Eck fistula in one animal suggests that the liver must play a part in the degradation of these hormones. No evidence was obtained that reducing steroids are excreted in the dog's bile.

The reduced excretion of corticosteroids that eventually occurs in the Eck fistula animal can best be explained on the basis of debility and inanition which developed during the postoperative period of study.

Summary. Normal values for neutral 17-ketosteroids and reducing corticoids have been presented for adult female mongrel dogs. Stimulation of the adrenal glands of the dog by continuous intravenous infusion of ACTH increased both the reducing corticoid and neutral 17-ketosteroid excretion. Additional observations are reported regarding factors that influence the excretion of reducing corticoids by the adult female dog.

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Antibiotic Combinations and Resistance: Response of *E. coli* to Antibiotics, Singly and in Pairs.* (20807)

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In a previous study(1) it was shown that the development of resistance in strains of staphylococci and in a strain of enterococcus to penicillin (Pen), streptomycin (S) or erythromycin (E) *in vitro* could be depressed and delayed by repeated subcultures in the presence of combinations of E with either Pen or S. Further observations(2) with strains of staphylococci and certain streptococci repeatedly subcultured in Pen, S, E, oxytetracycline (Terramycin, "T") and coloramphenicol (C) and in 8 additional pairs of these antibiotics confirmed these findings and indicated further that: 1) the rate and degree of resistance which developed against individual antibiotics to which the organisms were exposed varied with the antibiotics and sometimes with the strain of organism; 2) subcultures on the pairs of antibiotics resulted in increased resistance to the homologous mixture, but usually of lower degree than against the individual components when used separately; 3) when increases in resistance to the

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TABLE I. Sensitivity of 6 Parent Strains of *E. coli* to Antibiotics Singly and in Certain Pairs.

				Minimum inhibiting concentration, μg/ml									
S	C	T	P	S+C	S+T	C+T	S+P	C+P	T+P	A	N	Pen	B	E
													×1000	
6.3	6.3	6.3	1.6	3.1+3.1	6.3+3.1	3.1+1.6	6.3+.8	6.3+.8	6.3+1.6	12.5	3.1	200	15	100
6.3	25	"	1.6	6.3+6.3	"	6.3+3.1	"	"	"	"	12.5	"	"	200
6.3	6.3	"	.8	"	"	3.1+1.6	"	3.1+.4	3.1+ .8	"	"	400	"	"
6.3	12.5	"	.8	"	"	"	"	"	6.3+1.6	25	6.3	200	"	"
12.5	6.3	"	3.1	"	"	"	"	6.3+.8	"	"	"	"	"	"
6.3	25	"	1.6	"	"	6.3+3.1	"	"	"	"	"	"	"	"

S = streptomycin; C = chloramphenicol; T = Terramycin (oxytetracycline); P = polymyxin B; A = Aureomycin (chlortetracycline); N = neomycin; Pen = penicillin; B = bacitracin; E = erythromycin.

homologous individual components differed appreciably, the increase in resistance which developed against the pair corresponded more closely to that of the antibiotic which alone produced the smaller change; and 4) increases in resistance to the individual components of a mixture resulted from repeated exposures to the combination, but these increases were generally of smaller magnitude than those which developed against the same antibiotics when used separately. The present report deals with further extensions of these observations to strains of *Escherichia coli* using S, T, C and polymyxin B (Aerosporin, "P") separately and in mixtures of all 6 possible pairs.

Materials and methods. The 6 strains of *E. coli* used in this study were recently isolated from the blood or urine of patients and identified by Marion E. Lamb of the Mallory Institute of Pathology. The polymyxin B (Aerosporin) was furnished by Dr. Donald S. Searle of Burroughs Wellcome & Co.; the other antibiotics were similar to those employed in the previous studies(1,2). Heart infusion agar (Difco), pH 7.2±, was used with and without the incorporation of serial 2-fold concentrations of the antibiotics, singly and in combinations; pairs of antibiotics were always used in a constant ratio for each pair, the proportions being determined by the relative sensitivity of the parent strains to the respective antibiotics. The serial subcultures and tests for sensitivity and cross-resistance were carried out as in previous studies(1-3).

