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# SERUM DEPLETION OF CORTICOSTEROID BINDING ACTIVITIES, AN EARLY MARKER OF HUMAN SEPTIC SHOCK.

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## SUMMARY

We report that human sera within 24 hours after the onset of septic shock are virtually depleted of the corticosteroid binding activities characteristic for transcortin. By contrast, transcortin activities are not changed significantly in cases of acute inflammation, though septic shock and inflammatory sera are comparable in other respects : they show similar responses of their haptoglobin, thyroxine binding prealbumin, endogenous cortisol and progesterone levels. The physiological meaning and clinical interest of these results are discussed.

## INTRODUCTION

We reported recently (1) the 2-3 fold decrease of the plasma corticosteroid binding globulin (CBG or transcortin) concentrations in rats suffering a turpentine-induced acute inflammation. We now show that unlike the rat, human sera present no significant change of their corticosteroid binding activities in response to an acute inflammatory condition. However, sera originating from patients within 24 hours after the onset of septic shock are virtually devoid of the ability to interact with cortisol or corticosterone.

Inflammatory and septic shock sera are also compared with respect to endogenous cortisol and progesterone levels, as well as to the concentrations of two well known acute phase plasma proteins, the haptoglobin and the prealbumin (2).

Our results indicate that the serum depletion of corticosteroid binding activities may be a valuable early marker of septic shock in man.

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## MATERIAL AND METHODS

Sera : Three groups were studied : inflammatory, septic shock, and control sera.

The inflammatory sera originated from 19 adult patients of both sexes, undergoing one of the following acute inflammatory disorders : abcesses (n=11) ; appendicitis (n=4) ; orchiepidymitis (n=2) : peritonitis (n=1) ; panaris (n=1). At the time of blood sampling no antibiotics had been administered, no surgery applied and no clinical sign of septic shock observed.

The septic shock sera originated from 10 patients of both sexes (7 males, 3 females) within 24 hours following clinical onset of septic shock. Criteria for the diagnosis of shock were based at least on cold and mottled skin and urine flow under 30 ml/h. The septic state was also supported by positive (at least three) hemocultures ; the identified agents were E. Coli (n=3) : Staphylococcus aureus (n=2) ; Enterobacter cloacae (n=1) ; Streptococcus B (n=1) ; Clostridium perfringens (n=1) ; Salmonella typhimurium (n=1). No vasoactive drug or antibiotic had been administered at the time of blood collection. Hematocrit values were normal or slightly diminished. Arterial pressure was maintained only by saline perfusion. One patient, a 63 years old male, could be followed up at intervals, from about 10 h. after onset of shock up to 23 days later.

Control sera were collected from 12 healthy male and female adults receiving no medication.

Steroids : The following radioactive steroids of 97-98% regularly checked purity were used : 1,2,6,7-$^{3}$H corticosterone, 81 Ci/mmol ; 1,2,6,7-$^{3}$H cortisol 52 Ci/mmol ; 1α, 2α (n)-$^{3}$H progesterone. (Radiochemical Centre, Amersham). Radioinert steroids were from Roussel-Uclaf, France.

Binding studies : We measured the binding activities of the various sera for cortisol, corticosterone and progesterone, using a batchwise equilibrium dialysis technique, with a suspension of Sephadex G-25 as the semi-permeable membrane (3). Binding activities were expressed as "1/P" indices (L/g), where P is the concentration of serum proteins corresponding to an equilibrium ratio of steroid unbound/steroid bound =1. Protein concentration was assayed according to LOWRY et al. (4). Stripping of endogenous steroids was performed by addition of charcoal (5) : no difference was found between "1/P" binding indexes measured on native and stripped homologous sera.

Electrophoretic studies : Analytical electrophoresis in 10% polyacrylamide gels was performed on whole sera previously incubated with the labelled steroids, in order to locate the bound radioactivity at the different protein fractions. Detailed descriptions of this technique have been given elsewhere (6).

Haptoglobin and prealbumin determination : Haptoglobin was assayed by immunonephelometry (7). The thyroxine binding prealbumin was measured by radial immunodiffusion using the immunodiffusion plates M. Partigen-Prealbumin BEHRING.

Endogenous steroid determination : The serum concentrations of cortisol and progesterone were measured by radioimmunoassay with the specific rabbit antisera from the Institut Pasteur, Paris.

