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## **Studies on Conjugated Mono- and Dihydroxy Metabolites of Progesterone in Plasma, Bile and Urine in a Human Subject**

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The main conjugated metabolite of progesterone, pregnanediol ( $5\beta$ -pregnane- $3\alpha,20\alpha$ -diol), was isolated as a glucuronide from human pregnancy urine more than thirty years ago by VENNING and BROWNE (1936). During thirty years numerous reports have been published on the metabolism of progesterone in the human organism, but few of the investigators have been interested in the problems of the conjugation of pregnanediol and its isomers in the human organism. CREPY et al. (1962) have studied the distribution of  $5\beta$ -pregnane- $3\alpha,20\alpha$ -diol and  $3\alpha$ -hydroxy- $5\beta$ -pregnan-20-one and their epimers in the glucuronide and sulphate fractions of late pregnancy urine. Recently, HARKNESS et al. (1969) determined the proportions of metabolites excreted in urine as glucuronide- and sulphate conjugates after small and large doses of injected progesterone in human subjects. In both cases pregnanediols in sulphate form were observed in the urine.

After intravenous injections of progesterone-4- $^{14}\text{C}$ , not only  $5\beta$ -pregnane- $3\alpha,20\alpha$ -diol but also  $5\beta$ -pregnane- $3\beta,20\beta$ -diol and  $3\alpha$ -hydroxy- $5\beta$ -pregnan-20-one were identified in the glucuronide fraction of the plasma (THIJSSEN and ZANDER 1966). A part of the radioactivity was found in the sulphate fraction, too. The nature of the metabolites in this fraction was not reported. In pregnancy plasma  $5\alpha$ -pregnane- $3\beta,20\alpha$ -diol has been identified in the mono- and disulphate fractions and  $3\beta$ -hydroxy- $5\alpha$ -pregnan-20-one in the monosulphate fraction (SJÖVALL et al. 1968). Two additional pregnanolone and pregnanediol isomers were present, but their exact configuration was not determined.

The sulphate fraction of human late pregnancy bile was shown by ADLERCREUTZ and LUUKKAINEN (1964) to contain  $5\beta$ -pregnane- $3\alpha,20\alpha$ -diol and three of the epimeric  $5\alpha$ -pregnanediols. Recently, LAATIKAINEN et al. (1968) identified several neutral steroids in the mono- and disulphate fractions of human bile, and it was found that  $5\beta$ -pregnane- $3\alpha,20\alpha$ -diol and  $5\alpha$ -pregnane- $3\alpha,20\alpha$ -diol were present in both fractions.

The metabolites of progesterone are excreted to a great extent in the bile. SANDBERG and SLAUNWHITE (1958) recovered 30% of the radioactivity in the fistula bile after an intravenous injection of labelled progesterone. The nature of the metabolites carrying the radioactivity was not investigated in detail. Therefore, the nature of the biliary progesterone metabolites was felt to deserve further elucidation.

The technique developed for the fractionation of neutral steroid conjugates by chromatography on Sephadex LH 20, and separation of the conjugates into a glucuronide and mono- and disulphate fractions (SJÖVALL and VIHKO 1966; LAATIKAINEN and VIHKO 1969) also makes it possible to quantitatively determine the metabolites of progesterone conjugated in these three different ways. In the present study the mono- and dihydroxy metabolites of progesterone were investigated simultaneously in plasma, urine and bile before and during a cumulative load of progesterone, and their distribution in the glucuronide fraction as well as in the mono- and disulphate fractions was determined.

#### *Material and Methods*

The patient in the study was a woman aged 58 years who had undergone a cholecystectomy for gallstones. She had T-tube drainage of the common bile duct. 7 days after the operation, urine and fistula bile were collected for 24 hours and the plasma sample was obtained after overnight fasting. During the next 24 hours the patient received 400 mg of progesterone intramuscularly twice. The following 24-hour period was divided into two 12-hour collection periods of bile and urine, and plasma samples were obtained at the beginning of both 12-hour periods. The patient again received progesterone, 400 mg intramuscularly at the beginning of each of these two 12-hour periods.

