THE HYDROGEN ION CONCENTRATION OF JOINT EXUDATES IN RHEUMATIC FEVER AND OTHER FORMS OF ARTHRITIS.

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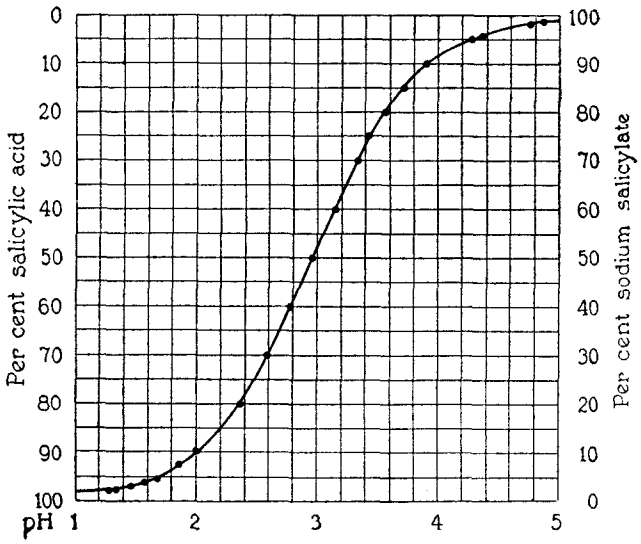
In connection with a study of acute rheumatic fever we have determined the hydrogen ion concentration of exudates aspirated from the inflamed joints of patients ill with this disease and also of exudates of patients with certain other forms of arthritis. This was done (1) to compare the reactions of the exudates in these arthritic diseases, and (2) to determine whether an acidity occurs in the inflamed joints in acute rheumatic fever sufficient to permit the liberation of free salicylic acid following salicylate therapy.

One explanation of the action of salicylates in patients with rheumatic fever has been based upon the hypothesis that free salicylic acid is liberated in the inflamed joint. If the inflammation in the joint results from the local irritation of bacteria, a bactericidal action of free salicylic acid might explain the improvement that ordinarily follows the administration of this drug. This theory originated with Binz (1), who realized that free salicylic acid could not exist in normal blood and tissues, and also that the salts of salicylates are not bactericidal in weak solutions. In subjecting sodium salicylate solutions to high CO_2 tensions *in vitro*, he found that sufficient acid was liberated to be bactericidal. The reason for the liberation of free salicylic acid in Binz' experiments is obvious: the increased CO_2 tension caused sufficient acidity to allow dissociation of the acid. He thought that a similar increased CO_2 tension occurred in the inflamed tissues in acute rheumatic fever because Ewald (2) had previously shown that such an increase could occur in certain types of inflammatory exudates. Although Binz' theory seemed unsatisfactory, it has never been conclusively disproven and is quoted in most pharmacologies.

Hanzlik and his collaborators (3), in an extensive investigation of the pharmacology of salicylates, examined joint fluids directly by the ferric alum test for the presence of free salicylic acid; the patients with rheumatic fever received full therapeutic doses of the drug. These authors found no free salicylic acid present. A criticism of this work was made by the authors themselves who state that: "Objection might be raised to the method used because of the possibility that

some CO_2 is lost when the fluid is exposed to the atmosphere, but this is to a considerable extent prevented by the presence of 'buffer' or protective substances."

Reaction at Which Free Salicylate Acid Occurs.—Hanzlik (4) also found that salicylic acid was released from mixtures of sodium salicylate and buffer salts only when the acidity was greater than pH 6.7. The iron test for salicylic acid was found to be faintly positive at a pH of 6.7 and progressively stronger with an increase in acidity from pH 6.5 to 1.0; on the alkaline side of pH 6.7 no salicylic acid was liberated. Mixtures containing 25 per cent serum



TEXT-FIG. 1. Salicylic acid-sodium salicylate curve at 40°C.

or plasma did not show any free salicylic acid between pH 7.4 and 5.9; tests were not carried out at a higher acidity. He states that: "It is conceivable that very low degrees of acidity, *i.e.*, in the neighborhood of pH = 6.8 or 6.9, might occur in closed cavities with sluggish circulation as in the articulations," but "fluids of joints and similarly enclosed regions would need to be more highly acid and freer from protein and other constituents than is probably the case in order to contain free salicylic acid and explain the therapeutic relief from salicylate medication according to the antiseptic theory."

