According to the authors, "the free metal (barium) is not prepared commercially and has no uses"; the fluorine molecule is "stable at all temperatures"; "the oxide (of aluminum) is infusible"; "the decomposition of potassium chlorate into potassium chloride and oxygen . . . (is) . . . endothermic"; "barium melts at 850° C and boils at 1140°."

R. A. BAKER

Brief College Chemistry. By LEON B. RICHARDSON and ANDERW J. SCARLETT. vi + 385 pp. 128 figs. New York: Henry Holt and Company. 1942. $3.00.

WRITTEN in the refreshing style already associated with these authors, this brief text is no scissors-and-paste abstract, but a paraphrase of their earlier works. Because it is scholarly it happily stands apart from those "science survey" texts which dilute science to the level of the tabloid.

There are five introductory chapters on valence, atomic structure and the periodic table; eight chapters on physical chemistry, including energy, states of matter, equilibrium—introduced probably too early for an elementary course—chemical calculations, the ionic properties of solutions, and a logical use of the Brønsted treatment of acids and bases; eight chapters on the non-metals and seven on the metals, with two concluding chapters on organic chemistry. Sandwiched into physical chemistry is the chemistry of water and its constituent elements; electrochemistry is interposed between aluminum and iron; colloids are inserted between phosphorus and carbon. Although these special topics are arbitrarily located, at least they are consistently handled.

This brief, elementary, but authoritative text should supplant its several inferior predecessors.

HUBERT N. ALYEA

Introductory College Chemistry. Second edition. By HORACE G. DEMING. 521 pp. 176 figs. New York: John Wiley and Sons, Inc. 1942. $3.00.

NOW written in collaboration with Professor Hendricks, this well-known, attractive and particularly reliable elementary text has been entirely reset in larger, clearer type. Chapter headings remain the same as in the first, 1933, edition. Cuts of modern industrial products and processes, new sections on the structure of liquids and solids, x-ray studies, photography, plastics, vitamins and animal nutrition considerably improve and modernize the subject-matter.

HUBERT N. ALYEA

PRINCETON UNIVERSITY

SPECIAL ARTICLES

INCREASED SYNTHESIS OF p-AMINOBENZOIC ACID ASSOCIATED WITH THE DEVELOPMENT OF SULFONAMIDE RESISTANCE IN STAPHYLOCOCCUS AUREUS

THE means by which bacteria become resistant to the bacteriostatic action of the sulfonamide drugs has remained obscure, although this phenomenon has been known for several years[1] and has been observed in a number of bacterial species.[2,3,4,5,6] While a sizable body of information on the technique of development of resistance *in vitro* has accumulated and its clinical analogue has been described, careful study has failed to disclose significant differences (in morphology, carbohydrate fermentation, growth rates, virulence, etc.) between sulfonamide-resistant and susceptible organisms. With the failure to solve the problem by the use of orthodox bacteriological procedures, the method of attack has shifted to the biochemical.

It has been postulated[7] and indirect evidence suggests[8,9,10] that increased p-aminobenzoic acid (PAB) synthesis by sulfonamide-resistant organisms may account for their lack of sensitivity to sulfonamides. This explanation has not been proved heretofore, since no adequate test for PAB was available. The development by Landy and Dicken[11] of a suitable microbiological method for the determination of PAB made possible a quantitative comparison of the amounts of PAB synthesized by sulfonamide-sensitive and resistant bacteria. Evidence presented in this report indicates that sulfonamide-resistant strains of *Staphylococcus aureus* produced significantly more PAB than the corresponding sensitive strains.

