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The use of material labelled with soft β-emitters in the Laurell immunoelectrophoretic technique

A two-dimensional immunoelectrophoretic technique in which the immune precipitates take the form of gaussian or near-gaussian peaks was described originally by LAURELL[1] and subsequently modified by CLARKE AND FREEMAN[2]. Using whole serum and an antiwhole human antiserum forty proteins may be identified by this means using a single plate and up to seventy if concentrated fractions of human serum are used[3]. Nevertheless certain proteins still cannot be identified due to their low concentration in serum; an example is thyroxine-binding globulin (TBG). In order to identify this protein FREEMAN AND PEARSON further modified the method[4] by soaking the dried immunoelectrophoretogram in a large excess of [^{125}I]thyroxine (T_4*) solution, rinsing it free of excess T_4* and subjecting it to autoradiography before staining. This method has disadvantages since it requires large amounts of radioactive material emitting radiation of a relatively penetrating nature and no satisfactory results could be obtained using soft β-emitters even with very prolonged times of exposure.

To overcome these disadvantages a technique has been developed whereby much smaller quantities of material emitting soft β radiation (^{14}C) may be used. This uses the principle of fluorography, where β emission is converted to electromagnetic radiation by means of a scintillating agent. This principle previously has been successfully employed by RANDERATH for polyacrylamide gel electrophoretograms[5]. It allows the Laurell technique with its unrivalled ability for identifying proteins in complex mixtures to be used to investigate the transport of certain substances by serum proteins. In this study, transport of [C^{14}]thyroxine has been studied.

Materials and methods

Materials. [^{14}C]Thyroxine (130 μCi/mmole) was obtained from the Radiochemical Centre, Amersham. Diphenyloxazole (PPO) and diethyl ether were obtained from BDH Ltd. and Kodirex X-ray plates from Kodak Ltd.; other materials were used as described by CLARKE AND FREEMAN[2].

Experimental procedure. Laurell two-dimensional immunoelectrophoresis was carried out as described by CLARKE AND FREEMAN[2] with two important modifications, *viz.*

(a) Equilibration of sample—A small volume of serum (4 to 40 μl) was equilibrated with ^{14}C-labelled thyroxine at 38° for 1 h before electrophoresis and on completion of the latter process, excess antiserum and salt were removed and the plate dried thoroughly in a stream of warm air.

(b) Application of scintillating agent—A 7% solution of PPO in diethylether (10 ml) was poured rapidly and evenly over each plate and the ether allowed to evaporate, giving a smooth layer over the surface of the plate. When the ether had fully evaporated the immunoelectrophoretogram was placed on a Kodirex X-ray film and fluorography allowed to proceed for several days (between 7 to 30), after which the film was developed in the usual manner.