The dissociation curve for mixtures of salicylic acid and sodium salicylate is given in Text-fig. 1. It is evident that at reactions more

alkaline than pH 5.0 the free acid constitutes less than 1 per cent or the total salicylate. Since it has been estimated that following full therapeutic doses of salicylate the concentration in the blood or joint fluid is about 0.02 per cent (3), the amount of free acid present at a pH of 6.0 would be less than 0.0002 per cent; *i.e.*, an amount that could exert no bactericidal action. This conclusion is in agreement with Hanzlik's experimental findings.

EXPERIMENTAL.

The reactions of twenty-six joint exudates have been determined. The majority of these were from patients ill with acute rheumatic fever; a number were from patients having arthritis of unknown origin (chronic arthritis, intermittent hydrarthrosis); two were from patients with definite bacterial arthritis; and one, a simple effusion, was from a patient with myocardial insufficiency and generalized edema.

In each instance the joint was aspirated with a tightly fitting Luer syringe containing a small amount of sterile paraffin oil to prevent the admission of any air bubbles. During the entire determination the fluid was prevented from coming in contact with the air.

Owing to the fact that a considerable quantity of fluid was necessary for electrometric pH determinations and that in each instance several additional cubic centimeters were needed for culture, colorimetric determinations were made in the majority of instances. When large quantities of fluid were available, both colorimetric and electrometric determinations were made, in order to obtain the factor necessary to convert colorimetric readings at room temperature to electrometric at body temperature (38°C.).

Electrometric Measurements.[1]—The electrometric determinations were made at 38°C. in the Clark cell, in a hydrogen atmosphere containing CO_2 at the tension existing in the joint fluid. The determinations were corrected for partial pressures of CO_2 and H_2O to one atmosphere of dry H_2. The solution used in the standardization of the hydrogen electrode was 0.1 N HCl whose pH at 38° was assumed to be 1.09. This brings the pH determinations to the basis of Sören-

[1] A number of these determinations were made by Dr. A. B. Hastings.

sen's standards. A detailed description of the technique employed has been recently published by Cullen (5).

Colorimetric Measurements.—The method was that described by Cullen (5) for the colorimetric determination of the hydrogen ion concentration of blood. 1 cc. of the aspirated joint fluid (usually within 4 minutes of withdrawal and before coagulation occurred) was added to a standard tube containing 20 cc. of 0.9 per cent sodium chloride solution and 7 drops of indicator; the addition was made under paraffin oil to prevent the escape of any CO_2. 1 cc. of the same joint fluid was added to another tube containing 20 cc. of saline solution without any indicator to serve as a turbidity control in the comparator. The pH determination was then made by placing the tube in a comparator block and comparing it with standard color tubes. This was done at room temperature. Phenol red was used as indicator for determinations above pH 6.8 and brom-cresol purple for those below pH 6.8. All glassware, mineral oil, and salt solution were previously tested for neutrality. As standards, Sörensen's phosphate standards were prepared in steps of pH 0.2 from pH 5.6 to 6.8 and in steps of pH 0.05 from pH of 6.8 to 8.0. To those above pH 6.8, 5 drops of 0.03 per cent phenol red solution were added to each 15 cc. of standard; to those below pH 6.8 a similar quantity of brom-cresol purple was added.

The colorimetric determination at room temperature was corrected to 38°C. by the following formula:

$$pH_{38^\circ} = \text{colorimetric pH} + 0.01\ (t - 20^\circ)\ \text{pH} - 0.21\ \text{pH}$$

in which t = room temperature and 0.21 pH represents the empirical correction for temperature ($38^\circ - 20^\circ$), dilution, and protein errors. This correction represents the average of the differences between electrometric determinations at 38°C. undiluted, and the colorimetric determinations, diluted, at 20°C.; it corresponds closely with the correction similarly found for correcting colorimetric determinations of human blood. The room temperature in these experiments varied within $\pm$ 3°C. from 20°C. and a temperature change of 0.01 pH was made for each degree of variation.

