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The autonomic nervous system and bronchial asthma

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APPENDIX "A"

METHODS OF BIOCHEMICAL ANALYSIS AND HORMONE ASSAY

1. PLASMA GLUCOSE

Plasma glucose levels were measured either with the Beckman Glucose Analyser I (Beckman Instruments, Fullerton, California, USA) or with an automated state routine analyser (Astra 8, Beckman Instruments, Brea, California, USA).

2. PLASMA POTASSIUM

Plasma potassium levels were measured either with a flame photometer (IL543, Instrumentation Laboratories, Milan, Italy) or with an automated state routine analyser (Astra 8, Beckman

3. PLASMA GROWTH HORMONE ASSAY

Principle

The principle of the assay is based on the competition for a fixed and limited number of antibody sites between growth hormone labelled with iodine^{125} and growth hormone contained in standards or in the specimens to be assayed.

Assay precision and sensitivity are increased by pre-incubating the unlabelled growth hormone in the specimens and standards with the antiserum before adding I^{125}-labelled growth hormone to the reaction mixture. After incubation the amount of

labelled growth hormone bound to the antibody is inversely related to the amount of unlabelled growth hormone in the sample. The antibody-growth hormone complex is allowed to react with a second antibody, and after a further incubation period, distilled water is added to the mixture.

The tubes are centrifuged and the supernatant fluids aspirated. The radioactivity in each tube is measured with a gamma scintillation counter. A graph is drawn of the percentage of relative binding obtained for each standard (y-axis) against its hormone concentration (x-axis) using semilog paper. The amount of growth hormone (ng/ml) in each sample is read by interpolating its percentage binding on the standard curve.

Expected Values: Adults 0-1 ng/ml.
Children 0-10 ng/ml

Molecular Weight: 21500.

Conversion Factor to SI Units: x 0.047 = nmol/l.

Sensitivity: The assay has a lower limit of sensitivity of 0,2-0,4 ng/ml and a measurable range up to 20 ng/ml.

Specificity: The percentage of cross-reaction measured at 50% binding shows the specificity of the growth hormone antiserum used in the radioimmunoassay kit to be 100% for growth hormone, 0,48% for prolactin

and absent up to high concentrations of various other hormones.

Precision: Each sample was analysed in duplicate and the mean of the two radioactivity counts used to calculated the final value. For each study, all samples were tested in a single assay to avoid inter-assay variability.
Laboratory control pooled plasma was included in the growth hormone assays to determine the intra-assay and inter-assay variability.

In the present studies, measurements of the laboratory control pooled plasma gave the following results:

	Intra-assay variability	Inter-assay variability
Mean (ng/ml):	4.7	4.4
Standard deviation:	0.16	0.36
Coefficient of variation (%):	3.4	8.1

Manufactured by: Serono Biodata, Milan, Italy.

Radioactivity: Approximately 1,5 uCi (55,5 k Bq) per kit.

Reference: Melani et al. 1968.

4. PROLACTIN ASSAY

Principle

The principle of the assay is based on the competition for a fixed and limited number of antibody binding sites between prolactin labelled with iodine^{125} and prolactin contained in standards or in the specimens to be assayed.

After incubation the amount of labelled prolactin bound to the antibody is inversely related to the amount of unlabelled prolactin present in the sample. The antibody-prolactin complex is allowed to react with a second antibody and a solution of polyethylene glycol.

Separation is effected by centrifugation and aspiration of the supernatant fluids. The radioactivity in each tube is measured with a gamma scintillation counter.

A graph is drawn of the percentage of relative binding obtained for each standard (y-axis) against its hormone concentration (x-axis) using semilog paper. The amount of prolactin (ng/ml) in each sample is read by interpolating its percentage binding on the standard curve.

Expected Values: Women 0-15 ng/ml
Men 0- 9 ng/ml

Molecular Weight: 23000

Conversion Factor to SI Units: X0.043 = nmol/l

Sensitivity: The assay has a lower limit of sensitivity of 2 ng/ml and a measurable range up to 150 ng/ml.

Specificity: The specificity of the antiserum used in the radioimmunoassay kit is such that high levels of various hormones result in measured levels of prolactin of less than 2ng/ml.

