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Thetin-Homocysteine Transmethylase

THE DISTRIBUTION OF THE ENZYME, STUDIED WITH THE AID OF TRIMETHYLSULPHONIUM CHLORIDE AS SUBSTRATE

By G. A. MAW*

Department of Biochemistry, St Thomas's Hospital Medical School, London, S.E. 1

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Thetin-homocysteine transmethylase, which catalyses the transfer of a methyl group from dimethylthetin or dimethyl- β -propiothetin to homocysteine, was first identified in rat, pig and guinea-pig liver and kidney by Dubnoff & Borsook (1948*a, b*). Kensler & Langemann (1954) detected the transmethylase in the livers of a number of other mammalian species, including man, and it was subsequently found in horse liver (Durell & Anderson, 1956) and in pigeon liver (Sloane, Boggiano, Coulomb & Hutchings, 1956). Thoai & Robin (1956) also showed the presence of the enzyme in turtle and mullet liver and produced evidence for its occurrence in several invertebrates. According to Schlenk & DePalma (1956), the yeast *Torulopsis utilis* is unable to utilize the above-mentioned thetins as methyl donors for homocysteine and, presumably, does not possess the enzyme. Shapiro (1958) has recently obtained similar findings for *Aerobacter aerogenes*, *Saccharomyces cerevisiae* and some strains of *Escherichia coli*.

In assays of thetin-homocysteine transmethylase carried out previously by other workers, the activity of the enzyme has been expressed in terms of the amount of methionine formed in a given time, the product being determined colorimetrically. It is not possible to compare these published findings on a quantitative basis, since in most cases optimum substrate concentrations were not

employed, and no attention appears to have been paid to whether methionine formation was linear with respect to time or whether the experimental conditions chosen gave a linear relationship between enzyme activity and amount of tissue preparation.

In this paper a manometric method has been used for the study of the transmethylase. This method measures enzyme activity in terms of the rate of carbon dioxide formation from a bicarbonate medium, since the methylation reaction involves the formation of acid (Maw, 1955). With either dimethylthetin chloride or dimethyl- β -propiothetin chloride as methyl donor, it was found that neither the reaction rates nor activity/concentration relationships obtained with rat-liver thetin-homocysteine transmethylase were linear (Maw, 1956). However, it was shown (Maw, 1958) that trimethylsulphonium chloride acts as a methyl donor for the enzyme and, with this compound as substrate, reaction rates as well as activity/concentration relationships were linear. Trimethylsulphonium chloride was therefore suggested as a possible substrate for the assay of the transmethylase.

Further evidence is now presented that trimethylsulphonium chloride can act as a substrate for the enzyme and can be used in its assay. This compound has been employed in a manometric study of the transmethylase in various tissues of the rat, mouse and guinea pig, and in a survey of its occurrence in twenty-four species of plant and

* Present address: Chemist's Laboratory, Arthur Guinness, Son & Co. (Dublin) Ltd., St James's Gate, Dublin, Eire.

animal. The transmethylase has been detected in significant amounts only in the higher animals tested, where it is located mainly in the liver. This is in qualitative agreement with the findings of other workers, referred to above. The enzyme appears to be present only in negligible quantities, if at all, in the green plants, moulds and invertebrates examined.

MATERIALS AND METHODS

Dimethylthetin chloride, trimethylsulphonium chloride, triethylsulphonium chloride and the other sulphonium salts employed were prepared as described in a previous paper (Maw, 1958). DL-Methionine was demethylated to homocystine with sulphuric acid (Butz & du Vigneaud, 1932) and reduced to homocysteine with sodium in anhydrous NH_3 (Riegel & du Vigneaud, 1935-36).

The various species of animal under study, with the exception of the dog, were killed by a blow on the head and the required tissues were excized and placed in ice. Samples of dog liver were obtained from animals which had been bled to death under cyclopropane anaesthesia. Suspensions of the tissues were prepared as soon as possible in 0.02M- NaHCO_3 with a glass homogenizer (Potter & Elvehjem, 1936), and filtered through several layers of muslin. Plant-tissue suspensions were obtained by grinding the tissue with fine sand in 0.02M- NaHCO_3 . When moulds were examined, the pellicle was washed with the bicarbonate solution and sliced before being homogenized. Baker's yeast was used in the form of suspensions of the fresh organism and also cell-ruptured preparations were obtained by freezing in solid CO_2 , followed by thawing at 37° (Krebs, Gurin & Eggleston, 1952).

