Society for Endocrinology, Metabolism and Diabetes: Abstracts of Papers

The following are abstracts of papers read at the meeting of the Society for Endocrinology, Metabolism and Diabetes, held in Bellville, CP, on 24 September 1973:

A SIMPLE METHOD FOR THE ESTIMATION OF PLASMA PROGESTERONE*

M. KATZ AND P. J. CARR, *Department of Obstetrics and Gynaecology, University of Cape Town*

A simple, rapid non-chromatographic method for the assay of progesterone in human peripheral plasma is presented. A single petroleum ether extract of plasma was partitioned twice with 28% aqueous ethanol, and progesterone was then measured by a competitive protein-binding assay, using plasma from women receiving high-oestrogen oral contraception.

Mean recovery (± SD) of tritiated progesterone in 80 samples was 75,0% ± 6,9. Coefficient of variation calculated for both inter- and intra-assay determinations of quality control plasma at two levels of progesterone was 7% or less, showing a high degree of precision. The sensitivity of the assay is 200 pg/ml. Accuracy was determined by measurement of known amounts of authentic progesterone added to progesterone-free plasma. The correlation coefficient (r = 0,995) and regression line (y = 9,46x − 0,08) show the assay to be extremely accurate, although accuracy diminishes in the sub-nanogram/ml range. Of numerous steroids tested only 20α-hydroxyprogesterone was shown to contribute to any significant degree (13,1%) to the assay.

The method is short, simple and satisfactory for measurement of plasma progesterone in the luteal phase of the menstrual cycle.

*Published in full elsewhere in this issue.

STRUCTURE-FUNCTION RELATIONSHIP OF SMALL AND LARGE POLYPEPTIDE HORMONES

A. I. VINIK, A. HARDCASTLE AND B. J. GRANT, *Endocrine Unit, Department of Medicine, University of Cape Town*

We have previously shown that the structural determinants of large polypeptide hormones which contain the antigenic activity reside mainly in the tertiary configuration of these hormones. With the smaller peptides, for example gastrin and glucagon, primary sequence seems to be a major factor in the antigenicity of these hormones. The data presented revolve around four main points:

1. Gut glucagon-like activity which does not react with a pancreas specific glucagon antiserum can be resolved after electrophoresis on polyacrylamide gel into several peptides which do cross-react with the pancreas-specific glucagon antiserum.

2. There is a critical locus in the glucagon molecule in the region of the amino acids 18-21 which determines its antigenicity. There are however areas in both the N terminal and C terminal portions of the molecule which are shared with gut glucagon peptides. Although gut glucagon itself appears to be devoid of insulin-stimulating properties, the peptides found after gel electrophoresis do possess this property. This may be of significance since the incretin, the factor which stimulates insulin release from the pancreas, has not yet been identified and one of these peptides may be responsible.

3. Gastrin, too, has specific antigenic determinants which differ from the biological locus. The C terminal portion of gastrin is responsible for biological activity, while the centre portion of the molecule and the N terminal region are immunologically active.

4. A circulating mini-gastrin has been identified in the plasma of normal subjects. The relevance of this observation remains to be resolved.

A SIMPLE METHOD FOR THE EXTRACTION AND PURIFICATION OF HUMAN GROWTH HORMONE AND ITS ASSAY BY PAPER CHROMATOGRAPHY

S. W. STROUD, *National Chemical Research Laboratory, CSIR, Pretoria*

A simple method for the extraction and purification of human growth hormone has been developed, by which, after the initial alkaline extraction of the acetone-dried pituitary powder, the bulk of the impurities is precipitated at pH 4,8 and the crude hormone subsequently precipitated with alcohol at 25% v/v concentration. The crude hormone is then purified by a single Sephadex G-100 gel filtration, 75% of the activity of the crude being obtained as virtually pure monomer and the remaining 25% in the form of two fractions containing two separate polymers.

A simple and rapid paper chromatographic assay method has also been developed for following the extraction and purification stages, which gives good agreement with the corresponding radio-immunoassays.

DICHOTOMY BETWEEN GROWTH HORMONE AND SOMATOMEDIN (SULPHATION FACTOR) IN PROTEIN CALORIE MALNUTRITION

B. L. PIMSTONE, D. J. BECKER, D. GRANT AND D. HAMBLEY, *Departments of Medicine and Paediatrics and Child Health, University of Cape Town and Clinical Research Centre, Middlesex, England*

In protein calorie malnutrition (PCM) plasma human growth hormone (HGH) levels are substantially elevated. Using the chick pelvis assay (Hall) we have found that HGH-dependent somatomedin is nevertheless substantially depressed. During the course of recovery somatomedin gradually rises towards normal at approximately the same rate as the rise in serum albumin and transferrin, while HGH levels fall towards normal. This remarkable dichotomy between elevated HGH and low somatomedin may account for the observation of marked depression of cartilage growth in PCM, in spite of HGH elevation.

