Demonstration of Thyroglobulin in the Presence of Other Proteins by the Precipitin Reaction

RECENTLY Doniach and Roitt[1] found that the sera of patients with Hashimoto's disease contained auto-antibodies to human thyroglobulin. They demonstrated this by diffusion-precipitation reactions in agar gels. Positive precipitin reactions were obtained by allowing the sera to react with saline extracts of human thyroid or purified thyroglobulin. We have investigated the possibility of using this precipitin reaction in an immuno-electrophoretic method for the demonstration of thyroglobulin in the presence of other proteins.

A saline extract containing radioactive thyroglobulin was prepared from a section of a thyrotoxic gland removed at operation from a patient who had previously been given radioactive iodine. The tissue was frozen, thinly sliced and shaken for 30 min. with 3 parts normal saline, and allowed to stand overnight at 2–3° C. The extract was centrifuged, and the supernatant liquid stored in the deep freeze. Our immuno-electrophoretic technique was similar to that employed by Kohn[2], except that electrophoresis was carried out on Whatman 3 *MM* paper. 30 μl. of the radioactive extract was electrophoresed in barbitone buffer, *p*H 8·6. Two strips were run. Immediately after electrophoresis, one strip was placed on an agar slab (1 per cent Davis standard agar in normal saline), 0·5 cm. away from a piece of filter paper 3 mm. × 13 cm., impregnated with serum known to give a strong precipitin reaction. The agar slab was placed in a moist chamber, and left to stand at room temperature. The proteins diffused into the agar, and precipitin lines developed four to eight days later. When the zones were clearly visible the agar slab was dried, and stained for six hours in a solution of bromphenol blue[3]. After staining, the preparation was washed in 5 per cent acetic acid until the background was clear. It was then placed in a solution of 10 per cent glycerine in 2 per cent acetic acid for 30 min., before drying at room temperature.

The radioactivity on the second strip was recorded, using two scintillation counters. Afterwards, it was stained in bromphenol blue and used as a marker strip.

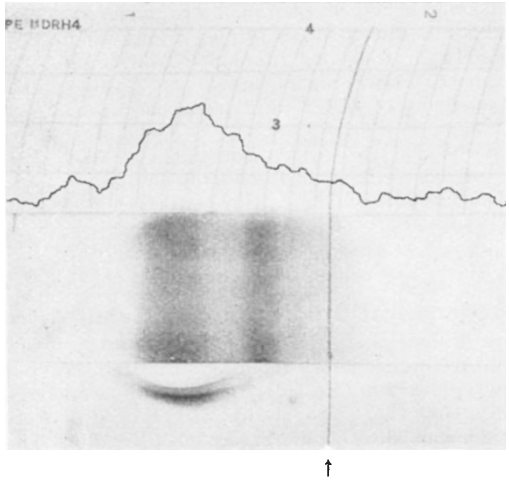

Fig. 1. Paper electrophoresis of thyroid extract at *p*H 8·6. The marker strip (*B*) stained with bromphenol blue is aligned with record of radioactivity (*A*) and stained agar film (*C*) showing precipitin zone. The arrow indicates the origin

From Fig. 1 it will be seen that the maximum radioactivity recorded is associated with the fastest moving fraction, and this is also where the precipitin zone is found. The radioactivity of the thyroid extract was not extractable with butanol, and the radioactive fraction was found to have a mobility at *p*H 8·6 between that of the α_1 and α_2 serum globulins. Robbins *et al.*[4] found that the sera of patients after iodine-131 therapy contained a protein which, by salting-out procedures, and ultracentrifugal sedimentation was indistinguishable from thyroglobulin. Electrophoresis at *p*H 8·6 did not differentiate between thyroxine-binding protein and a thyroglobulin-like protein[5].

After electrophoretic separation of the α-globulins in selected sera, it is proposed to apply the technique described in an attempt to demonstrate the presence of circulating thyroglobulin, particularly after iodine-131 therapy.

We wish to thank Miss Helen E. A. Farran for the measurement of radioactivity.

R. BIRD
DOROTHY JACKSON

Department of Endocrinology,
New End Hospital,
London, N.W.3. April 1.

[1] Doniach, D., and Roitt, I. M., *J. Clin. Endocrinol. Metab.*, **17**, 1293 (1957).
[2] Kohn, J., *Nature*, **180**, 986 (1957).
[3] Henry, R. J., Golub, O. J., and Sobel, C., *Clin. Chem.*, **3**, 49 (1957).
[4] Robbins, J., Peterman, M. L., and Rall, J. E., *J. Biol. Chem.*, **208**, 377, 387 (1954).
[5] Robbins, J., and Rall, J. E., *Proc. Soc. Exp. Biol. and Med.*, **81**, 530 (1952).

Effect of a Dithiol on Survival Time after Irradiation

CONTRARY to previous reports[1], Doherty *et al.*[2] obtained partial protection of X-irradiated mice with 2,3-dimercaptopropanol. If a foreign dithiol compound is effective perhaps the dithiol form of an essential metabolite might also prove beneficial in radiation injury. Mice were exposed to 550 r. acute whole body X-irradiation under our usual conditions[3]. The animals received 50 mgm./kgm. of DL-6,8-dithioloctanoic acid, the reduced form of thioctic acid, according to the schedule in Table 1. It is evident that the compound significantly increased the *ST*50 day, particularly if administered prior to irradiation. However, it has no real effect in reducing total mortality. The mechanisms involved in such partial protection are obscure, but dithiols are known to form resonance-stabilized radicals which could interact with the various oxidizing radicals produced by

Table 1. EFFECT OF 6,8-DIHYDROTHIOCTIC ACID ON SURVIVAL TIME IN IRRADIATED MICE

Treatment	*ST*50* and range (days)	Slope and range	Mortality Ratio	Mortality Day
Saline control	5·7 (4·9–6·6)	1·41 (1·27–1·56)	20/20	12
6,8 Dihydrothioctic acid†				
1 day pre-irradiation	9·3 (8·2–10·5)	1·33 (1·22–1·45)	20/20	19
10 min. pre-irradiation	9·3 (8·6–11·2)	1·35 (1·23–1·49)	20/20	20
10 min. post-irradiation	6·8 (6·2–7·6)	1·31 (1·21–1·42)	20/20	14
1 day post-irradiation	7·7 (6·8–8·8)	1·36 (1·25–1·49)	19/20	20
Control	—	—	0/19	20

* *ST*50, day upon which 50 per cent of animals are expected to be still alive. Confidence limits are calculated at $P = 0{\cdot}05$, ref. 4.
† 50 mgm./kgm. intraperitoneally.