of bloodstain antigens for a period of time following deposition. Thus, we routinely allowed stains to stand for 48 hours before analysis on the reasonable assumption that most crimes of violence are discovered and reported within that period. Nevertheless, it was of interest to obtain some tentative indication of antigenic stability over longer periods. Accordingly, stains from three subjects (A, E, and G) were allowed to stand for 16 days and analyzed as above. The major findings were that as many as 5 of the 22 antigens were not resolved but individualization was still possible. In fact, in all three comparisons the number of peaks with nonoverlapping ranges was equal to or greater than the number in comparisons of 48-hour stains.

Aside from our evaluation of CEID as a means for individualization, we considered it of interest to examine the value of the technique to distinguish female from male bloodstains because the best available procedure, Y chromosome fluorescence, is reliable in only about 50 percent of the cases (*7*). We found (*8*), as did Clarke and Freeman in studies of fresh serum (*9*), significant (Student's *t*-test) sexual differences in the amounts of several antigens, most notably α_1-lipoprotein ($P < .02$) and ceruloplasmin ($P < .01$). These results suggest a serious consideration of CEID as a way to judge the probability of a stain having come from one sex or the other.

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Human Pancreatic Islets in Culture: Effects of Supplementing the Medium with Homologous and Heterologous Serum

Abstract. *Islets of Langerhans, isolated from the human fetal pancreas and cultured in media supplemented with serums from human umbilical cord or adult donors, remained intact, free-floating, and functional for 2 to 5 months. Substitution of fetal calf serum for the human serum usually resulted in attachment of islets, monolayer formation, and relatively early (3 weeks) functional arrest.*

The ability to isolate pancreatic islets of Langerhans and maintain them in culture in a functional state for extended periods of time should be useful for the investigation of their normal and abnormal physiology. It would provide tissue for eventual attempts at transplantation. Here we report a technique that has allowed intact islets from the human fetal pancreas to remain functional for as long as 150 days and, by varying the condition of the culture medium, to produce functional monolayers of islet cells.

There are many methods for the isolation of islets and the preparation of functional monolayers of endocrine cells of the pancreas of rat, mouse, and guinea pig, but the monolayers either undergo

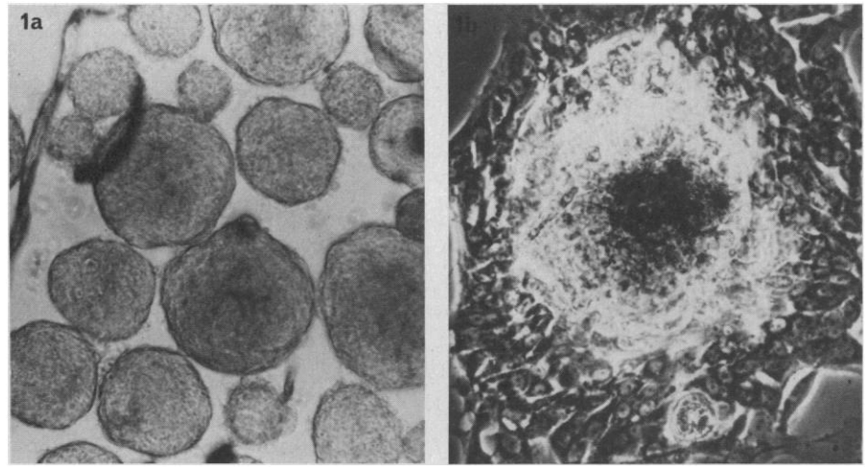

Fig. 1. (a) Intermediate-power phase micrograph of a cluster of intact free-floating islets isolated from the pancreas of a 12-week-old human fetus. The culture is 2 weeks old and has been maintained in CMRL-1066 enriched with 10 percent human cord serum. Note the islet capsule and the variety of islet size (×320). (b) Intermediate-power phase micrograph of an islet from a 12-week-old human fetus; the islet was exposed for 24 hours to CMRL-1066 medium supplemented with 10 percent fetal calf serum. The capsule of the centrally located attached islet has disappeared and epithelioid granule-containing cells have fanned out to form a monolayer. There are no contaminating fibroblasts (×320).

