states, and it may be the adaptation to these conditions that enables organisms to survive in the harsh environments of both of the endolithic desert habitats.

The finding of endolithic blue-green algae in the dry valley region provides direct visual evidence of the presence of indigenous microbial life in this area and, so far as we can determine, also constitutes the first record of primary producers in the Antarctic cold desert ecosystem (*11*). We suggest that, beyond the relevance of our finding to terrestrial biology, scientists making future efforts to detect life in extreme planetary (for example, martian) environments, which have so far centered exclusively on soft soils (*12*), should also consider the interiors of exposed rocks as possible substrates for pioneer life forms.

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Placental Localization of Human Pregnancy–Associated Plasma Proteins

Abstract. *Frozen sections of human placenta were examined for the presence of four human pregnancy proteins, pregnancy-associated plasma proteins A and C (PAPP-A and PAPP-C), human chorionic somatomammotropin (hCS), and pregnancy zone protein (PZP), by the indirect immunofluorescence technique. Monospecific rabbit antiserums to PAPP-A, PAPP-C, and hCS all stained the trophoblast cytoplasm equivalently in a continuous layer, suggesting that the same trophoblast cells synthesize all three pregnancy proteins. In contrast, PZP was localized in blood vessel walls, parenchymal structures within the villous, as well as in the trophoblast cytoplasm. Its distribution in the latter was relatively inhomogeneous, tending to be more intense on the basement membrane side.*

Our laboratory has demonstrated that in addition to human chorionic somatomammotropin (hCS) [also called human placental lactogen (hPL)], three other proteins, which are not seen in plasma of nonpregnant women, men, or cord blood, become detectable by immunodiffusion methods in plasma during late pregnancy (*1, 2*). These pregnancy-associated plasma proteins (PAPP-A, -B, and -C) were distinct from each other immunologically and physicochemically (*2, 3*). They were unrelated to numerous other pregancy-associated hormones, enzymes, or antigens (for example, prolactin, human chorionic gonadotropin, placental alkaline phosphatase, oxytocinase, α_1-fetoprotein, and carcinoembryonic antigen), as well as to the pregnancy zone protein (PZP). The latter is a pregnancy-associated protein which has been studied under ten different names (*3–5*). The sharp increase of PAPP-A, -B, -C, and hCS during the latter portion of pregnancy and their rapid disappearance after birth have been documented (*6, 7*), as well as certain differences in their biological behavior from that of PZP (*8*).

In previous studies, analysis of saline extracts of placenta demonstrated that all of the PAPP's are present in placental tissue in amounts significantly greater than the quantities attributable to their maternal blood content (*9*). In sharp contrast, all of the PZP found in the placental extracts was apparently derived from the maternal blood there. Because this evidence suggests that the PAPP's are synthesized in the placenta, we felt that immunofluorescence analyses should help to clarify the cellular localization of each PAPP. As a follow-up of preliminary data (*10*), the present report documents findings on human placental tissue sections when monospecific antiserums to PAPP-A, PAPP-C, hCS (hPL), and PZP were used. Since monospecific antiserum to PAPP-B is not yet available, it could not be investigated at this time.

Five placentas were obtained at the time of term delivery of normal Caucasian and Negro infants at the Jackson Memorial Hospital, Miami, Florida. Venous blood from each of the respective mothers was also obtained, the serum being harvested and stored at −20°C until use. Placental tissues were first rinsed with cold 0.01*M* phosphate-buffered saline (PBS) (*p*H 7.1) to remove blood clots from the surface. Small pieces of each placenta were cut from the villous side and frozen on Dry Ice or in a mixture of isopentane and Dry Ice. These frozen tissue blocks were wrapped in aluminum foil and stored at −50°C. Sections of each block were cut at a thickness of 2 to 4 μm with a cryostat. (They were air dried, since preliminary studies revealed that acetone fixation resulted in blurred patterns.) Sections were sequentially treated at room temperature for 1 hour with monospecific rabbit antiserum to each protein under study, rinsed with PBS twice (each 5 to 10 minutes), stained with fluorescein-labeled goat antibody to rabbit immunoglobulin (1 hour), rinsed with PBS as above, and mounted in a 50 percent solution of glycerine in PBS. Stained sections were examined with a Leitz Ortholux microscope with a dark-field condenser and a 200-watt ultra-high–pressure mercury lamp (HB-200) and were photographed with a Polaroid camera. Controls for specificity of the staining included parallel sections treated with (i) unlabeled goat antibody to rabbit immunoglobulin in place of labeled antibody; (ii) normal rabbit immunoglobulin, as well as rabbit antiserums to human albumin, immunoglobulin M, immunoglobulin A, and transferrin, in place of the monospecific antiserum to the PAPP's and PZP; and (iii) absorption of antiserum to each PAPP with plasma from pregnant or nonpregnant women prior to use.

