pelvic irradiation of women of childbearing age should be restricted to the 2 weeks following menstruation when the chance of an unsuspected pregnancy is small. This recommendation is based on the fact that the stages found most vulnerable in the mouse correspond developmentally to weeks 2-7 in human embryonic development, *i.e.*, to stages so early that pregnancy may still be unsuspected. The earlier work was primarily in the 200-300 r range(1-4) and recommendations for the human case were made mainly by downward extrapolation, supported, however, by some data from 100, 50, and 25 r irradiations(9). The present data, forming an extension to the former meager information at 25 r, are of especial interest to the human hazard problem since they deal with a dose level that is, on occasions, actually encountered in medical practice.

Summary. Mouse embryos of the *BALB/c* strain were irradiated 7½ or 8½ days postfertilization with 25, 50, or 100 r of X rays (doses lower than any heretofore investigated in detail), allowed to come to term, and then observed for changes in vertebral column and thorax. It was found (a) that there are different types of dose-effect curves for different abnormalities, and (b) that a dose as low as 25 r gives effects that are detectable even in relatively small samples.

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Elemental and Amino Acid Composition of Purified Plague Toxin. (23159)

D. F. BENT, H. ROSEN, S. M. LEVENSON, R. B. LINDBERG AND SAMUEL J. AJL
(Introduced by Geoffrey Edsall)

Department of Bacteriology, University of Maryland, College Park, and Departments of Surgical Metabolism and Bacteriology, Walter Reed Army Institute of Research, Washington, D.C.

During the past several years, we have been concerned with studies on the production, purification, characterization, and mechanism of action of the murine toxin of *Pasteurella pestis*(1,2). The isolation of the toxin in purified form(1) has led to an interest in its chemical composition. A detailed analysis of the purified material is presented in this report.

Materials and methods. Strain "Tjiwidej" (TJW) of *Pasteurella pestis* was employed for toxin production. The organisms were grown either in trypticase soy broth and the toxin extracted from the cells or in a casein hydrolysate-mineral-glucose medium(3) and the toxin salted out from solution after autolysis of the bacteria. For all analytical determinations, the toxin was purified either by the method described by Ajl, *et al.*,(1) or by the large scale ionophoresis procedure of Karler. Criteria of purity included homogeneity of the material in the ultracentrifuge and electrophoresis cell(1). By the gel precipitation technic there were a major component and a small minor component in the preparation used for most of this work; a sub-