## RESULTS

Binding of cortisol, corticosterone and progesterone by inflammatory and septic shock sera. We show in table I the "1/P" binding indices measured

TABLE I

Response of "1/P" serum binding indices for cortisol, corticosterone and progesterone in inflammation and septic shock.

| | N° of subjects | 1/P indices (Mean ± SE L/g) | | |
|---|---|---|---|---|
| | | Cortisol | Corticosterone | Progesterone |
| Inflammation | 19 | 1,96 ± 0,35 | 3,1 ± 1,3 | 2,7 ± 1,01 |
| Septic shock | 10 | 0,16 ± 0,28 | 0,2 ± 0,3 | 0,86 ± 0,7 |
| Control | 12 | 2,1 ± 0,6 | 3,15 ± 2,05 | 2,05 ± 0,7 |

at equilibrium for cortisol, corticosterone and progesterone on whole sera from patients with various inflammatory disorders, from patients within 24 hours after the onset of septic shock and from healthy controls. The cortisol and corticosterone "1/P" values express essentially the activity of the CBG, whereas those measured with progesterone involve both the CBG and, to a lesser degree, the orosomucoid or $\alpha_1$-acid glycoprotein (8,9).

Obviously, in the subjects undergoing an inflammatory reaction, the binding of the corticosteroids is similar to that of the control sera. Neither is significantly changed in these subjects the binding of progesterone, though slightly increased values may be noted, which probably result from the rise of the orosomucoid in response to inflammation (2).

In utter contrast to inflammatory patients as well as to healthy controls, the sera from patients in septic shock have lost their ability to interact with the glucocorticoids : the "1/P" cortisol and corticosterone binding indices measured in these cases have fallen to values between 0 and 0,5, from means of about 2 for cortisol and about 3 for corticosterone in the normal and inflammatory sera. As concerns progesterone, its binding is considerably decreased but not altogether lost in response to septic shock.

These results clearly demonstrate the disappearance of the binding activities characteristic for CBG in subjects undergoing a septic shock ; the weak interactions with progesterone which persist in their sera are plausibly contributed by the orosomucoid.

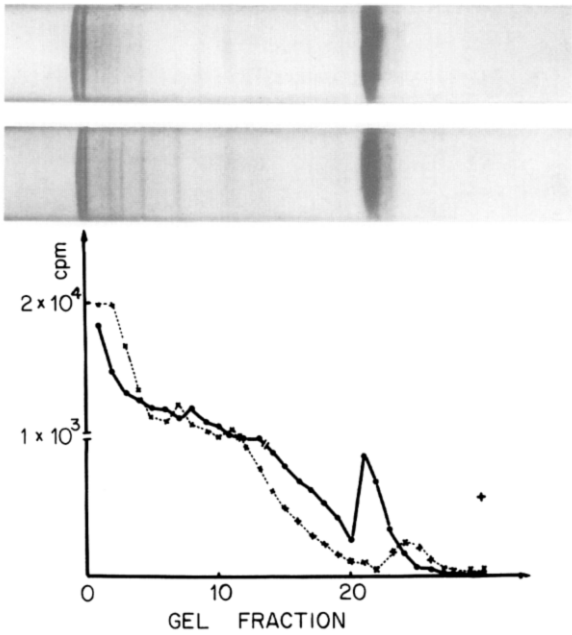


Figure 1 - Electrophoresis of control (.—. ; lower gel) and septic shock (x...x ; upper gel) sera preincubated with $^{3}H$-corticosterone. Ratios were about 350 µg serum protein/0,5 µCi tritiated steroid.

Electrophoretic studies in polyacrylamide gels.

Parallel electrophoretic runs of control and shock sera, pre-incubated with $^{3}H$ - corticosterone resulted in patterns essentially similar to those illustrated in Fig. 1. The significant trailing of radioactivity observed at the beginning of both gels is generally unavoidable when this non-equilibrium technique is applied to interactions with "1/P" binding indices inferior to about 3-4 (6). However, the two diagrams are clearly distinct : the one corresponding to the normal serum displays an obvious radioactive peak at the level of the $\alpha_1$-globulins, while no such peak is visible in the pathological case. Thus the main corticosteroid binding activity of the control sera appears located at a protein fraction with the electrophoretic characteristics of CBG (10) and the absence of this activity from the septic shock sera is specifically confirmed.

TABLE II

1/P binding indices for corticosterone measured on mixtures of control and septic shock sera. Means of determinations on 5 control and 5 septic shock serum samples.

| | 1/P indices (Mean ± SE L/g) | |
|---|---|---|
| | Measured | Calculated *) |
| Septic shock (S) | 0,2 ± 0,06 | - |
| Control (C) | 3 ± 0,3 | - |
| Mixtures of S + C | | |
| S + C (1:1 v/v) | 2,3 ± 0,3 | 1,9 |
| S + C (1:4 v/v) | 2,7 ± 0,4 | 2,6 |
| S + C (1:9 v/v) | 3,1 ± 0,2 | 2,9 |

*) Calculated for the actual concentration of control plasma proteins in the various mixtures.

Binding of corticosterone by mixed septic shock and control sera.

Taken together, our observations would plead a fall of transcortin levels in the septic shock sera, rather than an inhibition of its activity. We further investigated this point by measuring the "1/P" corticosterone binding indices on various mixtures of control and shock sera. Table II shows that addition of a patient non-binding serum to a normal one causes no inhibition of the control binding activity : variations of "1/P" values are indeed observed, but they are fully accounted for by the dilution of the control plasma proteins with different amounts of inert proteins. These results seem to rule out the possible presence, in the septic shock sera, of a factor inhibiting the CBG activity ; they favor the actual decrease of transcortin concentrations as the more plausible hypothesis.

