mild infection into a rapidly progressing, violent and uniformly fatal disease, and eliminates completely the natural refractoriness of the animal.

In the initial experiment both ACTH and cortisone were used. On further study it was found that cortisone alone was necessary for the effect, a single intramuscular injection of cortisone administered simultaneously with the intracranial inoculation of the virus sufficing for the production of an equally marked enhancement of susceptibility to the infection, (Groups 5 and 6). It may be noted, however, that hamsters which received 3 injections of ACTH alone in a total dose as large as 15 mg, (Group 7) failed to show enhanced susceptibility to the infection. These results suggest the possibility that in addition to cortisone, ACTH stimulates also the secretion of another factor which may be capable of reversing the enhancing effect of cortisone.

The following control series of experiments were done in order to ascertain that the results obtained were not due to enhancement by the treatment with the hormones of some latent animal virus, rather than to the enhancement of susceptibility to the poliomyelitis virus inoculated. The materials tested were obtained as follows:

1. Two mice received one injection of ACTH and two injections of cortisone at time intervals used in the experimental series. The brains were removed 2 hours after the second injection of cortisone; 2. brains of two normal mice, and 3. two mice were injected intracerebrally with 0.05 ml of broth diluted 1:10 with saline. The brains were removed 24 hours later.

Each brain emulsion diluted 1:10 with saline was inoculated intracerebrally into 10 untreated mice, 10 untreated hamsters, and 5 mice and 10 hamsters which received one injection of cortisone simultaneously with the inoculation. All animals remained well during the entire period of observation.

Summary and Conclusions. ACTH and cortisone in combination or cortisone alone produce a marked acceleration of poliomyelitis infection (strain MEF1) in mice and an extraordinary enhancement of susceptibility to this infection in hamsters giving rise to a violent and uniformly fatal disease. ACTH alone fails to produce this effect possibly due to elaboration of an unknown factor capable of reversing the enhancing effect of cortisone. The experiments seem to indicate the existence of a significant relation between adrenocortical function and susceptibility of mice and hamsters to experimental poliomyelitis.

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Effect of Succinate on Contractility and Phosphocreatine and Adenosine Triphosphate Content of the Dog Heart.* (18363)

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The oxidation of succinate is catalyzed in cardiac muscle with great rapidity. Addition of succinate in substrate quantities to excised cardiac muscle causes a striking acceleration of oxygen consumption, both at high(1-3) and at low(4-5) oxygen tensions. On this

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TABLE I. Effect of Succinate on the Heart-Lung Preparation of the Dog.

Dog	Succinate*			Heart rate, min.-1	Mean systemic arterial pressure, mm Hg	Pulmonary arterial pressure, mm H_2O	Right atrial pressure, mm H_2O	Systemic output, cc/min.	Coronary output,† cc/min.	Total output, cc/min.	Work, kg/m/min.
	Dose, g	Duration of inj., min.	Rate of inj., g/min.								
67	0	0	0	120	93	—	34	312	—	—	—
	1.97	10	.197	116	91	—	41	292	—	—	—
69	0	0	0	176	105	255	10	466	42	508	.851
	1.03	3	.344	156	101	229	39	282	35	317	.504
70	0	0	0	168	104	173	19	486	42	528	.833
	1.54	10.5	.147	156	105	168	19	489	39	528	.836
76	0	0	0	156	106	186	34	415	75	490	.792
	1.80	14	.129	144	106	164	34	430	69	499	.795
77	0	0	0	164	106	136	25	449	91	540	.844
	1.29	2	.645	156	104	150	47	329	102	431	.655
202	0	0	0	148	118	188	46	577	70	647	1.151
	1.93	4	.483	140	115	173	61	459	45	504	.868
205	0	0	0	174	110	191	47	444	102	546	.915
	1.71	6	.285	162	107	168	60	332	157	489	.740

* As unhydrated anion.
† Coronary sinus outflow × 10/6.

ground the use of the 4-carbon compound has been advocated as a possible therapeutic measure in myocardial weakness and anoxia (6). However, when the compound is administered to isolated heart preparations, the contractile power of the muscle is seen to diminish (7-9). A possible clue to this behavior is provided by the finding of Furchtgott and Shorr(5) that phosphorylation in cardiac muscle slices is depressed by succinate. The experiments reported below indicate that this may also be the case in the intact beating heart.

