Glucose

Although high blood glucose levels are commonly associated with diabetes, it has also been found to be associated with individuals possessing a hyperactive thyroid or adrenal gland. Conversely, a low blood glucose level (hypoglycemia) is usually associated with cases of insulin overdose, an underactive endocrine system, and some forms of liver disease. In general, blood glucose levels are useful in assessing the overall integrity of carbohydrate metabolism in an individual.

The assay used in the quantitation of blood glucose is one that you have become familiar with from an earlier experiment. It is a coupled assay that relates the reduction of NAD^+ (ΔA_{340}) to the concentration of glucose in the sample (see Experiment 11, Figure 11-3).

Urea Nitrogen

One of the major products of amino acid metabolism is ammonia (NH_3), a molecule known to be highly toxic to higher organisms. In the liver, ammonia and carbon dioxide are used to produce a water-soluble form of nitrogen, urea, via the urea cycle. The liver passes this urea to the blood, which carries it to the kidneys to be filtered out and excreted in the urine. Since one function of the kidney is to collect and excrete urea, increases in the concentration of this compound in the blood are an indicator of poor kidney function. Since urea is formed in the liver, low blood urea nitrogen is often the consequence of impaired liver function due to disease or as the result of infection (hepatitis).

The assay used to quantify blood urea nitrogen relies on coupling the production of ammonia and carbon dioxide from urea to the transamination of α-ketoglutarate to glutamate and the subsequent oxidation of NADH (A_{340}) (Fig. 16-2).

