considered as an absent response. Thyroid autonomy was detected by physical examination and verified by scintigraphy.

TSH response was not stimulated by TRH in 17 hyperthyroid patients with scintigraphically decompensated autonomous adenoma. In all these patients, basal TSH (IRMA) levels were below 0·15 mU/l. Comparison of TSH (IRMA) values and TSH response to intravenous TRH in 25 euthyroid patients with decompensated autonomous adenoma is given in the table.

Further thyroid hormone analysis was needed in 36/43 patients (84%) on the basis of the TRH test and in 38/43 (88%) on the basis TSH (IRMA) levels. Therefore, in patients with nodular goiter and suspected thyroid autonomy, TSH determination is not available as a screening test of thyroid function. However, the determination of TSH by a sensitive immunoradiometric assay may substitute for the TRH test.

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SIR,—Dr Caldwell and colleagues' proposal that a detectable TSH level on immunoradiometric assay (IRMA) would exclude hyperthyroidism should be revised to take into consideration hyperthyroid patients with inappropriate secretion of TSH. In these patients the supersensitive TSH-IRMA may be clinically important because several cases, of neoplastic or non-neoplastic origin, with serum TSH levels not above the normal range have been reported.[1,2] The finding by IRMA of measurable serum TSH in hyperthyroid patients would strongly suggest inappropriate secretion of TSH.

We have seen three acromegalic patients with signs and symptoms of hyperthyroidism whose serum TSH was normal by radioimmunoassay (RIA) and detectable by TSH-IRMA ('Sucrosep'; Boots-Celltech Diagnostics). In all of them the α-subunit/TSH molar ratio was supranormal (8·0, 31·2, and 6·1, respectively) and a pituitary tumour was revealed by computed tomography of the sella turcica. In two patients who underwent surgery, immunohistochemistry of the excised tumour revealed specific TSH secretory granules, and the hyperthyroidism remitted postoperatively.

The results of TSH assay (table) show that, in contrast to the TSH-IRMA and an in-house RIA, two commercial RIA kits would have not been able to suggest inappropriate secretion of TSH because of the overlap between the "hyperthyroid" and "euthyroid" ranges of TSH levels.

By contrast, four other hyperthyroid patients, referred to us with suspected inappropriate secretion of TSH because they had serum TSH levels above the "hyperthyroid" range by commercial RIA kits, did not have detectable serum TSH by TSH-IRMA (see table).

SERUM TSH CONCENTRATIONS (mU/l) IN PATIENTS WITH HYPERTHYROIDISM DUE TO INAPPROPRIATE SECRETION OF TSH (IST) AND IN 4 HYPERTHYROID PATIENTS WITH RIA MEASURABLE TSH DUE TO METHODOLOGICAL INTERFERENCES

Commercial RIA*		In house RIA	IRMA	FT4† (pmol/l)	FT3† (pmol/l)
A	B				
IST of neoplasia					
1·6	1·8	2·0	2·7	34·9	15·0
1·3	1·6	1·5	2·8	24·3	14·6
1·2	1·0	1·2	1·9	35·9	17·1
Anti-rabbit Ab					
5·3	5·2	<0·3	<0·07	42·5	19·8
5·8	4·4	<0·3	<0·07	26·4	14·8
6·4	4·2	<0·3	<0·07	25·6	9·7
Anti-hTSH Ab					
19·0	25·3	10·4	<0·07	22·4	18·5
Normal					
<1·0–10·0	<0·5–7·0	<0·3–4·6	0·15–7·0	9–20	3·8–8·9

*A = TSH kit PR method (Sorin, Saluggia); B = TSH MAIA kit (Biodata, Rome).
†'Lisophase' kits (Sclavo, Siena).

Three patients also had undetectable serum TSH by the in-house method, while the fourth would have been misdiagnosed by this method too. In the first three patients the source of the estimation error was the presence of circulating heterophilic antibodies cross-reacting with rabbit serum. These antibodies neutralise the rabbit anti-human serum in the two commercial RIA kits, but not in the in-house method, where the interference was prevented by adding normal rabbit serum, as suggested by Schaison et al.[3] The fourth patient had antibodies directly cross-reacting with human TSH. These antibodies[4] competed for the radiolabelled TSH by decreasing the radioactivity in the species-specific immunoprecipitate of TSH RIAs, thus affecting also the in-house method.