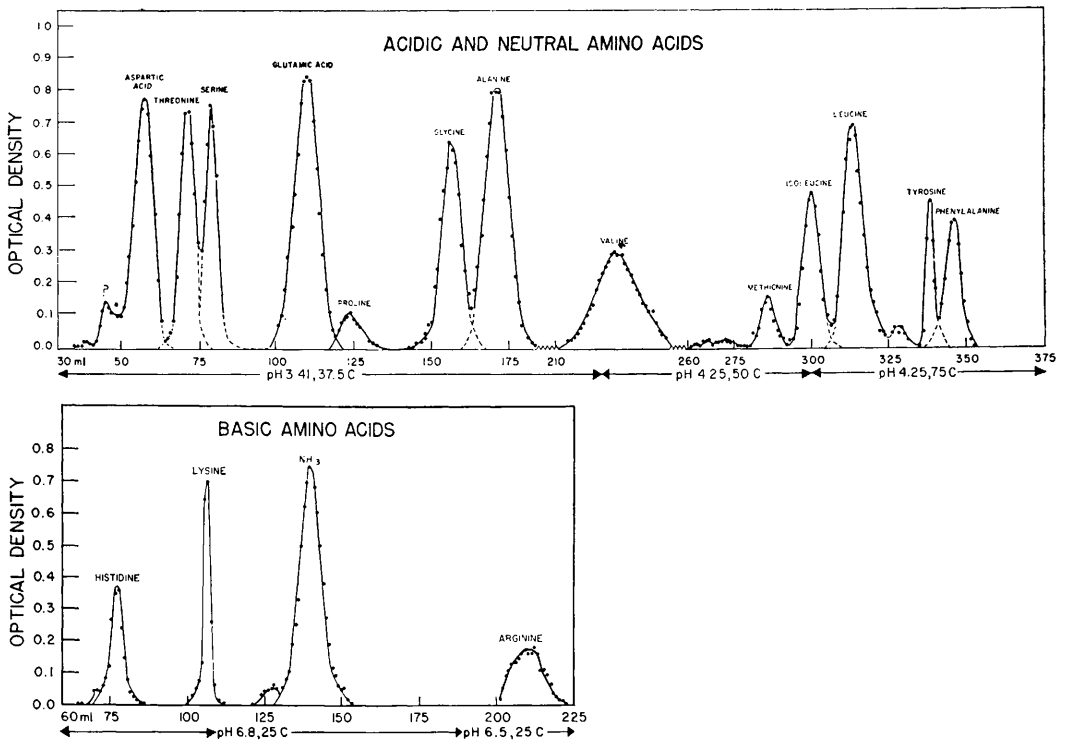

FIG. 1. Separation of amino acids of purified plague toxin by means of chromatography on ion-exchange resins.

sequent toxin preparation with only one band by the Oudin technic was analyzed for amino acid content and was found to differ in no measurable respect from the toxin lots used for the basic work reported here. Amino acid analysis was performed on weighed samples of toxin which were hydrolyzed in 6N HCl in sealed tubes at 110°C for 10 hours. The amino acids were separated on Dowex-50 ion-exchange resin columns according to the method of Moore and Stein(4) and quantitatively estimated by the ninhydrin procedure of Yemm and Cocking(5) as modified by Rosen.* To avoid the destructive action of acid hydrolysis, tryptophane was determined on the intact protein by the colorimetric method of Spies and Chambers(6). Cysteine plus cystine (calculated as cysteine) was estimated by first oxidizing these acids in the intact toxin molecule to cysteic acid with performic acid(7). The treated toxin was then hydrolyzed in the conventional manner and the cysteic acid separated on the Dowex-50 column. In several instances, the concentration of methionine was determined on the hydrolyzed protein by the nitroprusside method of Horn *et al.*(8). Moisture was determined by heating known samples of toxin to a constant weight in a drying pistol. Ash was determined by pre-charring under infra-red and ashing at 550°C. Nitrogen was determined by micro Kjeldahl. All inorganic elements were identified spectrographically.

Results. A typical analytical run representing the relative concentrations of each amino acid of hydrolyzed toxin is shown graphically in Fig. 1. The total concentration of each amino acid is represented by the area under each curve and was calculated by the use of a planimeter. Appropriate factors, determined from identical analyses of standard amino acid solutions, were applied in order to convert the planimeter readings to actual concentrations.

A summary of the bulk of the data obtained in this study is shown in Table I. The

* To be published.

TABLE I. Amino Acid Composition of Purified Plague Toxin.

Amino acid	Wt of amino acid/100 mg protein: Lot No. 101	Lot No. 103	Avg of other lots	Calculated % amino acid N.	g moles of amino acid per 10^4 g of protein	Min molecular wt of amino acid residues	Residues of each amino acid per molecule*
Aspartic acid	10.5	10.4	10.9	1.34	94.6	1007	76
Threonine	7.0	7.0	7.2	1.00	71.2	693	54
Glutamic acid	11.8	12.2	12.1	1.30	93.6	1060	71
Serine	4.2	5.1	5.4	.90	62.0	484	47
Proline	3.2	3.8	3.5	.50	36.1	340	28
Glycine	4.2	4.0	4.1	1.00	71.8	359	55
Alanine	6.8	6.4	6.9	1.40	97.0	605	74
Valine	5.4	5.9	5.5	.78	55.5	482	43
Methionine	1.1	1.7	1.5	.16	11.4	131	9
Isoleucine	4.3	4.3	4.3	.53	38.0	377	29
Leucine	6.8	8.1	7.8	.96	68.9	685	52
Tyrosine	3.2	3.2	3.2	.50	19.6	280	15
Phenylalanine	5.0	5.2	5.3	.98	36.0	465	28
Histidine	3.5	3.0	3.2	1.16	23.3	280	17
Lysine	6.1	5.3	5.3	1.79	41.4	465	31
Arginine	5.8	4.6	5.0	.28	32.0	439	24
Tryptophane	3.8	2.7	3.3	.50	17.7	279	13
Cysteine + cystine	1.3	0.9	1.3	.18	12.6	114	10
NH_3	2.1	2.3	2.3	2.0			
Totals	96.1	96.1	98.1	17.26		8445	676

Total ash content = 1.9%. Total moisture = 11.6%.
* Based on molecular wt of 74,000(1).

method of presentation of the results follows that suggested by Brand and Edsall(9). Data are presented for 18 amino acids which together with the ammonia represent a recovery of over 98% of the hydrolyzed toxin. The values given have been corrected for moisture and ash content. The threonine and serine values have been increased by 10% as is the usual procedure to correct for their partial loss during acid hydrolysis. In calculating the residues of each amino acid per protein molecule, a molecular weight of 74,000 was assumed(1).

