tion that such therapy would more rapidly and effectively terminate an infectious process. Such multiple chemotherapy has often been employed in the absence of accurate bacteriological diagnosis. In some instances it has been conclusively shown that several antimicrobial drugs administered together are more effective than one alone. The material presented here strongly suggests that the opposite may sometimes be the case. If antagonistic effects between antibiotic agents play any role in clinical practice it may become necessary for physicians to use such drugs only for specific indications, and abandon "shotgun therapy".

The observed antagonism between antibiotic agents may also provide a useful laboratory tool for the investigation of antibiotic action, provided it can be shown that it is not due to a simple chemical inactivation of one drug by another, but rather to an interference between the drugs at their site of action.

Addendum. Since this paper was submitted for publication additional experiments have been carried out with a separation of the two antibiotic agents in space and in time. Similar antagonistic results were obtained when the two drugs were injected subcutaneously at widely separated sites, or when they were given in different sites one hour apart.

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Resistance to Epinephrine Stress in the Dog.* (17821)

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Current concepts of the physiologic activation of the pituitary-adrenal mechanism suggest that epinephrine is the humoral agent responsible for the release of ACTH from the anterior pituitary(1,2). It is not known whether epinephrine acts directly on the anterior pituitary or by stimulating the hypothalamus(3,4). The hypothalamus in turn may secrete an adrenergic substance(5) into the hypothalamo-hypophysial portal system and thereby stimulate the anterior pituitary to release ACTH. Other evidence suggests that epinephrine released as a result of sympathico-adrenal discharge may act peripherally to increase the tissue utilization of 11-oxysteroids, leading to lowered plasma levels and activation of the anterior pituitary to release ACTH(6). The site of action of epinephrine is further disputed by conflicting reports in the hypophysectomized rat wherein epinephrine effects a lymphopenia in one laboratory(7) whereas another group reports that epinephrine effects no increase in adrenal cortical size or lipid content in the absence of the pituitary(8). It has been suggested that in man, large doses of epinephrine may act directly on the adrenals(2). Epinephrine is a key hormone in present thinking on pituitary-adrenal relationships. Pharmacologically speaking, it is believed that no true tolerance to epinephrine exists. This concept must be qualified. Continuous or repetitive administration of epinephrine fails to produce continuous hyperglycemia or gly-

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† Public Health Service Research Fellow of the National Heart Institute.

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cosuria(9,10). This tachyphylactic type of response is believed to be due to depletion of liver glycogen stores(11). Repeated daily subcutaneous injections of epinephrine to rats has been reported to produce no significant rise in blood pressure(12). Another example of epinephrine tolerance can be demonstrated with sub-lethal doses of the drug(13). Following administration of sub-lethal doses of epinephrine to dogs(14) and rabbits(15), lethal doses will no longer produce death. Clinically it is known that some asthmatic patients develop a refractoriness to epinephrine. The concept of epinephrine-histamine "see-saw" has been invoked to explain this phenomenon(16).

It has been demonstrated that repeated exposure to a single stress results in the development of a stage of resistance to that stress. In this stage of resistance, the animal is still sensitive to a different stress(17). Refractoriness of the pituitary-adrenal mechanism to epinephrine as a stress has been implied in the human(2). In view of the importance of epinephrine as a possible physiologic activator of the pituitary-adrenal mechanism, it was of interest to determine whether a "resistance" could be experimentally produced to epinephrine. It has been previously reported that repetitive daily administration of epinephrine to the unanesthetized dog resulted in a diminished eosinopenic response(18). The present paper presents further data on this interesting phenomenon.

Methods. One of the most sensitive and reliable criteria of the release of 11-17-oxysteroids from a functioning adrenal cortex is the depression of the circulating eosinophils in the blood(19). Thus the eosinophil response was chosen as a criterion of adrenal cortical discharge. The administration of epinephrine has been shown to effect an eosinopenia in man(2,19) and in the dog (4,18,20).

