Liver Storage

HAROLD SPILG, F.C.S. (S.A.), *Registrar* AND JOHN TERBLANCHE, CH.M., F.R.C.S., F.C.S. (S.A.), *Senior Lecturer, Department of Surgery, University of Cape Town*

SUMMARY

A brief history of the development of methods of organ storage with special emphasis on liver storage is presented. The current status of experimental liver storage is reviewed, emphasizing that hypothermia is at present the principal protective factor in all successful methods of liver storage.

S. Afr. Med. J., **45**, 1160 (1971).

The need for whole-organ preservation has become increasingly important, particularly with extension of human transplantation to single organs. The extreme sensitivity of the liver to anoxia makes it one of the more difficult organs to store.

Any method of storage is designed to either reduce the metabolic demands by the organ, or increase its metabolic supply; and most investigators who are currently storing

organs *in vitro,* are using one or more of the following modalities: hypothermia, hyperbaria, metabolic inhibitors and continuous or intermittent perfusion.

HISTORY

Hypothermia

John Hunter was probably the first to use hypothermia for purposes of tissue storage. In 1766[1] he froze 2 carp in ordinary river water in an attempt to keep fish 'alive' in a frozen state. Hunter thought that such an experiment could provide a stepping-stone to the prolongation of life. Fuhrman and Field[2] demonstrated that the physiological basis for its use was an exponential fall in oxygen uptake by the tissue associated with a fall in the core temperature. Subzero temperatures should theoretically provide the optimum reduction in metabolism, but the biological effects incurred during freezing[3] preclude successful whole organ preservation at temperatures below 0°C.

Hyperbaria

The mode of action of hyperbaric oxygen in extending *in vitro* preservation beyond that achieved with hypothermia alone has not been defined. Bloch *et al.*[4] showed that oxygen, under given conditions of temperature, can diffuse passively into heart muscle, but doubt has been expressed whether oxygen, under the same conditions, can pass through Glisson's capsule in the liver[5] or the renal capsule.[6] An alternate explanation to its mode of action is the inhibition of oxidation by virtue of its toxic effect on cell metabolism,[7,8] although Lyons *et al.*[9] feel that it is unlikely that oxygen poisoning occurs during the period of *in vitro* storage. Preservation with inert gases under hyperbaric conditions has shown that hyperbaric helium and nitrogen[9,10] are comparable to hyperbaric oxygen in heart and kidney preservation. Hence hyperbaria may exert its beneficial effect not by meeting the nutritional requirements of the organ nor by further inhibiting oxidative metabolism, but by preventing tissue oedema during hypothermic storage.[11]

Pharmacological Metabolic Inhibition

A reversible drug-induced inhibition of metabolism should prolong tolerance to ischaemic injury in the same way as hypothermia, but with the advantage that drugs could be administered before the moment of death with the hope of protecting the organ not only after death, but principally during agonal deterioration and before other organ-preserving measures can be instituted. Numerous pharmacological agents have been described which have either a cell-stabilizing action or a general inhibitory effect on cellular metabolism. These include steroids,[12] magnesium,[13] phenoxybenzamine[14] and phenothiazines.[15] It is clear, however, that no drug yet described is dramatically useful by itself, and that such agents may be useful in combination with more effective methods to enhance the quality of the preserved organ.

Perfusion

In 1812 Le Gallois[16] expressed his belief in the feasibility of artificially maintaining an organ indefinitely by substituting the heart with a perfusion system. A century later, Hooker took the concept of perfusion to its ultimate refinement by introducing a system which could both aerate the blood and pump it at a variable pulse pressure through an organ with a vascular pedicle. With the current surge of interest in organ transplantation, and the success achieved in perfusion storage of kidneys, both experimentally and clinically,[17-19] perfusion storage of the liver as a method of preservation preceding transplantation is currently being extensively investigated.[20-26]

Continuous perfusion should ideally fulfil the same purpose as the circulation of blood through the body, namely the continuous and adequate supply of oxygen and nutrients and the continuous removal of carbon dioxide and other end-products of metabolism. The factors which determine successful perfusion were set down by Carrel and Lindbergh.[27] They more than adequately describe the technical and mechanical problems involved in an artificially produced perfusion system. Numerous tests have been described to assess liver function in the isolated state. However, the only true test of successful storage is the ability of the organ to support life in a hepatectomized animal.

PRESENT STATUS OF SUCCESSFUL EXPERIMENTAL LIVER STORAGE

Hepatic hypothermia produced by the introduction of cold electrolyte solution into the portal vein will preserve the function of a canine liver for 2 hours.[28] Using a preserving solution of homologous plasma with added bicarbonate and dextrose, this period can be extended to 3·5 hours.[29] Recently, we reported successful hypothermic immersion storage for up to 8 hours in the pig.[30] Nine of 10 animals survived longer than 5 days; the longest-surviving animal is still alive 350 days after the transplant.

The addition of hyperbaric oxygen to hypothermic storage of the liver has not been well studied. Brettschneider *et al.*[31] preserved 3 canine livers for 24 hours at 4°C and 40 psig. Two recipients failed to survive the operation and a third died before the 4th postoperative day. When perfusion with diluted blood via the portal vein and hepatic artery was added to this system canine livers could be preserved for 8 - 9½ hours with good early function.

Belzer *et al.*,[25] using perfusion without hyperbaric oxygen, successfully stored pig livers for 8 - 10 hours and concluded that damage to the endothelium of the liver sinusoids when perfusing beyond this period precluded a successful result.

