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Serum Bilirubin Level and Steroid Excretion Following Progesterone Loads in New-born Infants

By

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In healthy full-term breast-fed male infants oestrogen excretion and serum bilirubin values have been determined daily for 10 days and on the 6th day of life the infants received a 1, 2, 5 or 10 mg dose of progesterone. Thereafter oestrogen excretion and serum bilirubin level increased. A correlation was found to exist between the dose applied and the degree of increase.

Arias et al. [1, 2], Holton and Lathe [5] and Krauer-Mayer et al. [8] could isolate 3α, 20α and 3α, 20β-pregnanediol from the milk of mothers of new-borns suffering from prolonged jaundice. The substance, inhibiting glucuronyl transferase, prevents glucuronic acid conjugation and this the excretion of bilirubin. Other steroids have been found to have a similar effect [3, 4, 9, 10, 11].

In previous investigations [13] we have observed a difference between artificially fed new-borns and new-borns offered solely mother's milk, inasmuch as steroid excretion and serum bilirubin values were lower in the first than in the second group. As reported previously [12], mother's milk contains a considerable amount of sex steroid which may inhibit the metabolism and excretion of bilirubin in the new-born.

To obtain direct evidence of this, the effect of progesterone loads has been studied in new-born babies.

Material and Methods

Sex steroid excretion and bilirubin level of 6 healthy male breast-fed new-borns weighing 3200—3600 g, born spontaneously following a normal pregnancy, was determined daily, and on the 6th postnatal day, when the bilirubin level usually undergoes a considerable decrease [13], 2, 5 mg or 10 mg of crystalline progesterone in oil were injected and the examinations were continued till the 9th or 10th day.

Serum bilirubin was determined by the photometric method of Jendrassik and Cleghorn [7], oestrogen according to the Ittrich column chromatography and photometric procedures [6]; and pregnanediol isomers by the thin-layer chromatography and photometric method of Schneider and Szereday [14]. 30 cm plates were used allowing the reliable isolation of (a) 5β-pregnane 3α, 20α-diol (3α, 20α-pregnanediol); (b) 5α-pregnane-3α,20α-diol (allo-

pregnanediol); and (c) 5β-pregnane-3α, 20-β-diol (3α, 20β-pregnanediol).

Neutral 17-ketosteroids were estimated according to ZIMMERMANN [15]. Urine was collected by means of a device applied to the penis and precision was controlled by determining creatinine content of the urine.

RESULTS

(a) *Pregnanediol excretion.* Table I shows the excretion of pregnanediol isomers. In one case (No. 49) it increased considerably, in two further new-

TABLE I

Case	Isomer	1	2	3	4	5	Loading	6	7	8	9	10
48th case B. I.	20α	0	0	10	10	10	2 mg i.m.	5	0	0	0	0
	20β	0	0	10	5	10		0	5	0	0	0
	Allo-	0	0	5	8	8		5	0	0	0	0
	Σ	0	0	25	23	28		10	5	0	0	0
49th case P. I.	20α	0	0	10	15	20	5 mg i.m.	5	5	15	5	0
	2β	0	0	10	12	20		15	0	10	0	0
	Allo-	0	0	8	10	10		30	15	25	0	10
	Σ	0	0	28	37	50		50	20	60	5	10
53rd case M. M.	20α	0	0	0	22	22	2 mg i.m.	22	12	12	12	—
	20β	0	0	0	12	12		12	5	12	12	—
	Allo-	0	0	0	15	0		15	10	0	10	—
	Σ	0	0	0	49	34		49	27	24	34	—
54th case V. J.	20α	0	0	22	0	14	2 mg i.m.	20	14	7	0	0
	20β	0	0	22	0	20		20	14	0	0	0
	Allo-	0	0	0	0	14		15	10	0	0	0
	Σ	0	0	44	0	48		55	38	7	0	0
55th case D. Sz.	20α	0	0	0	0	0	10 mg i.m.	0	0	0	0	—
	20β	0	0	0	0	0		0	0	0	0	—
	Allo-	0	0	0	0	0		0	0	0	0	—
	Σ	0	0	0	0	0		0	0	0	0	—
62nd case Sz. J.	20α	0	0	20	12	6	10 mg i.m.	6	6	0	0	0
	20β	0	0	20	12	0		6	12	0	0	0
	Allo-	0	0	15	13	20		12	20	0	0	0
	Σ	0	0	55	37	26		24	38	0	0	0