Precision: Each sample was analysed in duplicate and the mean of the 2 radioactivity counts used to calculated the final value. All samples were tested in a single assay to avoid inter-assay variability.
Laboratory control pooled plasma was included in the prolactin assay to determine the intra-assay variability.
In the present study, measurements of the laboratory control pooled plasma gave the following results:

	Intra-assay variability
Mean (ng/ml):	10.3
Standard deviation:	0.63
Coefficient of variation (%):	6.1

Manufactured by: Serono Biodata, Milan, Italy

Radioactivity: Approximately 1,7 uCi (63 k Bq) per kit.

Reference: Friesen et al. (1973).

5. INSULIN ASSAY

Principle

The principle of the assay is based on the competition for a fixed and limited number of antibody sites between porcine insulin labelled with iodine^{125} and insulin contained in standards or in the specimens to be assayed.

After incubation the amount of labelled insulin bound to the antibody is inversely related to the amount of unlabelled insulin present in the sample.

The bound and free fractions of plasma insulin are separated by the use of an immuno-precipitating reagent in which an excess of a second antibody is preprecipitated.

After a further incubation period, the tubes are centrifuged and the supernatant fluids aspirated. The radioactivity in each tube is measured with a gamma scintillation counter.

A graph is drawn of the percentage of relative binding obtained for each standard (y-axis) against its hormone concentration (x-axis) using semilog paper. The amount of insulin (uU/ml) in each sample is read by interpolating its percentage binding on the standard curve.

Expected Values: 5-25 uU/ml.

Molecular Weight: 5807.

Conversion Factor to SI Units: Report as mU/l (no multiplication required).

Sensitivity: The assay has a lower limit of sensitivity of 0.78 uU/ml and a measurable range up to 200 uU/ml.

Specificity: Because of the highly specific nature of the immunological reactions, most anti-insulin sera fail to combine equally strongly with insulin prepared from different species, with the result that the standard lines obtained with these different hormones are not superimposable. The anti-insulin serum used in the present study yields similar results with porcine and human insulin.

Precision: Each sample was analysed in duplicate and the mean of the 2 radioactivity counts used to calculate the final value. All samples were tested in a single assay to avoid inter-assay variability. Laboratory control pooled plasma was included in the insulin assay to determine the intra-assay variability and the inter-assay variability (between the present study and other studies).

In the present study, measurements of the laboratory control pooled plasma gave the following results:

	Intra-assay variability	Inter-assay variability
Mean (/uU/ml):	19.4	19.8
Standard deviation:	1.3	0.6
Coefficient of variation (%):	6.7	3.0

Manufacturers: Wellcome Research Laboratories, Beckenham, England, and Behringwerke AG, Marburg, Germany.

Radioactivity: Approximately 377 kBq ^{125}I per kit.

Reference: Welborn and Fraser (1965).

6. ASSAY OF NON-ESTERIFIED FATTY ACIDS

Principle

The principle of the assay is based on a titrimetric technique in which the non-esterified fatty acids are selectively extracted and titrated with dilute alkali using an ultramicroburette while nitrogen gas is bubbled through the solution.

Reference standards of known concentrations are similarly extracted and titrated, and a graph is drawn of the conc ntrations of standard (y-axis) and the volume of titrant used (x-axis) using linear graph paper.

The amount of non-esterified fatty acids (uEq/l) in each sample is read by interpolating the amount of titrant used on the standard graph.

Expected Values: Fasting 315-1210 uEq/l.

Sensitivity: The assay has a lower limit of sensitivity of 150 uEq/l and a measurable range up to 2400 uEq/l.

Precision: Each sample was analysed in duplicate and the arithmetic mean of the results used as the final value.

Laboratory control pooled plasma was included in every assay to determine the intra-assay and inter-assay variability.

In the present study, measurements of the laboratory control pooled plasma gave the following results:

	Intra-assay variability	Inter-assay variability
Mean (/uEq/l):	1015	1020
Standard deviation:	52	62
Coefficient of variation (%):	5.1	6.1

Reference: Kelley (1965).

7. CORTISOL ASSAY

Principle

The principle of the assay is based on the competition for a fixed and limited number of antibody binding sites between cortisol labelled with iodine^{125} and cortisol contained in standards or in the specimens to be assayed.

After incubation, the amount of labelled cortisol bound to the antibody is inversely related to the amount of unlabelled cortisol present in the sample.

The bound and free fractions of plasma cortisol are separated by the use of antibody-coated tubes. The supernatant fluid is aspirated and the radioactivity in each tube is measured with a gamma scintillation counter.

