

glutaraldehyde for 2 hours, washed in 0.1M potassium phosphate buffer (pH 6.8), and postfixed in osmium tetroxide for 2 hours or longer. After dehydration in an acetone series, the tissues were embedded in Spurr's epoxy resin (10). Ultrathin sections were cut on a Porter-Blum ultramicrotome, stained with uranyl acetate and lead citrate, and examined with an RCA EMU electron microscope.

Organisms similar to those seen in sections of naturally infected grapevines were observed in vessels of the plants exposed to leafhoppers injected with bacteria. The organism was not observed in the xylem vessels of the plants exposed to vectors that had been injected with sterile broth (Fig. 1c).

The same bacterium was recovered from vectors that had fed on plants experimentally infected. Identical bacteria were recovered by the same methods from naturally infected plants. As in the first experiment above, identical small white bacterial colonies grew on blood-dextrose agar and medium 523 agar. Again such colonies were absent on mediums streaked with extracts of the ground bodies of vectors that had fed on plants previously exposed to vectors injected with sterile medium 523 broth. The reisolated bacterium had the same morphology, size, and cultural and physiological characteristics as the original isolate.

The bacterium is Gram-positive, rod-shaped, 0.4 to 0.6 μm wide, 1.0 to 2.0 μm long, and nonmotile. It grows well at a temperature range of 20° to 32°C, with an optimum of 29° \pm 1°C as determined by the polythermostat method (11), and well but slowly on blood-dextrose agar, medium 523 agar, and medium D2 agar (minus LiCl)—a selective medium for Gram-positive bacteria (9). The bacterium grows profusely in mediums 523 and D2 broth (minus LiCl). On medium D2 the colonies are white to white-gray in color, slightly convex, circular with entire margins, and have a smooth shiny texture. On the basal medium recommended by Hugh and Leifson (12) it behaved as a facultative anaerobic bacterium; it also produced acid but not gas from glucose. Tests for production of indole and methyl red were negative.

Our experiments have demonstrated that a Gram-positive bacterium is the etiological agent of Pierce's disease in grapevines. We have cultured the organism on artificial mediums. By using the leafhopper vector injected with the cul-

tured and purified bacteria, we can consistently reproduce the disease symptoms in healthy grapevines and we can reisolate the same organism from clean leafhoppers fed on these plants and on naturally infected plants from the field. We have attempted to isolate and to culture the bacterium from diseased tissues without success. The reason for this is presently unknown. The characteristics of this bacterium, which in nature is apparently confined to its vectors and to the xylem tissues of its host plants, plus its morphological, cultural, and physiological features, suggest that the Pierce's disease agent belongs to a distinct group of plant pathogenic Gram-positive bacteria heretofore unrecognized.

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References and Notes

1. N. B. Pierce, *U.S. Dep. Agric. Div. Veg. Pathol. Bull.* **2**, 222 (1892).
2. J. L. Weimer, *J. Agric. Res.* **53**, 333 (1936).
3. W. B. Hewitt, *Phytopathology* **29**, 10 (abstr.) (1939); —, N. W. Frazier, B. R. Houston, *ibid.* **32**, 8 (abstr.) (1942); W. B. Hewitt, B. R. Houston, N. W. Frazier, J. H. Freitag, *ibid.* **36**, 117 (1946).
4. B. R. Houston, K. Esau, W. B. Hewitt, *ibid.* **37**, 247 (1947).
5. D. L. Hopkins and J. A. Mortensen, *Plant Dis. Rep.* **55**, 610 (1971).
6. A. C. Goheen, G. Nyland, S. K. Lowe, *Phytopathology* **63**, 341 (1973).
7. D. L. Hopkins and H. H. Mollenhauer, *Science* **179**, 298 (1973).
8. The medium consisted of a blood agar base containing 15 percent Bacto agar (Difco Laboratories, Detroit, Michigan), 20 percent tryptose, 5 percent NaCl, 5 percent dextrose, with 5 percent washed cow blood added, and pH adjusted to 7.6.
9. C. I. Kado and M. G. Heskett, *Phytopathology* **60**, 969 (1970).
10. A. R. Spurr, *J. Ultrastruct. Res.* **26**, 31 (1969).
11. C. I. Kado, M. G. Heskett, R. A. Langley, *Physiol. Plant Pathol.* **2**, 47 (1972).
12. R. Hugh and E. Leifson, *J. Bacteriol.* **66**, 24 (1953).
13. We thank Dr. A. C. Goheen of the U.S. Department of Agriculture, and Dr. G. Nyland and Dr. W. B. Hewitt of the Department of Plant Pathology, University of California, Davis, for their continued interest and assistance.

