[CONTRIBUTION FROM THE DEPARTMENTS OF NEUROLOGY AND MICROBIOLOGY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY, AND THE NEUROLOGICAL INSTITUTE, PRESBYTERIAN HOSPITAL]

Immunochemical Studies on Blood Groups. XIX. The Amino Acids of Blood Group Substances[1]

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The amino acid composition and the N-terminal amino acids of a variety of preparations of blood group A, B and O (H) substances of hog and human origin have been studied. Fourteen amino acids were identified and quantitatively determined. Large amounts of threonine and proline were found. Preparations of the same immunological activity, although grossly similar to one another, were found to show considerable heterogeneity with respect to the amino acid content. No correlation could be detected between amino acid composition and immunological specificity.

The presence of amino acids as an integral part of blood group substances with A, B and O specificity was discovered by Landsteiner and Harte.[3] Their purified preparations from commercial hog mucin contained both A and O (H) substances and had a N content of 5.5–6.3%, of which 35% was shown to be α-amino acid nitrogen. They also noted biuret and ninhydrin colors weaker than those for most proteins. Qualitative tests for individual amino acids were positive for arginine, histidine and alanine and negative for tyrosine, tryptophan, phenylalanine and hydroxyproline. Sulfur was absent.

These workers also reported 2.48, 2.35 and 2.91% amino acid N, respectively, in A, B and O group specific substances from human saliva.[4] In 1942 Freudenberg, Walch and Molter crystallized threonine from human A substance.[5] A polysaccharide–amino acid complex possessing blood group A specificity, isolated from hog gastric mucin by Morgan and King,[6] was found to be electrophoretically homogeneous. This complex contained 22% amino acid by weight and could not be dissociated with organic solvents into a polysaccharide and a peptide portion. Hence it was inferred that the amino acids are firmly linked by primary valence bonds to the carbohydrate portion.

Qualitative analyses by paper chromatography have shown the presence of the same eleven amino acids in human A and B blood group substances from pseudo-mucinous ovarian cyst fluids.[7,8] The only quantitative analysis to date was performed by microbiological assay on a purified hog gastric mucin preparation which contained both A and O (H) substances.[9]

The present report records analyses by the newer ion exchange technic of a number of preparations of blood group substances of different origin, specificity and mode of purification. These investigations were undertaken to determine whether immunological specificity might be correlated with chemical differences in the amino acid portion. A few N-terminal amino acid analyses are also reported.

Experimental

Blood Group Substances.—The hog blood group A and O (H) substances were preparations previously described. Five were A substances (36, 39, 58, 67, 73) and three were O substances (33, 40, 66). They were obtained from individual hog stomachs by peptic digestion. This was followed by extraction with phenol and precipitation with ethanol (58, 66, 67, 73)[10] or by isopropyl alcohol fractionation[11] (hogs 33, 36, 39, 40 and hog mucin). The B_2 and C fractions were precipitated from 0 to 41% and between 41 and 47% isopropyl alcohol (v./v.), respectively. The hog mucin fraction 2 was precipitated from 0 to 40% isopropyl alcohol (v./v.) and fraction 3 between 40 and 45%. Human A (A. B.5) and B (P.M.) substances were also prepared by peptic digestion and phenol extraction[12–14]; the preparations analyzed are the most active fractions obtained, *i.e.*, for the A substance the 10% precipitate (10% alcohol from 90% phenol) and for the B substance the phenol insoluble portion. All blood group substance preparations were extensively dialyzed prior to final precipitation.[15]

Hydrolysis.—In view of the high carbohydrate content, hydrolysis was carried out in large volumes of HCl in order to minimize losses through formation of humin.[16] Twelve-mg. samples of blood group substances were dried *in vacuo* to constant weight over P_2O_5 and hydrolyzed 16 hr.[7] in 100 ml. of 6 *N* HCl under reflux. The hydrolysates were taken to near dryness and made up to 5 ml. The solutions were clear, slightly yellow and showed only traces of precipitate. Aliquots corresponding to about 9 mg. blood group substance were transferred to ion-exchange columns.

