IV-4 gives a better yield than IV-1, and since the α-globulins are present in lesser amounts in IV-4 than in IV-1, one may suspect that the α-globulins are not responsible; contrariwise, the β_1-globulins are present in greater amount in IV-4, and could be suspected of containing the precursor. The electrophoretic experiment above, although not decisive, points to the β-globulins.

Summary. 1. The precursor of bradykinin is contained in Fraction IV of the bovine plasma proteins (Cohn's nomenclature). 2. Of the 2 main subfractions, IV-4 yields approximately twice as much bradykinin as IV-1.

I wish to thank Dr. C. A. Dragstedt for advice in preparation of the manuscript.

1. Rocha e Silva, M., Beraldo, W. T., and Rosenfeld, G., *Am. J. Physiol.*, 1949, v156, 261.

2. Beraldo, W. T., *Am. J. Physiol.*, 1950, v163, 283.

3. Neurath, H., Volkin, E., Erickson, J. O., Craig, H. W., Putnam, F. W., and Cooper, G. R., *Am. J. Syphilis, Gonorrhea and Venereal Dis.*, 1947, v31, 347.

4. Cohn, E. J., Gurd, F. R. N., Surgenor, D. M., Barnes, B. A., Brown, R. K., Derouaux, G., Gillespie, J. M., Kahnt, F. W., Lever, W. F., Liu, C. H., Mittelman, D., Mouton, R. F., Schmid, K., and Uroma, E., *J. Am. Chem. Soc.*, 1950, v72, 465.

5. Cohn, E. J., Strong, L. E., Hughes, W. L., Jr., Mulford, D. J., Ashworth, J. N., Melin, M., and Taylor, H. L., *J. Am. Chem. Soc.*, 1946, v68, 459.

6. Edsall, J. T., *Advances in Protein Chemistry*, 1947, v3, 383.

7. Rocha e Silva, M., *Bradicinina.* Thesis, Faculty of Medicine of the University of Brazil, Sao Paulo, 1951.

Received January 9, 1952. P.S.E.B.M., 1952, v79.

Cross-Resistance Studies with Streptomycin, Streptothricin, Neomycin, and Streptolin. (19378)

JOSEPH F. PAGANO, MARVIN J. WEINSTEIN, AND RICHARD DONOVICK.

From the Squibb Institute for Medical Research, New Brunswick, N. J.

Several studies have been reported concerning the cross-resistance of various bacterial species with streptomycin and streptothricin (1-4) and neomycin(5-8). In the present study, cross-resistance has been found among this chemically and biologically related group of antibiotics, but not with every organism studied.

Methods. Strains of *Escherichia coli* (D-56), *Streptococcus fecalis* (ATCC-9790), *Pseudomonas aeruginosa* (D-163), *Micrococcus pyogenes var. aureus* (209P), *Salmonella schottmulleri* (D-51), *Bodenheimer's bacillus* (D-78) and one strain of *Escherichia coli* previously made resistant to chloromycetin, aureomycin, and terramycin, designated *E. coli-R* (D-56-9), were used in this study. Several of these organisms were made resistant to either streptomycin hydrochloride (Squibb, 808 u/mg), streptothricin hydrochloride (Merck, 500 u/mg) or neomycin sulfate (Squibb, 175 u/mg) by repeated exposure to increasing amounts of the antibiotic in yeast beef broth medium (Difco, pH 6.8). Cultures were incubated at 37°C for 1-3 days. The minimal inhibiting concentrations (MIC) of antibiotic were determined by the agar dilution method, the organism being streaked on a series of yeast beef agar plates containing increasing concentrations of the antibiotic. Prior to streaking, the cultures were centrifuged for 30 minutes at 2000 RPM, washed and resuspended in saline. It was noted that the MIC in broth cultures was usually lower than the MIC observed on agar plates. To determine the degree of resistance and cross-resistance developed, an organism made resistant to one of the antibiotics was simultaneously streaked on agar plates containing the other antibiotics. Two-fold increases in concentration of antibiotic were utilized. When the organism grew well at one level and was inhibited completely by the next higher level of antibiotic, the MIC was considered as the mid-point between the two levels. Repeat testing of cross-resistance indicates that the variation

TABLE I. Sensitivity of Parent Culture to Various Antibiotics.

Organism	Strepto-mycin·HCl, Squibb, MIC (mg/ml)	Streptothri-cin·HCl, Merck, MIC (mg/ml)	Neomycin·SO_4, Squibb, MIC (mg/ml)	Strepto-lin, MIC (mg/ml)
E. coli	.0074	.012	.043	.09
S. fecalis	.046	.15	.17	.72
M. pyogenes var. aureus	.0025	.0044	.014	<.03
P. aeruginosa	.015	.02	.057	.24
S. schottmulleri	.0074	.009	.029	.06
Bodenheimer's bacillus	>1.2	.0044	.029	—
E. coli-R	.011	.024	.029	—

TABLE II. Cross-Resistance Behavior.

