Isolation of Cortisol from Guinea Pig Bile.* (23493)

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(Introduced by L. T. Samuels)
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Cortisol has been found to be the major adrenal hormone in the plasma and urine of the guinea pig(1,2). Wyngaarden *et al.*(3) find a considerable amount of chloroform-soluble compounds containing C^{14} in the bile from guinea pigs subsequent to the administration of cortisol-4-C^{14}. In view of the recent isolation of tetrahydrocortisol and tetrahydrocorticosterone from cattle bile after hydrolysis with β-glucuronidase(4) it seemed worthwhile to determine the nature of the metabolites in guinea pig bile. The present study deals with the isolation of cortisol in plasma and bile from guinea pigs under ACTH stimulation.

Methods. Normal albino guinea pigs weighing between 250-350 g were used. The animals were given 3 I.U. of ACTH in gel per 100 g body weight subcutaneously 6 hours before sampling of blood and bile. The control animals were handled in the same fashion as those given ACTH except for the administration of the tropic hormone. To another group of animals 1 mg/100 g body weight of an alkali-extracted polysaccharide complex from *K. pneumoniae* was given intravenously on 3 successive days, and at different time intervals after administration of the third dose blood and bile were collected. For the collection of blood and bile, the animals were sacrificed by clamping the neck with a forceps. This method gives lower levels of plasma 17-hydroxycorticosteroids (17-OHCS) than those seen in animals acutely anesthetized with ether(5). Plasma and bile samples were obtained from the same animal, extracted with methylene chloride, and processed as described by Eik-Nes(6). From 0.5-4 ml of plasma and from 0.1-1 ml of bile were used per determination. These volumes were made up to 10 ml with water before extraction. Only samples exhibiting a typical Porter-Silber chromogen with a higher absorbancy at 410 mμ than at either 390 or 430 mμ are included in this publication. Values of 17-OHCS were corrected on the basis of the recovery of 0.5 γ cortisol taken through the total procedure.

Results. When plasma levels of 17-OHCS in the guinea pig are elevated by ACTH stimulation, free 17-OHCS appears in the bile (Table I). Under the experimental conditions used, we failed to detect a typical Porter-Silber chromogen in the bile of "normal" guinea pigs. Animals exposed to the "stressful" environment associated with the administration of bacterial polysaccharide exhibit free 17-OHCS in the bile as soon as the plasma concentration of these steroids is elevated (Fig. 1). Free 17-OHCS seem to persist in the bile after the plasma levels have returned to the pre-stressed range.

To determine whether the typical Porter and Silber chromogen in plasma and bile was cortisol(7), the following experiment was done: Twenty-two guinea pigs were sacrificed 6 hours after subcutaneous administration of 3 I.U. ACTH per 100 g of body weight. From this group of animals a total of 10 ml of bile and 50 ml of blood plasma was obtained. Plasma and bile were extracted with methylene chloride, partitioned between benzene and water and the water phase re-extracted with methylene chloride. The methylene chloride extracts were evaporated under nitrogen. The Porter-Silber reaction was done on an aliquot of each extract and a typical chromogen found(8). Using cortisol as reference standard, the pooled bile sample contained 400 γ of 17-OHCS per 100 ml and the pooled plasma sample, 140 γ per 100 ml.

The remaining portions were subjected to paper chromatography using the formamide-chloroform system of Zaffaroni and Burton

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(9). Only one compound was found in plasma and bile; this compound showed the same mobility on paper as authentic cortisol and could be detected by the ultraviolet scanning technic(10). The paper strips were eluted and the concentrated eluates read in the ultraviolet part of the spectrum using a Beckman DK 2 Spectrophotometer. Maximal absorbancy was found at 241 mμ for both the plasma and bile eluates. Acetylation with radioactive acetic anhydride in pyridine was then performed(11). In both the plasma and the bile extracts a radioactive compound moving chromatographically like the acetate of cortisol was detected. Finally, the acetate of the compound in bile and plasma was oxidized with chromic trioxide. After oxidation the compound was rechromatographed and behaved now like cortisone acetate in the chloroform-formamide system of Zaffaroni and Burton.

Discussion. The data show that high levels of cortisol in the blood plasma of guinea pigs are associated with the presence of this steroid as a free alcohol in the animal's bile. The normal guinea pig excretes a large amount of cortisol in the urine(2) and the conjugated metabolites of adrenal steroids in guinea pig urine are not glucuronides(2) as in man(12). A radioactive chloroform-extractable compound has been found in guinea pig bile following the administration of about 100 γ cortisol-4-C^{14}(4); 60% of the total radioactivity in the bile from guinea pigs given cortisol-4-C^{14} was, however, conjugated, but probably not in glucuronide form. Conjugation by the liver, therefore, occurs in this animal species, and one may presume that reduction of the steroid nucleus precedes the conjugation. In man, hydroxylation in position 3 of the adrenal steroids has been reported to be the rate limiting step in their metabolism(13). The presence of cortisol in guinea pig bile at a time when the plasma concentration of this steroid is high, could indicate either that enzymatic reduction occurs at a very slow speed or that the high concentration of cortisol passing through the animal's liver exceeds the capacity of the enzymes.