Physical Characteristics of the Exudates Examined.—The fluids aspirated from the inflamed joints of patients ill with acute rheumatic

fever were never frankly purulent. They were viscous, slightly to distinctly turbid, and usually of a pale yellowish green color. Sometimes when the exudate was aspirated from a joint during the early stage of the arthritis, the greenish yellow shade was absent and the fluid had a grayish turbidity; this effect was probably due to a higher leucocyte content. The exudates contained considerable fibrin and formed soft clots on standing. Bacteriologically, they were sterile by ordinary culture methods. The fluids from the patients with arthritis of undetermined origin were indistinguishable from some of these exudates. The joint fluid from the patient with myocardial insufficiency was less turbid, did not clot so quickly, and had a lower leucocyte count than most of the rheumatic fever exudates. The exudates of the two patients with bacterial arthritis were easily distinguished as coming from infected joints. They were purulent fluids and contained bacteria.

Results.

Hydrogen Ion Concentration.—The results are shown in Table I. With the exception of the joint exudates of the two patients with bacterial arthritis, the reactions were all slightly alkaline and approximated the normal reaction of blood. The hydrogen ion determinations of sixteen fluids from patients with acute rheumatic fever varied between pH 7.27 and 7.42. Seven fluids were examined from three patients with arthritis of undetermined origin; one of these was diagnosed as intermittent hydrarthrosis, one as chronic arthritis, and the third was probably arthritis accompanying serum disease. Their reactions were approximately the same as those in acute rheumatic fever, varying between pH 7.33 and 7.47. The joint fluids of the two patients with bacterial arthritis were both definitely acid; the exudate from the knee joint infected with *Staphylococcus aureus* was pH 6.69; from the one with *Streptococcus hæmolyticus*, pH 6.19. The electrometric determination of this latter fluid cannot be considered accurate as the fluid was kept over night in the ice box and heated to 56°C. for 1 hour to kill the organisms; the result, however, is sufficiently accurate to show the fluid to be definitely acid. The simple effusion from the patient with myocardial insufficiency accompanied by general edema was pH 7.34.

TABLE I.

Hydrogen Ion Concentration of Joint Exudates.

Case No.	Patient's temperature at time of aspiration.	Joint.	Length of time joint aspirated was involved.	Degree of inflammation.*	Bacteriological examination.	Determinations.			
						Colorimetric at 20°C.	Electrometric at 38°C.	Difference between colorimetric at 20°C. and electrometric at 38°C.	Colorimetric calculated at 38°C.†
Acute rheumatic fever.									
	°F.		*days*			*pH*	*pH*	*pH*	*pH*
4444	104.5	Right knee.	3	+++	No growth.	7.65	7.39	0.26	7.39
4350	102	Left wrist.	5	+	" "	7.53‡			7.32
4453	102	Right knee.	1	+++	" "	7.58	7.34	0.24	7.34
	99	Left "	7	+	" "	7.58			7.37
4367	100.5	Right "	10	+	" "	7.63‡			7.42
	104	Left "	2	++	" "	7.56‡			7.35
	104	Right "	2	++	" "	7.57‡			7.36
4417	103	" "	8	+	" "	7.54‡			7.33
	103	Left "	3	++	" "	7.54‡			7.33
	102.5	" "	5	+	" "	7.55‡			7.34
	102.5	Right "	12	+	" "	7.57‡			7.36
4473	103	Left "	1	+++	" "	7.49	7.27	0.22	7.27
	103	" "	2	++	" "	7.60			7.39
4485	103.8	Right "	1	+++	" "	7.54			7.33
	103.8	Left "	3	++	" "	7.56			7.35
4493	103	" "	4	+++	" "	7.51	7.34	0.17	7.34

* + indicates swelling only; ++, moderate inflammation; +++, marked inflammation.

