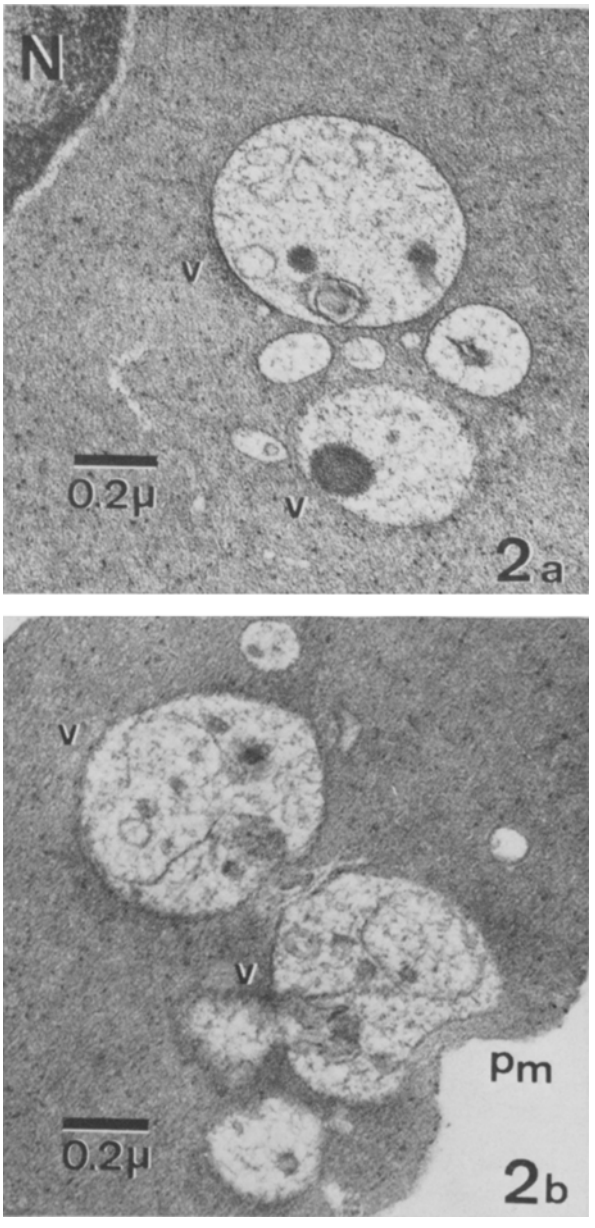

Fig. 2. Thin sections of mature erythrocytes. a) N, nucleus; V, free vesicles containing dense material. b) V, vesicles approaching the invaginated plasmic membrane (pm).

erythrocytes revealed the presence of labelled vesicles[16]. Often vesicles fuse among themselves, approaching the plasmic membrane which shows an invagination (Figure 2b); this suggests that vesicles or their content may be expelled from erythrocytes. Besides this, small Golgi complexes, and little of the smooth endoplasmic reticulum were also found. This peculiar mechanism of chromatin extrusion through vesicles of mitochondrial origin occurs only in the mature erythrocytic forms, i.e. those cells in which globin synthesis has ceased. These erythrocytes may still contain simple ribosomes, but no polysomes, which disintegrate after the globin molecule chain synthesis.

The functional relationship between the vesicles of mitochondrial origin, carrying nuclear material, and structures such as the Golgi complex, is still unknown. Further studies with labelled material will probably provide more information on this question, as well as on the significance of this phenomenon in the final erythrocytic maturation. The possibility of a correlation between this event and the nuclear extrusion, occurring in the orthochromatic erythroblasts of all mammals, may be considered.

Zusammenfassung. Chromatinhaltige feulgenpositive Bläschen wurden im Cytoplasma der Erythrocyten von *Cyprinus carpio* gefunden. Sie haben ihren Ursprung in den unmittelbar an der Kernmembran anliegenden Mitochondrien, die während der Chromatinaufnahme allmählich ihre Struktur verlieren.

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[13] A. GRASSO, H. SWIFT and G. A. ACKERMAN, J. Cell Biol. *14*, 235 (1962).
[14] E. SKUTELSKY and D. DANON, J. Cell Biol. *33*, 625 (1967).
[15] C. F. SIMPSON and J. M. KLING, J. Cell Biol. *35*, 237 (1967).
[16] H. MENEZES, C. Y. MITSUTANI and A. BRUNNER JR., unpublished results.
[17] The authors wish to thank Mrs. VERA MONDIN WEISZ and Mr. C. A. GONÇALVES SILVA for their technical assistance and Mrs. SIBYLLE HELLER for her editorial aid and translation. This research has been supported by the Conselho Nacional de Pesquisas (Proc. No. 10112/71) and Fundo Especial de Despesas do Instituto Butantan.

