EXHIBIT 2

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(19) (CA) **CANADIAN PATENT** (12)

(54) Production of Erythropoietin

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(73) Kirin-Amgen, Inc., U.S.A.

(30) (US) U.S.A. 561,024 1983/12/13
(US) U.S.A. 582,185 1984/02/21
(US) U.S.A. 655,841 1984/09/28
(US) U.S.A. 675,298 1984/11/30

(57) 3 Claims

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both) resulted in substantially homogeneous products having essentially identical molecular weight characteristics.

Purified human urinary EPO and a recombinant, CHO cell-produced, EPO according to the invention were subjected to carbohydrate analysis according to the procedure of Ledeen, et al. Methods in Enzymology, 83(Part D), 139-191 (1982) as modified through use of the hydrolysis procedures of Nesser, et al., Anal.Biochem., 142, 58-67 (1984). Experimentally determined carbohydrate constitution values (expressed as molar ratios of carbohydrate in the product) for the urinary isolate were as follows: Hexoses, 1.73; N-acetylglucosamine, 1; N-acetylneuraminic acid, 0.93; Fucose, 0; and N-acetylgalactosamine, 0. Corresponding values for the recombinant product (derived from CHO pDSVL-gHuEPO 3-day culture media at 100 nM MTX) were as follows: Hexoses, 15.09; N-acetylglucosamine, 1; N-acetylneuraminic acid, 0.998; Fucose, 0; and N-acetylgalactosamine, 0. These findings are consistent with the Western blot and SDS-PAGE analysis described above.

Glycoprotein products provided by the present invention are thus comprehensive of products having a primary structural conformation sufficiently duplicative of that of a naturally-occurring erythropoietin to allow possession of one or more of the biological properties thereof and having an average carbohydrate composition which differs from that of naturally-occurring erythropoietin.

EXAMPLE 11

The present example relates to the total manufacture by assembly of nucleotide bases of two structural genes encoding the human species EPO sequence of Table VI and incorporating, respectively "preferred" codons for expression in E.coli and yeast (S.cerevisiae) cells.