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Choleretic Response to Hydrocortisone in the Rabbit

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The influence of i.v. infusion of hydrocortisone sodium succinate on basal biliary secretion and maximal hepatic excretion of bilirubin was investigated in pentobarbitone anesthetized rabbits. Hydrocortisone caused an increase in bile flow and electrolyte secretion in basal conditions. When it was infused after a maximal load of bilirubin, its choleretic effect disappeared and the maximal excretion of bilirubin was not modified. Our data suggest that the effect of hydrocortisone on bile flow and composition takes place at canalicular level and is not directly related to the secretion of bile salts.

Key words: Choleresis, Hydrocortisone, Rabbit, Bilirubin.

Whilst a number of accounts of the adrenocortical steroid effects on biliary secretion in various species have been published, the results described conflict to a considerable extent. It is known that certain steroid hormones, such as estrogens or 19-alkylated androgens, are cholestatic and decrease bile flow by inhibiting both bile salt dependent and bile salt nondependent secretion (12, 18). This effect is accompanied by changes in the hepatocyte membrane bound Na-K ATPase (21). Variable effects have been attributed to cortisone and hydrocortisone (3), although in dog (15) and rat (4) hydrocortisone seems to have a clearly choleretic action. Furthermore, it has been demonstrated that in dog, this choleretic effect dissapears after the infusion of bromosulphthalein at maximal rates (15).

The aim of the present study was to determine the effect of i.v. infusions of hydrocortisone on the basal biliary secretion and maximal hepatic excretion of bilirubin in the rabbit, a species in which choleretic agents other than bile salts seem to be ineffective (13, 20).

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Materials and Methods

Experimental Protocols. Castilian rabbits were used with a weight range of 1.8-2.2 kg. Food but not water was withheld for 24 h before experiments. The rabbits were anesthetized with sodium pentobarbitone (Nembutal, 25 mg/kg i.v.). After tracheotomy, the cystic duct was ligated and the common bile duct cannulated with polyethylene tubing (PE 50). A femoral vein was cannulated for intravenous infusions. Rectal temperature was monitored and maintained at 38 ± 0.5° C by a thermostatically controlled table heater. Bile was collected in 20 min consecutive samples in tared tubes, cooled in ice and protected from light to avoid pigment oxidation.

Two kinds of experiment were carried out. In the first (Type I), after an initial control period of 60 min, hydrocortisone was infused at 1.5 mg/kg/min, and the collection of bile was then continued for a further recovery period of 60 min. In the second (Type II), following a control period of 60 min, bilirubin was infused for two hours at 0.6 mg/kg/min; this rate was chosen because higher doses did not further increase bilirubin excretion, indicating that a maximal excretion had been reached. During the second hour, an hydrocortisone infusion was added at 1.5 mg/kg/min.

Bilirubin (Sigma) was dissolved in distilled water alcalinized with 1M NaOH at pH = 7.5. Hydrocortisone sodium succinate (Sigma) was dissolved in 0.9 % NaCl.

Analytical Methods. The following determinations were carried out in bile: flow was determined gravimetrically; sodium and potassium by flame photometry and chloride by potentiometric titration with $AgNO_3$. Osmolarity was measured cryoscopically. Total bilirubin concentration was determined by the method of MALLOY and EVELYN (16); phospholipid concentration by the method of BARTLETT (1) in a biliary lipid extract (9). Deoxycholate, whose glycine conjugate is the most abundant bile salt in rabbit (11), was measured, previous precipitation of bile pigments, by the method of LEVIN *et al.* (14).

Results were expressed as mean values with standard error of the mean (SEM). The statistical significance of differences was determined by Student's *t* test.

