Analysis results and discussion. Elemental analysis of the microspheres yielded the following results:

	C%	N%	H%	atomic ratio C/N
20 kHz irradiation	12.22	7.95	2.49	1.80
800 kHz irradiation	9.75	7.23	3.65	1.57
ash about 70%				

After prolonged hydrolysis in 6 N HCl and evaporation of excess acid, the microspheres were subsequently subjected to TLC. The hydrolysates were first tested for the presence of amino acids, using cellulose plates, and n-butanol/acetone/diethylamine/water (10:10:2:5) as solvent[12]. The chromatograms were sprayed with ninhydrin, 2-4-6 collidine, acetic acid mixture[13] and the spots of glycine, α and β alanine, glutamic acid and a prominent unidentified violet spot were observed, but the amount of amino acids was moderate.

We searched unsuccessfully for nucleic acids, sugars and lipids. In aqueous suspension, the microspheres turned the water blue, but their shapes were unchanged, except that some particles were partially discolored. The resulting solution contained soluble compounds which yielded chromatograms similar to those of the microspheres.

An IR-spectrum showed a strong absorption at $2850-2920 \ cm^{-1}$ which indicated the presence of compounds rich in $-CH_2-and-CH_3$, but the characteristic bands of the peptide bond were not found. Mass spectrometry, by the desorption, chemical ionization technique, with the NH_4^+ ion, gave the following results:

m/e 44, 46, 59, 60, 73, 74, 77, 83, 87, 91, 98, 100, 103, 108, 114, 120, 128. Some of these values can be attributed to the protonated ions of glycine, alanine and methyl alanine or their fragmentation products, but heavier ions were not observed.

The analytical results suggest that the microspheres consist of a soluble derivative of amino acids mixed with an insoluble polymer, both containing molybdenum and other mineral elements.

To compare the synthesis described above with Bahadur's photochemical method, we exposed similar solutions to room light and UV-radiation, respectively. With room light, the UV is absorbed by the glass walls of the sealed flask containing the solution, which becomes slowly greenish. Microspheres appear after several weeks. When a solution, protected from evaporation by a quartz cover, is irradiated by a mercury lamp ($\lambda = 253,7$ nm), it turns blue and a rapid growth of microspheres is observed.

Analysis of these colored particles gives the same results as those previously obtained with US, whereas Bahadur[5] found more amino acids than we do, as well as a large number of other organic compounds. In these syntheses, we tried unsuccessfully to modify the composition of the solution; only the mixture noted above yields stable microstructures.

The formation of particles, under UV-radiation, has been observed with optical and electronic microscopes (figs 1 and 2). First the surface of the solution becomes coated with a hydrophobic film, made up of blue patches; subsequently the film wrinkles, breaks up and is finally transformed into microspheres.

In this paper, we have shown that sonolysis, like other sources of energy, can produce microparticles. Such structures could have been formed during the chemical evolution of early prebiotic times.

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The effect of a gastric mucus barrier on the dynamic response of a pH electrode

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Summary. Hydrogen ion mobility in gastric mucus has been found to be reduced to a greater extent than that hitherto suspected, though at low pH (< 4) and in buffer this mobility increases. Mucus, at an optimized pH, may therefore protect the gastric mucosa from acid digestion by providing a diffusion barrier.

