In vitro Incorporation of C^{14} Glycine and S^{35} Sulfate into Protein by Human Colon Mucosa.* (20245)

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(Introduced by E. S. West.)

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The incorporation of radioactive amino acids into protein and smaller peptides by *in vitro* technics has been reported from many laboratories, including our own(1-7). The bulk of the reports deals with liver tissues either sliced or fractionated into a variety of cell free systems. Winnick *et al.*(8) reported the incorporation of labeled glycine into protein of intestinal mucosa. The present report presents data concerned with the incorporation of radioactive glycine into the mucosa of resected human colon, into the insoluble protein and into a soluble fraction which has characteristics of a mucoprotein.

Materials and methods. Colon specimens were obtained from resections performed by the Surgical Service of Multnomah County Hospital. Pieces of normal bowel were used. The technic for handling the tissue after washing in 8-10 aliquots of buffer was the same as reported with liver biopsies(6) except that it was found necessary to dissect "strips" of mucosa from the colon tissue, since slicing was difficult. The "strips" were cut into small segments approximately 0.5 to 1.0 cm square, pooled, and then used for the incubation. Glycine-1-C^{14} (0.33 μc)† and 2 cc of a modified Krebs-Henseleit buffer‡ were added to 150-200 mg of wet weight of colon mucosa in each flask, which was then filled with 95% O_2-5% CO_2. The subsequent incubation, precipitation with boiling 1 M acetate, and isolation of protein for counting were the same as previously reported(6). In some experiments tissue was incubated with S^{35} labeled inorganic sulfate instead of glycine. In earlier experiments 1000 units of penicillin were added to each flask, and later aureomycin, 200 γ in 0.1 cc water, was substituted. The aureomycin solution was freshly made for each experiment. A mucoprotein fraction was obtained by a modification of the technic described by Glass and Boyd(9). Colon mucosa was homogenized, the protein precipitated with boiling 1 M acetate (pH 4.5), and 1.5 volumes of acetone were added to the supernatant. This solution was allowed to stand overnight at room temperature. The amorphous precipitate, after washing and drying with acetone, resembled a gastric mucoprotein described by Glass, contained 11.5% nitrogen and 0.95% sulfur by analysis, and gave a positive reaction to the uronic acid test described by Siplet, Komarov, and Shay(10). The 1 M acetate supernatants from incubated samples were dialyzed overnight against running tap water to remove unincorporated labeled glycine or sulfate. The mucoprotein was then precipitated with 1.5 volumes of acetone and prepared for counting. Tissue and labeled substrate were precipitated at zero time in each experiment as controls. Duplicate samples were run for each set of experimental conditions and the values were expressed as the means of duplicate pairs. Mucoprotein, however, was precipitated from the supernatants of duplicate pairs which had been pooled in order to obtain adequate amounts for handling. For the reprecipitation experiments, the dry protein was dissolved in 0.5 N sodium hydroxide to which 10-15 mg urea had been added. Protein was reprecipitated with 10% trichloracetic acid.

Results. Table I presents the effects of penicillin and aureomycin on incorporation. There was a consistent depression in the presence of penicillin and a more variable result with aureomycin. In order to evaluate the role

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† Glycine-1-C^{14}, 0.5 mc/millimole, purchased from Tracerlab, Boston, Mass.

‡ Composition previously described(6).

TABLE I. Effect of Penicillin and Aureomycin on Radioactive Glycine Uptake by Colon Mucosa.

Patient	Specific activity counts/min./mg — With penicillin, 1000 u	Without penicillin or aureomycin	With aureomycin, 200 γ
H.	822	1175	
O.	633	939	
S.	142	881	
R.	278	364	383
G.		568	326
B.		353	426
K.		457	666

of bacteria more definitively, slices of colon mucosa were compared to the same weights of tissue homogenized with the Potter glass homogenizer. This technic is known to destroy whole cells, and it is extremely unlikely that bacteria were destroyed. The homogenate was allowed to remain in the refrigerator overnight to insure destruction of the enzymes of the mucosa cells. The homogenate was incubated for 4 hours under experimental conditions and samples were then cultured in broth by the serial dilution technic. Table II demonstrates that incorporation into the mucosa was practically abolished by homogenizing. On the other hand bacteria were present in significant amounts, and a bacteriostatic effect of aureomycin was demonstrated.

In Table III are presented data which show no consistent effect as a result of omitting magnesium and calcium from the buffer. This is in contrast to the results previously obtained with liver slices(6). In the absence of citrate (patients K and B) the specific activities, although apparently higher, are probably of the same order of magnitude as with the complete buffer. Marked inhibition was observed in the presence of .005 M cyanide and in a nitrogen atmosphere. There was no apparent correlation between the colon site and the level of incorporation.

Data with respect to the incorporation of labeled glycine and labeled sulfate into both total protein and mucoprotein are presented in Table IV. Since the amount of S^{35} sulfate added varied, the specific activities cannot be compared with one another. In patients M and J the incorporation of labeled sulfate into the mucoprotein was obviously greater than in total protein. In contrast the incorporation of labeled glycine into the total protein was greater than into the mucoprotein in patients M, L and J. Solution of the colon mucosa protein in alkaline urea and reprecipitation with trichloracetic acid failed to remove any radioactivity when the labeling agent had been C^{14} glycine, whereas the sulfate label was entirely removed by the same procedure (Table V).

The figures presented are average values for duplicate pairs of observations for each set of experimental conditions except as noted. The variation of each observation from the mean of its pair of duplicates was less than 15% in 32 and less than 25% in 46 out of 58 pairs.

