mulation of citrate in male, female and castrated male rats after fluoracetate poisoning; it is also significant when male rats are exposed to X-radiation before injection of fluoracetate.

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Amino Acids and Peptides in the Oviduct of the Laying Hen.*† (20463)

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Inappreciable quantities of free peptides, other than certain specialized structures, appear in various tissues(1), even in tissues which are carrying out rapid synthesis of protein. This finding along with the sluggish capture(2) and utilization(3) of peptides by tissues has led us to question how extensively free peptides in general enter into protein synthesis. Anfinsen and Steinberg(4,5) however, have observed a large dilution of isotopic amino acids entering certain positions of ovalbumin, relative to other positions, during incubation of the minced oviduct of the laying hen. They have suggested that peptides present in the oviduct from the beginning of the experiment served as protein precursors, to produce the observed dilution of the labeled free amino acids. To assist in the interpretation of these results we have analyzed fresh and incubated oviducts for free and bound amino acids.

Experimental. The oviducts were excised promptly after sacrifice of the hen. In each case, except No. 4, the oviduct contained at removal an egg in the later stages of formation. The organ was divided into 2 similar samples; one part was immediately homogenized with saturated aqueous picric acid (10 ml per g) in a Waring blendor. The other portion was minced as described by Anfinsen and Steinberg(4), suspended in the medium they describe and incubated at 37°C for 2 to 4 hours. The suspension was then also treated with picric acid, 10 ml for each g of original tissue. The filtered extracts were immediately enclosed in a cellophane bag and dialyzed against measured volumes of water for 14 to 16 hours at 5°C on a rotating wheel; the dialysates were then analyzed immediately for glycine(6) and α-amino acid ("carboxyl") nitrogen at pH 2.5(7) both before and after acid hydrolysis(8).

Results. As shown in Table I, the fresh oviducts contained high levels of free amino acids. Calculation indicates that the cells were about 10 times as rich in α-amino acids in general, and in glycine, as was the plasma

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TABLE I. Amino Acids and Peptides in Picric Acid Extracts of Oviduct of the Hen. Concentrations are in mM per kilo fresh tissue.

Exp. No.	Condition	Glycine Free	Glycine Bound	α-amino acids Free	α-amino acids Bound	α-amino acids Non-glutathione bound*
1	Fresh	1.87	3.19	27.9	5.2	—1.2
2	Fresh	2.10	3.24	26.7	8.1	+1.6
	Minced and incubated 2 hr	4.44	2.85	45.8	5.4	— .3
3	Fresh	1.89	3.81	27.1	6.9	— .7
	Minced and incubated 4 hr	5.58	4.16	55.5	5.4	+2.9
4	Fresh	2.27	4.81	25.4	8.4	—1.2
	Minced and incubated 4 hr	6.11	2.86	45.1	4.6	—1.1

* Calculated as (bound α-amino acids) minus 2 × (bound glycine).

of the hen. Since these levels were maintained despite the rapid synthesis of egg-white protein which presumably had continued up to the moment of the sacrifice of the bird (except in No. 4) this organ clearly is quite active in concentrating free amino acids. The calculations show that close to one-half the bound amino acid residues are due to glycine. This proportion is characteristic of glutathione, which releases one bound glycine and a second bound α-amino acid structure due to cysteine upon hydrolysis. Hence the bound amino acids in the extracts appear to be due to glutathione; whatever substantial part may not be glutathione must contain glycine as at least half of its amino acid resdues, and any "pool" of peptides of random structure must be very small.

The difference between the bound α-amino acid nitrogen and twice the bound glycine is recorded as the "non-glutathione bound α-amino acids" in the last column of Table I, and has a value close to zero, with an average value of minus 0.5 millimole per kilo of tissue. This value in one case was slightly increased after incubation, but free amino acids were by far the chief product of the net protein breakdown during incubation. Because of the high potassium content of the medium, these free amino acids would be expected to be largely extracellular.

Discussion. The possibility must be kept in mind that larger intermediates in the synthesis of protein may be precipitated with the proteins by picric acid. Mixtures of smaller peptides, however, are recovered well following picric acid deproteinization(3); therefore, the amounts of lower peptides present in a free form must be small. These results appear to set an upper limit to the amount of dilution which can be attributed to free small peptides. Unless the total amount of albumin synthesized in the experiments of Anfinsen and Steinberg is very small, it appears necessary to look for other sources of the isotope dilution they report than the free peptides present in the oviduct at the beginning of an experiment.

Summary. The oviduct of the laying hen, either fresh or after incubation in a minced state, is rich in free amino acids, but the bound, dialyzable amino acids extracted by picric acid are largely limited to glutathione or similarly glycine-rich structures.

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