Effect of progesterone loading on 6th postnatal day on pregnanediol excretion of the new-born (μg per 24 hr.).

TABLE II

Day of life	1	2	3	4	5	Loading	6	7	8	9	10
48th case B. I.	—	714	720	900	720	Progesterone 2 mg i.m.	442	640	327	624	1045
53rd case M. M.	—	720	120	420	—	Progesterone 2 mg i.m.	400	275	280	120	119
49th case P. I.	—	—	748	495	789	Progesterone 5 mg i.m.	782	1040	780	550	756
54th case V. J.	492	1410	420	756	609	Progesterone 5 mg i.m.	540	637	165	160	525
55th case D. Sz.	640	180	170	520	480	Progesterone 10 mg i.m.	460	440	380	360	160
62nd case Sz. J.	120	243	728	283	300	Progesterone 10 mg i.m.	225	965	795	495	510

Effect of progesterone loading on the 6th postnatal day on 17-ketosteroid excretion of new-borns (μg/24 hr.).

TABLE III

Day of life	1	5	3	4	2	Loading	6	7	8	9	10
48th case B. I.	—	5.3	16.3	20.2	24.4	Progesterone 2 mg i.m.	47.7	43.2	58.9	79.1	69.4
53rd case M. M.	—	6.4	2.5	3.9	14.4	Progesterone 2 mg i.m.	22.8	12.6	3.3	2.8	—
49th case P. I.	1.0	—	12.1	11.5	14.3	Progesterone 5 mg i.m.	25.1	24.8	37.8	19.0	27.7
54th case V. J.	6.3	17.3	7.4	7.4	10.5	Progesterone 5 mg i.m.	27.0	46.8	28.7	22.9	43.6
55th case D. Sz.	4.2	2.3	5.0	13.7	24.0	Progesterone 10 mg i.m.	44.4	49.4	90.0	50.6	62.5
62nd case Sz. J.	3.7	5.8	—	36.7	33.8	Progesterone 10 mg i.m.	23.2	69.9	41.1	15.3	—

Effect of progesterone loading on 6th postnatal day on oestrogen excretion of the new-born (μg/24 hr.).

TABLE IV

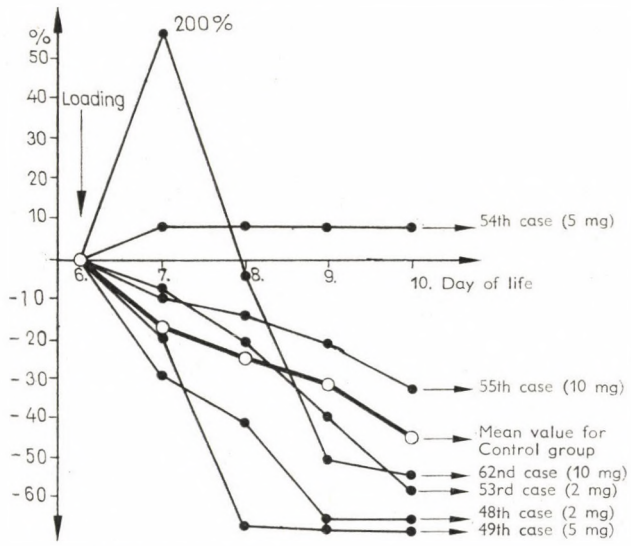
Day of life	1	2	3	4	5	Loading	6	7	8	9	10
48th case B. I.	2.4	3.0	3.0	3.0	4.0	Progesterone 2 mg i.m.	3.4	2.4	2.0	1.2	1.2
53rd case M. M.	3.0	3.7	4.2	6.3	7.9	Progesterone 2 mg i.m.	7.9	7.3	6.3	4.8	4.2
49th case P. I.	1.2	2.0	3.0	3.4	4.2	Progesterone 5 mg i.m.	3.7	3.0	1.2	1.2	1.2
54th case V. J.	3.0	3.0	3.7	4.2	4.2	Progesterone 5 mg i.m.	6.3	6.8	6.8	6.8	6.8
55th case D. Sz.	3.0	3.0	4.2	4.6	4.2	Progesterone 10 mg i.m.	4.3	3.9	3.7	3.4	2.9
62nd case Sz. J.	3.0	3.4	4.2	4.2	6.0	Progesterone 10 mg i.m.	6.5	13.0	6.4	3.4	3.0