Eosinophil counts were made by a modification of the acetone-eosin method of Dunger (21). Four Spencer Bright-Line chambers were counted in each determination. The average of 4 chambers was multiplied by a factor of 11.1 to give the cells/mm^3 of blood. Two separate control counts were done at the start of each experiment, if these failed to check within 10% of each other, a third count was made and the average of the 3 taken as the base line. Eosinophil counts were made at the conclusion of the epinephrine infusion and thereafter at hourly intervals for 5 hours. Repetitive, hourly, eosinophil counts on unanesthetized trained dogs under controlled conditions in our laboratory yielded a mean adjusted coefficient of variation (twice the usual coeff. of var.) of +11.1%(22). The mean of a given dog's control counts plus or minus the adjusted coefficient of variation would approximately include 95% of the dog's control counts. All experiments were conducted on trained unanesthetized female dogs weighing between 15 and 25 kg. The hyperglycemic effect of epinephrine was used as an additional criterion of pharmacologic activity of the drug. Serial plasma glucose determinations were made by Nelson's method(23) using a

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TABLE I.
Development of Epinephrine Resistance in the Dog.

Dog	Max. depression of eosinophil count (%)† Day of experiment 1	2	3	4	5	6	7	8
	A. 5 μg/kg/min. Dose of Epinephrine.*							
G.	57	18	6	(+1)	(+12)	—	—	—
Q.	67	41	37	23	8	—	—	—
C.	47	6	5	7	(+6)	16	+9	—
T.	63	64	50	27	—	32	—	—
S.	32	18	23	47	—	—	82	16
H.	40	—	—	29	40	30	13	18
	B. 10 μg/kg/min. Dose of Epinephrine.*							
G.	66	18	24	12	—	—		
G.	42	32	58	52	—	—		
H.	50	—	95	—	—	—		
C.	44	7	8	—	12	—		
Q.	71	62	69	44	55	—		

* Infused for 60 minutes.
† Calc. as 100 —% of pre-treatment control counts.

Somogyi filtrate(24). Parke-Davis adrenalin hydrochloride‡ was used as a source of epinephrine. In preliminary experiments it was found that the dog was strikingly more resistant than man in the terms of the doses of epinephrine required to effect eosinopenic reactions. Single doses of 5-30 μg/kg injected intravenously produced marked circulatory and hyperglycemic responses but no eosinopenia. The continuous intravenous infusion of 5 μg/kg/min for 60 minutes depressed the eosinophile counts in excess of 30% in 16 out of 20 experiments in 11 dogs. In 5 experiments on 4 dogs, an infusion of 10 μg/kg/min for 60 minutes produced an eosinopenic response in excess of 30% in all runs. Therefore, epinephrine was administered daily in doses of 5-10 μg/kg/min for 60 minutes in an attempt to produce a state of resistance. A dose of 20 μg/kg/min was fatal to one dog in which it was tried.

Animals that developed a resistance to epinephrine were exposed to other different stresses in an attempt to elucidate the mechanism of epinephrine refractoriness. Hyperglycemia as a stress was produced by the intravenous infusion of 15% glucose in water at the rate of 8 cc/min for up to 5 hours. This procedure effects marked eosinopenia in the dog(22). Histamine§ was administered intravenously at a dose of 5 μg/kg/min for 60 minutes. This dose of histamine results in both an immediate and delayed eosinopenic reaction in the dog(25). In addition, exposure to cold (3°C for 1 hour) or starvation were employed to activate the pituitary-adrenal mechanism. ACTH‖ equivalent to 50 mg of Armour Standard was injected intramuscularly to determine possible "exhaustion" of the adrenal cortex following repeated exposure to epinephrine stress.

Results. The data in Table IA represent maximum depressions of the eosinophil count occurring in a series of 6 dogs following the repeated administration of epinephrine at a dose of 5 μg/kg/min. In 3 out of 6 dogs a definite resistance to epinephrine developed. In the remaining 3 dogs the eosinopenic response was appreciably diminished.

