G-100 gel chromatography into 2 fractions: a low and high molecular one[19].

*Results.* The lysosomal hydrolases of CLM showed an average increase in specific activities over the cell homogenate 4–5 fold. The results of guinea-pig lymphocyte transformation in PHA, CLM and PMN leucocytes plasma material stimulated cultures are summarized in the Table. Lysosomal material separated from macrophages, lymphocytes and liver cells do not shown similar transformation activity in vitro. The heterogeneic crude lysosomal material of PMN leucocytes does not stimulate lymphocytes cultures in vitro (unpublished). The lymphocyte suspension was adjusted to a concentration $2–4 \times 10^6$ cells/ml in all experiments (Figure).

Fractions eluated from DEAE cellulose column were dialyzed. Material from these fractions was added to the lymphocyte cultures in quantities of 5 µg/ml. Studies with DEAE cellulose eluated fractions indicated that stimulatory effect on lymphocyte cultures was shown by PMN leucocytes lysosomal material. This suggests that only the PMN leucocytes possess the lymphocyte transforming activity.

*Discussion.* The factor described above stimulating lymphocyte transformation in vitro, can also perform some important functions in vivo. The response of cells of the lymphoid system in the course of phagocytosis may be unspecifically intensified after the release of stimulators known to be present in lysosomes of the PMN leucocytes. This assumption is supported by investigations on the role and mechanism of the adjuvants in immunological processes. Some adjuvants are labilizators of lysosomal granule membranes and activiting their contents[20]. The results obtained with fraction eluated from DEAE cellulose column indicate that lymphocyte transformation factor does not possess high activity level of the proteases which play an important role in blastic transformation of lymphocytes[17,21]. Activity of lymphocyte transforming factor described above cannot therefore be of an enzymatic nature.

The findings reported above indicate that the extracellular release of lysosomal constituents may contribute, not only as is generally believed, to the induction of vascular phenomena of inflammation but also to the lymphocyte transformation.

*Résumé.* On décrit le facteur protéique stimulant in vitro la transformation blastique des lymphocytes allogènes. La présence de ce facteur ne put être établie à l'intérieur des lysosomes des macrophages, des lymphocytes, des cellules hépatiques, ni dans les fractions des granulocytes polymorphonucléaires contenant le plasma cellulaire dépourvu d'autres structures à la suite d'une centrifugation. On discute le rôle présumé du facteur étudié in vivo.

H. Tchórzewski, Zofia Sułowska, A. Denys

*Department of General and Experimental Pathology, Pl. 9-go Maja 1, Lódž (Poland), 7 August 1972.*

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## Disappearance of Radioactivity from Plasma Following Intravenous Administration of Tritiated Thyrotrophin-Releasing Hormone (Ro 8-6270) to Man

Thyrotrophin-releasing hormone (TRH) is a simple tripeptide, pyroglutamyl-histidyl-proline-amide, secreted by the hypothalamus which stimulates the release of thyrotrophin (TSH) from the anterior pituitary into the peripheral circulation. Since the recent isolation and synthesis of TRH[1–5] considerable interest has been directed towards the importance of the hormone both clinically and in the research laboratory.

Studies on the distribution and excretion of radioactivity following administration of labelled TRH to rats and mice have been reported[6,7]. The results of these studies have shown that the highest accumulation of radioactivity occurred in the pituitary, liver and kidneys. Assays of urinary radioactivity has indicated that up to 40% of the radioactive dose was excreted in the urine within 1 h. Redding and Schally[8] have recently shown that, following i.v. injection of TRH labelled with $^{14}$C-histidine to rats, the plasma $^{14}$C-activity had a disappearance half-life of about 4 min up to 6 min following administration.

We wish to report here on studies carried out to determine the disappearance rate of radioactivity in plasma and its excretion in urine following intravenous administration of tritium labelled TRH to man.

*Materials.* Pyroglutamyl-2,5-$^3$H-histidyl-proline-amide hydrochloride with a specific activity of 9.15 mC/µ*M* was supplied by F. Hoffmann-La Roche and Co. AG., Basel, Switzerland. The preparation and purification of the radioactive tracer will be reported elsewhere. The radioactive doses for i.v. injection were prepared by adding 120 µC of the tracer in 0.1 ml of ethanol to vials containing 200 µg of unlabelled TRH (Ro 8-6270) as a solution in 2 ml of isotonic saline.

*Methods.* Two normal males and two normal females each received 200 µg (120 µC) of tritium labelled TRH by rapid i.v. injection. Blood samples were collected in heparinized tubes at the times indicated in the Figure and the plasma separated by centrifugation and stored at –20° until analysis. Urine was collected daily for two days.

