2–4 litres and low concentration of bulk ions in the sweat of fully acclimatized subjects.

The relevance of these findings to the post-operative management of surgical patients in hot climates is discussed.

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Lactobacillus acidophilus (Enpac) in Treatment of Hepatic Encephalopathy

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The routine treatment of chronic hepatic encephalopathy is successfully accomplished in many patients by restriction of dietary protein and by the continuous use of oral non-absorbable antibiotics, such as neomycin (Dawson *et al.*, 1957). This type of treatment, though often producing clinical improvement, has the following disadvantages: (*a*) neomycin is still relatively expensive (a week's treatment of 1 g. three times a day costs £6 6s.) ; (*b*) the treatment often becomes less effective as liver-cell function diminishes ; (*c*) there is a small risk of toxic effects (particularly deafness) in patients who have impaired renal function (Last and Sherlock, 1960) ; and (*d*) resistant staphylococcal infections in patients or contacts may also occur (Alder, personal communication, 1965). Various other types of treatment have therefore been suggested and used, including that of "medical colectomy"—the colonization of the colon with non-ammonia-producing bacteria. This type of therapy was reported by Macbeth *et al.* (1965) as being successful in the management of hepatic encephalopathy—the bacteria used for colonization being *Lactobacillus acidophilus*. In the two cases reported the patients were fed 1 or 2 litres daily of inoculated skimmed-milk medium containing 2.9×10^{12} organisms/l. The protein content of this medium was 26–30 g./l. This amount of protein may be toxic to the patient with hepatic encephalopathy (Phillips *et al.*, 1952).

We have used a freeze-dried preparation of *L. acidophilus* (Enpac) in an attempt to assess the value of this treatment in moderate dosage in hepatic encephalopathy. We have compared the effect of Enpac with neomycin and tried to determine whether any benefit was to be gained from using combined (Enpac and neomycin) therapy in this disorder. The lactobacilli in this preparation are resistant to 7 μg. of neomycin per ml.

Methods

Enpac was administered in a dose of 20–40 g. in four doses daily mixed with cold water. Enpac contains 1×10^7 organisms/g. Three patients also received 90 g. in nine daily doses. Treatment was continued for one to four weeks. Patients also received lactose, 60 g. four times daily. Enpac on analysis has a protein content of 31.17% and also contains lactose 39.25% and dextrose 16.5%. The dietary protein intake of all patients observed in hospital was maintained at 40 g./day, and in one patient at 20 g./day. Patients followed outside hospital were on a daily intake of 40 g. protein. Control subjects took a normal diet.

Assessment of the effects of treatment was made by E.E.G. recordings, blood ammonia estimation, and bacterial examination of faeces.

E.E.G. Recordings.—Use was made of the technique described by Laidlaw (1959) and Laidlaw and Read (1963) in which E.E.G. rhythms are fed through an electronic wave-form analyser. Frequency distribution graphs of analysed rhythmic activity allow the calculation of mean frequency, slowing of which occurs in hepatic encephalopathy. Normal mean frequency is 8 or more c./s. In hepatic encephalopathy it slows, and in hepatic precoma it is 4 c./s. or less. Each patient had several E.E.G. records, the mean frequency on each occasion being obtained from analysis of five or more 15-sec. periods. Care was taken to make sure that basal E.E.G.s did not vary significantly before treatment was started.

Blood Ammonia.—Arterial blood was drawn from the brachial artery of the fasting and resting patient, and ammonia estimated by a modification of the method of Seligson (Seligson and Seligson, 1951) (normal arterial ammonia up to 100 μg./100 ml.).

Bacterial Examination of Faeces.—Freshly passed specimens of stool were collected daily and cultured aerobically and anaerobically on blood agar, McConkey agar, and tomato agar. Colony counts of lactobacilli and coliforms were made by dilution of a known weight of faeces in broth and subsequent plate culture. Bacterial urease activity of total faeces and of coliform organisms was detected by culture in urea broth containing phenolphthalein. The following criteria were used for assessing *L. acidophilus* concentration in faeces:

Very scanty: 50,000 colonies per g. faeces
Scanty: 50,000–200,000 colonies per g. faeces
Moderate: 200,000–1,000,000 colonies per g. faeces
Profuse: >1,000,000 colonies per g. faeces.