CIRCADIAN RHYTHM IN THE URINARY EXCRETION OF CYCLIC 3'5' ADENOSINE MONOPHOSPHATE (cAMP) IN MAN

J. SAGEL,* G. LIZARRALDE, C. LOADHOLT, A. GREEN AND J. COLWELL, *Department of Medicine, University of South Carolina and VA Hospital, Charleston, SC, USA*

The existence of circadian variations in the urinary excretion of cyclic 3'5' - adenosine monophosphate (cAMP) was investigated. After a control day of standard food intake, 8 normal volunteers were placed on a constant 3-hourly feeding and urine collection schedule for a 48-hour period. Urinary cAMP was estimated by radio-immunoassay. Results were expressed as a % of the total 24-hour excretion and as % cAMP/g creatinine (cAMP/g Cr). In all subjects the peak daytime excretion occurred around noon and the nadir after 1800. A post-midnight rise occurred in 6 of the 8 individuals.

Period	*Mean % cAMP**	*Mean % cAMP/g Cr**
900 - 1200	16,58	16,10
1800 - 2100	9,39	9,27
0000 - 0300	13,65	12,93

*From a least-square analysis a difference between means of 3,64 (cAMP) and 3,82 (cAMP/g Cr) was required for significance ($P<0,05$).

The relationship of phosphate clearance (ClP) and tubular reabsorption of phosphorus (TRP) to cAMP excretion during the period 0600 - 1800 was analysed.

Independent	*Dependent*	*Regression coefficient*	*Standard deviation*	*Correlation coefficient*
% cAMP	ClP	0,011†	0,004	0,556†
% cAMP/g Cr	TRP	−1,480*	0,656	−0,434*

*$P<0,05$. †$P<0,01$.

In summary, we have demonstrated the presence of a circadian rhythm in the pattern of urinary cAMP excretion and have shown significant correlation between these fluctuations and renal phosphate handling.

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ASSESSMENT OF ADH RESERVE

F. BONNICI, *Endocrine Service, Department of Paediatrics and Child Health, University of Cape Town*

A test combining the effects of dehydration and vasopressin administration was used to assess the ADH reserve status of 20 patients. After a variable period of dehydration, urine was collected hourly until it reached a constant osmolality. Plasma osmolality (Posm) was then determined and the percentage change in urine osmolality (Uosm) 1 hour after the subcutaneous injection of 5 units aqueous vasopressin was calculated.

In 8 patients (controls or patients with anterior pituitary dysfunction), Uosm was much greater than Posm after dehydration, but showed no significant increase after vasopressin, never more than 5%. Two patients previously diagnosed as diabetes insipidus failed to increase Uosm above Posm on dehydration but exhibited a rise of at least 72% (mean 150%) after vasopressin. Four patients with ADH resistance failed to increase Uosm above Posm and showed no response to vasopressin. In 6 patients with polyuria (all with established pituitary disease) Uosm was greater than Posm after dehydration (mean 387, maximum 513 m-osm/kg) but showed a further increase in Uosm after vasopressin of at least 12% (mean 29%). These last patients were considered to have partial ADH deficiency. In addition, a significant relationship was demonstrated between the ability to decrease free water clearance during water deprivation and the subsequent response to therapy with clofibrate or carbamazepine.

These studies may permit the recognition of patients with partial ADH deficiency who will be likely to respond to oral drug therapy.

CALCIUM-BINDING PROTEIN AND VITAMIN D METABOLISM IN EXPERIMENTAL PROTEIN MALNUTRITION

W. J. KALK, *Endocrine Unit, Department of Medicine, University of Cape Town*

Intestinal and renal vitamin D-dependent calcium-binding protein (CaBP) was measured in young, vitamin D-supplemented, protein-deficient (4% casein diet) and control rats (20% casein diet). After 3 weeks on the respective diets, intestinal CaBP activity in the protein-depleted animals was 65% that of the controls. Renal CaBP was similar in both.

Protein-depleted and control rats were fed vitamin D-deficient diets for 21 days. On day 18, half of each group of animals received parenteral vitamin D; the remaining rats in each group were untreated; all were sacrificed on day 21, and intestinal CaBP activities were compared. The intestinal CaBP level in the vitamin D-deficient control animals was 50% of the level in vitamin-D supplemented controls; CaBP levels in the vitamin D-deficient and protein depleted animals was 15% of the control vitamin D-treated rats, and increased to 30% with vitamin D supplementation.

