ACTH. Additional studies employing various doses of both DCA and cortisone are contemplated.

The metabolic and toxic actions of DCA in the intact organism are a result, in part at least, of its inhibitory effect upon pituitary adrenocorticotrophic activity. The steroid induces insulin hypersensitivity(11). Furthermore, the elevation of electroshock threshold and hypernatremia, normally produced by DCA, are prevented by the simultaneous administration of ACTH(12).

In addition to a pituitary inhibitory effect, it is quite probable that DCA induces certain changes characteristic of adrenocortical insufficiency by competing with cortisone-like compounds for strategic loci in peripheral effector cells. This aspect of the problem is undergoing study in our laboratory at the present time.

The data of the present report have an important bearing on the mechanism of action of DCA in inducing pathological changes in experimental animals similar to those observed in the collagen diseases in man. It has been suggested by Cheng and Sayers(11) and by Woodbury *et al.*(12) that this toxic action of DCA is a result, in part, of a steroid hormone imbalance, namely, an excess of DCA (exogenous synthetic steroid) and a deficiency of the secretion of the adrenal cortex. The recent demonstration of Woodbury *et al.*(13) that ACTH given simultaneously with DCA can inhibit the development of pathological changes, which are normally induced by the synthetic steroid when administered alone, lends strong support to this interpretation.

Summary. DCA pellets increase the concentration of ACTH in the adenohypophysis of the intact rat. The steroid inhibits the marked depletion of pituitary stores of ACTH which normally occurs 24 hours after adrenalectomy.

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Cortisone Acetate and Terramycin in Polyarthritis of Rats.*† (18013)

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The L_4 strain of pleuropneumonia-like organism will produce in rats an arthritis which has been used as a method of evaluating various therapeutic agents(1-5). Pleuropneumonia-like organisms are evidently species specific as far as pathogenicity is concerned so that human strains cannot be used in animals. Dienes and his associates

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‡ With the assistance of Pelagio S. Tabar.

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TABLE I.
Preventive Effect of Cortisone in Polyarthritis of Rats.

Exp. No.	No. of rats	No. of inj.*	Dosage (mg/kg)	Avg max arthrogram score	Incidence of arthritis, %	Survival rate, %
V	20	14	2.5	1.3	70	85
	20	control	—	1.7	60	60
VI	18	13	2.5	4.0	89	66
	17	control	—	3.5	70	88
X	20	14	20.0	2.5	90	65
	20	control	—	2.0	70	100

* Injections were given once daily beginning on the day of infection.

have repeatedly isolated such organisms from the human genitourinary tract and feel that they "may be related etiologically to an acute infectious type of arthritis and to Reiter's syndrome(6)." Brown and his group have reported on the favorable effects of aureomycin treatment of rheumatic patients harboring pleuropneumonia-like organisms(7). In this laboratory human strains of pleuropneumonia-like organisms have been isolated from conjunctivae, urethral discharge, penile lesions, and joint fluid in 5 cases of Reiter's syndrome, and there has been one case of cross-infection which is to be separately reported.

In the polyarthritis of rats the use of gold salts will either prevent the development of the arthritis or will promote more rapid healing if given after the joints are swollen. Since gold salts often favorably influence the clinical course of rheumatoid arthritis in man various compounds have been tried in this rat infection in an effort to find agents which have the same therapeutic effect, but with less toxicity, for possible therapeutic trials in man.

Since cortisone acetate (Kendall's Compound E or 11-dehydro-17-hydroxycorticosterone-21-acetate) has produced such striking alterations in the clinical status of patients with rheumatoid arthritis(8) an attempt has been made in this study to evaluate its preventive and therapeutic properties in the polyarthritis of rats which is a specific infection. Terramycin has been evaluated in this study since aureomycin was shown to be effective in a previous study of the polyarthritis of rats(3).

Methods. Cortisone was given to the infected rats by daily intramuscular injection. The cortisone was prepared by Merck and was supplied in a saline suspension containing 25 mg per cc. The controls received daily intramuscular injections of the same volume of physiological saline solution. The terramycin used was the commercial product of the Charles Pfizer Co. It was administered by mixing with the diet (ground Purina laboratory chow), in the drinking water, and by stomach tube. The criteria used in evaluating these drugs included the per cent incidence of arthritis in each group, the extent of the joint involvement scored numerically, the per cent survival rate, and in some instances the per cent weight gain during the experimental period. The extent of the joint involvement was evaluated according to a modification of the arthrogram of Sabin and Warren(9) which assigns a numerical value of 4 to each front leg and 5 to each hind leg, giving a total of 18 points per animal assuming maximum joint involvement of all joints of the extremities. The average arthrogram scores were determined by adding the highest scores for each individual animal and dividing the totals by the number of animals in the group irrespective of per cent incidence of arthritis.