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Patients Studied

Ten patients suffering from recurrent episodes of acute hepatic encephalopathy or from chronic symptoms were investigated. One patient had chronic cerebellar and basal ganglion involvement (Victor *et al.*, 1965). Eight were in-patients and two out-patients. All but one (Case 3) suffered from hepatic cirrhosis, and all demonstrated various degrees of liver-cell failure (Table I). The patient without cirrhosis had portal hypertension, associated with slowly growing secondary hepatic cancer. Seven patients—five of the in-patients and both out-patients—were on continuous neomycin therapy, which they had had for periods varying from two weeks to three years. This was given daily to all but one patient (Case 8), who had neomycin on alternate days. Care was taken to make sure that all diuretic therapy and sedatives were stopped for the duration of the studies so that changes could be attributed to the treatment under investigation alone. One of the four control subjects had mild cirrhosis without encephalopathy.

TABLE I

	Age and Sex		Diagnosis	Serum Bilirubin (mg./100 ml.)	Serum Albumin (g.%/100 ml.)	Dietary Protein (g./24 hr.)	Neomycin Therapy
			Cases				
1	45	M	Cirrhosis. Portacaval anastomosis	2·4	2·1	20	3 months
2	57	F	„ „ „	5·1	2·5	40	3 months
3	65	F	Portal hypertension. Secondary hepatic cancer	0·8	2·9	40	6 months
4	65	M	Alcoholic cirrhosis	1·2	2·3	40	—
5	65	M	Cirrhosis	1·3	2·2	40	—
6	72	M	„	0·8	2·7	40	—
7	53	M	Cirrhosis. Portacaval anastomosis	1·5	3·3	40	3 years
8	59	F	Cirrhosis	0·85	2·6	40	6 months alt. days
9	38	M	Cirrhosis. Portacaval anastomosis	3·2	1·5	20	6 months
10	59	F	Cirrhosis	1·8	2·5	40	2 weeks
			Controls				
11	53	F	Chronic diarrhoea	—	—	Normal	—
12	56	M	Myocardial ischaemia	—	—	„	—
13	59	M	„ „	—	—	„	—
14	40	M	Cirrhosis	—	—	„	—

Results

The E.E.G. (Table II)

The seven patients on long-term neomycin received therapy with Enpac, both alone and combined with lactose. E.E.G. comparisons were made only while they were on the combined treatment, except in one out-patient (Case 7). Three patients not on continuous neomycin also received the combined treatment for seven days, and it was then compared with treatment with neomycin 1 g. three times daily for seven days without Enpac. Six out of seven patients on long-term neomycin showed on Enpac a mean E.E.G. frequency greater than that on neomycin alone. One patient on neomycin (Case 7) showed only a small increase in E.E.G. mean frequency after seven days' Enpac without lactose. Accompanying this E.E.G. change in four patients there was a clinical improvement, as judged by serial tests of mental function and general awareness. The three patients treated with Enpac and lactose for seven days and subsequently with neomycin showed an increase in E.E.G. mean frequency on neomycin greater than that obtained with Enpac. In two patients on neomycin, withdrawal of this drug was carried out while Enpac and lactose were continued—in both there was E.E.G. deterioration, so that the mean frequency was 4 c./s., which necessitated restarting neomycin. Two patients given initially large doses of Enpac (90 g. daily) without neomycin deteriorated, became stuporous, and showed gross E.E.G. slowing and a rise in the arterial ammonia level.

