that certain tumor cells produce their own growth factors which are secreted into the growth medium. In support of this hypothesis, EGF-like growth factors (termed sarcoma growth factors or transforming growth factors) have been isolated from culture fluids from a number of malignant cells (4, 5). Most of the cell lines which produce EGF-like factors bind reduced amounts of EGF (4, 5), perhaps due to interaction of the endogenously produced growth factors with EGF receptors.

However, loss of EGF requirement by all tumor cells is not readily explained in terms of production of endogenous factors. Cherington et al. (1) have shown that polyoma virus-transformed Syrian hamster cells no longer require EGF for growth in serum free medium, although polyoma virus transformed 3T3 cells bind normal amounts of EGF (6). Certain chemically transformed cells possess reduced numbers of EGF-receptors even though no EGF-like activity is present in culture supernatants (7) and SV40-transformed BHK cells produce factors which reduce EGF binding to normal cells without directly binding to the EGF-receptor (8).

Despite this general interest in growth factor interaction and neoplastic transformation, little is known about the role of growth factor systems in avian sarcoma virus (ASV)-transformed cells.

In this report EGF binding and EGF-dependent *in vitro* membrane protein phosphorylation are examined in ASV-transformed cells.

## Materials and Methods

Cell Cultures. 3Y1 normal rat cells, Rous sarcoma virus Schmidt-Ruppin subgroup, strain A (SRA) transformed 3Y1 cells, and the ts225 3Y1 line infected by a temperature sensitive mutant of Fujinami sarcoma virus were generously provided by Dr. H. Hanafusa (The Rockefeller University). The LA23 NRK line infected with a temperature sensitive mutant of Prague A Rous sarcoma virus was a gift from P. Vogt (University of Southern California). RR1022 Schmidt-Ruppin strain subgroup D transformed rat cells were from A. Goldberg (The Rockefeller University). All cells were grown in DME containing 10% calf serum (Flow Laboratories). For the

tion of the fructose 1,6-bisphosphatase (6). In the liver of the adult rat its level is high when glucose is abundant and low in the state of hypoglycemia.

This paper shows that Fru-2,6-$P_2$ is already present in the fetal liver. It undergoes significant changes which can be correlated to alterations in carbohydrate metabolism.

MATERIALS AND METHODS: Substrates and auxiliary enzymes were purchased from Boehringer (Mannheim, FRG). NADH was from VEB Arzneimittelwerk Dresden (GDR) and dicyclohexylcarbodiimide from Merck (Darmstadt, FRG). All other chemicals were of analytical grade.

The experiments were performed with rats of a Wistar albino strain. The animals were fed ad libitum and the gestational age was determined as previously described (7). Fetuses were delivered by caesarean section after ether anaesthesia of the dam. Fetuses of the -1st hour were artificially delivered, when natural delivery of the first one to three of its litter mates were finished. The whole birth process was assumed to proceed within approximately one hour. In the experiments in which the postnatal metabolic changes were studied the age of the newborns was precisely estimated from the time of natural birth.

The livers were removed and immediately freeze-clamped in liquid nitrogen.

For standardization of Fru-2,6-$P_2$ the compound was synthesized according to (8). It was determined with the pyrophosphate-dependent phosphofructokinase from potatoes by following the procedure recently published by Van Schaftingen et al. (9). The other metabolites were determined by means of established enzymatic procedures (10).

RESULTS AND DISCUSSION: Fig. 1 shows the levels of Fru-2,6-$P_2$ in rat liver during development. For comparison, the changes in the hepatic levels of glycogen, Glc-1-P and Fru-6-P are included. From the -6th to the -3rd day Fru-2,6-$P_2$ decreases and reaches a minimum on the -3rd day. Then it increases again transiently and shows a second minimum at birth. It remains comparatively low during suckling and increases after weaning to reach the level of the adult liver. In the adult, the hepatic level of Fru-2,6-$P_2$ was found at least four-fold higher than in the fetal stage. This may be due to differences in the enzymic patterns between the two developmental stages. In the liver of the adult Fru-2,6-$P_2$ is involved both in the control of glycolysis and gluconeogenesis, in the fetal liver however only its regulatory action on the very sensitively responding phosphofructokinase is required.