Physical Evidence of a Plasmid in *Rhizobium japonicum*

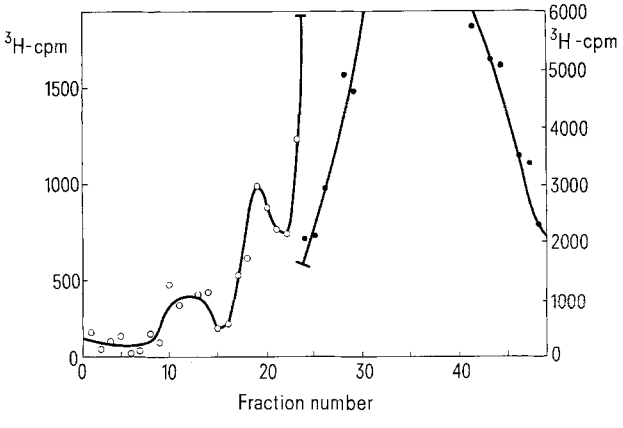
Many genetic observations, but little physical data, suggest the existence of plasmids in the genus *Rhizobium*. The transfer of clover infectivity from *Rhizobium trifoli* to *Rhizobium phaseoli* and a loss of infectivity upon treatment of the recipient culture with acridine orange has been observed[1]. Recombination has been demonstrated in conjugation experiments using strains of *Rhizobium lupini*[2] and *Rhizobium leguminosarum*[3], but could not be found in strains treated with acridine orange. Treatment of the antibiotic resistant strain 10324 of *Rhizobium japonicum* with acridine orange has been reported to increase its antibiotic sensitivity[4]. This presumptive R-factor was transmissible to *Agrobacterium tumefaciens*. The similarities between this observation and R-factor mediated antibiotic resistance in enteric bacteria, and the suggestion of plasmid control of the symbiotic nitrogen-fixing property[5] led us to examine *Rhizobium japonicum*, ATCC strain 10324, for the presence of plasmid DNA.

[1] S. HIGASHI, J. gen. appl. Microbiol. *13*, 391 (1967).
[2] W. HEUMAN, A. PUHLER and E. WAGNER, Molec. gen. Genet. *113*, 308 (1971).
[3] P. BOSE and G. S. VENKATARAMAN, Experientia *25*, 772 (1969).
[4] M. A. COLE and G. H. ELKAN, Antimicrob. Agents Chemother. *4*, 248 (1973).
[5] L. K. DUNICAN and F. C. CANNON, Plant and Soil, Special Volume *1971*, 73.

Bacteria growing in nutrient broth (*8* g/l) supplemented with 0.2% mannitol were used to inoculate the labeling medium, either nutrient broth with mannitol or minimal media consisting of 3.6 g K_2HPO_4, 0.4 g KH_2PO_4, 0.05 g $MgSO_4$-$7H_2O$, 0.5 g NaCl, 1.0 g $(NH_4)_2SO_4$ and 4.0 mg ferric citrate in 1 l of double-distilled water.

The pH of the minimal media was adjusted to 7.0 with NaOH and 10 g/l of mannitol was added after sterilization. The radioactive labels utilized were $^{32}PO_4$ (0.1 mCi/ml) or ^{3}H-adenine (0.001 mCi/ml). ^{3}H-thymidine was used but good incorporation was not obtained with this strain.

The bacteria were allowed to grow to late log or stationary phase before harvesting by centrifugation. The pellet was washed with TKE buffer (0.05 *M Tris*-HCl, pH 8.0, 0.6 *M* KCl, 0.05 *M* EDTA) and resuspended in a small volume of the same buffer. The suspension was quick frozen on acetone dry-ice and thawed in water at room temperature 2 times. Lysozyme (100 µg) was added to the suspension and the freeze-thaw repeated twice. The lysate was brought to 1% Sarkosyl and the freeze-thaw repeated again. The lysate was then layered on a gradient (Sarkosyl lysate) or cleared by centrifugation at 27,000 × *g* for 30 min (cleared lysate). Caesium chloride, ethidium bromide (200 µg/ml final concentration) and 0.01 *M Tris*-HCl buffer, pH 8.0 were added to the cleared lysate to a final volume of 4 ml and a refractive index of 1.393. A gradient was then formed by centrifugation in a SW 50 rotor at 29,000 rpm for 48 h at 20 °C.



