from the apple was an artefact produced from the sugars present. A little may perhaps have been present in the original extract. Bryant and Overell[1], using the strongly basic resin 'Amberlite IRA 400', obtained traces of an 'unknown' acid from the juice of apples and carrots which had an R_F in butanol – acetic acid – water similar to that of our lactic acid spots. They, however, used the resin in the less-alkaline carbonate form. In our experiments, in which a solution of sugars and acids similar to the one mentioned earlier was passed down 'Amberlite IRA 400 (OH)' in the carbonate form instead of 'Dowex 2', no sign of lactic acid was observed. It appears, therefore, that acids may be adsorbed with safety from plant extracts rich in sugars by strongly basic resins in the carbonate form. In this form, however, the resins have a low capacity, and the concentration of the acid in the solutions passing down them must be very low to prevent evolution of carbon dioxide, with subsequent break-up of the column.

For the separation and isolation of small amounts of acids in the presence of large amounts of other acids, it appears preferable to separate the acids *in toto* from the sugars by a preliminary adsorption on weakly basic ion-exchangers followed by displacement; followed by fractionation on strongly basic anion-exchangers in the 'hydroxyl' form in which they have their highest capacity and resolving power (because of 'tighter' bands) for acids.

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[1] Bryant, F., and Overell, B. T., *Nature*, **167**, 361 (1951).
[2] Hulme, A. C., *J. Exp. Bot.*, **2**, 298 (1951).
[3] Wadham, W. H., *J. Chem. Soc.*, 3051 (1952).

Percentage of Binucleate Cells in the Livers of Adult Rats

Several workers have suggested that the percentage of binucleate cells seen in histological sections is not a true measure of the percentage of such cells in the liver. The cells of the liver are so large, varying between 12 and 25 μ in diameter, that a considerable number of the cells in a section 6–8 μ thick must have been cut through, and in some cases two nuclei may have been separated. Pfuhl[1] corrected for this error in counts made on the livers of rabbits by assuming that the percentages of binucleate cells in sections 5 and 7·5 μ thick were one-third and one-half the absolute value, respectively. Later, Müller[2] elaborated a formula for converting the observed counts to the real percentages. It would be preferable to count the binucleate cells in a suspension of liver cells; but the difficulty has been to prepare a suitable suspension. Recently, Bucher and Glinos[3] reported finding three times as many binucleate cells in a cell suspension as in a section 6–8 μ thick. This suspension had been prepared by agitation of tissue slices in saline (private communication by Dr. N. Bucher).

A simpler method for preparing a cell suspension has now been developed and was used for counting the binucleate cells in the livers of adult male and female hooded rats of the Lister strain. A sample of fresh liver was homogenized for one minute at a low speed in the machine described by Folley and Watson[4] with ten times its volume of the medium described by Robinson[5]. A drop of the suspension so obtained was then mounted and stained with toluidine blue, and the number of binucleate cells in at least 1,000 cells counted. Duplicate counts varied by less than 1 per cent, that is, by less than 3 per cent of their actual value. The results have been listed in the accompanying table together with values for the percentage of binucleate cells in histological sections of livers from rats of similar weight, age and strain. These sections were kindly prepared by Dr. A. M. Barrett, Department of Pathology, Cambridge.

Percentage of Binucleate Cells in the Livers of Adult Male and Female Rats

Sex	Counts made on			
	(*a*) Cell suspension		(*b*) Histological section	
	Weight of rat (gm.)	Binucleate cells (per cent)	Weight of rat (gm.)	Binucleate cells (per cent)
Male	270	32	267	10
	266	30	273	11
	268	29		
	290	29		
Female	202	29	200	11
	220	29	216	12
	235	29		
	200	31		

Some cells were inevitably fractured in the preparation of the suspensions, but these formed only a small proportion of the liver cells, for drops of the suspensions contained mostly intact cells and remarkably little debris. The cells remained intact and separate for at least seven days when the suspensions were stored at 0–2° C. It seems reasonable, therefore, to assume that the mononucleate and binucleate cells had been broken in the same proportion in which they occurred in the liver.

Between 10 and 12 per cent of the cells in histological sections of the livers of both sexes appeared binucleate, in agreement with values reported by earlier workers. It was obvious that many of the cells in the sections had been cut, for all the lobules contained many 'cells' without any nuclei at all. Even though these non-nucleated 'cells' were not included in the counts, the proportion of binucleate cells in the sections was only one-third of that in the cell suspensions. There was no difference between the percentages of binucleate cells in the livers of adult male and female rats: and despite variations in the weights of the rats these values were almost constant at 30 per cent. This figure was required as a step in calculating the chemical composition of the average liver cell[6]; but its agreement with that found by Bucher and Glinos[3] for albino rats is worth recording.

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[1] Pfuhl, W., "Die Leber. Handbuch der mikroskopischen Anatomie des Menschen", Ed. by W. V. Mollendorf, **2**, 235 (Berlin: Springer 5, 1932).
[2] Müller, H. G., *Z. mik.-anat. Forsch.*, **41**, G 296 (1937).
[3] Bucher, N. L. R., and Glinos, A. D., *Cancer Res.*, **10**, 324 (1950).
[4] Folley, S. J., and Watson, S. C., *Biochem. J.*, **42**, 204 (1948).
[5] Robinson, J. R., *Biochem. J.*, **45**, 68 (1949).
Harrison, M. F., *Proc. Roy. Soc.*, B (in the press).