These results show that TSH-IRMA is a sensitive and specific method for serum measurement which is unaffected by the presence of heterophilic anti-rabbit serum antibodies and anti-human TSH antibodies.

We conclude that TSH-IRMA used as a first-line thyroid function test in association with free thyroid hormone measurement, may help in disclosing patients with inappropriate secretion of TSH. This syndrome may be more common than is usually thought and its recognition is clinically important because such patients are managed differently from those whose hyperthyroidism is due to other causes.

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INACTIVATION BY WET AND DRY HEAT OF AIDS-ASSOCIATED RETROVIRUSES DURING FACTOR VIII PURIFICATION FROM PLASMA

SIR,—We have shown that the mouse xenotropic type C retrovirus can survive and remain infectious after procedures used in the preparation of factor VIII (FVIII) cryoprecipitates or concentrates.[1] Heating at 68°C for more than 48 h eliminated essentially all of the infectious virus. We have now extended these studies of FVIII purification procedures and heating to examine the influence of virus titre and to include human AIDS-associated retrovirus (ARV).[2]

The mouse retrovirus was obtained from infected mink lung cells.[3] ARV-2 and ARV-3 were obtained from infected HUT-78 cells or infected human peripheral mononuclear cells (PMC).

The mouse xenotropic virus was assayed using mink S+L− cells or mink lung cells pretreated with diethylaminoethyl dextran.[3] Monolayer cells were passaged weekly for 3 weeks and then supernatants were assayed for infectious virus. This increases assay sensitivity 10–100 fold. ARV was detected by induction of reverse transcriptase activity in the culture fluid of normal human PMC maintained for up to 1 month after virus inoculation.[2] Infectious virus titres were measured by 10 fold and, where indicated, 2 fold dilutions.

Dry heating (68°C) of lyophilised FVIII concentrates was conducted as previously described.[1] Wet heating of FVIII filtrates was done at 60°C for 10 h in the presence of sucrose (1·2 g/ml). When high or low titre mouse retrovirus was added to plasma or filtrate, no substantial reduction of infectious virus was noted (table 1). On subsequent lyophilisation, the infectious virus titre, whether in concentrates receiving large or small quantities of virus, was reduced similarly (100-fold) (table 1A,B). These results indicated that the degree of recovery of infectious virus after lyophilisation is not substantially influenced by the initial quantity of virus in the filtrate.

When lyophilised FVIII product containing high-titre mouse virus had been heated at 68°C for 48 h or longer mouse virus was not detectable (table 1A). Previously[1] we found, with high titre mouse retrovirus, very low levels of residual infectious particles (less than 1

TABLE I—INFECTIOU
FACTOR VIII PRODU

Experiment
(A)
alone
plus filtrate
lio lyophilised product
Dry heat (68°C), at 24 h
Dry heat (68°C), at 48,72, 96 h
(B)
alone
plus filtrate
lio lyophilised product
Dry heating (68°C), at 24, 48 h

(a) infectious virus particles; separate experiments and reflec ed in two or three individual

TABLE II—EFFECT ON

(A) Purification
ARV-2 alone
ARV-2 + plasma (5°C)
ARV-2 in cryoprecipitate
ARV-3 alone
ARV-3 + plasma (5°C)
ARV-3 in cryoprecipitate
Treatment: $Al(OH)_3$+ $CH_3 \cdot COOH$*
Treatment: glycine precipitation†

*Aluminium hydroxide plus suspension, and filtration (0 from four to five separate expe

per ml) at 72 h. This s periods of heating mos initial virus titre. Whe mouse virus was heate Earlier[1] we found that i 30 min eliminated all i we looked at the effect (60°C, 10 h) used in t products (eg, albumin treatment of FVIII fil infectious virus, despit to protect FVIII (table inactivation could be e When ARV-2 and A sensitivity[4] and in repl human plasma (5°C), n ma). Subsequently, a infectious ARV at a retrovirus.[1] Purificatio precipitation and filtrat in a further 10-fold red Lyophilisation of a F infectious virus titre ab preparation was then after 10, 24, and 34 h b tissue culture fluid con for 30 min no infectiou containing FVIII filtrat at 60°C for 10 h befo detected in the liquid o Our results indicate t and human) if present in infectious form in F to FVIII purification p input titre (table 1). Bec culture as the mouse x particle may have been