In assays of thetin-homocysteine transmethylase, 0.5 ml. of 10% (w/w) tissue suspension in 0.02M- NaHCO_3 was used in the side arm of each Warburg flask, unless otherwise stated. The main compartment of each flask contained DL-homocysteine (0.03M) and either dimethylthetin (DMT; 0.1M) or trimethylsulphonium chloride (TMS; 0.2M). In other experiments, such as studies of the effects of inhibitors, the methyl donors were added at lower concentrations, as described in subsequent sections. The substrates and other substances used were prepared in 0.02M- NaHCO_3 , as described previously (Maw, 1958). The total fluid volume in each flask was 2.5 ml. Flasks were gassed with $\text{N}_2 + \text{CO}_2$ (95:5, v/v), and formation of CO_2 was followed for 40-60 min. after mixing of the tissue suspensions with the substrates.

Enzyme activity has been calculated in terms of the initial reaction rate, this being taken as the volume of CO_2 liberated during the 10 min. period commencing 5 min. after the enzyme has been mixed with the substrates. The rate obtained was then expressed as a metabolic quotient, Q_{CO_2} , i.e. $\mu\text{l. of CO}_2/\text{mg. dry wt. of tissue preparation/hr.}$ The use of the Q notation is strictly of value only when TMS has been used as the methyl donor, i.e. under conditions which yield a linear enzyme activity/concentration relationship. However, rates obtained with DMT as substrate have also been expressed in this way because, in almost all the experiments reported, enzyme activities have been compared at a fixed tissue concentration.

RESULTS

Trimethylsulphonium chloride as a substrate

Tissue preparations which give rise to carbon dioxide formation in the presence of homocysteine and DMT behave similarly when the thetin is replaced by TMS. Although this may be taken to indicate that TMS is a substrate for thetin-homocysteine transmethylase, there is also the possibility that the compound is being demethylated by a second transmethylase present. A number of experiments have therefore been carried out to determine whether or not DMT and TMS are acted on by the same enzyme.

(i) *Parallelism of substrate activity.* The methylation of homocysteine by the two sulphonium salts has been studied in the presence of various tissue suspensions obtained from a wide range of plants and animals (see Table 2). Whenever carbon dioxide formation was observed with DMT as substrate, there was a concomitant activity detectable with TMS. In no case was transmethylase activity demonstrable with one of the methyl donors and not the other.

(ii) *Distribution of transmethylase activity in rat-liver preparations.* Rat-liver suspensions were subjected to ammonium sulphate fractionation (see Maw, 1956). Four fractions were obtained, namely, material precipitated at 0-0.35, 0.35-0.45 and 0.45-0.55 saturation and the final supernatant fluid. These fractions were dialysed against running tap water for 15 hr. at room temperature and then centrifuged at 1600 g for 10 min. The precipitates

Fig. 1. (a) Distribution of thetin-homocysteine transmethylase activity in fractions of rat liver obtained by ammonium sulphate precipitation. Liver suspension, 10% (w/w); substrates: DL-homocysteine (0.03M), DMT (0.1M); 0.02M-bicarbonate medium; flasks were gassed with $\text{N}_2 + \text{CO}_2$ (95:5, v/v); temp. 37.1° ; P, precipitate; S, supernatant fluid. (b) Distribution of thetin-homocysteine transmethylase activity in fractions of rat liver obtained by ammonium sulphate precipitation. Substrates: DL-homocysteine (0.03M), TMS (0.2M). Other details were as given for (a).

from each fraction were suspended in 0.02M-NaHCO₃, the supernatant portions made 0.02M with respect to NaHCO₃ and all samples were tested for transmethylation activity, DMT and TMS being used as substrates. Figs. 1*a* and 1*b* show the activities of the various fractions obtained with the two substrates, expressed as percentages of the total activity present, and it will be seen that TMS gives an activity-distribution pattern similar to that obtained with DMT.