Methods. Starling heart-lung preparations of dogs weighing 8.1 to 12.0 kg were used. The weight of the hearts ranged from 51 to 75 g. The amount of circulating blood was approximately 800 cc, making the weight of the entire heart-lung system approximately 1 kilogram. The preparations were set up and their activity was measured as specified in a previous report(10). Succinate was injected as a 10% aqueous solution of the hexahydrated disodium salt over periods ranging from 2 to 14 minutes into the tube connecting the venous reservoir with the heart. The quantities injected are given in grams of the unhydrated anion, 0.43 g of which are equivalent to 1 g of the hydrated salt. Within one minute after the end of the injection a piece from the apex of the left ventricle was cut off and immediately immersed into liquid nitrogen. The frozen muscle was analyzed for adenosine triphosphate (ATP), phosphocreatine, inorganic phosphate, and total acid-soluble phosphate according to procedures outlined or referred to previously(10).

Results. Effect on Cardiac Function. The effect of succinate on the activity of seven heart-lung preparations is shown in Table I. The cardiodynamic measurements were taken shortly before the administration of the compound and then again at the completion of the administration, immediately before a sample of the heart was taken for the analysis of the phosphate compounds. In the majority of the experiments the hearts went into failure when succinate was injected, as indicated by

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TABLE II. Concentration of Some Acid-soluble Phosphates in the Hearts of Dog Heart-Lung Preparations Following Administration of Succinate.*

The figures in the italic print lie outside the fiducial limits of the respective control values (mean ± S.D. x t) for P = 0.05.

Dog	Time of survival, min.	Degree of heart failure‡	P, mg per 100 g ventricle			
			Inorganic phosphate	Phospho-creatine	Labile P of ATP	Total acid-soluble phosphate
8 controls,						
Mean†	82.6	—	28.15	14.33	35.21	104.10
± S.D.			±3.51	±1.64	±2.66	±6.47
67	152	Slight	25.9	*10.1*	31.9	97.5
69	138	Severe	32.7	*9.5*	*28.8*	94.4
70	67	None	33.4	*8.6*	30.1	100.7
76	67	"	28.5	11.9	35.2	100.0
77	77	Severe	*36.6*	*4.4*	32.7	106.1
202	72	Moderate	30.6	*8.9*	*25.0*	*87.0*
205	124	"	28.2	14.0	30.7	101.0

* For amounts of succinate administered, see Table I.
† For individual values, see (10).
‡ For details, see Table I.

a rise of the right atrial pressure and by a fall of the systemic arterial pressure, minute output, and work. However, the succinate had to be supplied to the heart at a fast rate in order to make it fail. The compound was without effect on myocardial contractility when administered at rates of 0.129 and 0.147 g per minute (hearts No. 76 and 70). Slight failure ensued at a rate of administration of 0.197 g per minute (heart 67). In the other experiments, in which the rate of administration was 0.285 g per minute and higher, the ensuing heart failure was fairly severe, although the total amount given was not much larger or was even smaller than in the experiments in which failure was absent or slight. That the changes observed were entirely due to the succinate anion is evidenced by the fact that administration of equivalent amounts of sodium chloride had not the slightest effect. A slight decrease in heart rate by succinate was observed in all preparations. Pulmonary arterial pressure was likewise slightly decreased. The effect on the coronary circulation was variable and apparently unrelated to the intensity of the inotropic effect.

Effect on Phosphocreatine and ATP Content. In all hearts, with one exception (No. 205), succinate produced a fall of the phosphocreatine level (Table II). Comparison with figures obtained in 8 control experiments shows that according to the statistical criterion selected in Table II this fall was of significant magnitude in 5 of the 7 succinate experiments, the phosphocreatine values lying from 30 to 67% below the mean of the controls. The concentration of ATP was significantly decreased in 2 hearts. A close parallelism between the impairment of contractility and the depletion of phosphocreatine or of ATP is not apparent from the data. The phosphocreatine was low in one of the 2 hearts in which contractility remained unimpaired. In heart 202 the total acid-soluble phosphate was low, but this does not account for its low content of phosphocreatine and ATP. For even if the phosphate of these 2 compounds is expressed in percent of the total acid-soluble phosphate, the figures remain below the lower fiducial limit of the respective control values.

