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 Blood samples were withdrawn slowly to prevent embolism.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.

(1) J. T. Doluisio, N. F. Billups, L. W. Dittert, E. T. Sugita, and J. V. Swintosky, *J. Pharm. Sci.*, **58,** 1196(1969).
(2) G. Levy and A. Perälä, *ibid.*, **59,** 874(1970).
(3) H. S. Schanker, D. J. Tocco, B. B. Brodie, and C. A. M. Hogben, *J. Pharmacol. Exp. Ther.*, **123,** 81(1958).
(4) G. L. Mouzas and G. Smith, *Int. Surg.*, **51,** 495(1969).
(5) K. J. V. Hartiala, P. Leikkala, and P. Savola, *Acta Physiol. Scand.*, **42,** 36(1958).
(6) W. H. Barr and S. Riegelman, *J. Pharm. Sci.*, **59,** 154(1970).

KATHLEEN S. PELZMANN
RUTH N. HAVEMEYER
Institute of Pharmaceutical Sciences
Syntex Research
Stanford Industrial Park
Palo Alto, CA 94304

Received August 13, 1970.
Accepted for publication September 9, 1970.
The authors thank Dr. Satoshi Tojo of the Institute of Hormone Biology, Syntex Research, for his suggestions.

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.

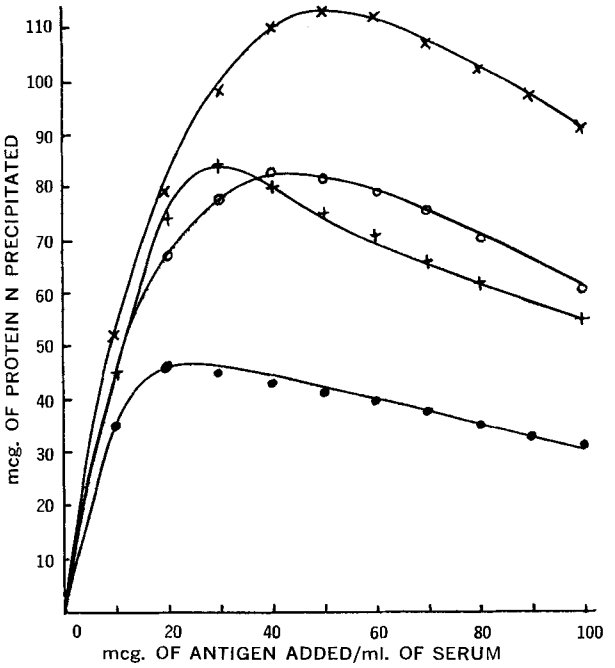

Figure 1—*Precipitin curves. Key:* × = *TGVGly against anti-TGVGly sera,* + = *TGAGly against anti-TGAGly sera,* ○ = *TGVGly against anti-TGAGly sera, and* ● = *TGAGly against anti-TGVGly sera.*

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

saline, and collected by centrifugation. The total amount of protein precipitated was estimated by the Kjeldahl method. Similarly, the crossreaction between poly-(tyr.-glu.ala.gly)gly-1-C^{14} ethyl ester and the antisera to poly-(tyr.glu.val.gly)gly-1-C^{14} ethyl ester was performed, and similar series of precipitin reactions were observed (Fig. 1).

Since both of these antigenic polypeptides give similar precipitin reactions with the antisera to poly-(tyr.-glu.ala.gly)gly-1-C^{14} ethyl ester, it has been concluded that these atibodies are unable to differentiate between the alanyl and valyl residues. It has, therefore, been concluded that the alanyl residue is not part of the active site of its respective antigen. However, antisera to poly-(tyr.glu.val.gly)gly-1-C^{14} ethyl ester does seem to show more specificity for the sterically larger valyl residue than for the alanyl moiety.

(1) B. J. Johnson and E. G. Trask, *J. Chem. Soc. (C)*, **1969**, 2644.
(2) B. J. Johnson, *J. Pharm. Sci.*, **59**, 1849(1970).
(3) B. J. Johnson and E. G. Trask, *ibid.*, **59**, 724(1970).
(4) B. J. Johnson and E. G. Trask, *J. Chem. Soc. (C)*, **1970**, 2247.

BRIAN J. JOHNSON
Department of Chemistry
Tufts University
Medford, MA 02155

Received August 10, 1970.
Accepted for publication September 24, 1970.
This work was supported by a grant from the National Science Foundation.