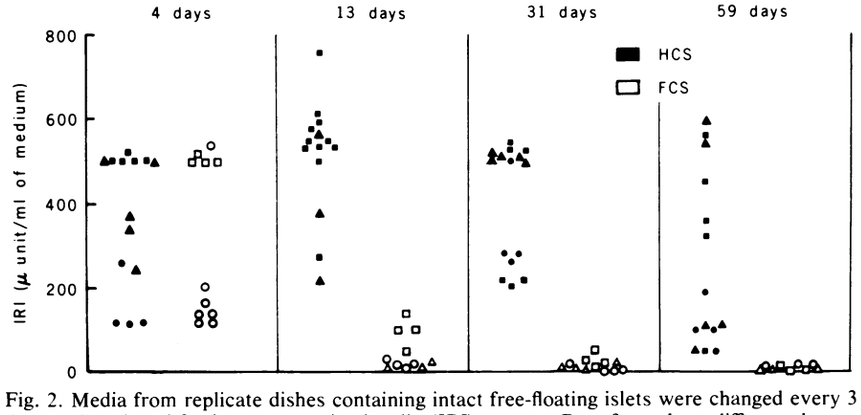

Fig. 2. Media from replicate dishes containing intact free-floating islets were changed every 3 days and analyzed for immunoreactive insulin (*IRI*) content. Data from three different donors (represented by different symbols) are summarized. Differences were significant ($P < .01$) at 13, 31, and 59 days. Glucagon content of the media was assayed at 7, 38, and 80 days. There was no difference between cultures maintained in human cord serum (HCS) and fetal cord serum (FCS) at 7 days. At 38 days, the mean glucagon content for the HCS replicates were 422, 760, and 347 pg/ml, and for the FCS replicates 33, 155, and 133 pg/ml. At 80 days all media that contained insulin still had measurable glucagon.

an unexplained disruption within 3 or 4 weeks after explantation or are overgrown by fibroblastoid cells (*1, 2*). We have adapted these methods for the isolation and long-term culture of islets from the human fetal pancreas. By changing the serum used to supplement the commercially prepared synthetic growth media, preparations of either intact floating islets or of functional monolayers have been obtained.

Pancreas from previable fetuses (9 to 20 weeks) delivered by hysterotomies approved by an accredited hospital committee were removed within 2 hours postmortem. The gland was suspended in Hanks balanced salt solution (BSS) containing 2.8 m*M* glucose and infiltrated with the same solution to loosen the parenchyma from the connective tissue. Large ducts, blood vessels, and connective tissue were dissected from the pancreas, and the parenchyma was cut into approximately 1-mm pieces. These were dissociated with gentle stirring in a solution of BSS containing collagenase (10 mg/ml) (Worthington; CLS IV) and 2.8 m*M* glucose at 37°C for 45 to 60 minutes. The dispersed pancreas, which includes isolated islets, exocrine cells, ductal epithelial cells, and fibroblasts, was washed three times with BSS, then explanted into a number of 100-mm plastic petri dishes (Falcon) and suspended in 15 ml of CMRL-1066 medium (Gibco) supplemented with 2 m*M* L-glutamine, penicillin (100 μunit/ml), and streptomycin (60 μg/ml). This medium was enriched with either (i) 10 percent pooled human serum obtained from umbilical cord (HCS), (ii) 10 percent pooled human serum from adult donors (HAS), (iii) 10 percent fetal calf serum (Flow Laboratories) (FCS), or (iv) 10 percent FCS for 48 hours, followed by 10 percent HCS. The cultures were maintained at 37°C in an atmosphere of 95 percent air and 5 percent CO_2 with high humidity. During the initial 48 hours after explantation, the exocrine cells completely disappear and a variable number of fibroblastoid cells become attached, whereas the islets remain intact and free-floating if maintained in media with homologous serum.

The final number of intact islets available for subculturing varied from 100 to 2000 and was a function of a number of factors such as the gestational age and the condition of the fetus, the postmortem time that elapsed prior to removal and dissociation of the pancreas, and the conditions during the technique of pancreatic dispersal and islet isolation.

In spite of the variability of islet size (Fig. 1a), it is on the basis of initial islet count that relatively equal numbers of from 25 to 200 variably sized islets were then distributed into each of four to eight replicate cultures (60-mm plastic petri dishes) by means of a dissecting microscope and small-bore Pasteur pipet. These subcultured replicates were now almost completely free of other cellular contamination.

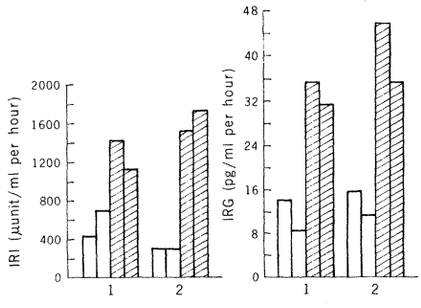

Fig. 3. Four replicate dishes of intact floating islets from a 14-week-old single donor were established and maintained in culture with CMRL-1066 and 10 percent HCS for 60 days. The media were changed 16 hours prior to and decanted at the beginning of the experiment. Two replicates were fed with CMRL-1066 and 10 percent HCS (open bars) and two were fed with CMRL-1066 plus 10 percent HCS and 10 m*M* theophylline (hatched bars). The media were collected at the end of the first hour and replaced with fresh media for an additional hour and collected. Portions were assayed for insulin (*IRI*) (left panel) and glucagon (*IRG*) (right panel). Hormonal content was greater in replicates treated with theophylline ($P = .006$ for insulin and $< .001$ for glucagon.

