

## PROGRESS REPORT

## Probiotics in human medicine

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Probiotics have been with us for as long as people have eaten fermented milks, but their association with health benefits dates only from the turn of the century when Metchnikoff drew attention to the adverse effects of the gut microflora on the host and suggested that ingestion of fermented milks ameliorated this so called autointoxication. Later work, based on the assumption that colonisation of the gut was essential for the maximum effect, used intestinal strains of *Lactobacillus acidophilus* for treatment of constipation.<sup>1</sup>

The use of the term 'probiotic' to describe food supplements specifically designed to improve health, however, dates from 1974 when Parker used it to describe growth promoting animal feed supplements. He defined the term as 'organisms and substances which contribute to intestinal microbial balance'. I have recently<sup>2</sup> revised this definition to read, 'A live microbial feed supplement which beneficially affects the host animal by improving its microbial balance'. This definition stresses the importance of viability and avoids the use of the too broad term 'substances' which could even include antibiotics.

This report uses the revised definition and considers the evidence for the effect of live preparations on the human host. It also presents some evidence from work with animals which is helpful in understanding the potential benefits of this sort of treatment.

**Protecting effect of the gut microflora**

The belief in the beneficial effects of the probiotic approach is based on the knowledge that the intestinal microflora provides protection against various diseases. The evidence for this is incontrovertible and comes from several sources.

Firstly, it can be shown that germ free animals are more susceptible to disease than are their conventional counterparts who carry a complete gut flora. This difference has been shown for infections caused by *Salmonella enteritidis*<sup>3</sup> and *Clostridium botulinum*.<sup>4</sup> For obvious reasons this sort of comparison cannot be made in human subjects, but it is reasonable to assume that at least some of these results would apply also to man.

Another source of evidence that supports the protective effect of the gut flora is the finding that antibiotic treated animals, including humans, can become more susceptible to disease. In humans the disease pseudomembranous colitis, caused by *Clostridium difficile*, is almost always a consequence of antibiotic treatment.<sup>5</sup> In rodents antibiotics have been shown to predispose to

infections with *Salmonella typhimurium*,<sup>6</sup> *Shigella flexneri*, *Vibrio cholerae*,<sup>7</sup> and *C botulinum*.<sup>8</sup>

The third source of supporting evidence comes from experiments in which dosing with faecal suspensions has been shown to prevent infection. In humans it has been shown that *C difficile* infection can be reversed by administering faecal enemas derived from a healthy human adult.<sup>10,11</sup> In chickens it has long been known that dosing newly hatched chicks *per os* with faecal suspension from adult hens can prevent the establishment of salmonellae in the gut.<sup>12</sup>

It is also possible to show under in vitro conditions that isolates of intestinal bacteria inhibit pathogenic bacteria. For example, gut isolates of bifidobacteria, lactobacilli, propionibacteria, and enterococci inhibited *C botulinum*<sup>13</sup>; *C difficile* was inhibited by a variety of intestinal bacteria.<sup>14</sup> Although it is frequently impossible to transpose in vitro results to the in vivo situation, these findings do suggest that specific organisms in the gut have the potential to control the growth of pathogens.

Although there seems no doubt that the gut microflora is protective, in order to produce an effective probiotic we must know which microorganisms are responsible for this effect. Here the evidence is less convincing. When defined microfloras are tested in gnotobiotic animals, the resistance produced is often less than that obtained in the fully conventional animal.<sup>15,16</sup> We should remember, however, that under practical conditions the gut microflora may be deficient only in one or a few of the many bacterial species which are necessary to provide the full resistance to disease. Although lactobacilli established in chickens as monoassociates will not protect against colonisation by *Salmonella*,<sup>17</sup> their omission from the protective microflora results in decreased resistance.<sup>18</sup> So we may not need to add all the bacteria involved in protection in order to restore the full resistance state.

**Factors affecting the natural gut microflora**

Under natural conditions a protective gut microflora develops and there is no need for a probiotic supplement; but humans and farm animals live under rather unnatural conditions. We eat a great deal of processed and in many cases sterile food which may affect our access to, and colonisation by, certain types of bacteria. We also consume antibacterial substances ranging from vinegar to antibiotics. Probiotics have a role in alleviating post antibiotic treatment syndromes. *B breve* is an organism that has given encouraging results in this area.<sup>19,20</sup>

But perhaps the trouble starts earlier when the baby is born. In the wild state the neonatal animal gets its intestinal microflora from its mother and its surroundings. Of these two sources the mother is by far the more important since she has intimate contact with the baby and provides bacteria which are already adapted to growth in the intestinal tract. In the hospital and home environment, however, great efforts are made to prevent the transfer of micro-organisms from the mother to the infant. Indeed, if contact with the mother is restricted by caesarian delivery and maintenance in an incubator, the incidence of lactobacilli in the gut is measurably reduced.<sup>21</sup> Under normal conditions of delivery there is transfer of faecal bacteria from the mother to the baby in spite of high standards of hygiene.<sup>22</sup> Even so the transfer will be less than that under completely natural conditions. Of course we cannot relax the high standards of hygiene that are currently practised in hospitals, but we can consider restoring the gut flora by administration of specific bacteria.

