Fig. (5): A photomicrograph showing weak-positive insulin-containing cells between the muscles of the tongue, 8 weeks after transplantation. (Immunoperoxidase stain for insulin; x 250)

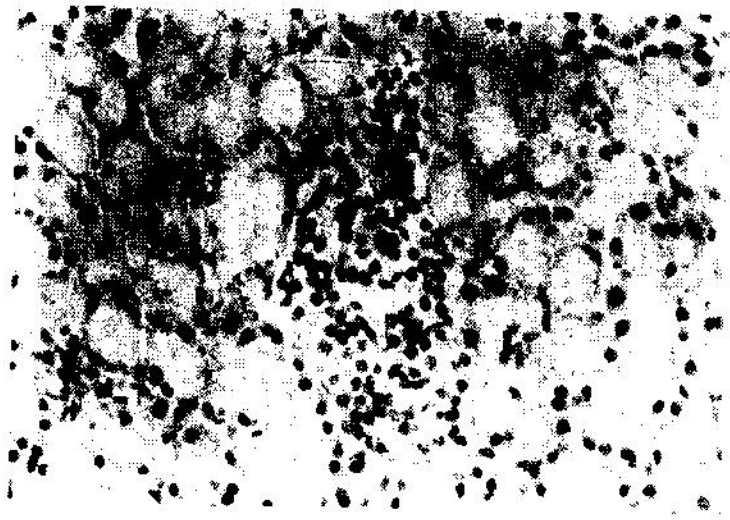

Fig. (6): A photomicrograph showing an islet of a pancreas of rat, 4 weeks following transplantation of islets into the tongue. The islet is composed entirely of non beta-cells. (Immunoperoxidase stain for insulin; x 300)

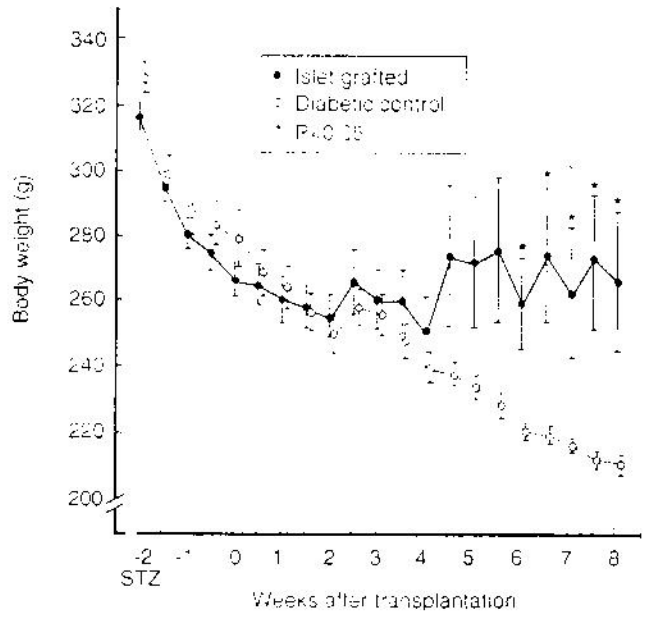

Fig. (7): A graph showing the body weight of the rats after transplantation of islets into the tongue, compared with that of the diabetic control rats after induction of diabetes with STZ. Values are expressed as mean ± SEM. The number of rats used for transplantation was 18 at the start of the experiment, 12 and 6 at 2 and 4 weeks post-transplantation, respectively. The number of the diabetic control rats was 10 at the start of the experiment, and 5 at 2 weeks post-experimentally.

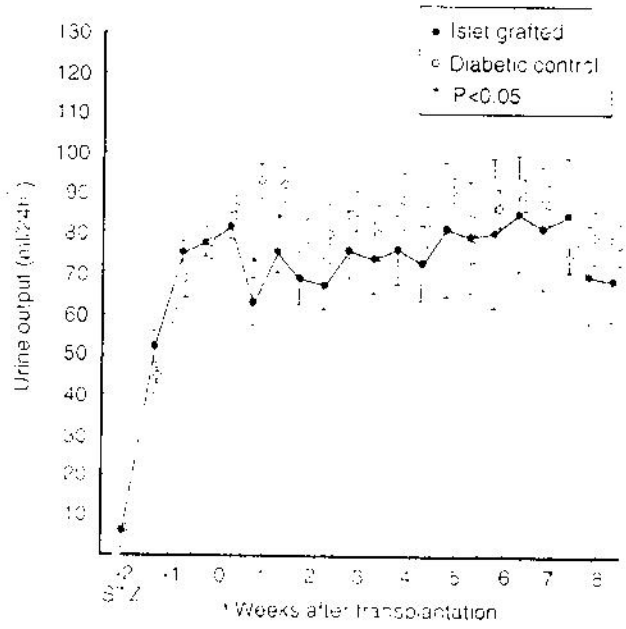

Fig. (8): A graph showing the urinary output per 24 h of the rats after transplantation of islets into the tongue and diabetic control rats, following induction of diabetes with STZ. Values are expressed as mean ± SEM. The number of rats is the same as above mentioned in fig. (7).