All solvents were of reagent grade and were redistilled before use. Reference steroids were obtained from W. KLYNE (Steroid Reference Collection, London, England) or Ikapharm (Ramat-Gan, Israel). The plasma, urine and bile samples were processed essentially as described previously (JÄNNE et al. 1969; JÄNNE and VIHKO 1968; LAATIKAINEN et al. 1968). The conjugates were divided into glucuronide, mono- and disulphate fractions according to LAATIKAINEN and VIHKO (1969). After the hydrolysis of the glucuronides with Ketodase (R) or solvolysis of the sulphates, they were fractionated on a silicic acid column as reported by LAATIKAINEN et al. (1968).

Gas-liquid chromatography (GLC) of the steroids was carried out on QF-1 and SE-30 liquid phases after conversion of the steroids to trimethylsilyl (TMS) derivatives.

*Gas-liquid chromatography-mass spectrometry* (GLC-MS) of the TMS derivatives was carried out with the LKB Model 9000 gas chromatograph-mass spectrometer, using a QF-1 column at a temperature of 210° C. The ionizing energy was 70 eV.

#### *Results*

The  $C_{21}O_2$  metabolites of progesterone identified and quantitatively analyzed in the present study were the following:  $3\alpha$ -hydroxy- $5\beta$ -pregnan-20-one,  $5\alpha$ -pregnane- $3\alpha,20\alpha$ -diol,  $5\beta$ -pregnane- $3\alpha,20\alpha$ -diol and  $5\alpha$ -pregnane- $3\beta,20\alpha$ -diol. They were all present during the progesterone load in plasma (Table I), in urine (Table II) and in bile (Table III).

During the control period the only sulphate conjugate present in measur-

Table I

The  $C_{21}O_2$  metabolites of progesterone in plasma before and during the progesterone load as different conjugates. The following symbols are used:  $3\alpha$ - $5\beta$ P-20-one =  $3\alpha$ -hydroxy- $5\beta$ -pregnan-20-one,  $5\alpha$ P- $3\alpha$ , $20\alpha$ -diol =  $5\alpha$ -pregnane- $3\alpha$ , $20\alpha$ -diol,  $5\beta$ P- $3\alpha$ , $20\alpha$ -diol =  $5\beta$ -pregnane- $3\alpha$ , $20\alpha$ -diol,  $5\alpha$ P- $3\beta$ , $20\alpha$ -diol =  $5\alpha$ -pregnane- $3\beta$ , $20\alpha$ -diol. Plasma samples were obtained 2 hours after the start of collection of urine and bile

Conjugate and metabolite	Control period $\mu\text{g}/100 \text{ ml}$	1st loading period $\mu\text{g}/100 \text{ ml}$	2nd loading period $\mu\text{g}/100 \text{ ml}$
<i>Monosulphate</i>			
$3\alpha$ - $5\beta$ P-20-one	—	7	18
$5\alpha$ P- $3\alpha$ , $20\alpha$ -diol	—	5	7
$5\beta$ P- $3\alpha$ , $20\alpha$ -diol	—	9	21
$5\alpha$ P- $3\beta$ , $20\alpha$ -diol	—	44	59
<i>Disulphate</i>			
$5\alpha$ P- $3\alpha$ , $20\alpha$ -diol	—	8	13
$5\beta$ P- $3\alpha$ , $20\alpha$ -diol	—	14	23
$5\alpha$ P- $3\beta$ , $20\alpha$ -diol	—	53	97
<i>Glucuronide</i>			
$3\alpha$ - $5\beta$ P-20-one	—	16	28
$5\alpha$ P- $3\alpha$ , $20\alpha$ -diol	—	—	—
$5\beta$ P- $3\alpha$ , $20\alpha$ -diol	—	39	60
$5\alpha$ P- $3\beta$ , $20\alpha$ -diol	—	—	—

