CYSTEINE SYNTHESIS BY SKIN HOMOGENATES

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The enzymatic systems, which provide for the synthesis of sulfur-containing amino acids through the use of inorganic oxidized sulfur, are found not only in plants and microorganisms but also in tissues from higher animals. Work published in 1962 indicated that such an enzyme had been isolated from liver and muscle in the rat and chicken but that the enzyme was not present in the skin [2].

Sheep, in contrast to numerous other animals such as the rat, are capable of utilizing sulfate sulfur for synthesis of wool keratin [1]. Results from autoradiographic studies demonstrated that within 10 or even 5 min after giving $Na_2S^{35}O_4$ by mouth, sulfur radioactivity could be observed in the skin of lambs in the form of methionine and cysteine. It was theorized that synthesis of these amino acids could take place in sheep skin. In connection with this hypothesis it was of interest to investigate sheep skin for sulfhydrase activity. A demonstration in vitro of cysteine formation from serine and labeled sulfate by skin homogenates would constitute the most convincing evidence.

In the present work an investigation was made of the cysteine synthesizing enzyme systems in sheeps and rabbit skin. Rabbit liver was studied for comparison since earlier work had demonstrated the presence of such enzymes in this organ [2, 3].

METHODS AND RESULTS

Skin samples weighing 350-500 mg were taken from sheep of the Romni-Marsh strain and rabbits of the Chinchilla breed; these were ground with 3-4.5 ml of phosphate buffer (pH 7.8) on ice using a quartz pestle in an agate mortar.

Series I Experiments. Serine was added to the homogenate to serve as the carbon skeleton for cysteine synthesis (4 mg per 0.1 ml of buffer), and other additions were: labeled sodium sulfate (25 mC per 0.1 ml buffer) as source of sulfur, the coenzyme for the synthesis, namely pyridoxal (20 μg per 0.1 ml buffer), and ATP (10 mg).

Series II Experiments. Only buffer and labeled sodium sulfate in the same quantities used in Series I were added to the homogenate.

Series III Experiments. Serine, sulfate, pyridoxal and ATP were added to tissue homogenate which had been boiled for 5 min.

Series IV Experiments. Buffer and radioactive sodium sulfate only were added to the boiled homogenate.

Series III and IV served as controls.

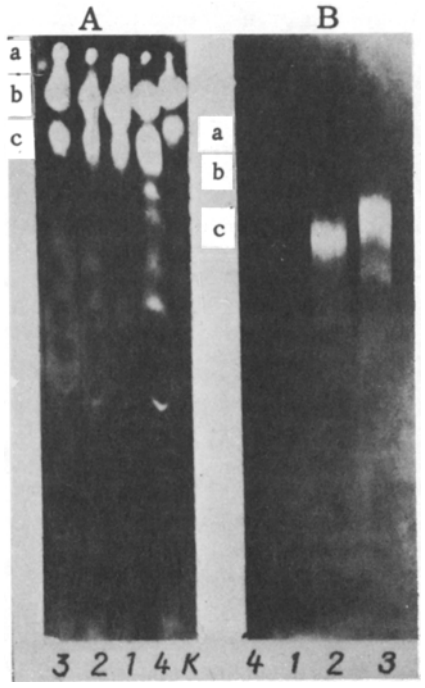
Samples were placed in a thermostat and incubated under aerobic conditions at 38° for 3 h. The reaction was halted by adding 5 ml of hot trichloroacetic acid (22% solution) to the homogenate. The homogenates were hydrolyzed in order to examine the amino acids.

The presence of radioactive cysteine and methionine was determined by radioautographic examination of the paper chromatograms obtained by descending flow with the system butanol: acetic acid: water (1:4:5), and passing the solvent 3 or 4 times in succession. After location with ninhydrin and fixation of the chromatogram it was exposed to an x-ray plate for one month.

Results from the Autoradiochromatographic Examination of Hydrolyzates of Sheep and Rabbit Tissues

Tissue	Without barium chloride precipitation			After barium chloride precipitation		
	point of sample application	sulfate area	cysteine area	point of sample application	sulfate area	cysteine area
Series I. Incubated with all components						
Sheep skin	+	+	+	−	−	+
Rabbit skin	+	+	+	−	−	+
Rabbit liver	+	+	+	−	−	+
Series II. Incubated without pyridoxal phosphate and serine						
Sheep skin	+	+	+	−	−	−
Rabbit skin	+	+	+	−	−	−
Rabbit liver	+	+	+	−	−	−
Series III. Incubated with boiled homogenate (control)						
Sheep skin	+	+	+	−	−	−
Rabbit skin	+	+	+	−	−	−

Note: + indicates presence of radioactive sulfur; − indicates absence of it.



Autoradiochromatogram of the hydrolyzates of rabbit and sheep skin. A) Without precipitation with barium chloride; radioactivity found in all samples at the point of their application, at the point corresponding to sodium sulfate, and in cystine. B) After precipitation with barium chloride; only cysteine is radioactive on incubation with serine and pyridoxal phosphate. 1, 2, 3) Sheep-skin hydrolyzates; 4) rabbit-skin hydrolyzates; K) control; a) point of application of hydrolyzate; b) sulfate front; c) cysteine-cystine point; d) methionine front.

Before chromatographic separation one of the hydrolyzates in a duplicate set of samples was precipitated with barium chloride to remove the radioactive sulfate not utilized during the reaction; the other hydrolyzate was examined without precipitation.

The results we obtained are presented in the table and in the diagram.

The autoradiochromatograms on all tissues under study, when hydrolyzates which had not been precipitated (with $BaCl_2$) were used, constantly showed three intensely radioactive spots: at the point of hydrolyzate application, at the point corresponding to sodium sulfate, and at the cysteine–cystine point. Traces of radioactivity were noted also in spots of other amino acids. The same distribution of radioactivity was noted in samples incubated with buffer and $Na_2S^{35}O_4$ but without serine or pyridoxal phosphate.

In samples which were incubated with pyridoxal phosphate and serine, and which following hydrolysis but prior to chromatography were precipitated with barium chloride, radioactivity was encountered only in the cysteine–cystine spot. For samples incubated without pyridoxal phosphate and serine there was no radioactivity. The methionine evidenced no radioactivity.

The disappearance of radioactivity, after barium chloride precipitation, from all spots except the cysteine–cystine indicates that radioactivity (in the other spots) was caused by unstable complexes of the various amino acids with sodium sulfate, which precipitated on addition of barium chloride, the sulfate coming down with the sediment.

The results of these experiments show that in sheep and also in the rabbit synthesis of cysteine can take place in skin isolated from the organism, i.e., skin has the necessary tissue enzymes.

From available information [3, 4], the sulfur of hydrogen sulfide is known to be incorporated into the carbon skeleton of serine. Therefore, inasmuch as the present experiment provided sulfate rather than sulfide as the substrate for reaction, it follows that sheep and rabbit skin must contain reducing enzymes to provide for the preliminary reduction of the sulfate sulfur.

LITERATURE CITED

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