Effect of progesterone loading on 6th postnatal day on serum bilirubin values (mg per 100 ml) of the new-born.

TABLE V

Day of life	6		7		8		9		10	
Mean value for control group	8.4	100%	7.0	83%	6.6	75.2%	5.8	69%	4.6	54.8%
48th case 2 mg	3.4	100%	2.4	70.6%	2.0	58.8%	1.2	35.3%	1.2	53.3%
53rd case 2 mg	7.9	100%	7.3	92.4%	6.3	79.8%	4.8	60.8%	4.2	53.2%
49th case 5 mg	3.7	100%	3.0	81.1%	1.2	32.4%	1.2	32.4%	1.2	32.4%
54th case 5 mg	6.3	100%	6.8	107.9%	6.8	107.9%	6.8	107.9%	6.8	107.9%
55th case 10 mg	4.3	100%	3.9	90.7%	3.7	86%	3.4	79%	2.9	67.4%
62nd case 10 mg	6.5	100%	13.0	200%	6.4	98.5%	3.4	50%	3.0	46%

Decrease of serum bilirubin following progesterone loads.



Precentual decrease of serum bilirubin level following progesterone loads.

FIG. 1

borns (Nos. 53 and 54) pregnanediol was still present in the urine on the 8th and 9th days, in contrast to the untreated new-borns. One infant (55th case) did not excrete pregnanediol.

(b) *Neutral 17-ketosteroid excretion.* The response to loading was not unequivocal, but in 50% of the cases excretion increased following the load (Table II), and at this time the value-were higher than in untreated news borns [13].

(c) *Oestrogen excretion* increased in all new-borns after the loading (Table III). On the day of the injection the increase was moderate; the highest

values were recorded 2 to 4 days later.

(d) *Serum bilirubin level.* After a 2 mg dose of pregnanediol the bilirubin level did not change. After 5 mg it rose in one case and after 10 mg it increased in both cases (Table IV).

As to bilirubin excretion, the value determined on the 6th postnatal day was considered 100% and to this were compared the following values (Table V). The course of the curves is presented in Fig. 1. This shows that the 2 mg dose did not influence the bilirubin excretion, whereas the 5 and 10 mg doses increased it considerably, and in 2 cases they were prolonging it.

Discussion

The moderate and not uniform rise of pregnanediol excretion was only natural as according to data in the literature and to own observations the foetus and the new-born form an insignificant amount of pregnanediol from progesterone which is metabolized in the foetus to oestrogens and 17-ketosteroids. This was supported by the above finding of an increase of oestrogen excretion following loading.

The increase in 17-ketosteroid excretion is theoretically conceivable, as in the course of its metabolism progesterone is transformed via androstendione in smaller amounts to 17-ketosteroids.

The bilirubin level increased on increasing the dose of progesterone. Progesterone per se could, however, hardly have caused the rise of the bilirubin level. It seems likely that when progesterone is decomposed into oestrogens (possibly 17-ketosteroids) it interferes with bilirubin metabolism.