The metabolic fate of an intravenous dose of tritiated cholecalciferol was examined in control and protein-depleted vitamin D-deficient rats. Hepatic hydroxylation of cholecalciferol was normal in the malnourished animals, and there was indirect evidence that renal hydroxylation was also normal.

It is concluded that the low levels of intestinal CaBP in this model of protein malnutrition may explain the reduced intestinal absorption of calcium previously described in a similar model. The decrease in CaBP activity probably resulted from reduced CaBP synthesis caused by amino acid substrate deficiencies, and not as a consequence of deranged vitamin D metabolism.

OUR TAMIL FAMILY

W. P. U. JACKSON, W. VAN MIEGHEM, N. MARINE AND I. EDELSTEIN, *MRC Endocrine Research Group, Department of Medicine, University of Cape Town*

A community of Tamil-speaking Indians in Cape Town was found to have an extremely high prevalence of diabetes, e.g. 37% aged over 25 years. They were considerably inbred, with close-kin marriages; other social and dietary factors did not appear relevant when comparison was made with other Indian groups. The newly-discovered diabetics were younger than the already known diabetics. Re-examination 5 years later indicated that 11 (34%) of 32 subjects who were normal initially had become diabetic, and that the mean GTT values of originally normal and originally diabetic subjects had greatly increased. Prolonged (5-hour) GTTs did not indicate any particular tendency to 'reactive hypoglycaemia' in this potentially diabetic community. We conclude that consanguinity (presumed multiple genetic influences) may be extremely important in determining the prevalence of maturity-onset type diabetes, at least among certain communities.

GROWTH HORMONE LEVELS IN A POTENTIALLY DIABETIC COMMUNITY

P. KELLER AND W. P. U. JACKSON, *MRC Endocrine Research Group, Department of Medicine, University of Cape Town*

Human growth hormone (HGH) was assayed in 51 members of the extended Tamil family mentioned in the previous abstract during a prolonged oral GTT, and also in 24 Tamil

non-family controls. On the whole the mean HGH values were higher in the family, with a lesser post-glucose fall. In particular, the 7 family subjects with 2 diabetic parents showed no HGH suppression after glucose. Other subgroups are analysed.

The pattern of response is consistent with the suggestion that prediabetic or potentially diabetic groups show less suppression of HGH than normal. The evidence, however, is certainly not conclusive.

HOW NOT TO RUN A DIABETIC CLINIC

R. GOLDBERG, I. BERSOHN, B. I. JOFFE, L. KRUT AND H. C. SEFTEL, *Diabetic Clinic, Johannesburg General Hospital and South African Institute for Medical Research, Johannesburg*

A study of middle-aged and elderly patients attending a diabetic clinic has revealed a disturbing state of affairs. Hyperlipidaemia and obesity were very common but little attention was paid to implementing appropriate dietary regimens. Management was largely confined to the control of hyperglycaemia with oral hypoglycaemic agents, especially combinations of sulphonylureas and diguanides.

This situation is deplored. Firstly, it ignores the correction of factors which are as, if not more, important than hyperglycaemia in regard to the development of the most lethal complication of maturity-onset diabetes, namely occlusive atherosclerosis. Secondly, it substitutes for dietary therapy which is physiological, treatment by drugs which are potentially harmful. It is probable that a similar situation exists in many other diabetic clinics.

METABOLIC RESPONSES TO SELECTIVE BETA-ADRENERGIC STIMULATION IN MAN

R. GOLDBERG, B. I. JOFFE, M. VAN AS, H. C. SEFTEL, L. KRUT AND I. BERSOHN, *Carbohydrate and Lipid Metabolism Unit, Department of Medicine, University of the Witwatersrand and South African Institute for Medical Research, Johannesburg*

The metabolic effects produced by intravenous administration of the predominantly β_2-adrenergic stimulant salbutamol were studied in 9 normal subjects. Blood samples were taken before and at frequent intervals after salbutamol injection for measurement of free fatty acid, insulin, growth hormone, glucose and lactate levels. In addition, a control study using saline was carried out on some of the subjects, while in others salbutamol was given together with practolol, a predominantly β_1-blocking agent.

Significant rises in free fatty acid, insulin, lactate and glucose levels were produced by salbutamol. Growth hormone levels did not change. Practolol had no effect on the free fatty acid response, diminished but did not abolish the insulin response, and suppressed the lactate and glucose rises.

It was tentatively concluded that β_2-receptors mediate lipolysis; that insulin release may be subserved by both β_1 and β_2-receptors; and that the lactate and glucose rises may reflect muscle and liver glycogenolysis, respectively, the receptors involved being of the β_1 type.