TABLE I—INFECTIOUS MOUSE RETROVIRUS (V) RECOVERY FROM FACTOR VIII PRODUCTS AND EFFECT OF HEAT TREATMENT

Experiment	$\log_{10}$ IP/ml	Experiment	$\log_{10}$ IP/ml
(A)		(C)	
V alone	6·4	V alone	6·4
V plus filtrate	6·0	V plus filtrate	6·0
V in lyophilised product	4·0	Wet heating (60°C, 10 h)	NV
Dry heat (68°C), at 24 h	1·0	Lyophilised product (after heating)	NV
Dry heat (68°C), at 48,72, 96 h	NV		
(B)		(D)	
V alone	3·6	V alone	3·6
V plus filtrate	3·0	V plus filtrate	3·0
V in lyophilised product	1·0	Wet heating (60°C, 10 h)	NV
Dry heating (68°C), at 24, 48 h	NV	Lyophilised product (after heating)	NV

IP = infectious virus particles; NV = no detectable virus. Results are from two or three separate experiments and reflect the average virus titre found in fluids tested at least twice and in two or three individual lyophilised ampoules of FVIII.

TABLE II—EFFECT ON ARV OF (A) FACTOR VIII PURIFICATION AND (B) HEAT

(A) Purification	$\log_{10}$ IP	(B) Heating	$\log_{10}$ IP/ml
ARV-2 alone	5	ARV-2 alone	4
ARV-2 + plasma (5°C)	5	ARV-2 in FVIII filtrate	4
ARV-2 in cryoprecipitate	4	ARV-2 in lyophilised concentrate, zero time	2·8
ARV-3 alone	4	Dry heating (68°C)	
ARV-3 + plasma (5°C)	4	10 h	0·9
ARV-3 in cryoprecipitate	3	24 h	0·3
Treatment: $Al(OH)_3$ + $CH_3 \cdot COOH$*	2	34 h	0·1
		48 h, 56 h	NV
Treatment: glycine precipitation†	2	ARV-2 in FVIII filtrate, Wet heating (60°C) 10 h	4 NV

*Aluminium hydroxide plus acetic acid (pH 6·3–6·6). †Glycine precipitation, resuspension, and filtration (0·45–0·22 μm). NV = no detectable virus. Results are taken from four to five separate experiments.

per ml) at 72 h. This small variation in virus survival for the two periods of heating most probably reflects slight differences in the initial virus titre. When lyophilised product containing low-titre mouse virus was heated no infectious virus was detected (table IB). Earlier[1] we found that in liquid culture medium heating at 56°C for 30 min eliminated all infectious mouse retrovirus particles. When we looked at the effect on infectious virus of a wet heat treatment (60°C, 10 h) used in the preparations of FVIII and other plasma products (eg, albumin) in clinical use we found that this heat treatment of FVIII filtrate completely eliminated over 7 logs of infectious virus, despite the fact that the solution contained sucrose to protect FVIII (table IC,D). In the absence of sucrose, wet heat inactivation could be expected to be even more potent.

When ARV-2 and ARV-3, which differ in restriction enzyme sensitivity[4] and in replicative ability (unpublished), were added to human plasma (5°C), no reduction in virus titre was observed (table IIA). Subsequently, a cryoprecipitate formed which contained infectious ARV at a 10-fold reduced titre, as with the mouse retrovirus.[1] Purification of cryoprecipitate by acid and glycine precipitation and filtration to achieve a sterile FVIII filtrate resulted in a further 10-fold reduction in virus titre (table IIA).