(iii) *Transmethylation activity with mixed substrates.*

If carbon dioxide production occurring in the presence of DMT and TMS is brought about by two separate transmethylation enzymes, then when the two compounds are added together to enzyme preparations there should be a summation of carbon dioxide formation. If, on the other hand, only one enzyme is involved, there should be competition between the substrates for the enzyme, and the observed reaction rate obtained with the mixed substrates should not be greater than the rates obtained with either substrate alone (the mixed substrate reaction rate should, in fact, be somewhere in between these two values, depending on the relative affinities of the two substrates for the enzyme). That one enzyme only is operating is suggested by the results given in Figs. 2*a* and 2*b*. These were obtained with a mouse-liver suspension and an eel-liver suspension respectively. They show that no summation of carbon dioxide production occurred when the mixed substrates were used. Furthermore, although the more active substrate was different in each case, the combined rate was in both cases closer to the rate for DMT alone, indicating that the enzyme has a greater affinity for the thetin. Findings confirming this lack of any summation of reaction rates have also been obtained with liver homogenates from the rat, pigeon and guinea pig. With some of the enzyme preparations used, e.g. rat- and mouse-liver homogenates, TMS was a markedly less effective substrate than the thetin, and in order to provide the maximum opportunity for competitive effects to make themselves apparent, the concentration of DMT was reduced well below the optimum value.

(iv) *Effects of structural inhibitors and inorganic salts.* A similarity has been observed between the types of compound which inhibit carbon dioxide formation with DMT as methyl donor and those which inhibit in the presence of TMS. As would be expected, the effects were more marked with the latter substrate, on account of its lower affinity for the transmethylation enzyme. Inhibitions produced by the various compounds used were small or negligible with an optimum concentration of DMT, but became more apparent when lower concentrations of the thetin were employed. Table 1 shows the effects of a series of inhibitors on the activity of

the mouse-liver transmethylation enzyme. The compounds have been arranged in order of their effectiveness in the presence of 0.01M-DMT, and it will be seen that this corresponds closely to the order of their effectiveness when 0.1M-TMS was the substrate. Parallel results have been obtained with guinea-

Fig. 2. (a) Formation of carbon dioxide by a mouse-liver suspension, DMT and TMS being used as methyl donors. Tissue suspension, 2% (w/w); DL-homocysteine (0.03M), DMT (0.025M), TMS (0.2M); 0.02M-bicarbonate medium; flasks were gassed with N₂+CO₂ (95:5, v/v); temp. 37.1°. ■, DMT alone as methyl donor; ●, TMS alone as methyl donor; ▲, DMT+TMS as methyl donor. (b) Formation of carbon dioxide by an eel-liver suspension, DMT and TMS being used as methyl donors. Tissue suspension, 1% (w/w); DL-homocysteine (0.03M), DMT (0.1M), TMS (0.2M). Other details were as given for (a).

Table 1. *Inhibition of mouse-liver thetin-homocysteine transmethylation by various sulphonium and quaternary ammonium halides*

Mouse-liver suspensions, 2% (w/w); DL-homocysteine (0.03 M); dimethylthetin chloride (DMT; 0.01 M); trimethylsulphonium chloride (TMS; 0.1 M); 0.02 M-sodium bicarbonate medium; flasks were gassed with $N_2 + CO_2$ (95:5, v/v); temp. 37.1°.

Inhibitor (0.1 M)	Percentage inhibition	
	With DMT	With TMS
Betaine chloride	70.3	86.0
Triethylsulphonium chloride	34.9	58.0
Sulphocholine iodide	31.8	26.7
Diethylthetin chloride	25.3	41.7
Choline chloride	23.6	41.2
Tetramethylammonium bromide	14.5	24.4
Ammonium chloride	8.9	3.6

pig-liver homogenates. By the method of Lineweaver & Burk (1934), the inhibitions produced by betaine chloride, tetramethylammonium bromide and triethylsulphonium chloride have been shown to be strictly competitive in character.

Mercuric ions markedly inhibit the rat-liver transmethylation in the presence of DMT (Maw, 1958), and this is also true when TMS is the methyl donor. It is also significant that bromide and iodide ions (as sodium salts) produce a slight increase in transmethylation activity, which is apparent when either sulphonium compound is used as substrate. Thus in the presence of 0.1 M-TMS or -DMT, 0.1 M-sodium iodide increases the activity of the transmethylation of mouse and guinea-pig liver by approximately 25%.

In a single experiment, determinations of the inhibitor constant, K_i , for betaine chloride were carried out with the mouse-liver transmethylation, DMT and TMS being used separately as substrates (Lineweaver & Burk, 1934). K_i values so obtained should be equal if the substrates employed are acted upon by the same enzyme (see Dixon & Webb, 1958). They were, in fact, found to be comparable in magnitude, namely 6.4×10^{-2} with DMT as substrate, and 3.7×10^{-2} in the presence of TMS.