The elemental analysis of purified plague toxin† is shown in Table II. In addition to the relatively large amounts of sulfur(1), 19 elements have been positively identified. They are grouped and listed in order of decreasing concentration. Sodium, calcium and magnesium, therefore, can be considered of quantitative significance. Others such as lead, tin, silver, etc., are present in such small amounts that they could be considered of little or no importance as constituents of plague toxin.

It was reported(1) that upon hydrolysis, the toxin yields, in addition to the various amino acids, materials which absorb and fluoresce in the ultraviolet range. The problem of the nature of the fluorescent components was further investigated in conjunction with the amino acid analyses. A fluorescent compound was eluted from the Dowex-50 column along with the basic amino acids. Tryptophane, alone of the basic amino acids, is destroyed by acid hydrolysis and is not recoverable as such by the chromatographic procedure employed. Accordingly, a pure solution of tryptophane was hydrolyzed and chro-

TABLE II. Elemental Analysis of Purified Plague Toxin.

Elements		
Major (1-10%)	Minor (.1-1%)	Traces (.01-.1%)
Sodium	Boron	Silver
Calcium	Barium	Cobalt
Magnesium	Chromium	Manganese
Silicon	Copper	Titanium
Strontium	Nickel	Cobalt
Zinc	Phosphorus	
Aluminum	Lead	
Iron	Tin	

† Performed by the National Bureau of Standards, Washington, D.C.

matographed in the same manner as the toxin; this yielded a fluorescent fraction which emerged at the same position as the unknown fluorescent material. This suggests that the fluorescent product obtained from hydrolyzed plague toxin may be a degradation product of tryptophane.

Summary. A detailed elemental and amino acid analysis was performed on highly purified *Pasteurella pestis* murine toxin. Eighteen amino acids and a number of elements were identified. The high proportions of acidic amino acids found are in accordance with the previously observed isoelectric point of the toxin of 4.7(1). On a dry weight basis, 98% of the toxin molecule was accounted for by the organic analysis including ammonia and ash content.

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Electrophoretic Distribution of Serum Protein and Glycoprotein in the Tuberculous Rat, Rabbit and Guinea Pig.* (23160)

PAUL C. HUDGINS AND ROBERT A. PATNODE
(Introduced by M. M. Cummings)
Tuberculosis Research Laboratory, Veterans Administration Hospital, Washington, D.C.

Serum protein distribution changes in man and experimental animals during tuberculous infection have been the subject of several investigations(1-2). Likewise, alterations of serum glycoprotein concentration in tuberculosis have been of interest(3-5).

The present study attempts to compare serum protein and glycoprotein response of the natively resistant rat and the susceptible guinea pig and rabbit following infection with tubercle bacilli. In a previous study(6) we compared the response of these species to an attenuated (BCG) strain of tubercle bacilli.

Methods. Eight male albino rats, Sprague-Dawley (avg wt 350 g), 8 male albino guinea pigs (500 g) and 6 male albino rabbits (1200 g) were inoculated intraperitoneally with 0.1 mg (wet wt) of *Mycobacterium tuberculosis,* human strain H 37Rv. Six other rabbits were similarly injected with bovine strain (Ravenel) tubercle bacilli. All animals were bled before and 4 weeks following infection. The sera were stored at –20°C and control and experimental sera were analyzed in duplicate at the same time. Electrophoretic separation of serum protein and glycoprotein was done by a paper strip technic previously described (6), with the exception of using 8 Ma of current for electrophoresis instead of the former 10 Ma used. The electrophoretic strips for serum glycoprotein determination were stained by a method described by Roboz(7). Stained strips were scanned in the Spinco Analytrol with a green (Wratten XI) filter. Relative % distribution of glycoprotein was determined in this manner. Total (non-glucosamine) polysaccharide content of serum was measured by the method of Shetlar(8). Milligram % glycoprotein concentration of each protein fraction was determined from total polysaccharide and relative % distribution of glycoproteins

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