gen administered. Hormones of mammotropes stimulate body growth and growth of many organs. Somatotropic and mammotropic effects are marked in hypophysectomized rats bearing mammotropic tumors; thus they are independent of other pituitary factors.

Mrs. Lee Zompetti skillfully assisted in this work.

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## Binding of Novobiocin with Plasma Proteins. (23097)

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The antibiotic activity of novobiocin *in vitro* is inhibited by the presence of serum (1).* In *in vivo* experiments novobiocin is still present in the blood stream 24 hours after an oral dose(2).* These findings together with evidence from dialysis* have suggested that novobiocin must bind to plasma proteins.

The purpose of this work was to determine the nature and extent of this binding. Fractionation studies with plasma from a dosed dog showed that novobiocin is extensively bound to plasma albumin, and *in vitro* experiments with crystalline bovine albumin showed that the binding is a reversible equilibrium process.

*Plasma fractionation.* An adult female Beagle dog was given by mouth 100 mg of novobiocin (CATHOMYCIN sodium, Merck) per kg of body weight. After 3 hours blood was withdrawn from the jugular vein, mixed with .15 volume of sodium citrate-citric acid-dextrose (ACD) solution(3), and centrifuged. The clear plasma, amounting to 38 ml, was fractionated by Cohn's Method 10(3). A second dog, not dosed, supplied a like amount of plasma as a control. The plasma fractions were assayed for novobiocin by the microbiological procedure of Frost and Valiant(4).

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TABLE I. Novobiocin Concentrations in Plasma Fractions.

| Fraction | Novobiocin content as γ/ml of original plasma: Dosed dog | Control dog |
|---|---|---|
| Original plasma | 164 | 0 |
| Fractions I, II, & III | <2 | 0 |
| " IV & V | 62 | 0 |
| " VI* | ++++ | ++++ |
| " IV | 4.2 | 0 |
| " V | 35 | 0 |

* Fraction VI contained $Zn^{++}$ which interfered with assay.

The results are shown in Table I. The first precipitate, consisting of fractions I, II, and III, did not contain appreciable amounts of novobiocin. Fractions IV and V, precipitated together by the addition of $Zn^{++}$ reagent, contained 38% of total plasma novobiocin. The excess $Zn^{++}$ reagent present with fraction VI interfered with microbiological assay of this fraction. On separation of fractions IV and V it was found that almost all of the novobiocin was bound to fraction V, which consists of the plasma albumin. Quantitative recovery cannot be expected in this fractionation when binding is reversible, first because not all of the novobiocin originally present in the plasma is bound, and second because of additional dissociation brought about by volume increases on reagent addition and washing of precipitates.

TABLE II. Novobiocin Concentrations in Dialysates.

| Dialysate of | | Novobiocin conc., γ/ml Microbiological | Colorimetric |
|---|---|---|---|
| .5% albumin | a | 62.5 | 59 |
| | b | 46 | 58 |
| .05% " | a | 92 | 93 |
| | b | 100 | 88 |
| .005% " | a | 86 | |
| | b | 120 | |

*Binding with bovine albumin.* One mg of novobiocin and 500 mg of crystalline bovine albumin were dissolved in 10 ml of M/15 pH 7.4 phosphate buffer and dialyzed in a cellophane sack against one liter of the same buffer for 64 hours in the cold. Microbiological assays at the end of this time showed that concentration of novobiocin inside the sack was 90 γ/ml and that in the dialysate was below the threshold of the assay procedure. Thus novobiocin will bind to bovine plasma albumin.

*Equilibrium concentrations.* In duplicate experiments 10 ml volumes of M/15 pH 7.4 phosphate buffer containing 100 γ of novobiocin per ml and either 0.5%, 0.05%, or 0.005% bovine albumin were dialyzed at 37°C for 64 hours in cellophane sacks against 30 ml volumes of buffer containing 100 γ of novobiocin per ml. At the end of this time the solutions outside the sacks were assayed by microbiological assay and by the colorimetric procedure of Boxer and Shonk(5). Volume changes were negligible. The results are presented in Table II.

Ignoring any effect of the Donnan equilibrium, which would be minimized by the buffer salts present, we may assume that concentration of novobiocin outside the sack is identical with concentration of unbound novobiocin inside the sack. For the sacks containing 0.5% albumin amount of bound novobiocin can be calculated as follows:

Total novobiocin introduced, $100 \times 40 = 4000\ \gamma$
" free novobiocin, $58.5 \times 40 = 2340$
Novobiocin bound to 50 mg of albumin $= 1660$

Similarly, in the sacks containing 0.05% albumin:

Total novobiocin introduced, $100 \times 40 = 4000\ \gamma$
" free novobiocin, $90.5 \times 40 = 3620$
Novobiocin bound to 5 mg of albumin $= 380$

The amount of novobiocin bound per milligram of albumin was greater when concentration of free novobiocin was higher, an indication that the binding is an equilibrium process.

This was further investigated by placing the sacks containing the 0.5% albumin solution in fresh portions of buffer, containing no novobiocin this time, dialyzing for another 24 hours, and again analyzing the dialysates by the colorimetric procedure. They contained 24 γ, 25 γ of novobiocin per ml.

For the second dialysis the calculation is as follows:

Total novobiocin in system, $1660 + 10 \times 58.5 = 2245\ \gamma$
" free novobiocin, $24.5 \times 40 = 980$
Novobiocin bound to 50 mg of albumin $= 1265$

Thus the second dialysis produced a decrease of 395 γ in the bound novobiocin, and therefore the binding is reversible.

*Summary.* 1. Fraction studies with plasma from a dog treated with novobiocin showed that novobiocin is extensively bound to plasma albumin. 2. *In vitro* experiments with crystalline bovine albumin showed that the binding is a reversible process.

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