The microbe was cultured in a broth which

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was prepared as follows, per liter distilled water: 3 g yeast extract, 7 g nutrient broth concentrate, 10 g tryptose phosphate concentrate (Difco), and 2 g dextrose, C.P. For solid media 2% agar agar and for broth 0.75 g agar agar per liter were added. The pH was adjusted as follows before autoclaving: for solid agar to 8.23, and for broth to 8.1. The media was then sterilized by autoclaving at 15 lb pressure for 20 minutes. Sterile, Seitz-filtered horse serum was then added aseptically in the concentration of 20%.

Albino rats were used in all experiments with an equal number of males and females in each group. The weight of the animals at the time of infection was between 60 and 100 g. The infection was induced by the intraperitoneal injection of 2 cc of a broth culture of the microorganism. This injection was a mixture of equal parts of 24- and 48-hour cultures of pathogenic strains. Since the arthritic pathogenicity of strains varies considerably it was necessary in each instance to run separate controls.

Results. Cortisone. Table I shows the preventive effect of cortisone administered once daily beginning on the day of infection. The average maximum arthrogram scores show that in one experiment the joint involvement was slightly less and in the other slightly more than in the control groups when the cortisone dosage was 2.5 mg per kg. Likewise, in one experiment at this dosage the per cent incidence of arthritis was less and in the third group more, and the per cent survival rate showed the same inverse relationship in the latter 2 groups. The dosage of 20 mg per kg in one experiment was accompanied by a slightly more extensive joint involvement, increased incidence of arthritis, and a decreased survival rate.

Table II shows the evaluation of the therapeutic effect of cortisone in 8 separate experiments. The medication was begun 6 to 8 days following the infection and at a time when the involved joints were beginning to swell. Five of the experiments were run with a daily dosage of cortisone of 2.5 mg per kg. In these groups generally the per cent incidence of arthritis was less in the controls than in the medicated animals at the

TABLE II. Therapeutic Effect of Cortisone in Polyarthritis of Rats.

Exp. No.	No. of rats	No. of inj.*	Dosage (mg/kg)	Incidence of arthritis (%)		Avg arthrogram score		Survival rate, %	Avg weight		Wt gain during treatment, %
				Beginning of treatment	End of treatment	Beginning of treatment	End of treatment		Beginning of treatment, g	End of treatment, g	
II	40	10	2.5	35	23	0.7	.38	98	87	118	36
	19	control	—	32	16	0.5	.1	100	88	126	43
III a	10	15	2.5	60	20	1.1	.4	100	70	107	53
	9	control	—	44	22	1.1	.3	100	80	133	66
b	10	15	2.5	70	20	1.9	.2	100	73	113	55
	9	control	—	56	22	0.7	.2	100	76	121	60
IV a	9	16	2.5	56	44	1.7	.66	89	80	114	43
	9	control	—	56	33	0.5	.33	100	72	111	54
b	9	16	2.5	67	33	1.7	.33	100	77	126	64
	9	control	—	67	11	1.0	.22	78	67	119	78
VII	19	13	10	89	84	2.4	1.8	95	76	84	11
	19	control	—	63	79	2.0	2.7	100	78	106	36
IX a	9	14	10	78	78	2.7	2.8	100	88	97	10
	9	control	—	89	77	2.4	2.3	89	99	129	30
b	8	14	10	75	63	3.0	2.5	63	61	64	5
	10	control	—	78	78	1.9	3.6	78	60	73	22

* Injections were given once daily beginning 6 to 8 days following infection.

TABLE III.
Therapeutic Effect of Terramycin in Polyarthritis of Rats.*

No. of rats	Dosage	Incidence of arthritis (%)		Avg arthrogram score		Survival rate, %
		Beginning of treatment	End of treatment	Beginning of treatment	End of treatment	
19	0.3% in drinking water 4 days	68	47	1.9	0.8	100
20	Control	75	80	2.7	3.2	90

* Medication began 8 days following infection.