The experimental data serve to confirm that hypothermia is at present the principal protective factor in all currently successful methods of liver storage. Its use is simple and the result is comparable to that obtained with complicated techniques. It should furthermore provide a more practical application in the procurement and short-term preservation of liver grafts for clinical hepatic transplantation. The addition of hyperbaria, pharmacological agents and even perfusion may prolong the period of preservation by a few hours, but will not provide the answer to long-term storage of the liver.

The experimental work referred to was supported in the University of Cape Town by the Medical Research Council, the C. L. Herman Bequest and the Cape Provincial Administration.

REFERENCES

1. Hunter, J. (1766): Cited by Armstrong-Davidson, M. H. (1959): Brit. J. Anaesth., **31**, 134.
2. Fuhrman, F. A. and Field, J. II (1944): J. Biol. Chem., **153**, 515.
3. Farrant, J. (1965): Nature (London), **205**, 1284.
4. Bloch, J. H., Manax, W. G., Eyal, Z., and Lillehei, R. C. (1964): J. Thor. Cardiov. Surg., **48**, 969.
5. In discussion, in Read, A. E., ed. (1967): *The Liver, Colston Papers*, p. 317. London: Butterworths.
6. Matloff, D. B., Mobley, T. L. and Schwartz, S. I. (1966): Arch. Surg., **92**, 83.
7. Dickens, F. (1946): Biochem. J., **40**, 171.
8. Haugaard, N. (1946): J. Biol-Chem., **16**, 265.
9. Lyons, G. W., Dietzman, R. H. and Lillehei, R. C. (1966): Trans. Amer. Soc. Int. Organs., 12, 236.
10. Groenewald, J. H., Weber, H. W., Van Zyl, J. J. W. and Murphy, G. P. (1970): Rev. of Surg., **27**, 170.
11. Huntley, R., Bartholemew, B., Bielanski, E., Homatas, J., and Eiseman, B. in Norman, J. C., ed. (1968): *Organ Perfusion and Preservation*, p. 305. New York: Appelton-Century Crofts.
12. Weissmann, G. (1964): Fed. Proc., **23**, 1038.
13. Webb, W. R., Dodds, R. P., Unal, M. O., Karow, A. M. J., Cook, W. A. and Daniel, C. R. (1966): Ann. Surg., 164, 343.
14. Manax, W. G. and Lillehei, R. C. in Cooper, P., ed. (1969): Surgery Annual p. 222. New York: Appelton-Century Crofts.
15. Dawkins, M. J. R., Judah, J. D. and Rees, K. R. (1959): Biochem. J., **73**, 16.
16. Le Gallois, C. J. J. (1812) cited by Carrel, A. and Lindbergh, C. A. (1938): *The Culture of Organs* p. 6. London: Hamish Hamilton Medical Books.
17. Humphries, A. L. Jr, Russel, R., Stoddard, L. D. and Moretz, W. H. (1968a): Surgery, **63**, 646.
18. *Idem* (1968 b): Invest. Urol., **5**, 609.
19. Belzer, F. O. and Kountz, S. L. (1970 a): Ann. Surg., **172**, 394.
20. Kestens, P. J., Mikaeloff, Ph., Haxhe, J. J., Dureau, G., Alexandre, G., Rassat, J. P., Culleret, J., Hassoun, A., Dubernard, M., Descotes, J. and Morelle, J. (1966): Bull. de la Soc. Int. de. Chir., **6**, 647.
21. Brettschneider, L., Daloze, P. M., Hugent, C., Porter, K. A., Groth, C. G., Kashiwagi, N., Hutchison, D. E. and Starzl, T. E. (1968): Surg. Gynec. Obstet., **126**, 263.
22. Hobbs, K. E. F., Hunt, A. C., Palmer, D. B., Badrick, F. E., Morris, A. M., Mitra, S. K., Peacock, J. H., Immelman, E. J. and Riddell, A. G. (1968): Brit. J. Surg., **55**, 696.
23. Mieny, C. J. and Eiseman, B. (1967): Surg. Forum., **18**, 374.
24. Abouna, G. M., Ashcroft, T., Hull, C., Hodson, A., Kirkley, J. and Walder, D. M. (1969): Brit. J. Surg., **56**, 289.
25. Belzer, F. O., May, R., Berry, M. N. and Lee, J. C. (1970 b): J. Surg. Res., **10**, 55.
26. Hickman, R., Saunders, S. J. and Terblanche, J. (1970): S. Afr. Med. J., **44**, 869.
27. Carrel, A. and Lindbergh, C. A. (1938): *The Culture of Organs* London: Hamish Hamilton Medical Books.
28. Starzl, T. E., Kaupp, H. A., Brook, D. R., Lazarus, R. E. and Johnson, R. V. (1960): Surg. Gynec. Obstet., **111**, 733.
29. Schalm, S. W. (1968): A simple and clinically applicable method for preservation of a liver homograft. M.D. Thesis. University of Leiden, Holland.
30. Spilg, H., Uys, C J., Hickman, R., Saunders, S. J. and Terblanche, J. (1971): Transplantation, **11**, 457.
31. Brettschneider, L., Groth, C. G. and Starzl, T. E. in Norman, J. C., ed. (1968): *Organ Perfusion and Preservation* p. 271. New York: Appelton-Century Crofts.