The cultures investigated were *S. aureus* strains 7 and 14 supplied by Dr. Wesley Spink, of the University of Minnesota Medical School, who has described their susceptibility to sulfonamide inhibition *in vitro*.[12]

[1] I. H. MacLean, K. B. Rogers and A. Fleming, *Lancet*, I: 562, 1939.
[2] C. M. MacLeod and G. Daddi, *Proc. Soc. Exp. Biol. and Med.*, 41: 69, 1939.
[3] L. Westphal, R. L. Charles and C. M. Carpenter, *Ven. Dis. Inform.*, 21: 183, 1940.
[4] E. Strauss, J. H. Dingle and M. Finland, *Jour. Immunol.*, 42: 313, 1941.
[5] E. Strauss, J. H. Dingle and M. Finland, *Jour. Immunol.*, 42: 331, 1941.
[6] H. N. Green, *Brit. Jour. Exp. Path.*, 21: 38, 1940.
[7] D. D. Woods, *Brit. Jour. Exp. Path.*, 21: 74, 1940.
[8] C. M. MacLeod, *Jour. Exp. Med.*, 72: 217, 1940.
[9] H. N. Green and F. Bielschowsky, *Brit. Jour. Exp. Path.*, 23: 1, 1942.
[10] G. S. Mirick, *Jour. Clin. Invest.*, 21: 628, 1942.
[11] M. Landy and D. M. Dicken, *Jour. Biol. Chem.*, 146: 109, 1942.
[12] J. J. Vivino and W. W. Spink, *Proc. Soc. Exp. Biol. and Med.*, 50: 336, 1942.

Resistance had been induced by exposure of the parent strains to increasing concentrations of sulfathiazole. These cultures were maintained by us in a chemically defined, PAB-free medium[13] which supported growth equivalent to that obtained with meat infusion broth. This medium was used in all the following experiments. The results of the determination of the sensitivity and resistance to sulfonamides of the *S. aureus* strains are given in Table I. Although

TABLE I

BACTERIOSTATIC ACTION OF THE SULFONAMIDE DRUGS ON PARENT AND RESISTANT STRAINS OF STAPHYLOCOCCUS AUREUS GROWN IN SYNTHETIC MEDIUM

Sulfonamide drug	Staphylococcus aureus 7		Staphylococcus aureus 14	
	Parent inoculum 620 org.	Resistant inoculum 315 org.	Parent inoculum 1860 org.	Resistant inoculum 124 org.
Sulfanilamide	1 = N 100 = P 500 = C	2,000 = N 12,500 = P 15,000 = C	50 = N 500 = P 1000 = C	20,000 = N
Sulfaguanidine	1 = N 10 = P 500 = C	2,000 = N 20,000 = P	10 = N 500 = P 1000 = C	20,000 = N
Sulfadiazine	10 = N 50 = P 100 = C	10,000 = N 12,500 = C	10 = N 50 = P 100 = C	10,000 = N 12,500 = C
Sulfathiazole	0.1 = N 0.5 = P 1.0 = C	1,000 = N 4,000 = P 8,000 = C	0.5 = N 1.0 = P 10 = C	4,000 = N 6,000 = P 8,000 = C

The concentration of sulfonamides is expressed in γ per 10 cc culture.
N = None, no inhibition of growth, growth same as control.
P = Partial inhibition of growth compared with control at 72 hours.
C = Complete inhibition of growth. No growth or cloudiness in 72 hours.

originally exposed only to sulfathiazole, it is evident that a high degree of resistance to the other sulfonamides has been established as well in both strains of *S. aureus*. More marked resistance to sulfadiazine and sulfathiazole than to sulfanilamide and sulfaguanidine appears to have been developed. It is possible, however, that this is more apparent than real, since fairly high concentrations of sulfanilamide and sulfaguanidine are tolerated by the parent strains while they are more sensitive to sulfadiazine and particularly to sulfathiazole.