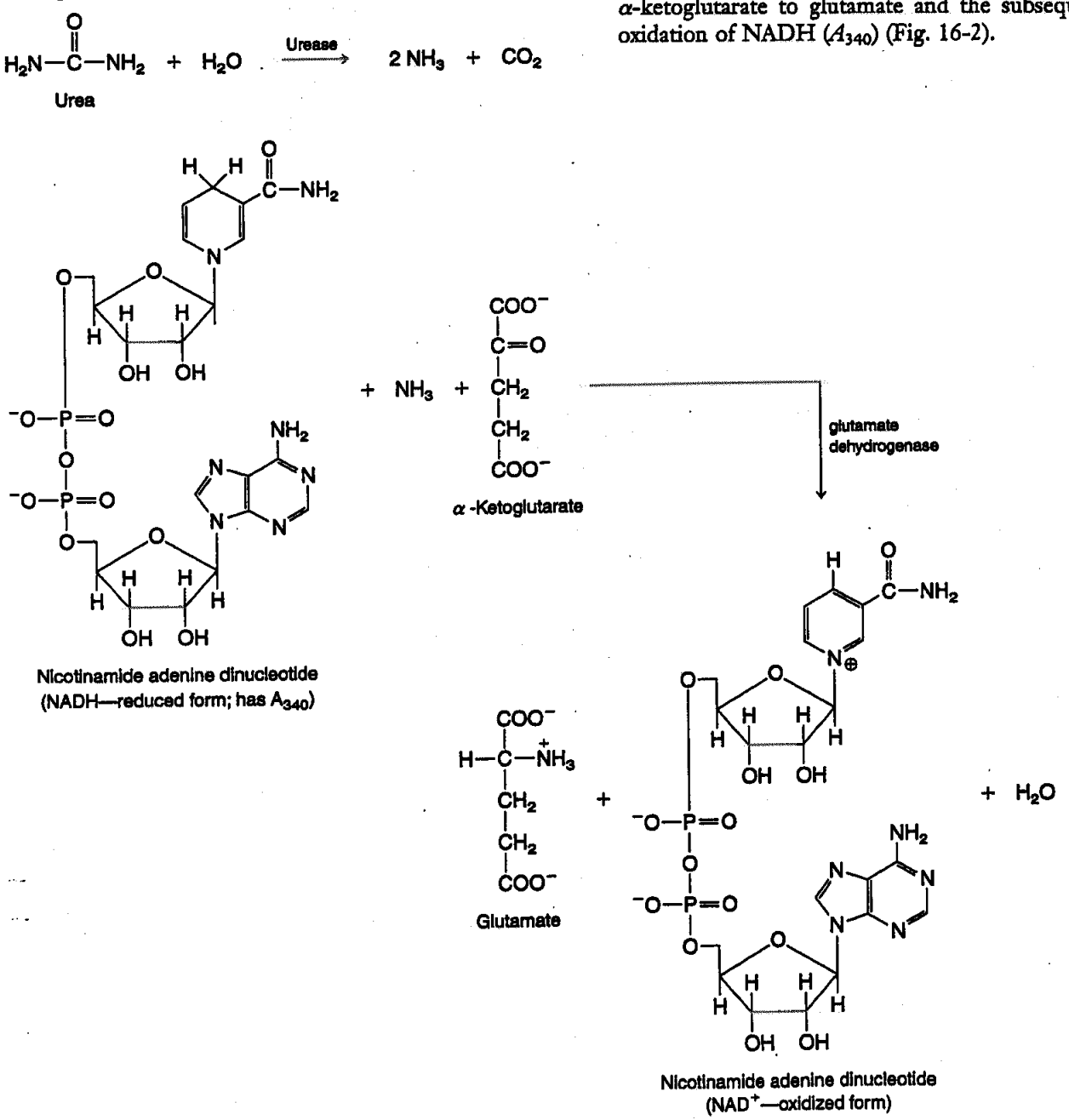

Figure 16-2 Quantitative assay for serum urea nitrogen.

Blood Glucose Assay

1. Add 2.0 ml of glucose reagent to five 13-by-100-mm test tubes labeled G1 through G5.
2. Add 20 μl of the following to each of these tubes and shake gently to mix:

   G1 Water
   G2 Plasma sample
   G3 Glucose standard (3.00 mM)
   G4 Glucose standard (11.10 mM)
   G5 Glucose standard (16.65 mM)

3. Incubate the tubes at room temperature for 10 min. It is not critical that the incubation time be exact. Rather, it is important that the reaction be allowed to run to completion (no change in A_{340}). When the reaction is complete, add 1 ml of water to each of the five tubes before taking a final A_{340} reading.
4. Blank your spectrophotometer at 340 nm against a water reference. Record the absorbance of all of your samples, G1 through G5.
5. Subtract the absorbance value of G1 (blank) from the rest of the samples. Construct a standard curve of A_{340} versus micromoles of glucose for your three glucose standard solutions (G3, G4, and G5).
6. Based on the A_{340} of your plasma sample and the *volume* of plasma used in the assay, determine the *concentration* of glucose in your plasma sample.

   NOTE: The normal or expected concentration of blood glucose in an adult human is ~4.0 mM to 6.5 mM. How does the concentration of glucose in the pig serum sample compare to that expected in human serum?

Blood Urea Nitrogen Assay

1. Add 2.0 ml of urea nitrogen reagent to five 13-by-100-mm test tubes labeled U1 through U5.
2. Add 20 μl of the following to each of these tubes and shake gently to mix:

   U1 Water
   U2 Plasma sample
   U3 Urea standard (3.57 mM)
   U4 Urea standard (8.92 mM)
   U5 Urea standard (10.71 mM)
   U6 Urea standard (35.70 mM)

3. Incubate the tubes at room temperature for 5 min. It is not critical that the incubation time be exact. Rather, it is important that the reaction be allowed to run to completion (no change in A_{340}). When the reaction is complete, add 1 ml of water to each of the five tubes before taking a final A_{340} reading.
4. Blank your spectrophotometer at 340 nm against a water reference. Record the absorbance of all of your samples, U1 through U6.
5. Subtract the absorbance value of U1 (blank) from the rest of the samples. Construct a standard curve by plotting the *absolute value* of A_{340} versus micromoles of urea nitrogen for your four urea standard solutions (U3, U4, U5, and U6).
6. Based on the A_{340} of your plasma sample and the *volume* of the plasma sample used in the assay, determine the *concentration* of urea nitrogen in your plasma sample.

   NOTE: The normal or expected concentration of blood urea nitrogen in an adult human is