Ion-exchange Chromatography.—Dowex 50-X8 (200–400 mesh) was cleaned, air dried and sieved through a 325 mesh sieve.[17,18] The air-dried resin (45 g.) in *N*/1 HCl was poured into a column of 1.2 cm. diameter. This gave a column height of approximately 78 cm. The column was washed free of acid and the sample was allowed to drain into the resin. Elution was carried out with HCl of increasing concentration,[18,19] beginning with 1 *N*. As soon as the

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(19) W. H. Stein and S. Moore, *Cold Spring Harbor Symp. on Quantitative Biol.*, **14**, 179 (1949).

first amino acid, aspartic, had emerged, elution was continued with 2.5 *N* HCl removing sequentially serine, threonine, glutamic acid, glycine, alanine, valine and proline. After emergence of proline, elution was finished with 4 *N* HCl. This removed isoleucine, ornithine, leucine, lysine, histidine and arginine. The rate of flow was adjusted to one drop per 90 seconds by raising or lowering the reservoir which consisted of a separatory funnel, connected through a long piece of rubber tubing and a T-tube to the top of the column. Samples of approximately 1 ml. during elution with 1 and 2.5 *N* HCl and of 2 ml. during elution with 4 *N* HCl were collected with a drop counting fraction collector. As a matter of convenience the exact volumes were determined during each run in tubes that contained no amino acids, by titration of the standardized acid with standard alkali. This is neither more nor less accurate than the drop counting. Since the resin is initially saturated with H^+, the acidity remains constant on passing through the column.

Quantitative Determination.—The amino acid concentration in each tube was quantitatively determined using the ninhydrin method of Troll and Cannan.[20] Proline was estimated with 1,2-naphthoquinone-4-sulfonate[21] and ornithine in some instances by the ninhydrin method of Chinard[22]; for these analyses the samples were taken to dryness in a vacuum desiccator over P_2O_5 and solid NaOH to remove excess HCl.

Samples which contained ammonia (following and slightly overlapping alanine) were taken to dryness in the same way. One drop of 0.1 *N* NaOH, 1 ml. of water and one drop of phenolphthalein were added. The tubes were then placed in a vacuum desiccator over H_2SO_4 under reduced pressure to allow the ammonia to diffuse and taken to dryness *in vacuo*. The residues were taken up in 0.5 ml. of *N* HCl and subjected to analysis by the ninhydrin method.[20]

Identification.—The position of the individual amino acids in the eluate was established by chromatographing a synthetic mixture of amino acids. In some instances known amounts of individual amino acids were added to the blood group substance hydrolysate. Furthermore, in every analysis the portions of the collected fractions for any one amino acid remaining after quantitative analysis were pooled, taken to dryness *in vacuo* and subjected to paper chromatography as previously described.[18] Paper chromatograms were run in phenol–ammonia–water and frequently also in phenol–water and in benzyl alcohol–water, using Whatman no. 1 filter paper.

Recoveries.—A known mixture containing a total of 100 μ/moles of the amino acids known to be present in the blood group substances (10 or 20 μmoles each) was hydrolyzed in 100 ml. of 6 *N* HCl under reflux for 16 hr. in the presence of 30 mg. each of glucose and galactose. Essentially all of the amino acids were recovered. When blood group substance with added amino acids (0.1–4 μmole of an individual amino acid), however, was subjected to hydrolysis, recovery of lysine as well as ornithine was 78–80%, while that of arginine was only 66%; other amino acids were quantitatively recovered.

End Group Analyses.—The blood group substances were preparations from individual hog stomachs described previously.[10] They were treated with dinitrofluorobenzene by the method of Sanger,[23] in order to give the dinitrophenyl (DNP) derivatives. After addition of sodium acetate, they were precipitated with ethanol, washed with ethanol and with ether and dried. They were kept in the dry state several years.[24]

Twenty to thirty mg. of each of four such DNP-blood group substances were dissolved in about 1–2 ml. of water and extensively dialyzed. Dialysis was continued until no free DNP-amino acids were present in a concentrate of the dialysate, as determined by spectrophotometric analysis. The DNP-blood group substances were hydrolyzed under reflux with 100 ml. of 6 *N* HCl for 16 hr. A second sample of DNP-blood group substance from hog 27 was hydrolyzed for only 8 hr. The hydrolysate was then concentrated to one-half its volume *in vacuo* (below 50°). After four extractions each with 50 ml. of ether, the ether was evaporated, the aqueous fraction taken to dryness *in vacuo*[23] and both fractions were separately subjected to paper chromotography.

