skipper, mealy bug and stink bug suggests absence of *Wolbachia* in these pests of rice. *Wolbachia* has recently been reported in white-backed planthopper in Japan[11]. Leaffolder and caseworm are new host records, while *Wolbachia* infection in white-backed planthopper and mosquito is reported for the first time from India.

Although *Wolbachia* is classified as monophyletic origin bacteria, recent molecular analysis using PCR primers that target faster-evolving and *Wolbachia*-specific genes such as *ftsZ*, a cell cycle gene[2] and *wsp*, a surface protein gene[12] has resolved variability existing among members of the *Wolbachia* genus and explained the role of the bacteria in host evolution and speciation. Further work on the horizontal and vertical distribution of *Wolbachia* in rice insects may help to understand the interaction and usefulness of the association.

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Selective loss of exocrine tissue during islet storage at –70°C

Since two and half decades[1] it has been shown that frozen–thawed–transplanted islets were able to normalize blood sugar in streptozotocin-diabetic rats. In experiments with adult rat islets, a number of different freezing protocols have been used, with viability demonstrated both *in vitro*[2] and *in vivo*[3]. Effective islet transplant programme promotion requires different forms of storage, such as cold storage of the pancreas during transport to a centre for islet isolation; tissue culture to reduce tissue immunogenicity and cryopreservation for long-term storage. It is also mandatory to have pure islet preparation without exocrine cell contamination for islet transplantation into a diabetic recipient.

In our ongoing project on islet isolation[4] and cryopreservation[5] we came across an interesting feature on differential susceptibility and sensitivity of pancreatic cell populations to cryo storage at –70°C. Islets were isolated from Balb/C mice of either sex following the protocol developed by us[4] and the resulting islets along with acinar cells were cryopreserved at –70°C (Deep Freezer, SO LOW, Cincinatti, Ohio, USA). After one month of storage at –70°C, islet preparations were rapidly thawed at 37°C and revived as described earlier[5]. Revived islets were stained for their viability and specificity employing trypan blue dye exclusion test and dithizone staining[6], respectively. It was observed that islets showed 92 ± 2.5% viability and remained unstained with trypan blue, whereas the acinar cells present in the preparation showed almost zero viability, turning blue in colour. The viability of frozen–thawed islets was comparable to that of freshly isolated islets (94 ± 3.1) The viable islets stained positive (brick-red in colour) with dithizone indicating their specificity and identity, whereas acinar cells remained unstained with dithizone.

These results clearly indicate that the protocol used for cryopreservation of islets at –70°C leads to selective killing of acinar cells, permitting survival of islets only. We repeated these experiments 8 times and obtained similar results on all the occasions, indicating susceptibility of acinar cells and resistance of islets to cold storage at –70°C under similar set of conditions.

Islet purity has been considered to be of major importance in islet transplantation programme as it determines the long-term survival of islets on transplantation. Several methodologies have been used to get pure islet preparations free of acinar-cell contaminants[7,8]; however they are laborious and affect islet yield adversely.

Our results indicate that impure islet preparations containing acinar cells could be safely subjected to cryopreservation at –70°C leading to selective killing of acinar cells, without complicating the purification procedure. The method described in this communication provides an alternative approach to purification of islets without affecting islet yield and viability. These cryopreserved and revived islets have been shown to retain their insulin secretory activity in response to glucose challenge[5], indicating their functional state. Cryopreserved pancreatic microfrag-

ments have been shown to normalize carbohydrate metabolism when autotransplanted into totally pancreatomized dogs. Measuring the insulin and amylase content of the grafts revealed that cryopreservation purified the pancreatic microfragments by selectively destroying the unwanted exocrine tissue[9].

Dog and porcine pancreatic fragments have been shown to be viable following slow cooling to $-40^{\circ}C$ or $-75^{\circ}C$ (refs 10 and 11). The insulin/amylase ratio and histologic data show a purification of the pancreatic microfragments with cryopreservation, which occurs with a hypersensitivity of exocrine tissue to cold conditions[12]. Further, it was found that dogs transplanted with cryopreserved tissue do better than those transplanted with fresh tissue, as reflected by improved glucose tolerance[13]. The purification obtained with cryopreservation may account for the observation that recipients of frozen–thawed allotransplants survived longer than the nonfrozen controls[14]. Such selective destruction has been reported in leukocytes during cryopreservation[15]. This feature is of great importance in reducing the tissue immunogenicity of tissue allografts prior to transplantation, as it would facilitate greater acceptance of the graft, even in the absence of immunosuppression. It is now recognized that antigen recognition alone is not sufficient for lymphocyte activation in the host. 'Passenger' leukocytes (antigen-presenting cells) in the donor tissue are now recognized as a major immunogenic stimulus. Removal of these contaminating leukocytes is essential to enhance the survival of tissue allografts. A variety of techniques have been used to deplete donor tissues of contaminating leukocytes, thereby reducing immunogenicity which results in a marked prolongation of tissue allografts[16,17].

In all these situations, whenever there is a selective loss of certain cell populations during cryopreservation procedures, it is uncertain whether most of these cells are lost during freezing and/or after the cells are thawed[15]. However, it is certain that exocrine cells die during the process of cryopreservation by necrosis[18,19].

All these studies cited above support our findings and indicate that exocrine and endocrine pancreas of mouse, dog and pig are differentially susceptible to cold shock, affecting only exocrine tissue while keeping the islets unaffected. The ability of mammalian islets to withstand cold shock seems to be well conserved during evolution with some survival value, over a wide spectrum from mouse to dog to pig. However it remains to be seen what happens in human islets and exocrine tissue.

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