Equilibrium density gradient centrifugation of a cleared-lysate of *R. japonicum* 10324 DNA. Fractions were collected from the bottom of the tube. 50 µl samples were removed from each fraction for refractive index determination and KOH hydrolysis. The KOH-hydrolyzed TCA-precipitable material was collected on GF/A filters, washed with ethanol and ether, and its radioactivity determined.

The Figure shows the satellite peak (fractions 16–22) found at the expected location for covalently-closed circular plasmid DNA. The chromosomal DNA banded at the location expected for linear DNA (fractions 30–40). The peak observed in fractions 11–14 was present occasionally and has not yet been identified. Similar profiles were obtained from either late-log or stationary cultures and with either the $^{32}PO_4$ or ^{3}H-adenine label. Gradients formed with cleared lysates as in the Figure were less ambiguous than gradients formed with Sarkosyl lysates. Treatment of the lysate with 50 µg/ml DNAase I (Sigma) in 10 m*M* $MgCl_2$ for a short time resulted in a decrease in the plasmid DNA peak and a porportional increase in the main genome peak. CsCl gradients in the absence of ethidium bromide showed a single peak of DNA at a density of 1.72.

The plasmid peak was observed in numerous independent experiments carried out in a variety of conditions. Quantitative determinations of molecular weight and percent plasmid DNA (of total DNA) were hampered by the low level of plasmid observed and the resulting difficulty in reproducibility. The upper and lower limits of plasmid DNA/chromosomal DNA are estimated to be 0.5% and 0.2% respectively.

Treatment of cultures under a variety of conditions with acridine orange or ethidium bromide had no effect on their antibiotic resistance. Treatment of cultures growing in minimal medium with 60 µg/ml atabrine for 3 to 4 days resulted in an increased frequency of susceptibility to the antibiotics chloramphenicol (less than 1% increased to 3%) and streptomycin (1% increased to 10%), as would be expected if atabrine acts to select cells which have lost an R-factor[6]. However, no effect of atabrine treatment on the presence or absence of plasmid DNA or the ability to produce nodules on soy beans was observed.

Résumé. L'ADN de *Rhizobium japonicum* 10324 montre une bande plasmide lors d'une centrifugation à l'équilibre sur CsCl/bromure d'éthidium. Nous ne pouvons pas corréler la présence de cette bande d'une part, la résistance aux antibiotiques ou la production de nodules de l'autre part.

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[6] M. Yoshikama and M. G. Sevag, J. Bact. *93*, 245 (1967).

[7] We are grateful to Dr. D. A. Phillips, Indiana State University, for assaying some of our bacterial isolates for the ability to produce nodules.

Intense Sweetener from Lo Han Kuo (*Momordica grosvenori*)

The search for non-sugar sweeteners from natural sources has led to the discovery of many interesting substances which possess either intense sweet taste[1] or taste-modifying properties[2]. This communication reports another source of a natural sweetening material.

Lo Han Kuo (Lo Han fruit), from *Momordica grosvenori* Swingle, is a dried fruit produced in Southern China. The fruits are gourd-like, 6–11 cm long by 3–4 cm broad, dark brown, broadly ellipsoid, ovoid or subglobose, with broadly rounded ends. The rind is very thin (0.5–0.8 mm

[1] For example: Glycyrrhizin, R. E. Muller, Forest Prod. J. *16*, 41 (1966). Monellin, J. A. Morris, R. Martenson, G. Deibler and R. H. Cagan, J. biol. Chem. *248*, 534 (1973). Osladin, J. Jizba, L. Dolejs, V. Herout and F. Sorm, Tetrahedron Lett. *1971*, 1329. Phyllodulcin, M. Yamato, K. Sato, K. Hashigaki, M. Oki and T. Koyama, Chem. Pharm. Bull., Japan *22*, 475 (1974). Stevioside, F. Bell, Chemy Ind. *1954*, 897. Thaumatin, H. van der Wel, FEBS Lett. *21*, 88 (1972).

[2] For example: Miraculin, K. Kurihara and L. M. Beidler, Nature, Lond. *222*, 1176 (1969). Gymnemic acid, G. P. Dateo and L. Long, J. agric. Food Chem. *21*, 899 (1973).