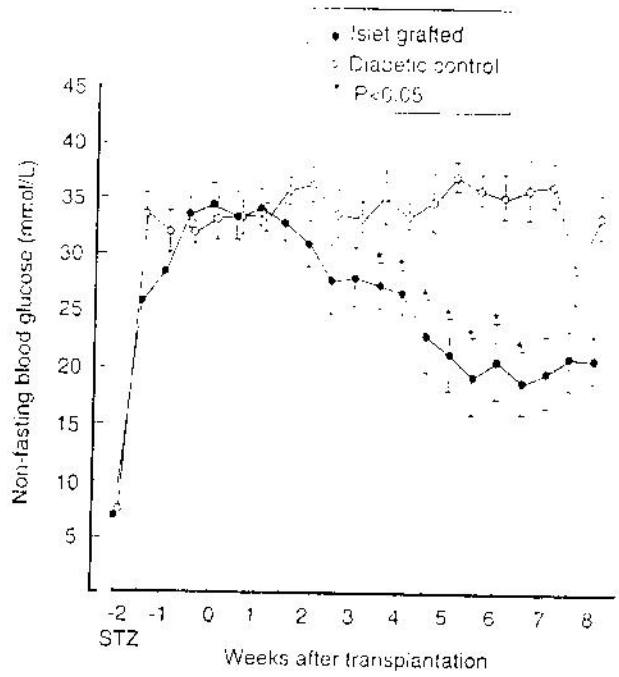

Fig. (9): A graph showing non-fasting blood glucose levels in rats after transplantation of islets into the tongue and diabetic control rats, following induction of diabetes with STZ. Values are expressed as mean ± SEM. The number of rats is the same as mentioned in fig. (7).

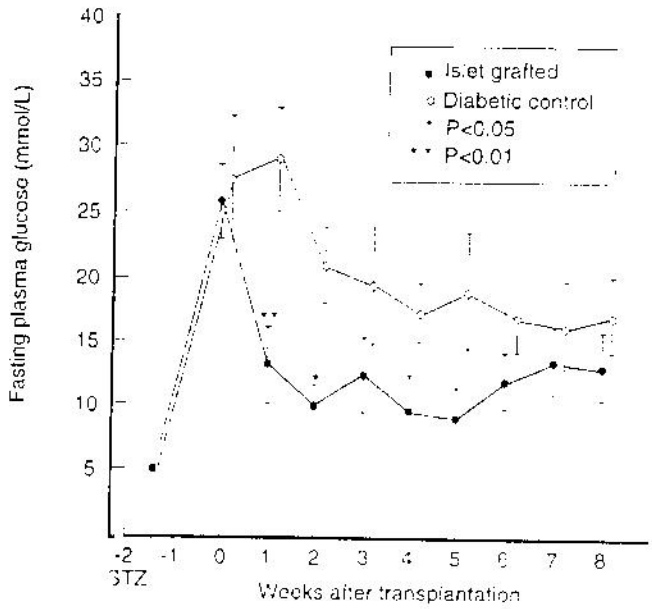

Fig. (10): A graph showing overnight-fasting plasma glucose levels in rats after transplantation of islets into the tongue and diabetic control rats, following induction of diabetes with STZ. Values are expressed as mean ± SEM. The number of rats used for transplantation and diabetic control groups was 6 and 5, respectively.

DISCUSSION

The results of the present study demonstrated an improvement in STZ-induced diabetes by implanting isologous islets into the tongue of diabetic rats. The improvement in the diabetic state following islet isograft could not be attributed to spontaneous recovery of the original beta-cells. This was supported by immunohistochemical examination of the pancreas of the islet recipient rats that showed absence of insulin-producing beta-cells. The results of the functional study correlate well with the immunohistochemical detection of insulin-positive beta-cells in the tongue of the recipient rats up to 8 weeks post-transplantation.