CIRCULATING INSULIN-LIKE COMPOUNDS IN THE PLASMA OF INSULIN-TREATED DIABETICS

A. I. VINIK AND A. HARDCASTLE, *MRC Endocrine Research Group, Department of Medicine, University of Cape Town*

On the assumption that measurement of circulating pro-insulin would provide a measure of residual pancreatic function in diabetics treated with exogenous insulin, a system employing polyacrylamide gel electrophoresis and immunoassay of insulin in the sliced gels was devised. It was found that insulin-treated diabetics all had circulating insulin peptides which were neither pro-insulin nor insulin itself. The question as to whether these peptides were actually secreted or formed part of the commercial insulin administered, was examined by dissociation of insulin bound to antibodies which appear in the plasma of all treated diabetics, and electrophoresing the freed insulin-like peptides. It was found that the antibodies bound considerable proportions of these peptides which were, in fact, derived from the commercial insulin preparation. To elucidate whether or not these peptides may also be derived from the pancreas, these diabetic patients are now being treated with monocomponent insulin and their sera will be examined from time to time.

EFFECT OF ADP, EPINEPHRINE, AND COLLAGEN ON PLATELET AGGREGATION IN DIABETES

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There is increased ADP-induced platelet aggregation (ADP pl.agg.) in diabetics with micro-angiopathy. No data are available in diabetics of lesser severity. We studied peak (P) and 4-min (4) ADP pl. agg. in citrated platelet-rich plasma in 15 normals (I), 7 prediabetics (II), 12 latent diabetics (III), and 20 frank diabetics (IV). Platelet aggregation after epinephrine and collagen was also studied.

% Decrease in Optical Density (OD) after ADP

Group	*0.12*		*0.25*		*0.50*		*1,0 μM ADP*	
	P	*4*	*P*	*4*	*P*	*4*	*P*	*4*
I	6	4	14	5	24	10	40	29
II	9	7	19	10	32	24	52*	43
III	12	11	24*	18	38*	35*	60*	62*
IV	12*	15*	24*	22*	41*	37*	58*	58*

*Significant difference from I ($P<0,05$). In II, second phase (2nd) pl.agg. was clearly accentuated in 3 subjects. Slope of initial decrease in OD after 0,5 μM ADP in I<III<IV (2.0<2,5<2,8% agg./sec/pl.; $P<0,5$).

% Decrease in OD after Epinephrine and Collagen (4 min)

	Epinephrine (μM)		*Collagen (μg/ml)*	
Group	*0,25*	*2.0*	*0,25*	*0,50*
I	14	52	7	26
III + IV	46	88	43	58
P <	0,005	0,005	0,01	0,05

Initial slope after collagen in I<III+IV (4<1,3 at 0,25 μg/ml and 1,2<2,2 at 0,50 μg/ml; $P<0,05$).

The conclusions were that increased platelet aggregation is detected at an early stage of diabetes mellitus; that the second phase of platelet aggregation is affected; and that further studies on platelet and plasma nucleotide levels and turnover are indicated in diabetics

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EVIDENCE FOR A SELECTIVE GLUCO-RECEPTOR DEFECT OF PANCREATIC BETA CELLS IN CHRONIC PANCREATITIS

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The early (10-minute) immunoreactive insulin release in response to various stimuli in patients with severe pancreatic

exocrine insufficiency was compared with controls.

The mean 10-minute incremental area of the insulin response to combined oral glucose, intravenous glucagon and tolbutamide was severely reduced in patients (728 ± 287,7; controls 2 714 ± 479,9 μU/min; $P<0,0025$).

Mean insulin responses to oral glucose were similar in patients and controls, but the insulin response to intravenous glucose was absent in 4 of 6 patients (patients 79,0 ± 51,2 *v.* controls 874 ± 171,9 μU/min; $P<0,0025$).

All patients retained some insulin responsiveness to intravenous arginine.

Intravenous secretin produced an insulin response in all patients (57,6 ± 17.6 and controls 220 ± 70,8 μU/min; $P<0.05$).

Pancreozymin produced an insulin response in 5 of 6 patients (38 ± 17,9 and controls 158 ± 30,7 μU/min; $P<0,005$).

It is concluded that, in patients with pancreatitis: (*a*) the beta-cell glucose-specific receptor mechanism may be defective, while the glucose-stimulated pathway of the entero-insular axis is maintained; (*b*) arginine-induced insulin secretion is mediated differently from the intravenous glucose-induced response; (*c*) insulin responses to secretin and pancreozymin are retained, which may explain the insulin response to oral glucose.

