Dehistaminization of Organ Extracts by Ion-Exchange Chromatography

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Abstract □ A method for the dehistaminization of organ extracts was studied using ion-exchange column chromatography. Resin A, a methacrylic polymer, was found to be the most efficacious resin used in the study. However, Resin C, another methacrylic polymer, gave a high yield since its retention of natural histamine was total while retention of other substances was negligible. Resin C, therefore, was well adapted to the preparation of organ extracts as well as to the analytical determination of histamine concentration.

Keyphrases □ Dehistaminization—biological extracts □ Extraction—using ion-exchange chromatography □ Resins—preparation, capacitance, regeneration, efficacy, comparison

Histamine, an amine base, is present in all organic liquids and tissues of an organism in the active free form as well as combined with certain compounds having acidic functions. Histamine can provoke smooth muscle contraction and capillary dilation which, if diffuse, can result in arterial hypotension and collapse. Increased cellular and capillary permeabilities resulting from histamine action cause plasmatic transudation and edema. An innocuous yet complete dehistaminization process for injectable organ extracts is required if these complications are to be avoided.

The chemical methods applied for dehistaminization of solutions, homogenates, and organic liquids can be divided into two types: (*a*) extraction by means of organic solvents, and (*b*) chromatography by means of adsorption on columns or by ion exchange.

Belonging to the first type are the methods of extraction using methanol (1), ethanol (2), and butanol (3, 4). Such extractions, however, are investigative and do not always give satisfactory results. More interesting and more applicable to the dehistaminization of biological extracts are the methods based on adsorption and ion exchange.

In 1923, Whitehorn (5) used synthetic zeolites as a means of removal of some amines; they were found to be capable of fixing 100 mcg. of histamine/g. of synthetic zeolite (6, 7). The synthetic zeolite adsorbs histamine and other amines, in the pH range of 3–5, almost quantitatively (8, 9).

Good results also were obtained in dehistaminization using charcoal (10), gel filtration with Dextran G 25 (11), and cation exchange with Dextran SE (12).

The ion-exchange resins, cationic in their acid forms, have the capacity to remove histamine quantitatively. The most frequently used exchanger, cotton acid succinate, is optimal in the retention of histamine but is not available on the market (13–15). It is best to use a weak cationic resin[1] which, when used in its sodium salt form, has a much higher activity (16, 17).

Starting with these premises, the author studied a method for the dehistaminization of biological extracts by means of ion-exchange chromatography which is efficient and causes a minimal alteration of extract composition. The resins selected for the experiments were all of the weak cationic type: methacrylic, carboxylic, and phenolic forms. This type of resin is, in fact, most selective for histamine (18). For purposes of comparison, a strong sulfonic cation and a synthetic zeolite were incorporated into the study.

In each case, saturation tests of the resin with standard histamine were performed prior to successive controls by means of bidimensional paper chromatography. The quantity and quality of amino acids eventually adsorbed by the resin from hepatic extracts of medium concentration were thus determined.

MATERIALS AND METHODS

Resins—The resins utilized in the experimentation are reported in Table I.

Regeneration of Resins—*Resin D*—The resin was treated with 2 *M* HCl for 2 hr. under agitation. A successive washing with demineralized water to remove all chlorides was followed by a 2-hr. treatment in 0.68 *M* acetic acid. After exhaustion of the resin, it was washed first with water and then with a saturated solution of sodium chloride until a clear supernate was obtained. The resin was then treated with 2 *M* ammonia to eliminate the bulk of adsorbed impurities. It was then regenerated using hydrochloric acid and acetic acid in the manner already described.

Resin C—The resin was treated with hydrochloric acid and acetic acid in the manner described for Resin D. In the case of tests performed with a resin having an optimal pH of 6.5, the treatment of acetic acid was followed by a treatment with 1 *M* NaOH for 2 hr. under agitation. The resin was then washed twice with water and buffered with 0.05 *M* sodium phosphate buffer (pH 6.5). The resin was then washed twice with water, using two volumes of water to one volume of resin in each washing. After exhaustion, the resin was washed with water and then with a saturated sodium chloride solution. It was then washed a third time with 2 *M* ammonia until the original brilliant white color returned. The resin was then regenerated in the manner already described.

