Case 2. Testis tissue from 25-year-old man

Crystallization	Tubule [^{14}C, ^{3}H]-testosterone (dpm/mg)			[^{14}C, ^{3}H]-androstenedione (dpm/mg)			Interstitium [^{14}C, ^{3}H]-testosterone (dpm/mg)			[^{14}C, ^{3}H]-androstenedione (dpm/mg)		
No.	^{3}H	^{14}C	ratio ^{3}H/^{14}C	^{3}H	^{14}C	ratio ^{3}H/^{14}C	^{3}H	^{14}C	ratio ^{3}H/^{14}C	^{3}H	^{14}C	ratio ^{3}H/^{14}C
1	17,757	902	19.69	562	36	15.61	13,485	25	539	251	1287	0.195
2	17,251	891	19.36	488	33	14.79	13,598	27	504	256	1360	0.188
3	17,732	901	19.68	440	31	14.19	13,101	26	504	249	1299	0.192
4	17,098	874	19.56	420	30	14.00	12,240	24	510	250	1286	0.194

Table II. Separated interstitium and seminiferous tubules of human testis tissue incubated with equimolar concentrations of [7α-^{3}H]-testosterone and [4-^{14}C]-androstenedione

Tissue	Time	Case 1. Testis tissue from 72-year-old man: % ^{3}H label recovered as		% ^{14}C label recovered as		Case 2. Testis tissue from 25-year-old man: % ^{3}H label recovered as		% ^{14}C label recovered as	
	(min)	[^{3}H]-A[a]	[^{3}H]-T[b]	[^{14}C]-A	[^{14}C]-T	[^{3}H]-A	[^{3}H]-T	[^{14}C]-A	[^{14}C]-T
Interstitium per 100 mg	5	4.6	88.1	91.4	6.7	4.3	89.4	98.6	0.8
	15	4.7	88.4	89.8	8.1	4.4	87.6	98.3	0.9
	30	5.0	88.5	88.7	8.2	4.5	87.9	97.8	1.1
	60	5.9	86.8	89.4	8.7	4.6	86.6	97.5	1.8
	120	–	–	–	–	4.9	86.3	97.3	2.3
	150	6.5	84.9	85.9	11.9	–	–	–	–
	180	–	–	–	–	4.8	82.6	96.3	3.1
	240	6.1	86.7	84.9	12.4	4.7	82.5	95.0	4.3
Tubule per 100 mg	5	2.1	90.9	83.1	15.0	0.9	90.9	83.7	14.7
	15	2.6	91.2	67.7	30.2	1.0	90.9	73.2	22.9
	30	3.9	90.3	50.7	46.9	1.3	89.7	51.4	42.5
	60	4.1	90.1	35.3	62.0	1.6	89.1	31.0	53.4
	120	–	–	–	–	1.6	88.9	23.7	55.2
	150	4.2	89.4	10.3	87.1	–	–	–	–
	180	–	–	–	–	1.7	88.3	23.5	56.4
	240	4.5	89.6	5.1	90.7	2.1	87.6	22.1	60.5

[a] Denotes androstenedione. [b] Denotes testosterone.

much more efficient than the interstitium in carrying out the enzymic reduction of androstenedione. The difference in the results obtained from the 2 cases may be attributed to the great difference in age between the two individuals, which suggests that, in the older man, higher levels of testosterone are needed to maintain normal spermatogenesis in the tubules.

These results indicate that the human testis shows the same differences in the relative activities of the 17β-hydroxysteroid dehydrogenase enzymes in the interstitium and seminiferous tubules as previously demonstrated in the rat. It is also apparent that the separation technique applied to the human testis in these experiments produces preparations with little or no cross-contamination between the tubules and the interstitium, and, like the rat, the human testis possesses at least two sites of steroid metabolizing enzymes, the interstitium and the seminiferous tubules[10].

Zusammenfassung. Nachweis, dass im menschlichen Hoden sowohl das Interstitium wie auch das tubuläre System befähigt sind, Androstendion-4-^{14}C und Testosteron-7α-^{3}H zu konvertieren.

