

RELATION OF pH OF ALLERGENIC EXTRACTS TO STORAGE AND SKIN REACTIVITY*

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A SURVEY of the literature has revealed a lack of extensive studies into the stability and storage of allergenic extracts, particularly in regard to pH. In 1922, Coca¹ found that extracts prepared with physiological salt solution and phenol had certain disadvantages, particularly a lack of stability of activity. "Coca's solution" was proposed to correct this. It consisted of sodium chloride (0.5 per cent), sodium bicarbonate (0.275 per cent), and phenol (0.4 per cent). According to Coca, the bicarbonate provided the buffer action to maintain a pH of 8.2.

Alles and co-workers,² studying the stability of some of the more important food allergens, adopted a phosphate buffer of pH 7.4 as an extracting fluid. A number of extracts were prepared and stored under various conditions for seven years, after which time they were tested on skin-sensitive patients. It was found that storage in the cold resulted in little or no apparent change in allergenic activity, while storage at room temperature caused, in some cases, a slight decrease in skin activity in comparison with fresh extracts.

In an investigation of the stability of fruit juices, Tuft and Blumstein³ reported that the rate of deterioration of peach juice was not affected by changing the pH to 4.5, but a pH of 8.5 tended to prolong activity.

With these few reports in mind, it occurred to us that there were many questions still unanswered in regard to pH and its relation to the storage and skin reactivity of extracts in general. In an attempt at answering some of the questions, experiments were designed to study the following:

1. The changes in pH during storage of allergenic extracts at refrigerator temperature.
2. The changes in pH during storage in the cold and at room temperature in the case of extracts which have a variety of initial pH values.
3. The effect of pH of extracts on the reactions in the skin of allergic and nonallergic patients.

In these experiments there has been no attempt to compare the merits of the various extracting fluids, but rather merely to observe the changes that could occur in pH under the usual conditions of storage of routinely prepared extracts and what effect such changes might have on skin reactivity.

EXPERIMENTAL PROCEDURES AND RESULTS

Changes in pH of Regular Extracts During Storage in the Cold.—Samples of sixty-six freshly prepared extracts were placed in sterile bottles and stored

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TABLE I. AVERAGE pH VALUES FOR ALL EXTRACTS ON REFRIGERATOR STORAGE

EXTRACT GROUPINGS*	MONTHS					TOTAL CHANGE
	0	1	2	4	6	
Extracts in Coca's solution	7.7	7.7	7.7	7.7	7.7	0
Extracts in buffered saline	6.8	6.8	6.8	6.8	6.7	-0.1
Extracts in sulfoxylate solution	6.7	6.4	6.3	6.2	6.1	-0.6

*Extracts are grouped according to the extracting medium used.

in the refrigerator under conditions simulating the routine storage of bulk extracts. The extracts were prepared according to the methods of Cooke.⁴ Twenty-nine extracts were prepared in Coca's solution,¹ eighteen in buffered saline,⁵ and nineteen in buffered sulfoxylate solution.⁶ They included inhalants, such as dust, epithelia, pollens, and molds, and foods, such as fruits, vegetables, fish, and meats. Controls were run by refrigerator storage of each of the extracting and diluting solutions alone. The pH of each extract and of the controls was measured at intervals of 0, 1, 2, 4, and 6 months after preparation. This determination was performed with a Beckman pH meter, model H-2.

TABLE II. pH VALUES OF EXTRACTING FLUIDS ALONE ON REFRIGERATOR STORAGE

SOLUTIONS	MONTHS					AVERAGE	TOTAL CHANGE
	0	1	2	4	6		
Coca's	8.1	8.6	8.7	8.6	8.7	8.5	+0.6
Buffered saline	7.2	7.2	7.2	7.3	7.2	7.2	0.0
Sulfoxylate	8.4	8.2	7.9	7.7	7.5	7.9	-0.9

Our findings are summarized in Tables I, II, and III. Extracts prepared in Coca's solution or buffered saline (Table I) tended to attain an average constant pH, while those in sulfoxylate solution underwent an average decrease of 0.6 of a pH unit over a period of six months. Table II indicates the change

TABLE III. AVERAGE pH VALUES OF VARIOUS TYPES OF EXTRACTS ON REFRIGERATOR STORAGE

EXTRACTS	MEDIUM	MONTHS					MAXIMUM VARIATION
		0	1	2	4	6	
Pollens	Coca	7.6	7.6	7.7	7.6	7.7	0.1
Other inhalants	Coca	8.2	8.2	8.2	8.1	8.1	0.1
Other inhalants	Saline	7.4	7.6	7.4	7.4	7.4	0.2
Fruits	Saline	6.7	6.6	6.7	6.6	6.6	0.1
Fruits	Sulfox.	6.5	6.5	6.3	6.4	6.4	0.2
Vegetables	Saline	6.5	6.6	6.6	6.6	6.5	0.1
Vegetables	Sulfox.	6.7	6.4	6.3	6.2	6.0	0.7
Spices	Sulfox.	6.9	6.7	6.4	6.1	6.1	0.8
Meats	Coca	7.6	7.9	7.8	7.8	8.0	0.4
Fish	Coca	7.5	7.8	7.9	7.9	7.7	0.4

