J. Cell Sci. 42, 323–327 (1980)
Printed in Great Britain © *Company of Biologists Limited 1980*

SECRETION OF NEWLY SYNTHESIZED PROTEINS BY ISOLATED LIVER CELLS

R. J. VAN KOOIJ AND C. POORT
Laboratory of Histology and Cell Biology, State University Utrecht, Nicolaas Beetsstraat 22, 3511 *HG Utrecht, The Netherlands*

SUMMARY

Suspensions of isolated liver cells were incubated with [^{3}H]leucine as a precursor. The appearance of radioactive albumin and α_1-acid glycoprotein in the incubation medium was determined. After about 20 min both albumin and α_1-acid glycoprotein were secreted in a linear way. When fucose and galactose were used as radioactive precursors, time lags of about 10 min could be observed.

INTRODUCTION

In the hepatocyte, albumin and serum glycoproteins are synthesized. After intercellular transport from their site of synthesis these proteins are secreted into the serum. In *in vivo* experiments in rats it was found that, following an injection with radioactively labelled leucine, it takes about 16 min before radioactive albumin can be detected in the serum (Peters & Peters, 1972). Other investigators, using labelled sugars as precursors, found that radioactivity in macromolecular form can be detected earlier in the serum after the administration of the radioactivity: 5–6 min after galactose injection and 8 min after fucose injection (Bauer, Lukaschek & Reuther, 1974).

Experimental conditions can be kept better defined in an *in vitro* system than in an *in vivo* experiment. Moreover an *in vitro* system is preferable for more precise time measurements. Isolated hepatocytes are capable of synthesizing and secreting albumin and serum glycoproteins (Crane & Miller, 1974). In such a system a time lag between the addition of radioactive leucine and the appearance of labelled albumin in the medium was observed of about the same order as found *in vivo*, viz. 19–21 min (Feldhoff, Taylor & Jefferson, 1977).

We have used isolated hepatocytes to examine whether the times of transportation through the cell of 2 well defined secretory proteins: albumin, a non-glycosylated protein, and α_1-acid glycoprotein, a heavily glycosylated protein (Schmid *et al.* 1977) are different when [^{3}H]leucine is used as a radioactive marker. We used radioactive fucose and galactose to determine at what time before secretion these sugars are incorporated in the growing glycoprotein.

MATERIALS AND METHODS

Antisera

The method of Shibata, Okubo, Ishibashi & Tsuda (1977) was used to isolate α_1-acid glycoprotein from rat serum. After SDS-polyacrylamide gel electrophoresis of the final product only one band could be stained with Coomassie Brilliant Blue and with PAS. One precipitation line was formed with rabbit anti-rat serum (Rara ielfo, Nordic, Tilburg, Holland) on immunoelectrophoresis. This purified α_1-acid glycoprotein was injected in a rabbit for the generation of an antibody. Rabbit anti-rat albumin was purchased from Nordic, Tilburg, Holland.

Immunoprecipitation

The optimal precipitation conditions of rat serum albumin and purified α_1-acid glycoprotein with their antisera were determined. The secreted albumin and α_1-acid glucoprotein were precipitated by the addition of a constant amount of albumin and α_1-acid glycoprotein to a fixed quantity of medium of the cell suspension, followed by the addition of adequate volumes of the antiserum. Precipitates were washed twice with physiological saline, dissolved in 0·1 M formic acid and counted in a Liquid Scintillation Spectrometer (Packard Instruments). The amount of precipitated radioactivity was expressed in dpm/mg DNA.

Perfusion and cell isolation

Rat liver cells were obtained with the method of Berry & Friend, with some modifications. The liver of a rat (225–300 g) was perfused via cannulation of the portal vein *in situ* with calcium-free Hanks' Balanced Salt Solution (BSS) containing 0·5 mg/ml collagenase (Worthington, type II) and 10 mg/ml BSA. When the liver disintegrated, the tissue was minced, the suspension filtered through nylon cloth (mesh width 140 μm). A mixture of carbogen (95 % CO_2/5 % O_2) was bubbled through the suspension and the separated cells were washed twice with BSS and finally with Basal Medium Eagle. Viability of the cells was tested by trypan blue exclusion. As a rule, 85–98 % of the cells excluded a 0·5 % solution of trypan blue in 0·9 % NaCl.

