SUMMARY

When hydrochloric acid is added to an isoelectric solution of ovalbumin in a dilatometer an expansion of about 11 ml per bound proton is observed. The expansion is caused by the neutralization of carboxylate ions by protons (WEBER AND NACHMANSOHN). A similar experiment with bovine serum albumin yields much smaller expansions when the pH is below 4, where the optical rotation and the viscosity also undergo anomalous changes. Evidently the molecular changes that occur below pH 4, bring about a decrease in the partial specific volume, $\bar{v}$, similar to that observed in protein denaturation, and this decrease in $\bar{v}$ is superimposed on the increase in $\bar{v}$ that normally occurs when carboxylate ions are discharged by protons. It is significant, however, that whereas 0.15 *M* potassium chloride almost completely eliminates the optical rotation change below pH 4, it is much less effective in preventing the anomalous contribution to $\bar{v}$. This fact raises questions about the hypothesis of protein structure recently made by YANG AND DOTY.

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A CELLULAR THYROXINE-BINDING PROTEIN FRACTION

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The observation of GORDON, GROSS, O'CONNOR AND PITT-RIVERS[1] that thyroxine is bound to a specific serum protein fraction in man, has been followed by extensive work on diverse aspects of this binding[2,3]. The specific protein fraction has also been detected in the blood of various mammals and reptiles and in other human extracellular fluids[2,4]. It is commonly referred to as thyroxine-binding protein or TBP. This protein fraction has the same electrophoretic mobility as serum α-globulin and is also capable of binding 3:5:3′-triiodo-L-thyronine and structurally related substances to a lesser degree. When the thyroid hormones are added in larger amounts they are then "spilled over" and bound to serum albumin. But, on the other hand, except for the precipitation of tissue iodine with proteins, only very little is known as yet about the interaction between thyroid hormones and tissue proteins[4,5]. This

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report is concerned with presenting evidence for the presence of a specific tissue protein (different from serum TBP) that is capable of binding thyroxine. Rat and rabbit skeletal muscle, liver, kidney, and brain were examined; more detailed experiments performed with rat muscle extracts are described below.

Fragments of rat skeletal muscle, washed to remove adhering blood, were homogenized in a medium of low ionic strength (0.1 M phosphate buffer, pH 6.8) using 1 g tissue/3 ml buffer. After centrifugation of the cell-free suspension at 20,000 $\times$ g for 30 min, the supernatant fluid was dialyzed against 0.05 M phosphate buffer (pH 6.8) for 24 h at 0–2°. The dialysate, after removal of the sediment, contained 7–10 mg protein/ml and constituted the extract used for electrophoretic and other studies.

Proteins of the muscle extract were fractionated by paper (Whatman No. 3) electrophoresis following the addition of small amounts of L-thyroxine labelled with ^{131}I. Of many buffers tried for this fractionation the most suitable was the veronal–acetate–KCl buffer described by KOTTER, LENZ AND PRÄNDL[5]. Recordings of distribution on paper strips of radioactivity from thyroxine are shown in Fig. 1, where the conditions of electrophoresis are also described.

Thyroxine alone, which shows no appreciable electrophoretic mobility (Fig. 1A), is found to migrate towards the anode in the presence of muscle extract (Figs. 1B and C). The position of the fraction of mobile thyroxine on the strip corresponds to a position intermediate to that of fractions "l" and "m" of Group I of the globular muscle proteins[6,7,8]. The radioactivity remaining immobile in the presence of muscle extract is considered to represent "free" or non-protein-bound thyroxine. The amount of thyroxine accompanying the muscle protein(s) in Fig. 1, B and C is determined by the concentration of this hormone. A high sensitivity of protein-binding to the concentration of the substance bound is here observed. The difference between the cellular and extracellular thyroxine-binding proteins is apparent from a comparison

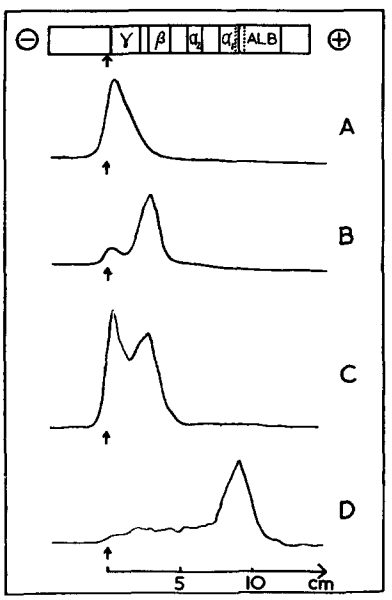

Fig. 1. Radioactivity distribution in electropherograms of ^{131}I-labelled L-thyroxine added to rat skeletal-muscle extract and rat serum. Electrophoresis with veronal–acetate–KCl buffer, μ = 0.1, pH 7.4; 7 V/cm for 7.5 h. Horizontal strip at top of the figure shows distribution of rat serum α_1-, α_2-, β- and γ-globulins, and albumin under these conditions of electrophoresis. Vertical arrows indicate the points of application of samples.

A: L-thyroxine alone;
B: Muscle extract + L-thyroxine ($3.5 \cdot 10^{-8}$ M);
C: Muscle extract + L-thyroxine ($7.0 \cdot 10^{-8}$ M);
D: Rat serum + L-thyroxine ($6.0 \cdot 10^{-8}$ M).

