40 cm.) using a buffer (0.05 *M* KH_2PO_4 corrected to pH 8.0) as eluent. Acidification and dialysis of the high molecular weight fractions yielded the pure polypeptide, Compound 1.

Anal.—Calcd. for $C_{19}H_{24}N_4O_6$: C, 55.3; H, 6.1. Found: C, 55.0; H, 6.0.

Filtration of the polymer through a calibrated column (11) of Sephadex G-100 (2.5 × 45 cm.), using a solution of 0.05 *M* KH_2PO_4 corrected to pH 8.0 as eluent, indicated a molecular weight of at least 1×10^5.

Two rabbits were immunized with poly-(phe.glu.ala.gly)gly methyl ester (Compound 1) using the same protocol as that previously described (6). To aliquots of the sera obtained from each rabbit was added up to 500 mcg. of the synthetic polypeptide (Compound 1). No precipitin reaction was observed. Thus, substitution of the tyrosyl residue in the antigen poly-(tyr.glu.ala.gly)gly-1-^{14}C ethyl ester with the phenylalanyl moiety caused a loss of antigenicity, as shown by the precipitin reaction. It has been concluded that it is the phenolic hydroxy group of tyrosine that confers antigenicity to the molecule.

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Portal Vein Blood Sampling in Intestinal Drug Absorption Studies

Keyphrases ☐ Portal vein sampling—intestinal drug absorption ☐ Cannulation method—portal vein

Sir:

In an attempt to study the *in situ* absorption of drugs from the rat small intestine, a method of sampling portal vein blood was devised.

Generally, *in situ* drug absorption from the small intestine has been studied by determining the reduction in concentration of drug in the lumen (1–3). To complete the picture of intestinal absorption, simultaneous measurements of drug appearance in the portal blood and in the bile are required.

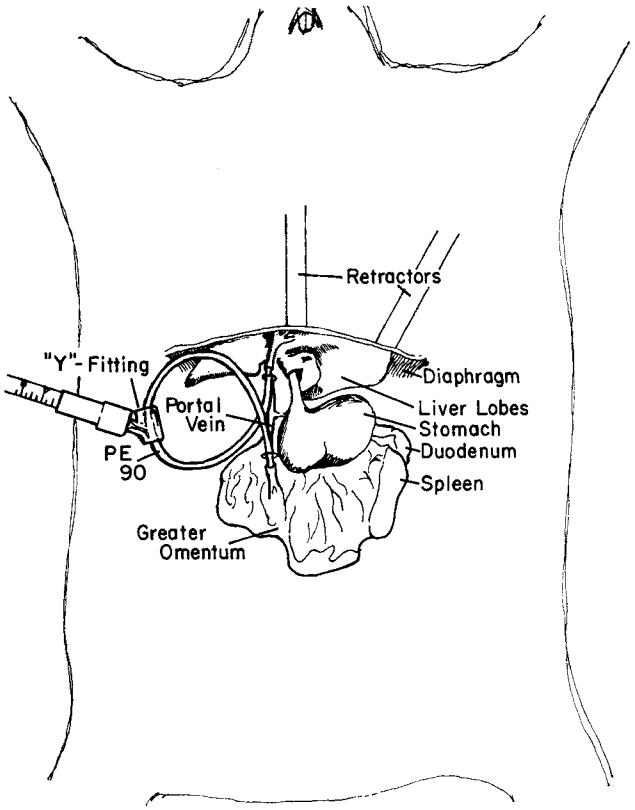

Figure 1—*Portal vein cannulation. Stomach, liver, and mesenteries were retracted to show the cannulation.*

In this communication, we report a method for sampling portal blood. The methods presented in previous work on dogs (4, 5) and rabbits (6) are not practical for the portal vein of a 200-g. rat since this portal vein is quite fragile. We tried direct venous puncture, using a needle point with tubing attached for sampling. The major problems were placing and securing the needle among the liver lobes, the mesenteries, and the small intestine, all of which should remain in their normal positions. The method described here consists of a shunt of polyethylene tubing cannulated in opposite directions in the portal vein. The surgery is delicate, requiring speed and accuracy to prevent excessive engorgement of the intestinal veins and acute loss of blood in the liver.

The viscera were exposed by midline abdominal incision. The portal vein was exposed by gentle retraction of the stomach just above the pyloric sphincter. The distal cannulation was prepared first. A Johns Hopkins bulldog clamp was placed around the portal vein, deep in the greater omentum. Fat and mesentery were included in the clamp to cushion the vein, thus preventing its collapse. The vein should not be cleaned of connective tissue. A cut in the vessel was then made approximately 3 mm. proximal to the clamp, and a slightly beveled length of PE 90 polyethylene tubing [i.d. 0.086 cm. (0.034 in.), o.d. 0.13 cm. (0.050 in.), length 27.9 cm. (11 in.)],[1] containing heparin solution, was inserted

[1] PE 90 Intramedic, Clay Adams.

and secured by silk. The clamp was released, and the heparin was mixed with blood by gentle pumping with a syringe attached to the free end of the tubing. The syringe was removed; the tubing was looped around, beveled, and used as the second cannula (Fig. 1). The sample port, consisting of a Y-fitting, was attached just before the second cannulation was made. The proximal cannulation was done approximately 3 mm. before the portal vein bifurcation into the liver.