INSULIN RESERVE AND IRON STORAGE

A. I. VINIK, D. J. MILLER, J. ROSSOUW AND S. J. SAUNDERS, *MRC Liver and Endocrine Research Groups, Department of Medicine, University of Cape Town*

The association of iron overload with glucose intolerance is well recognised. We have previously shown that with progressive glucose intolerance there is a sequential fall-off in pancreatic insulin secretion accounting for the eventual emergence of diabetes. In this study we have examined the relationship between body iron stores and insulin reserve. Insulin reserve was measured by the rise in serum insulin concentration following intensive beta cell stimulation by oral glucose followed by the intravenous administration of glucagon and tolbutamide simultaneously. In haemosiderotic patients who were as yet untreated there was no difference in the insulin response to the intensive stimulus. Following repeated venesection to remove body iron there was a significant decrease in the insulin responsiveness. Withdrawal of these patients from the venesection programme caused a return in insulin secretion to the earlier pattern within one month. These changes in insulin responses occurred without parallel changes in serum iron or total iron-binding capacity. Twelve iron-deficient patients had responses which were impaired compared with the normal controls. It seems that: (*i*) insulin responses are a sensitive index of the effect of venesection on body iron stores, reflecting a therapeutic effect before changes in serum iron are apparent; (*ii*) the excess iron stored must affect the cellular insulin secretory mechanism and not the iron *per se*. We are in the process of treating the iron-deficient patients with iron to reassess their insulin status when iron-replete.

INSULIN RELEASE AND PANCREATIC ISLET VOLUME IN MALNOURISHED RATS

C. WEINKOVE, E. A. WEINKOVE AND B. L. PIMSTONE, *Department of Medicine, University of Cape Town*

Human protein calorie malnutrition is now a well-established cause of diminished insulin release. In a protein calorie-deficient rat model we have shown a diminished early insulin response to oral glucose, intravenous glucose and intravenous tolbutamide. Light and electron microscopy of the pancreas showed no difference between the islets of the malnourished and control rats. Quantitative histology, however, showed a 40% reduction in total pancreatic islet volume in the malnourished rats.

The very low insulin values after intravenous glucose and tolbutamide in malnourished rats cannot be entirely explained by this reduction in total islet volume. It appears, therefore, that both release mechanisms, as well as pancreatic islet volume, must be affected by protein calorie malnutrition.

INVESTIGATIONS IN TYPE I HYPERLIPOPROTEINAEMIA

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Type I hyperlipoproteinaemia (HLP) is a rare autosomal recessive disorder characterised by marked fasting hyperchylomicronaemia, severe hypertriglyceridaemia and a normal to moderately raised plasma cholesterol level. The plasma cholesterol : triglyceride ratio is <0,2 and may be <0,1. Post-heparin lipase activity (PHLA) measured in the plasma is generally moderately or severely reduced. We present 4 cases from 3 families, 3 of whom (from 2 families) show the classical features of type I HLP enumerated above. The fourth child, however, a 6-year-old Coloured male, whether on a normal or low fat diet, showed a lipoprotein pattern on cellulose acetate electrophoresis, suggesting a type IV or V HLP. The cholesterol : triglyceride ratio was >0,2 on all but one occasion on which it was tested and his PHLA was normal with the screening method used by our laboratory. His mother and only sibling have normal lipids, the father was not tested prior to this report. In view of the fact that type IV and V HLP are considered not to occur in childhood we have tentatively classified this patient as a type I but final diagnosis remains to be established.

These 3 families (together with 1 other discovered subsequently) represent an unusually large collection of cases suffering from a rare disorder. The biochemical heterogeneity revealed on the routine diagnostic investigation of these patients may reflect genetic differences and are being investigated further.

TRH IN THE INVESTIGATION OF THYROID FUNCTION

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Synthetic TRH has been under clinical investigation since 1970. The main field of interest has been the release of TSH induced by intravenous TRH and its correlation to thyroid function. The results have been very encouraging, so that attempts are now being made to use a TRH test not only in addition to, but also instead of, the radio-iodine study (uptake, scintigraphy, PBI-131), which for a number of years appeared to be the method of choice. Although still widely employed, it is generally accepted that radio-iodine study does not sufficiently clarify the state of thyroid function. This may be due to the fact that the iodine uptake is only an indirect measurement of TSH activity.

The determinations of the T_3-binding capacity and thyroxine in the serum have therefore increased in value and in most clinics they are employed as a routine, yet it has remained difficult in many cases to differentiate between normal thyroid function and mild hyperthyroidism or hypothyroidism. A T_3 suppression test or a TSH stimulation test will be helpful in such cases but will mean greater radiation dosage to the thyroid gland, side-effects in a number of patients and more time and work expended.