The various findings outlined above are considered to point strongly to the existence of a single transmethylation utilizing both DMT and TMS as methyl donors, but having a greater affinity for the former compound.

Assay of the transmethylation

With TMS as the methyl donor, a brief examination has been made of carbon dioxide formation corresponding to thetin-homocysteine transmethylation activity in various tissues. With 0.1- or 0.2 M-TMS, carbon dioxide evolution was generally strictly linear for 1 hr. at least, as found previously

for preparations of the rat-liver enzyme (Maw, 1958), although for some tissues, notably pigeon liver, there was occasionally a slight falling off in rate after about 30 min. It was therefore possible to make accurate determinations of initial reaction rates.

Plots of initial reaction rate against the volume of tissue suspension used have been carried out for liver homogenates from eight animal species, namely the dog, eel, goldfish, guinea pig, mouse, pigeon, rabbit and rat. In every case a strictly linear relationship was obtained with tissue concentrations ranging up to 2% (w/w) of the reaction mixture. It is therefore considered that, provided the amounts of tissue taken do not greatly exceed this value, the manometric procedure embodying the use of TMS as substrate may be reliably employed for the assay of thetin-homocysteine transmethylation.

Distribution of the transmethylation

Table 2 gives the results of a survey of thetin-homocysteine transmethylation activity in 24 plant and animal species, each value reported being the average of at least three separate determinations. The choice of substrate concentrations, namely 0.1 M-DMT and 0.2 M-TMS, was based on previous experiments with the rat-liver transmethylation. DMT was originally used only for the qualitative examination of the enzyme in the various source materials, but for comparative purposes activities obtained with this substrate have been expressed as Q_{CO_2} values.

Under the conditions of the transmethylation assay there was some carbon dioxide formation with homogenates from the majority of the green plants, moulds and samples of baker's yeast examined. This was small, however, and comparable with that observed in the absence of substrates. Moreover, it fell off sharply after 30 min. and in no way resembled the steady carbon dioxide production obtained with transmethylation preparations from animal liver. It is therefore assumed that transmethylation activity is negligible or absent in these species. Of the four invertebrates studied, only the common shore crab appeared to possess some slight transmethylation activity.

Significant activity was detected in the liver of all the vertebrates examined. Rat liver was a poor source of the enzyme, liver from the mouse, dog and guinea pig being two to three times as active. The most active tissue examined was eel liver, which contrasted markedly with goldfish liver in this respect. The enzyme was definitely present in rat and guinea-pig kidney, although its activity was low. Negligible amounts were found in mouse kidney and in the muscle, spleen and brain of the rat, mouse and guinea pig.

Table 2. *Thetin-homocysteine transmethylation activity of various tissues*

Tissue suspensions, 2% (w/w); DL-homocysteine (0.03M); dimethylthetin chloride (DMT; 0.1M); trimethylsulphonium chloride (TMS; 0.2M); 0.02M-sodium bicarbonate medium; flasks were gassed with $N_2 + CO_2$ (95:5, v/v); temp. 37.1°. Activity is expressed as Q_{CO_2} (μ l. of CO_2 /mg. dry wt. of tissue/hr.).

Species	Tissue	Q_{CO_2} (DMT)	Q_{CO_2} (TMS)
Rat	Liver	13.2	6.9
	Spleen, muscle, brain	0	0
	Kidney	4.3	2.0
Mouse	Liver	27.7	15.8
	Spleen, muscle, brain	0	0
	Kidney	0.9	0.5
Guinea pig	Liver	9.2	15.5
	Spleen, muscle, brain	0	0
	Kidney	2.4	1.8
Dog	Liver	14.5	20.4
Rabbit	Liver	17.0	11.2
Chicken	Liver	6.1	9.3
Pigeon	Liver	7.9	8.8
Goldfish	Liver	8.4	8.1
Eel	Liver*	25.5	65.6
Frog	Liver	11.7	15.3
Tortoise	Liver	2.9	2.8
Locust (adult)	Hepatic caecae, fat body, whole viscera	0	0
Cockroach	Whole viscera	0	0
Common mussel	Whole viscera	0	0
Common shore crab	Liver	1.6	0.4
	Gonads	0.8	0.5
	Gills	2.8	1.2
French kidney bean	Bean, leaf, root	0	0
Antirrhinum	Leaf	0	0
Lupin	Leaf, root	0	0
Cabbage	Heart, outer leaves, root	0	0
<i>Aspergillus niger</i>	Pellicle	0	0
<i>Penicillium chrysogenum</i>	Pellicle	1.4	0.9
<i>Scopulariopsis brevicaulis</i>	Pellicle	0.5	0.5
<i>Neurospora crassa</i> (three strains)	Mycelia	1	1
Baker's yeast	Whole cells, broken cells	0	0

* Tissue suspension (1%, w/w) was used.