Resin B—The resin was treated for 2 hr. with 2 *M* HCl. It was then washed until all chlorides were removed. After a treatment with 1 *M* NaOH for 2 hr., it was washed to a pH of 7 with water, treated with 2 *M* HCl for 1 hr., and then washed to a pH of 5 with water. The entire cycle was repeated two or three times, stopping in the final phase at the hydrochloric acid washing.

Resin A—The resin was treated with 0.5 *M* H_2SO_4 for approximately 1 hr. It was then washed with demineralized water to a pH of 5. A subsequent treatment with 1 *M* NaOH for 1 hr. was followed by washing with demineralized water to a pH of 7. It was then buffered with 0.05 *M* sodium phosphate buffer (pH 6.5) for 1 hr. and washed three times with water. Three volumes of water to one volume of resin were used for each washing.

Resin E—The resin was treated with 0.68 *M* acetic acid under agitation and boiled for 3 min. It was then decanted to remove the fine residue and dried at 80°. After exhaustion, the resin was treated with 2 *M* ammonia to remove impurities. It was subsequently treated with acetic acid in the manner already described.

[1] Amberlite IRC-50, Rohm & Haas Co.

Table I—Resins Utilized in Dehistaminizations

Resin	Type of Polymer	Functional Group	Type Resin	Total Exchange Capacity, meq./ml. Wet Resin
Resin A[a]	Cross-linked methacrylic	—COOH	Weak acid	3.50
Resin B[b]	Cross-linked polystyrene	—SO_3H	Strong acid	1.90
Resin C[c]	Cross-linked methacrylic	—COOH	Weak acid	3.00
Resin D[d]	Phenolic	—OH, —COOH	Weak acid	1.00
Resin E[e]	Sodium alumino-silicate	Silico-aluminic acid	Weak acid	0.53

[a] Amberlite IRC-50, Rohm & Haas Co. [b] Amberlite IR-120, Rohm & Haas Co. [c] Zeokarb 226, Permutit Co., Ltd. [d] Zeokarb 216, Permutit Co., Ltd. [e] Decalso (synthetic zeolite), Merck Darmstadt.

Determination of Histamine—The determination of histamine was made using the fluorometric method of Shore *et al.* (3). The capacity tests were performed using standard histamine dichlorhydrate[2]. In the tests on biological extracts, histamine was determined using a biological method (in which guinea pig ileum was used) as well as the fluorometric method of Kremzner and Wilson (19).

Chromatographic Determination of Amino Acids in Dehistaminized Extracts with the Resins—The technique of bidimensional paper chromatography was employed utilizing the following mobile phases: first phase—butanol–glacial acetic acid–water (80:20:20 ml.); and second phase—phenol–water (75:25 g.). For visualization, 0.019 *M* ninhydrin in a 0.68 *M* glacial acetic acid in butanol solution was employed. The chromatograph was sprayed and heated in a 60° oven for 30 min. The evaluation of the spots was made using the visual comparison method of Gänshirt (20).

Charge Tests of the Resins—Four grams of the resin under study was exposed to increasing concentrations of histamine in aqueous solution and maintained in agitation for 30–60 min. at room temperature. At the end of this time, the quantity of histamine residue in the supernate was measured fluorometrically. From these data, the "histamine adsorption capacity" (or "capacitance") of each resin was determined; that is, the maximum quantity of histamine adsorbed per gram of resin used. The charge and capacitance tests were performed using standard histamine in the dichlorhydrate form and were reported as base histamine.

RESULTS

Table II shows that Resin A demonstrated the highest efficacy in the capacitance tests. The order of decreasing capacitance for the remaining resins is: Resin C in the sodium salt form at pH 6.5, Resin B, Resin C in the acid form at pH 4, and Resin D.

From the chromatographic control of the amino acid composition of a 10% dried residue of liver extract performed before and after dehistaminization, it was determined that only Resins A and C in the acid form did not alter the: (*a*) qualitative or quantitative composition in amino acids of the extract, (*b*) content in amine or total nitrogen, and (*c*) absorbance at 450 nm.