Thus, the findings allowed to conclude that

(i) progesterone loading increases the excretion of oestrogens and ketosteroids in the new-born infant, and

(ii) it raises the bilirubin level depending on the dose applied.

In these changes a general phenomenon associated with steroid excretion is presumably involved, inasmuch as both oestrogens [13] and progesterone prevent the conjugation of bilirubin to glucuronic acid by a competitive inhibition. The same could be observed after loading with pregnanediol isomers.

These findings may also have a clinical meaning. To avoid threatening premature birth, progesterone in doses of 50—100 mg or even more have been applied with favourable results. In some cases, however, the treatment was unsucessful and premature birth ensued.

In the course of progesterone treatment, the compound passes through the placental membrane and following premature birth it, or its metabolites, are excreted with milk. In both cases the new-born ingests the steroid and this may intervene with bilirubin metabolism in the manner described above.

Literature

1. Arias, I. M., Gartner, L. M., Seifer, S., Furman, M.: Neonatal unconjugated hyperbilirubinemia associated with breast feeding and a factor that inhibits glucuronide formation in vitro. J. clin. Invest. **42,** 913 (1963).
2. Arias, I. M., Gartner, L. M., Seifer, S., Furman, M.: Prolonged neonatal unconjugated hyperbilirubinemia associated with breast feeding and a steroid, pregnane-3α,20β-diol in maternal milk that inhibits glucuronide formation in vitro. J. clin. Invest. **43,** 2037 (1964).
3. Hsia, D. Y. Y., Dowben, R. M., Shaw, R., Grossman, A.: Inhibition of glucuronyl transferase by progestational agents from serum of pregnant women. Nature (Lond.) **187,** 693 (1960).
4. Hsia, D. Y. Y., Dowben, R. M., Riabov, S.: Inhibitors of glucuronyl transferase in the newborn. Ann. N. Y. Acad. Sci. **111,** 326 (1963).
5. Holton, J. B., Lathe, G. H.: Inhibitors of bilirubin conjugation in newborn infant serum and male urine. Clin. Sci. **25,** 499 (1963).
6. Ittrich, G.: Eine neue Methode zur chemischen Bestimmung der oestrogenen Hormone im Harn. Hoppe—Seyler's physiol. Chem. **312,** 1 (1958).
7. Jendrassik, L., Cleghorn, R. A.: Photometrische Bilirubinbestimmung. Biochem. Z. **289,** 1 (1937).
8. Krauer-Mayer, B., Keller, M., Hottinger, A.: Über den frauenmilchinduzierten Icterus prolongatus des Neugeborenen. Helv. paediat. Acta **23,** 68 (1968).
9. Lauritzen, C., Lehmann, W. D.: Einfluß von Steroidhormonen auf die Bilirubinwerte beim Neugeborenen. 2. Mitteilung. Geburtsh. u. Frauenheilk. **26,** 17 (1966).
10. Lauritzen, C., Lehmann, W. D.: Die Bedeutung von Steroidhormonen in der Pathogenese der Hyperbilirubinämie und des Icterus neonatorum. Z. Kinderheilk. **95,** 143 (1966).
11. Lauritzen, C., Lehmann, W. D.: Ausscheidung von Pregnandiol und Pregnanolon im Neugeborenen. Stimulierung durch Choriongonadotropin und ACTH. Arch. Gynäk. **204,** 197 (1967).
12. Sas, M., Viski, S., Gellén, J.: Steroidgehalt der Frauenmilch. Arch. Gynäk. **207,** 452 (1969).
13. Sas, M., Gellén, J., Viski, S.: Steroidausscheidung und Serum-Bilirubinwerte bei mit Muttermilch und bei künstlich ernährten Neugeborenen. Zbl. Gynäk. **91,** 1296 (1969).
14. Sas, M., Herczeg, J.: Újszülöttek serum-bilirubin szintje és steroid-kiválasztása progesteron-terhelés után. Orv. Hetil. **110,** 2502 (1969).

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