Results. Resistance to homologous antibiotics. The sensitivity of the 6 parent strains of *E. coli* to all of the antibiotics used in this study are listed in Table I. It is seen that the antibiotics used in the various combinations exerted no important synergistic or antagonistic effects on the parent strains. In general the minimum inhibiting concentration (M.I.C) of each of the antibiotics in the combinations was the same as that of the component which was most active, except for mixtures C + T and C + P which appeared to be approximately additive, *i.e.*, about ½ the M.I.C. of each of the components was required when they were used together. The changes in the M.I.C. of the homologous antibiotics resulting from 20 successive subcultures on agar containing S, C, T or P, individually or in pairs, are depicted in Fig. 1 and 2. There were some individual variations among the strains in the rate and degree to which resistance increased. Marked and rapid increases in resistance occurred regularly with S and C; increases in resistance to T also occurred in all strains but these were of smaller magnitude, while little or no increases in resistance to P occurred, except in strain 2. The increases in resistance to homologous pairs of antibiotics were most marked with C + T and only slightly less with S + C; only small increases in resistance or none at all resulted from the subcultures in each of the other 4 combinations.

Resistance and cross-resistance to individual antibiotics. The changes in resistance to individual antibiotics that resulted from the 20 subcultures of each of the 6 strains of *E. coli* in S, C, T or P, separately and in pairs, are listed in Table II. No significant changes in sensitivity or resistance to Pen, bacitracin (B), or E resulted from these subcultures; the results of the tests with these antibiotics are, therefore, not listed in this table. The exposures to S alone resulted in slight increases in sensitivity of most of the strains to C, T and chlortetracycline (Aureomycin,

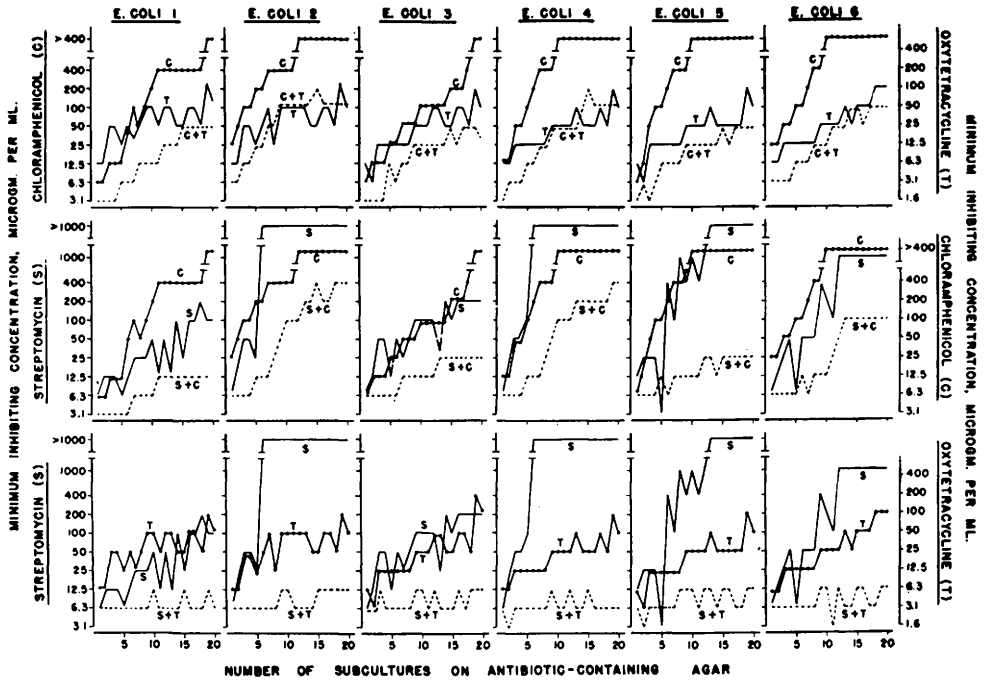

FIG. 1. Resistance developing during successive subcultures on streptomycin, chloramphenicol or oxytetracycline, alone and in pairs.