Monospecific rabbit antiserums to PAPP-A, PAPP-C, hCS (hPL or PAPP-D), and PZP immunoglobulin reagents were prepared as described (*3*). All were rendered monospecific by absorption with plasma from nonpregnant women. They also reacted only with their respective protein in plasma from pregnant women and in placental tissue extracts, and not with plasma from nonpregnant women or extracts of normal human heart, liver, kidney, and pancreas. A pool of preimmunization serums from the rabbits was processed as the control normal rabbit immunoglobulin, in which

Table 1. Pregnancy protein level in the maternal venous plasma of five placental donors. Protein was measured by the rocket electroimmunodiffusion methods, with a fourfold concentrate of a pool of plasmas from ten women in the third trimester of pregnancy as a reference system. The concentration of each pregnancy protein in this reference concentrate was arbitrarily given a value of 100 units per milliliter.

Donor placenta	Concentration (unit/ml) in maternal plasma			
	PAPP-A	PAPP-C	hCS (hPL)	PZP
230-1-1	7	48	27	2
230-1-2	60	50	51	35
230-1-3	47	56	70	9
230-1-4	81	44	45	6
230-1-5	6	36	31	18

the same procedures were used as in the preparation of the monospecific antibodies. (Rabbit antiserums to albumin, transferrin, immunoglobulin A, and immunoglobulin M were purchased from Behring Diagnostics; goat antibodies to rabbit immunoglobulin with and without fluorescein labeling were obtained from Sylvana.) The placental extracts were prepared as described (*9*). Rabbit and goat antibodies were used undiluted or at a 1:3 dilution, without critical differences being noted. The goat antibodies were absorbed with mouse or human liver powder, or both, to remove nonspecific reactions (*11*).

Monospecific antiserums to PAPP-A, PAPP-C, and hCS (hPL or PAPP-D) all reacted strongly and rather evenly with the cytoplasm of the villous trophoblast cells, each demonstrating a continuous fluorescent layer (Fig. 1). Other placental tissue components, as well as the nuclei of the trophoblast cells, were essentially unstained. It could not be ascertained whether staining was limited to the syncytiotrophoblast, since the cytotrophoblast cells in term placentas are so greatly reduced in size and number. Antiserums to PAPP-C and hCS gave brighter and sharper patterns than did antiserum to PAPP-A.

Sections treated with antiserum to PZP showed fluorescence in two major tissue components—blood vessels and trophoblast cells—but additional irregular streaks of fluorescence were seen in the villous connective tissue (see Fig. 2). Fluorescence of the blood vessels appeared as several concentric wavy rings, being brighter near the intima. The cytoplasmic trophoblast stained with antiserum to PZP was less homogeneous than that seen with antiserums to the PAPP's, often being discontinuous and brighter at the basement membrane or villous surface, or both.

Treatment of sections with antiserums to albumin, transferrin, immunoglobulin A, and immunoglobulin M produced relatively diffuse patterns of irregular fluorescence (as in Fig. 3). The fluorescence seen with antibodies to the normal plasma proteins in many cases was appreciably brighter than the fluorescence seen with antiserums to the PAPP's.