Table IB summarizes the eosinopenic responses to the 10 μg/kg/min dosage level in 4 dogs. In the dog G., one experiment resulted in development of resistance whereas a repeat run was negative. Only 1 of the re-

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‡ Obtained through the kindness of Dr. E. A. Sharp of Park-Davis and Co.
§ Obtained through the kindness of Dr. M. J. Schiffrin of Hoffmann-LaRoche, Inc.
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‖ We are indebted to Dr. Edwin Hays of Armour Laboratories for a generous supply of ACTH.

TABLE II.
Hyperglycemic Responses to Repeated Administration of Epinephrine in the Dog.

Dog	Day of epinephrine infusion	Max. decrease in eosinophils (%)	Blood sugar (mg%) Time after onset of infusion (min.) Control	60	120	180	240
	A. 5 μg/kg/min. Dose of Epinephrine.						
Q.	1	67	69	325	63	—	82
	3	37	78	339	—	—	78
C.	1	47	58	165	112	92	89
	3	5	82	275	148	95	74
	5	+6	90	294	170	112	96
T.	1	63	51	175	50	49	85
	3	50	53	265	99	53	66
	6	32	55	168	—	70	58
S.	1	32	74	260	75	49	69
	3	23	50	228	81	—	75
	7	82	50	114	98	84	81
H.	1	40	62	234	86	80	69
	4	29	65	173	79	82	—
	6	30	50	276	98	107	113
	8	8	85	260	106	121	128
	B. 10 μg/kg/min. Dose of Epinephrine.						
G.	1	66	69	291	131	82	59
	3	24	75	341	166	96	64
G.	1	42	82	359	140	83	79
	3	58	86	205	185	114	103
C.	3	8	75	383	99	71	74
Q.	1	71	90	245	—	184	101
	4	44	73	285	158	114	75

maining 3 dogs demonstrated the epinephrine resistance phenomenon. There was no demonstrable cumulative eosinopenic effect of the daily administration of epinephrine at the two dosages used. The control pre-infusion eosinophil counts on successive days were comparable in magnitude to those eosinophil levels occurring prior to any epinephrine therapy. The hyperglycemic responses to the epinephrine infusions are presented in Table II. The pharmacologic effectiveness of the repetitive epinephrine infusions is apparent in the hyperglycemic responses. Hyperglycemia was produced by infusions of epinephrine that failed to produce marked eosinopenia. Dogs in the epinephrine refractory state were exposed to secondary stresses, larger doses of epinephrine, or ACTH. The eosinophil and hyperglycemic responses to these stresses are presented in Tables III, IV, and V. From Tables III and IV it can be seen that the resistant state is probably not due to a depletion of endogenous ACTH since secondary stresses effected eosinopenic responses. Further evidence in this direction is presented in Table IV where it can be seen that an increased dose of epinephrine effected an eosinopenic response in an animal resistant to a dose of 5 μg/kg/min. Had endogenous ACTH supplies been depleted, neither the higher dose of epinephrine nor the subsequent glucose stress should have produced eosinopenia.

The data in Table V, although somewhat extraneous to the thesis of this paper, are presented for their theoretical implications. Initially an epinephrine dose of 5.0 μg/kg/min produced a questionable eosinopenic response. The animal was then starved for 2 weeks with the production of a state of resistance to starvation as a primary stress. In keeping with present concepts

TABLE III.
Effects of Stresses Superimposed on a State of Epinephrine Resistance.
Dog Co, ♀, wt 16 kg.

Date	Epinephrine stress,* μg/kg/min.	Second stress	Max. decr. in eosinophils %	Blood sugar (mg%) Time after onset of infusion (min.) Control	60	120	180	240
6/13/49	5	—	47	57	165	112	92	89
14	5	—	6.5	—	—	—	—	—
15	5	—	5.5	84	275	148	95	74
16								
A.M.	5	—	7.7	—	—	—	—	—
P.M.	—	Exposure to cold†	20	—	—	—	—	—
17								
A.M.	5	—	(+0.9)	91	294	170	112	—
P.M.	—	ACTH‡	40.5	—	—	—	—	—
18	5	—	15.6	—	—	—	—	—
20	5	—	(+9.6)	—	—	—	—	—
21	—	Hyperglycemia§	54	105¶	1240¶	895¶	800¶	800¶
22								
A.M.	5	—	18.5	—	—	—	—	—
P.M.	—	Histamine‖	34.8	—	—	—	—	—