Effect on Hearts Poisoned with Anesthetics. In 1943 Soskin and Taubenhaus(11) reported that succinate counteracts depression of central nervous system activity by pentobarbital. Subsequently, however, the usefulness of succinate as an antidote to barbiturates was questioned by others and became the subject of considerable controversy in the pharmacological literature. Since pentobarbital and other anesthetics are also capable of depres-

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TABLE III. Effect of Succinate on a Heart-Lung Preparation Poisoned with Pentobarbital.

Time of survival, min.	Compound admin.	Heart rate, $min.^{-1}$	Mean systemic arterial pressure, mm Hg	Pulmonary arterial pressure, mm H_2O	Right atrial pressure, mm H_2O	Systemic output, cc/min.	Coronary output,* cc/min.	Total output, cc/min.	Work, kg/m/min.	Competence index
67		156	106	262	18	420	87	507	.859	.87
71	0.07 g pentobarbital sodium									
80		144	102	274	40	303	130	433	.715	.57
91-95	0.64 g succinate									
96-97		140	97	269	53	185	114	299	.472	.38

* Coronary sinus outflow × 10/6.

TABLE IV. Effect of Succinate on a Heart-Lung Preparation Poisoned with Chlorobutanol.

Time of survival min.	Compound	Heart rate, $min.^{-1}$	Mean systemic arterial pressure, mm Hg	Right atrial pressure, mm H_2O	Systemic output, cc × $min.^{-1}$	Competence index
23		148	113	29	485	.92
25	0.15 g chlorobutanol					
52		144	113	30	385	.49
53	0.64 g succinate					
69		148	110	71	260	.25
71	0.64 g succinate					
73		154	106	86	155	—
81		156	109	76	215	.00

sing the activity of the heart(12), it seemed of interest to examine the effect of succinate on hearts poisoned with these agents. Table III summarizes an experiment in which 0.64 g succinate was given to a heart-lung preparation failing as a result of previous administration of pentobarbital. In addition to the cardiodynamic variables listed in Table I the response of the right atrial pressure to an increase in blood supply was determined in this experiment as a sensitive measure of its competence(13), and the results are expressed as the competence index(10). It is seen that succinate aggravated the heart failure caused by pentobarbital. The work done diminished by 34 percent, and the competence index which had been lowered by the barbiturate from 0.87 to 0.57, fell to a value of 0.38. In heart failure produced in another heart-lung preparation by the anesthetic chlorobutanol, succinate likewise exerted a strong negative inotropic action (Table IV). Again a dose of 0.64 g of succinate caused further impairment of contractility, and shortly following injection of a second dose of 0.64 g 18 minutes later the heart was in a state of very severe failure. After 10 more minutes had elapsed, it had slightly recovered from the impact of the succinate injection, but was still unable to handle an increase in load, as reflected in a competence index of zero. To judge from the data in Table I, the administration of 0.64 g succinate over a period of more than 4 to 5 minutes to a non-failing heart probably would have been without effect on its contractile power. It appears that poisoning the heart with an anesthetic makes it more susceptible to the negative inotropic action of succinate.

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Discussion. Although the heart in the heart-lung preparation is well provided with oxygen, its phosphocreatine store becomes depleted in the presence of large amounts of succinate, and it may go into failure. The decrease in phosphocreatine distinguishes this failure from that occurring in this preparation as the result of spontaneous deterioration or of poisoning with anesthetics(10). The present data do not constitute proof that succinate heart failure is actually due to depletion of energy-rich phosphate, particularly since the ATP content was found to be only slightly diminished and a close correlation between the decrease in contractility and in phosphocreatine was not apparent. However, they at least are suggestive of such a mechanism.