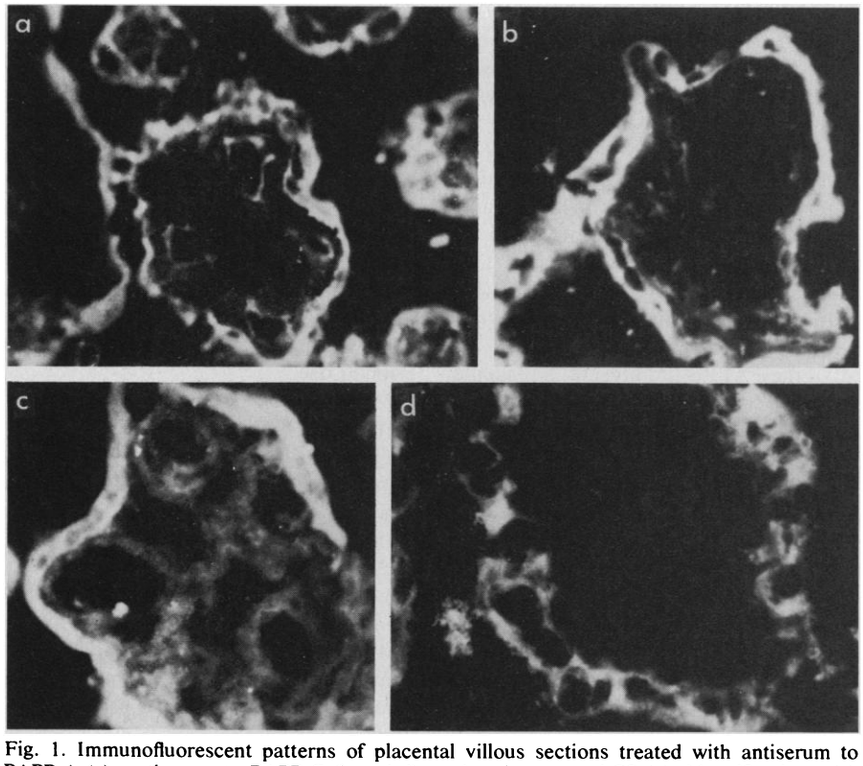

Fig. 1. Immunofluorescent patterns of placental villous sections treated with antiserum to PAPP-A (a), antiserum to PAPP-C (b and d), and antiserum to hCS (c). Magnifications: (a) × 340, (b) and (c) × 350, and (d) × 625.

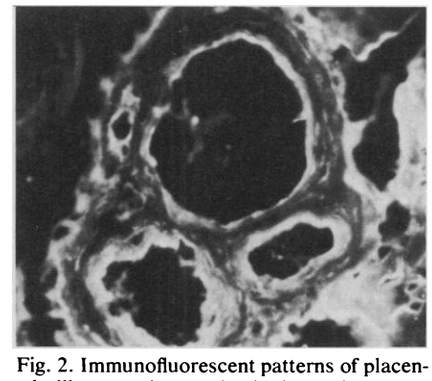

Fig. 2. Immunofluorescent patterns of placental villous sections stained with antiserum to PZP (× 340).

Because of large variations in the concentrations of these pregnancy proteins in maternal plasma (*6*, *12*), placental specimens from five individuals were examined, but no differences were found in the fluorescence patterns observed. Plasma samples from these women at delivery all contained the pregnancy proteins at readily measurable levels. However, the highest levels of PAPP-C and hCS were about twice the lowest value, while the highest levels of PAPP-A and PZP were ten to 20 times greater than the lowest one (Table 1). In spite of this wide range in the levels of pregnancy protein, the placental immunofluorescence patterns were undistinguishable, even for PAPP-A and PZP.

Placental tissue sections that were exposed to antiserums to the PAPP's and then to unconjugated goat antibody to rabbit immunoglobulins showed no fluorescence, indicating no appreciable autofluorescence of the tissue preparations. Direct application of fluorescein-labeled goat antiserum to rabbit immunoglobulin to placental sections, without prior treatment with rabbit antibody, rendered the superficial villous surface very faintly fluorescent in the form of a thin line; other parts of the villi were not fluorescent.

Tissue sections treated with normal rabbit immunoglobulin, especially at concentrations two- to threefold that found in serum, revealed only dull fluorescence in the trophoblast, as well as in larger blood vessels, after application of fluorescein-labeled goat antibody.

The trophoblast-restricted fluorescent

patterns seen with antibody to PAPP-A, PAPP-C, and hCS were abolished when the antibody had been previously absorbed with plasma from pregnant women. Completeness of absorption was confirmed by immunodiffusion tests. Absorption with plasma from nonpregnant women and those taking contraceptives failed to remove the activity of antiserum to PAPP, and concomitantly left the immunofluorescence patterns essentially unchanged. The PZP pattern was greatly reduced in intensity when antiserum to PZP had been absorbed with PZP-containing plasma from women taking contraceptives. Use of antiserum to PZP absorbed with plasma from women taking contraceptives resulted in fluorescence slightly weaker than with untreated antiserum to PZP.

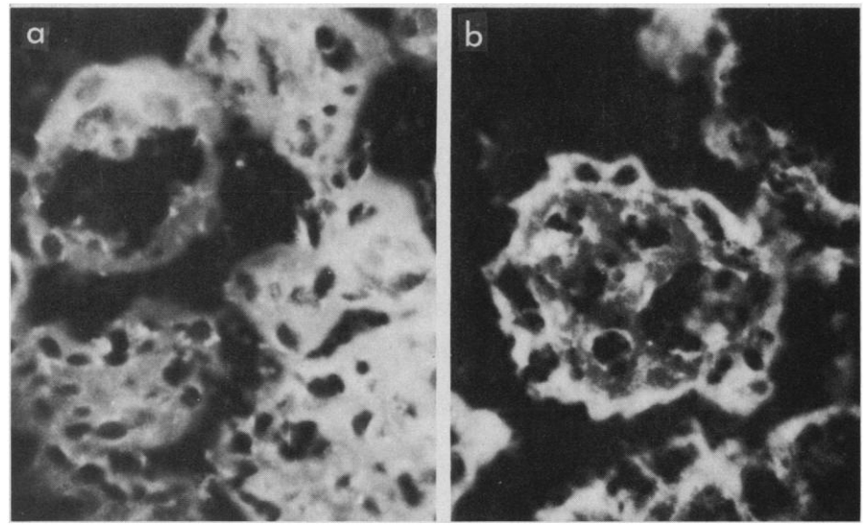

Fig. 3. Immunofluorescent patterns of placental villous sections treated with antiserum to albumin (a) and antiserum to transferrin (b) (× 410).

The present study revealed that PAPP-A and PAPP-C, as well as hCS, are localized in the trophoblast, and not in other portions of the placental villi. It also confirms the observations of other investigators which indicate that hCS is localized in and synthesized by the trophoblasts (*13, 14*). Since these three different proteins of pregnancy showed the same distribution, it seems probable that all trophoblast cells are active in their production, probably simultaneously. Although the syncytiotrophoblast was clearly rich in these proteins, we could not determine whether the cytotrophoblast was as well. Our data support a brief independent report by Tatarinov *et al.* (*15*), who showed that the pregnancy-specific β-glycoprotein (identical to PAPP-C) was localized in trophoblasts. Their examination of placentas obtained at 6 to 12 weeks of pregnancy suggested that the cytotrophoblast contains less PAPP-C than does the syncytiotrophoblast.

Human chorionic somatomammotropin [hCS (hPL or PAPP-D)] was discovered independently by Ito and Higashi in 1961 and Josimovich and MacLaren in 1962 (*16*), and it has since been studied by many investigators, although its primary biological function remains to be elucidated (*14, 17*). In previous studies from our laboratory, it was shown that PAPP-A, PAPP-C, and hCS had similar kinetics during gestation and the postpartum period (*6, 7*) and that none are detectable in cord blood by the gel diffusion methods used. The present study revealed that they also share a similar distribution in placental tissue.