The amelioration of the diabetic state in the present study was less impressive than that reported in islets transplanted into the liver (**Kemp et al., 1973-a & 1973-b; Pipeleers et al., 1975; Matas et al., 1977; Sutherland et al., 1980; Hiller and Klempanauer, 1989; Scharp et al., 1992; Van Suylichem et al., 1994**), spleen (**Reckard et al., 1978; Warnock et al., 1983; Hesse et al., 1986; Scharp et al., 1992; Van Suylichem et al., 1994**), or under the kidney capsule (**Reece-Smith et al., 1981; Toledo-Pereyra et al., 1984; Amiel et al., 1987; Mendola et al., 1994**) where transplanted pancreatic islets were reported to completely normalize the diabetic state. However, functional exhaustion of the grafted islets was reported in long term transplantation studies and was characterized by sudden deterioration of the islet function (**Sutherland et al., 1980; Alejandro et al., 1986; Hiller and Klimpnauer, 1989; Hiller et al., 1991; Rilo et al., 1994**). This could be controlled by repeating the transplantation procedure, provided that the graft site is easily accessible; which is an advantage provided by the tongue as a site of transplantation. The results of the present study are reasonably promising compared with the results reported following experimental transplantation of islets into other easily accessible sites such as intramuscular or subcutaneous. The reported data following transplantation of islets into the above-mentioned sites are controversial. Decreased hyperglycemia has been reported after implantation of isolated islets to the anterior thigh muscle pocket (**Ballinger and Lacy, 1972**), fetal pancreatic tissue under the neck skin (**Usadel et al., 1974**), neonatal islets into the subfascial spaces of the anterior abdominal wall of experimentally induced diabetic rats (**Weber et al., 1978**) or unpurified islets in the skeletal muscle of the dog (**Al-Abdullah et al., 1995**). Transplantation of isolated islets into a subcutaneous pocket of the skin of the anterior abdominal wall (**Kemp et al.,**

1973-a), fetal pancreatic tissue into the brachioradialis muscle (Usadel et al., 1980), or dispersed pancreatic endocrine cells into the muscles of the left thoracic area **(Tze and Tai, 1988)** were, however, reported to show no evidence of physiological functioning.

Various factors have been contemplated for the occurrence of graft inability to normalize diabetes. A recent body of work has suggested that a primary reason for nonimmune graft inability to normalize diabetes may be transplantation or survival of an inadequate mass and/or number of islets **(Alejandro et al., 1986; Warnock and Rajotte, 1988; Munn et al., 1989; Sutherland et al., 1989; Montana et al., 1993; Van Suylichem et al., 1994)**. In the present study, the number of the islets grafted into each recipient's tongue was 2500-3000. It was greater than that proved experimentally capable of normalizing diabetes when transplanted intraportally into the liver **(Kemp et al., 1973-b; Van Suylichem et al., 1994)** or into the spleen **(Reckard et al., 1978; Lacy and Davie, 1984)**. Progressive decrease in the islet mass during and after transplantation into the tongue was evidenced by the decrease in the number of cells per islet section and the decrease in the profile diameter of the islets. It seems that the number of the transplanted islets that could survive in the tongue was probably below the level required to normalize the diabetic state. The possibility of immunorejection as a cause of the decrease in the islet mass grafted in the tongue could be obviated, in the present work, by the use of an inbred strain of rats both as islet donors and recipients. This was supported by successful skin grafting as a test for the genetic quality control of the inbred strain throughout the experiment (results not shown). However, the possibility of minor interaction between the host immune system and the cells of the transplanted islets cannot be completely ruled out. The graft ability to normalize diabetes could also be affected by the functional viability **(Scharp, 1984)** and purity **(Hesse et al., 1986; Al-Abdullah et al., 1995)** of the transplanted islet cells. Examination of aliquots from the isolated islet preparations showed that the islets were viable and functioning, with 83.4±2.3% purity that is higher than the currently accepted purity of 60% that allows safe transplantation (Gotoh et al., 1986).

The mechanism of impairment of islet implantation is open to speculation. One interpretation of the histological findings would be that the contaminant acinar tissue dies resulting in the release of exocrine enzymes. The released exocrine enzymes, along with the mechanical trauma of the transplantation procedure, resulted in non-specific cellular reaction and death of the adjacent islet tissue. The persistent

hyperglycemic state, although moderately ameliorated, may affect the process of revascularization of the survived islets leading to further loss of beta-cells. This was proved by **Andersson et al. (1989)** who found decreased revascularization of islets 2 weeks after intraportal implantation in hyperglycemic mice, as shown by fewer islet sections containing microspheres, compared with their normoglycemic counterparts. The results of the present work showed that the hyperglycemic state is reflected on the histological picture of the beta-cells. They were enlarged, with partial or completely degranulated cytoplasm and prominent nucleoli, clearly indicating that the beta-cells were viable and responding to hyperglycemia.

