tigate this question, two types of experiments were performed: In the first, mouse spleen lymphocytes were cultured in the presence of optimal concentrations of Con A (2μg/ml), a T-cell mitogen, or LPS (40 μg/ml), a B-cell mitogen, and the rates of ^{14}C-lactate production (from ^{14}C-glucose) and ^{3}H-thymidine uptake were compared after various intervals of culture. In the second, T- and B-cells were isolated from mouse spleen by passage through columns of nylon-wool or by treatment with anti-Thy 1.2 monoclonal antibody and complement, respectively, and the rates of ^{3}H-thymidine uptake and the activities of the glycolytic enzymes, lactate dehydrogenase (LD), pyruvate kinase (PK) and phosphofructokinase (PFK), were compared after 48 hr of culture. Lactate production progressively increased after stimulation of unfractionated spleen cells with both Con A and LPS, but the increase in the presence of Con A was appreciably greater (about 3-fold after adjusting for the rate of ^{3}H-thymidine incorporation). In the experiments with fractionated cells, the activities of all three glycolytic enzymes were greater in T-cells stimulated with Con A than in B-cells stimulated with LPS. After adjustment for the rate of ^{3}H-thymidine incorporation, the activity of LD was about 4-fold greater in T-cells than in B-cells, whereas the activities of PK and PFK were only 1.4 and 1.7-fold greater, respectively. These results suggest that proliferating T-cells have a greater capacity for glycolysis than proliferating B- cells and raise the possibility of utilizing LD activity as a marker to differentiate between B- and T-cell proliferation.

321 LABORATORY DIAGNOSIS AND THERAPY OF SEVERE HYPOPHOSPHATEMIA IN A HOSPITALIZED POPULATION

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Severe hypophosphatemia (SHP) leads to significant disorders including cardiomyopathy, muscle weakness and rhabdomyolysis, neurological abnormalities, acute respiratory failure, platelet and granulocyte dysfunction, and hemolytic anemia. In order to establish the incidence of SHP, we examined the serum phosphorus levels AST, ALT, ALP, albumin, globulin, Ca, and Fe performed by SMAC on 6,656 specimens. Hemoglobin was assayed on selected samples. We followed patients with SHP ($PO_4 \leq 15$ mg/l) for diagnosis and therapy.

Of 222 patient samples with phosphorous levels of ≤ 20 mg/l, 93 measured ≤ 15, 35, ≤ 10, and 7, ≤ 5 mg/l. We followed those patients whose levels were ≤ 15 mg/l, and found that 64% were male and 36%, female. The age distribution showed 2%, ≤ 19 yrs old; 34%, 20-40 yrs; 43%, 41-60 yrs; and 21% ≥ 61 yrs. The principle diagnoses were: 57%, alcoholic withdrawal; 15%, sepsis; 8%, other liver disease, and 7%, trauma. Several cases of diabetic ketoacidosis, nutritional deficiency, and disturbances of calcium metabolism also were observed. Phosphorus therapy in 40% of patients with ≤ 10 mg/l was parenteral. For patients with levels of 11-15 mg/l, 63% received no therapy. We conclude that SHP is a frequent finding in our inpatient group. It needs to be identified, especially in the susceptible population, for adequate therapy to avoid clinical complications.

322 AUTOMATED TESTING OF SLOWLY CHANGING LEVELS OF ANALYTES IN MIXTURES.

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A fully automated method of analyzing the dissolution pattern of sustained release tablet preparations is described. The USP paddle apparatus was used in the dissolution studies. Data comparing this automated method to the previous manual method for dissolution testing of Vitamin C will be presented.

For the Vitamin C test, a sustained release tablet is placed in USP simulated gastric juice. At timed intervals, aliquots are taken, buffers added, and the sample is titrated. After exactly 3 hours pancreatin pH 7.2 is added. During a six hour dissolution study, the ascorbic acid is protected from air oxidation by blanketing under an argon atmosphere.