The topographical distribution of trophoblasts seems to favor the secretion of hCS, PAPP-A, and PAPP-C into the intervillous space rather than through basement membrane and villous stroma into the umbilical cord and fetal circulation. At any rate, all available data seem to indicate that PAPP-A and PAPP-C, like hCS, are synthesized by the trophoblasts and are released primarily into the maternal circulation.

Our unpublished quantitative studies of amniotic fluid from six women at term showed that the hCS concentrations in these specimens amounted to 10 percent of that in maternal plasma, while PAPP-A and PAPP-C were (if present at all) found in the amniotic fluid at levels less than 1 percent of the maternal values. This apparent difference between hCS and the other PAPP's (A and C) may be due to the differences in their molecular weights, both PAPP-A (m.w., 750,000) and PAPP-C (m.w., 110,000) being appreciably larger than hCS (m.w., 20,000).

The significance of the staining pattern for PZP is not clear. Its apparent partial localization in trophoblast cells suggests that it may be performing some local function, although our previous data indicated that all the PZP in the placenta is derived from the maternal blood in the placenta. It will be necessary to determine whether other tissues also contain PZP in similar patterns, since Stimson and Blackstock (*18*) have reported that leukocytes may synthesize this protein.

Another fact that must be considered regarding the localization and function of PZP in pregnancy is the demonstration that PZP analogs in some species of monkey are present at high concentrations prior to conception and actually decrease during gestation. Bohn and Weinmann (*19*) first noted this phenomenon in cynomolgus monkeys. We have confirmed such a decrease in PZP in three additional species of Old World monkeys, as well as in one species of New World monkey (*8*). On the other hand, we have found that the gestational kinetics of PZP in two species of apes (chimpanzee and orangutan) was similar to that in humans, although the serum concentrations of PZP in the chimpanzee reached levels three to five times higher than those seen in humans during late pregnancy.

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Long-Term Survival of Skin Allografts in Mice Treated with Fractionated Total Lymphoid Irradiation

Abstract. *Treatment of recipient Balb/c mice with fractionated, high-dose total lymphoid irradiation, a procedure commonly used in the therapy of human malignant lymphomas, resulted in fivefold prolongation of the survival of C57BL/Ka skin allografts despite major histocompatibility differences between the strains (H-2^d and H-2^b, respectively). Infusion of 10^7 (C57BL/Ka × Balb/c)F_1 bone marrow cells after total lymphoid irradiation further prolonged C57BL/Ka skin graft survival to more than 120 days. Total lymphoid irradiation may eventually prove useful in clinical organ transplantation.*

The immunosuppressive effects of ionizing radiation have been well known since the beginning of the century (*1*). Treatment of prospective allograft recipients with whole body radiation (WBR) became customary in organ transplantation studies of both laboratory animals and humans. However, the high dose of WBR required to prevent allograft rejection in animals with major histocompatibility differences produced severe injury to the bone marrow and gut (*2, 3*). By 1965, almost all clinical centers had ceased using WBR to prepare patients for kidney transplantation (*2–5*). Nevertheless, extensive research was devoted to overcoming the adverse consequences of radiation by limiting its effects specifically to immunocompetent lymphoid cells. These experimental procedures included local irradiation of the graft site (*4*), local irradiation of the allotransplant (*2–4*), intralymphatic administration of radioactive isotopes (*3–6*), intravascular implantation of a high-energy beta-emitting source (*4, 7*), and extracorporeal irradiation of blood (*2–5, 8–10*) and lymph (*2–5, 8, 11*). Technical difficulties, primarily in estimating the distribution of the radiation dose in the tissues, and the limited success in prolonging allograft survival have restricted the clinical use of these techniques (*3–5*).