19 February 1974

Control of Experimental Diabetes Mellitus in Rats by Transplantation of Fetal Pancreases

Abstract. *Experimental diabetes mellitus in young adult Lewis rats was successfully treated by transplantation of fetal pancreases from syngeneic fetuses. Complete or partial control lasting up to 165 days was achieved in 64 percent of recipients by using two to three pancreases of fetal age (15 to 18½ days) placed under each kidney capsule. Islets of Langerhans without exocrine elements were present in the transplants.*

Transplantation of the pancreas in patients with diabetes mellitus has been complicated in most cases by technical problems related to the exocrine secretions of the organ (pancreatitis) and to immunological rejection of the pancreatic elements plus intestinal tissues transplanted to drain the digestive enzymes. Survival has usually been measured in months or even weeks. In most cases, however, insulin treatment could be stopped, indicating at least temporary function of the endocrine elements of the pancreas (1).

Transplantation of isolated islets of Langerhans apparently avoids some of these problems. When 400 to 600 islets obtained from two to three rat pancreases were injected intraperitoneally into syngeneic inbred rats made diabetic with streptozotocin, the diabetic state was somewhat ameliorated (2). Injection of the islets into the portal vein was followed by lowering of blood glucose and urine volume to normal (3). When the islets from 20 to 35 rat pan-

creases were placed into the peritoneal cavity of syngeneic alloxan-diabetic rats, the rats showed return of blood sugar to normal (4).

An alternative approach to pancreas transplantation for therapy of experimental diabetes is the use of fetal or neonatal pancreases. Such tissues have survived, grown, and continued to synthesize insulin, although only barely detectable effects on diabetes in the recipient have resulted (5). When fetal pancreas is transplanted, atrophy of the exocrine cells usually occurs, and the epithelial cells of the ducts show mitoses and form new islets of Langerhans (6). This tissue may thus afford distinct advantages for the treatment of diabetes, although to date no one has reported a surgically feasible (with respect to humans) technique for transplantation of fetal pancreas which has had a physiologically significant impact on the diabetic state. The following preliminary studies were initiated in order (i) to identify a site for transplantation

Table 1. Transplantation of fetal pancreases into syngeneic diabetic recipients. Three fetal pancreases were placed beneath each kidney capsule of recipient rats 3 to 4 days after injection of streptozotocin. Success as judged by amelioration of the diabetes was apparent 9 to 19 days after transplantation. Current survival indicates the duration of function of the pancreatic transplants at the time of writing this report. In experiment No. 4, only two fetal pancreases were used in one of the completely successful results and in two of the three partial successes.

Experiment	Current survival (days)	Fetal age (days)	Success		Failure
			Complete	Partial or temporary	
No. 1	165	17½	4	0	3
No. 2	120	15½ to 17½	0	2	3
No. 3	105	18½	3	0	0
No. 4	75	17½ to 18½	2	3	0

which is surgically accessible, which will not interfere with normal body function, and from which transplanted material can be readily recovered; (ii) to determine whether fetal pancreas can establish vascular contact and function metabolically in such a site; and (iii) to determine a stage of fetal pancreatic development suitable for donor material. In order to study these variables free from complications due to rejection, a syngeneic donor-recipient combination has been used throughout these experiments.

Diabetes was produced in young adult male Lewis rats by intravenous injection of streptozotocin at a dose of 70 mg per kilogram of body weight. The rats were allowed free access to food and water, and beginning 18 to 20 hours later, daily measurements of urine

volume and glucose content and weekly measurements of serum glucose were made. Mean serum glucose in 58 untreated diabetic (fed) rats was 431 ± 8 mg per 100 ml; in 40 rats urine volume was 129 ± 6 ml per day and urine glucose was 8.5 ± 0.4 g per day. Rats which were clearly diabetic were subsequently used as recipients for transplantation. Fetal pancreases were removed from syngeneic embryos at varying gestational ages and immediately placed beneath the kidney capsule. Four units of NPH insulin was injected subcutaneously each day into transplanted diabetic rats and controls for 4 days beginning the day after transplantation, and 2 units was injected daily for four additional days thereafter. At various intervals transplant sites were observed under ether anesthesia

and photographed, and in some cases the transplants were removed for histological examination and electron microscopy, and to observe the effects of removal on the diabetic state of the recipient.

A completely successful transplant is here defined as one in which the blood glucose concentration becomes normal (less than 140 mg/100 ml), urine volume is normal (less than 10 ml/day), and urine glucose content is less than 0.2 g/day. A partial or temporary response is one in which these indices of diabetes are significantly less than in untreated diabetic controls.