For the determination of PAB synthesis by the parent and resistant strains of *S. aureus*, the cultures were transferred a number of times in the synthetic medium, thus reducing the possibility of carrying over any PAB. Plate counts after 24 hours incubation revealed that the populations of the four cultures were approximately equal. Small inocula of the cultures, averaging several thousand organisms, were introduced into 50 cc volumes of synthetic medium, incubated for 24 hours at 37° C, filtered through Seitz filters and the filtrates autoclaved and assayed for PAB by the method of Landy and Dicken.[11] The results of the assay of the *S. aureus* culture filtrates[14] are given in Table II. It will be seen that the data

TABLE II

p-AMINOBENZOIC ACID CONTENT OF STAPHYLOCOCCUS AUREUS FILTRATES AT VARYING ASSAY LEVELS
Parent Strains

Amount of filtrate per assay flask	p-Aminobenzoic acid			
	Found		Content	
	No. 7	No. 14	No. 7	No. 14
cc	γ	γ	γ per cc	γ per cc
1.00	.042	.047	.042	.047
0.50	.024	.024	.048	.048
0.25	.014	.013	.056	.051
0.10	.005	.005	.050	.050
Average			.049	.049

Resistant Strains

Amount of filtrate per assay flask	p-Aminobenzoic acid			
	Found		Content	
	No. 7	No. 14	No. 7	No. 14
cc	γ	γ	γ per cc	γ per cc
0.025	.080	.082	3.20	3.28
0.010	.033	.033	3.30	3.30
0.005	.016	.017	3.20	3.40
0.0025	.008	.009	3.20	3.60
Average			3.22	3.40

are in good agreement both as regards PAB content at various levels of assay and in the amounts found for strains 7 and 14. The quantity of PAB produced by resistant strains in contrast to that by parent strains is particularly impressive.

Since PAB was found in the culture filtrates in considerable amounts it was deemed advisable to measure its concentration by chemical as well as by microbiological means. Using the Litchfield-Marshall colorimetric test,[15] with crystalline PAB as the standard, values were obtained which were in fair agreement with those obtained by microbiological assay (Fig. 1). It should be pointed out that the diazo reaction could be employed here only because relatively large quantities of PAB were present,[16] since the method does not possess the sensitivity characteristic of the microbiological assay. It is well known that the chemical test is not specific for PAB, but is a measure of the concentration of primary aromatic amines.

S. aureus cultures were grown in the presence of

[13] The medium of M. Landy and D. M. Dicken, *Jour. Lab. and Clin. Med.*, 27: 1086, 1942, omitting sodium acetate, asparagine, guanine, xanthine and uracil. pH is readjusted to 7.5.

[14] Comparative assays performed on *S. aureus* filtrates and acid hydrolyzed whole cultures revealed that the bulk of PAB produced is secreted into the medium, since the filtrates contained almost as much as did the culture hydrolysates.

[15] J. T. Litchfield, Jr. and E. K. Marshall, Jr., SCIENCE, 88: 85, 1938.

[16] It is likely that the values obtained for PAB synthesis by the parent strains are in error since the quantity found for these strains by microbiological assay is known to be too small for detection by the chemical method.

sulfonamides[17] and the filtrates assayed for PAB. While the presence of sulfonamide in the culture filtrates made the subsequent quantitative measurement of PAB impossible (since sulfonamides inhibit the growth of *Acetobacter suboxydans*)[18] we were definitely able to ascertain that PAB synthesis was greater by resistant than by parent strains.

It is clear that sulfonamide-resistant staphylococci grown in a synthetic, PAB-free medium synthesize far more PAB than do the parent strains of the same

