TABLE II. The Effect of Subcutaneous TP and Local Cortisone on Weight of Chick's Comb.

Group	Mean body wt (g)	Mean comb wt (mg)	Comb wt (mg) / Body wt (g)
TP + cortisone	71 ± 8.1*	62.6 ± 12.9 P<.3†	.86 ± .15 P<.5
TP + ethanol	78 ± 8.5	73.6 ± 25.6	.94 ± .30
Ses. oil + ethanol	73 ± 11.4	23.3 ± 36.6	.31 ± .06

* Stand. dev. † Fisher student t test.

periments adrenocortical steroids failed to suppress the growth-stimulating action of sex hormones on secondary reproductive organs as revealed by hyperplasia and cornification of the vaginal epithelium which resulted from direct application of ACE, by failure of locally applied cortisone to suppress estrogenic stimulation of the vagina and of androgenic stimulation of the chick's comb.

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Comparison of *in vitro* Serum Protein Binding of Thyroxin and Triiodothyronine.* (20696)

WILLIAM P. DEISS, EDWIN C. ALBRIGHT, AND FRANK C. LARSON.
(Introduced by Ovid O. Meyer.)

From the Department of Medicine, University of Wisconsin Medical School, and Veterans Administration Hospital, Madison, Wisc.

It has been observed that 3:5:3′ L triiodothyronine disappears more rapidly than L thyroxin from blood following intravenous administration(1). It is possible that this observation may be explained by a difference in serum protein binding of these two amino acids inasmuch as thyroxin, but not triiodothyronine, has been shown to associate with a specific alpha globulin *in vivo*(2).

The purpose of this paper is to report on the *in vitro* binding of L thyroxin and 3:5:3′ L triiodothyronine by serum proteins.

Methods and materials. The radioactive amino acids† employed in these experiments were of sufficiently high specific activity to permit the use of quantities that were approxi-

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† The radiothyroxin‡ used in these studies contained on chromatographic analysis nearly 100% of its radioactivity as thyroxin. The radio triiodothyronine‡ was found to contain approximately 5% of its radioactivity as thyroxin.

‡ Obtained from Abbott Laboratories.

TABLE I. Mixtures of Labelled and Unlabelled Amino Acids Added to Serum in Serum Protein Binding Experiments.

	μg of amino acid/ml serum			
Exp.	Radioactive thyroxin	Thyroxin	Radioactive triiodothyronine	Triiodothyronine
I	.1	—	—	—
II	.1	—	—	.1
III	.1	—	—	1.0
IV	—	—	.1	—
V	—	.1	.1	—
VI	—	1.0	.1	—

mately physiological. The labelled amino acids were dissolved in sodium hydroxide. Stock solutions were then prepared to contain 1.0 μg/ml in 0.001 N NaOH. Solutions of unlabelled amino acids§ containing 1.0 and 10 μg/ml in 0.001 N NaOH were prepared. As a preliminary experiment 0.1 ml of the labelled amino acid solution was added to 1.0 ml of saline and subjected to electrophoresis as described below. In the serum experiments quantities of the radioactive and unlabelled amino acid seen in Table I were added to 1 ml of serum. In each instance the unlabelled analogue was added 15 minutes after the labelled amino acid. This amino acid serum mixture was allowed to stand for 15 minutes, following which 0.05 ml was subjected to electrophoresis as previously described(3). Whatman No. 3 MM paper was used for these studies. The electrophoresis was carried out at pH 8.6, using 0.075 M veronal buffer and at pH 7.4, using 0.075 M phosphate buffer. Upon completion of electrophoresis the paper strips were dried, sectioned into 1 cm segments and counted. Protein zones were localized by bromphenol blue staining.

Results. In the electrophoresis of the amino acids in saline solution (Fig. 1), triiodothyronine showed no movement while thyroxin moved slightly toward the anodal end of the paper strip. The electrophoresis of the amino acid-serum mixtures at pH 7.4 and 8.6 disclosed a definite and consistent localization of both amino acids to an alpha globulin (thyroxin-binding protein) just ahead of the alpha-2 globulin zone (Fig. 2, I and IV). A small fraction of radioactivity remained at the starting point of electrophoresis and in the thyroxin experiment a small fraction moved with albumin. There was much greater trailing of the triiodothyronine than of the thyroxin. It is believed that this trailing of triiodothyronine represents dissociation of the amino acid from the thyroxin-binding globulin as this protein moved along the paper. That it is not "nonspecific" binding with the various proteins in this zone is indicated by the lack of correlation between the distribution of the radioactivity

§ Thyroxin was kindly supplied by E. R. Squibb & Sons. Triiodothyronine was kindly supplied by Smith, Kline & French Laboratories.

