was maintained over phosphorus pentoxide in an evacuated desiccator.

A sample (1.1 mg) of the dry C^{14}-urea-urease preparation (containing 1 μc of C^{14}) was weighed on a glass planchet, and the radioactivity was measured in a chamber monitored with a Geiger-Mueller gas-flow tube connected to a scaler and to a rate meter. The integrated amount of $C^{14}O_2$ in the chamber was recorded on a strip chart. The atmosphere in the chamber was equilibrated at the desired humidity before the reactants were introduced, and the humidity was maintained in the chamber with appropriately diluted sulfuric acid (*2*) placed in an open dish.

Measurements of the reaction rate of the lyophilized urea-urease mixture with water vapor showed that, at 100-percent relative humidity (20°C), there was a maximum hydrolysis of urea in about 2 hours. With decreasing humidity, the rate decreased, and there was no measurable release of $C^{14}O_2$ below 60-percent relative humidity (Fig. 1). Similar results were also obtained with an NF-grade urease (*1*), and both sets of values of relative activities are shown in Fig. 2.

Isotherms of water-vapor adsorption were determined separately for urease and urea. Recrystallized urea and urease (*1*) were dried under vacuum and over phosphorus pentoxide. After the samples containing 50 to 100 mg of material reached a constant weight, they were placed in moisture chambers at 20°C and weighed again after constant weight was reached (*3*). The water-vapor adsorption isotherms of the substrate (urea) and the enzyme (urease) (Fig. 2) were compared with the relative reaction rates at the respective humidities.

The relative reaction rates follow the sorption isotherm of the enzyme rather than that of the substrate. In the range of relative humidities where there is no measurable water-vapor adsorption on the substrate (relative humidity 60 to 75 percent), a significant amount of urea becomes hydrolyzed. Evidently, the limiting factor in this reaction is the availability of water molecules to the active sites of the enzyme: at 60-percent relative humidity, the amount of water sorbed by urease is only 1.3 moles per mole of side-chain polar groups (*4*), corrected for side-chain amide. Thus, only loosely bound water can be utilized in hydrolysis, that is, water sorbed in excess of the stoichiometric minimum of one molecule of water per polar site (*3*).

Soils exhibit urease activity, and there is evidence that a certain amount of urease may be present in soils in a free state (*5*). Our results with soils containing added urea and equilibrated with water vapor show that considerable hydrolysis of urea may occur in "air-dry" soils at 80-percent relative humidity and above. Applications of enzymatic behavior at low humidities in food technology have been reviewed (*6*).

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Solid-Phase Radioimmunoassay in Antibody-Coated Tubes

Abstract. *The adsorption of antibody to polymeric surfaces has been used to develop a new method of solid-phase radioimmunoassay. Incubation is performed in antibody-coated, disposable tubes that are finally washed-out with water and counted for quantitation of the bound tracer. The method is simple, rapid, inexpensive, and suitable for automation.*

The principle of solid-phase radioimmunoassay (*1*) is based on the ability of antibody-coated polymers specifically to bind radioactive tracer antigen. The use of antibody in this form allows rapid removal of the free radioactive tracer antigen by washing of the solid phase with water on completion of the immune reaction. The solid-phase material is then counted for quantitation of the bound tracer, which varies inversely with the total quantity of antigen present in the original incubation mixture. This simple and sensitive procedure can be used to measure nanogram quantities of protein hormones in plasma.

The materials originally used for solid-phase radioimmunoassay were prepared in powder form as either poly-(tetrafluoroethylene-*g*-isothiocyanatostyrene) (*2*) or Sephadex-isothiocyanate (*3*). More recently the former polymer has been prepared in the form of small discs (*4*), each of which, when coated with antiserum, represents a portion of specific antibody. Such discs have been used for radioimmunoassay of human growth hormone (*5*) and luteinizing hormone (*6*) in plasma; they are an improvement on the powder form of this solid phase. The discs have also been used in this laboratory for radioimmunoassay of human chorionic gonadotrophin, human placental lactogen, bovine luteinizing hormone, fibrinogen, and tetanus toxin; they appear to be suitable for radioimmunoassay of proteins in general.

During examination of various polymeric materials for applicability to solid-phase radioimmunoassay, it became apparent that certain unsubstituted polymers may adsorb antibody that can then bind an adequate quantity of radioactive tracer antigen for use in the assay. In contrast, adsorption of antibody to glass was negligible. Adsorption of antibody by polymer surfaces, from antiserums of moderately high titer, has been used to develop a simple and inexpensive form of radioimmunoassay.