Lyophilisation of a FVIII filtrate containing ARV-2 lowered the infectious virus titre about 10-fold (table IIB). When this lyophilised preparation was then heated, very low titre virus was detectable after 10, 24, and 34 h but not after 48 h of heating (table IIB). When tissue culture fluid containing ARV at 10^4 IP/ml was kept at 56°C for 30 min no infectious virus remained. Furthermore, when ARV-containing FVIII filtrate fortified with sucrose 1·2 g/ml was heated at 60°C for 10 h before lyophilisation, no infectious virus was detected in the liquid or lyophilised product (table IIB).

Our results indicate that lipid-enveloped retroviruses (both mouse and human) if present in sufficient amount in plasma can be found in infectious form in FVIII lyophilised products. Their sensitivity to FVIII purification procedures appears to be independent of virus input titre (table I). Because ARV is not as easy to measure in tissue culture as the mouse xenotropic virus, not every infectious ARV particle may have been detected. Nevertheless, the results confirm the ability of infectious retroviruses to withstand the procedures used to purify FVIII from plasma.[1] The comparable results with the mouse and human retroviruses suggest that heating lyophilised FVIII for 72 h at 68°C or the liquid product for 10 h at 60°C will eliminate infectious ARV if it is not present in the plasma at more than 10^6 infectious particles/ml. The data indicate that wet heat procedures used to prepare other plasma products, such as albumin, should inactivate infectious ARV.

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EFFECT OF HEAT TREATMENT OF PLASMA AND SERUM ON BIOCHEMICAL INDICES

SIR,—Like Dr Goldie and colleagues (May 18, p 1161) we have been examining the effects of heating serum and plasma on biochemical test results. Plasma or serum from patients was incubated at 56°C for 30 min in screwcapped glass bottles completely immersed in a water bath. Heat-treated plasma is turbid and has to be centrifuged (3000 *g* for 5 min) before the supernatant can be analysed. Samples which were not centrifuged gave erroneous results. Centrifugation of heat-treated plasma does not fully clear the turbidity but heat-treated serum remains clear and does not require centrifugation. Heat-treated samples were analysed in the same batch as the corresponding unheated samples.

Analyses were done on lithium heparin samples, except for glucose (fluoride oxalate) and immunoglobulins (serum). Results were compared by paired t-tests (table).

We can confirm Goldie and colleagues' findings for creatine kinase and alkaline phosphatase activities, which were almost completely destroyed. Free T_4 levels were much increased by heat treatment. The decrease in plasma total protein is partly due to the disappearance of fibrinogen (Goldie et al demonstrated no

SELECTED* ANALYTICAL RESULTS ON UNHEATED (N) AND HEAT-TREATED (HT) PLASMA OR SERUM (n = 25–37 FOR NON-MANUAL ASSAYS)

Analyte	N	HT	Δ(%)	p
Na (mmol/l) (S)	137·6	137·5	−0·08	NS
HCO_3 (mmol/l) (S)	26·1	22·2	−14·95	<0·001
Cl (mmol/l) (S)	100·2	101·4	1·19	<0·001
AST (IU/l) (C)	42·6	44·4	4·2	<0·05
GGT (IU/l) (C)	57·2	46·4	18·9	<0·001
Ca (mmol/l) (C)	2·21	2·15	−2·71	<0·001
P (mmol/l) (C)	1·0	0·99	−1·0	NS
Total protein (g/l) (C)	68·0	64·0	−5·9	<0·001
Albumin (g/l) (C)	37·4	37·4	0·0	NS
Cortisol (nmol/l)	500	437	−12·6	NS
Free T_4 (nmol/l)	17·8	28·3	59·0	<0·001
IgG (g/l)	11·32	11·43	0·97	NS
IgA (g/l)	1·87	1·88	0·53	NS
IgM (g/l)	1·4	1·35	−3·6	NS

*This summary table is restricted to analytes significantly affected by heat treatment by Goldie et al and/or by US. (In our study significant differences were not found for K, urea, creatinine, glucose, total bilirubin, hydroxybutyrate dehydrogenase, cholesterol, triglyceride, amylase, or urate.) Full data and details of methods are available on request. S = Technicon SMA; C = 'Centrifichem'; AST = aspartate transaminase; GGT = γ-glutamyltranspeptidase.