BIOCHEMICAL INDICES OF REPARATIVE SKIN TRANSPLANTS IN HUMAN FETUSES AND ADULTS

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Homotransplantation of skin is associated with the difficult-to-solve problem of the tissue incompatibility which exists in the human organism throughout all phases of its life. However, according to certain data, [1, 4], this incompatibility is not as marked when the donor is younger. It is believed that the antigenic properties of embryonic tissue are weaker than in an adult and that the immunological protective reactions which develop in the recipient are less marked when embryonic skin is transplanted; the skin itself is antigenically less sensitive and this creates more favorable conditions for its acceptance. The fact that homoplastic skin transplants from aborted human fetuses are accepted better than those from adults is explained by the greater regenerative capacities of fetal skin and the more rapid restoration of nutrition in the transplant.

TABLE 1. Content of Nitrogenous Substances in Embryonic and Adult Skin (% of dry residue)

Subject	Moisture	Dry residue	Total nitrogen	Protein nitrogen	Residual nitrogen	Polypeptide and basic nitrogen	Amine nitrogen (total)	Free amino acids
Embryonic skin	81.5	18.5	12.0	11.0	0.98	0.54	0.28	0.23
Adult skin	69.1	30.9	13.4	13.1	0.28	0.06	0.24	0.15

The present investigation was intended as a study of the nitrogen composition of the skin and the biochemical changes which occur in transplants of embryonic and adult skin.

EXPERIMENTAL METHOD

In our experiments we investigated skin from 23-24 week male human fetuses and from adults. We determined the protein, residual, and polypeptide nitrogen, total amine nitrogen, free amino acids, and the amino acid composition of the total skin proteins. We simultaneously investigated the moisture content of the skin and its total nitrogen content by Kjeldahl's micromethod. This same method was used to determine nitrogen content after precipitation of proteins with trichloracetic acid and after precipitation of polypeptides and bases with phosphotungstic acid. The total amine nitrogen was determined colorimetrically by the ninhydrin method. The free amino acid content was determined by single-pass descending paper chromatography. Chromatography was also used to investigate the amino acid composition of the total skin proteins, which were isolated by the method adopted in the laboratory of the Department of Biochemistry of the 1st Moscow Medical Institute [2]. The protein preparation was

TABLE 2. Amino Acid Composition of Embryonic and Adult Skin (in dry residue)

Amino acid	Free amino acids (in mg-%)		Total protein amino acids (in %)	
	Embryonic skin	Adult skin	Embryonic skin	Adult skin
Cystine + + cysteine	24.4	7.6	11.4	5.7
Lysine	12.6	6.7	3.6	2.9
Histidine	21.1	8.4	2.0	2.3
Arginine	18.7	9.0	5.5	6.6
Aspartic acid	22.9	6.9	9.9	11.5
Serine	11.3	12.7	9.1	10.9
Glycine	9.1	9.8		
Glutamic acid	26.4	13.2	10.4	11.6
Threonine	5.2	5.7		
Alanine	13.4	8.8	4.3	5.2
Tyrosine	9.8	8.2	11.6	13.8
Methionine	7.5	13.5	10.5	11.3
Valine	2.9	7.5		
Tryptophan	24.9	21.6	—	—
Phenylalanine	—	—	9.8	10.0
Leucine + isoleucine	17.9	13.0	8.2	8.9

subjected to hydrochloric acid hydrolysis after complete lipid extraction in a boiling mixture of alcohol and acetone in sealed ampules at 132° for 3 h. In addition, we determined the cleavability of the skin proteins from the increase in residual nitrogen content during autolysis in a heater for 72 h at 37° and pH 5.8 and studied the changes in the biochemical indices of the transplants on heterotransplantation to rats.

EXPERIMENTAL RESULTS

It may be seen from the data given in Tables 1 and 2 that embryonic skin contains more moisture and somewhat less total and protein nitrogen. It contains substantially more water-soluble nitrogenous substances than the skin of an adult. The residual nitrogen content as a percentage of total protein is 8.9% for embryonic skin and 2.1% for adult skin.

Embryonic skin also differs from adult skin in its content of free amino acids and total protein amino acids It contains 2-3 times more cystine, histidine, aspartic acid, and alanine and less methionine and valine. The contents of the other free amino acids differ only slightly. The total protein amino acids of embryonic skin include far more cystine and lysine.

In another series of experiments we studied the products of protein decomposition of the skin under the influence of autolytic and proteolytic enzymes.

TABLE 3. Composition of Protein Decomposition Products in Embryonic and Adult Skin (% of dry residue)

Subject	Autolysis			Proteolysis			Hydrolysis		
	Residual nitrogen	Increase during autolysis	Coefficient of autolysis	Residual nitrogen	Increase during proteolysis	Coefficient of proteolysis	Residual nitrogen	Increase during hydrolysis	Coefficient of hydrolysis
Embryonic skin	1.8	0.83	7.5	1.6	0.62	2.9	1.3	0.30	2.7
Adult skin	0.63	0.35	2.7	0.51	0.23	0.91	0.39	0.12	0.92

The data given in Table 3 show that the proteins of embryonic skin are substantially more subject to the action of autolytic and proteolytic enzymes than those of adult skin. Thus, after 72 h of autolysis the content of free amino acids in embryonic skin increases by 500%, that of methionine increasing by a factor of 35, that of valine by a factor of 24, and that of tyrosine, lysine, and glutamic acid by a factor of 6-7; the cystine level also rises and a substantial quanity of phenylalanine, which is lacking in fresh skin, appears. While the quantity of the majority of amino acids increases, the aspartic acid and serine contents are somewhat reduced; this apparently results from deamination and decarboxylation during autolysis, a change in pH and in the enzymatic action of microorganisms being noted (Table 4 and figure).