The adsorption phenomenon has been applied to radioimmunoassay by coating of the interior of plastic tubes with uniform quantities of specific antibody. The two most commonly available disposable plastic tubes are manufactured from polypropylene or polystyrene, both of which give satisfactory results in the assay. Tubes (*7*) suitable for use in an automatic gamma counter were used without washing or other treatment; each was coated by addition of a uniform volume (1.0 or 2.0 ml) of diluted antiserum. Antiserum dilutions between 1:100 and 1:5000 proved to be satisfactory, the optimal *p*H of the buffer solution being 9.0 to 10.0. The duration of exposure to antiserum was not critical; results were identical with times ranging from 1 minute to 16 hours.

After removal of the coating solution, the tubes were washed-out three times with 0.15*M* NaCl and once with

a protein diluent solution consisting of 10 percent aged human plasma and 0.01 percent merthiolate in 0.15*M* NaCl.

For performance of an assay, serums or standards were diluted 1:5 and incubated in coated tubes for 16 hours at 37°C with 100,000 count/min of ^{125}I tracer antigen (*8*). The incubation volume (1.2 or 2.2 ml) slightly exceeded that used for coating the tubes. After aspiration of the contents, each tube was washed twice with tap water and counted for 1 minute in an automatic gamma counter.

The antigens used to establish the tube method of solid-phase radioimmunoassay were human growth hormone and human placental lactogen; antiserums to them, prepared by immunization of rabbits, were diluted to from 1:500 to 1:1000 before being added to the plastic tubes, which were coated in batches of from 100 to 200. After 16 hours, the coating solution was aspirated and stored at 5°C; stored solutions have have been reused ten times satisfactorily.

When applied to the radioimmunoassay of human growth hormone and human placental lactogen, the coated tubes gave standard curves of sensitivity comparable to that obtained by other methods of radioimmunoassay. Figure 1 shows the standard curve obtained in 20 percent serum by the use of tubes, coated with antiserum to human growth hormone, incubated for 16 hours at 37°C with 140,000 count/min of ^{125}I–human growth hormone in the presence of increasing quantities of unlabeled human growth hormone. Measurement of human growth hormone in samples of plasma with antibody-coated tubes under these conditions has given the same results as has the disc solid-phase method. The procedure has also been applied to measurement of human placental lactogen in plasma during pregnancy; it showed a rise from 36 ng/ml at 9 weeks to 3300 ng/ml at 26 weeks.

The notable features of this new procedure are the simplicity and economy resulting from use of antibody-coated plastic tubes. Attachment of antibody to the polymer surface appears to occur by a process of adsorption similar to that described by Leininger *et al.* (*9*) in their studies of blood-compatible plastics. The strength of this adsorption is shown by the quantity of tracer that attaches to the tubes after thorough