Explanation of Above Groupings

1. Pollens: 22 different pollens, including trees, grasses, and weeds.
2. Other inhalants, Coca: dust, *Aspergillus*, and *Hormodendrum*.
3. Other inhalants, saline: Cattle epithelium and chicken feathers.
4. Fruits, saline: pineapple, orange, lemon, and watermelon.
5. Fruits, sulfoxylate: strawberry, grapefruit, apple, banana, and peach.
6. Vegetables, saline: 10 different vegetables, including tomato, carrot, cabbage, and green pea.
7. Vegetables, sulfoxylate: 9 different vegetables, including spinach, celery, and white potato.
8. Spices: black pepper, red pepper, and vanilla.
9. Meats: lamb and beef.
10. Fish: clam and cod.

following storage over a period of six months of the extracting solutions alone. It is seen that where the sulfoxylate showed a decrease of 0.9, the Coca's solution showed an increase of 0.6, and the buffered saline showed no change at all.

Since it was not practical to give the data on each of the sixty-six extracts studied, they were grouped according to types of materials and extracting media. The average values are listed in Table III. It is to be noted from this table that spices, vegetables, meats, fish, fruits, and inhalants tend to show the greatest changes in pH in the order in which they are mentioned. However, the same types of extracts show a difference in pH variation depending on the extracting medium. For example, vegetables extracted with sulfoxylate solution, which is used for the purpose of minimizing color formation, show a greater pH variation than those extracted with buffered saline.

Effect of Storage at Refrigerator and Room Temperature on pH of Ragweed Extract.—To observe the changes that might occur if extracts are stored when not at the usual pH values, the following experiment was performed. A freshly prepared extract of ragweed (a 1:1 mixture of high and low ragweed) was diluted with buffered saline to a concentration of 0.01 mg. total nitrogen/c.c. and divided into a series of samples. The pH values of these samples were adjusted to the following: 4.0, 4.5, 5.0, 5.5, etc., up to 9.0, by the addition of acid or base in amounts small enough to have negligible effect on the nitrogen concentration. Each sample was then divided into two portions; one was stored at room temperature, the other in the refrigerator. A set of controls consisting only of buffered saline at each of the above pH levels was prepared simultaneously with the ragweed samples and portions stored in the refrigerator as well as at room temperature. The pH of each sample and control was tested at intervals of 0, 1, 2, 4, and 6 months. The data for the 4, 7, and 9 pH samples are given in Table IV. It is seen that the general trend in all series was toward a slight increase in pH. Greater increases were observed in the lower pH ranges, evidently due to the poor buffering capacity of the saline-phosphate solution in that region. These variations are comparable to those of the saline controls.

TABLE IV. EFFECT OF TEMPERATURE ON pH OF STORED RAGWEED EXTRACTS

TEMPERATURE	MONTHS									
	0		1		2		4		6	
	ROOM	COLD	ROOM	COLD	ROOM	COLD	ROOM	COLD	ROOM	COLD
Ragweed (0.01	4.0	4.0	4.7	5.2	5.0	5.4	5.1	5.2	4.9	5.0
mg. total	7.0	7.0	7.1	7.3	7.2	7.6	7.3	7.6	7.2	7.2
nitrogen/c.c.)	9.0	9.0	8.9	9.2	8.9	9.4	9.1	9.4	9.0	9.2
Buffered saline	4.0	4.0	4.1	4.1	4.2	4.3	4.3	4.0	4.3	4.4
controls	7.0	7.0	7.2	7.3	7.2	7.3	7.3	7.3	7.2	7.5
	9.0	9.0	8.9	9.2	9.0	9.1	9.1	9.0	9.0	8.9

Effect of Varied pH on Skin Sensitivity.—This experiment was carried out in order to determine whether artificially induced changes in the pH of a ragweed extract would alter its ability to elicit skin reactions on known ragweed-sensitive patients.

A freshly prepared ragweed extract (a 1:1 mixture of high and low ragweeds) was diluted with buffered saline to concentrations of 0.01 and 0.001 mg. total nitrogen/ml. and each divided into a series of samples. The pH values

EFFECT OF pH ON SKIN SENSITIVITY OF RAGWEED-SENSITIVE PATIENTS

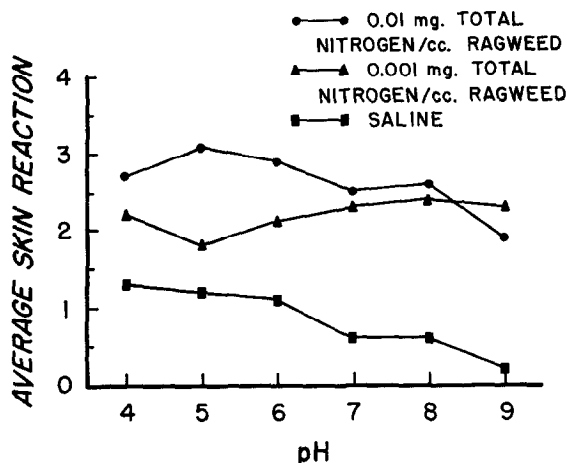


Fig. 1.