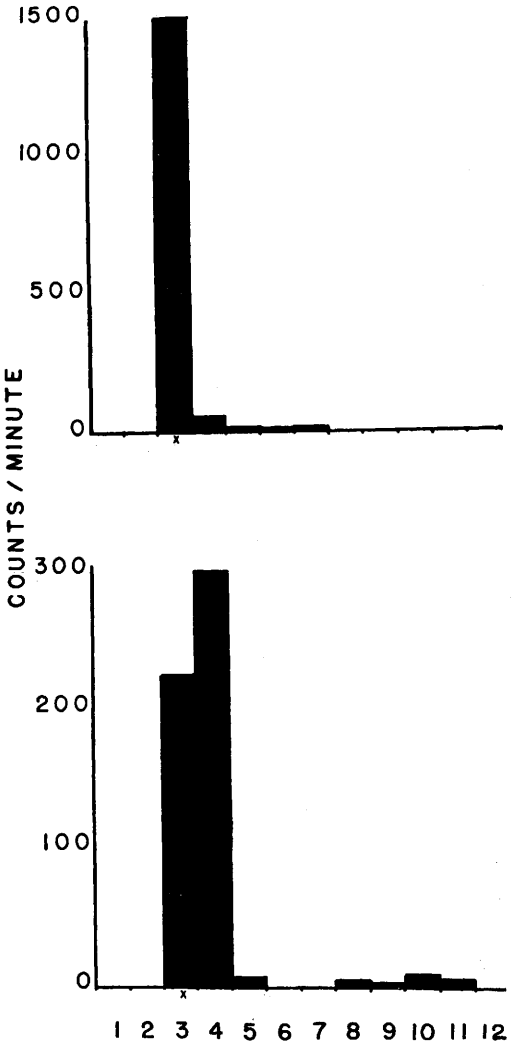

FIG. 1. Radioactivity in 2 cm segments of paper after electrophoresis in veronal buffer of saline solutions of radioactive triiodothyronine (top graph) and radioactive thyroxine (lower graph). (×) marks the starting point of electrophoresis.

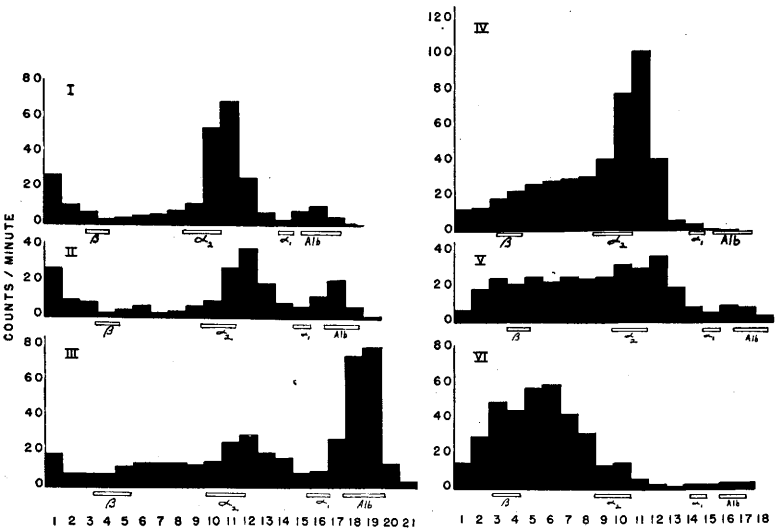

FIG. 2. Distribution of radioactivity in 1 cm segments of paper after electrophoresis in veronal buffer of the amino acid-serum mixtures described in Table I. The protein fractions are marked schematically by the small boxes. Only the paper on the anodal side of the starting point is shown since there was never any radioactivity in the gamma globulin, which moves by electro-osmosis toward the cathode.

and the concentration of the protein in this zone.

Fig. 2 (II and V) represent the effect of the addition of equal quantities of the unlabelled analogue to serum containing the radioactive thyroxin or triiodothyronine. The presence of unlabelled thyroxin greatly increases the dissociation of triiodothyronine whereas the binding of radioactive thyroxin is only slightly affected by the addition of triiodothyronine.

Fig. 2 (III and VI) represent the effect of the addition of excess quantities of the unlabelled analogue to serum containing the radioactive thyroxin or radioactive triiodothyronine. The addition of unlabelled thyroxin in quantities 10 times as great as radioactive triiodothyronine prevents entirely the association of radioactive triiodothyronine with the thyroxin-binding globulin while the presence of 10 times the quantity of unlabelled triiodothyronine fails to displace radioactive thyroxin completely from this protein.

There is a notable difference in the behavior of the displaced labelled amino acids. While displaced radioactive triiodothyronine does not associate with any specific protein, displaced radioactive thyroxin becomes associated with albumin.

Discussion. The demonstration of the *in vitro* association of thyroxine with the thyroxin-binding globulin is consistent with previous observations that thyroxin is associated with this protein *in vivo*(2). It is not unexpected that triiodothyronine also associates with this protein *in vitro* in view of the similarity of their molecular structures.

It appears that the thyroxin-binding protein has a greater affinity for thyroxin than for triiodothyronine, as thyroxin will displace triiodothyronine from this linkage. It is possible, in view of the known presence of thyroxin in serum in quantities greater than triiodothyronine, that thyroxin blocks the binding of triiodothyronine with this protein or replaces it once bound. This may account for a greater diffusibility of triiodothyronine and hence its more rapid disappearance from serum.

Summary. 1. *In vitro* experiments on the serum protein binding of thyroxin and triiodothyronine were performed using paper electrophoresis to separate the protein fractions and I^{131} labelled triiodothyronine and thyroxin to trace the localization of these amino acids. 2. Although both thyroxin and triiodothyronine were shown to be bound by alpha globulin, thyroxin was more firmly bound and not easily displaced by triiodothyronine whereas triiodothyronine was less firmly

bound and readily displaced by thyroxin. 3. Displaced triiodothyronine was found to trail the thyroxin-binding globulin, whereas displaced thyroxin was seen to associate with the albumin fraction. 4. These observations are possibly related to the rapid disappearance of triiodothyronine after intravenous injection and to the relatively low levels of triiodothyronine in blood.

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Erratum

Vol. 84, No. 1, Article 20539, Transfusion of Separated Leucocytes into Irradiated Dogs. . ., by Brecher *et al.*, page 56, left column, 12th line from bottom should read, "agranulocytic tonsillar lesion . . ."