Because the microvascular system of the islet is lost soon after isolation **(Stagner and Samols, 1990)** the cells of the islet must depend on the process of diffusion for nutrition and gaseous exchange. Although the tongue is richly supplied with blood, it is possible that the size of the grafted islets was so large that it exceeded the diffusion distance for oxygen, and the tongue was not fully capable of supplying the implanted islets with sufficient nutrients by diffusion. This could lead to early ischaemia of the islet cells, which is aggravated by the compactness of the muscules of the tongue. This early ischemia has been claimed to be the cause of failure of islets transplanted beneath the renal capsule in dogs and humans **(Hesse et al., 1986; Toledo-Pereyra, 1986; Evans et al., 1989)**. The early ischemia of the grafted islets, the released exocrine enzymes, the mechanical trauma, added to the high mobility of the tongue, could explain early loss of islet mass.

The results of the present study showed that transplanted pancreatic islets could be detected in the tongue of diabetic recipient rats up to 8 weeks after transplantation. Functional studies showed improvement, but not complete cure, of the diabetic state of the recipient rats. It appears, however, that further investigations are required before this site could lend itself to transplantation studies as an easily accessible site for curing diabetes. The tongue has the advantage of being easily accessible and the islets could be implanted into it by simple injection through a needle, with no requirement for invasive surgical procedure. Transplanting the islets into the tongue in small successive doses is possible, and might lead to accumulation of a number of islets large enough to ameliorate the diabetic state. Further improvement in the purity of the islet preparations has to be contemplated to improve the success rate in this site.

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SUMMARY

This study was designed to test the tongue as a suitable site for transplantation of pancreatic islets in streptozotocin-diabetic rats. Pancreatic islets were isolated from inbred Lewis rats by the intraductal collagenase digestion technique and were purified by the discontinuous Ficoll gradient method together with manual picking-up of the non-islet tissue. Recipient rats, from the same inbred strain, were made diabetic by a single dose of intravenous injection of streptozotocin (40 mg/kg) under light ether anesthesia. A total of 2500-3000 islets was injected into the tongue of each recipient diabetic rat using 23-gauge needle. Intact pancreatic islets could be detected in the tongue 2 h after transplantation. Cellular reaction at the site of the grafted islets was detected 24 h after tansplantation, and the islets were identified by positive immunoperoxidase reaction to insulin. Islets could be easily detected in the tongue at 2, 4 and 8 weeks after transplantation. Morphometric analysis showed progressive reduction in transplanted islet mass with the increasing duration of time. Functional studies showed improvement, but not complete cure, of the diabetic state of the recipient rats. This was demonstrated by the maintenance of body weight, decreased urinary output, decreased blood glucose concentrations in the fed and overnight-fasting states, together with elevated plasma insulin and C-peptide levels. Further improvement in the purity of the pancreatic islet preparation is required to improve the success rate of transplantation in this site.

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الملخص العربى

اللسان كمكان لزراعة جزر البنكرياس : تقييم مناعى نسيجى كيميائى ووظيفى

مصطفى النجار* ، أحمد الصياط* ، محمد صالح مرضاوى** محمد طاهر*
من قسمى التشريح* والكيمياء الحيوية السريرية**
كلية الطب والعلوم الطبية – جامعة الملك عبد العزيز – جدة
المملكة العربية السعودية

صممت هذه الدراسة لأختبار اللسان كمكان مناسب لزراعة جزر البنكرياس "جزر لانجر هانز" فى الفئران المصابة معملياً بالسكرى . وقد تم فصل جزر البنكرياس من سلالة متقاربة من فئران "لويس" وذلك بأستخدام طريقة الهضم بالكولاجينيز . ثم تم تنقية الجزر المفصولة بأستخدام طريقة "فايكول" المتدرجة بالأضافة الى الالتقاط اليدوى للأنسجة الأخرى .