† Average correction of pH 0.21 used except where actual difference is given.

‡ Room temperature not recorded. Variation not more than ± 3°C. from 20°C.

TABLE I—*Concluded.*

Case No.	Patient's temperature at time of aspiration.	Joint.	Length of time joint aspirated was involved.	Degree of inflammation.*	Bacteriological examination.	Determinations. Colorimetric at 20°C.	Electrometric at 38°C.	Difference between colorimetric at 20°C. and electrometric at 38°C.	Colorimetric calculated at 38°C.†
Arthritis of undetermined origin (includes chronic arthritis and intermittent hydrarthrosis).									
	°F.		*days*			*pH*	*pH*	*pH*	*pH*
4451	101	Right knee.	2	+++	No growth.	7.54			7.33
	102	Left "	5	+++	" "	7.65			7.44
4428	99	Right "	?	+	" "	7.61	7.43	0.18	7.43
	100	" "	2	+	" "	7.67	7.42	0.25	7.42
	99	" "	2	+	" "	7.62	7.42	0.20	7.42
	98.5	" "	30 *mos.*	+	" "	7.68			7.47
4449	99	Left "	5	+	" "	7.60	7.42	0.18	7.42
Bacterial arthritis.									
			days						
4262	104	Right knee.	8	+++	*Staphylococcus aureus.*	6.90			6.69
4467	104.2	Left "	?	+++	*Streptococcus hæmolyticus.*	6.40§ 6.10‖	5.89	0.21	6.19
Joint effusion, myocardial insufficiency.									
4458	102	Left knee.	?	+	No growth.	7.55			7.34

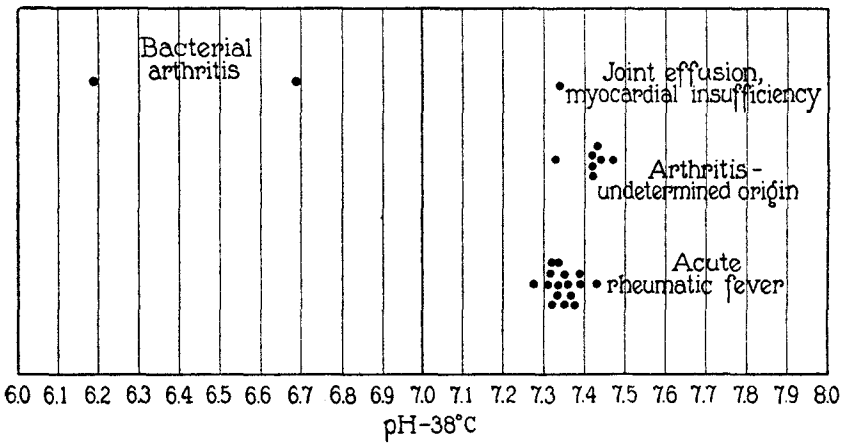
§ Within 4 minutes of aspiration.

‖ Determined after standing in ice box over night and heated at 56°C. for 1 hour to kill bacteria.

DISCUSSION.

A comparison of the results is indicated in Text-fig. 2. It shows that the joint exudates on the basis of their reactions fall into two groups; the one having a slightly acid, the other a slightly alkaline reaction. The fluids containing bacteria fell into the first, the sterile fluids into the second. In general, these findings correspond with those of other investigators who have recently made hydrogen ion

determinations on various types of exudates. Shearer and Parsons (6) found the purulent spinal fluid of epidemic cerebrospinal meningitis to be about pH 6.9. Lord (7) found the pH of pneumonic lung exudates to be as low as 5.4. Shade and his coworkers (8) determined the reactions of many exudates electrometrically; pus from acute infections varied in pH between 5.96 and 6.57; pus from chronic inflammations such as tuberculosis between 6.58 and 7.00; serous exudates as in tuberculous pleurisy between 7.00 and 7.09; and non-inflammatory transudates between pH 7.17 and 7.24.