An explanation for the loss of phosphocreatine in the heart supplied with large quantities of succinate may be furnished on the basis of existing information about the coupling of succinate oxidation with phosphorylation. The occurrence of this coupling has been demonstrated in extracts of heart muscle and other tissues(14-17). However, the efficiency of phosphorylation linked to succinate oxidation is low. According to Ochoa(17) no more than one phosphate bond is formed per atom of oxygen taken up. Using whole heart homogenates Potter(18) was unable to obtain a net uptake of inorganic phosphate with creatine as phosphate acceptor when succinate was the substrate. In slices of dog myocardium incubated in 20% oxygen Furchtgott and Shorr(5) found that substrate quantities of succinate, while greatly stimulating oxygen consumption, depressed phosphorylation and produced a fall in the concentration of phosphocreatine and ATP. The succinate apparently inhibited the oxidation of endogenous substrates and was itself oxidized for the most part only to fumarate. The authors conclude that the energy furnished in this step was largely or completely wasted, the number of phosphate bonds formed per molecule succinate oxidized being much below the value of 1 postulated for heart extracts(19). In view of the fact that succinate had the same effect in the heart-lung preparation as it did in Furchtgott and Shorr's study in cardiac slices, namely, a lowering of the energy-rich phosphate concentration in the myocardium, it is quite likely that their conclusions are likewise applicable, at least in a qualitative fashion, to the intact beating heart. At any rate, the heart-lung experiments indicate, in agreement with earlier observations on substrate-depleted myocardium(9,20), that succinate is a poor source of energy for the contraction of the heart.

Of further interest in this connection is the finding that poisoning the heart with pentobarbital or chlorobutanol seems to make it more susceptible to the negative inotropic action of succinate. It had been shown previously(3) that on addition of succinate to cardiac slices whose respiration is severely depressed by pentobarbital or chlorobutanol, the oxygen uptake rises to the same level as in unpoisoned slices, and that for a considerable period almost the total oxygen uptake can be accounted for by the oxidation of the succinate to fumarate. While there is evidence (10) that heart failure produced in the heart-lung preparation by these anesthetics is not due to impairment of oxidative metabolism, there is, nevertheless, reason to believe(cf. 21) that in this failure the oxidation of substrates normally available to the heart, such as glucose and lactate, is inhibited to some extent. If inferences may be drawn from the behavior of heart slices with respect to the situation obtaining in the intact beating organ, it would appear that when succinate is administered to hearts poisoned with these anesthetics, the

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succinic oxidase system, the activity of which is not affected by these agents, is able to compete even more successfully with other oxidation systems for oxygen than in unpoisoned hearts, with the result that the negative inotropic action of succinate becomes more intense and prolonged.

Summary. When succinate is administered to the heart-lung preparation of the dog at sufficiently high rates (0.2 g or more of unhydrated succinate anion per kg heart-lung system per minute) the phosphocreatine concentration in the myocardium declines markedly and the heart goes into failure. The concentration of ATP declines to a lesser extent or remains unchanged. Heart failure in the heart-lung preparation caused by pentobarbital and chlorobutanol is intensified by succinate. These findings are discussed in the light of existing information on the coupling of succinate oxidation with phosphorylation and on the metabolism of cardiac tissue poisoned with anesthetics.

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Amino Acids of the Shope Papilloma Virus. (18364)

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Among the symptoms attributable to viral infections is the production of tumors, such as those, for example, of infectious papillomatosis (Shope papilloma) of rabbits(1). Interest in the Shope papilloma virus is heightened by the possibility that findings gained from studies of it may, in addition to contributing to knowledge of host-virus relationships, possess implications for the cancer problem(2). A desirable basis for further studies on rabbit papillomatosis is provided by investigation of the physical and chemical nature of the agent inducing the disease. Progress in this direction has been made by Beard and associates who, from studies made on purified preparations, have reported the virus to be composed of desoxypentose nucleic acid, protein, and perhaps a small amount of lipid(3-8).

In the present investigation, several purified preparations of papilloma virus have been obtained, and the 2 largest of these have been analyzed for amino acid content by means of microbiological methods of assay. It was not possible to perform so exhaustive an analysis as was done with some strains of tobacco mosaic virus(9), owing to the relatively greater difficulty in procuring substantial quantities of highly purified virus; nevertheless, this report is presented with the hope that the data given may guide others working or proposing to work in this field.

Methods and Findings. Preparation of virus for assay. The source of virus for the experiments described herein was the warty growths removed from cottontail rabbits trapped in Kansas. The papillomas, which had been stored in glycerin-saline solution(4) upon harvest were drained on a fluted filter in a

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