The sodium salt of Resin C, buffered at pH 6.5, demonstrated a much higher capacitance than the acid form (optimal pH range 6–9) but was inefficient inasmuch as it showed notable affinity for a few strongly basic amino acids (arginine, lysine, and ornithine) which are completely adsorbed. This resin also demonstrated a lesser affinity for methionine, phenylalanine, and valine which resulted in the 40, 25, and 30% adsorption of these amino acids, respectively.

Resin B showed a net affinity for all amino acids, particularly for histidine, proline, tryptophan, and arginine which are completely fixed by this resin.

Resin D showed a net affinity (other than for histamine) for histidine, arginine, cystine, and valine. It left other amino acid concentrations unchanged.

In accordance with the determination made by Whitehorn (5), Resin E showed a net affinity for cystine, arginine, and lysine which are completely captured. It also showed a discrete affinity for ornithine and asparagine, as demonstrated by a 50% reduction of their initial concentrations.

The data are tabulated in Table III.

DISCUSSION AND CONCLUSIONS

Resin A has the greatest selectivity and capacitance for histamine. The sodium salt form of Resin C, although having the same matrix composition and the same exchanger group, exchanges with the amine group of strongly basic histamine as well as with the amine group of the diaminodicarboxylic amino acids (arginine, lysine, and ornithine) and with some semiamides of dicarboxylic amino acids (asparagine and L-glutamine), in which the imidation of a carboxyl group increases the basicity of the amino acid and, therefore, the affinity for the resin.

The retention of methionine, valine, and phenylalanine is probably dependent on the percentage of transverse bonds of the matrix and, therefore, on the diameter of the pores of the resin and the steric configuration of the reacting molecules.

The better performance of Resin C in the acid form (which captures only histamine) is obviously due to a limited dissociation at an acid pH of the carboxyl groups and, therefore, a limited exchange with substances particularly basic such as histamine. The optimum pH of this resin is, in fact, 6–9.

Of the two resins giving the better results in the dehistaminization of the extracts, Resin C in the acid form is preferable over Resin A for the following reasons:

1. There is selectivity on the part of Resin C in the acid form for natural histamine which is, however, retained by Resin A less easily than is standard histamine dichlorhydrate with which the capacitance tests were performed. This activity of Resin A may result from the fact that not all the histamine present in the tissues is in the "free histamine" form (9, 10).
2. The low percentage of Resin A that is necessary and sufficient for the dehistaminization of an extract requires longer agitation

Table II—Capacitance Determinations of the Resins

Resin	Agitation Time, min.	Capacitance (or Adsorption Capacity), mcg. Base Histamine/g. Resin
Resin D	30	130
Resin C (pH 4)	30	188
Resin B	30	207
Resin C (pH 6.5)	30	350×10^1
Resin A	30	780×10^2
Resin E	60	185×10^2

[2] Merck.

Table III—Variation of Concentrations of Individual Amino Acids in Dehistaminized Extracts Using Various Resins

Amino Acid	Resin D	Resin C	Resin C Sodium Salt (pH 6.5)	Resin B	Resin A	Resin E
Alanine	Unchanged	Unchanged	Unchanged	Diminished	Unchanged	Unchanged
Serine	Unchanged	Unchanged	Unchanged	Diminished	Unchanged	Unchanged
Cysteine	Unchanged	Unchanged	Unchanged	Zero	Unchanged	Unchanged
Cystine	Zero	Unchanged	Unchanged	Zero	Unchanged	Zero
Phenylalanine	Unchanged	Unchanged	Diminished	Diminished	Unchanged	Unchanged
Tyrosine	Unchanged	Unchanged	Unchanged	Zero	Unchanged	Unchanged
Tryptophan	Unchanged	Unchanged	Unchanged	Zero	Unchanged	Unchanged
Histidine	Zero	Unchanged	Unchanged	Zero	Unchanged	Zero
Methionine	Diminished	Unchanged	Diminished	Zero	Unchanged	Unchanged
Threonine	Unchanged	Unchanged	Unchanged	Diminished	Unchanged	Unchanged
Valine	Zero	Unchanged	Diminished	Zero	Unchanged	Unchanged
Leucine	Diminished	Unchanged	Unchanged	Diminished	Unchanged	Unchanged
Arginine	Zero	Unchanged	Zero	Strongly diminished	Unchanged	Zero
Lysine	Unchanged	Unchanged	Zero	Strongly diminished	Unchanged	Zero
Ornithine	Unchanged	Unchanged	Zero	Strongly diminished	Unchanged	Diminished
Citrulline	Unchanged	Unchanged	Unchanged	Absent	Unchanged	Unchanged
Aspartic acid	Unchanged	Unchanged	Unchanged	Diminished	Unchanged	Unchanged
Glutamic acid	Unchanged	Unchanged	Unchanged	Diminished	Unchanged	Unchanged
Proline	Unchanged	Unchanged	Unchanged	Zero	Unchanged	Unchanged
Semiamides of Monoaminodicarboxylic Amino Acids						
Asparagine	Unchanged	Unchanged	Diminished	Zero	Unchanged	Diminished
L-Glutamine	Unchanged	Unchanged	Diminished	Zero	Unchanged	Unchanged

