LOW BINDING CAPACITY OF PORCINE CORTICOSTEROID-BINDING GLOBULIN

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In experiments on the hypothalamic-pituitary-adrenal axis in piglets [1] low basal concentrations of cortisol in the plasma were noted. In a recent series of experiments three piglets were chronically catheterised by a method described elsewhere [2], and the mean basal cortisol concentration was found to be 2.6 µg/100 ml plasma (S.E.M. ± 0.3). This figure was based on thirty-four separate samples from the three animals, all taken in the early afternoon to allow for diurnal variation. Our assay technique was the competitive protein-binding method of Murphy [3] adapted for ultramicro use by using dog plasma as the binding protein.

Dvorak [4] quotes the normal plasma 17-hydroxycorticosteroid (17OH CS) concentration in eight week-old piglets as 8.2 µg/100 ml (S.D. ± 3.4), using the Porter-Silber reaction; and Donald et al. [5] found the basal plasma 11-hydroxycorticosteroid (11OH CS) concentration in 20 kg piglets to be between 3 and 6 µg/100 ml, using the fluorimetric assay technique of Mattingly [6]. These workers used animals of the same age and weight as those in our experiments, and considering differences in assay methods their results confirm the relatively low plasma cortisol concentrations in piglets.

It has been noted in our laboratory that pig plasma is unsuitable as a binding agent for use in the protein-binding cortisol assay of Murphy [3]. Plasma from dog or man (which have higher plasma cortisol concentrations), however, give excellent standard curves in this assay system; and we therefore postulated that porcine corticosteroid-binding-globulin (CBG) may be of low cortisol-binding capacity.

Binding dilution curves were therefore prepared for human, canine, and porcine plasma samples. Aliquots of 0.5 ml of serially diluted plasma (concentrations 1 in 10 to 1 in 1280) were equilibrated for thirty minutes at 4°C with 0.5 ml of 0.05 M phosphate buffer pH 7.4 containing [3H]corticosterone ([1,2,6,7-3H]corticosterone 10 µCi/ml; Radiochemical Centre, Amersham, Bucks, England) 0.4 ml per 50 ml buffer. Unbound steroid was separated by
agitation with 40 mg portions of Florisil (British Drug Houses, Poole, Dorset, England), and radioactivity measurements were carried out using a scintillation cocktail containing Triton X-100 as emulsifier on a Tracerlab CM. 200 liquid scintillation counter.

The results are shown in Fig. 1 and show a remarkably flat binding curve for porcine CBG as compared to human and canine CBG. This could be due to either porcine CBG being present in very low concentrations, or having a very poor binding affinity for cortisol. Further work is in progress to elucidate the problem, but either way the result of this observation would be that a very high proportion of the total circulating cortisol is present in the unbound, physiologically active form. This may explain why pigs tolerate concentrations of total plasma cortisol which are low in comparison to human levels.

References