Table II

The  $C_{21}O_2$  metabolites of progesterone in urine before and during the progesterone load. Symbols as in Table I

Conjugate and metabolite	Control period $\mu\text{g}/24 \text{ h}$	1st loading period $\mu\text{g}/12 \text{ h}$	2nd loading period $\mu\text{g}/12 \text{ h}$	1st + 2nd loading period $\mu\text{g}/24 \text{ h}$
<i>Monosulphate</i>				
$3\alpha$ - $5\beta$ P-20-one	—	124	492	616
$5\alpha$ P- $3\alpha$ , $20\alpha$ -diol	—	6	17	23
$5\beta$ P- $3\alpha$ , $20\alpha$ -diol	—	161	604	765
$5\alpha$ P- $3\beta$ , $20\alpha$ -diol	—	34	109	143
<i>Disulphate</i>				
$5\alpha$ P- $3\alpha$ , $20\alpha$ -diol	7	39	104	143
$5\beta$ P- $3\alpha$ , $20\alpha$ -diol	—	66	250	316
$5\alpha$ P- $3\beta$ , $20\alpha$ -diol	—	286	1001	1287
<i>Glucuronide</i>				
$3\alpha$ - $5\beta$ P-20-one	81	2450	5800	3250
$5\alpha$ P- $3\alpha$ , $20\alpha$ -diol	—	90	177	267
$5\beta$ P- $3\alpha$ , $20\alpha$ -diol	172	6820	11800	18620
$5\alpha$ P- $3\beta$ , $20\alpha$ -diol	—	620	890	1510

Table III

The  $C_{21}O_2$  metabolites of progesterone in bile before and during the progesterone load.  
Symbols as in Table I

Conjugate and metabolite	Control period μg/24 h	1st loading period μg/12 h	2nd loading period μg/12 h	1st + 2nd loading periods μg/24 h
<i>Monosulphate</i>				
3 $\alpha$ -5 $\beta$ P-20-one	—	140	290	430
5 $\alpha$ P-3 $\alpha$ ,20 $\alpha$ -diol	—	150	270	420
5 $\beta$ P-3 $\alpha$ ,20 $\alpha$ -diol	—	400	720	1120
5 $\alpha$ P-3 $\beta$ ,20 $\alpha$ -diol	—	100	200	300
<i>Disulphate</i>				
5 $\alpha$ P-3 $\alpha$ ,20 $\alpha$ -diol	—	450	560	1010
5 $\beta$ P-3 $\alpha$ ,20 $\alpha$ -diol	—	530	740	1270
5 $\alpha$ P-3 $\beta$ ,20 $\alpha$ -diol	—	350	560	910
<i>Glucuronide</i>				
3 $\alpha$ -5 $\beta$ P-20-one	23	7100	7500	14600
5 $\alpha$ P-3 $\alpha$ ,20 $\alpha$ -diol	—	—	—	—
5 $\beta$ P-3 $\alpha$ ,20 $\alpha$ -diol	27	6400	4430	10830
5 $\alpha$ P-3 $\beta$ ,20 $\alpha$ -diol	—	540	325	865

able amounts in the biological fluids studied was 5 $\alpha$ -pregnane-3 $\alpha$ ,20 $\alpha$ -diol as a disulphate in the urine. However, because of the low concentration of this compound in urine, a mass spectrometric identification was not possible. The identification is, therefore, tentative. In the glucuronide fraction in the bile and urine relatively small amounts of 3 $\alpha$ -hydroxy-5 $\beta$ -pregnan-20-one and 5 $\beta$ -pregnane-3 $\alpha$ ,20 $\alpha$ -diol were excreted. The total glucuronide/total sulphate ratio could be determined only in the urine where it was 36.

