UNUSUAL BEHAVIOUR OF FROG AND HUMAN SERUM PROTEINS LOADED WITH ^{131}I-LABELLED THYROID HORMONES DURING PAPER ELECTROPHORESIS

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It has been reported earlier that the serum proteinbound iodine in the South African toad (*Xenopus laevis*) is higher than that of most animals.[1] Since it is thought that the serum proteins to which thyroid hormones are bound, determine to some extent the speed with which the hormones enter the cells,[2] a greater affinity of the specific binding proteins for thyroid hormones or a slower turnover rate of the binding proteins in cold-blooded animals could explain in part the relatively high serum protein-bound iodine and, therefore, the decreased metabolic rate in the frog.

During studies on the binding properties of serum proteins with ^{131}I-labelled thyroid hormones, the association of ^{131}I-labelled tri-iodothyronine ($^{131}T_3$) and thyroxine ($^{131}T_4$) with the proteins of frog serum was compared with that of human serum. The specific binding proteins, in most cases, were overloaded with $^{131}T_3$ and $^{131}T_4$. The hormones were labelled with ^{131}I chemically by exchange[3] and also endogenously by injecting 200μc of ^{131}I into a mouse. After 48 hours the thyroid was digested enzymically. The hydrolysate was chromatographed, radio-autographed, and the radioactive bands corresponding to tri-iodothyronine and thyroxine markers were eluted (Fig. 1*). The ^{131}I-labelled thyroid hormones were added *in vitro* to serum and electrophoresed on paper in barbiturate-acetate buffer at pH 8·6.

The electrophoresed frog serum, unlike human serum, gave only 3 distinct bands on the anode side of the origin (Fig. 2). The albumin band of frog serum shows a greater electrophoretic mobility than that of human serum. Midway between the albumin and the origin, a broad band appeared in frog serum which corresponded to the area between the α_1 and the α_2 globulin of human serum. This protein band is referred to as the β-globulin, although it may not necessarily be the same as β-globulin of human serum. In some cases the γ-globulin is fairly well defined, but in most cases it is just visible when 20μl. of frog serum is used.

The radioactivity from endogenously prepared $^{131}T_4$ added to serum was associated with both the inter-alpha globulins and albumin when human serum was used. On the other hand, in frog serum it was mainly associated with β-globulin and to a lesser extent with albumin (Fig. 3).

In order to gain some information about the binding capacity of frog and human serum proteins, chemically prepared $^{131}T_4$ was added in known concentrations to serum, and electrophoresed. At a concentration of 0·05μg. $^{131}T_4$/ml. of serum the bulk of radioactivity was again associated with the inter α-globulins in human serum and with the β-globulins in frog serum (Fig. 4). However, when the chemically prepared $^{131}T_4$ was increased to 0·5μg. $^{131}T_4$/ml. of serum, most of the radioactivity shifted onto the human serum albumin, whereas the albumin of frog serum had not taken up much $^{131}T_4$. In frog serum such high concentrations of $^{131}T_4$ were mainly associated with β-globulin and another radioactive peak appeared ahead of the albumin (Fig. 5).

When endogenously prepared $^{131}T_3$ was added to frog and human sera, an unusual electrophoretic migration of the radioactivity was noted. With human serum the major portion of the radioactivity moved during electrophoresis for a distance of about 5 cm. from the origin towards the cathode, while smaller portions were associated mainly with albumin and pre-albumin. With frog serum the major portion of the radioactivity was similarly located as an intense band about 5 cm. on the cathode side of the origin whereas the rest of the activity coincided with the positions of albumin and β-globulin (Fig. 6).

Staining of the electrophoretograms showed no protein bands corresponding to the ^{131}I-labelled substance which migrated towards the cathode. Electrophoresis of the same sera loaded with endogenously prepared $^{131}T_3$ was repeated and the same results were obtained.

In an attempt to identify the radioactive substance which migrated towards the cathode, the human and frog sera containing the endogenously prepared $^{131}T_3$ and $^{131}T_4$ were chromatographed one-dimensionally in butanol: dioxan: 2-N NH_4OH (4:1:5) with carriers T_3 and T_4 (Fig. 7). The darkened areas on the radio-autograph of the chromatogram indicated that the $^{131}T_4$ corresponded exactly with carrier T_4 in frog and human sera, but that the $^{131}T_3$ was slightly ahead of the carrier T_3 spots. The concentration of carrier T_3 was greater than that of carrier T_4, and this may have caused the $^{131}T_3$ to move slightly ahead of carrier T_3.

The section of the electrophoretogram corresponding to the ^{131}I-labelled substance, which migrated towards the cathode, was cut out, eluted, and the extract chromatographed two-dimensionally in butanol:dioxan:2-N NH_4OH and in butanol:acetic acid:water (120:30:50). Most of the activity corresponded to non-radioactive T_3 and iodide (Fig. 8). Other carriers tested out, like 3-mono-iodothyronine, 3:3'-di-iodothyronine, and 3:5:3'-tri-iodothyroacetic acid, did not coincide in two-dimensional chromatograms with the radioactive spots.

It is concluded that (1) frog serum, unlike human serum, carries thyroxine on the β-globulins, (2) the binding capacity of the β-globulins of frog serum for thyroid hormones is greater than that of the inter-α-globulins of human serum, and (3) the bulk of the ^{131}I-labelled substance, which migrated towards the cathode during electrophoresis of frog and human sera under the conditions of the experiment, was in fact $^{131}T_3$.

* Figs. 1 - 8 are on p. 1047.