Measurements

As a routine, cells were preincubated for 30 min before addition of the radioactive precursor in Basal Medium Eagle. As radioactive precursors were used L-[4,5-^{3}H]leucine (sp. radioact. 110 mCi/mmol), D-[1-^{14}C]galactose (10 mCi/mmol) and L-[1-^{3}H]fucose (25 mCi/mmol), obtained from the Radiochemical Centre, Amersham. At certain points of time aliquots of the suspension were taken out and the cells were spun down at 100 *g*. In the supernatant products were determined by immunoprecipitation; the DNA content of the pellet was determined according to the method of Burton (1956).

RESULTS AND DISCUSSION

A suspension of liver cells is able to synthesize and secrete proteins and glycoproteins. As no storage of exportable proteins occurs in the liver, a linear increase in amount of secreted product with time can be expected after a certain time lag. Indeed, radioactive albumin is found to be secreted in a linear way, 30 min after addition of radioactive amino acid (Feldhoff *et al.* 1977). Extrapolation of the obtained secretion slope to the time axis gave a 'secretion time' of about 20 min. We confirmed the findings on rat serum albumin. When we applied the same technique in the study of the secretion of α_1-acid glycoprotein, we obtained a similar pattern (Fig. 1). The non-glycosylated protein albumin and the heavily glycosylated α_1-acid glycoprotein appear to have the same secretion time. Accordingly we can conclude that attachment

of sugars to the growing glycoprotein during transport from RER via SER and Golgi system to the extracellular space does not prolong the transport time as compared to the time required for transport of a pure polypeptide.

In *in vivo* work of Bauer *et al.* (1974) it is supposed that the time of incorporation of galactose and fucose would differ. So it seemed of interest, if we could confirm this by

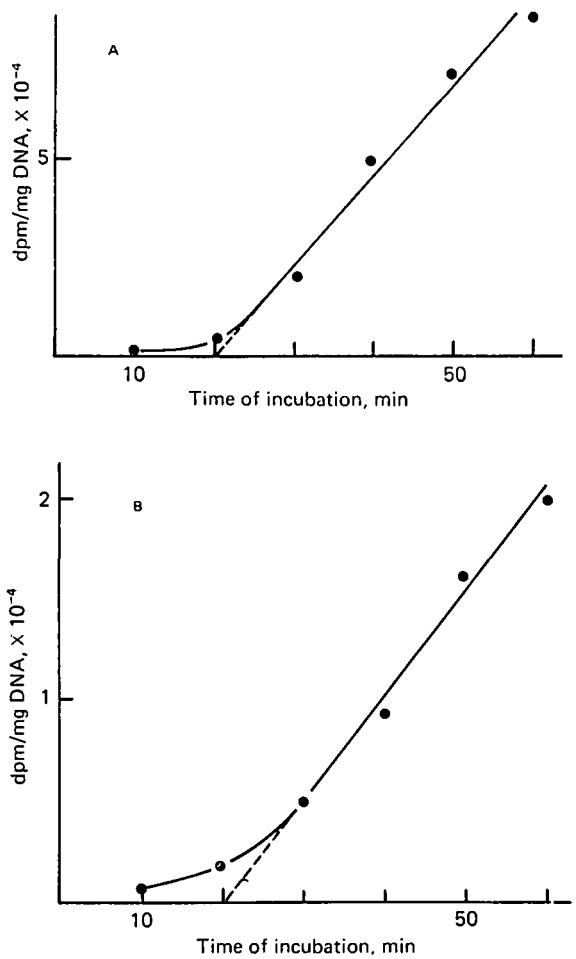

Fig. 1. A, typical secretion line of albumin with linearity after 30 min. B, the secretion line of α_1-acid glycoprotein linear after 30 min. Incubations in these experiments were performed in the presence of [^{3}H]leucine.

working here with a cell suspension and following a well defined single glycoprotein in its secretion.

The results of typical experiments are given in Fig. 2. A linear appearance with time of secreted α_1-acid glycoprotein was again observed. When these lines are extrapolated to the x-axis we obtain points of time of incorporation, expressed as min prior to secretion. Table 1 gives time lag data from the secretion experiments.