Results

Type I experiments. Within the first 20 min of the infusion of hydrocortisone a clear increase in bile flow was observed (fig. 1). This increase gradually disappeared in the following one hour recovery period. Deoxycholate concentration, like that of phospholipid and bilirubin, decreased (table I) and there was a slight decrease in the output of all these solutes (fig. 2). With respect to the electrolyte concentrations, chloride rose significantly ($p < 0.05$) and sodium to a lesser extent (table I). The output of chloride, sodium and potassium increased, although to different degrees (fig. 3). There was no

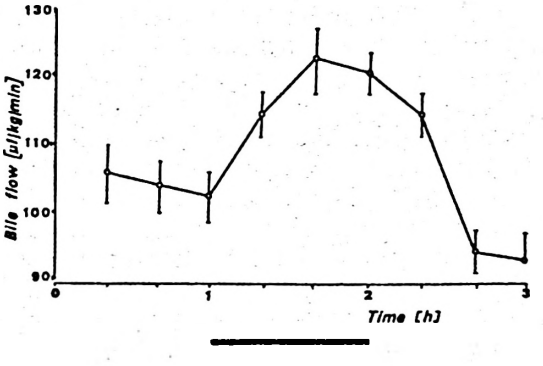

Fig. 1. *Effect of hydrocortisone on bile flow in Type I experiments.*
———: hydrocortisone infusion at 1.5 mg/kg/min.
Mean ± SEM (n = 5).

Table II. *Effect of bilirubin infusion (0.6 mg/kg/min) and hydrocortisone infusion (1.5 mg/kg/min) on bile composition (Type II experiments).*

Period	Sample, 20 min	Deoxycholate, mEq/l	Bilirubin, mg/100 ml	Phospholipid, mg/100 ml	Chloride, mEq/l	Sodium, mEq/l	Potassium, mEq/l
Control	1	4.5 ± 0.9	6.2 ± 1.0	36 ± 2	94 ± 4	147 ± 4	3.6 ± 0.2
	2	4.3 ± 0.7	6.0 ± 0.9	38 ± 3	94 ± 2	136 ± 4	3.3 ± 0.2
	3	3.9 ± 0.5	5.3 ± 1.1	35 ± 2	98 ± 4	149 ± 5	3.3 ± 0.1
Bilirubin	4	3.1 ± 0.3	116.6 ± 25.0	30 ± 4	101 ± 4	157 ± 4	3.6 ± 0.2
	5	3.0 ± 0.2	368.2 ± 45.7	33 ± 4	104 ± 6	168 ± 2	3.8 ± 0.1
	6	3.4 ± 0.5	397.3 ± 37.2	31 ± 4	104 ± 8	161 ± 2	3.9 ± 0.3
Bilirubin plus hydrocortisone	7	2.9 ± 0.3	387.9 ± 11.9	31 ± 4	102 ± 6	168 ± 2	3.9 ± 0.4
	8	2.8 ± 0.3	368.8 ± 21.1	33 ± 2	107 ± 4	171 ± 7	3.9 ± 0.2
	9	2.9 ± 0.3	365.1 ± 26.1	33 ± 3	105 ± 6	168 ± 3	4.1 ± 0.3

Values are mean ± SEM of five animals.

Table I. *Effect of hydrocortisone infusion (1.5 mg/kg/min) on bile composition (Type I experiments).*

Period	Sample, 20 min	Deoxycholate, mEq/l	Bilirubin, mg/100 ml	Phospholipid, mg/100 ml	Chloride, mEq/l	Sodium, mEq/l	Potassium, mEq/l
Control	1	4.3 ± 0.5	4.0 ± 1.3	39 ± 3	91 ± 6	147 ± 8	3.3 ± 0.1
	2	4.0 ± 0.4	4.0 ± 1.4	35 ± 1	93 ± 5	143 ± 6	3.5 ± 0.1
	3	4.2 ± 0.6	3.6 ± 0.9	36 ± 2	99 ± 8	146 ± 8	3.1 ± 0.2
Hydrocortisone	4	3.3 ± 0.3	3.2 ± 0.8	31 ± 4	109 ± 9	151 ± 4	3.0 ± 0.1
	5	3.2 ± 0.5	2.5 ± 0.7	26 ± 2	112 ± 10	151 ± 7	3.1 ± 0.1
	6	3.0 ± 0.3	2.5 ± 0.5	26 ± 2	117 ± 7	155 ± 3	3.1 ± 0.2
Recovery	7	3.2 ± 0.3	2.8 ± 0.7	29 ± 2	110 ± 11	150 ± 5	3.2 ± 0.1
	8	3.7 ± 0.4	2.7 ± 0.4	28 ± 1	103 ± 6	147 ± 5	3.0 ± 0.2
	9	3.5 ± 0.3	2.7 ± 0.5	25 ± 2	104 ± 6	150 ± 3	3.3 ± 0.1