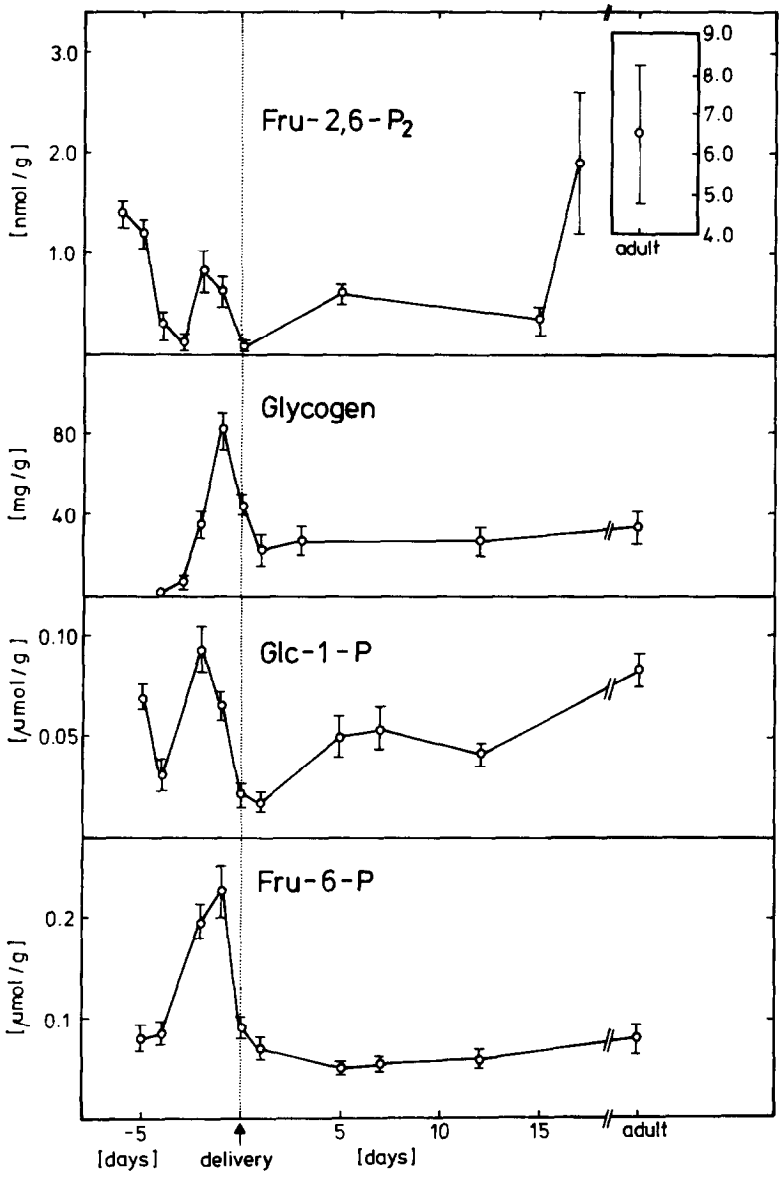


Fig. 1 Levels of Fru-2,6-$P_2$ and of other metabolites in rat liver during development.
Mean values of 4-10 analyses from different animals are presented, vertical bars indicate ± S. E. M.

Consequently, around the -5th day Fru-2,6-$P_2$ may be assumed to permit glucose utilization proceeding via the glycolytic pathway. The Fru-2,6-$P_2$ minimum three days before birth coincides with the beginning of glycogen synthesis. Apparently, the extreme low level of Fru-2,6-$P_2$ causes a diminution of the phosphofructokinase activity and owing to this it contributes to the shift of the flow of substrate from glycolysis to glycogen

synthesis. This temporary inhibition of glycolysis gives rise to an increase of Fru-6-P and Glc-6-P (latter not shown; Glc-6-P is always in quasi-equilibrium with Fru-6-P).