The thin layer of mucus which lines the gastric mucosa has long been thought to be a defence against the damaging effects of acid gastric juice. The rate of H^+ diffusion, previously observed to be as great as that in water[2], has recently been shown to be reduced in mucus[3,4]. The diffusional resistance has been attributed in turn to the gel-like nature of mucus, with its ability to immobilize water molecules, and to a possible steric hinderance from the mucus glycoprotein itself. Undoubtedly gastric mucus can maintain steep pH gradients in acid if left in contact with the gastric epithelium[5-7] but the capability has been considered mainly to be due to the neutralizing action of bicarbonate secreted into mucus by the surface epithelial cells[8] rather than to the effect on diffusion. We have examined H^+, Na^+ and K^+ mobility in isolated pig gastric mucus using glass ion-selective electrodes. Our study shows that
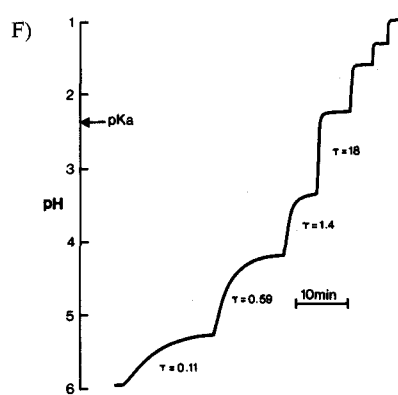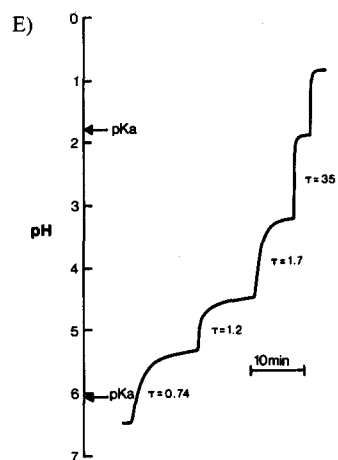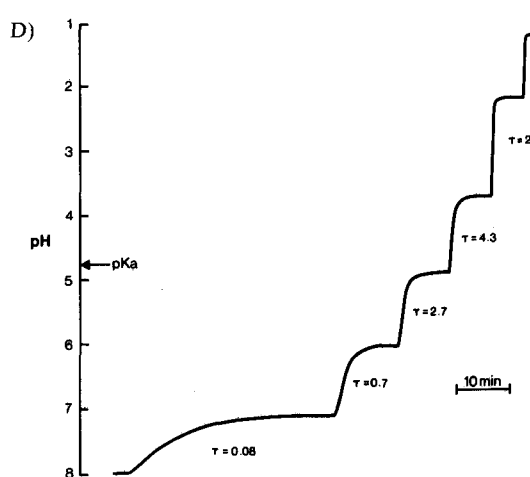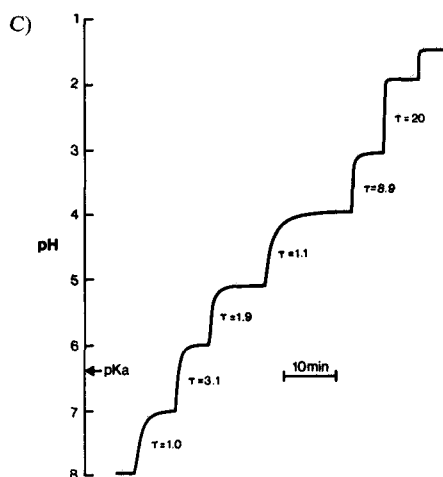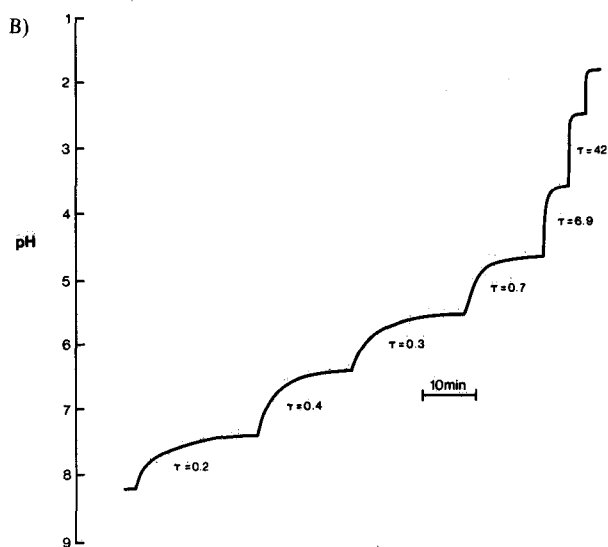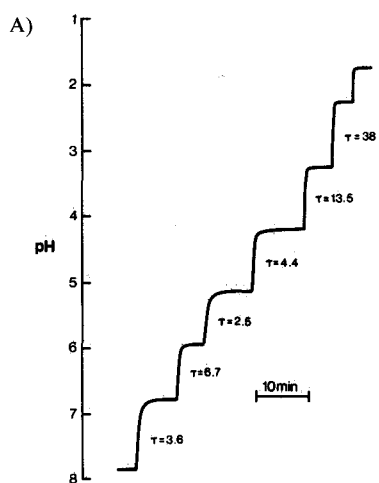
A Response of an acrylamide gel-coated pH electrode to HCL added in 1 mM Na HCO₃. Response of gastric mucus-coated pH electrodes to HCl additions made in *B* 1 mM Na HCO₃, *C* 10 mM Na HCO₃, *D* 10 mM CH₃COONa, *E* 1 mM histidine and *F* 1 mM glycine. τ (min⁻¹) is the time constant for the electrode response (see text).