For sampling, a modified Hanson Dissoette™ sampling system controlled by an Apple II™ computer is used. The aliquoted samples are titrated with iodine using a Brinkmann E 526 Titration™ system fitted with a Brinkmann™ E 624 Sample Transport Handler™.

The system is controlled by an Apple II™ basic language computer through a 32 bit IO interface. Results are read through a BCD interface from the digital Dosimat™. Final calculations, archival storage and graphical display of the dissolution patterns are performed after the run by the computer. Our system can run six assays simultaneously unattended.

Other applications for testing of analytes in other mixtures are discussed.

323 DEVELOPMENT OF A RAPID QUALITATIVE RIA FOR THE DETECTION OF HCG IN URINE.

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We wish to report the development of a rapid qualitative RIA for the detection of hCG in urine. The protocol utilized is as follows: to 100 μl of urine standard or unknown urine and 100 μl of hCG free serum is added 200 μl of I-125 labeled β-hCG and 200 μl of diluted antiserum. After incubation at 37°C for 30 minutes, the antibody-antigen complex is precipitated with second antibody-polyethylene glycol-silica gel and centrifuged for 15 minutes. The pellet is counted and the unknown urine sample is compared to a reference urine standard containing 100 mIU/ml hCG.

The assay was found to be unaffected by high glucose (8 mg/ml), protein (2.7 mg/ml), salt (35 mg/ml) and variations of pH (pH 5.0 to 9.0). High concentrations of hLH (5000 mIU/ml), hTSH (20,000 μIU/ml) and hFSH (2500 mIU/ml) were shown not to give values equivalent to the 100 mIU/ml hCG standard. The inter- and intra-assay variation for high and low levels of hCG is between 4 and 5%.

324 DEVELOPMENT OF A NEW, 24 H PROLACTIN ASSAY

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A new RIA for prolactin has been developed which offers greater sensitivity, requires smaller sample volume and has faster turnaround time than our routine assay which was previously published by this laboratory (Clin. Chem. 23:1564, 1977).

In our routine assay, we used a commercial antiserum, incubated for 3 days, used 100 μl serum sample and obtained as assay sensitivity of 0.16 ng/tube. In the new assay, we used an antiserum developed in-house by immunizing white New Zealand rabbits with human prolactin, incubated for 24 h c 50 μl sample and obtained a sensitivity of 0.08 ng/tube. Second antibody was used for both methods to separate free and bound fractions.

We compared the prolactin results of 132 patients determined by both assays and obtained a correlation coefficient of 0.970, a slope of 1.031 and a Y intercept of -0.135.

At present, since a good prognosis of transphenoidal surgery to remove pituitary adenoma generally is observed in patients whose pre-surgical prolactin levels can be suppressed to normal by bromocreptin, a fast prolactin assay would be helpful in making a decision for that surgery.

325 DIRECT NO-EXTRACTION ASSAY FOR SERUM PROGESTERONE BY SOLID PHASE ^{125}I RADIOIMMUNOASSAY

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A direct no-extraction assay for serum progesterone has been developed. The procedure calls for adding 100 μl of (unextracted) patient serum to antibody-coated tubes, followed by 1.0 ml of buffered ^{125}I Progesterone. The tubes are incubated for 3 hours at ambient temperature, then decanted and counted. The assay is sensitive to as little as 0.15 nmol/l. The intra-assay C.V. is less than 8%, inter-assay less than 10%. Recovery of added progesterone (ranging from 0.8 to 64 nmol/l) averaged 105%. Scaling efficiency based on patient samples was 90 to 105%. There were no matrix effects due to bilirubin, lipemia, hemolysis or protein. Specificity of the immobilized antiserum is excellent. Cross-reactivity with 20α-dihydroxyprogesterone is less than 2%, with 17α-hydroxyprogesterone less than 0.3%. All other major circulating steroids show cross-reactivity of less than 1%. Preliminary reference ranges for serum progesterone using the present method are as follows: