cardiogram and fluorocardiogram, both in the case with a physiological opening sound and in that with a pathological opening snap, indicates the following:

The part of the fluorocardiogram which corresponds to the isometric relaxation period

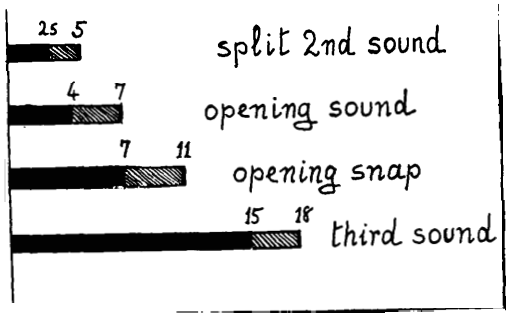

FIG. 3.
Scheme indicating the distance in hundreds of seconds between the two phases of a split or reduplicated second sound and between second sound and third sound. The black bar indicates the minimal distance, the striped bar, the maximal distance between the two phases of the reduplicated sound or between second and third sound. *Split second sound,* recorded over the pulmonic area. *Opening sound,* recorded over the mitral area in normal subjects. *Opening snap,* recorded over the apex or mitral area in patients with mitral stenosis. *Third sound,* recorded over the apex in normal subjects.

lies between the lowest point of the ventricular wave and the beginning of the rebound, and not that ending with the lowest point of the rebound, as suggested by others.[10] However, this period cannot be measured routinely on the various fluorocardiographic records because the diastolic rebound is not a constant feature; it may be represented by a less steep rise of the tracing or may be completely absent. According to our data, the figures found recently by others[2] for the isometric relaxation period are too long, especially because they did not study patients with mitral stenosis; as known, these may present the longest duration of the isometric relaxation period because of impaired opening of the mitral valve.

The results of our study, sketched in Fig. 3, facilitate basing the classification of an extra-sound on the above temporal data.

Summary. The isometric relaxation period of the left ventricle in normal subjects and in patients with mitral stenosis was studied. This period was measured on the phonocardiogram as the interval between the main vibration of the second sound and the opening sound or snap of the mitral valve.

The findings in 7 normal subjects varied between 0.04 and 0.07 seconds; 24 mitral patients had figures of from 0.07 to 0.11 seconds. Additional data on the split second pulmonic sound and the third heart sound are presented.

Simultaneous phonocardiograms and fluorocardiograms in a normal subject and in a patient with mitral stenosis, both having an opening sound or snap of the mitral valve, allowed correlation of the ventricular fluorocardiogram with the sound tracing in regard to the isometric relaxation period.

[10] Boone, B. R., Chamberlain, W. E., Gillick, F. G., Henny, G. C., and Oppenheimer, M. J., *Am. Heart J.*, 1947, **34**, 560.

16606

Staphylococcus aureus: Drug-fastness Studies with Penicillin and Sulfactin.*†

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Sulfactin, a new antibiotic produced by an Actinomyces, was described by Junowicz-Kocholaty, Kocholaty, and Kelner.[1] Its bacterial spectrum, toxicity, and therapeutic action in mice were described by Morton.[2] It was found that there was a very favorable ratio between

the toxic dose and the therapeutic dose in mice which had been infected with *Diplococcus pneumoniae,* type 1. Sulfactin is active against gram-positive microorganisms, primarily, and in this respect it resembles penicillin. We were interested to learn if a culture of a gram-positive microorganism such as *Staphylococcus aureus* readily acquired an increase in its resistance to sulfactin, whether a sulfactin-resistant strain of staphylococci showed any change in its susceptibility to penicillin, and whether a penicillin-resistant strain of staphylococci showed any increase in its resistance to sulfactin.

The supply of pure sulfactin was that mentioned previously.[2] The commercial sodium salt of penicillin was used. Stock solutions of both antibiotics were prepared in sterile distilled water and stored in the dry-ice box. Bacto-nutrient broth, adjusted to pH 7.4 was employed as the culture medium.

Penicillin-resistant and sulfactin-resistant staphylococcal cultures were developed *in vitro* by growing the test organism in the presence of varying concentrations of the antibiotics. From the stock solutions of each antibiotic serial two-fold dilutions were prepared in Bacto-nutrient broth, pH 7.4. All of the dilution tubes were then seeded with one drop of broth culture of *Staphylococcus aureus* and the tubes incubated at 37°C. Observations were usually made after 24 hours, but in some instances the incubation period was 48 or 72 hours. The tubes in the penicillin series and in the sulfactin series containing the highest concentration of the antibiotic which showed visible growth of staphylococci were selected for subculturing. New series of dilutions of penicillin and sulfactin were prepared and inoculated from the tubes in the previous series which had been selected for subculturing. This procedure was repeated until penicillin-resistant and sulfactin-resistant cultures of staphylococci were obtained. The extent of the tolerance of the cultures for the two antibiotics is shown in Table I. Parallel transfers of the parent culture of staphylococci were made in the Bacto-nutrient broth for a control.

Tests to determine the sensitivity to penicillin and to sulfactin were then made with the penicillin-resistant strain, the sulfactin-resistant strain and the normal culture of staphylococci. In becoming resistant to penicillin the staphylococcal culture did not change in its sensitivity towards sulfactin. Likewise when the culture of staphylococci became resistant to sulfactin it did not change in its sensitivity towards penicillin. Typical results of repeated tests are presented in Table II.

Results. After 7 serial transfers of a staphylococcal culture in Bacto-nutrient broth containing varying amounts of sulfactin, the culture grew in the presence of 10 μg of the drug. This represents an increase in resistance of the culture to the drug of over 1100 times that of the original strain (Table I). Under similar conditions the staphylococcal culture developed a resistance to penicillin of slightly over 32 fold. The acquisition of a tolerance for either penicillin or sulfactin by the staphylococcal culture was not accompanied by an increase in tolerance for the other antibiotic.