diffusional effects may be important, and we present a modified theory of gastric mucosal protection based upon our findings.

Ion-selective electrodes respond more sluggishly to changes in analyte concentration when covered with acrylamide gel[9], enzyme[10] or gas-permeable[11] membranes, due mainly to the reduce rate of solute transfer within such membranes. We have measured the response times of electrodes covered with thin mucus layers in order to determine the diffusion limitations imposed by mucus in this type of system.

Materials and methods. Mucus, obtained from pig gastric mucosa[3], was used to produce covering layers over the sensor surface of a Beckman Type S41263 pH electrode. Coatings of a standard thickness were obtained by the use of 70 μm thick nylon netting as spacer and an overlying dialysis membrane[12]. Acrylamide gel[13] was also used to cover the electrode in a similar way. Electrode responses to stepwise reductions in pH were measured in several weakly-buffered aqueous solutions containing 155 mmoles/l NaCl as background electrolyte. Responses were determined at room temperature $(21 \pm 2\,°C)$ in vigorously stirred 40 ml solutions after rapid manual addition of HCl. These additions were controlled by monitoring pH changes at an uncoated pH electrode. For bicarbonate solutions, the reaction vessel was closed from the atmosphere to leave a 2 ml dead space. The rest of the system comprised a Radiometer PHM 72 meter and a Servoscribe chart recorder, and gave response times of < 30 sec with an uncoated Beckman electrode.

Results and discussion. The responses of the coated pH electrode were hyperbolic (fig.), and largely an agreement with the equation used by Müller[14] to characterize dynamic ion-selective electrode response:

$$\Delta E = t/(a + bt) \qquad (1)$$

where ΔE is the change in emf, t is time after an increment in ion concentration and a and b are constants. With ΔpH substituted for ΔE, plots of $t/\Delta pH$ against t allowed estimation of a by extrapolation to the $t/\Delta pH$ axis; some curvature, observed in most cases, indicated deviation from equation 1 at the early part of the response. Constant a is related to the speed of response, and 1/a was used as a response time constant, τ, to facilitate comparison of the different electrode responses.

The response times of the acrylamide gel-coated electrode (fig.A) are longer than those of the underlying sensor. By substituting a mucus layer, there is a considerable further increase in response times (fig.B). These were reproducible with the same as well as different electrodes in 1 mmole/l NaHCO₃ and denote a barrier property of mucus which cannot be due simply to its providing an unstirred layer. The diffusional resistance provided by mucus is, however, diminished at low pH; mucus-pH electrode responses become much faster and the difference between mucus and acrylamide gel then disappears. This could explain why previous studies, using strongly acidic conditions, demonstrated no difference in diffusion resistance between mucus and water in an unstirred system[2], or when water was absorbed into filter paper pads[3].