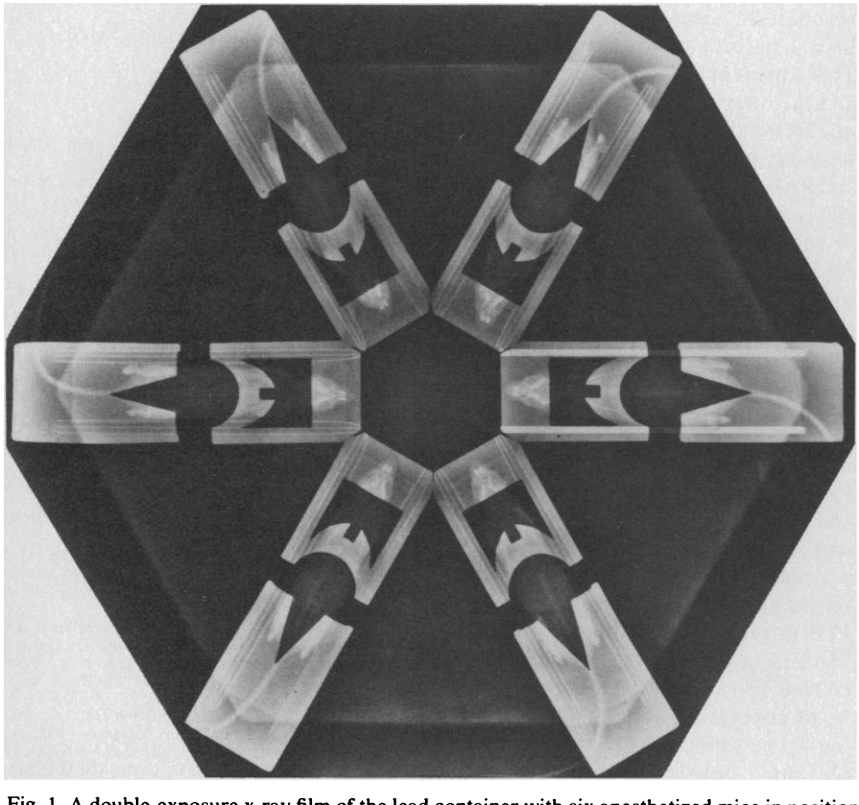

Fig. 1. A double-exposure x-ray film of the lead container with six anesthetized mice in position for irradiation.

We have studied the effect of fractionated high-dose total lymphoid irradiation (TLI) on the survival of skin allografts between mice with major histocompatibility differences. In humans, TLI has been used extensively for treating malignant lymphomas (*12*). High, cumulative doses of irradiation to the lymphoid tissues can be used without causing the severe side effects of WBR, by selectively localizing the radiation to lymphoid tissues and fractionating the total radiation dose. In humans, TLI is immunosuppressive (*13*). If the radiation is selectively restricted to lymphoid tissues and the total dose is fractionated, high cumulative doses of irradiation do not have the severe side effects of WBR.

Adult (8-month-old) Balb/c (H-2^d) male and female mice were anesthetized with pentobarbital and positioned in an apparatus designed to expose the major lymphoid organs to x-rays but to shield the skull, chest (lungs, heart, ribs, and dorsal spine), hind legs, and tail (Fig. 1). Radiation was administered with a Phillips x-ray unit (250 kv at 15 ma, 40 r/min) 60 cm from source to skin; filter, 0.25-mm Cu plus 1.0-mm Al. The dosimetry was verified with a calibrating ionization chamber and by lithium fluoride thermoluminescence dosimeters. All mice received 200 r/day five times a week to a total dose of 3400 r. One day after completion of irradiation, full-thickness skin from C57BL/Ka (H-2^b) mice was grafted onto the flank of the Balb/c recipients (*14*). Grafts were checked daily and considered rejected when they had completely sloughed off or an eschar had developed. The mean survival time of C57BL/Ka skin grafts on 12 multiply anesthetized but not irradiated Balb/c control mice was 10.7 days (range, 10 to 13 days). The mean survival time of skin allografts on 16 mice receiving TLI was 49.1 days (range, 35 to 67 days) (Table 1). Full hair growth was noted on all experimental grafts at about 20 to 25 days. The combination of TLI and a single intravenous injection of 1×10^7 (Balb/c × C57BL/Ka)F_1 bone marrow cells on the day of skin grafting resulted in long-term acceptance (>120 days) of skin by seven of eight recipients. Although