Discussion. The value of using drug-fast strains of microorganisms in studying new chemotherapeutic compounds was described by Ehrlich[3] and was advocated in the study of new antibiotics by Stansly,[4] Eisman *et*

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2 Morton, H. E., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 345.

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4 Stansly, P. G., *Science,* 1946, **103**, 402.

TABLE I.

Development of Resistance *in Vitro* of a Strain of *Staphylococcus aureus* (P 210) to Sulfactin and Penicillin.

Antibiotic	Sulfactin														Penicillin										
Tube No.	c	1	2	3	4	5	6	7	8	9	10	11	12	13	c	1	2	3	4	5	6	7	8	9
Approximate conc. of antibiotic per ml of medium	0	10 μg	5 μg	2.5 μg	1.25 μg	0.625 μg	0.312 μg	0.156 μg	0.078 μg	0.039 μg	0.019 μg	0.009 μg	0.004 μg	0.002 μg	0	10 U	5 U	2.5 U	1.25 U	0.625 U	0.312 U	0.156 U	0.078 U	0.039 U
Subculture 1	4	—	—	—	—	—	—	—	—	—	—	—	4	4	4	—	—	—	—	—	—	3	3	3
2	4	—	—	—	—	—	—	—	—	—	3	3	4	4	3	—	—	—	—	—	1	2	2	
3	3	—	—	—	—	—	—	—	—	2	3	3	3	3	4	—	—	—	—	—	1	2	2	
4	4	—	—	—	—	—	—	4	4	4	4				3	—	—	—	—	+	1	2	2	
5	4	—	—	—	—	—	4	4							4	—	—	2	2	3	3	4	4	
6	4	—	—	—	—	+	2	3							4	—	+	2	3	4				
7	4	3	3	3											4	+	2	3	4					

— = no growth.
4 = maximum amount of growth.
3 and 2 = proportionate amount of growth between 4 and 1.
1 = smallest amount of growth readily detected macroscopically.
+ = trace, or growth barely detectable macroscopically.

TABLE II.

Comparison of Typical Results Obtained by Testing the Parent Culture of *Staphylococcus aureus*, P 210, and the Penicillin-adapted and Sulfactin-adapted Strains of This Culture Against Penicillin and Sulfactin.

Antibiotic	Sulfactin										Penicillin									
Tube No.	c	1	2	3	4	5	6	7	8	9	c	1	2	3	4	5	6	7	8	9
Concentration of antibiotic per ml of medium	0	10 μg	5 μg	2.5 μg	1.25 μg	0.625 μg	0.312 μg	0.156 μg	0.078 μg	0.039 μg	0	10 U	5 U	2.5 U	1.25 U	0.625 U	0.312 U	0.156 U	0.078 U	0.039 U
Staphylococcus aureus																				
Parent culture	4	—	—	—	—	—	—	—	3	4	4	—	—	—	—	—	—	1	2	2
Penicillin-adapted strain	4	—	—	—	—	—	—	—	3	4	4	+	2	3						
Sulfactin-adapted strain	4	4	4	4							4	—	—	—	—	—	—	1	2	3

—, 1, 2, 3, and 4 = amount of growth as described in footnote to Table I.

al.,[5] Graessle and Frost,[6] and Sullivan *et al.*[7]

Therapeutic failures, as a result of a particular strain of an infecting parasite acquiring or possessing a tolerance to the drug, have been known since the advent of chemotherapy. The superior therapeutic action effected by the use of more than one chemotherapeutic agent was pointed out by Laveran[8] in his work with trypan red and arsenous acid in experimentally induced trypanosomiases. The rational of a plan of drug rotation in the case of certain bacterial infections was pointed out by Feirer, Meader and Leonard[9] and again by Duemling and Horton.[10]

Ungar[11] first demonstrated the synergistic effect *in vitro* and *in vivo* of chemotherapeutic agents (para-aminobenzoic acid and sulfapyridine) on an antibiotic (penicillin) and Carpenter, Bahn, Ackerman, and Stokinger[12] pointed out the advantages of combining 3 chemical substances with penicillin in order to prevent the development *in vitro* of drug-fast strains of the gonococcus. Although sulfactin is active against the same types of microorganisms against which penicillin is active, it nevertheless has a possible role in the field of chemotherapy.

It will be noted that the sensitivity of the staphylococcal strain to sulfactin at the beginning of the studies (Table I) is slightly different from that found for the parent culture later in the studies (Table II). A possible factor in explanation of this may be that a different lot of stock solution was employed in the later tests but this is not a complete explanation.

The magnitude of the resistance to sulfactin developed by a strain of staphylococci was intermediate between the increase of the same strain of staphylococci to penicillin and the increase in resistance of a strain of *Escherichia coli* to streptomycin.[13]

Summary. A strain of staphylococci in becoming resistant to penicillin did not show an increase in its resistance to sulfactin. A similar strain of staphylococci in becoming resistant to sulfactin did not show an increase in its resistance to penicillin. The increase in resistance to sulfactin developed by the strain of staphylococci was intermediate between the increment in resistance developed by staphylococci to penicillin and the increase in resistance developed by a strain of *E. coli* to streptomycin.

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16607

Quantitative Chemical Analysis of the Adrenal Glands of Wild Norway Rats.

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Rogers and Richter[1] pointed out that the wild Norway Rat has an adrenal gland 2-3 times as large as that of the domestic albino Norway Rat. This difference in size is limited to the cortex. Since the cortical hor-

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