interest was the persistence of a positive titer in the presence of YP antigen in the sera of rabbits with histoplasmosis after gross external symptoms of the disease had disappeared and blood cultures were no longer positive.

During the course of these studies on laboratory animals, two human patients appeared from whom *H. capsulatum* was isolated.‡ Their sera fixed complement in the presence of YP antigen in dilutions of 1:32 and 1:128. More than 30 "normal" persons from whom *H. capsulatum* was not isolated had no complement-fixing antibodies in the presence of YP antigen.

Summary. An antigen from the yeastlike phase of *Histoplasma capsulatum* fixed complement in the presence of antisera from experimentally infected rabbits from the 2nd to the 23rd weeks following infection. In contrast to histoplasmin, this antigen did not react with several other fungal antisera.

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16087

Sulfactin. Bacterial Spectrum, Toxicity and Therapeutic Studies.*

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A new antibiotic, sulfactin, has been described by Junowicz-Kocholaty, Kocholaty, and Kelner.[1] A description of the actinomyces, the method for growing the organism, and the purification and chemical properties of the antibiotic substance were given by the above authors. It is the purpose of this publication to present results of studies on the bacterial spectrum and the toxicity and therapeutic action in mice.

Experimental. The sterile crude culture filtrate of Actinomyces, R-30, was employed for determining the bacterial spectrum. In general, the *in vitro* antibacterial tests were carried out at the time similar tests were being made with actinorubin and lavendulin employing Bacto-nutrient broth adjusted to pH 7.3 and by the technic described previously by Kelner and Morton.[2] Since sulfactin is more inhibitory for gram-positive than for gram-negative organisms, it was standardized against *Staphylococcus aureus,* P210. The smallest amount of sulfactin per ml of Bacto-nutrient broth, pH 7.3 which prevented growth of *S. aureus,* under the conditions of the test, was designated as one dilution unit.

The crude culture filtrate of Actinomyces, R30, was titrated against *S. aureus,* P210, in Bacto-nutrient broth, pH 7.3, and in the same medium to which had been added 0.5% NaCl. In each medium the activity titrated to the same tube, representing 6,400 dilution units. Thus the antibacterial action of sulfactin is not appreciably reduced in the presence of 0.5% NaCl. The presence of 10% sterile, defibrinated, normal horse blood in the Bacto-nutrient broth with salt necessitated 10 times more sulfactin to inhibit *S. aureus.*

There was received on June 20, 1946, from Dr. Junowicz-Kocholaty about 60 mg of recrystallized sulfactin which had been dried over $CaCl_2$. By the agar streak technic of assay it was estimated that 0.0254 μg of this material equalled one unit. The material was put in solution as follows: To 10.43 mg of sulfactin was added one ml of chloroform and warmed to 45°C until dissolved. One ml of

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1 Junowicz-Kocholaty, R., Kocholaty, W., and Kelner, A., *J. Biol. Chem.,* 1947, **168**, 765.

2 Kelner, A., and Morton, H. E., *J. Bact.,* 1947, **53**, 695.

TABLE I.
Results of *in vitro* Antibacterial Tests with Sulfactin.

Organism	Strain	Amt of sulfactin/ml of medium inhibiting the strain
Aerobacter aerogenes	P41	>32
Alcaligenes faecalis	P61	>32
*Brucella abortus**	P62, P18A	>32
" *melitensis**	P80	>32
" *suis**	P64	>32
Chromobacterium violaceum	P104	>32
Eberthella typhosa	P11, P115	>32
Escherichia coli	P216	>32
" *communior*	P218	>32
" *neapolitania*	P161	>32
Klebsiella pneumoniae	ATCC No. 8050	>32
Mycobacterium smegmatis	P49	>32
Neisseria catarrhalis	P66	>32
Proteus vulgaris	P98, P188	>32
Pseudomonas aeruginosa	P2, P186A	>32
Salmonella enteritidis	P51	>32
" *paratyphi*	P198A	>32
" *schottmuelleri*	P35	>32
Sarcina lutea	P6	>32
Serratia marcescens	P4	>32
Shigella dysenteriae	P52A	>32
" *paradysenteriae*, Flexner	P21	>32
Streptococcus faecalis†	P122	>32
Trichophyton interdigitale		>32
Vibrio comma	P215	>32
Alpha streptococcus†	P26	32
Gamma streptococcus†	P169	32
Bacillus anthracis	P119	16
" *cereus*	P109	16
" *mycoides*	P34A, P58A	16
" *anthracis*	P60	8
Gaffkya tetragena	P140A	8
Bacillus subtilis	P219	4
Streptococcus pyogenes†	P24	4
Bacillus circulans		2
" *megatherium.*	P97	2
" *mycoides*	P33A	2
" *subtilis*	P23A	1
Diplococcus pneumoniae, type 1†	P27	1
Staphylococcus aureus	P210	1
Bacillus mesentericus	P112	0.5
" *mycoides*	P156	0.5
" *subtilis*	P7	0.5
Diplococcus pneumoniae, type 3†	P29	0.5
Gaffkya tetragena	P43	0.5
Neisseria sicca	P141A	0.5
Micrococcus aurantiacus	P103	0.25
" *lysodekticus*		0.25
" *roseus*	P120B	0.125
Corynebacterium xerose	P82	0.06
" *diphtheriae*	P1	0.03