The aqueous fraction was chromotographed in butanol–ammonia–water (4:1:5) using Whatman no. 1 paper, descending, and in butanol–acetic acid–water (80:20:20)[25] using Whatman 3 MM paper, ascending. The ether fraction was chromatographed on buffered paper in phthalate buffer, *p*H 6, according to the procedure of Blackburn and Lowther.[26] The solvents used were propanol–cyclohexane (30:70) and ethanol–benzyl alcohol (10:90), equilibrated with the same buffer.[26] Whatman no. 1 paper was used for the former solvent, no. 50 for the latter. Authentic samples of all dinitrophenyl (DNP) amino acids present in blood group substances were run simultaneously.

For semi-quantitative measurements the yellow spots in the chromatograms of the hydrolysates were eluted and read in a Beckman DU spectrophotometer against appropriate blanks. Elution was carried out with water for the aqueous fraction and with 0.1 *M* bicarbonate for the ether fraction. The spots were cut out and soaked in 1 ml. of the appropriate solvent for 15 minutes in a water-bath at 60°.[27] Blanks cut from the same paper were treated in the same way. Beckman readings were taken at 360 mμ for ϵ-DNP-lysine,[28] DNP-leucine[29] and DNP-valine.[29]

Results

With the concentrations of HCl used for elution, the amino acids emerged well separated from one another in the eluate, with the exception of aspartic acid and serine which overlapped substantially and serine and threonine which overlapped slightly.

As seen in Table I, duplicate analyses (hog mucin fraction 3 and hog 66) showed good reproducibility. Those amino acids which emerge last from the column gave somewhat poorer results than the others because they were present in only small amounts and spread out over relatively many tubes in the eluate.

Table I also shows an over-all similarity of all preparations. Fourteen amino acids were found in all samples of blood group substances analyzed, despite differences in the mode of preparation, the source or the immunological specificity. The total amount of amino acids is about 20–21% for all blood group substances from individual hog stomachs. For the preparations from human saliva and for the hog mucin the total amount of amino acids is lower.

The nitrogen content of all the amino acids accounts for 80 to 110% of non-hexosamine N with the individual hog and human substances prepared by the phenol–ethanol procedure. With the substances prepared by isopropyl alcohol fractionation, values of only 70 to 80% of the non-hexosamine N were obtained. This may be in part caused by the relatively large errors in the hexosamine determinations (8–10%) using the modified Elson–Morgan method[30] and perhaps due to the somewhat lower purity of the substances prepared by isopropyl alcohol. Some error is also inherent in the addition of fourteen analyses for amino acids, the high nitrogen content of basic amino acids, which can be estimated with the least precision, perhaps further accentuating the variation. In

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(23) F. Sanger, *Biochem. J.*, **39**, 507 (1945).
(24) Two of these samples had been prepared by Dr. Harold Baer.
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TABLE I