The free-floating islets were fed twice weekly with 8 ml of medium. At each feeding, they were centrifuged (600 rev/min for 5 minutes), and the supernatant medium was carefully removed so that the sedimented islets remained undisturbed. Portions of the medium were assayed for insulin by means of ^{125}I-labeled porcine insulin and an antiserum against porcine insulin (*3*). Glucagon was assayed with 30K antiserum (*4*) and ^{125}I-labeled glucagon (*5*).

Pancreases from 32 fetal donors have been cultured continuously in HCS, and the islets of 29 of these pancreases failed to attach to the dishes. They remained morphologically intact (Fig. 1a) and floated freely in the media. Nine of the donors were 11 weeks or less in age (< 70-mm crown-rump length), and insulin was detected in the media from only two of these preparations. Insulin and glucagon were identified in media from all other donors and, in some replicates, insulin secretion continued for as long as 150 days. Electron micrographs confirmed the presence of an islet capsule and β, α2, and α1(D) cells. The hormonal content was greater in islets maintained in HCS than in the replicate dishes maintained in FCS (Fig. 2). In cultures established from three donors, supplementation of the medium with HCS was compared to supplementation with HAS. Hormonal content did not differ after 45 days in culture, and most of the islets cultured continuously in HAS also failed to attach to the dish and remained intact and floating. During the extended culture period there was a progressive loss of islet mass, but this decrease in the number of islets appeared similar in all replicates. This was reflected in the steady decrease of hormonal content assayed in the media.

Islets from six donors were cultured in medium supplemented with 10 percent FCS. Most of the islets anchored to the petri dish, their capsular integrity was broken, and a monolayer of epithelioid cells resulted (Fig. 1b). A few islets remained intact and floating while a few anchored but did not form monolayers. Insulin and glucagon could be identified in the media for 14 to 21 days. Usually after 21 days, peeling appeared at the periphery, and the monolayers subsequently broke apart into a single cell suspension. Fibroblastoid overgrowth was rarely a contributing cause to monolayer disruption.

Islet monolayers established from two donors were cultured in medium containing HCS after an initial 48 hours in FCS to attach the islets. They secreted more insulin into the medium than did replicates maintained continuously in FCS. At 2 days, mean insulin was as follows: for FCS, 290 μunit/ml; for HCS, 225 μunit/ml; *P*, not significant. At 10 days, mean insulin was for FCS, 44 μunit/ml; for HCS, 105 μunit/ml; *P*, $< .05$. At 14 days the mean insulin was for FCS, 24 μunit/ml, for HCS, 90 μunit/ml; *P*, $< .05$. At 21 days, peeling had occurred in some replicates. There was no difference in the content of glucagon in the media at any of the times. Peeling of the monolayers occurred in both groups between 3 and 4 weeks.

Theophylline enhances the release of insulin induced by glucose. To test the responsiveness of the islet cells, theophylline was used in replicate dishes from single pancreases. All replicates were initially fed with fresh medium 16 hours before the experiment, and this medium was decanted at the beginning of the experiment. Then replicate dishes received either control medium (CMRL with 10 percent HCS) or control medium supplemented with 10 m*M* theophylline, and the hormonal content of the medium was determined at the end of 1 and 2 hours. Data were analyzed by analysis of variance. Floating intact islets in culture

for 60 days secreted more insulin and glucagon when theophylline (10 m*M*) was present in the medium (Fig. 3). Likewise, attached islets with monolayer formation subjected to theophylline (10 m*M*) after 10 days in culture secreted more insulin in each of two 1-hour periods than those in control media.

It is customary to supplement nutrient medium used for the culture of living cells with serum that contains macromolecular components. These components, studied primarily in fibroblast cultures, have been shown to affect attachment of cells, cell stretching, DNA synthesis, mitosis, and cell multiplication, RNA synthesis, protein synthesis, and survival of cells (*6*). Fractionation of fetal calf serum has revealed the presence of fetuin and other α-globulins that are important for attachment and growth-promoting activity, as well as smaller molecular weight components, such as nonsuppressible insulin-like activity, multiplication activity, epidermal growth factor, and the somatomedins (*1*, *7*). Fetal calf serum, chick embryo extract, and rooster serum have been used as sources of these components in pancreatic endocrine cell culture systems (*8*).

The major advantage conferred by the use of homologous serum is either its ability to maintain the integrity of the islet capsule or to inhibit islet attachment. The result is a preparation suitable both for the relatively short-term maintenance of islets prior to their transplantation, and for the long-term maintenance of islets for use in extended physiologic studies.