The mucus-coated electrode gives faster responses at 10 times higher bicarbonate concentration (fig. C), with the responses in the pH region of bicarbonate pK_a showing the greatest increase. The response profile is changed in acetate solution, with responses now faster in the vicinity of acetate pK_a (fig. D). Both these observations can be explained by the development of a buffer shuttle, postulated by Engasser and Horvath[15], in which protons are transported along a pH

gradient after reacting with the conjugate base (B⁻) of a buffer[16]:
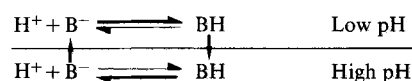
The rate of H⁺ transfer is governed on this model by the concentration gradients of B⁻ and BH, and increases with increasing buffer concentration. For a fixed buffer concentration it will be greatest when the pH gradient occurs close to the pK_a of the buffer. This arises since changes in B⁻ and BH concentrations are maximal when pH is varied near pK_a. Thus, at high pH, shorter electrode response times are obtained with histidine (fig.E) than with glycine buffer (fig.F) because of the additional pK_a of histidine at 6.04.

The 95% response times of a Na⁺- and a K⁺-selective electrode were determined in 2 mmoles/l Tris buffer at pH 6.0; the cation concentration was changed from 10^{-5} to 10^{-4} mmoles/l. The mucus-coated Na⁺ electrode had a 6-min 95% response time $(\tau = 2.7\ \text{min}^{-1})$ compared with 4.5 min when acrylamide gel was employed $(\tau = 7.0\ \text{min}^{-1})$, and the K⁺ electrode had a 27-min response with mucus $(\tau = 0.3\ \text{min}^{-1})$ compared with 26 min $(\tau = 0.3\ \text{min}^{-1})$ when acrylamide gel was used. This particular, much used, underlying K⁺ electrode had a somewhat sluggish (15.5 min) response when uncoated. In the same buffer, a mucus-coated pH electrode had a 7-min response $(\tau = 1.2\ \text{min}^{-1})$ for a pH change from 4.93 to 4.06 compared with a 1-min response for an acrylamide gel-coated electrode $(\tau = 30\ \text{min}^{-1})$ for a pH change from 4.95 to 4.06. The large difference in response times observed when the pH electrode was coated respectively with mucus and acrylamide gel contrasts with the situation for the Na⁺ and K⁺ electrodes and demonstrates the selective nature of the mucus barrier. Furthermore, it suggests that the reduction in H⁺ mobility is unlikely to be due to charge, steric effects, or the immobilization of water molecules, as Na⁺ and K⁺ are larger cations than H⁺, and a primary effect on water would be expected to affect the 3 ions to a similar extent.

In our electrode system, mucus only presents a significant barrier to proton diffusion at pH > 4; however, in the stomach it needs to function at pH < 4. The apparent anomaly is resolved by invoking the current hypothesis of a combined mucus-bicarbonate barrier. However, its basic premise that acid is neutralized in the mucus layer, and that HCO_3^- needs to be secreted at a molarity approximately equal to that of H⁺, which enters mucus[8], requires modification; as luminal pH falls, the increased bicarbonate concentrations envisaged, with steeper pH gradients in the mucus layer, would generate accelerated buffer shuttles. In any case, the concentration of bicarbonate in the non-parietal component of gastric juice is probably quite low, one estimate in dogs being 8 mmoles/l bicarbonate[17], and that for man approximately 25 mmoles/l[18]; these are much lower concentrations than the ~ 150 mmoles/l H⁺ considered to be produced by oxyntic cells. Furthermore Na⁺ and Cl⁻ are present in non-parietal secretions at well over 100 mmoles/l[19]. We propose therefore that non-parietal secretions into mucus are designed, in part, to dilute the acid which diffuses in; this would reduce the amount of bicarbonate which had to be secreted to raise pH, and so would reduce CO_2/HCO_3^- gradients and the speed of operation of the buffer shuttle within the mucus layer. Also if, instead of a complete neutralization, mucus pH was elevated only to ~ 5, then a pH of high mucus resistance would be attained (fig. C) with the deployment of very little bicarbonate, and the mainly unreacted bicarbonate at deeper levels in mucus

could maintain a near-neutral pH at the epithelial surface. Bicarbonate thus still has a vital role to play in adjusting mucus pH, but the combined defence provided by mucus and bicarbonate is likely to work by restricting proton diffusion and minimising the buffer shuttle, rather than by generating an unstirred layer within which a complete neutralization takes place.