AMINO ACID COMPOSITION OF BLOOD GROUP A, B AND O (H) PREPARATIONS

Substance	Hog Mucin A, O Mixture			Hog A						Hog O (H)				Human saliva	
	Frac. 2 Isopropyl alc.	Fraction 3 Isopropyl alc.		36B₂ Isopropyl alc.	36c Isopropyl alc.	39B₂ Isopropyl alc.	58 (4% ppt.)[a] phenol	67 (4% ppt.)[a] phenol	73 (4% ppt.)[a] phenol	33B₂ Isopropyl alc.	40B₂ Isopropyl alc.	66(8% ppt.)[a] phenol		A A.B.₅ (10% ppt.)[a] phenol	B P.M. (phenol insol.) phenol
Sample no Method of prepn.	fractionation	fractionation		fractionation	fractionation	fractionation	extraction	extraction	extraction	fractionation	fractionation	extraction		extraction	extraction
Total N %	6.1[b]	5.3[b]		6.1[b]	6.1[b]	6.1[b]	5.9[c]	6.0[c]	5.9[c]	5.8[b]	5.8[b]	6.0[c]		5.0	3.3
Hexosamine %	32[b]	33[b]		28[b]	27[b]	28[b]	35[c]	35[c]	35[c]	30[b]	27[b]	34[c]		33	20
Non-hexosamine N %[d]	3.6	2.7		3.9	4.0	3.9	3.2	3.3	3.1	3.5	3.7	3.3		2.4	1.7
Amino acids (g./100 g. blood group substance)															
Aspartic acid	0.70	0.90	0.76[e]	0.69	0.66	0.51	0.86	0.82	0.86	0.71	0.71	0.82	0.86[e]	0.80	0.72
Serine	2.28	1.58	1.64	2.13	2.19	1.61	2.50	1.78	3.17	2.05	2.36	2.30	2.52	1.96	1.81
Threonine	5.27	4.38	4.78	5.43	5.56	5.18	5.47	6.54	6.96	6.02	5.74	6.89	6.78	6.53	4.62
Glutamic acid	1.47	1.04	1.12	1.81	1.91	1.74	1.28	1.31	1.47	2.07	1.76	1.16	1.29	1.34	0.79
Glycine	0.62	0.32	0.39	1.16	1.23	1.27	0.49	0.59	0.48	0.68	1.28	0.75	0.69	0.67	0.65
Alanine	1.17	1.06	1.05	1.44	1.45	1.42	1.11	1.23	1.44	1.29	1.74	1.43	1.50	1.08	1.05
Valine	1.08	1.01	0.96	1.04	1.03	0.93	1.24	1.13	0.86	1.22	0.74	1.27	1.19	0.66	1.01
Proline	3.53	2.03	2.09	3.58	3.74	3.27	3.99	3.49	4.66	3.44	2.50	3.82	4.17	2.12	2.39
Isoleucine	0.57	0.11	0.27	0.14	0.20	0.71	0.25	0.49	0.15	0.59	0.35	0.43	0.27	0.27	0.36
Ornithine				.08[f]	.07[f]		.31		.07[f]		.17			.05[f]	.05[f]
Leucine	.72	.59	.50	.51	.57	.65	.49	.50	.64	.33	.42	.62	.61	.57	.59
Lysine	.49	.48	.40	1.23	.89	.74	.93	.27	.41	.39	.51	.51	.26	.54	.40
Histidine	.58	.28	.24	0.57	.63	.60	.95	.68	1.05	.59	.61	.57	.78	.89	.48
Arginine	.50			1.44	1.27	1.06	.42	.40	0.51	.95	.73	.70	.46	.31	.39
Total amino acid %	19.0	13.8	14.2	21.3	21.4	19.7	20.3	19.2	22.7	20.3	19.6	21.3	21.4	17.8	15.3
Total amino acid N %[g]	2.5	1.8	1.8	3.1	3.1	2.8	2.8	2.6	3.2	2.8	2.7	2.9	2.9	2.4	1.8
Amino acid N / Non-hexosamine N	0.7	0.7	0.7	0.8	0.8	0.7	0.9	0.8	1.0	0.8	0.7	0.9	0.9	1.0	1.1

[a] % precipitate means the % ethanol used to precipitate the blood group substance from 90% phenol solution after peptic digestion. [b] Unpublished data, S. Leskowitz and E. A. Kabat. [c] Analyses by Mr. P. Z. Allen. [d] Total N minus hexosamine N. [e] Duplicate analysis. [f] Analysis by Chinard's ornithine method. See text reference 22. [g] % N is the sum of the N for the individual amino acids.

the case of hog mucin fraction 3 the lower amino acid values are attributable to the substantially smaller amount of non-hexosamine N in this preparation; the proportion of the non-hexosamine N recovered is no lower than that for the other isopropyl alcohol fractionated materials.