and contact time between the resin and the extract. An increase in the quantity of Resin A in the dehistaminization is not advised since any increase causes a decrease of natural substances such as amino acids in the extract, with a consequent reduction of the adsorbance.

3. Resin A regeneration is laborious since it has a tendency to flake off during agitation. Such flaking is very limited in the dehistaminization if it takes place in the optimal resin concentration of 0.1% (w/v). If the resin concentration is greater, the flaking can alter the dried residue of the extract.

4. Resin C, although necessary in concentrations of 10% (w/v) relative to the extract, gives a much higher yield since its retention of natural histamine is total. The retention of other substances is negligible (the decrease of the dry residue is 1% or less, and absorbance is unchanged). Such behavior renders Resin C adaptable to analytical purposes.

5. The contact time between the acidic form of Resin C and the extract during dehistaminization is relatively short (30 min. if all the resin is maintained in constant agitation).

6. Resin C is easily and rapidly regenerated.

REFERENCES

(1) O. S. Sager and Horowitz, *J. Ass. Offic. Agr. Chem.*, **40,** 892(1957).

(2) F. C. McIntire, "Ciba Symposium on Histamine," Little Brown, Boston, Mass., 1956, p. 170.

(3) P. A. Shore, A. Burkalter, and V. H. Chon, Jr., *J. Pharmacol. Exp. Ther.*, **127,** 182(1959).

(4) F. C. McIntire, L. W. Roth, and J. L. Shaw, *J. Biol. Chem.*, **170,** 537(1947).

(5) J. Whitehorn, *J. Biol. Chem.*, **56,** 751(1923).

(6) M. Tabak, *Rev. Fac. Cienc. Quim.*, **22,** 205(1949).

(7) A. Schwartz and A. Riegert, *C. R. Soc. Biol.*, **123,** 219 (1936).

(8) O. H. Lowry, H. T. Graham, F. B. Harris, M. K. Priehat, A. R. Marks, and R. Bregman, *J. Pharmacol. Exp. Ther.*, **112,** 116(1954).

(9) M. Roberts and H. Adam, *Brit. J. Pharmacol.*, **5,** 526(1950).

(10) H. M. Adam, *Quart. J. Exp. Physiol.*, **35,** 281(1949).

(11) H. Weissbach, W. Lovenberg, and S. Udenfriend, *Biochim. Biophys. Acta*, **50,** 179(1961).

(12) D. Aures, R. Hakanson, and L. Spotter, *Anal. Chem.*, **243,** 483(1968).

(13) F. C. McIntire, F. B. White, and M. Sproull, *Arch. Biochem.*, **29,** 376(1950).

(14) N. Kantor, J. Levine, and H. Fieschabach, *Antibiot. Chemother.*, **1,** 579(1951).

(15) H. Weissbach, T. P. Waalkes, and S. Udenfriend, *J. Biol. Chem.*, **230,** 865(1958).

(16) S. Bergström and G. Hansson, *Acta Physiol. Scand.*, **22,** 87(1951).

(17) J. S. Oates, personal communications, 1961.

(18) F. C. McIntire, *Hand. Exp. Pharmacol.*, **18/1,** 57(1966).

(19) L. T. Kremzner and L. T. Wilson, *Biochim. Biophys. Acta*, **50,** 364(1967).

(20) E. Stahl, "Thin Layer Chromatography," Academic Press, London, England, 1966, p. 399 ff., p. 44 ff.

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