During the cumulative load there was an increase in the concentration of every steroid estimated between the first 12-hour and the second 12-hour collection period in the plasma, urine and bile. The absolute amounts of glucuronides of 5 $\beta$ -pregnane-3 $\alpha$ ,20 $\alpha$ -diol and 5 $\alpha$ -pregnane-3 $\beta$ ,20 $\alpha$ -diol recovered from the fistula bile decreased during the second collection period. The glucuronide/sulphate ratio in the bile, which was 7 during the first 12 hours, decreased to 4 during the second 12-hour period. It therefore seems that this ratio decreased when the load increased. The same phenomenon is also observable in the urine, where the glucuronide/sulphate ratio decreased from 36 during the control period to 14 and further to 7 during the load. The individual steroids were distributed in the different conjugate fractions in the plasma, urine and bile as follows:

*3 $\alpha$ -hydroxy-5 $\beta$ -pregnan-20-one* (3 $\alpha$ -5 $\beta$ P-20-one). The concentrations of this metabolite in the plasma in the monosulphate fraction during the load were similar to those of 5 $\beta$ -pregnane-3 $\alpha$ ,20 $\alpha$ -diol. Of all the metabolites in the total sulphate fraction (mono- + disulphate) it was present in the lowest

amount, but its proportion increased during the experiment. This metabolite was excreted as the monosulphate in the urine in about the same amounts as the monosulphate of  $5\beta$ -pregnane- $3\alpha,20\alpha$ -diol. If the total excretion of the epimeric pregnanediols is calculated from both the mono- and the disulphate fractions in the urine, four times as much  $3\alpha$ - $5\beta$ P-20-one as  $5\alpha$ -pregnane- $3\alpha,20\alpha$ -diol is excreted, but the other two pregnanediols are present in considerably greater amounts. In the bile the excretion of  $5\alpha$ -pregnane- $3\alpha,20\alpha$ -diol in the monosulphate fraction is similar to that of  $3\alpha$ -hydroxy- $5\beta$ -pregnane-20-one, but because the pregnanediol sulphates in the bile are mainly disulphates,  $3\alpha$ -hydroxy- $5\beta$ -pregnane-20-one formed only 10% of the total sulphates of the four metabolites determined. In the plasma glucuronide fraction the concentration of  $3\alpha$ - $5\beta$ P-20-one increased during the load.

In the urinary glucuronide fraction the amount of  $3\alpha$ - $5\beta$ P-20-one excreted during the control period was half that of  $5\beta$ P- $3\alpha,20\alpha$ -diol. This ratio was about the same during progesterone administration. In the urine,  $3\alpha$ - $5\beta$ P-20-one was mainly excreted as the glucuronide. From the quantitative point of view  $3\alpha$ - $5\beta$ P-20-one was the second most important of the urinary metabolites determined.

In the bile during the control period the amounts of  $3\alpha$ - $5\beta$ P-20-one and  $5\beta$ P- $3\alpha,20\alpha$ -diol excreted in the glucuronide fraction were equal. During the load  $3\alpha$ - $5\beta$ P-20-one was the main constituent of the bile glucuronide fraction and quantitatively the most important metabolite.

*$5\alpha$ -pregnane- $3\alpha,20\alpha$ -diol* ( $5\alpha$ P- $3\alpha,20\alpha$ -diol). The concentrations of this steroid in the plasma sulphate fractions were relatively low. The bulk of this compound was in the disulphate fraction.

During the control period the only sulphate conjugate found in the urine was the disulphate of  $5\alpha$ P- $3\alpha,20\alpha$ -diol. During the load small amounts were excreted as monosulphate and disulphate conjugates.

The excretion into the bile sulphate fractions was relatively much greater. The amount of  $5\alpha$ P- $3\alpha,20\alpha$ -diol in the monosulphate fraction was equal to that of  $3\alpha$ - $5\beta$ P-20-one and in disulphates to that of  $5\alpha$ P- $3\beta,20\alpha$ -diol during the load.