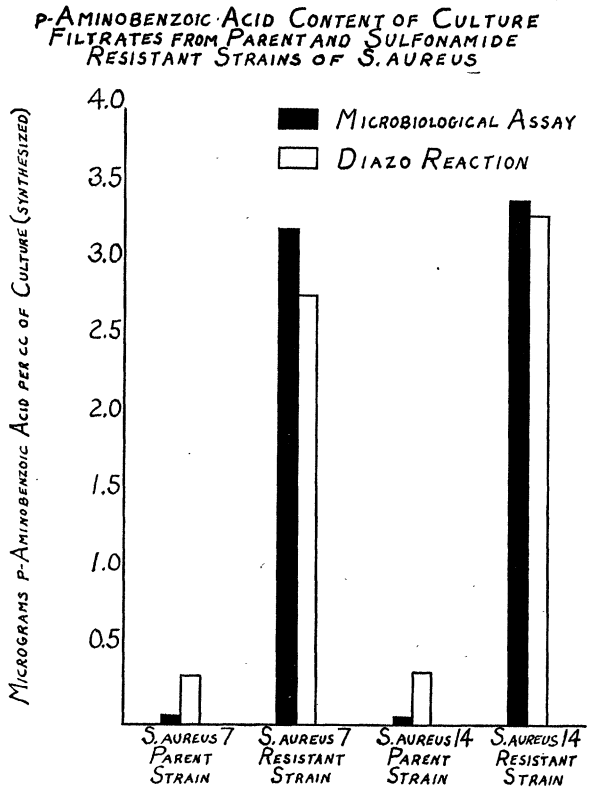

FIG. 1

organism. Under our test conditions this ratio is consistently in the order of 70 to 1. This great output stands in contrast to the amount of PAB synthesized by a variety of organisms representative of twelve bacterial genera.[19] None of these organisms studied produced PAB in quantities approaching that made by sulfonamide-resistant staphylococci. The average for all other organisms was 0.033 micrograms PAB per cc of culture, while the resistant staphylococci produced 3.3 micrograms of PAB per cc, or 100 times as much. Based on their inhibition by sulfonamides *in vitro* (Table I), the quantity of PAB synthesized by sulfonamide-resistant staphylococci is in considerable excess of that known to be required for reversal of the inhibitory action of the quantity of sulfonamide to which these organisms are resistant.[7]

Sulfonamide-resistant staphylococci continue to synthesize PAB far in excess of that normally produced by *S. aureus* for many generations following exposure to sulfonamides. It has been stated[6,20] that although sulfonamide fastness may be lost when only partially developed, well-established resistance is apparently retained indefinitely. Continued increased production of PAB by these resistant staphylococci may be considered as additional evidence for the permanence of sulfonamide fastness.

As a result of these and other data obtained from our PAB studies, we are of the opinion that the staphylococcus cell, becoming resistant to sulfonamides, undergoes fundamental changes in its PAB metabolism. That organisms may acquire resistance to sulfonamides without increasing their production of PAB is indicated by our studies[21] on *Escherichia coli, Vibrio cholerae, Shigella dysenteriae* and *Diplococcus pneumoniae*. Sulfonamide-resistant strains of these organisms failed to synthesize greater amounts of PAB than did their parent, non-resistant, strains. The possibility exists, however, that future investigation will reveal that these resistant organisms produce other "antisulfonamide metabolites" as yet unidentified, which do not support growth of our test organism, *A. suboxydans*.

SUMMARY

Sulfonamide-resistant strains of *Staphylococcus aureus* produce greater amounts of p-aminobenzoic acid than do their parent strains. This synthesis occurs both in the absence and in the presence of sulfonamides. The quantity of p-aminobenzoic acid synthesized by resistant strains appears sufficient to account for their resistance to sulfonamide drugs.

On the basis of this evidence, it is suggested that the development of ability to synthesize p-aminobenzoic acid in excess of the normal metabolic requirements, as a result of continued exposure to sulfonamides, explains the phenomenon of sulfonamide fastness in *Staphylococcus aureus*.

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17 Sulfanilamide and sulfadiazine were employed. The amounts used varied from 1/10 to the maximum concentration which would still allow growth equal to that of controls (*cf.* Table I).

18 M. Landy, N. W. Larkum and E. J. Oswald, *Jour. Bact.*, in press.

19 M. Landy, N. W. Larkum and E. J. Oswald, *Jour. Bact.*, in press.

20 L. H. Schmidt, C. Sesler and H. A. Dettwiler, *Jour. Pharmacol. and Exp. Therap.*, 74: 175, 1942.

21 M. Landy, N. W. Larkum, E. J. Oswald and F. Streightoff, to be published.