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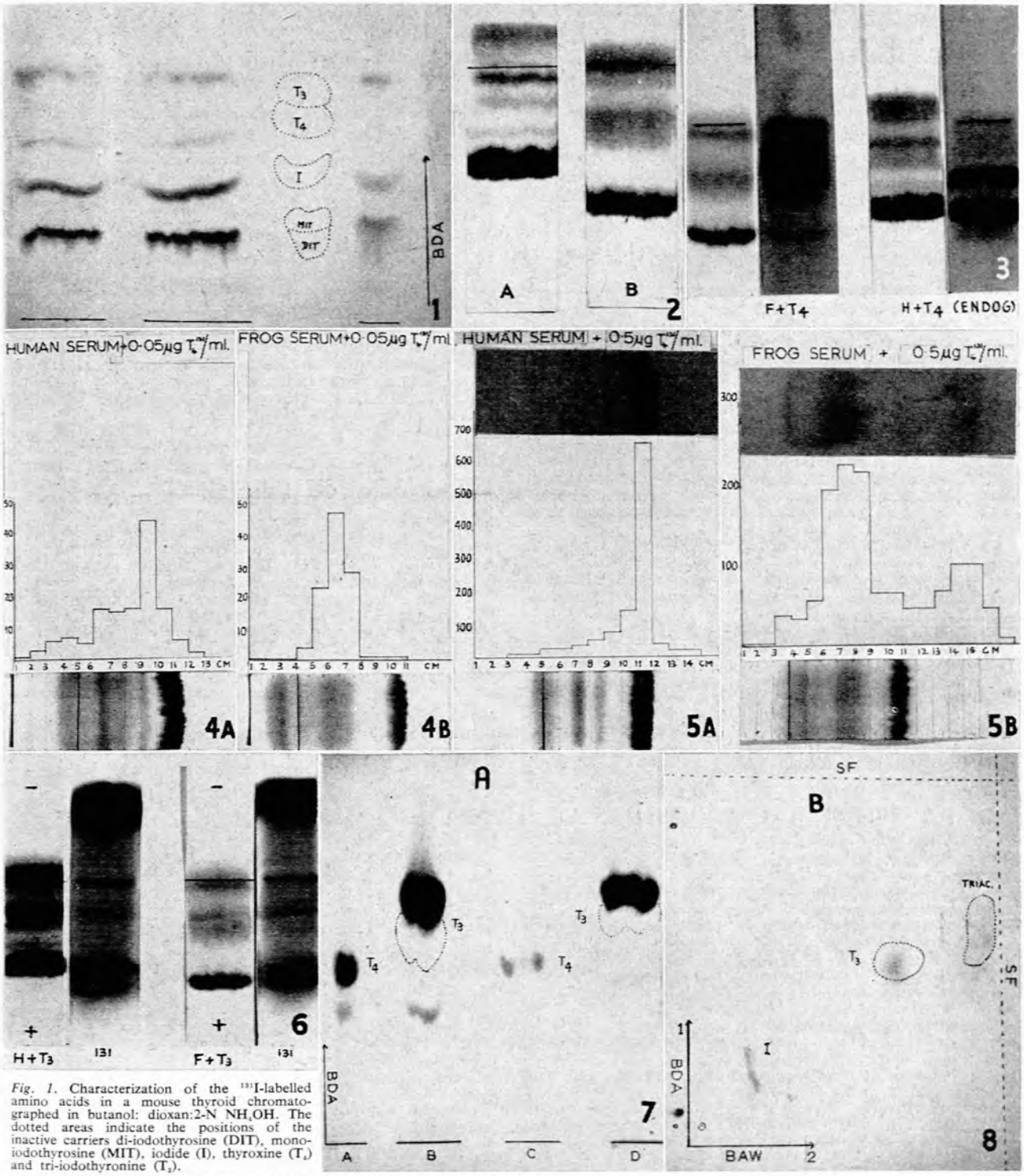

Fig. 1. Characterization of the ^{131}I-labelled amino acids in a mouse thyroid chromatographed in butanol: dioxan:2-N NH_4OH. The dotted areas indicate the positions of the inactive carriers di-iodothyrosine (DIT), mono-iodothyrosine (MIT), iodide (I), thyroxine (T_4) and tri-iodothyronine (T_3).

Fig. 2. Stained electrophoretic patterns of (*A*) normal human serum, and (*B*) frog serum.

Fig. 3. The association of endogenously prepared $^{131}T_4$ with frog ($F+T_4$) and human ($H+T_4$) sera. The radio-autograms are shown to the right of the stained electrophoretograms in each case.

Fig. 4. The association of 0·05 μg. $^{131}T_4$/ml. with (*A*) human serum, and (*B*) frog serum. The histograms represent the radioactive counts per cm. length of the electrophoretograms.

Fig. 5. The association of 0·5 μg. T_4/ml. with (*A*) human serum, and (*B*) frog serum. The radio-autogram (above) and the graph indicate qualitatively and quantitatively the activity associated with the various protein fractions on the electrophoretogram below the graph.

Fig. 6. The association of endogenously prepared $^{131}T_3$ with human (H) and frog (F) serum proteins. The stained electrophoretogram is indicated on the left of each pair while the radio-autogram is on the right.

Fig. 7. Radio-autogram of chromatographic analyses of frog (A and B) and human (C and D) sera to which endogenously prepared $^{131}T_4$ and $^{131}T_3$ had been added.

Fig. 8. Characterization of the endogenously prepared radioactive substance which migrated to the cathode on electrophoresis, indicating the positions of non-radioactive carriers:iodide (I'), tri-iodothyronine (T_3) and tri-iodothyroacetic acid (TRIAC). Solvents: 1. Butanol:dioxan:2-N NH_4OH (BDA) 2. Butanol:acetic acid:water (BAW).