It will be noted that, for the liver of some species, e.g. the rat, mouse and rabbit, initial rates were greater with 0.1M-DMT as substrate than with 0.2M-TMS, whereas with liver from the guinea pig, dog, eel, etc., the reverse was the case. This apparent variation in substrate activity is possibly due to species differences in the shapes of the activity/substrate concentration curves for the two methyl donors.

DISCUSSION

This work, coupled with that of previous investigators, has revealed the presence of thetin-homocysteine transmethylation in the livers of a wide

range of vertebrates. The thetin transmethylation activity of the various bacteria, moulds, yeasts and green plants tested has, on the other hand, been found to be negligible. Thoai & Robin (1956) detected the enzyme in tissues from certain invertebrates, including the hemichordate *Glossobalanus*, the sea-urchin *Sphaerechinus* and the brittle star *Ophiothrix*, but found it absent from the thorn-backed crab *Maia* and the squid *Sepia*. This may be compared with the findings given here for the locust, cockroach, common mussel and common shore crab. The apparently variable occurrence of the transmethylation among marine invertebrates may possibly be linked with their feeding habits, for dimethyl- β -propiothetin, which

is a good substrate for the transmethylase, is known to be present in various marine algae (Challenger & Simpson, 1948; Bywood & Challenger, 1953), and is therefore a likely constituent of their diet.

The thetin transmethylase appears to be a characteristic component of animal liver, but its role in metabolism is not an obvious one, despite the fact that it is present in this tissue in appreciable amounts, and in the horse, for example, accounts for 1% of the total liver proteins (Durell, Anderson & Cantoni, 1957). It is unlikely to be concerned solely with the demethylation of simple thetins, such as dimethylthetin, since these compounds are not usually constituents of an animal diet, and they are apparently not present in detectable amounts in animal tissues (Dubnoff & Borsook, 1948*b*). The transmethylase could, however, participate in the catabolism of *S*-methylmethionine, which is a substrate for the rat liver enzyme (Maw, 1958), and which also occurs widely in plants (Challenger & Hayward, 1954; McRorie *et al.* 1954). According to Durell *et al.* (1957), the enzyme does not act on *S*-adenosylmethionine and, furthermore, it is distinct from the adenosylmethionine-homocysteine transmethylase of bacteria (Shapiro, 1958) and from the transmethylases requiring as methyl acceptors compounds other than homocysteine, e.g. glycocyamine and nicotinamide.

One possibility is that it normally acts on some other intermediate in transmethylation, such as betaine. Purified thetin transmethylase preparations from horse liver have been shown to possess betaine transmethylase activity (Durell *et al.* 1957), and in the present studies betaine was found to be a potent competitive inhibitor of the transmethylase, which would be explained if betaine were acting as a substrate. It is not possible to test this suggestion by means of the manometric method employed here, but direct determination of the amount of methionine formed from dimethylthetin chloride and betaine by the purified enzyme with the mixed substrate technique, together with a further study of the kinetics of the transmethylase, would be of value in this respect.

Recent work on betaine-homocysteine transmethylase by Ericson (1958*a*) has shown that highly purified preparations of this enzyme from pig liver possess marked thetin transmethylase activity. The betaine transmethylase and thetin transmethylase activities of these preparations were destroyed at the same rate by heat treatment and, in addition, both activities were inhibited by dimethylglycine. There are therefore close similarities between the properties of thetin-homocysteine transmethylase and betaine-homocysteine transmethylase. This applies also to their occur-

rence in tissues, for Ericson (1958*b*) has reported that the betaine transmethylase is present in the liver of vertebrates, but is absent from other organs and from the tissues of invertebrates and plants, a distribution resembling that now found for the thetin transmethylase. These similarities emphasize the desirability of obtaining more detailed comparative information about these two transmethylases, since it is possible that they may even be identical.