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The effect of weak electrical potential gradients on the transport of water in broad bean plants under stress

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Summary. Adequate moisture was transported by an electrical potential gradient of 1 V/cm of the correct polarity to maintain the turgid state of broad-bean plants subjected to severe stress conditions.

The effect on growth of plants of small direct currents, driven by low voltage gradients has been reviewed by several authors[2-4]. Studies on the transportation of water in plants by low voltage gradients in relation to the movement and metabolism of plant hormones have also been reported[5-7]. In a communication[8] which dealt with transmission of viruses to plants by small currents driven by low voltage gradients, several preliminary control experiments were conducted to confirm the observations of the previous authors[5-7].

In these experiments[8] use was made of 9 V dry cell batteries, either singly, or, as 2 batteries connected in series. The poles of the batteries were connected to the plants via short pieces of platinum (Pt) wire inserted in their stems and the opposite poles via Pt wires to the soil in the vicinity of the roots. A voltage gradient of 1 V/cm was used. Young bean plants (Phaseolus vulgaris cv. van Zyls) could be dehydrated, or wilted, in 20 h by connecting their stems at positions 9 cm from the soil to the positive ('positive plants') and the soil to the negative pole ('negative soil') of a 9 V battery. The wilting or, dehydration of the plants, judging from their appearance compared to control plants, kept under the same conditions of temperature (22 °C) and relative humidity (approximately 50%), occurred despite adequate water in the soil. Using bean plants in which the polarities were reversed ('negative plants') no dehydration occurred as witnessed by their turgidity after 20 h.

The dehydration of the plants, 'positive plant' 'negative soil' could be counteracted by slicing off the tips of bean plants, immersing the damaged leaves into weak buffer, 10 mM phosphate, pH 7.5, and passing a current of approximately 1 V/cm for 20 h through the plants having the plant positive and the soil 'negative'. Having confirmed the effect of weak direct currents on plants the effect of weak potential gradients on plants subjected to severe stress conditions was investigated.

Seeds from broad beans (Vicia faba cv. aqualdulce) were germinated and grown in autoclaved sandy loam soil in plastic pots of 150 cm diameter. Four pots, each with 2 plants were used in the experiment. When the seedlings emerged from the soil the pots were transferred to a glass house. The containers with the plants were watered daily to saturation. The glass house was fully exposed to the sun and the internal temperature fluctuated between 25 °C at 06.00 h to 40 °C at 14.00. When the plants reached a height of approximately 10 cm they were subjected to the 'voltage gradient' experiment. One of 2 plants in 1 container was made 'negative' and its soil 'positive' (gradient 1 V/cm). The 2nd plant was the control. In a 2nd pot, 1 plant was made 'positive' and its soil 'negative'. The 2nd plant served as control. The 4 plants in the remaining 2 containers were additional controls.

The plants were well watered at the start of the experiment, but, it must be emphasized that the pots received no water during the next 4 days. The plants were examined daily and their conditions noted.

After 1 day the 'positive plant' (soil negative) was severely wilted, while the control in the same pot and the additional controls were slightly dehydrated. In contrast, the 'negative' plant (soil 'positive') was turgid. On the 4th day the 'positive' plant (soil negative) was dark brown and dry. At this stage the 6 control plants were wilted to such a degree that when watering was resumed on the same day they did not recover, despite daily watering to saturation. They were dry and brown on the 6th day.

From this experiment it was concluded that residual moisture in the soil of the 4 pots on the 4th day could not be mobilized for maintenance of the 6 control plants; the