Haptoglobin and prealbumin levels in inflammatory and septic shock sera.

Whereas it is well established that a number of plasma proteins increase or decrease in concentration in response to an acute inflammatory process (2) little is known on the possible variations of these "acute phase reactants" (APRs) in the septic shock. In table III we present the levels of two APRs, haptoglobin and thyroxine binding prealbumin, measured comparatively in sera

TABLE III

Response of haptoglobin and thyroxine binding prealbumin serum levels in inflammation and septic shock.

| | N° of subjects | Haptoglobin (Mean ± SE g/L) | Prealbumin (Mean ± SE mg/L) |
|---|---|---|---|
| Inflammation | 19 | 3,3 ± 1,4 | 103 ± 44 |
| Septic shock | 10 | 3 ± 1,3 | 98 ± 39 |
| Control | 12 | 0,84± 0,26 | 212 ± 64 |

from inflammatory and septic shock patients. It may be seen that these proteins are similarly affected in the two categories of injury and do not allow to discriminate between them : indeed comparable increases are observed for haptoglobin and comparable decreases are observed for prealbumin as a result of either inflammation or septic shock.

These results further support the notion that the serum CBG depletion described above might constitute in the human a specific response to the septic shock.

Endogenous cortisol and progesterone serum levels in inflammation and septic shock. To evaluate the in vivo impact of the responses of CBG it was important to assess how its main hormone ligands are affected by the pathological conditions under study. We present in table IV the concentrations of cortisol and progesterone in inflammatory, septic shock and normal sera.

In all the studied categories, the progesterone levels are fairly comparable. By contrast the cortisol levels are clearly increased in the inflamma-

TABLE IV

Response of serum cortisol and progesterone levels in inflammation and septic shock.

| | N° of subjects | Cortisol (Mean ± SE nmoles/L) | Progesterone (Mean ± SE nmoles/L) |
|---|---|---|---|
| Inflammation | 10 | 620 ± 370 | 2,2 ± 0,4 |
| Septic shock | 10 | 810 ± 500 | 2,4 ± 1,4 |
| Control | 12 | 390 ± 190 | 1,4 ± 1,2 |

tory and in the septic shock subjects. It must be stressed that our plasma samples were not collected at the same hour, and thus possible variations due to circadian rythms of corticosteroid secretion (11) should be considered. Our data constitute nevertheless preliminary evidence that, on an average, the responses of corticosteroid concentrations do not markedly differ in septic shock and in acute inflammation.

On the other hand, as discussed in more detail below, the concomitant rise of cortisol and fall of CBG might be one of the important mechanisms appealed to by the organism to face the septic shock.

## DISCUSSION

We demonstrate that human sera originating from patients in the early phase of a septic shock are virtually depleted of the ability to bind cortisol and corticosterone. Several lines of evidence, in particular the comparative electrophoretic behaviour of control and septic shock plasma proteins, strongly suggest that this dramatic collapse of corticosteroid binding activities results from the decrease of serum transcortin (CBG) levels. By contrast, sera from patients with acute inflammatory diseases display normal CBG indices, similar to those of healthy controls. This contrast is the more striking, as all other tested parameters, i.e. the concentrations of the "acute phase reactants" haptoglobin and thyroxine binding prealbumin, as well as the levels of endogenous cortisol and progesterone, are likewise affected by the septic shock and by the acute inflammation.

From these results a picture of the serum response in the septic shock would evolve, made up by features of the acute inflammation plus specific effects of the septic shock. The loss of CBG activities described here might thus be considered - by analogy to the "acute phase reactants" - as a " septic shock reactant ".

The present finding that CBG activities are not changed in the human inflammatory sera must be opposed to our previous demonstration of a marked

CBG decrease in the turpentine injected rats (1). This lays stress on the fact that extrapolations from experimental turpentine-treated animal models to clinical cases of acute inflammation should be made with considerable caution.

Further studies are necessary to explain the mechanisms controlling the disappearance of CBG in the early phase of septic shock. The rise of cortisol coinciding with the fall of CBG suggests an endocrine regulation, possibly an involvment of the adrenal function. Be that as it may, the unquestionable fact that increased amounts of corticosteroids are carried by the blood stream in a wholly free state is biologically important. It is now generally accepted that any decrease of the high affinity interactions between hormones and their specific serum binders enhances the strength of the hormone effects (12,13). Therefore the loss of CBG must considerably emphasize the impact of the elevated glucocorticoid secretion, and the simultaneous cortisol rise and CBG decline might represent an emergency response, triggered by the shock condition.

In the light of these consideration we also think that the serum levels of CBG activities ought to be taken into account when deciding upon a corticoid therapy.

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