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This study shows that sphingosine-1-phosphate (SPP) significantly inhibits melanin synthesis in a concentration-dependent manner, and that the activity of tyrosinase was also reduced in SPP-treated cells. In contrast, a specific extracellular signal-regulated protein kinase (ERK) pathway inhibitor, PD98059 increased tyrosinase activity and melanin production, and PD98059 restored the reduced tyrosinase activity and pigmentation induced by SPP. We also found that SPP induces the sustained activation of ERK and the subsequent degradation of microphthalmia-associated transcription factor (MITF), which plays a key role in melanogenesis. Thus, we further studied the relationship between the ERK pathway and melanin synthesis. PD98059 was found to prevent the MITF phosphorylation and degradation induced by SPP and to abrogate reduced tyrosinase and tyrosinase-related protein 1 (TRP1) production by SPP. These results indicate that the ERK pathway is potently involved in the melanogenic signaling cascade, and that SPP-induced ERK activation contributes to reduced melanin synthesis via MITF degradation.

[PA1-71] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

A Collaborative Study to Establish a Korea National Biological Standard for Antithrombin Concentrate

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We have carried out collaborative study to evaluate a preparation of antithrombin concentrate whether or not it was suitable to serve as the candidate for a Korea National Biological Standard. Six laboratories, including three manufacturers and three National Control Laboratories, participated in this study. The potency of this candidate preparation was determined using the heparin cofactor chromogenic method. The method is described in the Minimum Requirements for Biological Products and the European Pharmacopoeia. The candidate gave excellent intra- and inter-laboratory correlations when assayed against the second international standard for antithrombin concentrate, coded as 96/520. The participants contributed data from a total of 88 assays and the results were accepted as statistically valid when the outcome of the analysis was for linearity of dose-response relationships and for intersection at a common point at zero dose in slope-ratio model. Combined potency estimates were obtained by taking geometric means of results from all assays at each laboratory, and overall potency estimates were calculated as geometric means of results from all laboratories. The results were expressed in the form of histograms and 95% confidence intervals. Based on the results of the collaborative study described here, the candidate reference standard is judged to be suitable to serve as the Korea National Biological Standard for antithrombin concentrate.

[PA1-72] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

A NAT for reliable HBV DNA Screening of Plasma

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The safety of blood and blood products is ensured by careful selection of donors, screening of

donated blood and the use of validated viral inactivation and/or removal steps during the manufacture of blood products. Serologic screening procedures have substantially reduces the risk of transmission of blood-borne viruses. However, there are still residual risks despite these measures due to the inclusion of 'window period' donations. To reduce the durations of the window period, nucleic acid amplification technology (NAT) is being instituted in Europe, Japan and the United States. Standardization of NAT assay is necessary before the introduction of such an assay for routine screenings of blood and blood products and can be achieved by use of international standard of HBV DNA for NAT assays. Validation characteristics are described as specificity, detection limit and robustness. The specificity was established by studying conditions that might be expected to cause cross-reactivity or interference in the analysis. The conditions that cause cross-reactivity or interference by other relevant blood-borne viruses include human immunodeficiency virus(HIV), hepatitis C virus(HCV), hepatitis A virus(HAV) and parvovirus B19 and other human DNA viruses including human papilloma virus(HPV) 18 & 6b, cytomegalovirus(CMV) and human herpesvirus 1 & 2. In order to validate the specificity of the analytical procedure, at least 100 HBV DNA-negative plasma pools were tested and shown to be non-reactive. To determine the positive cut-off point, a diluted series of the WHO HBV international standard (97/746) were tested under these conditions, and the detection limit was calculated to be approximately 50 IU/mL. To demonstrate robustness, at least 20 HBV DNA negative plasma pools, and spiked with HBV DNA to a final concentration of 3 times the previously determined 95% cut-off value, were tested and found positive.

[PA1-73] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

Temperature Regulates Melanin Synthesis in Mel-Ab Cells

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Temperature change is one of the major environmental factors to influence human skin. However, the relationship between temperature and melanogenesis has received little attention. In the present study, we investigated the effects of temperature change including heat shock on melanogenesis using a mouse melanocyte cell line, Mel-Ab. Our results demonstrated that cells maintained at 37°C showed maximal melanin synthesis. Cells cultured at low temperatures produced less melanin than cells at 37°C. Heat treatment for 1 h also decreased melanin production. The melanin production is accompanied by tyrosinase activity at each temperature, indicating that the tyrosinase activity is regulated by temperature. To examine how heat shock decreases melanin synthesis, we treated cell with suramin (an inhibitor of growth factor receptors) or N-acetyl-L-cysteine (a free radical scavenger) before heat shock. However, neither suramin nor N-acetyl-L-cysteine restored heat-induced depigmentation. It has been reported that activated ERK is responsible for MITF degradation, leading to decreased melanin synthesis. Our results showed that heat shock induces sustained ERK activation, which may inhibit melanogenesis.

[PA1-74] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

Characterization of Human Epidermal Stem Cells and Living Skin Equivalents

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Human epidermal keratinocytes consist of stem cells, transit amplifying cells, and postmitotic differentiating cells. Among them, stem cells play a critical role in cell renewal, wound healing, and neoplasia. However, till now, specific markers of human epidermal keratinocytes are not clearly defined. In the present study, we separated putative stem cells from other cells using fluorescence activated cell sorting (FACS), based on differences in a6-integrin and CD71 expression. We next analyzed keratinocytes obtained from young and old donors. We found that stem cell portion