

## 5

AB, Sweden) at a cell density of  $0.4$  to  $0.6 \times 10^6$  vc/ml with a viability of  $>90\%$  and incubated at  $37^\circ\text{C}$ . in an atmosphere of  $5\%$  carbon dioxide in air in spinner culture flasks. After 2 days the total cell number and viability were determined. The cells were then resuspended in the corresponding cell culture medium and incubated for a further 2 days, and the total cell number and viability determined daily. The population doubling time (dt) was determined for each culture. The results are shown in FIG. 2 and Table 1 below. No significant difference was seen in the growth characteristics of the cells in media containing the different supplements.

TABLE 1

Growth rate	dt (h)
HSA	20.7
Dextran T10 (Mw 10,000)	20.9
Dextran T40 (Mw 40,000)	23.7
Dextran T70 (Mw 70,000)	19.4
Ficoll 70 (Mw 70,000)	22.2
Ficoll 400 (Mw 400,000)	29.3

## EXAMPLE 4

## Production of rVIII

CHO cells containing the gene encoding rVIII SQ (as for Example 1) were cultivated in serum-free medium containing HSA. Cells were then transferred into medium in which HSA was replaced by Dextran T10, Dextran T40, Dextran T70, Ficoll 70 or Ficoll 400 (all from Pharmacia Biotech AB, Sweden) at a cell density of  $0.4$  to  $0.6 \times 10^6$  vc/ml with a viability of  $>90\%$  and incubated at  $37^\circ\text{C}$ . in an atmosphere of  $5\%$  carbon dioxide in air in spinner culture flasks. After 2 to 3 days the cell number increased to approximately  $1.2$  to  $2.0 \times 10^6$  vc/ml with a viability of  $>90\%$ . rVIII activity for each culture was determined regularly using the Kabi Coatest Factor VIII kit (Pharmacia AB, Sweden). The results are shown in FIG. 3. rVIII was produced in each of the media containing the various polysaccharide supplements. It was found that the rVIII activity in medium containing dextran T-70 and T-40 was comparable to that found with medium containing HSA. Lower levels of rVIII were found, however, in medium supplemented with the other polysaccharide compounds.

## EXAMPLE 5

## Cultivation in stirred tank bioreactor

In order to show that serum-free medium in which HSA had been replaced with a polysaccharide compound was also applicable to cultivation in a stirred tank bioreactor where the cells were known to be exposed to greater shear forces the following was performed. CHO cells containing the gene encoding rVIII SQ (as for Example 1) were cultivated in serum-free medium containing Dextran T-70 in replacement for HSA in spinner flasks (as described in Example 2 above). The cells were then transferred into a 2L working volume stirred tank bioreactor (Belach AB, Sweden) at a cell density of  $0.6$  to  $0.9 \times 10^6$  vc/ml in 2L of fresh serum-free media containing Dextran T-70 ( $M_w$  70,000) in replacement to HSA and cultivated for 12 days. The bioreactor cultivation was compared with that in a corresponding bioreactor in which the CHO cell was cultivated in serum-free media

## 6

containing HSA under the same conditions. No significant difference was seen between the growth characteristics in a stirred tank bioreactor in medium in which HSA was replaced with Dextran T-70 to that seen where the medium contained HSA, as is shown in FIG. 4. This bioreactor cultivation also confirmed that rVIII was produced.

In Examples 1 to 5 above corresponding results were obtained where Dextran T-40 and T-70, respectively, were replaced by the corresponding pharmaceutical grade products.

We claim:

1. A method of cultivating mammalian cells expressing recombinant Factor VIII in a serum-free cell culture medium that requires the presence of human serum albumin (HSA) and which is essentially free from fatty acids, fatty acid esters and lipids, which comprises replacing HSA with at least one glucose or sucrose based polysaccharide comprising dextrans, partially hydrolyzed native dextran or Ficoll, and having an average molecular weight of from about 10,000 to about 450,000, with the exception of polycationic and polyanionic glucose or sucrose based polysaccharides.

2. The method according to claim 1 wherein said polysaccharide has an average molecular weight of from about 40,000 to about 400,000.

3. The method according to claim 2, wherein said mammalian cells are CHO cells.

4. The method according to claim 2, wherein said polysaccharide has an average molecular weight of from about 40,000 or about 70,000.

5. The method according to claim 1, wherein said mammalian cells are CHO cells.

6. The method according to claim 1, wherein the cells are cultivated in a suspension cell culture.

7. The method according to claim 6, wherein the cells are cultivated in a stirred tank bioreactor.

8. The method according to claim 1 wherein recombinant Factor VIII is an active truncated derivative of recombinant Factor VIII.

9. The method according to claim 8, wherein the active truncated derivative of recombinant Factor VIII is recombinant Factor VIII SQ (rVIII SQ).

10. A method of cultivating mammalian cells expressing recombinant Factor VIII in a serum-free cell culture medium that requires the presence of human serum albumin (HSA) and which is essentially free from fatty acids, fatty acid esters and lipids, which comprises replacing HSA with at least one glucose or sucrose based polysaccharide selected from dextran and copolymers of sucrose and epichlorhydrin, having an average molecular weight of from about 10,000 to about 450,000.

11. The method according to claim 10, wherein said dextran has an average molecular weight of from about 40,000 or about 70,000.

12. The method according to claim 10, wherein said polysaccharide has an average molecular weight of from about 40,000 to about 400,000.

13. The method according to claim 10, wherein said mammalian cells are CHO cells.

\* \* \* \* \*