It was interesting to observe that  $5\alpha$ P- $3\alpha,20\alpha$ -diol was at no time during the experimental period present in the biliary glucuronide fraction, nor was it found as glucuronide in the plasma either. In contrast, in the urine  $5\alpha$ P- $3\alpha,20\alpha$ -diol was excreted both as mono- and disulphate and as a glucuronide following the progesterone load.  $5\alpha$ P- $3\alpha,20\alpha$ -diol was thus present in plasma and bile after the load exclusively as sulphate conjugates, preferably as the disulphate.

*$5\beta$ -pregnane- $3\alpha,20\alpha$ -diol* ( $5\beta$ P- $3\alpha,20\alpha$ -diol). The highest plasma concentration of  $5\beta$ P- $3\alpha,20\alpha$ -diol was found in the glucuronide fraction during progesterone administration. The concentrations of mono- and disulphate conjugates in the plasma were equal.

In the urine the amount of  $5\beta$ P- $3\alpha,20\alpha$ -diol excreted as the monosulphate conjugate exceeded that excreted in the disulphate form. This is in sharp con-

trast to the pattern found for the  $5\alpha$ -pregnanediol sulphates. The main conjugate in urine was, as expected, the glucuronide of  $5\beta$ P- $3\alpha,20\alpha$ -diol. In bile also,  $5\beta$ P- $3\alpha,20\alpha$ -diol was excreted mainly as glucuronide, although the excretion of mono- and disulphate conjugates relative to glucuronide was greater in the bile than in the urine. However, in the plasma the relative concentration of  $5\beta$ P- $3\alpha,20\alpha$ -diol sulphates as compared with glucuronides was about three times as high as in the bile and ten times as high as in the urine. The same amounts of  $5\beta$ P- $3\alpha,20\alpha$ -diol were excreted in the bile as mono- and disulphate conjugates, whereas  $5\alpha$ -pregnanediols were excreted more as disulphates.

*5 $\alpha$ -pregnane- $3\beta,20\alpha$ -diol* ( $5\alpha$ P- $3\beta,20\alpha$ -diol). This steroid was present during the load in the mono- and disulphate fractions in plasma, preferably as the disulphate. It was not found as glucuronide in the plasma.

In the urine  $5\alpha$ P- $3\beta,20\alpha$ -diol was present during the progesterone load in the glucuronide, mono- and disulphate fractions. The excretion during the load increased most in the disulphate fraction and  $5\alpha$ P- $3\beta,20\alpha$ -diol was then the main disulphate in the urine. The excretion as disulphate was almost 10-fold as compared with the excretion as monosulphate. The combined amounts in the two sulphate fractions were the same as in the glucuronide fraction, where the increase of the excretion during the progressive load was lower than in the sulphate fractions.

In bile more  $5\alpha$ P- $3\beta,20\alpha$ -diol was excreted as sulphates than as glucuronides. The ratio of monosulphate conjugate to disulphate in bile was 1:3, whereas in urine it was 1:10.

*Mass spectrometry.* The mass spectrometric fragmentation of  $3\alpha$ -hydroxy- $5\beta$ -pregnan-20-one and  $5\beta$ -pregnane- $3\alpha,20\alpha$ -diol has been reported earlier (ADLERCREUTZ et al. 1966), and the mass spectra of these compounds obtained from urine (ADLERCREUTZ et al. 1966), and bile (LAATIKAINEN and VIHKO 1969) have been presented.

Analysis of the fractions by GLC-MS revealed the identity of the compounds estimated by GLC with the corresponding derivatives of the reference standards. The present investigation thus has demonstrated the presence of the following compounds in bile, plasma and urine following a progesterone load:  $3\alpha$ -hydroxy- $5\beta$ -pregnan-20-one,  $5\alpha$ -pregnane- $3\alpha,20\alpha$ -diol,  $5\beta$ -pregnane- $3\alpha,20\alpha$ -diol and  $5\alpha$ -pregnane- $3\beta,20\alpha$ -diol.