EFFECT OF pH ON SKIN SENSITIVITY OF NON-RAGWEED-SENSITIVE PATIENTS

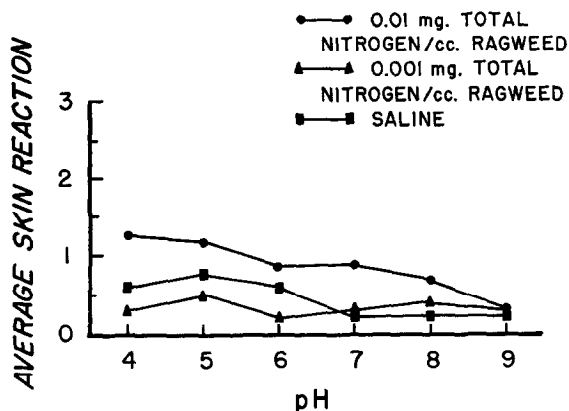


Fig. 2.

of these samples were adjusted as above to 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0. Eleven untreated ragweed-sensitive individuals and eleven nonragweed-sensitive persons (as established by previous testing) were used. The tests were performed in the deltoid area. In the first row, 0.001 mg. total nitrogen/ml. ragweed

was injected intracutaneously at six sites with samples of increasing pH from above downward. The volume injected in each site was 0.05 ml. In the next, 0.01 mg. total nitrogen/ml. was injected and, in the last row, buffered saline in like manner, with samples of similar pH at the same horizontal levels on the arm. Injections were performed by one, measurements made by another, and data recorded by a third person. Each reaction wheal was traced and measured after ten minutes and judged as negative, doubtful, slight, moderate, marked, and very marked according to the diameter and presence of pseudopods. These scores were translated to a scale of 0, $\frac{1}{2}$, 1, 2, 3, and 4, respectively. The averages for each group of patients are plotted in Figs. 1 and 2. It may be seen that changes in pH have little effect on the skin reactivity of the patients in either group, although there would appear to be a slight trend toward decreasing reactivity with increasing pH.

Passive transfer tests were performed with sera from two of the ragweed-sensitive patients who had shown very marked reactions above. Here again the results indicated very little difference in skin reactivity between ragweed extracts of pH 4, 7, and 9.

DISCUSSION

The preparation of desirable allergenic extracts presents many problems. One of these is the pH of the extract, for it has been thought that variations in pH may affect the antigenicity of the material or may render it nonspecifically irritating when employed for skin testing. The literature contains references to the possible effect of changes in pH on antigens employed in experimental sensitization. Landsteiner⁷ and Boyd⁸ cite a number of these references, some dealing with antigen-antibody reactions, others with proteins in general. It must, of course, be understood that a protein will be altered by any change in pH by virtue of its fundamental structure. Since a protein is composed of many amino acids, there will be found on its surface a large number of acidic and basic groups, the relative ionizations of which govern the extent of the electrical charge on the surface. Any small amount of added acid or base will affect the ionization of these groups and thus cause a change in the surface charge, resulting in altered physical properties.^{9, 10, 11, 12, 13} This does not necessarily mean a change in antigenicity. The extent to which the surface charges may be altered without affecting the antigenicity depends on the nature of the particular protein. It must be stressed, however, that we have been speaking of relatively small amounts of acid or base. In general, if the pH of a protein solution is kept within a range of from 6.0 to 8.0,^{9, 14, 15, 16} the protein will exhibit maximum antigenicity as determined in precipitin reactions. It will exhibit considerable antigenicity throughout an even wider range, namely, from 4.5 to 9.5.¹⁷ Beyond these limits the antigenicity disappears and, if one proceeds further toward the extremes in pH, denaturation of the protein occurs. On the basis of these statements, it would appear that the changes in pH which occur on storing extracts for periods of time extending to six months are of little significance in their effect on antigenicity. Such changes as do occur are apparently insufficient to affect the specificity of the material used in direct or indirect skin testing or to produce nonspecific or irritating positive reactions.

SUMMARY

1. Samples of a variety of stock allergenic extracts were stored in the refrigerator and tested for changes in pH over a period of six months. On the average, extracts prepared with buffered saline or Coca's solution exhibited the greatest pH stability, while those prepared with sulfoxylate showed a general decrease in pH. However, no samples were found to change beyond the limits of antigenic activity.

2. Samples of dilute ragweed extract were adjusted in pH to a variety of values and then stored at both room and refrigerator temperatures. Changes in pH were observed over a period of six months. The effect of temperature on the change of pH was not significant.

3. Changes in pH of ragweed extract had little effect on the skin reactivity of either allergic or nonallergic patients.

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