* The tests with *Brucella* were made in Bacto-tryptose broth.

† Tests with *Streptococci* and *Diplococci* were carried out in Bacto-nutrient broth, pH 7.3, to which had been added 0.5% NaCl and 5% sterile, normal defibrinated horse blood.

95% ethyl alcohol was added then distilled water to make 10 ml. When assayed by the technic employing broth, the material appeared considerably more active than when assayed by the agar streak technic. In broth 0.0048 μg of sulfactin per ml inhibited *S. aureus*. It was found that a concentration of 0.0097 μg per ml of Bacto-nutrient broth to which had been added 0.5% NaCl and 5% sterile, normal, defibrinated horse blood prevented visible growth of *Diplococcus pneumoniae*, type 1, P28 for 48 hours at 37°C.

TABLE II.
The Toxicity of Sulfactin for White Mice by Intraperitoneal Injection.

Dose of sulfactin	No. of mice injected	No. of mice living	No. of mice dying
0.1 mg	5	5	0
0.5	5	5	0
1.0	5	4	1*
5.0	4	0	4†

* The animal was found dead on the morning of the 8th day. A gram-negative rod was isolated from the heart's blood. The tissues were too badly autolyzed for a critical pathological examination.

† All animals died within 3.5 hours after injection. All animals showed a reaction within 2 minutes after injection; one mouse had violent tremors. It was not entirely satisfactory working with concentrations of 5 mg of sulfactin per ml of water because of the insolubility of the product at that concentration. The sulfactin was put into solution with chloroform at 45-50°C, distilled water added, and most of the chloroform evaporated by bubbling a small stream of sterile air through the liquid. This resulted in a heavy white suspension, relatively free of chloroform vapor. It is not certain that the early deaths of the mice receiving 1 ml of the suspension containing 5 mg of sulfactin can be attributed to the pharmacologic action of the drug. Nothing abnormal could be detected by gross examination, except, perhaps, that the liver and kidneys appeared a bit pale. Microscopic examination revealed that the capillaries in the lungs contained an excessive number of polymorphonuclear leukocytes. The pancreas, at the periphery of the lobes in 3 of the 4 mice, showed well marked degeneration of the outer zone of acini. Large numbers of fragmented nuclei were found in the "reaction centers" of the thymus and lymphoid follicles of the spleen. Other tissues appeared essentially normal. Pathological studies did not permit an opinion of the mechanism of death of the animals.

Because of the high virulence of our strain, this organism was selected for therapeutic studies.

The toxicity of sulfactin was determined by intraperitoneal injection into white mice weighing 17 to 20 g each. The results are summarized in Table I. By applying the formula of Reed and Muench[3] to the results, the LD_{50} is calculated to be about 2.75 mg. The necessity for employing the LD_{50} is set forth in the footnotes to Table I.

The mice which survived the injections of sulfactin were sacrificed for pathological studies. Two mice which received one mg each of sulfactin were sacrificed 14 days after the injection of the drug. Possible slight enlargement of the liver was the only gross pathological change detectable. These changes were in no ways as extensive as those observed in mice following the injection of 0.75 mg of actinorubin.[4] Two mice which received 0.5 mg each of sulfactin were sacrificed 14 days after the injection and a possible enlargement of the liver was the only gross pathological change observable. Two mice which received 0.1 mg each of sulfactin were sacrificed 14 days after the injection and no gross pathological changes were detectable.