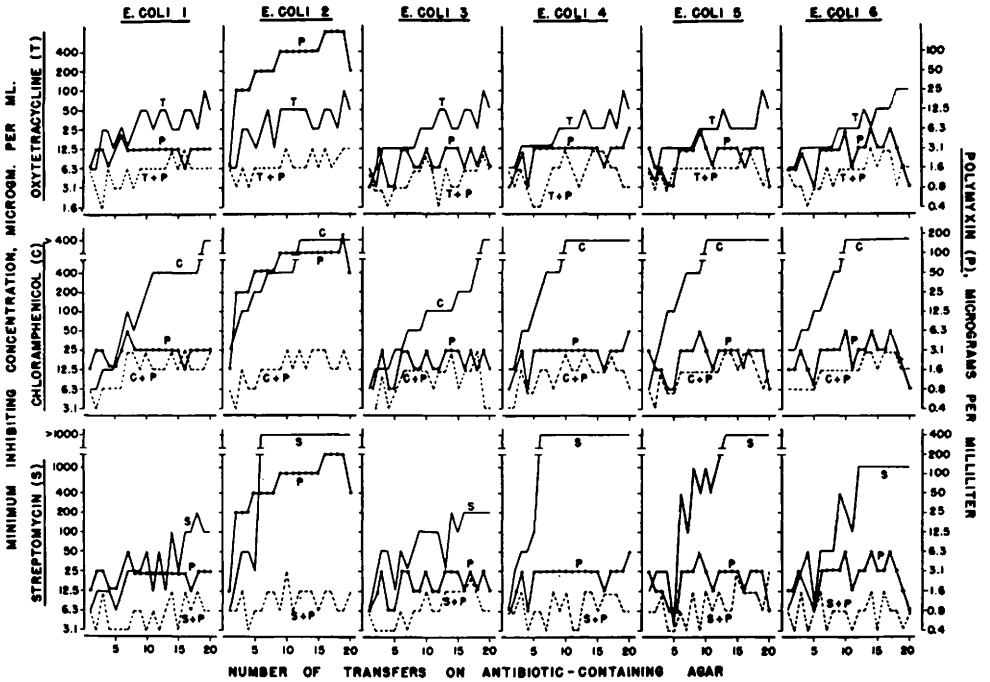

FIG. 2. Resistance to polymyxin combined with streptomycin, chloramphenicol, or oxytetracycline and to each antibiotic individually.

TABLE II. Changes in Sensitivity of *E. coli* after Subcultures on Antibiotic-Containing Media.*

Antibiotic used in tests†	*E. coli*, strain No.	Antibiotic(s) on which strain was subcultured 20 times: S	C	T	P	S+C	S+T	C+T	S+P	C+P	T+P
S	1	16	½	1	1	4	800	1	2	1	1
	2	800	¼	¼	2	800	800	½	40	½	½
	3	32	1	½	1	4	16	½	8	1	1
	4	800	½	½	1	800	8	1	8	½	1
	5	800	¼	½	1	16	160	1	32	½	½
	6	80	½	1	½	800	8	1	2	½	½
C	1	½	128	4	1	8	1	128	1	2	8
	2	¼	64	4	¼	64	2	64	½	4	2
	3	½	64	2	½	4	1	64	1	4	1
	4	¼	64	8	1	64	1	64	1	4	8
	5	½	64	2	1	8	4	64	½	8	4
	6	½	64	8	½	32	2	64	1	2	2
T	1	¼	32	16	1	1	2	16	¼	4	2
	2	¼	8	8	1	2	1	32	¼	4	2
	3	½	2	8	½	1	1	8	½	2	2
	4	⅛	4	8	1	2	1	32	½	1	2
	5	½	8	8	1	2	1	8	¼	1	2
	6	¼	4	16	1	2	2	32	1	1	2
P	1	1	¼	1	2	1	1	1	½	4	1
	2	1	¼	1	32	2	1	2	1	1	8
	3	1	½	1	1	1	1	½	1	2	1
	4	2	2	1	4	2	1	2	2	8	1
	5	1	½	½	1	2	1	2	1	1	1
	6	½	½	1	2	1	1	1	1	½	2
A	1	1	64	8	1	4	4	16	½	2	2
	2	¼	8	8	1	4	2	16	½	8	2
	3	¼	2	8	1	1	2	8	½	2	2
	4	½	8	4	2	1	1	8	1	4	2
	5	¼	32	4	1	1	1	4	1	2	2
	6	¼	8	16	1	2	2	16	1	4	4
N	1	32	¼	1	1	1	4	½	4	1	1
	2	2	⅛	¼	2	2	8	1	½	1	1
	3	32	1	¼	½	8	16	1	2	½	1
	4	2	¼	1	1	2	4	1	2	1	1
	5	1	¼	1	1	8	4	1	2	1	1
	6	32	¼	½	2	2	8	2	4	1	1