Values are mean ± SEM of five animals

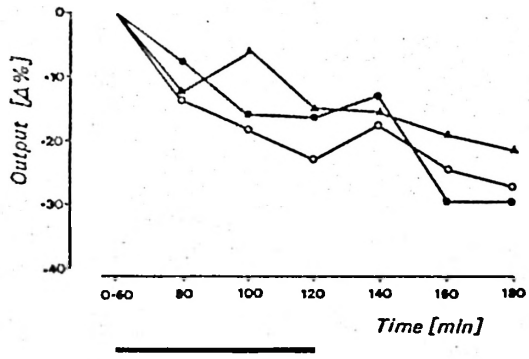

Fig. 2. *Mean change in deoxycholate (▲), bilirubin (●) and phospholipid (o) output as % of control in Type I experiments.*
———: hydrocortisone infusion at 1.5 mg/kg/min (n = 5).

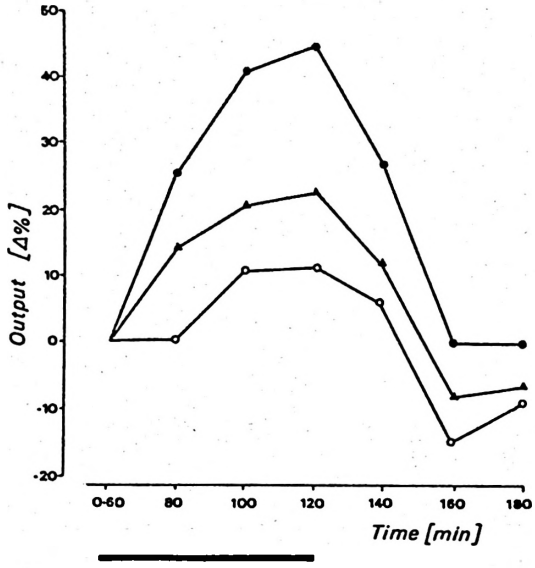

Fig. 3. *Mean change in chloride (●), sodium (▲) and potassium (o) output as % of control in Type I experiments.*
———: hydrocortisone infusion at 1.5 mg/kg/min (n = 5).

significant change in osmolality, with values from 280 ± 4 to 289 ± 5 mOsm/l.