Discussion. There is considerable evidence indicating that the incorporation of amino acids into protein is an enzymatic process fundamentally requiring oxidative phosphorylation(11,12). The data presented do support the hypothesis that the incorporation of radioactive glycine into colon protein is probably enzymatic, especially since homogenizing the tissue abolished incorporation, and since both a nitrogen atmosphere and small concentrations of cyanide produced marked inhibition. The evidence rules out bacteria as being responsible for the incorporation. The percentage of labeled glycine incorporated is many times greater than the low levels of incorporation obtained by Brunish and Luck with their histone system(13). If the incorporation observed in our experiments were due to some artefact or adsorption rather than to a true enzymatic process, one would expect much higher levels of incorporation in the homogenized tissue (Table II) than in tissue with intact cells. Furthermore, solution with alkaline urea and reprecipitation with trichloracetic acid or treatment with mercaptoethanol has been shown to remove some radioactivity from labeled protein obtained from particulate systems and not from systems with intact cells. We were unable to alter the specific activity of our C^{14} glycine labeled protein by such treatment.

The marked variation in specific activities obtained from patient to patient cannot be readily explained. The same variation has been noted with liver tissue. The lack of sensitivity of the system to the presence or ab-

TABLE II. Radioactive Glycine Uptake and Bacterial Growth in Colon Mucosa Homogenate.

Patient	Specific activity: Slices with aureomycin	Specific activity: Homogenate With aureomycin	Specific activity: Homogenate Without aureomycin	Highest dilution showing growth: With aureomycin	Highest dilution showing growth: Without aureomycin
S.	279	3	3.	10	10^8
R.	444	3	12.	10^5	10^7

TABLE III.

Patient	Colon site	Specific activity: Complete buffer	No Ca	No Mg	No Ca or Mg	No citrate	N^2	CN^-
O.	Transverse	632	694	278	134			11
R.	Sigmoid	244	355	180	125			22
S.	"	279	278	225	210			17
M.	Splenic flexure	230‡*	581*	238*	358*			
L.	Rectosigmoid	124	106	127	610			
B.	"	177	723	172		248		
K.	Sigmoid	725				824		
G.	Rectosigmoid	326					23	
F.S.	"	142†					32†	43†
O.	"	634†					55†	
N.	Sigmoid	215*		147				
		65						
B.	Transverse	426						
A.S.	Rectosigmoid	364						
M.S.	Transverse	889						
H.	Rectosigmoid	822†						
Zero time mean ± S.E.		6.3 ± 1.3						

* No antibiotic present. All others contained 200 γ aureomycin.
† 1000 units penicillin added. No aureomycin.
‡ Single observation. All others are averages of duplicate pairs.

TABLE IV. Incorporation of Labeled Substrate into Protein and Mucoprotein of Colon Mucosa.

Patient	Specific activity: Total protein C^{14} glycine	Total protein S^{35} sulfate	Mucoprotein†‡ C^{14} glycine	Mucoprotein†‡ S^{35} sulfate
A.	364	155		
B.	410	177		
	353*	207*		
N.	65	47		
	215*	50*		
M.	230†	27	185	440
	580		427	
	358		270	
	238		200	
L.	124		40	
	610		265	
	127		61	
J.	240	100	140	2820

* Without aureomycin. All other exp., 200 γ aureomycin present.
† Single observation. All others are averages of duplicate pairs.
‡ In each exp. mucoprotein was isolated from supernatant from which total protein had been precipitated.

sence of added Ca^{++} or Mg^{++} in the buffer in contrast to the more easily demonstrable sensitivity to these ions in the liver might be explained by a more variable concentration of these ions in the colon. We have no data to substantiate or refute this possibility.

The incorporation of S^{35} sulfate into the protein and mucoprotein is a little more difficult to defend as a true enzymatic process, although such a process is certainly compatible with the data presented. Boström and

TABLE V. Reprecipitation of Labeled Colon Protein.

Patient	Label	Specific activity: Before solution	Specific activity: After reprecipitation
C.S.	C^{14} glycine	274	284
A.S.	C^{14} "	404	464
C.G.	C^{14} "	326	530
Pooled protein A	S^{35} sulfate	96	7
" B	S^{35} "	96	2

Mansson(14) have shown that cartilage slices will incorporate labeled sulfate into its mucoprotein, and that this process is inhibited by a nitrogen atmosphere. Dziewiatkowski(15) demonstrated that after giving S^{35} sulfate intravenously, intestinal mucosa was found to have significant radioactivity. We have obtained a protein with labeled inorganic sulfate which cannot be dialyzed away, yet is easily removed by solution and reprecipitation. This is compatible with the concept that the protein contained a coprecipitated mucoprotein which was easily removed by solution and reprecipitation. Winzler *et al.*(16) have demonstrated that serum mucoprotein is coprecipitated with serum protein.

The specific activity of the total crude protein, when labeled with C^{14} glycine, was consistently higher than that of the mucoprotein obtained from the same experimental conditions. The data are not sufficiently quantitative to draw any inferences with respect to precursor relationships. Since the mucoprotein contains only 12% nitrogen, the larger proportion of non-protein components in the mucoprotein might well account for the lower specific activities.

Summary. 1. The incorporation of carboxyl C^{14} labeled glycine into the protein of human colon mucosa has been studied *in vitro*. The incorporation was inhibited by nitrogen and cyanide. There was no consistent effect from omitting calcium and/or magnesium from the buffer. Penicillin depressed the incorporation; aureomycin produced no consistent results. Homogenized tissue failed to incorporate even though viable bacteria were present. 2. A mucoprotein was isolated from colon mucosa and both carboxyl C^{14} glycine and inorganic S^{35} sulfate were found to be incorporated into protein and mucoprotein. 3. Solution and reprecipitation of total crude protein removed the S^{35} label, but not the C^{14} label.

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