All blood group substance preparations contain threonine and proline in amounts unusually large for a protein. The blood group substances from human saliva (Table I) however contain less proline than those from individual hog stomachs. In comparing these values with those of other proteins, it should be remembered that the values in Table I are in grams per 100 grams blood group substance of which the amino acids comprise only about 20%. Thus in hog blood group substances in relation to the total amino acids the proportions of threonine (average 29%) and proline (average 18%) are substantially higher than in γ-globulin (8.8% threonine),[31] some crystalline enzymes (pepsin 9.6%, chymotrypsinogen 11.4% and ribonuclease 9% threonine)[32] or in gelatin and collagen (15% proline),[33] respectively. Threonine and proline together account on the average for 47% of the amino acids in the samples of blood group substances analyzed.

Only traces of ornithine and isoleucine are present. Inasmuch as ornithine is rarely a component of proteins, its presence was verified in three different ways: (1) ornithine was added to the blood group substance hydrolysate before applying it to the ion exchange column. The added ornithine emerged simultaneously with the ornithine of blood group substance and followed the isoleucine. (2) The Chinard ninhydrin method[22] (specific for ornithine and proline) was applied in several instances to the fractions in question. The spectrum typical of the ornithine ninhydrin reaction product was obtained. (3) Paper chromatograms of the pooled eluate fractions in phenol–water, phenol–ammonia–water and benzyl alcohol–water showed a substance with the same R_f as that of an authentic sample of ornithine run simultaneously. Furthermore the chromatograms indicated that the unknown was a dibasic amino acid. In this connection it is of interest that only recently a small amount of ornithine was found in a protein, namely, in salmine.[34] However, because of the small amounts, it cannot be excluded that the ornithine is only a contaminant and not a constituent of the blood group substances themselves.

The results as shown in Table I roughly correspond with the analysis of Brand and Saidel.[9] The absence of the sulfur-containing amino acids confirms the work of previous investigators on substances from hogs[3] and from humans.[4,7,8] The absence of the aromatic amino acids is also in agreement with published results obtained by chemical[3] and by chromatographic methods,[7,8] as well

(31) E. L. Smith and R. D. Greene, *J. Biol. Chem.*, **171**, 355 (1947).

(32) E. Brand in J. H. Northrop, M. Kunitz and R. M. Herriott, "Crystalline Enzymes," Columbia Univ. Press, New York, N. Y., 1948, p. 26.

(33) A. C. Chibnall, *J. Intern. Soc. Leather Trades Chemists*, **30**, 1 (1946).

(34) D. M. P. Phillips, *Biochem. J.*, **60**, 403 (1955).

as by lack of specific light absorption of blood group substances around 280 mμ.[35,36]

The results for the N-terminal amino acids of four blood group substances are given in Table II. As can be seen, the two A substance preparations differ from one another in their end groups as do the two O (H) substances, and in hog 30 (A) a trace of a second N-terminal amino acid was found. These results were obtained in both solvent systems used. Since it was thought that too much destruction might occur in a 16-hour hydrolysis, DNP-blood group substance 27 (O) was also subjected to 8 hr. hydrolysis, but no DNP-amino acid other than DNP-valine could be found.

TABLE II

N-TERMINAL AMINO ACIDS OF BLOOD GROUP A AND O (H) PREPARATIONS

Sample	Specificity	N-Terminal amino acid	Mole/300,000 g.
Hog 18	A	Leucine	0.5[a]
23 (insol.)	O	Leucine	
27	O	Valine	.5[a]
30	A	Lysine, trace histidine	

[a] These values would be increased by 20% if the correction used by Sanger (see text reference 23) for insulin is employed.

Quantitatively the DNP leucine (hog 18) and the DNP-valine (hog 27) found amounted to about 0.5 mole per mole blood group substance, using molar extinction coefficients[29] of 1.88×10^4 and 1.90×10^4, respectively, and a molecular weight of 300,000[37a] for calculation. In view of the uncertainty of the amount destroyed and of the high molecular weight of blood group substances, these results can be taken only as an order of magnitude. Too little of the other two preparations was available to make a quantitative estimate of the terminal amino acid.

