concentration of the mesenchyma.

Our findings emphasize the importance of androgens in differentiation of the mammary gland. Malformations in male fetuses, induced by antiandrogens, differ fundamentally from those that are induced by estrogens(3). In contrast to the estrogens, the antiandrogens induce a process which is already inherent in the biological "blueprint," the realization of which apparently is prevented only by the androgens of the male fetus. Our data suggest that the female pattern of development will be expressed up to puberty, irrespective of sex, in the absence of androgens.

Summary. In male fetuses of the mouse, mammary gland tissue is stimulated and frequently also the development of teats is observed under the influence of the androgen antagonist cyproterone acetate (1,2α-methylene-6-chloro-$\Delta^{4,6}$-pregnadiene-17α-ol-3,20-dione-17α-acetate). The continuity of the glandular process, normally lost in male fetuses because of a destruction of the epidermal sector, is maintained by cyproterone acetate. Inhibition of the endogenous androgens thus results in a female organogenesis of the mammary glands.

We wish to express our thanks to Miss Böhnisch, Miss Klemme-Wolff, Miss Fleischer and Miss Blank for assistance in the experiments.

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Received April 20, 1966. P.S.E.B.M., 1966, v123.

Unequal Amino Acid Incorporation into Rabbit Serum Albumin Synthesized *in vivo*.* (31565)

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In recent studies on the mechanism of protein synthesis by *in vitro* preparations, it has been observed that short-term exposure of the synthesizing system to isotopic amino acid results in a pattern of amino acid incorporation compatible with the idea that the biosynthesis of the polypeptide chains proceeds from the amino terminal end. This confirmation of an hypothesis of Dagliesh(1) has come from work with hemoglobin(2), lysozyme(3), ribonuclease(4) and recently rat serum albumin(5).

The purpose of the present work is to describe unequal labeling of the moieties of an amino acid in a protein obtained with an *in vivo* preparation. Furthermore, persistence of the unequal labeling over relatively long periods has been observed. A preliminary report of this work has appeared(6).

Materials and methods. Biosynthesis of labeled albumin. 1.0 mc DL-1-C^{14} lysine (Orlando Research Chemicals, Inc.) dissolved in 0.1 ml saline was injected into a mesenteric vein of an anesthetized (nembutal), laparotomized male albino rabbit. The wound was covered with saline packs and anesthesia was continued. After 4 hr 10.0 ml blood samples were taken by heart puncture with heparinized syringes and the plasma was isolated by centrifugation. The plasma albumin was separated by starch block electrophoresis at pH 8.6, as described by Kunkel(7). The separated albumin was eluted with 0.5 N NaCl from starch segments cut from the central section of the albumin band, and the solution was dialyzed and lyophilized. The isolated albumin was homogeneous by the criteria of ultracentrifugation and paper electrophoresis.

* This work was supported by NIH grant AM-02457 and NSF grant GB-3344 and was taken in part from an M.S. thesis submitted by K. E. Neet.

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TABLE I. Specific Activities of Lysine Residues from Rabbit Serum Albumin Peptides.

Peptide No.*	Specific activity†	
	Mesenteric vein injection‡	Intraperitoneal injection§
I	387	84
III	630	76
IV	340	73
VI	807	71
VII	331	83
IX	87	80
X	168	77
Total hydrolysate	357	78

* Peptides were numbered sequentially in order of elution from the ion exchange columns.

† Millivolts/min/μmole of lysine.

‡ 1 meurie, 3.1 kg rabbit, sample obtained 245 min post injection.

§ 1 meurie, 3.4 kg rabbit, sample obtained 241 min post injection.

Oxidation and hydrolysis of plasma albumin. The dialyzed albumin sample was lyophilized, oxidized with performic acid and hydrolyzed with trypsin, as described by Fried and Putnam(8). The mixture of peptides resulting from the proteolysis was resolved into individual peaks on ion exchange columns of Dowex 50X2 by a modification (9) of the procedure of Hirs *et al*(10). The peptide peaks were numbered in order of their elution from the column. Only peptides recovered from large, well-separated peaks were used for the subsequent analyses.