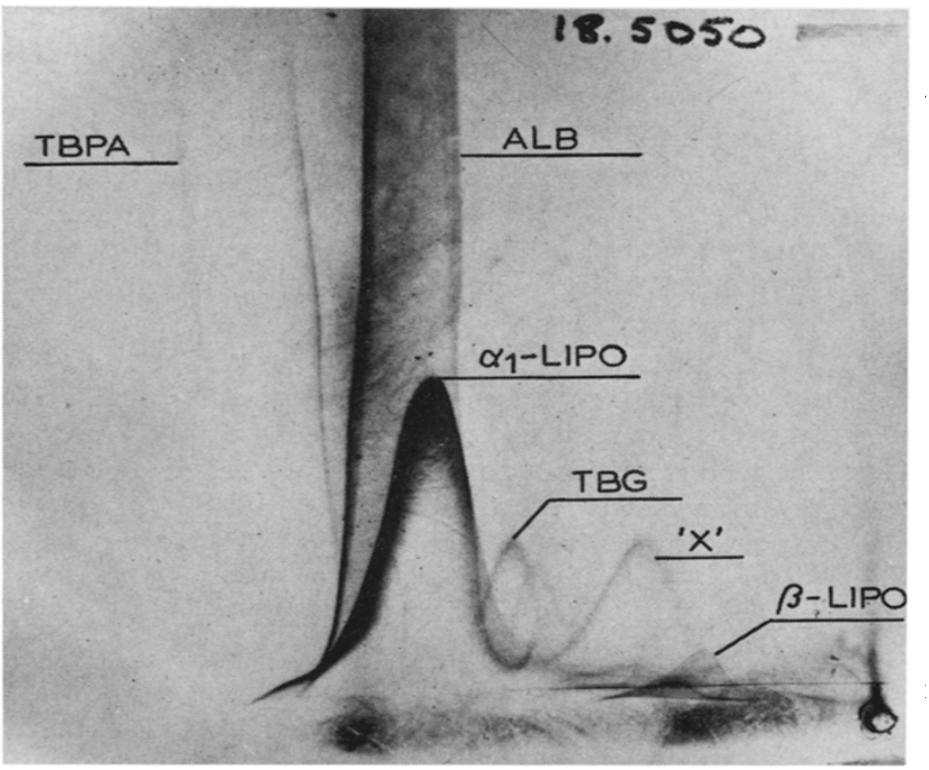

Fig. 1. Fluorograph of human serum equilibrated with [^{14}C]thyroxine prior to immunoelectrophoresis. TBPA = Thyroxine binding prealbumin; TBG = thyroxine globulin; 'X' = unnamed protein.

Results

The results of such a fluorographic procedure employing a ratio of 3 μl of serum and 3 μl of ^{14}C-labelled T_4 [0.04 μCi/film exposure 14 days] may be observed in Fig. 1. This clearly shows thyroxine binding globulin and the other proteins which bind thyroxine. The apparently greater intensity of the α_1 lipoprotein peak compared to TBG is due to the excess of T_4 used compared to the capacity of TBG and is typical of the results obtained using ^{125}I-labelled T_4 (unpublished observations). The excess of thyroxine although somewhat different from physiological conditions does clearly reveal the sensitivity of the method by showing the spill-over onto other binding proteins.

Discussion

By using a scintillating agent to convert β-emission to electromagnetic radiation, it was possible to detect human serum proteins, which bound [^{14}C]thyroxine, using the Laurell two-dimensional immunoelectrophoretic technique. No satisfactory results had previously been obtained using such soft emitters and conventional autoradiography. Further, in the method described here, the serum is equilibrated with thyroxine prior to electrophoresis thus allowing the use of small amounts of labelled material which may be varied, in relation to a fixed amount of serum to study the effect of load on binding. This compares favourably with the previously described technique of FREEMAN AND PEARSON[4] which relies essentially on saturating the whole plate in a large excess of labelled thyroxine.

Several other substances labelled with ^{14}C, which bind to serum proteins, have been investigated using this modified version of the Laurell technique. These include testosterone and cortisol (unpublished observations). It is difficult to formulate universal conditions which will be successful for the detection of these and similar material on Laurell plates. This is due to a number of variables, some not readily apparent, which must be taken into account, *viz.* (a) the total amount of radioactivity added to the plate; (b) the length of time the plate is developed; (c) the nature and amount of the antigen which transports the labelled substance and (d) the amount of specific antibody present in the whole antiserum.

It should be borne in mind that the area of a peak is directly proportional to the amount of antigen and inversely proportional to the amount of specific antibody. Consequently an antigen present in low concentration, which raises antibody poorly and hence also in low amounts, may well result in a peak of much greater area than that of an antigen present in large amount raising an antibody of high titre in the antiserum. Further the nature of the binding to the antigen is important; some antigens bind in a 1:1 molecular ratio while in other cases the ratio of bound material to antigen is much higher. All these points are exemplified by comparing thyroxine binding prealbumin and α_1 lipoprotein.

Two final points should be considered and these are: (e) the affinity of antigen for the substance bound, and (f) the effect of any of the experimental conditions (buffer, pH etc.) on (e).

All of the above factors make it necessary to determine individual conditions by trial and error. Nevertheless, this combination of fluorography and Laurell immunoelectrophoresis should allow the investigation of large numbers of compounds such as drugs, hormones and organic ions which are transported by serum proteins. Also by incorporating a reference protein (acetylated albumin) into the original serum a degree of quantification may be obtained by comparing an individual serum with either a standard serum or a range of normal sera.

Conclusions

A method for identifying and quantifying serum proteins transporting ^{14}C-labelled thyroxine has been described. This combines the principles of two-dimensional immunoelectrophoresis and fluorography. Its wider significance for investigating the transport of many other biologically significant molecules has been pointed out.

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