### Discussion

Because most of the bile was collected and the enterohepatic circulation thus interrupted to a great extent, this experiment does not give a true picture of the metabolism of progesterone *in vivo*. Some of the bile most probably by-passed the T-tube collection and entered the intestinal canal. Therefore, any transformation that may take place in the steroid nucleus or changes in conjugation during passage through the intestine and reabsorption are only partly reflected in the pattern of conjugated metabolites in

plasma and urine. In addition, it must be taken into account that only one patient was used for the present investigation.

It was observed by HARKNESS et al. (1969) that the urinary sulphate fraction increased after the larger dose of progesterone. In the present study their observation in urine was confirmed, and it was observed that in plasma and bile the sulphate conjugates also increased with the higher load. With increased concentration of metabolites in bile more  $3\alpha$ -hydroxy- $5\beta$ -pregnan-20-one was excreted than  $5\beta$ -pregnane- $3\alpha,20\alpha$ -diol in the glucuronide fraction. This may indicate saturation of the 20-keto reduction in the liver when the progesterone load is increased.

Investigating the urinary conjugates, CREPY et al. (1962) found that at term of pregnancy 0.1% of  $3\alpha$ -hydroxy- $5\beta$ -pregnan-20-one, 0% of  $5\alpha$ -pregnane- $3\alpha,20\alpha$ -diol, 0.1% of  $5\beta$ -pregnane- $3\alpha,20\alpha$ -diol and 99.9% of  $5\alpha$ -pregnane- $3\beta,20\alpha$ -diol were present as sulphates. In the present study the corresponding metabolites were present as sulphates in the following proportions: 7%, 38%, 5% and 48%.

Furthermore,  $5\alpha$ -pregnane- $3\alpha,20\alpha$ -diol was not excreted in the bile as glucuronide conjugate, whereas the proportion of the total bile monosulphates was 18% and of the total bile disulphates 32%, the corresponding percentages in urine being 1.5% and 8% respectively. This may suggest that the biliary mono- and disulphates of  $5\alpha$ -pregnane- $3\alpha,20\alpha$ -diol could be partly transformed to glucuronides during reabsorption from the intestine. The absence of the glucuronide conjugate of this steroid in bile and its presence in urine may be taken to suggest that in the human organism there are two glucuronyl transferases with different steric requirements for pregnanediols. Of these conjugating enzymes, the one conjugating  $5\alpha$ P- $3\alpha,20\alpha$ -diol may be present in some other organs than the liver. Another possibility is that the liver specifically excludes the glucuronide of  $5\alpha$ -pregnane- $3\alpha,20\alpha$ -diol from the biliary excretion route.

It has recently been shown by LAATIKAINEN and VIHKO (1969) that in bile the  $C_{19}O_2$  and  $C_{21}O_2$  steroids with a  $5\beta$  structure predominate in the glucuronide fraction, whereas steroids with a  $5\alpha$  structure are excreted mainly as sulphate conjugates.

### Summary

The  $C_{21}O_2$  metabolites of progesterone were investigated by gas-liquid chromatography and mass spectrometry in the bile, plasma and urine of a 58-year-old woman with biliary T-tube drainage of the common bile duct both before and after administration of progesterone. During the control period no metabolites were detected in the plasma, but in the bile  $3\alpha$ -hydroxy- $5\beta$ -pregnan-20-one and  $5\beta$ -pregnane- $3\alpha,20\alpha$ -diol were identified in the glucuronide fraction, and in the urine  $5\alpha$ -pregnane- $3\alpha,20\alpha$ -diol was tentatively identified in the disulphate fraction, and  $3\alpha$ -hydroxy- $5\beta$ -pregnan-20-one and  $5\beta$ -pregnane- $3\alpha,20\alpha$ -diol were identified in the glucuronide fraction.