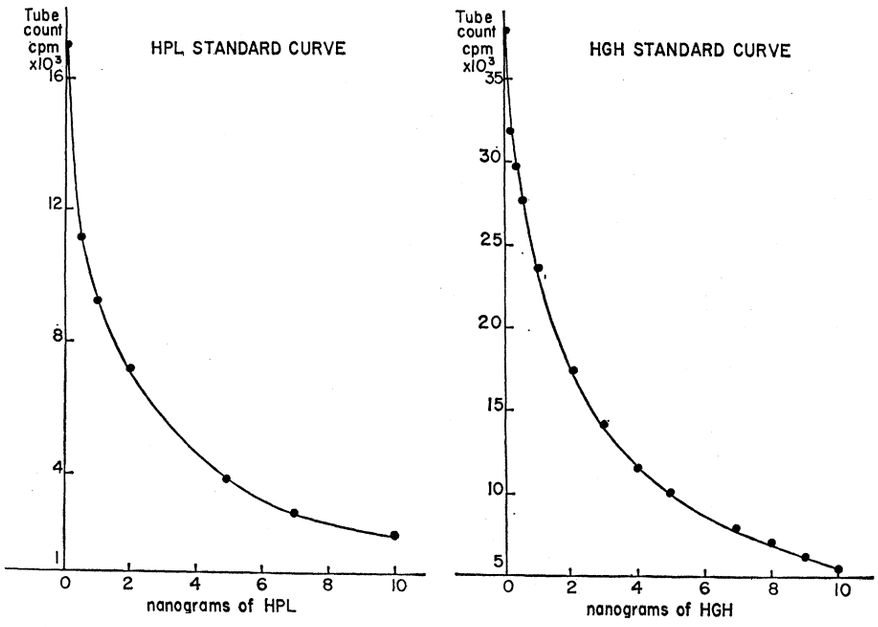

Fig. 1. Standard curves obtained with antibody-coated polypropylene tubes. (Left) Tubes coated with 2.0 ml of antiserum to human placental lactogen (*HPL*), diluted 1:250. Incubation performed in quadruplicate for 64 hours at 37°C with 0 to 10 ng of HPL and 118,000 count/min ^{125}I–HPL in 2.2 ml of 20-percent horse serum. (Right) Tubes coated with 1.0 ml of antiserum to human growth hormone (*HGH*), diluted 1:500. Incubation performed for 20 hours at 37°C with 0 to 10 ng of HGH and 95,000 count/min ^{125}I–HGH in 1.2 ml of 20-percent horse serum.

preliminary washing of the antibody-coated surface with saline and protein solutions. On completion of the immune reaction, repeated washing with water does not significantly reduce the amount of radioactivity bound to the antibody-coated tubes.

Four additional washing steps reduced the count rate of assay tubes by only 2 percent below the value obtained after a single wash with water. Even washing-out of the tubes with 0.2*M* NaOH and 0.2*M* HCl has not greatly reduced the radioactivity remaining after the initial wash with water. Physical stress, such as vigorous rubbing of the tube interior with the tip of a pipette during aspiration of the water washes, also has had no effect on the tracer binding.

Our findings show that antibody is strongly adsorbed to the tube surface by forces that are unaffected by conditions far more rigorous than those occurring during the assay. The adsorbed specific antibody provides the site for competitive binding of labeled and unlabeled forms of antigen to the inner surface of the tube. In contrast to the substantial binding of tracer human growth hormone to tubes coated with specific antiserum, only negligible binding (about 0.5 percent of added counts) occurred when tubes were untreated, coated with nonimmune rabbit serum, or coated with rabbit antiserum to human chorionic gonadotrophin.

The use of antibody-coated tubes provides the simplicity inherent in all solid-phase forms of radioimmunoassay, because the isolation of bound tracer is readily effected by washing-out of the tube on completion of the immune reaction. The assay is performed in tubes suitable for use in a gamma counter, adequate uniformity of replicates is achieved, and satisfactory results are obtained by overnight incubation without initial disequilibration. Expense is minimized by use of the same tube for all phases of the assay procedure; thus incubation, separation of bound and free tracer, and counting of the bound tracer are all performed in a tube costing only a few cents. We believe that the tube method of solid-phase radioimmunoassay is of considerable value for measurement of protein antigens against which antiserums of suitable titer can be prepared. The advantages of use of antibody-coated tubes are currently being incorporated into a system for automated radioimmunoassay.

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Carbohydrate Supply as a Regulator of Rat Liver Phosphoenolpyruvate Carboxykinase Activity

Abstract. *Administration of glucose, fructose, and glycerol to fasted rats produced a significant depression of liver phosphoenolpyruvate carboxykinase activity within 4 to 8 hours; galactose and ribose were much less effective. All the compounds yielded appreciable quantities of liver glycogen. The depression of phosphoenolpyruvate carboxykinase activity by glucose and glycerol was diminished by the concomitant administration of 2-deoxyglucose. The latter depressed glycogen formation from administered carbohydrate in muscle but not in liver. In rats made diabetic by alloxan, depression of elevated phosphoenolpyruvate carboxykinase activity by insulin was dependent upon a dietary source of carbohydrate. These results were interpreted to indicate that depression of certain gluconeogenic enzymes after carbohydrate ingestion is initiated by the metabolism of carbohydrate in some extrahepatic site.*

Homeostatic mechanisms in animals maintain blood sugar within a narrow range except when assaulted by massive carbohydrate intake or when endocrine balance is disturbed. It has frequently been proposed that part of the regulation may be mediated by induction and repression of enzyme formation (*1*) like that so well documented for microorganisms (*2*). In the case of the enzymes involved in gluconeogenesis by rat liver, the "adaptive" changes of enzyme activity are readily demonstrable, but whether these changes are the cause or result of altered rates of carbohydrate synthesis is not yet established (*3, 4*).

