As a control, glutamic acid was isolated from protein obtained from a kidney neoplasm (dog). We are indebted to Dr. E. T. Bell, Head of the Department of Pathology, University of Minnesota Medical School, who examined sections of this tumor and found it to be an undifferentiated sarcoma.

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Configuration of Glutamic Acid Isolated from Proteins of Pig and Chick Embryo Tissues.

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(Introduced by F. H. Scott.)

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Since Kögl and Erxleben[1] announced the isolation of partly racemized glutamic acid from malignant tissue protein, numerous confirmatory and non-confirmatory articles have appeared. The only laboratories, other than Kögl's, that have investigated carcinoma protein by the Kögl-Erxleben procedure have reported the isolation of partly racemized glutamic acid.[2] Dittmar[3] obtained only 1(+)glutamic acid from Rous sarcoma and from Jensen sarcoma free of necrosis, but later[4] found racemic glutamic acid in mouse sarcomas and carcinomas containing some necrotic material. Johnson[5] reported the isolation of glutamic acid containing small percentages of racemate from acid hydrolysates of Jensen sarcoma, but claimed that similar percentages were obtained also from normal mouse liver protein hydrolysates.

Kögl, Erxleben, and Akkerman[6] found only 1(+) glutamic acid in the hydrolysate of protein obtained from two- to three-months-old calf embryos. The embryonic tissues of other animals have not been investigated from this point of view. Since the metabolism of malignant tissue is similar in many respects to that of embryonic

1 Kögl, F., and Erxleben, H., *Z. physiol. Chem.*, 1939, **258**, 57.

2 Arnow, L. E., and Opsahl, J. C., *Science*, 1939, **90**, 257; White, J., and White, F. R., *J. Biol. Chem.*, 1939, **130**, 435; Dittmar, C., *Z. Krebsforsch.*, 1939, **49**, 441.

3 Dittmar, C., *Z. Krebsforsch.*, 1939, **49**, 397.

4 Dittmar, C., *Z. Krebsforsch.*, 1939, **49**, 441.

5 Johnson, J. M., *J. Biol. Chem.*, 1940, **132**, 781.

6 Kögl, F., Erxleben, H., and Akkerman, A. M., *Z. physiol. Chem.*, 1939, **261**, 141.

tissue, obviously it is important to investigate the configuration of the glutamic acid obtained from the latter.

We have isolated glutamic acid from the proteins of pig and chick embryo tissues. The isolations proved unexpectedly difficult, and glutamic acid hydrochloride crystallized only after standing in saturated hydrochloric acid solution for several weeks. Nine percent hydrochloric acid was used as a solvent in the determinations of optical rotation. The specific optical rotations reported in Table I have been calculated for free glutamic acid (literature: + 31.7° for l(+)glutamic acid). The melting point (more properly, the decomposition point) of glutamic acid hydrochloride varies somewhat, depending on the method used for its determination. By the method employed by us, known pure l(+) glutamic acid hydrochloride melts at 203-205°.

TABLE I.

	Protein, g	Glutamic acid HCl (Final yield) mg	Melting point	$[\alpha]_D$
Pig	9.2	37.4	203-204°	+31.8°
Chick	15.7	234.1	203-204°	+31.7°

Experimental. Pig Embryos. Pig embryos varying in length from 750 to 1000 mm, and in weight, from 20 to 50 g each, were used. The bones were just beginning to calcify. Two hundred g of fresh total embryo tissue were macerated to a brei in a hand coffee mill. The brei was placed in a heavy cloth bag, and immersed several times in about 500 cc of boiling water to which a few drops of concentrated hydrochloric acid had been added. After centrifugation, the precipitated protein was washed with aqueous alcohol solution (1:4) until the washings were free of chloride. The washed protein was dried at 110°, pulverized in a mortar, and washed 3 times with boiling ether. Yield, 9.2 g of protein. Hydrolysis and isolation of glutamic acid hydrochloride were carried out as described by Kögl, Erxleben, and Akkerman.[6]

Chick Embryos. The entire bodies of 704 seven-day-old chick embryos were removed from eggs incubated by a standard method, blotted carefully with filter paper to remove excess moisture, and weighed (397 g). After maceration, the tissue was covered with 0.1 *N* hydrochloric acid, and heated on a water bath until a majority of the material had gone into solution (6-8 hours). Sufficient trichloracetic acid was now added to make a concentration of 10%, and the precipitated protein was allowed to settle. After filtration with suction, the protein was washed repeatedly with alcohol and ether

(to remove trichloracetic acid and the majority of adsorbed lipids). The protein thus isolated weighed 15.7 g following drying at 110°. Hydrolysis and isolation of glutamic acid hydrochloride again were carried out by the procedures used by Kögl, Erxleben, and Akkerman.[6]

Summary. Proteins obtained from the embryonic tissues of chicks and pigs have been hydrolyzed, and the glutamic acid hydrochloride obtained from the hydrolysates examined in the polarimeter. The glutamic acid hydrochloride isolated was entirely the 1(+) variety.

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Effect of Trypsin on Development of *Rana pipiens.*

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Proteases secreted by the embryos of numerous species of fish and amphibia are believed to be important factors in bringing about hatching by digestion of the egg membranes. It has been asserted that in teleosts this protease is much like trypsin and so powerful that it attacks the embryo itself.[1] This statement conflicts with the dictum that living organisms are resistant to digestive enzymes. A striking confirmation of the latter view is Northrup's demonstration of the immunity of a wide variety of organisms to concentrated trypsin.[2]

These conflicting statements suggested the desirability of investigating the action of trypsin on the embryologically useful amphibian *Rana pipiens.* Since this anuran appears to possess a hatching enzyme the effect of trypsin on both membranes and embryonic viability was studied. The eggs were stripped of excess jelly on paper toweling and immersed in a 10% solution of dialyzed trypsin made up in 10% Ringer solution. Each experiment involved 25-50 eggs. Table I gives some typical results.

These results are in agreement with Northrup's work in their clear distinction between the susceptibility of the membrane proteins and the resistance of the living organism to trypsin. The possibility, suggested by work on teleosts, of a general embryonic sensitivity to

1 Needham, J., *Chemical Embryology,* 1931, **3**, 1597.
2 Northrup, J., *J. Gen. Physiology,* 1926, **9**, 497.