When newly isolated islets were exposed to a medium that contained heterologous serum (FCS), the majority of the islets anchored rapidly, capsular integrity was quickly interrupted, and the production of endocrine cell monolayers followed. Here again those monolayer replicates maintained with HCS secreted significantly more insulin, but not glucagon, during the first 2 weeks in culture as compared to the replicates maintained with FCS, although this advantage disappeared during the third week of culture. This suggests that a further favorable factor is present in homologous serum. During the fourth week in culture, the monolayers of both HCS and FCS replicates disintegrate almost simultaneously. Nevertheless, the monolayer remains a useful preparation for morphologic, histochemical, and immunofluorescent studies.

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4. Antiserum provided by Dr. Roger Unger; 30K is a specific pancreatic glucagon antiserum fraction derived from rabbit antiserum to beef-pork glucagon.
5. The antiserum to insulin was used at a final dilution of 1 : 600,000. Porcine insulin was used as a standard, and bound and free insulin were separated by charcoal. Identity of reaction between human and porcine insulin with this antiserum has been demonstrated. Dilution studies utilizing the media showed identity of displacement curves with the standard. Fractionation of the media on Sephadex G-100 indicated that more than 95 percent of the immunoreactive insulin was found in the same fractions as the ^{125}I-labeled porcine insulin. To assess the synthesis of insulin, 200 μc of [4,5-^{3}H]leucine (specific activity of 30 to 50 c/mmole; New England Nuclear) were added to the medium of islets maintained in culture as long as 60 days. After 24 hours under the usual conditions, the medium was removed, dialyzed against 0.05*M* phosphate buffer, *p*H 7, for 24 hours at 4°C, and then fractionated on columns of Sephadex G-100 (24 by 3 cm) at room temperature. Fractions corresponding to the second peak were combined and reacted with antiserum to insulin for 24 hours. (The amount of antiserum added is sufficient to bind 80 percent of ^{125}I-labeled insulin in the presence of 5 milliunits of insulin.) The reaction mixture was refractionated on Sephadex G-100, and 30 to 50 percent of the radioactivity of the second peak appeared in the bound form. This suggests synthesis of hormone rather than release of preformed insulin due to islet destruction.
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Direct Evidence for a Bone Marrow Origin of the Alveolar Macrophage in Man

Abstract. *Alveolar macrophages were obtained from 23 patients who had received marrow transplants for hematologic disorders. The presence of a Y body in macrophages of male origin was demonstrated by fluorescence microscopy. In those patients with a marrow donor of opposite sex the alveolar macrophages were shown to be of donor origin. The disappearance with time of host macrophages indicates a life-span, under the conditions, of approximately 81 days.*

Several studies of marrow transplantation in rodents (*1*) and one in the dog (*2*) have provided evidence that the lung macrophages derive from a bone marrow precursor. Other investigators, however, indicate that mouse alveolar macrophages may proliferate actively in vitro (*3*) and organ culture studies suggest that alveolar macrophages can be produced in vitro from a cell in the interstitium of the lung (*4*). In man, pulmonary macrophages exhibit a low but definite level of scheduled DNA synthetic activity in vitro, suggesting that they also

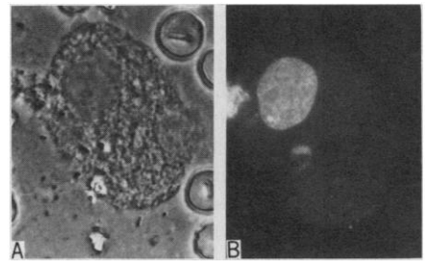

Fig. 1 (above). (A) Alveolar macrophage from a female patient given male bone marrow observed under phase contrast microscopy (×540). (B) The same macrophage observed under fluorescence microscopy. The Y body is the bright round spot at 7 o'clock in the cell nucleus (×540).

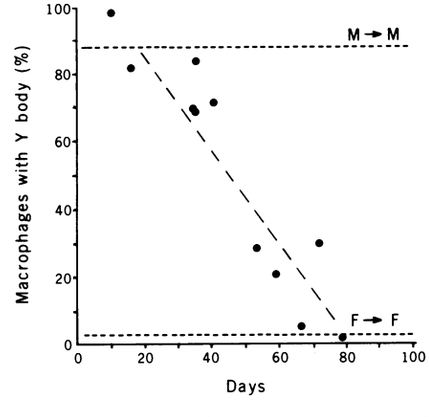

Fig. 2 (right). The dashed line $M \rightarrow M$ represents the average percentage of macrophages with a Y body in male to male transplants, and the dashed line $F \rightarrow F$, the average in female to female transplants. The closed circles represent the percentage of macrophages with a Y body in female to male transplants observed at various times after transplantation. The sloping dashed line was calculated by the method of least squares.