Arterial Ammonia (Table II)

Arterial ammonia values were followed in the five in-patients on long-term neomycin who were treated with Enpac. Ammonia values were also compared in the three patients given both Enpac and neomycin as two seven-day courses. In three patients in the former group ammonia values fell, and in two it was unchanged or showed an insignificant fall. In the three patients who had Enpac and neomycin in turn the basal ammonia level was unchanged by Enpac, but fell significantly with neomycin. Basal ammonia levels did not differ from ammonia levels on Enpac in the two patients in whom it was measured.

Faeces

Viable lactobacilli were recovered in the faeces of all patients and control subjects in either moderate or profuse concentration. Neomycin had no constant inhibiting effect on growth of lactobacilli, though growth usually became more pronounced when neomycin was stopped. Lactose had no constant effect, and control subjects and those on neomycin usually showed no change in the concentration of lactobacilli in the stools when lactose was added. There was one exception (Case 1) who, though on Enpac, showed no growth of lactobacilli in the faeces. The effect of adding lactose on this occasion was not assessed, but a previous course of Enpac and lactose had produced a profuse faecal growth. One patient (Case 3) showed a considerable drop in the concentration of coliform organisms (as klebsiellae) in the stools: all the others showed no change. Case 3 showed a drop from 4–10 million klebsiellae/g. faeces on neomycin to 0–40,000/g. on neomycin, Enpac, and lactose. The faecal urease remained unchanged by Enpac in all patients but became negative in those not on continuous neomycin who received this drug.

A prompt reduction of coliforms occurred in all three patients treated with neomycin for one week, there having been no change in coliform counts after one week's therapy with Enpac alone. All four control patients showed low coliform counts

TABLE II

Patients on Continuous Neomycin					Patients *not* on Continuous Neomycin				
	E.E.G. Mean Frequency (c./s.) N > 8 (c./s.)		Arterial Ammonia (μg./ml.)			E.E.G. Mean Frequency (c./s.)		Arterial Ammonia (μg./ml.)	
Case No.	On Neomycin	On Neomycin + Enpac	On Neomycin	On Neomycin + Enpac	Case No.	On Enpac	On Neomycin	On Enpac	On Neomycin
1	5·6	6·2	1·52, 1·36	0·93	4	6·8	7·5	1·6 (1·6)*	0·7
2	5·4	6·2	2·25	1·60	5	7·7	8·3	0·9 (0·9)*	0·5
3	5·8	6·6	1·23, 1·33	1·15	6	6·3	6·3	0·9	0·7
7	6·5	6·7	—	—					
8	5·1	6·4	—	—					
9	4·6	5·2	3·7	2·6					
10	7·7	7·7	1·6	1·5					

The E.E.G. changes in Cases 1, 2, 3, 8, and 9 are thought to be significant.
* Basal level on no treatment and 40-g. protein diet.

(<1 million/g. on Enpac), and in Case 12 counts became less than 800/g. after three weeks' treatment. A prompt increase in coliform concentration in the stools occurred in all three patients in whom continuous neomycin was stopped while Enpac was continued. Improvement in the E.E.G. on neomycin was often accompanied by disappearance of *Escherichia coli* (urease-negative) with persistence of klebsiellae (urease-positive).

Discussion

Enpac administered orally produces viable lactobacilli in the faeces and therefore it is a convenient way of attempting to colonize the colon. The number of organisms given cannot, however, exceed 4×10^8/24 hours unless treatment is given two-hourly, when it ceases to be a convenient means of administering long-term therapy. The substance is pleasant to take, and therefore has considerable advantages over the procedure described by Macbeth *et al.* (1965). Further, it contains less protein: if large amounts of Enpac are given there is no doubt that hepatic coma can be precipitated—90 g. of Enpac produced stupor with gross E.E.G. slowing. This amount of Enpac represents the addition of about 30 g. of protein/day. The organisms are also resistant to 7 μg. of neomycin/ml. This concentration is usually lethal to coliforms, so that survival of lactobacilli would tend to occur even in patients on neomycin therapy. Enpac could thus be partially effective in the patient on long-term neomycin.