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Duplicate 0.1 ml aliquots of plasma and urine were assayed for $^3$H-activity in a Packard Model 3375 liquid scintillation spectrometer and quenching corrected by means of the automatic external standard.

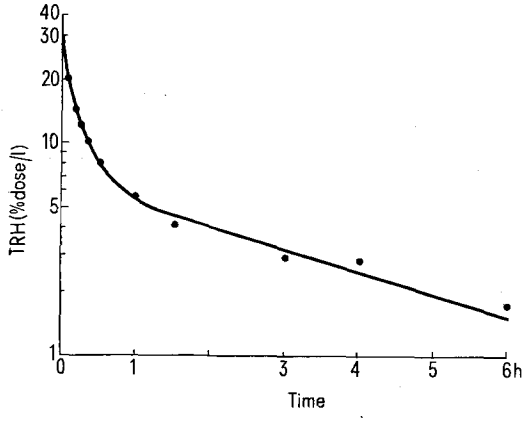
*Results.* The plasma concentrations ($C_p$) of $^3$H-activity, expressed as % dose/L plasma, apparently declined biexponentially and were fitted, by means of an iterative non-linear digital computer programme, to the equation: $C_p = Ae^{-\alpha t} + Be^{-\beta t}$, where A and B are the zero ordinate axis intercepts and $\alpha$ and $\beta$ are both hybrid rate constants reflecting all the individual rate processes. A typical disappearance curve is shown in the Figure. In each subject, the initial fast disposition phase had a half-life ($0.693/\alpha$) of about 10 min followed by a slower disappearance with a half-life ($0.693/\beta$) of around 3 h.

Of the total radioactive dose, 70–78% was excreted in the urine within 24 h after administration.

*Discussion.* It should be stressed, that the data reported in this communication concerns the disappearance of total plasma $^3$H-activity following administration of 2,5-$^3$H-histidyl TRH to man and does not necessarily reflect the overall disappearance of intact TRH. Since TRH undergoes rapid inactivation in both tissue and blood[9] our data represents the disappearance and excretion of TRH and its biotransformation products which contain the 2,5-$^3$H-histidyl moiety.

Recently LEPPALUOTO et al.[10] have reported that, after injection of 200 µg of unlabelled TRH to man, plasma TRH had a halflife of 5 min within the first 5 min after administration. This observation is in close agreement with the data reported here.

It is interesting to note that the mean maximum TSH response in normal individuals receiving 200 µg of TRH intravenously[11,12] occurs during the initial fast distribution phase (0–60 min) observed in the present study. It would not appear unreasonable to suggest that the TSH response is the result of TRH stimulation of the pituitary during this period following administration.




Total $^3$H-activity in plasma ($C_p$) following i.v. administration of 200 µg (120 µC) of tritium labelled TRH to man. (●), observed data; (—), computer calculated curve; $C_p = 18.9e^{-4.8t} + 6.6e^{-0.24t}$.

*Zusammenfassung.* Nach i.v. Verabreichung von Thyrotrophin-releasing Hormonen an Versuchspersonen ergibt sich eine biphasische Kurve für den Abfall der Tritiumaktivität im Plasma mit einer Exkretions-Halbwertszeit von 3 h.

R. DIXON, S. EAST, M. BUCKLEY and A. DARRAGH[13]

*Psycho-Endocrine Centre, Department of Psychiatry U.C.D., St. James's Hospital, Dublin 8 (Ireland),*
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## Effect of 2-Br-α-Ergokryptine (CB 154) on Lactation in the Bitch

Lactation and other prolactin-dependent mechanisms in the reproduction of the rat are inhibited by 2-Br-α-ergokryptine (CB 154 Sandoz, Basel)[1,2]. This action is based on inhibiting prolactin secretion at the pituitary level[3]. Suppression of serum prolactin levels by CB 154 have been shown by radio-immuno-assay methods in humans[4], cows[5] and in sheep[6]. Inhibition of lactation by CB 154 has been demonstrated, apart from the rat[1], in humans[7,8], in pigs[2], and in rabbits[2]. In contrast, even after repeated applications of CB 154, lactation could not be inhibited in cows[5] and sheep[6]. This means that prolactin is not essential in all mammalian species for the maintenance of lactation. Therefore it is of interest to test different mammalian species for the sensitivity of established lactation towards the action of CB 154.

It was the objective of the following experiments to find whether lactation in the bitch can be influenced by CB 154.

*Material and method.* Female beagles (6 groups, consisting of 5 animals each), aged 2–6 years, weighing between 9–18 kg were used. After parturition the animals were housed in single boxes at a temperature of 20–23 °C Only litters of 5 or 6 pups were used. In cases of more than 6 pups per litter, the supernumerary animals were removed on the 2nd day of life. From the 2nd–21st day

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