The chromatograms of the aqueous fractions showed only ε-DNP-lysine. In DNP-hog 18, 23, 27 and 30 the moles ε-DNP lysine found were 5, 6, 5 and 3, respectively, per mole blood group substance. These figures are based on a molar extinction coefficient[28] of 1.74×10^4 and a molecular weight of 300,000 for blood group substance. They would correspond with 0.15 to 0.3 g. lysine per 100 g. blood group substance. The amount of lysine found in various blood group substance preparations averages to about 0.6%, so that there is at best a 50% recovery of the lysine present. In view of the low recoveries of ε-DNP-lysine, it is hardly surprising that no δ-DNP-ornithine could be detected in the chromatograms. Ornithine is present in blood group substances in considerably smaller quantities than lysine (see Table I), and consequently its presence in the peptide chain could not be established.

Despite the over-all similarity apparent from Table I, there are distinct and significant differences in the amounts of individual amino acids in the various preparations of the same immunological activity and of the same species. As with the N-terminal amino acid residues, no consistent pattern in the quantitative values for the amino acids is apparent.

Hog A substances show large differences in the analyses for alanine and valine in preparations 58 and 73 and for proline in preparations 67 and 73. Hog O substances exhibit strikingly different values for glycine, alanine, valine and proline in preparations $33B_2$ and $40B_2$, prepared in the same manner. In view of these differences within a group of blood group substances of the same specificity and mode of preparation, it is doubtful whether valid comparisons can be made between samples of the same specificity, but differing from one another in the mode of preparation. There are also further discrepancies, among samples prepared by different methods. For example, more glutamic acid and glycine are found in hog A substances prepared by isopropyl alcohol fractionation ($36B_2$, 36C, $39B_2$) than in those prepared by phenol extraction (58, 67, 73). Because of these discrepancies in the analytical values for amino acids in substances of A or of O (H) specificity, no characteristic differences between them are evident.

Indeed, no correlation can be made between amino acid composition and blood group specificity, as substances with the same immunological activity show differences with respect to amino acid composition. The N-terminal amino acids do not seem to be the determinant group in blood group specificity.

Discussion

In interpreting the results, it is well to consider the steps in the purification of these blood group substance preparations, one of which involves treatment with proteolytic enzymes. The hog stomach linings are digested either by pepsin or by autolysis.[10] Lack of completeness in the enzymatic digestion may lead to preparations varying in their amino acid composition and in their terminal amino acid group. This possibility has already been considered by Kabat, Baer and Knaub[38] and by Kabat.[37b]

A second reason for the heterogeneity encountered may be that each of the purified materials is still a mixture of several molecular species. This view recently has been expressed by Gibbons, Morgan and Gibbons,[39] who found considerable differences in the non-hexosamine nitrogen in preparations from human ovarian cysts of the same specificity. This was true despite immunological or electrophoretic homogeneity. The inadequacy of electrophoresis for establishing homogeneity of A substance preparations was pointed out by Holzman and Niemann.[40] They were able further to fractionate preparations which were homogeneous electrophoretically into fractions differing in solubility. Preparations, homogeneous electrophoretically and in fractional solubility tests, were frequently found to be polydisperse when examined

(35) G. Holzman and C. Niemann, *J. Biol. Chem.*, **174**, 305 (1948).
(36) M. Ikawa and C. Niemann, *Arch. Biochem.*, **27**, 441 (1950).
(37) *Cf.* E. A. Kabat, "Blood Group Substances," Academic Press, Inc., New York, N. Y., 1956, (a) page 154; (b) page 128.
(38) E. A. Kabat, H. Baer and V. Knaub, *J. Exp. Med.*, **89**, 1 (1949).
(39) R. A. Gibbons, W. T. J. Morgan and M. Gibbons, *Biochem. J.*, **60**, 428 (1955).
(40) G. Holzman and C. Niemann, THIS JOURNAL, **72**, 2048 (1950).

in the ultracentrifuge,[8,41] as are polysaccharides generally.

With respect to immunological homogeneity it has been established by several investigators that proteins which are non-homogeneous in the ultracentrifuge,[42] electrophoretically[43] and in amino acid composition[31] may be immunochemically uniform.