TRH introduced new aspects and since TSH concentration in serum can now be determined radio-immunologically, we investigated the reliability of the determination of thyroid function based on the TSH concentration in serum before and after intravenous injection of synthetic TRH. The study is based on the evaluation of 191 cases within the clinical trial of Hoe 50 011, monitored by the Medical Department of Farbwerke Hoechst AG.

The substance under investigation was the tripeptide Hoe 50 011 — pyroglutamyl-histidyl-proline amide, which is a synthetic releasing hormone effecting the release of thyrotrophin (TSH) from the hypophysis.

The ages of the 191 patients investigated ranged between 13 and 75 years with a mean of 39,5 years. There were 98 women and 85 men. The sex was not recorded for the remaining 8 patients.

PATIENTS AND METHODS

Diagnosis

As practised in outpatient departments for thyroid diseases, the diagnoses were attained by physical examination, T_3-binding index and the quantitative determination of thyroxine and/or protein-bound iodine. In addition, a radioactive iodine test was performed in some cases.

We examined 112 euthyroid subjects, 40 hyperthyroid subjects and 39 primarily hypothyroid subjects.

Method

The investigators used the customary double-antibody method (Odell) for the radio-immunological estimation of TSH in patients who received 0.2 or 0.5 mg Hoe 50 011 intravenously. Blood samples were taken before the administration and 20 or 30 min, some also 60, 90 and 120 min, thereafter to determine the TSH level.

RESULTS

After the IV inection of the Hoe 50 011 (synth. TRH), in euthyroid subjects the TSH level in the serum increased, reached its peak after 20 min and decreased again 30 min after the injection. Some investigators took the second blood sample 20 min, others 30 min after administration of synth. TRH. As there is no significant difference between the delta's

$\triangle (TSH_{20'}) - TSH_{0'} = 9{,}85 \pm 6{,}66\ \mu U/ml$
$\triangle (TSH_{30'}) - TSH_{0'} = 8{,}67 \pm 6{,}46\ \mu U/ml$

both values were considered equally in the evaluation below.

Preliminary investigations had already shown that the TSH increase in healthy subjects is dose-dependent up to 0,2 mg synth. TRH/patient. Some investigators had conducted part of the trial with a dose of 0.5 mg/patient but did not observe a greater increment. The mean values were:

inj. of 0,2 mg synth. TRH (N = 53) $\triangle$ TSH = 10,6 ± 6,05 µU/ml
inj. of 0,5 mg synth. TRH (N = 59) $\triangle$ TSH = 8,67 ± 6,46 µU/ml

In further evaluation we therefore made no distinction between the two dosages.

If one compares the absolute TSH values in euthyroid, hyperthyroid, and hypothyroid patients before and after synth. TRH it becomes evident that the initial TSH values are increased far more in primarily hypothyroid subjects than in euthyroid or hyperthyroid cases. The TSH level after administration of synth. TRH exceeds 25 µU/ml in all cases of hypothyroidism, while in hyperthyroid patients there is almost no TSH increase. But since the initial TSH values and the TSH values after administration of synth. TRH clearly overlap, particularly in euthyroid and hyperthyroid patients, the absolute TSH values do not suffice for a definite diagnosis of thyroid function. If one calculates the quotient $TSH_{(20)30'}/TSH_{0'}$, and the difference between $TSH_{(20)30'}$ and $TSH_{0'}$, the hyperthyroid patients can be far better differentiated.

With the three parameters (*i*) absolute TSH values in the serum before and 20 - 30 min after administration of synth. TRH (TSH p. Rx); (*ii*) TSH increase 20 - 30 min after synth. TRH ($\triangle$); (*iii*) quotient (Q) $TSH_{(20)30'}/TSH_{0'}$, a diagnostic scheme can be set up which allows diagnosis of the patients' thyroid function with a security of more than 95% in this trial. The frequency of the respective parameters observed by us is given in parenthesis, in Table I.

The importance of the parameters differs in the various states of thyroid function. The increment of TSH, however, is most important in euthyroidism as well as in hyperthyroidism and in primary hypothyroidism. The order was established as shown.

Evaluation of Tolerance

Of the 191 patients, 66 reported transient subjective complaints. Nausea and the urge to micturate were the most common. Other complaints included the sensation of warmth, slight dizziness, a peculiar taste, and in 1 case cardiac palpitations. These sensations, which have also been recorded in the literature, in all cases lasted only a few seconds or minutes but could not be measured by the investigators. There was, for instance, no increased diuresis despite the sensation of urgency. The complaints which the patients did not regard as being severe, were milder and less frequent after a dose of 0,2 mg (23%) than after 0,5 mg Hoe 50 011 (42%).