Organism	Factor* of induced resistance to:	Factor of increased resistance to:		
	A. Streptomycin HCl (Squibb)	Streptomycin HCl (Squibb)	Streptothricin HCl (Merck)	Neomycin HCl (Squibb)
E. coli	>338		25	11
	30		33	11
S. fecalis	> 54		1	1
M. pyogenes var. aureus	>480		4	2
P. aeruginosa	>167		10	25
S. schott.	>338		1	2
	19		1	2
E. coli-R	>227		1	2
	B. Streptothricin HCl (Merck)			
E. coli	>250	62		16
S. fecalis	10	1		2
M. pyogenes var. aureus	114	37		24
	34	12		4
P. aeruginosa	38	31		12
Bodenheimer's bacillus	>682	1		12
E. coli-R	>125	42		12
	C. Neomycin SO_4 (Squibb)			
P. aeruginosa	90	8	5	
	16	6	8	
S. fecalis	27	3	2	

* Factor $= \frac{\text{MIC (mg/ml) of resistant strain}}{\text{MIC (mg/ml) of parent strain}}$.

in resistance observed by this assay method was never greater than 2-fold. An increase in resistance of 4-fold or more is therefore considered significant. The MIC of the parent sensitive strains are shown in Table I. The experimental results obtained are presented in Tables II and III. A number of the resistant organisms were also tested for cross-resistance with different preparations of neomycin [Neomycin sulfate, Pfizer (180 u/mg); Neomycin sulfate, Abbott (250 u/mg); Neomycin B hydrochloride, Squibb (280 u/mg); Neomycin C hydrochloride, Squibb (170 u/mg); a Squibb preparation of streptothricin sulfate (161 u/mg); and the antibiotic streptolin hydrochloride (30 u/mg)]. The results obtained are presented in Table III. With all organisms, attempts were made to induce the initial resistance to each of the individual antibiotics before testing for cross-resistance, however, where such strains are not reported, difficulty was encountered in inducing resistance.

Results. E. coli. Resistance to streptomycin or streptothricin resulted in cross-resistance (Table II). When the resistance to streptomycin was increased from 30-fold to greater than 338-fold, there was no detectable increase in the level of cross-resistance.

S. fecalis. An increase in resistance to either

TABLE III. Cross-Resistance Behavior to Streptolin and Different Preparations of Neomycin and Streptothricin.

Organism	Antibiotic* and factor† of increase in resistance		Factor of increased resistance to: Neomycin SO_4 (Pfizer)	Neomycin SO_4 (Abbott)	Neomycin B HCl (Squibb)	Neomycin C HCl (Squibb)	Streptothricin SO_4 (Squibb)	Streptolin HCl
E. coli	Stm	>338	10	6	6	5	40	8
	Str	>250	6	5	5	5	>129	32
S. fecalis	Stm	> 54	1	1	1	1	1	1
	Str	10	1	2	2	1	3	4
M. pyogenes var. aureus	Str	114	20	19	18	10	> 59	>24
P. aeruginosa	Stm	>167	9	10	8	4	10	4
	Str	38	10	8	>6	3	6	16
	Neo	90	>36	36	17	>4	2	—
S. schott.	Stm	>338	1	2	1	1	> 1.5	4

* Stm—Streptomycin·HCl (Squibb); Str—Streptothricin·HCl (Merck); Neo—Neomycin·SO_4 (Squibb).

† Factor $= \frac{\text{MIC (mg/ml) of resistant strain}}{\text{MIC (mg/ml) of parent strain}}$.

streptomycin, streptothricin, or neomycin was not accompanied by cross-resistance.

Micrococcus pyogenes var. aureus. Resistance to streptothricin resulted in a simultaneous increase in resistance to streptomycin and neomycin. Further increases in resistance to streptothricin resulted in an increase in the level of cross-resistance which was approximately proportional to the increase in streptothricin resistance. A greater than 480-fold increase in streptomycin resistance, however, did not result in any cross-resistance to neomycin or any significant cross-resistance to streptothricin.

P. aeruginosa. Resistance to streptomycin or streptothricin resulted in cross-resistance. When made resistant to neomycin (90-fold), only a 5 to 8-fold increase in resistance to streptomycin or streptothricin was observed. Increasing the level of neomycin resistance from 16-fold to 90-fold did not result in any increase in cross-resistance.