Twenty-one days post-injection 2 mice which had received one mg, 2 mice which had received 0.5 mg and 2 mice which had received 0.1 mg sulfactin each were sacrificed. Nothing abnormal could be observed microscopically. None of the organs of the mice sacrificed 14 days and 21 days post-injection showed anything abnormal by microscopic examination. We are indebted to Dr. Herbert Ratcliffe for the pathological studies on these animals.

For a preliminary test of the therapeutic potentialities of sulfactin it was tested against *D. pneumoniae,* type I in mice. Serial dilutions of a 24-hour-old blood broth culture of the test organism were made in nutrient broth. One ml portions of the various dilutions of the test organism were inoculated intraperitoneally into mice to determine its pathogenicity, and one ml of each dilution was made into a poured blood agar plate to estimate the number of organisms constituting a lethal dose. Those mice receiving sulfactin received it intraperitoneally within a few seconds after the injection of the culture. The results are summarized in Table II. The results of the protection test made on July 2nd appeared so promising that a second ex-

[3] Reed, L. J., and Muench, H., *Am. J. Hyg.,* 1938, **27**, 493.

[4] Morton, H. E., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 327.

TABLE III.
Ability of Sulfactin to Protect Mice Infected with *Diplococcus pneumoniae*, Type 1.

Diln. of culture, 1 ml/mouse	No. of organisms dose*	No. of mice inj.	Amt of sulfactin inj.	No. of mice living	No. of mice dying
		Experiment of July 2, 1946.			
10^{-5}	∞	5	0	0	5
10^{-6}	284	5	0	0	5
10^{-7}	28	5	0	0	5
10^{-8}	5	5	0	0	5†
10^{-9}	0	5	0	3	2†
10^{-5}	1000 M.L.D.	5	0.1 μg	0	5†
10^{-5}	" "	5	0.5 "	0	5†
10^{-5}	" "	10	1.0 "	5	5†
10^{-5}	" "	5	2.0 "	1	4‡
10^{-5}	" "	5	10.0 "	5	0
		Experiment of July, 10, 1946.			
10^{-5}	∞	5	0	0	5
10^{-6}	>300	5	0	0	5†
10^{-7}	51	5	0	0	5
10^{-8}	8	5	0	1	4†
10^{-9}	1	5	0	4	1†
10^{-10}	1	5	0	5	0
10^{-5}	<1000 M.L.D.	10	0.5 μg	0	10†
10^{-5}	" "	10	1.0 "	0	10†
10^{-5}	" "	10	5.0 "	4	6†
10^{-5}	" "	10	10.0 "	6	4†

* No. of organisms estimated by inoculating 1 ml, the same amount as was injected into each mouse, into a poured blood agar plate.

† *D. pneumoniae* isolated from the heart's blood at autopsy.

‡ Three mice died between the 2nd and 3rd day after injection and pneumococci were isolated from the heart's blood of each. One mouse was found dead in the cage during the 6th day with its nose chewed. The heart's blood culture was sterile, so it is doubtful that it died of pneumococcal infection.

periment was made eight days later. In the first experiment the amount of sulfactin which protected 50 per cent of the mice against 1,000 minimum lethal doses of culture was in the order of 1 or 2 μg. This compares quite favorably with the LD_{50} toxic dose of about 2,750 μg. In the second experiment the challenging dose of culture may have been slightly less than 1000 M.L.D. The sulfactin was another lot prepared from the original purified material. The amount of sulfactin which protected against nearly 1000 M.L.D. of culture was of the order of 7.5 μg. This is still a quite favorable comparison to the toxic dose. There was not sufficient material available for further toxicity and therapeutic studies.

Studies on drugfastness and comparison with penicillin in this respect have been made and will be reported later.

Summary. Sulfactin is active primarily against gram-positive microorganisms. The LD_{50} for mice of 17-20 g by intraperitoneal injection is about 2,750 μg. The amount of sulfactin required to protect mice against the intraperitoneal injection of approximately 1000 minimum lethal doses of *Diplococcus pneumoniae*, type 1, is of the order of 1 to 7.5 μg.