CONCLUSION

It may be stated that, according to the data presented, the diagnosis of normal or primarily impaired thyroid function may be established with a certainty of more than 95% by means of the radio-immunological determination of TSH in

TABLE I. DIAGNOSIS SCHEME

Diagnosis	TSH in serum (µU/ml) 0′ (before synth. TRH)	TSH in serum (µU/ml) 20′ - 30′ post inj.	Quotient $TSH_{(20)30'}/TSH_{0'}$	$\triangle (TSH_{(20)30'} - TSH_{0'})$	
Euthyroidism	$\leqslant 7$ (99%)	$\leqslant 25$ (96,4%)	$> 1{,}5$ (99%)	$\geqslant 2$ (96,5%)	< 25 (98%)
Hyperthyroidism	< 7 (97,5%)	< 7 (95%)	$\leqslant 1{,}5$ (85%)	< 2 (95%)	
Primary hypothyroidism	> 7 (95%)	> 25 (100%)	$> 1{,}5$ (82%)	$\geqslant 25$ (77%)	

Importance of the parameters (observed frequency):
Euthyroidism: 1. $\triangle$ TSH $\geqslant 2$ (96,5%) < 25 (98%). 2. Q $> 1{,}5$ (99%). 3. $TSH_{(20)30'} \leqslant 25$ (98%).
Hyperthyroidism: 1. $\triangle$ TSH < 2 (95%). 2. $TSH_{(20)30'} < 7$ (95%). 3. Q $< 1{,}5$ (85%).
Primary hypothyroidism: 1. $\triangle$ TSH $\geqslant 25$ (77%). 2. $TSH_0 > 7$ (95%). 3. $TSH_{(20)30'} > 25$ (100%).

the serum before and 20 - 30 min after 0,2 mg Hoe 50 011 (synth. TRH), taking into account the initial TSH values, the TSH increment and the ratio between these two parameters.

High TSH levels and increased release of TSH will only be seen in primary hypothyroidism. Secondary hypothyroidism which shows low TSH values, as does hyperthyroidism, may be recognised, apart from the clinical aspect, by simultaneous assessment of T_3 and/or T_4.

DEVELOPMENT OF A RADIO-IMMUNOASSAY FOR LUTEINISING HORMONE-RELEASING HORMONE (LH-RH)

R. MILLAR, S. HENDRICKS AND B. PIMSTONE, *Departments of Medicine and Chemical Pathology, University of Cape Town*

A radio-immunoassay capable of measuring LH-RH has been developed, using classical procedures.

LH-RH was conjugated to keyhole limpet haemocyanin and also to bovine serum albumin using carbodi-imide as condensing agent. Condensation presumably occurs between the free amino groups of the glycinamide or arginyl residues in LH-RH and the acid anhydrides of condensed free carboxyl groups in the carrier proteins.

About 0,5 mg of the conjugate was emulsified with Freund's complete adjuvant and injected into multiple intradermal sites in rabbits and guinea pigs. After bleedings at day 75, and after subsequent bleedings following booster injections, an antiserum was obtained from a single rabbit which had been injected with the LH-RH haemocyanin conjugate.

Both the chloramine T and lactoperoxidase methods of radio-iodinating LH-RH were attempted and the relative effectiveness of Sephadex G25, Biogel P2, and Cellulose CF11, in separating intact ^{125}I LH-RH from damaged ^{125}I LH-RH and free ^{125}I were tested. Best separation was achieved with CF11, resulting in a fraction with 80 - 90% of radio-iodinated LH-RH binding to excess antiserum.

The assay procedure employed a 24-hour pre-incubation of antiserum at final dilution 1 : 3 000 with LH - RH standards, followed by a second 24-hour incubation after addition of ^{125}I LH-RH, and finally separation of bound and free hormone, using a second precipitating antiserum to rabbit γ-globulin.

Cross-reaction with other peptides including TRH, oxytocin, glucagon and pentagastrin was negligible. The assay was fairly sensitive; a concentration of 10 pg/ml of LH-RH eliciting more than 10% decrease in binding of ^{125}I LH-RH, and 50 pg/ml eliciting a 50% decrease.

EFFECT OF CLOMIPHENE AND LUTEINISING HORMONE-RELEASING HORMONE (LH-RH) ON THE MENSTRUAL DISTURBANCE IN ANOREXIA NERVOSA

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Plasma levels of luteinising hormone and of oestradiol were depressed in 11 amenorrhoeic patients with anorexia nervosa. On refeeding, hormone levels rose, and 2 patients resumed spontaneous menstruation once their weight reached 80% of standard.