TABLE I. Levels of 17-OHCS in Plasma and Bile from the Same Guinea Pig.

Condition	17-OHCS γ/100 ml Plasma	Bile
Normal	20	0*
	31	0*
	10	0*
	43	0*
	17	0*
	22	0*
6 hr after ACTH	144	260
	209	315
	215	310
	212	375
	189	301
	210	298

* No detectable Porter-Silber chromogen.

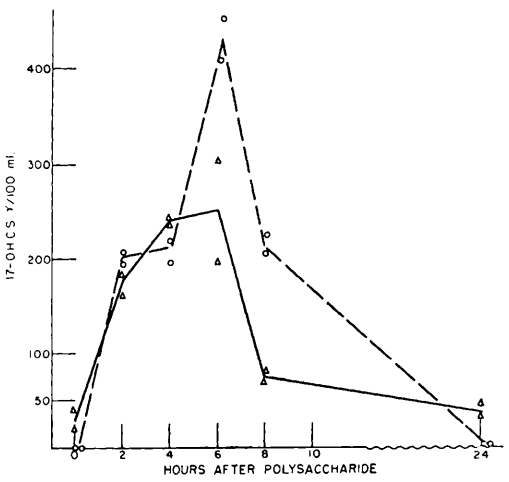

FIG. 1. △ = Plasma levels of 17-OHCS in individual guinea pigs sacrificed at different time intervals following intravenous administration of 1 mg *K. pneumoniae* polysaccharide complex/100 g body weight. ○ = Bile levels of 17-OHCS in the same animals. The average bile and plasma curves for 17-OHCS are plotted. All animals had received 1 mg *K. pneumoniae* polysaccharide complex/100 g body weight intravenously on the 2 preceding days.

Summary. Free cortisol has been isolated in the bile from guinea pigs with high plasma concentrations of this steroid. A limited hepatic ability to hydroxylate this hormone in position 3 of its nucleus has been suggested as a possible cause.

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Transformation and Culture Phase Dissociation of Staphylococcus (*Micrococcus pyogenes*) (23494)

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A distinctive pattern of dissociation evolved from studies on *Gaffkya tetragena*. A specific immunologic white colony type gave rise to yellow, pink, pink-yellow and brown types, each with its mucoid, smooth and rough phases, and in addition a translucent form (1,2,3). The variants emerged best in aging cultures in broth or on agar and seemed to appear by chance. Because of a close relationship between *G. tetragena* and *Staphylococcus,* tests were made with similar methods to see if a parallel dissociative pattern pertained.

Methods. Cocci from a single yellow colony of *Staphylococcus PCI 209 P* (U.S. Food & Drug Admin.) served as the inoculum of 100 ml nutrient broth in flasks. After incubation at room temperature for 3 to 7 months, subcultures were made by streaking or as 5 or 6 point colonies on nutrient agar 7-10 mm thick in plates sealed with a strip of parafilm to delay drying. The plates were similarly aged and continually inspected for visible evidence of colonial changes.

Results. Point colonies made from a 54-day flask culture were of the smooth(S) yellow phase 10 mm wide after 20 days. At first they were of the usual yellow butter color and consistence, but later faded until some were yellow in the center shading into white; others turned cream-color or white. On further aging a sharply defined, indented, colorless, translucent rim appeared around some; others were studded or rimmed or both with minute yellow and white daughter colonies. The white ones usually were yellowish by transmitted light. Subcultures from the yellow, white and translucent areas, and from the yellow and white nubbins, with exceptions to be described, grew as the original S-yellow form as if youth and crowding had delayed the formation of pigment. Shades from nearly white, cream, buff, pale yellow (citreus), yellow (aureus), to deep orange (auranticus) appeared in many streaked cultures and point colonies for no discernible reason. Subculture usually reproduced the original yellow tint. Growth at 10°C or 37°C made little difference in color change. One set of cream-colored point colonies 90 days old and 22 mm broad were studded and edged with pale daughter colonies. These in turn were surrounded by a translucent band with another rim of minute colonies (Fig. 1).

Cocci from the S-yellow colonies were coagulase-positive, fermented mannitol, liquefied gelatin and hemolized blood.

Origin of the Mucoid-Yellow Phase. Subcultures from the pale areas of a 58-day S-yellow colony grew as thick, glistening, pale yellow colonies, confluent where crowded (Fig. 2) in contrast with the usual discrete S-