* Expressed as quotient derived by dividing minimum inhibiting concentration (M.I.C.) of strain after 20 subcultures in the antibiotic by the M.I.C. of the same strain not previously exposed to antibiotic, when tested at the same time.

† S = streptomycin; C = chloramphenicol; T = Terramycin (oxytetracycline); P = polymyxin B; A = Aureomycin (chlortetracycline); N = neomycin.

"A"), no change in their sensitivity to P and cross-resistance of considerable degree in ½ of the strains to neomycin (N). The increase in resistance to C resulting from the exposures to that antibiotic was accompanied by cross-resistance of varying degrees to T and A and by some increases in sensitivity to S, P and N. The subcultures in T produced considerable cross-resistance to C and A, but no changes (or slightly increased sensitivity) to S, P or N. Repeated exposures to P did not affect the sensitivity to any of the other antibiotics. The effects of the subcultures in the pairs of antibiotics on the sensitivity of the strains to individual antibiotics varied considerably. The 3 combinations containing S all produced moderate or marked increases in resistance to that antibiotic; the combination of S with C may have reduced but did not prevent the development of resistance to the latter, but resistance to T or to P did not result from the exposures to each of these antibiotics in combination with S. Cross-resistance to N resulted irregularly from subcultures in each of the 3 combinations containing S, but the least change occurred when

TABLE III. Changes in Sensitivity to Pairs of Antibiotics Resulting from Subcultures in Both or Each.

Antibiotic(s) to which strain was exposed	Antibiotics with which strain was tested	Changes in M.I.C.* — *E. coli*, strain No. — 1	2	3	4	5	6
S	S+C	4	1	½	2	1	½
C	"	2	1	½	2	½	1
S+C	"	4	64	4	64	4	16
S	S+T	4	1	1	½	1	1
T	"	4	2	2	2	2	1
S+T	"	4	4	4	1	4	4
C	C+T	32	8	2	32	16	8
T	"	4	8	8	16	4	16
C+T	"	16	16	8	32	16	16
S	S+P	1	1	1	1	1	½
P	"	1	8	1	1	1	½
S+P	"	1	2	1	1	4	2
C	C+P	4	1	1	2	1	2
P	"	1	1	2	2	1	2
C+P	"	4	2	1	1	2	2
T	T+P	½	1	1	2	1	½
P	"	½	2	1	1	1	1
T+P	"	2	4	2	1	2	2

* See footnotes to Table II.

S was paired with P; some increase in sensitivity to T resulted from subcultures in the latter mixture. The combination C + T failed to prevent or depress the development of resistance to either of these agents. The strains exposed to C + P showed slight and irregular increases in resistance to each of these agents separately and also to T and A but no effect on the sensitivity to any of the other antibiotics. Subcultures in T + P resulted in only minor and irregular increases in resistance to these agents separately and also some cross-resistance to C and A.

Resistance to pairs of antibiotics. The changes in resistance to pairs of antibiotics which resulted from the 20 transfers in the homologous mixtures and in their respective components are listed in Table III. With minor exceptions, the sensitivity of each strain to the pairs of antibiotics was not altered by the exposures to the single constituents of the mixtures except in the case of the strains which had been subcultured in either C or T; almost all of these strains increased in resistance to the mixture of these 2 antibiotics.