In experiment 1 (Table 1), three fetal pancreases of gestational age 17½ days were placed under the capsule of each kidney. In four of the seven recipients there was a completely successful reversal of the diabetic state. In this experiment (shown in more detail in Fig. 1) success was evident within a few days after insulin injections were stopped, which was usually 9 days after transplantation. After 42 days the transplants were removed from two of the recovered rats and the indices of diabetes in these rats immediately returned toward the levels of the untransplanted controls, proving conclusively that recovery was due to the transplant and not to recovery of host pancreatic function. The other two successfully transplanted rats maintained normal urinary volume and glucose content, although there was a slight rise in blood sugar after 70 days in one animal and after 83 days in the other. The transplants have continued to function successfully for more than 9 months after implantation. Table 1 reveals that fetuses of gestational age 15 to 15½ days were less effective for transplantation than those of 17½ days. Improved results followed the use of more mature fetuses of 17½ to 18½ days (experiments 3 and 4) and care to remove the fetuses quickly and keep them in cold phosphate-buffered saline until placement of the pancreas under the kidney capsule. Partial or complete control of blood and urine glucose occurred in all diabetic recipients in this experiment, three of these rats receiving only four fetal pancreases. Successful treatment or improvement of the diabetic state has been achieved in 64 percent of all transplanted rats.

Visual observation of the transplant reveals a prominent vascular supply beginning a few days after transplanta-

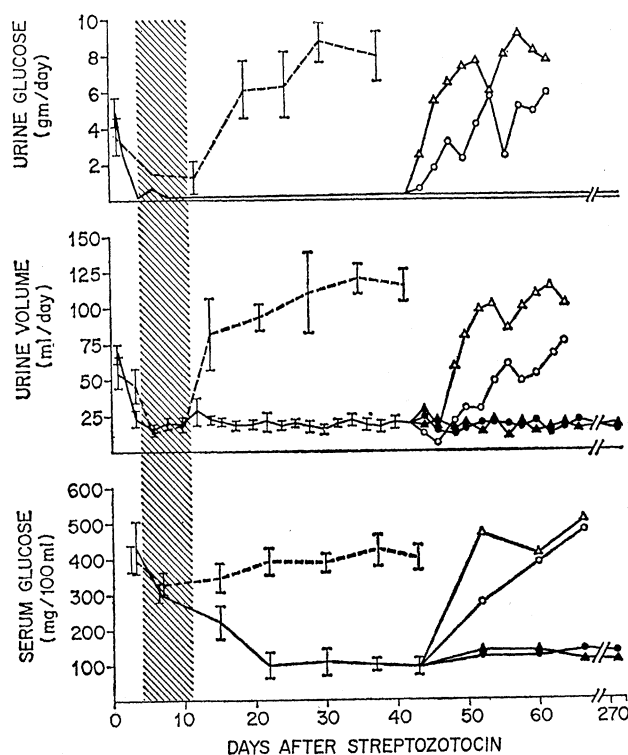


Fig. 1. Diabetes was induced by streptozotocin on day 0. On day 4, three 17½-day fetal pancreases were placed under each kidney capsule (Table 1, experiment 1). Results in four successful rats (—) are compared with diabetic controls (---). Both groups were treated with insulin for 8 days (shaded area). At 42 days the implants were removed from two of the four transplanted rats (open symbols). The time axis is condensed during the interval 70 to 270 days. Urine volume and glucose were measured daily; each point is the average of 2 days. Serum glucose was measured weekly.

tion. By 42 days (experiment 1) a multi-lobed "organ" had formed beneath the kidney capsule, consisting mostly of adipose tissue. Histologic sections revealed scattered foci consisting of islets of Langerhans of varying size and shape and variable numbers of ducts in a thin fibrous stroma with a rich vascular supply. No exocrine elements were apparent. Electron micrographs reveal normal-appearing beta and alpha cells; typical secretory granules are present in the beta cells, and the many empty envelopes suggest active insulin secretion (7). At the present time we cannot estimate the extent of growth in size or number of islets of Langerhans in situ after transplantation.

Our rate of success continues to increase in current experiments as various factors of the transplantation protocol are altered. These parameters include precise location and arrangement of the transplants on the kidney, insulin regimen around the time of transplantation, handling of the donor tissue, and so forth. The kidney has proved to be an ideal locus in terms of ease of accessibility for the initial placement of the fetal pancreases and subsequent evaluation, and in terms of attracting an early and vigorous vascularization.