The detection of clinical improvement in patients with chronic hepatic encephalopathy is far too crude a method of assessing the effectiveness of treatment. The same may be said of arterial ammonia values (Phear *et al.*, 1955). The most sensitive way of detecting improvement is with the E.E.G., and electronic analysis adds considerably to its sensitivity. All our patients had encephalopathy and all showed slow basal E.E.G. frequencies, and seven needed neomycin continuously. They were in this respect advanced cases in which a continuous and steady deterioration might be expected. An E.E.G. improvement was still, however, obtained in five patients despite the neomycin, thus demonstrating a synergistic effect of *L. acidophilus* therapy. The improvement was not only an E.E.G. one but was accompanied by a fall in blood ammonia in three patients and some clinical improvement in five. It is of interest that the one patient who showed no further improvement of the E.E.G. with combined Enpac and neomycin treatment had been on neomycin for only two weeks. Perhaps improvement after this short period was maximal.

Treatment with Enpac alone was not, however, as effective as neomycin in patients not on continuous antibiotic treatment. Both the E.E.G. improvement and the arterial ammonia showed a greater response to neomycin than to Enpac. This may have been due entirely to the fact that "colonization" as opposed to the presence of lactobacilli in the faeces takes several weeks, whereas neomycin is more rapidly effective. If this is so it would also mean that Enpac has little place in the treatment of acute bouts of encephalopathy unless these were resistant to conventional treatment with neomycin.

The precise way in which lactobacilli are able to improve hepatic encephalopathy is uncertain. That they can do so seems certain from the cases described by Macbeth *et al.* (1965) and those mentioned by Fenton *et al.* (1965). It would be reasonable to assume that lactobacilli interfere with the growth of colonic coliforms, which are subsequently reduced in number, with a resultant fall in the production of ammonia and other protein breakdown products in the gut. One of the patients of Macbeth *et al.* showed a fall in the faecal aerobic count as lactobacilli increased, while the other showed a decrease in coliforms which could have been due to antibiotics administered as well as to *L. acidophilus* therapy. Improvement in our patients was not necessarily correlated with alteration of faecal coliform count. Though all our control subjects showed low coliform counts on Enpac, only one of the seven patients followed up on neomycin showed this change, though four improved clinically. It may be that proliferation of lactobacilli in the gut produces a diminution of metabolic activity of coliform organisms, perhaps by changing bowel *p*H rather than by a reduction of coliform bacterial population alone. An additional diminution of coliform population could result if the administration of *L. acidophilus* was continued for some weeks. In this respect it may be noted that the improvement in hepatic encephalopathy seen after oral neomycin may persist despite the reappearance of coliform bacilli in the stools (Dawson *et al.*, 1957).

Whatever the modes of action, "medical colectomy" from the use of Enpac seems to be an effective way of improving chronic hepatic encephalopathy and is an extra therapeutic weapon to supplement neomycin and protein restriction, particularly when encephalopathy progresses with the onset of diminishing liver-cell failure. It is perhaps particularly suitable for the patient with chronic encephalopathy after portacaval anastomosis. Neomycin in the usual therapeutic dosage does not seem to interfere with its action, though an organism of higher neomycin resistance might be of increased value. Lactose administration seems also not to be essential unless bacilli do not appear in the faeces in a sufficient concentration. Enpac contains some lactose, which might have a beneficial effect on bacterial growth. Its cost per 60-g. tin—that is, one-and-a-half-days' treatment—is about 10 shillings.

Summary

In five out of seven patients with chronic hepatic encephalopathy on long-term neomycin, Enpac (a freeze-dried preparation of *Lactobacillus acidophilus*) produced an improvement in the E.E.G. and clinical status. A fall in blood ammonia occurred in three out of five patients tested. A short course of Enpac for seven days was less effective than neomycin in producing E.E.G., clinical, and ammonia changes. Enpac is a convenient way of attempting "medical colectomy" in hepatic encephalopathy and a worth-while therapeutic addition to treatment of this condition.

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