Type II experiments. Bile flow decreased continously throughout the ex-

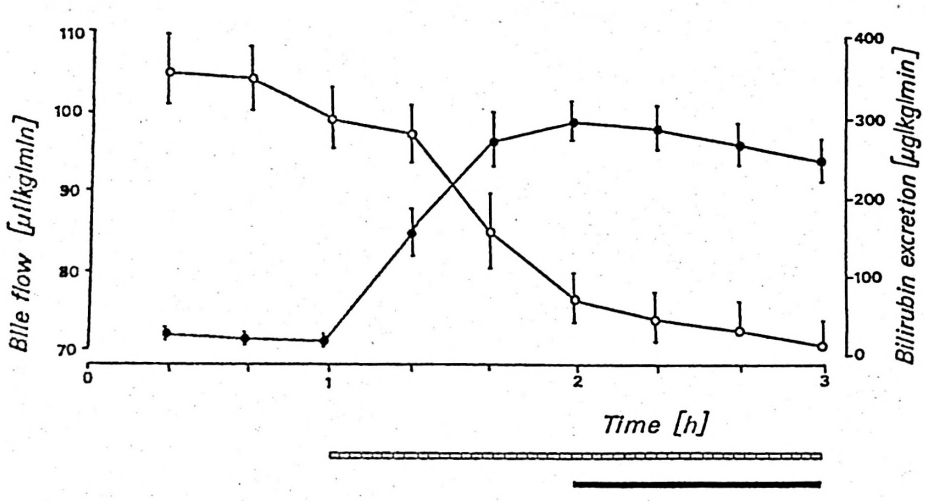

Fig. 4. *Effect of hydrocortisone on bile flow (o) and bilirubin excretion (●) after a maximal infusion of bilirubin. Type II experiments.*
═══: bilirubin infusion at 0.6 mg/kg/min. ———: hydrocortisone infusion at 1.5 mg/kg/min. Mean ± SEM (n = 5).

periments (fig. 4). Bilirubin concentration was elevated in response to its infusion ($p < 0.001$), but no further increases occurred when the hydrocortisone infusion was added (table II). A maximal excretion was reached 40 min after pigment infusion began and then showed only a slight decrease in response to the addition of hydrocortisone (fig. 4). The concentrations of sodium, potassium and chloride increased during bilirubin infusion and remained elevated during the additional infusion of hydrocortisone. The opposite was true for deoxycholate and phospholipid concentrations. Osmolarity showed no significant change.

Discussion

The present results agree with those of MACAROL *et al.* (15) and DUMONT and ERLINGER (4) in the sense that hydrocortisone infusion does induce a choleresis characterized by an increase in the concentration and output of electrolytes, especially chloride. No increase in the secretion of bile salts took place, thereby excluding the possibility that they could be responsible for the increase in bile flow when hydrocortisone was infused. The slight decrease in the output of bile salts, phospholipid and bilirubin, was probably related to the fact that in our experiments the enterohepatic circulation of bile salts was interrupted (8).

The possibility that hydrocortisone might act through the establishment of an osmotic gradient at canalicular level, as is the case with other coleretics, like methyl umbelipherone in the dog (6) or ethacrynic acid in the rat (2), is also remote. It has been demonstrated in the dog that, even through hydrocortisone is excreted into bile (15), the amounts found are insufficient to cause an increase in bile flow. Moreover, in our own experiments, no statistically significant differences were observed in bile osmolarity.

Nor it is likely that there could be a secondary rise in secretin levels due to an increase in gastric secretion, for in that case, much more concentrated secretion would be expected (19) and furthermore, in the rabbit, as in the rat (4), secretin seems to be ineffective except in massive doses (7).

The effects of bilirubin infusion at 0.6 mg/kg/min on bile flow and composition were described in a previous paper; bilirubin caused a decrease in bile flow when compared to control animals with the enterohepatic circulation interrupted, attributable to the inhibition of the bile salt nondependent fraction of secretion (10). The addition of an infusion of hydrocortisone to that of bilirubin (Type II experiments) left the effect of the latter essentially unchanged: the stimulatory action of hydrocortisone when infused alone (Type I experiments) was completely masked under these conditions. This fact, together with the positive effect on erythritol clearance obtained by other authors in the dog (15) and the rat (4) seems to indicate that the effect of hydrocortisone is exerted at canalicular level.

A possible explanation for a coleretic action at this site would be an increase in the permeability of the canalicular membrane to water and electrolytes, though studies carried out using saccharose clearance (4) suggest that this is unlikely. The increase observed in sodium output could lend weight to the idea of stimulation in some way of the bile salt nondependent fraction of secretion, as has been indicated in rat (17), but the data available cannot explain the exact nature of the mechanism and further work is evidently needed. The fact remains nevertheless, that hydrocortisone seems to be the first canalicular choleretic

other than bile salts to be described in the rabbit, unless we consider the contradictory reports on the effects of ethacrynic acid (5, 22).

