME DE BÚSQUEDA DE PATENTES PARA EL No de Radicación: 11-102361						
CRITERIOS PARA LA BÚSQUEDA (anexar pantallazo o reporte en PDF, junto con los boleanos utilizados)						
Palabras utilizadas		clave "B. longum R175" AND FOS Lactobacillus AND lactoferrin AND prebiotics Glutathione supplement AND prebiotic "potassium citrate" AND "magnesium gluconate" AND "zinc gluconate" supplements "diarrhoea" AND lactobacillus AND boulardii				
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		St. Justine's Hospital 2008	1 a 27	NI		X
		Laetitia et al. 2007	1 a 27	NI		X
		Coppa et al. 2006	1 a 27	NI		X
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		US2004/223956	1 a 27	NI		X
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		D'Souza et al. 2002	1 a 27	NI	X	
		Timmerman et al. 2004	1 a 27	NI	X	
Williams et al. 2008	1 a 27	NI		X		
DOCUMENTOS ADICIONALES ENCONTRADOS POR EL EXAMINADOR DE ACUERDO A LOS CRITERIOS DE BÚSQUEDA.						
US7101565						
Bengmark, S. and Gianotti, L. 1996. Nutritional Support to Prevent and Treat Multiple Organ Failure. World J. Surg. 20: 474 – 481						
Bezkorovainy. 2001. Probiotics: determinants of survival and growth in the gut. En línea: http://ajcn.nutrition.org/content/73/2/399s.full						
Henriksson & Mitchell. 2007. Prebiotics enhance survival and prolong the retention period of specific probiotic inocula in an in vivo murine model. En línea: http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2672.2007.03469.x/pdf						
Table of Active ingredients as vitamins and minerals in food supplements. 2006. http://goo.gl/MAS6JL						