kv. Although the photomultiplier tube (1P21) is more sensitive to the blue light from zinc sulfide than from the yellow-green light from zinc cadmium sulfide, the latter gave more response at 60 to 80 kv, presumably because of its greater absorption.

The experiments with the long Lucite rods were performed without any amplifier added to the photomultiplier tube. An amplifier of current gain of about 10^5 was constructed and used to observe the alpha particle pulses. This amplifier was not a d-c amplifier, so that it could not be used for measuring a steady luminosity level unless a mechanical chopper were used to interrupt the light entering the photomultiplier. However, it was easy to observe the half-wave pulses of X-rays from self-rectified X-ray tubes, even at extremely low levels. The intrinsic noise level, mostly due to shot effect from the thermionic emission of the photocathode of the photomultiplier tube, was easily observable with the full gain of the added amplifier. The extra gain is necessary to pick up alpha particle scintillations through the hypodermic needles and scintillations caused by beta rays.

Alpha particle scintillations on the zinc cadmium sulfide phosphor were so high as compared with the noise level that it was fairly easy to observe scintillations through the polished No. 15 needle. Further work is in progress to improve this technique and investigate the possibilities of measuring the local concentration of beta activity of radioisotopes *in vivo*. It has been found possible to turn Lucite rods of sufficiently small diameter to slide inside a No. 15 needle. These are coated with evaporated aluminum and a few crystals of fluorescent material are stuck on one end. The method of insertion is to slide them into the needle so that the screen end is slightly retracted from the beveled end of the needle. The needle is inserted to proper depth and then the Lucite rod is pushed on so that the screen end slightly protrudes into the tissue. One-mm-diam optically clear quartz rods gave even better results than the Lucite, but they have the disadvantage of being fragile.

Concerning the Specificity of Chicken Pancreas Conjugase[1]

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In a previous paper of this series (*1*), the conclusion was reached that chicken pancreas conjugase should be classified as γ-glutamic acid carboxypeptidase. The minimum requirements for the length of the peptide chain of the substrate could not have been established at that time. While it was suggested that pteroyl-γ-diglutamate was the end product of the reaction, the possibility of premature termination of the reaction, due to inhibition by the products formed, was not excluded.

We were able to study some recently synthesized derivatives of pteroylglutamic acid.[3] The methods used were the same as those previously described (*1*). The results are summarized below:

Substrate	*Equivalents of glutamic acid recovered*
Pteroyl-α-diglutamate	0 (or traces)
Pteroyl-γ-diglutamate	0 (or traces)
Pteroyl-α-α-triglutamate	0.0–0.2
Pteroyl-γ,γ-triglutamate	0.4–1.0
p-Aminobenzoyl-γ-γ-triglutamate	0.5–1.0

Since pteroyl-γ-diglutamate was not attacked by the enzyme, it represents the end product of digestion of the substrate by chicken pancreas conjugase. These data confirm our previous hypothesis and are in agreement with the findings of J. J. Pfiffner[4] who isolated the diglutamate from a digest of pteroylheptaglutamate with this enzyme. The minimum requirement for the number of glutamic acid residues for the substrate was, therefore, established at *three*.

In three out of five experiments with α,α-triglutamate, free glutamic acid was recovered. But the speed of hydrolysis of the alpha linkage was less than one-half that for the gamma linkage. Contrary to our previous conclusion, it appears that the chicken pancreas conjugase hydrolyzes the gamma linkage *preferentially*, but not *specifically*. The final decision must be withheld, however, until a purer enzyme is available.

The rate of liberation of the microbiologically active substance at various stages of purification of the enzyme was also studied. The ratio of the units of activity found with heptaglutamate[5] as substrate divided by the units found with triglutamate varied from 1: 2 to 1: 8, with an average value of 1: 4.2. The calculated value for the diglutamate as the end product of the reaction is 1: 5; for the monoglutamate, 1: 3. In spite of considerable variation in experimental results, there was no tendency for a change in this ratio with increasing purity of the enzyme. This indicates that the liberation of microbiologically active substance from heptaglutamate is achieved by a single chicken pancreas conjugase, in contrast to the hog kidney conjugase, which has two components (*2*).

References

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[2] On leave of absence from the Agricultural Institute, Pulawy, Poland.

[3] Obtained through the courtesy of E. L. R. Stokstad, Lederle Laboratories Division, American Cyanamid Company.

[4] Discussion at the Federation Meeting, Atlantic City, 1948.

[5] Heptaglutamate was obtained through the courtesy of Dr. J. J. Pfiffner, Parke, Davis and Company.