TABLE IV.
Preventive Effect of Terramycin in Polyarthritis of Rats.*

No. of rats	Dosage	Avg max. arthrogram score	Incidence of arthritis, %	Survival rate, %
18	50 mg/kg stomach tube 2 days	.11	5.5	67
10	0.1% diet 12 days	.5	40	100
10	0.3% diet 12 days	.0	0	100
20	Control	3.7	80	75

* Medication began on the day of infection.

end of treatment. The extent of the joint involvement was about the same. In one experiment the survival rate was less in the controls and in 2 others it was slightly less in the medicated animals. The remaining 2 experiments showed 100% survival of both groups. The per cent weight gain was less in the medicated rats than in the controls in these 5 experimental groups. At a dose of 10 mg cortisone per kg the per cent incidence of arthritis remained the same in one experiment and decreased slightly in 2 other groups during the period of medication. In their controls one group remained the same, one decreased slightly, and one increased slightly. The extent of joint involvement decreased in 2 and increased slightly in one of the medicated groups while in their controls the arthritis became more extensive in 2 groups and decreased slightly in another. The survival rate was slightly less in 2 and greater in one of the treated groups as contrasted with their controls. The per cent gain in weight was less in the medicated animals as compared with controls in each of the 3 groups on the 10 mg per kg dosage schedule.

Terramycin. Table III demonstrates the therapeutic effect of terramycin given for 4 days in the drinking water to the animals 8 days following infection. The incidence of joint arthritis decreased 21% in the medicated animals as compared with 15% in the controls; whereas, the extent of joint involvement decreased more than half in the treated while in the controls it increased slightly. All treated animals survived while 10% of the controls died. The addition of 0.3% terramycin to the ground food at the time of infection prevented the development of arthritis and permitted 100% survival as compared with 80% incidence of arthritis and 75% survival rate in the controls (Table IV). With 0.1% terramycin in the diet only half as much arthritis developed in the treated animals as in the controls and all survived. When terramycin was administered by stomach tube for 2 days beginning on the day of infection only 5.5% developed arthritis as compared with 80% of the controls. While 67% of those medicated via stomach tube survived as compared with 75% survival among the controls the difference may have been due to trauma incident to intubation. The controls were used for the other 2 groups as well and therefore were not intubated with a placebo material.

Discussion. Cortisone apparently does little to alter the course of the rat polyarthritis when it is used as a preventive agent, and when used therapeutically the differences were also small. Generally speaking the medicated animals looked worse at the end of the experiments and weighed less than the controls. Selye has reported that cortisone and ACTH

will inhibit in albino rats the development of arthritis after injection of formalin into the joints(10). We have previously reported that ACTH lacked a beneficial effect as a preventive in the polyarthritis of rats(4). Since there is no exact counterpart in experimental animals of human rheumatoid arthritis, it is interesting that in these 2 types of experimental arthritis the results with cortisone and ACTH are different. It is also of interest that gold salts will favorably influence the rat polyarthritis as well as many cases of rheumatoid arthritis in man. It would be interesting to check the use of gold salts in the formalin arthritis.

In view of the previous reports of the beneficial effect of aureomycin on pleuropneumonia infections in animals and man (3,7) it is interesting that terramycin is also effective in preventing the development of the rat polyarthritis. This finding suggests that terramycin might be given clinical trial in patients with Reiter's syndrome and in early cases of rheumatoid arthritis.

Summary. Cortisone failed to prevent the development of the polyarthritis of rats when its administration was begun on the day of infection or to improve it when given therapeutically. The animals receiving cortisone gained less weight than the unmedicated controls. Terramycin given in 0.3% in the diet prevented the development, and 0.3% in the drinking water favorably altered the course of the rat polyarthritis.

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Blood and Packed Cell Volume of the Adult Rat as Measured by Tagged Cells.* (18014)

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The blood volume of the rat derived from the dilution of intravenously administered dyes by plasma has been reported frequently. Estimates vary from about 4.3 to 8.0 ml per 100 g body weight(1-8), with a mean of approximately 7.0 ml. Berlin *et al.*(9), on the basis of the dilution of transfused blood containing cells tagged with radioactive iron, phosphorus, or both, reported a mean volume of about 4.6 ml per 100 g, a value considerably lower than most obtained by the plasma-dye method.

It seemed pertinent to measure adult rat blood and packed cell volumes employing both the transfused radio-iron tagged erythrocyte and hemoglobin extraction(10) technics and to compare these data with those of Berlin (9). The tagged cell transfusion method was also applied to the evaluation of the efficiency of perfusion with respect to the total rat as well as individual tissues.

Experimental. The rats used were adult males of the Sprague-Dawley strain bred in this laboratory and maintained on a Purina ration. They appeared in no way unusual.

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