Effect of antibiotic exposures on morphologic and biochemical characteristics. All of the strains which had been subcultured on agar containing antibiotics, singly or in pairs, were transferred 1 or 2 times on antibiotic-free broth and then studied simultaneously with their respective parent strains which had not been exposed to antibiotics. No changes were made out in the appearance of the different strains in gram-stained preparations,

TABLE IV. Summary: Resistance of *E. coli* Resulting from 20 Subcultures on Antibiotics, Singly and in Pairs.*

Tested with†	Subcultured 20 times in: S	C	T	P	S+C	S+T	C+T	S+P	C+P	T+P
S	++	0,—	0,—	0	+,++	+,++	0	+,++	0	0
C	0,—	++	+	0	+,++	0,+	++	0	+	0,+
T	—	+,++	+,++	0	0	0	+,++	0,—	0,+	0
P	0	0,—	0	0,++	0	0	0	0	0,+	0,+
S+C	0,+	0			+,++					
S+T	0,+		0,+			+				
C+T		+,++	+,++				++			
S+P	0			0,+				0,+		
C+P		0,+		0					0,+	
T+P			0	0						0,+
A	0,—	+,++	+,++	0	0,+	0,+	+,++	0	0,+	0,+
N	0,++	0,—	0,—	0	0,+	+	0	0,+	0	0
Pen B E	0	0	0	0	0	0	0	0	0	0

* Changes in resistance as compared with inhibiting concentration of parent strain not previously exposed to antibiotic and tested simultaneously; these changes are expressed as follows: 0 = no significant change, *i.e.* quotient described in footnote to Table II is ½, 1 or 2; + = 4- or 8-fold increase in resistance; ++ = 16-fold or greater increase in resistance; — = more sensitive, *i.e.* requiring ¼ or less of original inhibiting concentration.

† See footnote Table I.

nor in the appearance of the colonies which they formed on heart-infusion agar and on eosin-methylene-blue agar. All of the strains failed to utilize citrate, and showed negative Voges-Proskauer and positive methyl-red tests. Tests for penicillinase production were done by a method previously employed with *Staph. aureus*(4). *E. coli* strains 2 and 3 produced penicillinase in these tests while the other 4 did not; this was true of both the parent strains and those which had been subcultured in the antibiotics singly or in pairs. In order to visualize more clearly the changes that have been described, the results shown in Tables II and III are summarized in Table IV.

Summary and conclusions. The data presented confirm and extend the observations previously made with staphylococci and some streptococci using other antibiotic combinations. For *E. coli,* and within the limitations of the methods employed, it was shown that following repeated subcultures in pairs of antibiotics, resistance generally developed more slowly and to a smaller degree than when the same organisms were exposed to the same antibiotics individually. There were wide variations, however, depending mainly on the antibiotics used, but perhaps also with the strain of organism. Cross-resistance to neomycin frequently accompanied increases in resistance to streptomycin resulting from exposure to the latter, alone or in combinations. Likewise, cross-resistance to oxytetracycline and chlortetracycline from exposures to chloramphenicol and to chloramphenicol and chlortetracycline following subcultures on oxytetracycline accompanied the development of resistance to the homologous antibiotic, whether present alone or paired with another agent.

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Amyloidosis and Renal Lesion Induced in Mice by Injection with Freund-Type of Adjuvant.* (20808)

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During attempts to produce injury to collagen in the mouse by subcutaneous injections of homologous collagen incorporated in the Freund-type of adjuvant, it was noted that deposits of amyloid occurred in certain tissues of the animals. Subsequent studies revealed that collagen played no role in the induction of amyloidosis and that the injury was due to the adjuvant. The Freund-type of adjuvant has been shown to enhance in the animal host circulating antibody formation against a variety of antigens(1) and to potentiate the production of allergic encephalitis (2). Another reaction to this adjuvant has been the development of amyloidosis. Although numerous reports have appeared in which this type of adjuvant has been used in many animal species, amyloidosis has been reported only in the duck following injection of killed malarial parasites combined with paraffin oil and killed tubercle bacilli(3). Experimental amyloidosis has been induced in several species of animals with diverse materials but only after long periods of treatment and with highly variable incidence. The observation that repeated injections of adjuvant induce amyloid in the mouse may offer a more efficient method of inducing amyloid-

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