titer of the last three sera. However it is frequently found that only comparatively low titer sera are obtained when rabbits are immunized with human red cells.

These experiments definitely indicate that immunization with the CO_2-globulin prepared from the water-soluble portion of the red cells of the sheep, the pig and man leads to the appearance, in the blood stream of the treated animal, of a specific hemolytic sensitizer and of an agglutinin. It appears possible, although our experiments are incomplete on this subject, that the globulin from the water-soluble portion of the red cell is closely related to one of the proteins contained in the stroma, since by immunization with either of these antigens, the same antibody is obtained.

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The separation of the hexone bases from a protein hydrolysate by electrolysis.[1]

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In a previous communication describing a method for the preparation of glutamic acid, the need of developing cheaper methods for the production of amino acids in quantity was pointed out. This is especially true with respect to the amino acids arginin, histidin and lysin. The cost of reagents and the labor required for the preparation of these amino acids prohibits experimental work in which large quantities of these substances are required.

Some years ago Ikeda and Suzuki[2] described a method for separating certain fractions of the products of protein hydrolysis. Their method has apparently not come into general use and experimental data are not available. On passing direct current through a solution of the protein cleavage products, which is placed in the center of a three-compartment cell, the amino acids are separated into three fractions consisting of (*a*) the amino

[1] Aided by a grant from the Research Board of the University.

[2] Ikeda, K., and Suzuki, S., U. S. Patent No. 1015891, Jan. 30, 1912.

acids which are predominantly acid, including aspartic and glutamic acid, which migrate to the anode, (*b*) the basic amino acids which include arginin, histidin and lysin, and which wander to the cathode, and (*c*) the remaining amino acids, which on account of the fact that their acid properties are about equally balanced by their basic properties, remain in the center compartment.

Since this method eliminates the reagents which, in other methods, are required in order to separate the hexone bases, experimental work was carried out on a laboratory scale to determine the general applicability of this method to the isolation of the basic amino acids.

The electrolytic cell consists of a rectangular wooden box $3 \times 6 \times 4.5$ inches which was cut into three approximately equal vertical sections. The membranes separating the compartments consist of strips of linen cloth which were coated with gelatin by immersion in a 30 per cent. solution of this substance and the gelatin was subsequently fixed by allowing the strips to remain in formalin over night. After placing the membranes in position, the three parts of the cell are clamped by means of bolts. For the purposes of water-proofing, the cell was painted with asphalt. Thin sheets of carbon were used instead of the iron electrodes recommended by Ikeda and Suzuki. The latter were found to dissolve at the anode and $Fe(OH)_3$ to precipitate in the cathode solution, necessitating its subsequent removal. The fluid in the center compartment was kept at the P_H indicated in the table by the addition of small quantities of $Ba(OH)_2$ at frequent intervals. Similarly the reaction of the cathode liquor was kept approximately neutral by the occasional addition of H_2SO_4. The temperature of the solutions was kept below 35° C. by circulating a stream of water through a test tube which was placed in the center compartment, and by continuous agitation of the solution. Distilled water was placed in the anode and cathode compartments. Electrolysis was effected by passing 1.5 amperes of the 110-volt circuit through the cell.

The experiments were carried out with gelatin since this substance is a convenient source for arginin. The protein was hydrolyzed with the aid of 30 per cent. H_2SO_4 as suggested by Dakin,[3]

[3] Dakin, H. D., *J. Biol. Chem.*, 1920, xliv, 499.

the acid was neutralized by addition of $Ba(OH)_2$ in slight excess and the ammonia was removed by blowing air through the solution for several days. The electrolysis, which requires approximately three hours, is continued until a specimen of the fluid from the center compartment, on addition of phosphotungstic acid, gives no precipitate. The fluid in the cathode solution invariably contained approximately 15 per cent. of non-basic nitrogen. A small quantity of amino acids other than the hexone bases are carried over into the cathode compartment by cataphoresis. On reëlectrolysis of the cathode solution the non-basic nitrogen was reduced to an indeterminable quantity.

The table shows the results which were obtained in a number of experiments and indicates the applicability of the method for the separation of the basic group of amino acids. Of particular interest is the observation that when the reaction of the gelatin hydrolysate is more alkaline than P_H 7.5, histidin is not carried over into the cathode compartment while P_H 5.5 analysis of the cathode solution shows the presence of the three basic amino acids in approximately the same proportions as in the original hydrolysate. This is not unexpected when it is recollected that the isoelectric point of histidin is considerably lower than that of either arginin or lysin. A further advantage possessed by this method lies in the fact that the coloring matter contained in the protein hydrolysate migrates to the anode and a clear colorless cathode solution is obtained.

The cathode solution is freed from sulphates by the addition of $Ba(OH)_2$ in slight excess and the excess of barium is removed by means of CO_2. From the filtrate, containing arginin and lysin, the former can be quantitatively separated by the addition of a concentrated alcoholic solution of picrolonic acid in an amount equivalent to the arginin present. The filtrate, after removal of the arginin picrolonate, is freed from a trace of picrolonic acid by extraction with ether in the usual manner. Lysin can now be isolated from the concentrated solution by addition of picric acid.

Further work on the application of this method to the separation of amino acids is in progress.

DISTRIBUTION OF NITROGEN IN THE GELATIN HYDROLYSATE BEFORE AND AFTER ELECTROLYSIS.

	Nitrogen in original Hydrolysate.[1]		Distribution of Basic Nitrogen in Gelatin as Found by Van Slyke,[2] %.	Cathode Solution after Electrolysis at P_H 7.5.[3]		Cathode Solution after Electrolysis at P_H 5.5[4]	
	Nitrogen, Gms.	Distribution of Basic Nitrogen, %.		Nitrogen, Gms.	Distribution of basic Nitrogen, %.	Nitrogen, Gms.	Distribution of Basic Nitrogen, %.
Total nitrogen.....	61.0			6.21		0.99	
Total basic nitrogen....	18.5			6.19		0.99	
Arginin nitrogen.....	10.1	55	58	4.70	76	0.62	63
Histidin nitrogen.....	3.6	19	17	0	0	0.15	15
Lysin nitrogen.....	4.8	26	25	1.49	24	0.22	22

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Immunologic studies of actinomycetes, with special reference to the acid-fast species.

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The serums of six out of eight actinomycotic cattle fixed complement with four different antigens prepared from *Actinomycetes* isolated aërobically from lesions in cattle, and not with antigens prepared from saprophytic strains. The complement fixation test may be of diagnostic value in this disease.

The serum of a rabbit immunized against *A. bovis* fixed complement with antigens prepared from *A. bovis* and *A. maduræ*, but not with antigens prepared from the acid-fast varieties *A. asteroides* and *A. gypsoides*, or from *Mycobacterium tuberculosis*. The

[1] 500 gms. of gelatin were hydrolyzed and the volume was brought to 2,000 c.c.

[2] Van Slyke, D.D., *J. Biol. Chem.*, 1911, x, 49.

[3] 1,000 c.c. of the original hydrolysate were used for this experiment.

[4] 130 c.c. of the original hydrolysate were used in this experiment.