S. schottmulleri. A greater than 338-fold increase in resistance to streptomycin resulted in no increase in resistance to streptothricin or neomycin.

Bodenheimer's bacillus. A greater than 682-fold increase in resistance to streptothricin resulted in a 12-fold increase in resistance to neomycin.

E. coli-R. After being made resistant to streptothricin, a simultaneous increase in resistance to streptomycin and neomycin was observed. However streptomycin resistance did not result in cross-resistance to streptothricin or neomycin.

Cross-resistance with streptolin and various preparations of neomycin and streptothricin (Table III). *E. coli* resistant to streptomycin or streptothricin, *Micrococcus pyogenes var. aureus* resistant to streptothricin, and *P. aeruginosa* resistant to streptothricin, all showed cross-resistance with streptolin as well as with the other antibiotics used in this study. *S. fecalis* made resistant to streptomycin showed no cross-resistance and when made streptothricin-resistant showed minimal (4-fold) resistance to streptolin. Streptomycin-resistant *S. schottmulleri* showed a minimal (4-fold) increased resistance to streptolin. In no case do the latter two organisms show cross-resistance with any of the other antibiotics used in this study.

When the organisms shown in Table III were tested for cross-resistance to the different preparations of neomycin and a Squibb preparation of streptothricin, cross-resistance was confirmed in every case. Resistance levels are of the same order of magnitude and the slight variations in MIC levels are not considered significant.

Discussion. In the present study, and confirming previous reports with other strains of *E. coli*(5,7,9,10), *E. coli-R* (D56-9) made resistant to streptomycin showed no cross-resistance with streptothricin and neomycin. However. *E. coli* (D-56), resistant to streptomycin showed a simultaneous increase in resistance to streptothricin and neomycin. It is evident that within the same species there exists the potentiality for developing strains which may or may not show cross-resistance. The mechanism governing this phenomenon is now under investigation.

The strain of *S. fecalis* used in these studies appears to develop mono-resistance. Resistance to either streptomycin, streptothricin, or neomycin was not accompanied by a simultaneous increase in resistance to the other antibiotics considered in this investigation. Since a limited number of resistant cultures were examined, no extensive generalizations can be made concerning the observed lack of cross-resistance in this organism. Further study may reveal in *S. fecalis* the two types observed in strains of *E. coli.*

No cross-resistance with neomycin was observed when *M. pyogenes var. aureus* or *E. coli-R* was made initially resistant to streptomycin. The former organism showed only a minimal (4-fold) increase in resistance to streptothricin while the latter showed no resistance. However, when made initially resistant to streptothricin, both organisms showed cross-resistance to streptomycin and neomycin. It would appear that cross-resistance in these two organisms is related to the pathway of development of the initial resistance. In order to elucidate this behavior a more detailed study of the various types is necessary. The cross-resistance behavior observed provides evidence for considering streptomycin, streptothricin, neomycin, and streptolin as a biologically as well as a chemically related group of antibiotics. When a given organism was made resistant to one antibiotic, its cross-resistant behavior with the other 3 antibiotics was consistent. The organism either showed increased resistance to all of the other 3 antibiotics or to none of them. A biological relationship based on cross-resistance patterns is now well established for the penicillins (11, 12), streptomycins(13,14) and between aureomycin, chloromycetin, and terramycin(15-18).

In the present studies, the inhibiting concentration of an antibiotic for an organism was obtained by the agar dilution method. This procedure permits distinguishing total population growth from that of individual colonies appearing along the streak line. Such colonies may be mutants which arise in the population and do not represent the resistance level of the total population. Determination of culture sensitivity to an antibiotic in a liquid medium does not permit visual differentiation between the resistance of the total population and that of the individual cells.

Summary. Cross-resistance of a number of organisms to streptomycin, streptothricin, neomycin, and streptolin has been observed by the agar dilution method. Variability of cross-resistance behavior within a species is reported.

1. Sullivan, M., Stahly, G. L., Birkeland, J. M., and Myers, W. G., *Science,* 1946, v104, 397.

2. Hayano, S., *Jap. J. Antibiotics,* 1949, v2, 281.

3. Hosoya, S., Solda, M., Komatsu, N., Iwasaki, M., Sonoda, Y., and Uchida, M., *Jap. J. Exp. Med.,* 1949, v20, 117.

4. Kuroya, M., Ishida, N., Katagiri, K., Konno, J., Kikuchi, M., and Mizuguchi, R., *Jap. J. Antibiotics,* 1950, v3, 223.