Table 1. Effect of various hexoses, glycerol, and ribose on liver phosphoenolpyruvate carboxykinase activity and glycogen in rats fasted 24 hours. At completion of the fasting time, a solution containing 0.5 g of the compound was administered orally every 2 hours. After 8 hours, the animals were killed, and liver samples were assayed for enzyme activity and glycogen. Numbers in parentheses represent number of rats. Means and their standard deviation are given in columns 2 and 3.

Compound administered	PEP carboxykinase (nmole • min^{-1} • mg of $protein^{-1}$)	Glycogen (%)
None (9)	94 ± 5.6	0.012 ± 0.02
Glucose (5)	52 ± 8.3	5.7 ± .02
Fructose (4)	53 ± 13.6	
Glycerol (4)	37 ± 7.6	4.9 ± .10
Galactose (7)	73 ± 9.7	3.7 ± .07
Ribose (4)	87 ± 2.4	1.1 ± .05

This is an extension of previous reports (*5, 6*) on the influence of dietary or parenterally administered carbohydrate on rat liver phosphoenolpyruvate (PEP) carboxykinase activity. Male rats (*7*), some of which were made diabetic by intravenous injection of alloxan (50 mg/kg), were used in all experiments. Methods for preparing the supernatant fraction resulting from high-speed centrifugation of rat liver homogenates and for assay of the PEP carboxykinase have been described (*8*). Glycogen was determined by the anthrone procedure after the tissue was digested with hot potassium hydroxide and the glycogen was precipitated with ethanol (*9*). Protein was determined by the biuret method (*10*).

Glucose, fructose, and glycerol decreased the PEP carboxykinase activity in fasting rats, whereas galactose was less effective (Table 1). Extending the experiments to 12 hours or more brought out an increased effect of galactose which may have resulted from its gradual conversion to glucose in the liver. Although ribose yielded appreciable quantities of glycogen in the liver, it did not depress enzyme activity. Thus, there appears to be no inverse relationship between liver glycogen concentration and the activity of this gluconeogenic enzyme (*6, 11*).

The results showing little depression of PEP carboxykinase activity with galactose and ribose indicated that suppression of gluconeogenesis may possibly be initiated in peripheral tissue such as muscle, because neither of these compounds is metabolized there to any extent, if at all. As a test of this hypothesis, glucose and glycerol (the latter is metabolized primarily in the liver) were used in conjunction with 2-deoxy-D-glucose (2-DOG), a potent inhibitor of carbohydrate metabolism (*12*). The effectiveness of glucose and glycerol in depressing PEP carboxykinase activity is diminished by the concomitant administration of 2-DOG (Table 2). In that large amounts of liver glycogen were found in all experiments, including glycerol plus 2-DOG, there is apparently no impairment of carbohydrate formation or utilization in this organ. In the presence of 2-DOG, carbohydrate formed from glycerol in the liver was probably not effective in depressing hepatic PEP carboxykinase activity. Somewhat surprising were the amount of liver glycogen found when 2-DOG was administered alone and the increment brought about by 2-DOG in rats given glucose. Although it appeared unlikely that 2-DOG could be incorporated into glycogen, this possibility was checked directly by hydrolyzing the glycogen to glucosyl units with Diazyme (*13*) and assaying for 2-DOG with the quinaldine reagent (*14*). These analyses indicated that less than 0.1 percent of the hexose units in the glycogen could have been 2-DOG.

From Table 3, it is apparent that 2-DOG does not diminish glycogen accumulation in the liver, but it tends

Table 2. Relation of liver phosphoenolpyruvate carboxykinase to carbohydrate supply, studied with 2-deoxyglucose in animals fasted for 24 hours. The procedure was the same as in Table 1, except that 0.5 g of 2-DOG was given to alternate lots of rats 30 minutes prior to the glucose or glycerol, and the animals were killed 4 hours later. Numbers in parentheses represents number of rats.

Compound administered	PEP carboxykinase (nmole • min^{-1} • mg of protein)	Glycogen (%)
None (6)	108 ± 8.7	0.02 ± 0.01
2-DOG (6)	101 ± 13	1.6 ± .01
Glucose (11)	71 ± 13*	3.2 ± .03
Glucose plus 2-DOG (11)	84 ± 11 †	4.5 ± .08
Glycerol (11)	78 ± 12*	2.8 ± .06
Glycerol plus 2-DOG (11)	97 ± 11‡	2.7 ± .07

* Significantly different from lot 1 ($P < .001$). † Different from lot 3 ($P < .05$) and significantly different from lot 1 ($P < .001$). ‡ Significantly different from lot 4 ($P < .01$) and from lot 5 ($P < .001$).