* Epinephrine infused for 60 min.
† Exposure to 3°C for 1 hr.
‡ 50 mg of Armour Standard inj. i.m.
§ 15% glucose infused i.v. at 8 cc/min. for 5 hrs.
‖ 5 μg/kg/min. of histamine (calc. as free base) infused i.v. for 60 min.
¶ Arterial blood samples.

TABLE IV.
Effects of Increasing the Epinephrine Dose on a Pre-existing State of Epinephrine Resistance.
Dog Gi, ♀, wt 18 kg.

Date	Epinephrine stress.* μg/kg/min.	Second stress μg/kg/min.*	Max. decr. in eosinophils %	Blood sugar (mg%) Time after onset of infusion (min.) Control	60	120	180	240
7/12/49	5	—	57.5	—	—	—	—	—
13	5	—	17.6	86	220	55	67	68
14	5	—	5.8	—	—	—	—	—
15	5	—	(+0.8)	—	—	—	—	—
16	5	—	(+11.9)	—	—	—	—	—
17	—	10	64.5	67	291	131	82	59
19	—	10	19.2	—	—	—	—	—
20	—	10	33.6	75	341	165	96	64
21	—	10	3.7	—	—	—	—	—
22	—	Hyperglycemia†	58.0	69	752	935	1102	1022

* Epinephrine infused for 60 min.
† 15% glucose infused i.v. at 8 cc/min. for 5 hrs.

(17), the animal was markedly sensitive to a second stress, epinephrine, which was administered at a much lower dosage than initially. Of great interest was the ensuing phenomenon of the development of a refractory state to this second stress. That the adrenal cortex was not exhausted even in the face of these two stresses, is adequately demonstrated by the eosinopenic reaction to ACTH. That the endogenous supply of ACTH was probably not exhausted was demonstrated by the eosinopenic response to 5.0 μg/kg/min of epinephrine, although the magnitude of the response is admittedly moderate.

Discussion. In terms of one criterion of the endogenous release of 11-17-oxysteroids namely the eosinopenic response, the data indicate that the repetitive daily administration of epinephrine at a dose of 5.0 μg/kg/min infused for 60 minutes, results in a state of relative resistance or refractor-

TABLE V.
Epinephrine Resistance Superimposed on State of Resistance to Starvation Stress.
Dog Tr., ♀, wt 16 kg.

Date	Primary stress	Secondary stress	Max. decr. in eosinophils %	Blood sugar (mg%) Time after onset of infusion (min.) Control	60	120	180	240
5/16/49	5.0 μg/kg/min.*	—	20	—	—	—	—	—
18	Starvation—through 6/4/49 (Water fed *ad lib.*)							
31	,,	.5 μg/kg/min.*	95.5	60	173	160	155	137
6/ 1	,,	.5 ,,	32.	—	—	—	—	—
2	,,	.5 ,,	46.	58	135	108	98	81
3								
A.M.		.5 ,,	9	—	—	—	—	—
P.M.		ACTH†	64	—	—	—	—	—
6/4	,,	5.0 μg/kg/min.*	26	—	—	—	—	—

* Epinephrine infused for 60 min.
† 50 mg of Armour Standard inj. i.m.

iness. During this state, other pharmacologic effects of the drug such as the mobilization of glycogen and the production of hyperglycemia are not diminished. Higher dosages of epinephrine (10 μg/kg/min) fail to produce the same degree of resistance although a diminished eosinopenic response was noted in some animals. Neither dosage levels of epinephrine seemed to deplete liver glycogen stores under the conditions of these experiments. Resistance to epinephrine as a stress can be explained by several mechanisms. Depletion of endogenous ACTH would be the simplest explanation, however, the eosinopenic response to secondary stresses would seem to speak against this hypothesis. Exhaustion of the adrenal cortex might also explain the refractory state, but again this is unlikely in view of the marked eosinopenic response to exogenous ACTH. Admittedly the dose of ACTH is very large, however the clinical reactions of the dogs receiving both dosage levels of epinephrine were also striking in terms of the severity of the stress. The animals showed profound dyspnea, cardiac arrhythmias, frequently vomited and in some instances went on to a bloody diarrhea. One would expect that toxic stress of this severity would liberate maximal quantities of endogenous ACTH if such stress acted on the pituitary.