Determination of specific radioactivity. The peptide materials from the separated peaks were hydrolyzed in 6 N HCl in sealed evacuated tubes. Aliquots of each hydrolysate were analyzed for lysine on a Spinco amino acid analyzer. Lysine was isolated from the acid hydrolysate by the short column technique of Moore *et al*(11). The radioactivity of the lysine in each hydrolysate was measured by a procedure involving the conversion of the carboxyl carbon into CO_2 using ninhydrin(12). The radioactivity of the CO_2 liberated in this reaction was determined in a Cary Model 31 vibrating reed electrometer.

Results. In repeated experiments it was found that the specific activities of lysine residues isolated from peptides representing different portions of the albumin molecule varied significantly. These differences in specific activity were apparent for some hours following the injection of the labeled amino acid. Data from one such experiment are shown in Table I. The specific activities of the lysine from the individual peptides are compared with the specific activity of the lysine from the total hydrolysate. It can be seen that the variation is large (10-fold) and suggestive of different degrees of incorporation of isotopic amino acid into individual loci on the polypeptide chain. The actual number determined for the specific activity depends on the homogeneity of the chromatographic fraction and on the relative position of the lysine residue in the polypeptide chain.

Control experiments were carried out in which the C^{14} lysine was injected intraperitoneally. When the albumin resulting from such experiments was isolated, oxidized and hydrolyzed, and the specific activities of its individual lysine moieties determined, it was found that they did not differ from the specific activity of the total lysine in the albumin sample by more than 10%. These data are shown in Table I.

Discussion. Previous workers have shown that in short-term experiments using cell free systems it is possible to demonstrate sequential unequal incorporation of labeled amino acid into the primary structure of several proteins. There is some question whether in these cases such incorporation represents the synthesis of new albumin chains or the completion of pre-existing incomplete polypeptides.

The experiments described in the present work resulted in differential incorporation in an *in vivo* system which persists over a relatively long period. This difference from preceding work may be ascribed to the method of introduction of the isotope into the protein synthesizing system, which had many of the characteristics of pulse labeling. The isotope, administered in small volume, was swept directly through the liver in a very short period of time, which presented the liver with a sudden rise and equally sudden fall of specific activity of the lysine pool. Following equilibration and dilution, the specific activity of the lysine in the circulating serum within the animal would have fallen markedly.

Albumin molecules synthesized during the period of high specific activity would repre-

sent the addition of amino acids to incomplete polypeptide chains of different lengths (2). Any lysine incorporated at this time would be of high specific activity and would be incorporated unequally into different sites on the polypeptide. Subsequent synthesis, involving the diluted isotope on many later passages through the liver, would result in albumin of much lower specific activity with the isotopic lysines uniformly distributed. Such subsequent synthesis would not necessarily mask the unequal labeling resulting from synthesis during the brief period of high, and rapidly changing, specific activity of the lysine pool.

No attempt has been made to determine whether there is a regularity in the specific activity of individual lysine residues in the protein, that depends on the position of that amino acid moiety with respect to the N-terminal or C-terminal amino acid. In the light of the recent finding by Sargent and Campbell(5) that albumin is synthesized sequentially, starting at the N-terminal end in a cell free system, it would seem reasonable that further investigation could demonstrate this process with the *in vivo* system employed here, providing the isotope is introduced in the form of a pulse of high specific activity.

Summary. The mechanism of albumin biosynthesis in rabbit liver was investigated *in vivo* by observing the pattern of lysine incorporation in albumin isolated from a rabbit injected with DL-1-C^{14} lysine *via* a mesenteric vein. Peptides were obtained by chromatographing tryptic hydrolysates of the albumin. Radioactivity measurements were made on lysine chromatographically isolated from individual acid-hydrolyzed peptides and quantitative lysine determinations were made with the automatic amino acid analyzer. The results indicated nonuniform labeling of the lysine residues in serum albumin which persisted for as long as 4 hours after isotope injection. It is postulated that the unequal labeling and its long duration were demonstrable because of the "pulse" nature of the introduction of the isotope to the synthesizing system.

The expert technical assistance of Mrs. O. Newton and Mrs. S. P. Eoff is gratefully acknowledged.

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Received May 16, 1966. P.S.E.B.M., 1966, v123.