#### Composition of probiotics

The probiotic preparations currently on the market are in the main based on lactic acid bacteria (lactobacilli, streptococci, and bifidobacteria). These three genera have been shown to be important components of the gastrointestinal microflora and are all relatively harmless. It has also been shown experimentally that administration of *Escherichia coli* to babies can prevent the colonisation of the gut by antibiotic resistant strains of *E. coli*.<sup>23</sup> A probiotic preparation may contain one or several different strains of bacteria.

The strains of lactic acid bacteria used in probiotics are mostly intestinal isolates such as *L. acidophilus*, *L. casei*, *Enterococcus faecium* and *Bifidobacterium bifidum*. Yoghurt starter bacteria (*L. bulgaricus* and *Streptococcus thermophilus*) are also included because yoghurt has been associated with health benefits in the past. However, the ability of the yoghurt bacteria to colonise the gut is extremely doubtful, although they can persist and remain viable throughout the gastrointestinal tract of rats. The necessity for continuous ingestion in these cases is obvious. Even with intestinal isolates such as *L. acidophilus*, it is necessary to dose regularly rather than to assume that a few doses will allow the organism to colonise the gut permanently. Work in the USA illustrates this point: when *L. acidophilus* was fed to human patients there was a significant reduction in the activity of the bacterial enzymes  $\beta$  glucuronidase, nitroreductase, and azoreductase. This reduced activity, however, persisted only as long as the lactobacillus supplement was being fed; when it was stopped the enzyme activity slowly returned to the presupplementation values.<sup>24</sup>

Similarly, if lactobacilli are administered to newborn rats there is a significant decrease in intestinal coliform count, but on stopping the treatment this returns to normal after a few days.<sup>25</sup> It seems unlikely, therefore, that probiotic bacteria will permanently colonise the intestine; continuous feeding is necessary if a persistent effect is required.

#### How probiotics might work

The way in which probiotics work is not known, but our knowledge of gut microecology suggests four ways in which they may be operating.

##### PRODUCTION OF ANTIMICROBIAL SUBSTANCES

These might have the effect of reducing the number of viable cells or of affecting the metabolism or toxin production of the intestinal bacteria. There is some evidence for both of these, although it is often very difficult to rule out confidently a reduction in count.

Studies with defined floras in gnotobiotic mice have shown that although the flora introduced into the mouse failed to eliminate *C. difficile*, it did protect against colitis and presumably was suppressing toxin production.<sup>15</sup> Little is known about the antibacterial substances produced by lactic acid bacteria in the gut. Volatile fatty acids produced by the indigenous microflora, of which lactic acid bacteria form a part, are responsible for controlling the colonisation of the gut by *S. sonnei* and enteropathogenic *E. coli*.<sup>26</sup> The high molecular weight antibiotic like inhibitors which have been detected under in vitro conditions have never been found in the intestine. Their very existence has been questioned by some workers who suggest that they have been confused with primary metabolites like lactic acid and hydrogen peroxide.<sup>27</sup>

##### COMPETITION FOR ADHESION RECEPTORS

It is now accepted that many intestinal pathogens must be able to adhere to the gut wall if they are to colonise the gut and produce disease. Consequently, some probiotic strains have been chosen for their ability to adhere to the epithelial wall and thus to compete with pathogens for the adhesion receptors. The potential of this sort of approach was shown in pigs that had been dosed with a non-pathogenic adhering strain of *E. coli*.<sup>28</sup> When these pigs were challenged with a pathogenic strain of the same serotype they were more resistant to infection than the untreated control group and it was concluded that the non-pathogenic strain was occupying the same ecological niche as the pathogenic strain and that this was probably due to their binding to the same adhesion receptors on the gut wall. Although this trial was successful, it was a very specific effect that would operate only against *E. coli* with the k88 antigen, and unfortunately no more work was done to try to broaden the protective effect. In the chicken there is evidence that the protective microflora is gut wall associated because the bacteria washed from the wall of the caecum produced the effect.<sup>29</sup> Selection of probiotic strains on the basis of epithelial adhesion, however, did not ensure permanent colonisation of the intestinal tract of pigs,<sup>30</sup> although it was shown that adhering strains did persist, albeit in low numbers.<sup>31</sup>