Autolysis occurs more slowly in adult skin than in embryonic skin. Thus, after 72 h of autolysis the free amino acid content of the adult skin had increased from 16.5 to 31.7 mg-%.

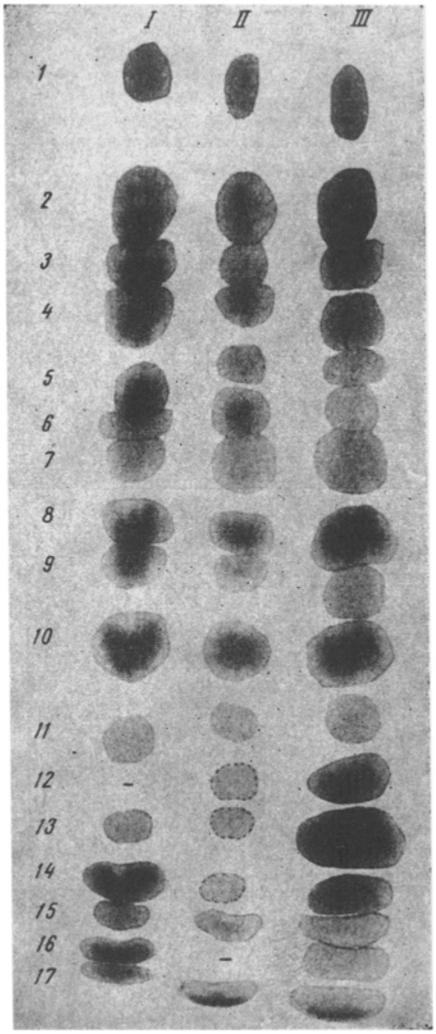
The fact that the proteins of embryonic skin are more labile than those of adult skin was also noted on heterotransplantation to rats. The biochemical indices of the adult-skin transplants changed substantially more slowly.

TABLE 4. Change in Quantitative Composition of Free Amino Acids during Autolysis of Embryonic Skin (calculated in terms of fresh weight)

Amino acid	Amino acid content (mg-%)		Change (%)
	Before autolysis	After autolysis	
Cystine + cysteine	2.7	6.2	+228
Lysine	1.4	7.9	+567
Histidine	2.3	6.5	+279
Arginine	2.1	6.2	+300
Aspartic acid	2.5	1.4	-185
Serine	1.3	0.92	-136
Glycine	1.0	2.5	+250
Glutamic acid	2.9	18.8	+645
Threonine	0.57	1.8	+307
Alanine	1.5	3.3	+218
Tyrosine	1.1	7.9	+731
Methionine	0.83	29.4	+3540
Valine	0.33	7.9	+2395
Tryptophan	2.8	9.5	+344
Phenylalanine	—	6.0	—
Leucine + isoleucine	2.0	5.2	+260

TABLE 5. Change in Free Amino Acid Content of Transplant after 72 h

Amino acid	Amino acid content (mg-%)	
	In fresh transplant	In transplant after 72 h
Cystine + cysteine	7.7	Traces
Tryptophan	21.6	"
Serine	12.7	2.6
Glycine	9.8	3.2
Valine	7.5	Traces
Leucine + isoleucine	13.0	4.4
Methionine	13.5	4.8
Glutamic acid	13.2	7.7



Content of free amino acids in human embryonic skin. I) Control; II) before autolysis; III) after autolysis; 1) cystine + cysteine; 2) lycine; 3) histidine; 4) arginine; 5) aspartic acid; 6) serine; 7) glycine; 8) glutamic acid; 9) threonine; 10) alanine; 1) tyrosine; 12) proline; 13) methionine; 14) valine; 15) tryptophan; 16) phenylalanine; 17) leucine + isoleucine.

Thus, all other conditions being equal, when adult skin was temporarily accepted its total nitrogen content decreased from 13.4 to 13.1%, while that of embryonic skin decreased from 12.0 to 6.3%. The detachment of the necrotic tissues and the cleaning and healing of the skin defect occurred more rapidly in this case.

During temporary acceptance of transplants the organism accumulates the free amino acids of the skin. It utilizes cystine, cysteine, tryptophan, serine, valine, methionine, and glycine to the greatest extent (Table 5).

The larger role of free animo acids and enzymes has also been shown by Hardin [5], who recommends injections of embryonic-skin extracts to increase transplant survival time.

The effectiveness of using embryonic skin for closing wounds has been described by V. I. Marchenko and S. P. Ushakova [3], who advise that wounds be treated with trypsinized embryonic-skin cells.

All the material presented above indicates that in a number of cases it is expedient to use human embryonic skin for homoplastic purposes.

The method employed in this work to study the nitrogen changes in the skin may also be used for evaluating the viability of transplants.

SUMMARY

The data presented characterize nitrogen composition and biochemical changes in transplants from the human embryo and adult man skin. As compared to adult, the human embryo skin contains more water soluble nitrogen substances, viz., residual and polypeptide nitrogen, free amino acids, as well as cystine and lysine. Protein disintegration of the skin characterized by autolysis, proteolysis, and hydrolysis coefficients is much more rapid in the human embryo than in the adult. Redistribution of nitrogen substances during the embryonic skin disintegration is attended by a considerable rise in the free amino acid content. High lability of proteins in the embryonic skin, as well as a high content of proteolytic enzymes in the latter, provides for intensive protein disintegration and resynthesis which is conductive to a more rapid desquamation of necrotic tissues, and to a more rapid healing of the skin defect. This shows the expediency of using the embryonic skin in homoplasty.

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