5. Waksman, S. A., Lechevalier, H. A. and Harris, D. A., *J. Clin. Invest.,* 1949, v28, 934.

6. Waksman, S. A., and Lechevalier, H. A., *Antibiotics and Chemotherapy,* 1951, v1, 125.

7. Demerec, M., and Demerec, R., *Bact. Proc.,* 1950, 101.

8. Gezon, H. M., and Fasan, D. M., *Science,* 1951, v114, 422.

9. Waksman, S. A., and Lechevalier, H. A., *Science,* 1949, v109, 305.

10. Kelner, A., and Morton, H. E., *J. Bact.,* 1947, v53, 695.

11. Eisman, P. C., Marsh, W. S., and Mayer, R. L., *Science,* 1946, v103, 673.

12. Dowling, H. F., Hirsh, H. L., and O'Neil, C. B., *J. Clin. Invest.,* 1946, v25, 665.

13. Rake, G., McKee, C. M., Pansy, F. E., and Donovick, R., PROC. SOC. EXP. BIOL. AND MED., 1947, v65, 107.

14. Donovick, R., and Rake, G., *J. Bact.,* 1947, v53, 205.

15. Herrell, W. E., Heilman, F. R., and Wellman,

W. E., *Ann. N. Y. Acad. Sci.*, 1950, v53, 448.
16. Pansy, F. E., Khan, P., Pagano, J. F., and Donovick, R., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 618.
17. Kaipainen, W. J., *Ann. Med. Exp. Biol. Fenn.*, 1951, v29, Suppl. I.
18. Fusillo, M. H., and Romansky, M. J., *Antibiotics and Chemotherapy*, 1951, v1, 107.

Received January 16, 1952. P.S.E.B.M., 1952, v79.

Blood Changes of Normal Dogs During Chronic Blood Volume Expansion with Dextran.* (19379)

L. H. MARSHALL, C. H. HANNA, HEINZ SPECHT, AND P. A. NEAL.

From the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, Federal Security Agency, Bethesda, Md.

Blood changes occurring during long continued administration of dextran have been studied in normal dogs.

Methods. Three litter mate mongrel bitches weighing 13-16 kg were fed equal parts dog chow and horse meat in amounts adjusted to maintain body weight. They received intravenously 10, 15 and 20 ml dextran solution (Macrodex†) per kg respectively, 3 times weekly for 13 weeks. This amounted to approximately 300, 500 and 600 g dextran respectively. A control dog from the same litter received 10 ml/kg physiological saline. Preceding the injections 8 ml of blood were withdrawn without stasis and heparinized. Hematocrit values were determined in Wintrobe tubes spun 30 min at 1050 G. Estimations of relative viscosity were made within 20 min of withdrawing the sample in an Ostwald viscosimeter at 3 different pressures. Specific gravities of whole blood and of plasma were measured by the copper sulfate method. Values for the sedimentation of the cells after standing undisturbed for 60 min were obtained within 3 hrs of withdrawing the sample. Hemoglobin was converted to acid hematin and read on the Klett-Summerson photoelectric colorimeter. Mean arterial blood pressures were recorded at weekly intervals by direct puncture of a femoral artery. Estimations of glomerular filtration rate (creatinine), effective renal plasma flow and maximal tubular transfer (p-amino hippurate) were made(1) on 2 dogs receiving dextran and on the control before and after 7 to 12 wks of injections.

Results. The repeated injections of dextran were well tolerated. Body weights, after an initial fall during the control period, were maintained with an increase in food intake of 25 g (10-12.5% of the total diet). Mean arterial blood pressures varied within normal limits. Plasma specific gravity was 1.022 to 1.024 in all dogs throughout the experiment. The renal function tests revealed no significant variations from the control. In agreement with the report of Thorsen(2) no significant pathological changes were found by microscopic examination‡ of the tissues of the dog that received 10 ml/kg Macrodex. The tissues of this dog were fixed in aqueous formalin and therefore were not suitable for demonstrating possible dextran deposits.

Average weekly changes in the hematocrit percentage and relative viscosity are shown in Fig. 1, where the mean of the preinjection

TABLE I. Preinjection Mean Values Taken as 100% in Fig. 1 for Hematocrit Volume and Relative Viscosity.

Subsequent treatment Sol.	Dose, ml/kg	Hematocrit vol, %	Relative viscosity
Saline	10	41.4	4
Dextran	10	46.9	4.68
	15	48.8	4.88
	20	41.3	4.01

* Presented before the American Physiological Society, Salt Lake City, Utah, Sept. 6-8, 1951.

† Solution used was the Swedish product "Macrodex," 6% dextran in 0.9% NaCl.

‡ We are indebted to Dr. Benjamin Highman of the Laboratory of Pathology and Pharmacology for this examination.