tissues[11,12] of various mammals. The urinary excretion of the oligosaccharide seems to be quite constant, ranging from 20 to 50 mg/24 h in normal men. The origin of neuraminlactose in human male urine is unknown. The only compounds known to contain the carbohydrate sequence of neuraminlactose are the gangliosides.[13] Consequently, the catabolism of these glycolipids might give rise to the formation of free neuraminlactose. On the basis of preliminary observations, the concentration of neuraminic-acid-containing oligosaccharides is markedly increased in the urine of lactating women. This finding is in good agreement with the observations of Date, who isolated milk-typical neutral oligosaccharides from female urine during lactation.[14,15]

The chemical characterization of the other substances isolated is in progress and will be reported separately.

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Studies of β-Glucuronidase Activity in Bile and Liver of Developing Chick Embryos and Chicks

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The β-glucuronidase activity was first described by Masamune in 1934.[1] Since 1948 when Fishman[2] described a colorimetric method for the determination of β-glucuronidase activity in serum many reports have been published about the nature and significance of the enzyme. β-Glucuronidase has been shown to catalyze the hydrolysis of β-glucuronides as well as the transfer of glucuronyl groups to acceptor alcohols.[3,4] As substrates of the enzyme can act, *e.g.*, β-glucuronides of steroid hormones,[5,6] bilirubin,[7,8] phenolphthalein,[2] menthol, and borneol. The enzyme does not hydrolyse either α-glucuronides or α- and β-glucosides. The β-glucuronidase has a wide distribution. It is found to occur in bacteria,[9] plants,[10] fish,[11] and in most tissues of mammals and other animals.[2,12]

In the course of a study on the bile pigment metabolism[13] in developing chick embryos and chicks it was noted that a considerable amount of bilirubin and biliverdin could occur in the gall bladders as the unconjugated pigments. In a report[14] concerning the β-glucuronidase activity in bile, the bile of healthy humans does not contain β-glucuronidase. Therefore it was of interest to check if the same holds true in developing chick embryos and chicks or if the presence of unconjugated pigments could be partly explained by a β-glucuronidase activity. For comparison the liver activity values were determined at the same time.

To obtain chick-embryo bile and liver, fertile White Leghorn eggs were incubated at 37.5 ± 0.5°C in a relative humidity of 65 ± 5 %. After a desired incubation time the embryos were removed from the eggs with a forceps and freed from adherent membranes. The gall bladders and livers were quickly removed and homogenised in a Potter-Elvehjem type homogeniser in tubes kept in ice cooled water, the bladders in 0.5 ml of water for 20 sec and the livers in two volumes of 0.154 M KCl

for 60 sec. One to seven (according to the age) gall bladders or livers were pooled in one sample.

β-Glucuronidase activity was measured according to the method by Fishman using phenolphthalein mono-β-glucuronic acid (Sigma Chemical Co.) as substrate.

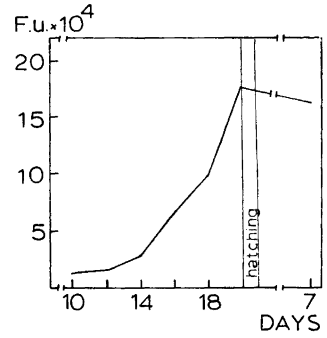

Fig. 1. The development of β-glucuronidase activity in chick-embryo and chick bile as a function of the age. One Fishman unit (F.U.) is the amount of phenolphthalein in μg, which is liberated per 100 ml of bile per hour.

For the measurement of β-glucuronidase the bile homogenate was diluted with water (1:5), and the liver supernatant (2000 *g*, 10 min) 1:40 with 0.154 M KCl.

The development of β-glucuronidase activity as a function of age in bile and liver is given in Figs. 1 and 2. A detectable β-glucuronidase activity is regularly

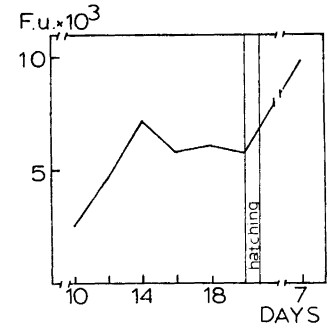

Fig. 2. The development of β-glucuronidase activity in chick-embryo and chick liver as a function of the age. One Fishman unit is the amount of phenolphthalein in μg, which is liberated per gram of liver (wet weight) per hour.

observed in chick-embryo bile at the tenth day of incubation. During the embryonic development the activity values increase to a peak at the time of hatching and decrease slightly after hatching. It is seen that a considerable β-glucuronidase activity in chick-embryo and chick bile is the rule, not an exeption as it is in man.

The high activity values in the bile indicate a possibility of enzymatic hydrolysis of bile pigment glucuronides. In the experiments the pH-value of the incubation system was kept at 4.5, conforming with the optimum pH-value for β-glucuronidase. It is highly improbable that the pH-value of bile *in vivo* should ever be as low as 4.5, and therefore the hydrolytic activity of β-glucuronidase would not be as high as one could assume from the values above. Perhaps the activity is around 20–30 % of the maximal at the pH-value of chick-embryo bile, 6.7 ± 0.3 (S.D.).

In search for the reason of the occurrence of unconjugated bilirubin and biliverdin in chick-embryo and in chick bile the possibility of a β-glucuronidase function must be taken into serious consideration.

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