However, in the period of high glycogen synthesis, when Glc-1-P is high and Fru-6-P is rising, Fru-2,6-$P_2$ again increases. During the last day before birth the mobilization of glycogen begins. Apparently, the elevated level of Fru-2,6-$P_2$ allows conversion of glycogen to lactic acid (11). Because of the very low activity of glucose 6-phosphatase at this developmental stage the hepatic release of glucose is assumed to be low (12). After birth glucose 6-phosphatase comes into appearance giving rise to the hepatic liberation of glucose. The diminished level of Fru-2,6-$P_2$ around birth contributes to a low activity of phosphofructokinase because of which the glycolytic degradation of Glc-6-P in the perinatal liver is small (11).

In Fig. 2 the perinatal variations of Fru-2,6-$P_2$ with higher temporal resolution than in Fig. 1 are presented. For comparison the levels of liver glycogen, blood glucose, and blood lactate are included. The Fru-2,6-$P_2$ remains extremely low within the first two hours after delivery, then it begins to increase. A delay in glycogen degradation within the first two hours of extrauterine life is observed before further mobilization of glycogen is taking place. The glucose level in the blood of the offsprings remains high within 30 minutes after delivery. Then it drops slightly to an intermediate plateau for again 30 minutes and declines thereafter before it increases again during the first day. This increase is apparently due to the commencing gluconeogenesis (3, 13). The blood lactate level increases before birth and decreases sharply in the first hours of extrauterine life. In this very early postnatal stage lactate is most probably still not utilized for gluconeogenesis, but is apparently oxidized via the tricarboxylic acid cycle (13, 14).

It is noteworthy that the decrease in the glycogen content after birth coincides with an increase in the Fru-2,6-$P_2$ level. This again suggests a temporal correlation between Fru-2,6-$P_2$ and glycogen metabolism as on the -3rd day. The regulatory significance of this relationship remains to be clarified.

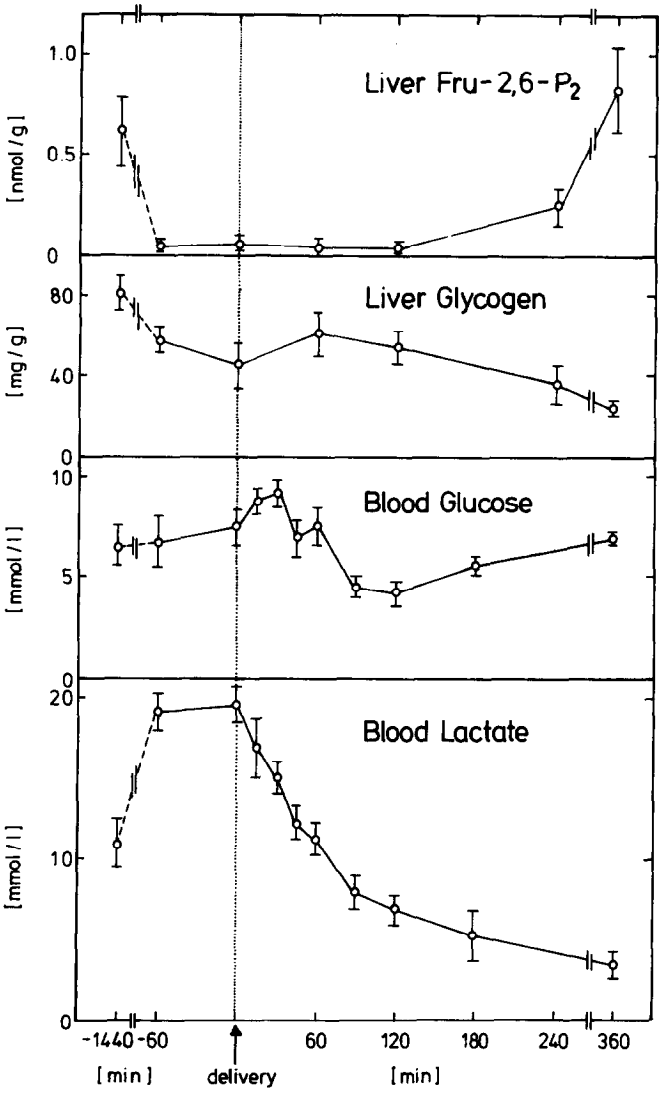


Fig. 2 Perinatal changes of hepatic Fru-2,6-$P_2$ in relation to liver glycogen, blood glucose and blood lactate. Mean values of at least 10-12 analyses from different animals are presented, vertical bars indicate ± S. E. M.

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