Five patients were treated with one or more courses of clomiphene citrate. In those instances in which clomiphene was administered to patients weighing less than 80% of standard, there was a poor response of plasma LH and menstrual bleeding did not occur. When the drug was administered to patients weighing more than 80% of standard, a normal response of LH and of oestradiol was found, and it was followed by menstrual bleeding.

The response of plasma LH and plasma FSH to LH-RH was assessed in 4 patients. A normal response to infused LH-RH was found in patients weighing more than 80% of standard, but severely emaciated patients showed a diminished response.

THYROTROPHIN RESPONSE TO INTRAVENOUS THYROTROPHIN-RELEASING HORMONE IN PATIENTS WITH HEPATIC AND RENAL DISEASE

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Immunoreactive TSH responses to intravenous TRH were studied in normal controls and in patients with chronic renal failure, established liver disease or after successful renal transplantation. Basal TSH levels and magnitude of the peak response to TRH were, with one exception, normal in liver and renal disease. However, though prompt, TSH elevation was sustained in liver disease, while the peak was delayed and sustained in chronic renal failure. Successful renal transplantation was associated with normal TSH responses. Presumably the sustained TSH levels after TRH reflect impaired plasma clearance of TSH and/or TRH in liver and renal disease.

LH AND FSH RESPONSE TO LH-RH IN NORMAL AND MALNOURISHED CHILDREN

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The recent advent of the immunoassay of LH and FSH and the synthesis of LH-releasing hormone (LH-RH) has provided another tool to assess pituitary function in children.

A total of 27 children aged 8 - 34 months was studied. Ten control children and 17 children suffering from protein calorie malnutrition (PCM) were given LH-RH. It was found that normal children at a young age have measurable basal levels of both LH and FSH. Their response to LH-RH shows a similar percentage increase to adults, although absolute levels are lower. Both basal and stimulated levels of FSH are usually greater than LH, unlike the situation in adults. In PCM the basal levels of both LH and FSH are normal, as is the FSH response to LH-RH. However, the LH response to LH-RH is statistically subnormal. This is in contrast with the other pituitary hormones which have normal or increased response to stimulation.

TSH RESPONSE TO THYROTROPHIN-RELEASING HORMONE (TRH) IN PATIENTS WITH HYPOTHALAMIC AND PITUITARY DISEASE

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The TSH response to TRH has been measured in 45 patients with hypothalamic or pituitary disease and compared with the measurement of circulating thyroid hormone levels and of

cortisol and human growth hormone (HGH) reponse to hypoglycaemia in the same patients. Of 18 patients with untreated pituitary tumours, the vast majority had normal tests of endocrine function, including TSH responses to TRH, except for the small number of untreated acromegalic patients who showed elevated HGH levels.

Patients with pituitary tumours treated by either deep X-ray or a combination of hypophysectomy plus deep X-ray had abnormal HGH reserve in the majority of instances and impaired cortisol reserve and basal thyroid function in about half. The TSH-TRH test was very variable in this group. It was often predictably subnormal when other tests of pituitary function were likewise impaired; rarely, a delayed TSH response was accompanied by clinical and biochemical evidence of hypothyroidism; and in three instances high basal levels with sustained responses after TRH were found in association with severe impairment of pituitary function and low circulating levels of thyroid hormones. This latter finding suggests the possibility that a treated pituitary tumour may secrete fragments or subunits of TSH which might be poorly active biologically but nevertheless cross-react in the radio-immunoassay.

A small group of patients with established hypothalamic disease had low basal TSH levels but responded to TRH in the classical delayed manner, while patients with isolated gonadotrophin or HGH deficiency had predictably normal TSH secretion. Patients with panhypopituitarism due to Sheehan's syndrome always showed low TSH levels after TRH.

The use of TRH to assess TSH reserve in patients with pituitary tumours is a less sensitive test of pituitary function than the measurement of HGH response to hypoglycaemia and about equal to the cortisol reserve test.

EFFECT OF ALPHA- AND BETA-ADRENERGIC BLOCKADE AND RESERPINE ON THE TSH RESPONSE TO TRH

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Little is known about the participation of catecholamines in the feedback control of TSH. In thyrotoxicosis basal TSH values and post-TRH are low. The effect of α- and β-adrenergic blocking drugs and reserpine on the TRH and TSH pathway in thyrotoxicosis was studied. Oral and intravenous propranolol had no effect on the flat TSH response to TRH. Similarly. phenoxybenzamine given orally and intravenously failed to alter TSH unresponsiveness to TRH. The administration of rogitine, which depletes both catecholamines and indole amines, also had no significant effect. It thus appears that catecholamines do not play a major role in the feedback suppression of TSH in thyrotoxicosis.