The glass Y, fitted with a needle support, provided a means of sampling the blood. A 27-gauge needle was placed in the tubing through the support, and the sampling syringe was attached to the needle. Blood samples were withdrawn slowly to prevent embolism. Heparin was occasionally injected through this port to ensure free blood flow.

The bile duct was then cannulated with PE 50 polyethylene tubing, and samples were introduced into the duodenum of the intestine. At this point, the incision was closed by clamp or sutures. If lumen samples are to be taken, the organs are kept warm with a high-intensity lamp and moist with wet gauze.

The procedure has proven useful in drug absorption studies now being conducted in our laboratories.

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Immunochemical Studies on Linear Antigenic Polypeptides of a Known Sequence of Amino Acids

Keyphrases □ Polypeptides, linear, antigenic—immunochemical properties □ Antibodies, polypeptide produced—specificity

Sir:

It has recently been shown that the linear polypeptide poly-(L-tyrosyl-L-glutamyl-L-alanylgycyl)glycine - 1 - C^{14} ethyl ester (1, 2) is antigenic, eliciting antibodies in rabbits (3). It was thought reasonable that if the alanyl residue of this antigen was part of the active site, then substitution of this residue with the sterically larger valyl moiety could possibly alter the immunochemical properties of the polypeptide. For this purpose, poly-(L-tyrosyl-L-glutamyl-L-valylglycyl)glycine-1-C^{14} ethyl ester was synthesized (4), and we wish to report some immunochemical studies using this material.

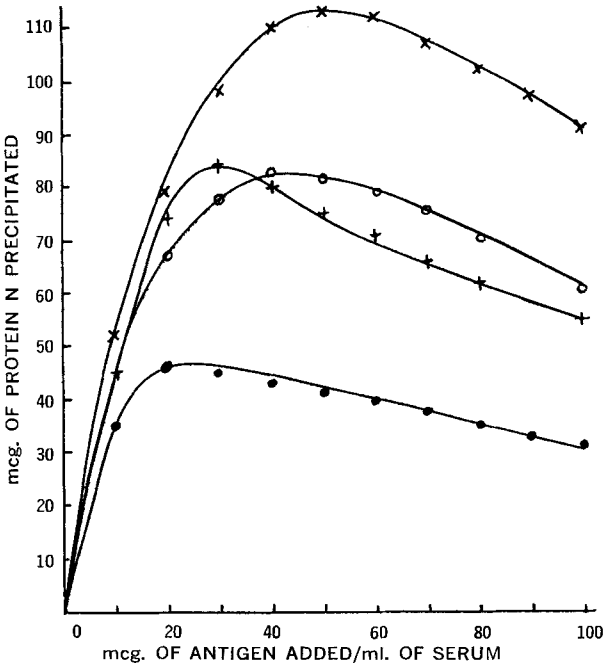

Figure 1—*Precipitin curves. Key:* × = *TGVGly against anti-TGVGly sera,* + = *TGAGly against anti-TGAGly sera,* ○ = *TGVGly against anti-TGAGly sera, and* ● = *TGAGly against anti-TGVGly sera.*

To investigate the antigenicity of poly-(tyr.glu.val.-gly)gly-1-C^{14} ethyl ester, two rabbits were treated at weekly intervals with 500 mcg. of the material. The first 2 weeks, they were injected interdermally using complete Freund's adjuvant as suspension medium; the 3rd week they were injected subcutaneously. The injection on the 4th week was done intravenously using buffered saline. Bleedings were conducted on the following week, and the serum from each animal was found to give a precipitin reaction with the polymer. The preimmunized sera under the same conditions gave a negative precipitin reaction. The quantitative determination of the precipitate was obtained by the addition of dilutions of poly-(tyr.glu.val.gly)gly-1-C^{14} ethyl ester to 1-ml. samples of the pooled rabbit sera. The precipitates were kept at 4° for 48 hr., washed twice with small volumes of buffered saline, and collected by centrifugation. The total amount of protein precipitated was estimated by analysis for nitrogen by the Kjeldahl method. The precipitin curve is shown in Fig. 1, and it would appear that the polymer containing valyl residues is a better antigen than that having alanyl residues.

To investigate the specificity of the antibodies produced by both of these antigenic polypeptides, the following series of crossreactions was performed. To 1-ml. aliquots of rabbit sera immunized against poly-(tyr.glu.ala.gly)gly-1-C^{14} ethyl ester were added dilutions of poly-(tyr.glu.val.gly)gly-1-C^{14} ethyl ester. After incubation at 37° for 1 hr., each aliquot had produced a precipitate. These precipitates were kept at 4° for 48 hr., washed twice with small volumes of buffered