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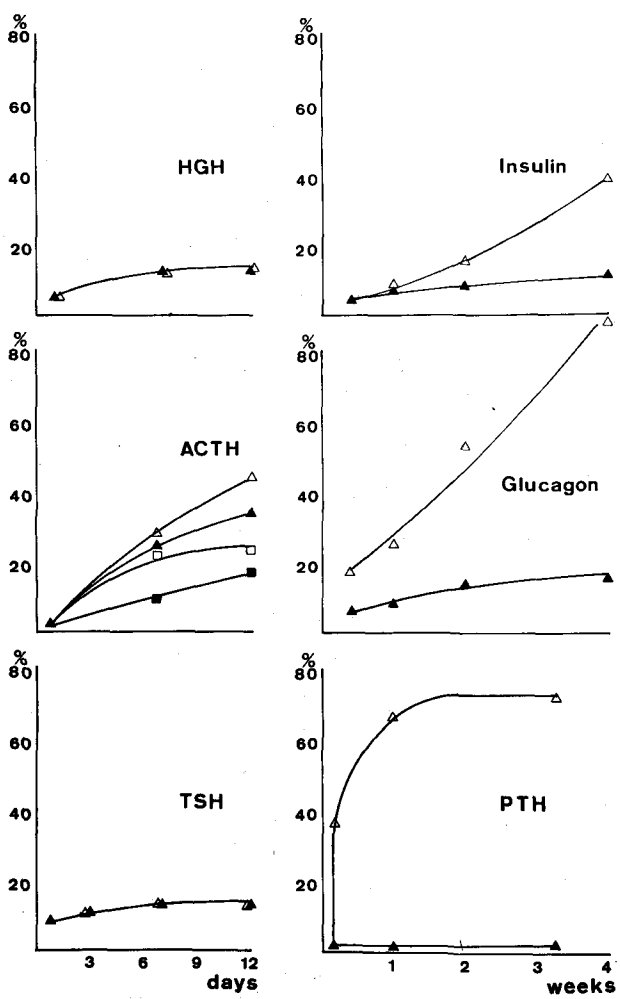
On the Use of a Peptidase Inhibitor (Trasylol) for Storage of I^{125}-labelled Peptide Hormones (HGH, TSH, ACTH, Insulin, Glucagon, PTH)

One of the main problems in radioimmunoassay is the degradation of I^{131} or I^{125}-labelled tracer hormones. The degradation may preexist due to extraction or storage of unlabelled preparation. It can occur during iodination (iodination damage) or on subsequent storage of the labelled hormone. Finally the degradation may markedly increase during incubation, especially in medium containing plasma or serum. This form of incubation damage

largely depends upon the degree of degradation at the beginning of the incubation period. As any form of degradation impairs physical and immunological properties of the preparation, it may lead to erroneous results in radioimmunoassay. The aim of the present study was to assess the usefulness of Trasylol for reducing the degree of degradation of I^{125}-labelled peptide hormones stored at +4°C.

Methods. Hormones studied: human growth hormone (HGH, prepared according to Roos et al.[1]), ACTH (synthetic $\alpha_P{}^{1-39}$ ACTH Ciba), TSH (human TSH from the NPA), Insulin (Actrapid Novo), Glucagon (porcine glucagon Novo, lot MC 6770), and PTH (highly purified bovine PTH Wilson, lot 43601). All hormones were iodinated with minor modifications according to the method of GREENWOOD et al.[2]. HGH and TSH were purified with Sephadex G 75 and ACTH, Insulin, Glucagon and PTH with cellulose. The tracer hormones were stored at +4°C in a 0.06 *M* veronal buffer pH 8.6 with addition of human serum albumin 2.5 g/l and merthiolat 10^{-4}. The enzyme inhibitor Trasylol (Bayer, Leverkusen) was added to give final concentrations of up to 1000 U/ml. For ACTH, the addition of mercaptoethanol 5 g/l was tested. The degradation was measured using both chromatoelectrophoresis on Whatman 3 MC paper[3] and charcoal dextran separation according to the method of HERBERT et al.[4] adapted individually for each hormone.



Storage damage with and without peptidase inhibitor (Trasylol). (△, without Trasylol; ▲, with Trasylol; □, with mercaptoethanol; ■, with Trasylol and mercaptoethanol). Increase in percentage damage to labelled hormones stored up to 4 weeks at 4° C with and without peptidase inhibitor (Trasylol).

Results. The results obtained with chromatoelectrophoresis or charcoal dextran were comparable for all 6 hormones. The effect of Trasylol (500 U/ml) on damage during prolonged periods of storage are shown in the Figure. Damage to labelled HGH and TSH incrased only very slightly during 12 days of storage and Trasylol had therefore no appreciable effect. An increase in damage to labelled insulin could, however, be inhibited by addition of Trasylol. The most marked effect of the peptidase inhibitor was seen on labelled PTH and glucagon. Damage to both hormones increased very rapidly during storage in diluent alone, which could be almost completely inhibited by addition of Trasylol. The increased degradation of ACTH during storage could only be effectively inhibited by a combination of 500 U/ml of Trasylol with 0.5% of mercaptoethanol.

A concentration of 500 U/ml of Trasylol was found to be optimal. An inhibition of antibody binding by this concentration of Trasylol was not observed with any of the hormones tested.

Conclusions. High specific activity of labelled peptide hormones is required for sensitive radioimmunoassays. Overloading with radioactive iodine may lead to rapid degradation[5]. Limited substitution with iodine may, however, also damage the hormone (due to largely unknown factors). Labelled peptides are much less stable than the unlabelled compound and the slight damage resulting from iodination may increase rapidly during storage, yielding an unusable labelled hormone. Apparently an enzymatic reaction is involved in the process of degradation. This type of damage to labelled hormones can be controlled by addition of the peptidase inhibitor Trasylol, as shown previously[5] and by the present study.

Zusammenfassung. Von den untersuchten J^{125}-markierten Peptidhormonen sind HGH und TSH auch ohne Trasylol gut haltbar. Die mässige Degradation von Insulin und die rasch zunehmende Degradation von PTH und Glukagon kann durch Trasylol (500 E/ml) verhindert werden. Für ACTH ist eine Kombination von Trasylol mit 0.5% Mercaptoethanol erforderlich.

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