##### COMPETITION FOR NUTRIENTS

The gut is such a rich source of nutrients that it may seem unlikely that this is the way in which the gut flora influences its own composition. We

should remember, however, that it requires only one nutrient to be limiting for this mechanism to operate successfully. In vitro results suggest that gut micro-organisms compete more efficiently than *C. difficile* for monomeric glucose, N-acetyl-glucosamine, and sialic acid found in the colonic contents.<sup>32</sup>

#### STIMULATION OF IMMUNITY

An interesting development in recent years has been the finding that lactobacilli administered by mouth can stimulate macrophage activity against several different species of bacteria. For example, *L. casei* given per os to mice increased phagocytic activity.<sup>33</sup> Presumably the effect is produced either by absorption of a soluble antigen or by translocation of lactobacilli through the gut wall into the blood stream. It is known that lactobacilli can translocate in this way,<sup>34</sup> and lactobacilli injected intravenously will survive in the liver, spleen, and lungs.<sup>35</sup> In the latter case, natural killer cell activity was enhanced.

An indirect piece of evidence comes from some work in which it was shown that *Enterococcus faecium* (a species used in probiotic preparations) established as a monoassociate in gnotobiotic mice reduced the count of *S. typhimurium* in the spleen after challenge with this organism.<sup>36</sup>

#### Beneficial effects of probiotics

Although there are claims for beneficial effects of probiotics in constipation, cancer, heart disease, and ulcerative colitis they will not be dealt with here because the evidence is almost exclusively from laboratory animals and this is not always conclusive. What is worth describing are two examples where the evidence for a positive beneficial effect in man is very good.

#### LACTASE DEFICIENCY

A large proportion of the world's population is unable to utilise lactose. The enzyme responsible for lactose digestion, although present in the suckling infant, disappears after weaning. In areas of the world where milk is not a staple food it causes no problems, but if people from these regions migrate to Europe or the USA problems arise because ingestion of lactose in some form is difficult to avoid.

It has been known for some time that lactase deficient subjects could tolerate lactose in yoghurt better than the same amount of lactose in milk; this has now been confirmed by hydrogen breath analysis experiments.<sup>37</sup> Although the mechanism remains unknown, it has been suggested that the yoghurt is supplying either preformed lactase or bacteria which produce lactase when they get into the small intestine. It is possible to show increased lactase activity in the small intestine of rats fed yoghurt; that this is of microbial origin is shown by the large increase in activity in the gut contents compared with the gut wall.<sup>38</sup> Early experiments with *L. acidophilus* improved lactose intolerance, but the results of subsequent trials have been

variable.<sup>39</sup> This variation may be due to differences among the strains of *L. acidophilus* used. Intestinal infection can produce lactase deficiency. Yoghurt has been used to restore lactase activity in the intestine of children with *Giardia lamblia* infection.<sup>40</sup>

#### PSEUDOMEMBRANOUS COLITIS

This disease, caused by *C. difficile*, can be cured by administration of faecal enemas from healthy adults.<sup>9-11</sup> It is, therefore, concluded that resistance to this disease is dependent on the presence in the gut of the right flora. There is now evidence that a particular strain of *Lactobacillus* is effective in preventing relapse in pseudomembranous colitis patients who have been treated with antibiotics.<sup>41</sup> Another approach that has shown some success is the administration of non-pathogenic strains of *C. difficile*,<sup>14</sup> which presumably occupy the niche that the pathogen would normally expect to find available. The use of probiotics in treatment of pseudomembranous colitis has potential because the 'at risk' patients can be readily identified and treatment can be started before the onset of the disease.

#### Future developments

While there is no doubt that gut flora can protect the host against intestinal disease, we lack good evidence from controlled clinical trials that the organisms currently being used as probiotics are those which are responsible for the beneficial effects of the gut flora. More trials are needed to establish the efficacy of those probiotics which are currently on the market. These trials should seek to show their effectiveness as prophylactic rather than therapeutic agents. Probiotics as formulated at the moment will not replace antibiotics as therapeutic agents, but they can be seen as a means of repairing deficiencies in the flora induced by dietary and environmental stress.<sup>42</sup> They might render the host more resistant to disease and reduce the frequency of antibiotic use.

What is also desperately needed is more research on the way probiotics work. When this is known we will be able to take a more rational approach to the selection of strains used in probiotic preparations. It will also open the way for genetic manipulation so that an organism's ability to colonise the gut may be brought together with the ability of that same organism to produce the factors responsible for the probiotic effect.

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