SUMMARY

1. Evidence is presented that trimethylsulphonium chloride is a substrate for thetin-homocysteine transmethylase, and may be used in the manometric assay of the enzyme.

2. A survey has been made of the distribution of thetin-homocysteine transmethylase in twenty-four species of plant and animal. The thetin transmethylase activity of the green plants, moulds and samples of baker's yeast studied was negligible. Of the four invertebrates examined, only the common shore crab showed possible slight activity. The enzyme was present in appreciable amounts in the liver of all eleven vertebrates tested, and also in small amounts in rat and guinea-pig kidney. It was found to be absent from the spleen, brain and muscle of the rat, mouse and guinea pig.

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An Explanation for the Difference between the Responses of Mammals and Birds to Thyroxine and Tri-iodothyronine

By J. R. TATA* AND C. J. SHELLABARGER†

National Institute for Medical Research, Mill Hill, London, N.W. 7

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It is now agreed that in various biological tests in mammals, and in amphibian metamorphosis, 3:5:3'-tri-iodo-L-thyronine is about two to six times as potent as L-thyroxine (Barker, 1955). This difference in potency in mammals is maintained over a wide variety of physiological potency tests involving basal metabolic rate, body growth, serum cholesterol and antioitrogenic action. However, Shellabarger (1955) and Newcomer (1957) reported that, in the chicken, tri-iodothyronine had the same potency as thyroxine whatever the parameter used for comparison. This is the only example of a lack of difference in the potencies of these hormones when measured by all the above tests and no explanation was produced to account for it.

In most mammals the higher potency of tri-iodothyronine is accompanied by a more rapid action, which can be attributed to a faster rate of diffusion from blood to tissues than that of thyroxine. This concept is upheld by the two to four times shorter biological half-life of tri-iodothyronine (Berson, 1956) and the comparatively feeble affinity for serum thyroxine-binding proteins (Robbins & Rall, 1957). In order to determine whether the similar responses of the chicken to the two hormones were due to similar rates of diffusion into tissues, the biological half-lives of thyroxine and tri-iodothyronine in the chicken were first determined. A study was then made of thyroxine-binding protein in chicken and duck sera, with special regard to its relative affinity for the two hormones. It was assumed that the rate of disappearance of ^{131}I from the whole body gave a satisfactory indication of the biological half-lives

of the ^{131}I -labelled hormones. The results show that both thyroid hormones have similar biological half-lives in the chicken; this can be accounted for by fundamental differences between the binding of thyroxine and tri-iodothyronine by chicken- and duck-serum proteins and by mammalian-serum proteins.

EXPERIMENTAL

Groups of five 21-day-old male chickens [Light Sussex $\times$ Rhode Island] were used for the determination of biological half-lives of L-thyroxine and 3:5:3'-tri-iodo-L-thyronine. Thyroxine (1 $\mu\text{g.}$) and tri-iodothyronine (0.82 $\mu\text{g.}$), labelled with ^{131}I with specific radioactivities of 22.1 mc and 24.3 mc/ μmole respectively, were injected into each bird. The rates of disappearance of the two hormones were measured by counting the residual radioactivity in a total body ring γ -counter as described by Campbell, Cuthbertson, Matthews & McFarlane (1956), against suitable standards of each labelled compound. Measurements were made at time intervals of 5 min.-15 hr. up to 72 hr. after the intravenous injection of ^{131}I -labelled compounds. The results are expressed as the percentage of initial radioactivity found in the birds at time intervals studied.

The association of thyroid hormones with serum proteins was studied qualitatively by zone electrophoresis on paper according to methods described by Robbins & Rall (1957). Whatman no. 3 MM paper was used with 0.075 M-veronal (sodium barbiturate-barbituric acid) buffer, pH 8.6 (Block, Durrum & Zweig, 1955); radioactivity was localized by an automatically recording end-window β -counter and protein fractions distributed on paper were stained with bromophenol blue. The binding of endogenously labelled thyroid hormones to serum proteins was studied in the blood of 31-day-old chickens, 24 hr. after the administration of 0.8 mc of carrier-free ^{131}I iodide per bird. Electrophoretic analysis of such serum samples was performed only after chromatographic methods had established that 90% of circulating organic ^{131}I in butanol extracts consisted of thyroxine and tri-iodothyronine. For binding studies in

* Beit Memorial Fellow.

† Special Fellow, NIAMD, U.S. Public Health Service. Present address: Brookhaven National Laboratory, Upton, L.I., N.Y.