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References and Notes

1. R. C. Lillehei, R. L. Simmons, J. S. Najarian, C. M. Kjellstrand, F. C. Goetz, *Transplant. Proc.* **3**, 318 (1971); ACS/NIH Organ Transplant Registry, "First scientific report," *J. Am. Med. Assoc.* **217**, 1520 (1971).
2. W. F. Ballinger and P. E. Lacy, *Surgery* **72**, 175 (1972).
3. C. B. Kemp, M. J. Knight, D. W. Scharp, P. W. Lacy, W. F. Ballinger, *Nature (Lond.)* **244**, 447 (1973).
4. R. J. Leonard, A. Lazarow, O. D. Hegre, *Diabetes* **22**, 413 (1973).
5. H. Browning and P. Resnick, *Yale J. Biol. Med.* **24**, 141 (1951-52); E. L. House, M. S. Jacobs, B. Pansky, *Transplant. Bull.* **28**, 435 (1961); A. E. Gonet and A. E. Renold, *Diabetologia* **1**, 91 (1965); O. E. Hegre, L. J. Wells, A. Lazarow, *Diabetes* **19**, 906 (1970).
6. R. E. Coupland, *J. Endocrinol.* **20**, 69 (1960).
7. Details are in preparation.
8. Supported by grants from the Kroc Foundation and the Diabetes Association of Southern California. We thank Celia Brown and Jim Dee for valuable technical assistance and Dr. William H. Carnes for assistance with histologic study. Dr. W. E. Dulin of the Upjohn Company, Kalamazoo, Michigan, kindly supplied streptozotocin.

11 February 1974

28 JUNE 1974

Geckos: Adaptive Significance and Energetics of Tail Autotomy

Abstract. *Coleonyx variegatus* is adapted to readily sacrifice its tail to predators. This adaptation is associated with characteristic tail behavior and rapid tail regeneration. There is no facultative metabolic increase associated with tail regeneration, and energy normally allocated to body growth and maintenance is diverted to tail regeneration. This supports the contention that tail behavior, autotomy, and rapid regeneration evolved as mechanisms promoting survival in terms of predator escape.

Tail autotomy in geckos and a number of other lizard species is considered a mechanism for reducing mortality from predation, although little quantitative experimental data have been presented in support of this hypothesis (1). The eublepharid geckos are remarkable in that a regenerated tail may be larger than the original (2). Moreover, the tail display of these lizards may be used to distract a predator's attention away from the head or body. It has been suggested that *Coleonyx* tails serve as fat stores contributing to increased survival over periods of fasting due to unpredictable environmental conditions (3). This effect appears secondary when the high incidence of regenerated tails (up to 74 percent in natural populations) is considered (4). These data and those reported here suggest that *Coleonyx* tails are adapted primarily for use in predator escape.

Behavioral experiments with a natural *Coleonyx* predator, the spotted night snake (*Hypsiglena ochrorhyncha*) (5), were designed to determine the advantage associated with having an autotomous tail. Predator-prey encounters took place in a 1.0 by 1.8 m arena with sand substrate interrupted by rocks and brush to simulate natural conditions. Red light was used for initial observations (6), but white light was subsequently used because it had no apparent effect on predator or prey behavior. We first tested for differences in predator attack frequency or prey behavior related to the presence or absence of tails on geckos [see (7)] and

found none ($\chi^2 = 0.288$, $P > .05$).

Second, we determined the advantage associated with possessing an autotomous tail. Eight tailed geckos were introduced into the arena and allowed 15 minutes to adjust. Then a night snake was introduced. Geckos responded to the night snake by carrying the tail high above the body and waving it back and forth. Occasionally, geckos seemed attracted to undulatory movements of the snake and approached it with the tail-waving display. The geckos responded to any sudden movement of the predator by rapid retreat. When a gecko was within striking range, the snake would orient to the thickest part of the prey or the waving tail. Attacks were scored as either capture or escape. In 30 such trials, 11 (37 percent) of the geckos escaped while losing portions of the tail, and 19 (63 percent) were captured by night snakes. In our tests (7), no tailless geckos escaped from snake attack.

Rapid regeneration of the lost tail would be expected in *Coleonyx* because of the adaptation of the tail for predator escape. Regeneration rates were monitored on 14 geckos after tails were removed at the base (8). In the first 5 weeks, tail growth of males ($N=7$) averaged 0.80 mm per day and that of females ($N=7$) averaged 0.60 mm per day (Fig. 1), as compared to an average of 0.82 mm per day in the field (both sexes) (4). More rapid regeneration in males suggests different selection pressures between sexes and can be attributed to several

Table 1. Mean caloric values and ash and water contents of *Coleonyx variegatus* tails and bodies.

Part	Caloric content		Ash (% of dry weight)	Water (% of wet weight)
	Per milli- gram of dry weight	Per milli- gram of ash-free dry weight		
Original tails ($N = 3$)	5.79	6.31	9.01	75.50
Regenerated tails ($N = 19$)	6.24	6.68	7.06	75.66
Bodies ($N = 22$)	5.06	5.90	16.70	73.74