TEXT-FIG. 2. Hydrogen ion concentration of joint exudates.

It seems to us that only in the instance of purulent exudates would the liberation of free salicylic acid be at all possible. If acid is liberated in these exudates it is not sufficient to be bactericidal, as clinical observations have never shown any improvement in purulent arthritis following salicylate treatment. As Hanzlik has previously concluded from direct examinations that free salicylic acid could not be demonstrated in the joint exudates of patients with acute rheumatic fever, and our findings indicate that free acid could not possibly exist, it is evident that the local antiphlogistic effect of the drug is due to some other factor than free salicylic acid.

The determination of the reactions of these fluids has thrown some light upon the nature of the pathological process in the arthritis of

acute rheumatic fever. The results of various workers already mentioned show that when bacteria such as hemolytic streptococci, staphylococci, pneumococci, meningococci, or tubercle bacilli are present in an exudate in sufficient numbers to be detected, either by cultural or microscopic examination, the reaction of that exudate is always more acid than blood. We have evidence to show that the exudates in experimental arthritis of animals inoculated with green streptococci are also acid. From these results it would seem that if green streptococci were growing in the joint fluids of rheumatic fever patients, one should expect those fluids to be acid. But our examinations have shown that this is not the case. It is highly improbable, therefore, that one could cultivate streptococci from such exudates. This is in agreement with our bacteriological studies.

Another possibility must be considered: the etiologic agent—whatever it may be—may exist only in the capsule or contiguous tissues; and the exudate may be similar in nature to the serofibrinous fluid often found in pleurisy secondary to pneumonia. In many instances these pleuritic fluids are sterile and the patients recover without developing empyema. While there are not sufficient data concerning the reaction of such fluids, from the report of Shade we would expect them to be more alkaline than pH 7.0. Further observations should be made concerning the reaction of sterile fluids in serous cavities contiguous to foci of inflammation resulting from bacterial infection. When this information is available we shall be in a better position to evaluate properly the relation of our findings to the etiology and pathology of rheumatic fever.

The slight variation in the reactions of the exudates of acute rheumatic fever was not dependent upon the severity of the inflammation as evidenced clinically. The exudates from acutely inflamed joints were of about the same pH as those from joints in which the clinical signs of acuteness had disappeared and the only remaining evidence of involvement was the presence of fluid. The most acid exudate, having a pH 7.27, was found in a highly inflamed joint; the most alkaline fluid, having a pH 7.42, was found in a joint from which all acute signs of inflammation had disappeared; one highly inflamed joint, however, had a pH of 7.39 (Table II).

TABLE II.

Acute Rheumatic Fever.

Comparison of Exudates from Acutely Inflamed Joints with Those in Which Signs of Acute Inflammation Had Subsided.

Exudates from joints acutely inflamed	$pH_{38°}=7.27-7.39$
" " " in which inflammation had subsided	$pH_{38°}=7.31-7.42$

SUMMARY AND CONCLUSIONS.

1. The hydrogen ion concentration of joint exudates aspirated from patients ill with acute rheumatic fever, arthritis of undetermined origin, and bacterial arthritis was determined. The hydrogen ion concentrations of the joint exudates from patients with acute rheumatic fever approximated the normal reaction of blood, varying from pH 7.27 to 7.42. Exudates from patients with arthritis of undetermined origin varied in pH from 7.33 to 7.47. The pH of a joint effusion occurring in a patient with myocardial insufficiency was 7.34. Bacteriologically, all of these fluids were sterile by ordinary means of cultivation. An exudate aspirated from a knee infected with *Staphylococcus aureus* had a pH of 6.69, while that from a patient having an arthritis due to *Streptococcus hæmolyticus* was also acid, having a pH of 6.19.

2. Since a definitely acid medium is necessary for the liberation of free salicylic acid and since all of the joint fluids from patients with acute rheumatic fever were slightly alkaline, no free salicylic acid could possibly exist in such joint fluids following the administration of salicylates.

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