Resumen

Se estudia la influencia de la infusión intravenosa de succinato sódico de hidrocortisona sobre la secreción biliar basal y excreción hepática máxima de bilirrubina en conejos anestesiados con pentobarbital sódico. La infusión de hidrocortisona origina un incremento en el flujo de bilis y secreción de electrolitos en condiciones basales. Tras una infusión máxima de bilirrubina, desaparece el efecto colerético de la hidrocortisona sin que se modifique la máxima excreción del pigmento. Estos resultados sugieren que el efecto de la hidrocortisona sobre el flujo y composición de la bilis tiene lugar a nivel canalicular y no se relaciona de forma directa con la secreción de sales biliares.

References

1. BARTLETT, G. R.: *J. Biol. Chem.*, **234**, 466-468, 1959.
2. CHENDEROVITCH, J., RAIZMAN, A. and INFANTE, R.: *Am. J. Physiol.*, **229**, 1180-1187, 1975.
3. CLIFTON, J. A., INGELFINGER, F. J. and BURROWS, B. A.: *J. Lab. Clin. Med.*, **51**, 701-708, 1959.
4. DUMONT, M. and ERLINGER, S.: *Biol. Gastroenterol. (Paris)*, **6**, 197-203, 1973.
5. ERLINGER, S., DHUMEAUX, D. P., BERTHELOT, P. and DUMONT, M.: *Am. J. Physiol.*, **219**, 416-422, 1970.
6. ERLINGER, S. and DUMONT, M.: In «The Liver. Quantitative Aspects of Structure and Function» (G. Paumgartner and R. Preisig, eds.). Karger, Basel, 1973, pp. 306-314.
7. ESTELLER, A., LÓPEZ, M. A. and MURILLO, M.: *Q. Jl. exp. Physiol.*, **62**, 353-359, 1977.
8. ESTELLER, A., JIMÉNEZ, R. and LÓPEZ, M. A.: *Q. Jl. exp. Physiol.*, **66**, 359-366, 1981.
9. FOLCH, J., LEES, M. and SLOANE-STANLEY, G. H.: *J. Biol. Chem.*, **226**, 497-509, 1957.
10. GONZÁLEZ, J., HIDALGO, F., ESTELLER, A. and LÓPEZ, M. A.: *Comp. Biochem. Physiol.*, **74A**, 67-70, 1983.
11. GREGG, J. A. and POLEY, J. R.: *Am. J. Physiol.*, **211**, 1147-1151, 1966.
12. GUMUCIO, J. J. and VALDIVIESO, V. D.: *Gastroenterology*, **61**, 339-344, 1971.
13. KAMINSKY, D. L., BROWN, B. H. and DESPHANDE, Y. G.: *Am. J. Physiol.*, **238**, G119-G123, 1980.
14. LEVIN, S. J., JOHNSTON, C. G. and BOYLE, A. J.: *Analyt. Chem.*, **33**, 1407-1411, 1961.
15. MACAROL, V., MORRIS, T. Q. and BAKER, K. J.: *J. Clin. Invest.*, **49**, 1714-1723, 1970.
16. MALLOY, H. T. and EVELYN, K. A.: *J. Biol. Chem.*, **119**, 481-487, 1937.
17. MINER, P. B., SUTHERLAND, E. and SIMON, F. R.: *Gastroenterology*, **79**, 212-221, 1980.
18. PETERSON, R. E. and FUJIMOTO, J. M.: *J. Pharm. Exp. Therap.*, **202**, 732-739.
19. PREISIG, R., COOPER, H. L. and WHEELER, H. O.: *J. Clin. Invest.*, **41**, 1152-1162, 1962.
20. PUGH, P. M. and RUTISHAUSER, S. C. B.: *Gen. Pharm.*, **9**, 119-120, 1978.
21. REICHEN, J. and PAUMGARTNER, G.: *Am. J. Physiol.*, **231**, 734-742, 1976.
22. SHAW, H. M., CAPLE, I. and HEATH, T. J.: *J. Pharm. Exp. Therap.*, **182**, 27-33, 1972.