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between strips B and C and strip D, the ^{131}I peak in the latter indicating the mobility of rat serum TBP. In many extracts both thyroxine-binding protein fractions were present in the same sample owing to inadequate washing of the tissue prior to extraction. Chromatographic analysis proved that all the radioactive peaks seen in Fig. 1 represented thyroxine.

The effect of an increase in the concentration of added thyroxine on its distribution between the protein-bound and "free" forms became even more obvious when dialysis and trichloracetic acid (TCA) precipitation of proteins were carried out. The agreement in results obtained by the three different methods can be seen in Table I.

TABLE I

CAPACITY FOR PROTEIN-BINDING OF ^{131}I-LABELLED L-THYROXINE IN RAT SKELETAL-MUSCLE EXTRACT (8.2 mg PROTEIN/ml)

Concentration of L-thyroxine $\times 10^{-7}$ *M*	% ^{131}I *protein-bound after*		
	Electrophoresis	*Dialysis*	*TCA precipitation*
0.25	84.4	89.7	94.1
0.40	80.1	87.6	95.3
0.80	57.8	66.5	70.0
1.50	31.3	40.6	38.5
2.50	12.0	19.9	23.4
5.00	6.6	12.5	16.0

The fact that an increase in thyroxine concentration from $5 \cdot 10^{-8}$ *M* to $2 \cdot 10^{-7}$ *M* resulted in the saturation of the binding protein(s) indicates either (a) a relatively low affinity of cellular TBP for thyroxine, or (b) a feeble concentration of this protein fraction in the extract. (The amount of thyroxine bound/mg protein was, however, higher in the extract than in the crude homogenate.) The concentration of non-radioactive thyroxine already present in muscle is not known, but from other studies[5] it could be assumed to be in the order of 10^{-9}–10^{-10} *M*. By comparison, in rat serum thyroxine was found to be protein-bound (either to serum TBP or albumin) up to a concentration of $5 \cdot 10^{-6}$ *M*. In Table I, the higher values for protein-bound thyroxine found by dialysis and TCA precipitation seem to indicate that a small fraction of what appeared to be "free" thyroxine could have been bound to another muscle protein fraction that is electrophoretically immobile.

The addition of increasing amounts of rat serum to samples of muscle extracts containing a trace amount of radiothyroxine further illustrated the relatively feeble thyroxine-binding power of cellular TBP (Table II).

TABLE II

EFFECT OF ADDING INCREASING AMOUNTS OF RAT SERUM TO MUSCLE EXTRACTS CONTAINING ADDED ^{131}I-LABELLED L-THYROXINE ($4.0 \cdot 10^{-8}$ *M*), AS MEASURED BY PAPER ELECTROPHORESIS

Final diln. of serum	% *of* ^{131}I *distributed in fractions*		
	"Free"	*Tissue TBP*	*Serum TBP*
—	21.0	79.0	—
1:25	14.0	63.2	14.8
1:15	8.7	51.1	40.2
1:10	3.2	36.0	61.8
1:5	0.8	2.6	96.6

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A transfer of thyroxine from cellular TBP to the serum TBP is thus brought about. This transfer is almost total, even at a final serum dilution of 1:7, a dilution at which serum and muscle-protein concentrations are almost equal. This difference in binding power or capacity between the specific protein fractions could be an important reason for the low concentration of iodine found in skeletal muscle. It would also explain further the recently observed inhibition of uptake of thyroxine and triiodothyronine by tissue slices in the presence of serum, serum TBP, or serum albumin[4,5].

As with serum proteins, triiodothyronine was much less firmly bound to cellular proteins than was thyroxine—an important fact in considering the more rapid disappearance *in vivo* of triiodothyronine from both serum and tissues. A similar cellular TBP was found in rabbit skeletal-muscle extract, but the presence of blood in liver, kidney, and brain rendered its detection difficult in these tissues. It was possible to isolate small quantities of rat and rabbit muscle TBP by electrophoresis of extracts on cellulose columns. None of the experiments here described gives any indication of the homogeneity of the TBP fraction in muscle.

Studies of a more quantitative nature are necessary for determining a more precise physiological significance of cellular thyroxine-binding protein. In this respect, the effect of this protein fraction on thyroxine "deiodinase" is of interest. Enzyme activity of rat muscle "deiodinase" preparations was non-competitively inhibited by the addition of electrophoretically isolated rat-muscle TBP, the inhibition being directly proportional to the fraction of thyroxine bound to cellular TBP[9]. This interaction suggests a role for cellular thyroxine-binding protein in the regulation of the intracellular level of thyroid hormones by controlling their availability to enzymes in the cell.

SUMMARY

Evidence is presented of the existence of a protein fraction in rat skeletal muscle that is capable of binding L-thyroxine and 3:5:3′-triiodo-L-thyronine in physiological concentrations. As revealed by electrophoretic analysis, the specific protein fraction has a mobility intermediate between those of fractions "l" and "m" of globular muscle proteins. The cellular thyroxine-binding protein has both lower affinity and lower capacity for binding of thyroid hormones than that of serum thyroxine-binding protein or of serum albumin.

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