وقد أستخدمت فئران من نفس السلالة لعملية الزراعة . وقد تم أحداث مرض السكرى فى هذه الفئران معملياً عن طريق حقنها بعقار " الأستربتوزوتوسين" وقد تم زرع مجموعة ٢٥٠٠ – ٣٠٠٠ جزيرة فى لسان الفار الواحد . وقد وجدت الجزر المزروعة فى لسان الفئران بعد ساعتين من عملية الزرع . وقد لوحظ وجود تفاعل خلوى فى مكان الزرع بعد ٢٤ ساعة . حيث تم التعرف على الجزر بوجود تفاعل "مناعى نسيجى كيميائى" موجب للأنسولين . وقد تم أيضاً العثور والتعرف على الجزر المزروعة بعد ٢ ، ٤ ، ٨ أسابيع من زراعتها . وبالتحليل القياسى وجد أن هناك نقص تدريجى فى كمية الجزر المزروعة وهذا النقص يزداد بمرور الوقت . كما أوضحت الدراسة الوظيفية أن هناك تحسن فى الحالة المرضية للفئران ولكن لم يصل الى درجة التحسن الكامل . وقد وضح ذلك من ثبات وزن الفئران ونقص كمية البول ونقص تركيز الجلوكوز فى الدم فى الفئران المغذاة والصائمة . وكذلك أيضاً زيادة مستوى الأنسولين وال "سى – ببتيد" فى البلازما .

مجلة التشريح المصرية ، ١٩ (١) ، يناير ١٩٩٦

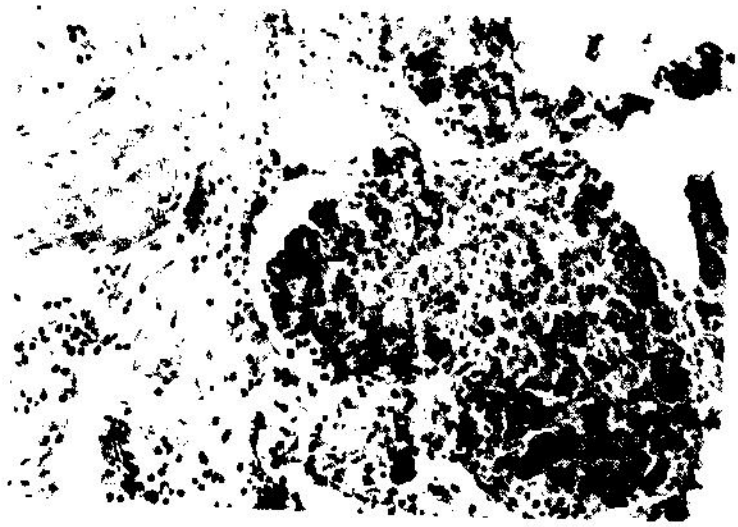

Fig. (1): A photomicrograph showing pancreatic islets, 2 h after transplantation into the tongue. Most of the islet cells reacted positively to anti-insulin serum. (Immunoperoxidase stain for insulin; x 200)

Fig. (2): A photomicrograph showing positively-stained clusters of insulin-containing cells scattered between the muscles of the tongue, 24 h after transplantation. The site of the graft showed non-specific cellular reaction. (Immunoperoxidase stain for insulin; x 250)

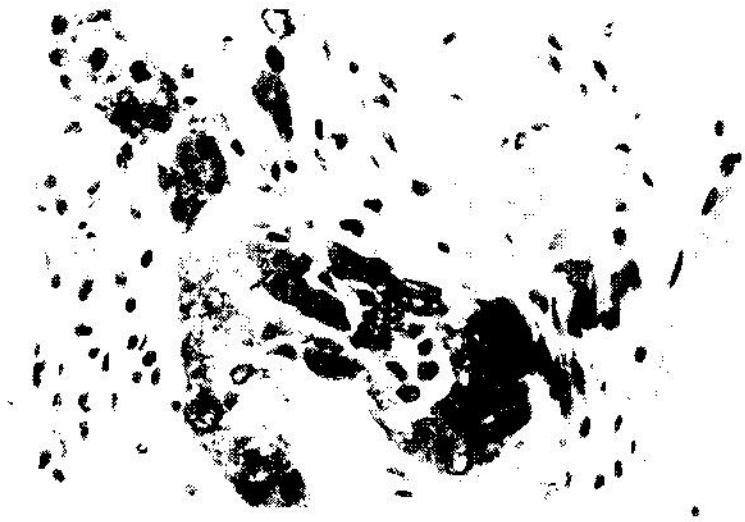

Fig. (3): A photomicrograph showing clusters of positively-stained cells between the muscles of the tongue, 2 weeks after transplantation. (Immunoperoxidase stain for insulin; x 500)

Fig. (4): A photomicrograph showing weak-positive insulin-containing cells within a clearly defined islet, 4 weeks after transplantation into the tongue. (Immunoperoxidase stain for insulin; x 250)