A graph is drawn of the percentage of relative binding obtained for each standard (y-axis) against its hormone concentration (x-axis) using linear graph paper. The amount of cortisol (ng/ml) in each sample is read by interpolating its percentage binding on the standard curve.

Expected Values: 8 am = 60-230ng/ml
4 pm = 10- 85 ng/ml

Molecular Weight: 363.5

Conversion Factor to SI Units: x 2.75 = nmol/l

Sensitivity: The assay has a lower limit of sensitivity of 4ng/ml and a measurable range of up to 800ng/ml.

Specificity: The percentages of cross-reaction of the cortisol antiserum used in the radioimmunoassay kit is 100% for cortisol, 51% for prednisolone and less than 3% for various other glucocorticoid hormones (calculated according to Abraham et al. 1977).

Precision: Each sample was analysed in duplicate and the mean of the 2 radioactivity counts used to calculate the final value. All the samples were tested in a single assay to avoid inter-assay variability. Laboratory control pooled plasma was included in the cortisol assay to determine the intra-assay variability and the inter-assay variability (between the present study and other studies).

In the present study, measurements of the laboratory control pooled plasma gave the following results:

	Intra-assay variability	Inter-assay variability
Mean (ng/ml):	82	84
Standard deviation:	2.7	2.9
Coefficient of variation (%):	3.2	3.5

Manufactured by: Sorin Biomedica, Italy.

Radioactivity: Approximately 3,4 uCi (126 k Bq) per kit.

References: Abraham et al. (1977), Rolleri et al. (1976).

8. ADRENOCORTICOTROPIC HORMONE (ACTH) ASSAY

Principle

The principle of the assay is based on the competition for a fixed and limited number of antibody sites between ACTH labelled with iodine^{125} and ACTH contained in standards or in the specimens to be assayed.

After incubation, the amount of labelled ACTH bound to the antibody is inversely related to the amount of unlabelled ACTH present in the sample.

The bound and free fractions of plasma ACTH are separated by the use of an immuno-precipitating reagent in which an excess of a second antibody has been preprecipitated.

After a second incubation period, the tubes are centrifuged and the supernatant fluid aspirated. The radioactivity in each tube is measured with a gamma scintillation counter.

A graph is drawn of the percentage of relative binding obtained for each standard (y-axis) against its hormone concentration (x-axis) using semilog paper. The amount of ACTH (pg/ml) in each sample is read by interpolating its percentage binding on the standard curve.

Expected Values: The normal range for the hormone assay laboratory of the Department of Medicine of the University of the Witwatersrand is 50-150pg/ml.

Molecular Weight: 4500.

Conversion Factor to SI Units: x 0.22 = pmol/l.

Sensitivity: The assay has a lower limit of sensitivity of 10 pg/ml and a measurable range up to 1000 pg/ml.

Specificity: The percentage of cross-reaction of the ACTH antiserum used in the radioimmunoassay kit is 100% for ACTH with negligibile cross-reactivity for other pituitary hormones (calculated according to Abraham et al. 1977).

Precision: Each sample was analysed in duplicate and the mean of the 2 radioactivity counts used to calculate the final value. All samples were tested in a single assay to avoid inter-assay variability. Laboratory control pooled plasma was included in the ACTH assay to determine the intra-assay variability and the inter-assay variability (between the present study and other studies).

In the present study, measurement of the laboratory control pooled plasma gave the following results:

	Intra-assay variability	Inter-assay variability
Mean (pg/ml):	99	65
Standard deviation:	7.4	5.4
Coefficient of variation (%):	3.3	8.4

Manufactured by: Compagnie ORIS Industrie SA, France.

Radioactivity: Approximately 2 uCi (74 k Bq) per kit.

References: Lee et al. (1961); Berson and Yalow, (1968); Abraham et al. (1977).

9. PLASMA CATECHOLAMINE ASSAYS

Principle

The principle of the assay is based on the catalytic conversion of norepinephrine and epinephrine to their corresponding meta ^{3}H-methoxy-derivatives by the enzyme catechol-O-methyl-transferase in the presence of S-adenosyl-L-methionine-[3H-methyl].

The resulting products are extracted and separated by thin layer chromatography. The isolated catecholamine derivatives, ^{3}H-normetanephrine and ^{3}H-metanephrine are converted by periodate oxidation, to ^{3}H-vanillin and extracted.

The radioactivity attributable to each catecholamine hormone is measured with a beta scintillation counter.