Antitumor Activity of An Acronycine–Polyvinylpyrrolidone Coprecipitate

Keyphrases ☐ Acronycine–polyvinylpyrrolidone coprecipitate—antitumor activity ☐ Antitumor activity—acronycine–polyvinylpyrrolidone coprecipitate

Sir:

Polyvinylpyrrolidone (PVP) is known to increase the solubility of aromatic compounds (1–3). This has been explained (1) as a result of hydrophobic bonding and an exothermic interaction between the solubilized compound and PVP. Application of this phenomenon to pharmaceuticals has been demonstrated (2, 3), and the advantages of PVP–drug coprecipitates as superior systems for pharmaceutical applications are known (4, 5).

In our work, pharmaceutical grade, 40,000 molecular weight, PVP was coprecipitated with acronycine from ethanol. The ratio of acronycine to PVP was 1:5 (w/w). Solubility of acronycine as the coprecipitate (75 mcg./ml., distilled water) was approximately 15 times that of noncoprecipitated acronycine (5 mcg./ml.). Acronycine,[1] a compound of known experimental antitumor activity (6), was compared with the acronycine–polyvinylpyrrolidone (Ac–PVP) coprecipitate against two of the most sensitive tumors to acronycine, X5563 plasma cell myeloma and C1498 myelogenous leukemia (Table I). These data indicate that PVP coprecipitated acronycine is more active than acronycine itself. Efforts are continuing in this area to optimize acronycine activity by adjusting the acronycine to PVP ratio and by employing other molecular weight grades of PVP.

(1) P. Molyneux and H. Frank, *J. Amer. Chem. Soc.*, **83**, 3169 (1961).
(2) T. Higuchi and R. Kuramoto, *J. Amer. Pharm. Ass., Sci. Ed.*, **43**, 393(1954).
(3) *Ibid.*, **43**, 398(1954).
(4) M. Mayersohn and M. Gibaldi, *J. Pharm. Sci.*, **55**, 1323(1966).
(5) A. P. Simonelli, S. C. Mehta, and W. I. Higuchi, *ibid.*, **58**, 538(1969).
(6) G. H. Svoboda, G. A. Poore, P. J. Simpson, and G. B. Boder, *ibid.*, **55**, 758(1966).

GORDON H. SVOBODA
MARTIN J. SWEENEY
W. DOUGLAS WALKLING
The Lilly Research Laboratories
Eli Lilly and Company
Indianapolis, IN 46206

Received July 16, 1970.
Accepted for publication September 21, 1970.

[1] The United States Adopted Names Committee (USAN) has approved acronine as the generic name for acronycine.

Table I—Antitumor Activity of Acronycine and an Acronycine–PVP Coprecipitate

Sample	Tumor[a]	mg./kg. × Days Dosed[b]	Route	ATD, mm.[c]	ALS, Days[d]	I, %[e]	PL, %[f]
Acronycine	X5563	30 × 9	i.p.	8.3	—	52	—
Ac–PVP	X5563	180 × 9	i.p.	0.9	—	95	—
Control	X5563	Emulphor × 9	i.p.	17.2	—	0	—
Acronycine	X5563	45 × 9	Oral	6.8	—	46	—
Ac–PVP	X5563	270 × 9	Oral	0.0	—	100	—
Control	X5563	Emulphor + 9	Oral	12.5	—	0	—
Acronycine	C1498	30 × 10	i.p.	—	20.4	—	40
Ac–PVP	C1498	180 × 10	i.p.	—	23.6	—	62
Control	C1498	Emulphor × 10	i.p.	—	14.6	—	0
Acronycine	C1498	45 × 10	Oral	—	19.4	—	24
Ac–PVP	C1498	270 × 10	Oral	—	24.2	—	55
Control	C1498	Emulphor × 10	Oral	—	15.6	—	0

[a] X5563 was implanted by trocar, subcutaneously, in C_3H mice, and therapy was begun 72 hr. later. C1498 was transferred by an intraperitoneal injection of tumor homogenate, and therapy was begun 24 hr. later. [b] Ac–PVP was coprecipitated in a 1:5 w/w basis. Emulphor (General Aniline and Film, New York, N. Y.) was the suspension vehicle for both acronycine and Ac–PVP. [c] ATD = average tumor diameter of 10 animals expressed in millimeters. [d] ALS = average life span of 10 animals expressed in days. [e] % I = ATD of the treated/control expressed as a percent inhibition. [f] % PL = ALS of the treated/control expressed as a percent prolongation of life.