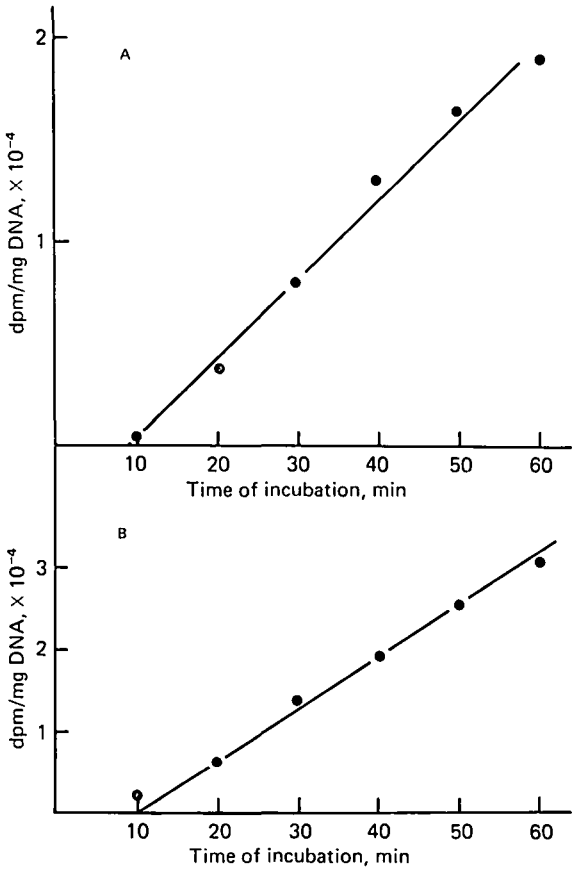

Fig. 2. Typical secretion lines of α_1-acid glycoprotein. The incubation medium in A contained [^{14}C]galactose and in B, [^{3}H]fucose.

Table 1. *Time lag data*

Precursor	Time lag ± S.D.	No. of experiments
[^{3}H]leucine	20·8 ± 1·8	6
[^{14}C]galactose	9·6 ± 1·1	4
[^{3}H]fucose	9·8 ± 1·2	4

That the incorporation of galactose and fucose takes place 9–10 min after the incorporation of leucine is in agreement with the fact that these sugars are added in the Golgi system (Molnar, 1975). Our results show a same point of time of incorporation of galactose and fucose in α_1-acid glycoprotein. These findings do not necessarily contradict those of Bauer *et al.*, as the latter determined the appearance of incorporated sugars in the whole mixture of glycoproteins synthesized *in vivo*. It is possible that not all glycoproteins, originating from different secreting glands, have the same secretion times.

REFERENCES

BAUER, C. H., LUKASCHEK, R. & REUTHER, W. G. (1974). Studies on the Golgi apparatus, cumulative inhibition of protein and glycoprotein secretion by D-galactosamine. *Biochem. J.* **142**, 221–230.

BERRY, M. N. & FRIEND, D. S. (1969). High-yield preparation of isolated rat liver parenchymal cells. *J. Cell Biol.* **43**, 560–520.

BURTON, K. (1956). A study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of DNA. *Biochem. J.* **62**, 315–323.

CRANE, L. J. & MILLER, D. L. (1974). Synthesis and secretion of fibrinogen and albumin by liver cells. *Biochem. biophys. Res. Commun.* **60**, 1269–1277.

FELDHOFF, R. C., TAYLOR, J. M. & JEFFERSON, L. S. (1977). Synthesis and secretion of rat albumin in vivo, in perfused liver and in isolated hepatocytes. *J. biol. Chem.* **252**, 3611–3616.

MOLNAR, J. (1975). A proposed pathway of plasma glycoprotein synthesis. *Molec. cell. Biochem.* **6**, 3–14.

PETERS, TH. & PETERS, C. (1972). The biosynthesis of rat serum albumin. IV. *J. biol. Chem.* **247**, 3858–3863.

SCHMID, K., NURNBERG, R. B., KIMURA, A., YAMAGUCHI, H. & BINETTE, J. P. (1977). The carbohydrate units of human plasma α_1-acid glycoprotein. *Biochim. biophys. Acta* **492**, 291–302.

SHIBATA, K., OKUBO, H., ISHIBASHI, H. & TSUDA, K. (1977). Rat α_1-acid glycoprotein: purification and immunological estimation of its serum concentration. *Biochim. biophys. Acta* **495**, 37–45.

(*Received* 9 *August* 1979)