A third explanation of the epinephrine resistance state might be that with adaptation to epinephrine as a stress, the tissue utilization of corticoids decreases. With the decreased utilization of corticoids, the stimulus for ACTH production is removed and no eosinopenia would result. This theory has been substantiated by experimental work in the rat(6) and would satisfactorily explain the epinephrine resistant state as well as the eosinopenic responses to secondary stresses and exogenous ACTH.

The experimental demonstration of an epinephrine resistant state does not exclude the possibility that stress effects the release of epinephrine which acts as the continuous physiologic activator of the pituitary-adrenal mechanism, but it makes such a hypothesis difficult to accept. Recent work in the human substantiates this concept that the sustained secretion of ACTH may not be due to the continuous action of epinephrine(2). It has been stated that epinephrine in large doses may act directly on the adrenal cortex. Under the conditions of these experiments, the lack of eosinopenia could be explained if epinephrine acted directly on the adrenal cortex, which then became refractory to this stress. The adrenal cortex was sensitive to endogenous ACTH since other stresses effected eosinopenia during epinephrine. Thus in addition to refractoriness of the adrenal gland to epinephrine, one must postulate that the pituitary is equally refractory so as not to release ACTH, to which the adrenal is sensitive, during the epinephrine resistant stage. It would therefore appear

that a direct action of epinephrine on the adrenal cortex is problematic.

Summary. 1. The repetitive daily administration of epinephrine in the dog results in a progressively diminished eosinopenic response.

2. During the state of epinephrine resistance, there is neither a depletion of endogenous ACTH nor an exhaustion of the adrenal cortex.

3. Adaptation of the organism to epinephrine stress with decreased tissue utilization of corticoids seems to most satisfactorily explain the phenomenon of epinephrine resistance at the present time.

4. Demonstration of resistance to epinephrine speaks against epinephrine as the only physiological stimulus for the continuous secretion of ACTH.

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Inhibition of Vascularization of the Rabbit Cornea by Local Application of Cortisone.* (17822)

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It has become apparent that one of the most striking properties of cortisone is its selective depression of certain mesenchymal cells, as evidenced experimentally in the effect on mouse lymphosarcoma(1) and on wound healing(2). Presumably, the antirheumatic effect of cortisone is likewise due to the selective suppression of cells of mesenchymal origin, involved in an as yet undetermined mechanism in the production of rheumatic diseases. It was thought of interest to investigate whether capillary endothelial cells were among those influenced by cortisone. The vascularization of the cornea appeared to be most suitable for the study of this effect, especially since cortisone might be expected to exert its action on capillary growth when applied locally. Such local application would allow use of the fellow eye as a control. The vascular responses to corneal injury are well known, having been investigated thoroughly by several authors (3,4).

As will be shown by the data here presented, the subconjunctival injection of cortisone did indeed inhibit the growth of capillaries into a chemical burn of the cornea to a high degree.

Method. Three groups containing 6 albino rabbits each were used. The first group sustained corneal burns with 0.2 N alkali, and the second and third groups received burns with 0.5 N alkali. The control eyes in the first and second groups were given no subconjunctival injection, while the third group's control eyes received a suspension of cholesterol in the same medium as the cortisone suspension. The experiments were carried out in the following manner: under topical nupercaine anesthesia, an injection of 0.03 cc NaOH in the concentrations mentioned above was made into the superficial corneal layers half way between the apex of the cornea and the superior limbus with a 30 gauge needle. This was done on both eyes of all animals. A burn about 3 mm in diameter resulted when the weaker solution

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