are asymptotic to extensions of 75–85 %[4]. The ratio of these asymptotic extensions is approximately 80/30, *i.e.* 2.67, and could be explained by assuming that the first 30 % extension is primarily due to the X component, while extension from 30 to 80 % is primarily in the Y component. Finally, chemical or physical modifications of the mechanical properties of single fibres in the postyield region correlate with the ability of the fibres to supercontract in the final supercontraction stage[5,6].

We believe that the evidence outlined above favours the concept of series zones differing in accessibility and/or chemical composition in wool fibres. The two zones are most likely components of microfibrils, the matrix being converted immediately upon entry of LiBr solution into an elastomer. A full account of the experimental details will be given in a future paper.

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The net synthesis of cytochrome *c* in calf-heart mitochondria

Previous studies have shown that rat-liver mitochondria can effect the incorporation of labeled amino acids into cytochrome *c*[1]. We now report that calf-heart mitochondria can carry out this process over a much longer period of time, permitting the demonstration of a net increase in cytochrome *c* (Table I). This was observed (a) by adding labeled cytochrome *c* to each incubation flask at the end of the reaction (isotope dilution), and (b) by weighing the cytochrome *c* isolated by a modification[2] of available procedures[3]. The amount of cytochrome *c* formed increased linearly with time; the results of the gravimetric and isotope-dilution procedures are in excellent agreement. Both zero-time and 12-h samples of cytochrome *c* were assayed at 550 mμ by reduction by dithionite and oxidation by cytochrome oxidase[4], and gave results identical to those given by an authentic sample. The synthetic process requires energy and amino acids; omission of the complete amino acid mixture or of L-leucine not only stops synthesis but leads to degradation of endogenous cytochrome *c*. The possibility that the observed increase in cytochrome *c* is the result of combination of heme with preformed protein

Abbreviations: FDNB, 1-fluoro-2,4-dinitrobenzene; DNP-, dinitrophenyl-.

TABLE I

THE NET SYNTHESIS OF CYTOCHROME *c*

In the net-synthesis expts., the reaction mixture consisted of KH_2PO_4 (750 μmoles), α-ketoglutarate (250 μmoles), $MgCl_2$ (250 μmoles), adenosine 5′-phosphate (30 μmoles), adenosine 5′-diphosphate (30 μmoles), ethylenediaminetetraacetate (100 μmoles), a complete mixture of protein L-amino acids (a.a.) (25 μmoles of each amino acid), and thrice-washed mitochondria obtained from about 100 g of calf heart in a final vol of 25 ml, pH 7.4. In the isotope-dilution expts., 123 μg cytochrome *c* (1046 counts/min/mg) were added to each reaction flask at the end of the incubation. In the amino acid incorporation expts., [1-^{14}C]DL-valine (10.7 μmoles containing $3.11 \cdot 10^6$ counts/min) replaced the unlabeled L-valine in the amino acid mixture. The final concentration of 2,4-dinitrophenol (DNP) was $3 \cdot 10^{-4}$ *M*. All flasks were oxygenated hourly except where otherwise indicated, were incubated at 37.5°, and contained 6000 units penicillin G and 1.2 mg streptomycin sulfate. The medium at the end of the 12-h incubation period showed no bacterial contamination.

Expt.	*Conditions*	*Incubation time*	*Increase in cytochrome c*		*Specific activity***	*Cytochrome c synthesized*§
			*I.D.**	*Gravimetric***		
		h	μg	μg	counts/min/mg cytochrome *c*	μg
1	Complete system	0	—	—	0.6	
	Complete system	4	118	119	2947	46
	Complete system	12	341	341	8782	156
	— a.a. mixture	12	— 229	— 218	116	
	— leucine	12	— 180	— 171	153	
	+ DNP, N_2	12			87	
2	Complete system	0	—	—		
	Complete system	12	196	206		
	+ DNP, N_2	12	33	27		

* Isotope dilution. Calculations were done according to RITTENBERG *et al.*[6] The zero-time value was 1192 μg in Expt. 1 and 812 μg in Expt. 2. The figures in this column represent increments above these values.

** The zero-time value was 1015 μg in Expt. 1 and 674 μg in Expt. 2. The figures in this column represent increments above these values.

*** The incorporation experiments were done under conditions similar to those of the net-synthesis experiments except that the tissue preparations were obtained on different days.

§ Calculated from previous column using the formula: $y = (S_c/S_v)(C/V)$ (1000) where y = μg of newly formed cytochrome *c*, S_c = specific activity of cytochrome *c* (counts/min/mg), S_v = specific activity of [1-^{14}C]valine used (counts/min/mg), C = total wt. (μg) of cytochrome *c* at end of incubation, and V = μg valine present in 1 mg cytochrome *c*. These calculated values are minimal since possible dilution of the added [1-^{14}C]valine has not been taken into account.

is rendered unlikely by (a) the parallel changes in valine incorporation and extent of net synthesis and (b) the agreement between the amount synthesized as calculated from valine incorporation and that derived from the gravimetric or isotope-dilution data. It should be added that several assumptions were made in the calculation from incorporation data; one of them is that the 3 valine residues of cytochrome *c* have the same specific activity. Recent experiments have shown, however, that this is not the case[2].

The unequivocal interpretation of incorporation studies in protein synthesis requires the demonstration that the labeled amino acid appears in the expected positions in a known portion of the amino acid sequence of a discrete protein. Cytochrome *c*, labeled with [1-^{14}C]valine and [1-^{14}C]lysine, was therefore subjected to peptic degradation[5] to yield the heme peptide whose amino acid sequence is: Val·Glu(NH_2)·Lys·CySH·Ala·Glu(NH_2)·CySH·His·Thr·Val·Glu[5]. The heme pep-

tide was isolated by ion-exchange chromatography on DEAE-cellulose and by partition chromatography; treatment with trypsin gave Val·Glu(NH_2)·Lys and the heme-containing octapeptide. On treatment of the tripeptide with FDNP followed by acid hydrolysis, only DNP-valine was found in the ether extract. The aqueous phase was treated with FDNB, and DNP-glutamic acid and di-DNP-lysine were isolated by paper chromatography. Only the valine and lysine derivatives were radioactive. Of the amino acids in the octapeptide, only the valine was labeled.

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Separate permeases for the accumulation of methyl-β-D-galactoside and methyl-β-D-thiogalactoside in *Escherichia coli*

Studies on the accumulation of β-D-thiogalactosides in cells of *Escherichia coli* led MONOD and his collaborators[1,2] to postulate the existence of a specific transport system termed "galactoside-permease".

The following facts were considered by the same authors to imply that galactosides and thiogalactosides entered the cell by the same transport system[1,2,3]: (a) the capacity to accumulate labeled thiogalactoside is specifically inducible and formation of β-galactosidase is simultaneously induced; (b) β-galactosides are found to inhibit competitively the accumulation of thiogalactosides; (c) the rate of hydrolysis of *o*-nitrophenyl-β-D-galactoside by intact cells and their capacity to accumulate thiogalactoside were correlated under a variety of conditions.

On the other hand, data obtained in this laboratory raised the possibility that a single permease might not mediate the entrance of all galactosides[4]. Therefore, an attempt was made to test whether the same system is responsible for the transport of galactosides and thiogalactosides. For this purpose the accumulation of methyl-β-D-galactoside and methyl-β-D-thiogalactoside, both 1-^{14}C labeled, was studied. Since methyl-β-D-galactoside, unlike the thiogalactoside, can be hydrolyzed by β-galactosidase, a mutant strain (K12-W2244, kindly supplied by Dr. J. LEDERBERG) which lacks the enzyme was used.