The relationship of amino acid composition to specificity among the blood group substances is not clear from the data presented. Work on the inhibition by oligosaccharides of the precipitation of blood group substances with specific antibody suggests that the carbohydrate presents binding sites for the immunological reaction.[44] In view of the general similarity of amino acid composition, it is possible that the amino acids function to maintain the structure of the blood group substances.[45] This assumes that the samples are molecularly homogeneous. Because of the differences in amino acid composition among samples belonging to the same group, it is more likely that at least a part of the amino acids found are remnants of enzymatic digestion and play no role in specificity. A less appealing possibility is that a variety of protein–carbohydrate molecules are identically specific as blood group substances. The isolated samples would thus be molecules having a variety of compositions. Further elucidation of the function of the amino acids in blood group substances will have to await the preparation of more uniform materials or a demonstration of their inherent heterogeneity.

(41) R. A. Kekwick, *Biochem. J.*, **46**, 438 (1950).

(42) E. A. Kabat and J. P. Murray, *J. Biol. Chem.*, **182**, 251 (1950).

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[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES AND THE CONVERSE MEMORIAL LABORATORY OF HARVARD UNIVERSITY]

The Total Synthesis of Lysergic Acid

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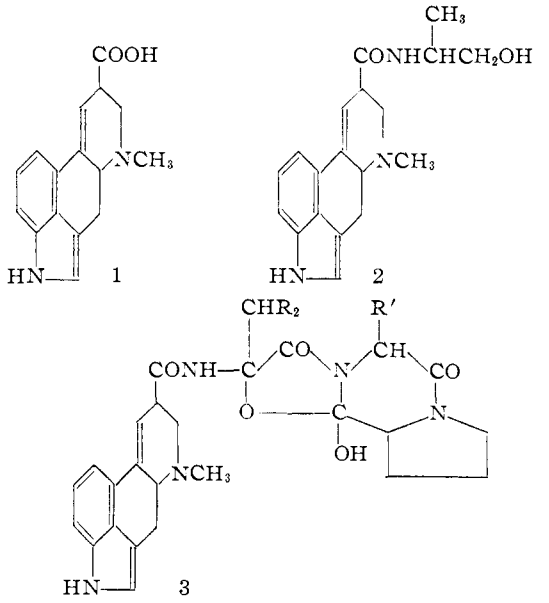
Lysergic acid, the basic fragment derived from the ergot alkaloids, has been synthesized in a fifteen-stage sequence beginning with 3β-carboxyethylindole. The starting material was converted to the intermediate 1-benzoyl-5-keto-1,2,2a,-3,4,5-hexahydrobenz[*cd*]indole (4), containing three of the four rings present in lysergic acid. This ketone in turn was transformed into the tetracyclic compound, 9-keto-7-methyl-4,5,5a,6,6a,7,8,9-octahydroindolo[4.3-*fe*]quinoline (69), and thence to lysergic acid. The synthetic acid was converted to *dl*-isolysergic acid hydrazide which had previously been resolved and converted to ergonovine. The present work, therefore, completes also the synthesis of this ergot alkaloid.

The striking physiological properties of ergot early directed attention to this remarkable product of the growth of the fungus *Claviceps purpurea* on rye grain. Pre-Christian allusions to its effects have been recorded, and it was identified in 1676 as the causative agent of the dreaded medieval gangrenous scourge, St. Anthony's Fire. The therapeutic powers of ergot were likewise recognized during the middle ages. Its capacity to induce uterine contractions was recorded as early as 1582, and crude preparations were introduced into orthodox medicine early in the nineteenth century.[2] However, its present important position in medical practice was made possible only by the extensive researches of the past forty years on the isolation and characterization of the pure active principles. These elegant investigations, in which Arthur Stoll has played a dominant role,[3] have led to the isolation of no less than six related bases all of which have been shown to be amides of the same key substance, lysergic acid (1).[4] Of the natural bases, ergonovine (2) is a particularly simple representative; the others—ergotamine (3, R = H; R′ = —CH_2-



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