A catecholamine standard of known concentration is added to a second aliquot of each sample, and is extracted and separated in the same way to provide an internal standard value. This is used to calculate the concentrations of the individual catecholamines.

Expected Values:	Norepinephrine	200-300 pg/ml
	Epinephrine	20- 50 pg/ml
Molecular Weights:	Norepinephrine	169
	Epinephrine	183

Conversion Factors to SI Units:

Norepinephrine	x 0.0059 = nmol/l
Epinephrine	x 0.0055 = nmol/l

Sensitivity: The assay has a lower limit of sensitivity of 2-5 pg/ml for norepinephrine and epinephrine, with a measurable range up to 3000 pg/ml/.

Specificity: The extent of cross-over of one catecholamine hormone into the assay of another (including dopamine) is less than 0.25% with the exception of epinephrine cross-over to norepinephrine which is 1.3%.

Precision: Each sample was analysed in duplicate and the mean of the 2 radioactivity counts used to calculate the final value. A control plasma specimen, provided by the manufacturers, with artificially high levels of catecholamines was included in each catecholamine assay to assess the efficiency of the kit by obtaining results within the ranges specified by the manufacturers, and to determine the inter-assay variability.

In the present study, the measurements of the control plasma specimen gave the following results:

	Inter-assay variability	
	Epinephrine	Norepinephrine
Mean (pg/ml):	671	729
Standard deviation:	71	52
Coefficient of variation (%):	10.6	7.1

Manufacturer: Upjohn Diagnostics, Kalamazco, Michigan, USA.

Radioactivity: Approximately 500 uCi ^{3}H per kit.

References: Cryer et al. (1974), Passon and Peuler, (1973).

APPENDIX "B"

STUDIES OF SERUM NEUTROPHIL CHEMOTACTIC ACTIVITY

1. Preparation of Polymorphonuclear Leukocytes (PMNL's)

PMNL's were prepared from blood obtained from normal adult donors which was treated with preservative-free heparin (5 units/ml blood). The PMNL's were separated from the mononuclear leukocytes by centrifugation at 400g for 15 minutes of the heparinised blood on cushions of Ficoll (Pharmacia, Uppsala, Sweden) metrizoate. The resultant erythrocyte/PMNL fraction was sedimented with 3% gelatin for 30 minutes at 37°C to remove most of the erythrocytes. The PMNL-rich supernatant was centrifuged at 250g for 10 minutes and the residual erythrocytes in the cell pellet lysed by exposure to 0.85% ammonium chloride. The PMNL's were centrifuged, washed once and resuspended at a concentration of 10^7/ml in Hank's balanced salt solution (HBSS, Grand Island Biological Co., New York, USA) - pH 7.4, supplemented with 0.1% bovine serum albumin (BSA).

2(a) Polymorphonuclear Leukocyte Migration Assay

The leukotactic potential of each serum specimen was measured using modified Boyden Chambers (Wilkinson, 1971). Two hundred microlitres of PMNL suspension (6 x 10^5 PMNL) were placed in the upper compartment of the Boyden chamber and separated from the lower compartment containing 20% serum diluted in BSA-supplemented HBSS by a 5 µm pore-size micropore filter (Sartorius

membrane filters, Göttingen, Federal Republic of Germany). After incubation at 37°C the filters were removed and processed and the number of cells that had migrated through the entire thickness of the filter were counted (4 fields/filter). The results were expressed as PMNL/microscope high-powered field. Corresponding controls for random migration in the absence of serum were included and subtracted from each result.

2(b) Activation of PMNL membrane-associated oxidative metabolism

The effects of each serum specimen on PMNL membrane-associated oxidative metabolism were measured by a myeloperoxidase-mediated iodination assay (Root and Stossel, 1974) as an alternative method of detection of serum-associated leukoattractants (Becker et al. 1974). 10^6 PMNL were incubated with 20% serum, 0.06 uCi of Na I^{125} (17 Ci/mmol, New England Nuclear Corp., Boston, Mass., USA) and 5mg BSA in a final volume of 1ml HBSS in 5ml polypropylene test tubes which were incubated on a rotator for 30 minutes at 37°C. The reactions were terminated and the protein precipitated by the addition of ice-cold 10% trichloroacetic acid. After washing, the I^{125} in the protein precipitates was measured in a solid state gamma counter. The results were expressed in fmoles I^{125}.

Each serum specimen was coded prior to storage and the code broken only once the results were available.

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