Following a load with progesterone, the metabolites  $5\alpha$ -pregnane- $3\alpha,20\alpha$ -diol,  $5\beta$ -pregnane- $3\alpha,20\alpha$ -diol and  $5\alpha$ -pregnane- $3\beta,20\alpha$ -diol were identified in the plasma, bile and urine in both the mono- and disulphate fractions, and in the urine in the glucuronide fraction also.  $5\beta$ -pregnane- $3\alpha,20\alpha$ -diol was identified in the plasma glucuronide fraction and, with the exception of  $5\alpha$ -pregnane- $3\alpha,20\alpha$ -diol, the above-mentioned pregnanediols were also identified in the bile glucuronide fraction.

With increasing load more sulphates occurred in relation to glucuronides and more pregnanolone (identified in the plasma, bile and urine both as sulphate and glucuronide) in relation to pregnanediol. The dominating type of conjugate of pregnanolone was the glucuronide, especially in the urine, while in the bile the sulphate represented about  $1/5$  of the metabolites and in the plasma about 40% occurred as sulphate. Following a progesterone load,  $5\alpha$ -pregnane- $3\alpha,20\alpha$ -diol was present in the plasma and bile mostly as the disulphate, while in urine both sulphates and glucuronides were excreted.  $5\beta$ -pregnane- $3\alpha,20\alpha$ -diol occurred in all fluids predominantly as glucuronides and  $5\alpha$ -pregnane- $3\beta,20\alpha$ -diol both as disulphate and glucuronide (not in the plasma) and to a lesser degree as monosulphate.

The results indicate that during the metabolism of administered progesterone the conjugation of the various metabolites with sulphate is important from the quantitative point of view.

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### Discussion

H. BREUER: I think the interesting point of your investigation is the demonstration that most of the  $5\beta$  compounds are excreted as glucuronides and that the  $5\alpha$  compounds are excreted predominantly as sulfates. Is that correct?

T. LUUKKAINEN: Yes, that is correct.

H. BREUER: So you would think that about 90% of the  $5\beta$ -reduced metabolites of progesterone are excreted in the urine, as the glucuronides and most of the  $5\alpha$  are excreted as sulphates?

T. LUUKKAINEN: Or equal amounts in glucuronide fraction during the load. Of course, if the load is very small then I think most of the  $5\alpha$ -pregnanediols are excreted as sulphates, as you saw that the  $3\alpha$ - $5\alpha$ -pregnanediol was not excreted in the bile as a glucuronide at all during the load. So it is perhaps later conjugated with glucuronic acid.

H. BREUER: How big was the load of progesterone in your experiments? You did not give the actual figure?

T. LUUKKAINEN: There was 4 times 400 mg.

H. BREUER: Would you say that the metabolic patterns of the glucuronides and the sulphates vary with increasing load?

T. LUUKKAINEN: Yes, very much, because in the urine of the control period the ratio between glucuronide and sulphate was 36, during the first loading collection it was 14 and in the second collection 7. The same was observed in the bile where it was 7 and 4 during the increasing load. It shifts more to sulphates during the higher load as HARKNESS could observe.

H. BREUER: Would you think that the increased amount of sulphates, excreted at a high load of progesterone, is due to the reduced capacity of the glucuronyl transferase to conjugate or do you think it is more an inhibition of the glucuronyl transferase by the steroid?

T. LUUKKAINEN: That is a very difficult question to answer. As you know, HARKNESS has discussed it recently and stated that both mechanisms could be involved. It seems that with this very high load the glucuronide transferase is somewhat saturated, but the interesting thing is that when you have sulphates, the ratio between mono- and disulphates is the same during the higher load. So you have no shift to more mono-sulphates than disulphates.