indirect effects upon renal function produced by generalized physiological disturbances in the organism.

Summary. Young chicks were exposed to 1000 r X radiation at dose rates of 6 or 43 r per minute. Determinations of excretion rate of urate and phenol red and of blood urate levels indicated that death in the initial period (within 8 to 24 hours) following high rate exposures was preceded by renal failure and a rapidly developing azotemia. Individuals that survived 24 hours or more after exposure, whatever the dose rate, continued to excrete at a normal rate. Phenol red retention invariably accompanied failure of urate excretory function.

1. Jacquez, J. A., and Karnofsky, D. A., *Am. J. Roentgenol. Radium Ther.*, 1950, v64, 289.
2. Stearner, S. P., *Am. J. Roentgenol. Radium Ther.*, 1951, v65, 265.
3. Brown, H., *J. Biol. Chem.*, 1945, v158, 601.
4. Folin, O., Berglund, H., and Derick, C., *J. Biol. Chem.*, 1924, v60, 361.
5. Levine, R., Wolfson, W. Q., and Lenel, R., *Am. J. Physiol.*, 1947, v151, 186.
6. Stearner, S. P., and Christian, E. J. B., Unpublished data.
7. Edson, N. L., Krebs, H. A., and Modd, A., *Biochem. J.*, 1936, v30, 1380.
8. Shannon, J. A., *J. Cell. Comp. Physiol.*, 1938, v11, 135.
9. Prosser, C. L., *Radiology*, 1947, v49, 299.

Received September 4, 1951. P.S.E.B.M., 1951, v78.

Effect of Cortisone on Anaphylactic Response of Guinea Pig Ileum. (19180)

WILLIAM D. GRAY, LAURA PEDRICK, AND ROBERT WINNE.
(Introduced by Raymond W. Cunningham.)
From the Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y.

A diminished antibody production(1), interference with antigen-antibody reaction(2), and altered reactivity of tissues(2) have been considered as possible mechanisms by which ACTH and cortisone influence certain hypersensitive states. The anaphylactic response of the isolated guinea pig ileum (Dale-Schultz Phenomenon) appeared to be a simple method of evaluating the importance of these postulated modes of action of cortisone on hypersensitivity of the Arthus type. In contrast to the intact guinea pig, the response of the isolated ileum is more easily standardized, and therefore tests of significance can be made with fewer animals. Other investigators have reported the inefficacy of cortisone treatment on guinea pig anaphylaxis(3,4). It is not known whether this is a species difference or a matter of dosage, since cortisone has been claimed to be effective against anaphylaxis in the mouse(5), rabbit(6), and the anaphylactoid reaction in the rat(7). Accordingly, experiments were performed on the isolated sensitized guinea pig ileum to study the effect of prolonged administration of cortisone acetate on (1) the level of tissue antibody, (2) the reactivity of tissue to histamine, and (3) the effect of cortisone acetate *in vitro* on the antigen-antibody reaction. The effects of sodium salicylate and of ethyl alcohol on the latter reaction were also investigated.

Method. Twelve virgin, female guinea pigs weighing between 260-350 g were divided at random into 4 groups of 3 animals each. Each group was injected intraperitoneally with 0.5 ml of undiluted horse serum, without preservative. Beginning on the day of sensitization and for a period of 20 days, one guinea pig in each group was injected subcutaneously with 5 mg/kg of a saline suspension of cortisone acetate, a second guinea pig with 1.0 ml/kg of 0.9% saline, and the third guinea pig in each group served as the untreated control. The saline suspension of cortisone acetate was prepared by dissolving the material in warm acetone, reagent grade, adding saline, and then removing most of the acetone by evacuation. The suspension was diluted so that each milliliter contained 5 mg of cortisone acetate. On the 20th day, all animals in

each group were injected with 0.1 ml of undiluted horse serum and 0.9% saline intradermally, and the sites of injection examined 24 hours later. No attempt was made to grade the Arthus reaction. The animals were sacrificed on the 21st day by a quick blow on the head. A section of ileum was immediately removed, washed free of blood with warm Tyrode's solution and duplicate strips about 4 cm in length were mounted in two chambers of 100 ml capacity in a constant temperature bath thermostatically maintained at 38°C. The tissues were bathed in Tyrode's solution continuously oxygenated with 100% O_2. The sections of ileum were run in parallel and the responses recorded kymographically. The experimental procedure was as follows: challenge by the addition of a standard dose of 10 γ histamine diphosphate, wash out; challenge by horse serum in a final chamber concentration of 1:2000, wash out; challenge by horse serum 1:2000, wash out; and finally challenge by the second addition of histamine diphosphate. The concentrations of cortisone acetate used in the *in vitro* experiments were prepared from a stock solution of 4 mg/ml in absolute ethyl alcohol by dilution with Tyrode's solution. Control concentrations of ethyl alcohol were prepared in a similar manner and expressed as the final concentration in the tissue chamber. The amounts of sodium salicylate (Merck) tested were added to the chamber in a volume of 0.5 ml Tyrode's solution. The experimental procedure for the *in vitro* studies was similar to that described previously, with the exception that an equilibration period of 5 or 10 minutes was allowed between the addition of the drug under test and the first challenge by horse serum 1:2000. In order to test the effect of the drug on histamine response, a wash out followed only the first addition of histamine diphosphate. In all experiments control sections of ileum from the same guinea pig were run in parallel. The guinea pigs used in these experiments were sensitized for a period of 21-30 days. All animals were housed in individual cages in air conditioned animal quarters (76°F) and maintained on Rockland stock guinea pig pellets fortified with ascorbic acid.

Results. The effect of prolonged cortisone acetate administration on the Arthus reaction, histamine reaction, anaphylactic response and the sources of variation isolated in the analysis of variance are given in Table I.

The histamine responses are expressed to the nearest millimeter and the anaphylactic response as a percentage of the mean of the histamine responses. It is evident that cortisone acetate treatment had no effect on histamine sensitivity and the reaction to a standard dose of histamine could be used as a standard to compare the anaphylactic responses. Since there was some variation between duplicate strips, the anaphylactic response was expressed as a percentage of the mean histamine response of each strip. Prolonged administration of cortisone acetate had no effect on the anaphylactic response of the isolated guinea pig ileum and did not abolish the Arthus reaction. The cortisone treated group did not gain as much weight as the two control groups but this difference was not analyzed statistically. Two guinea pigs sensitized to horse serum for 21 days and pretreated with 25 mg of cortisone acetate intraperitoneally 4½ and 6½ hours before sacrifice likewise showed no diminution in the anaphylactic response or histamine sensitivity of the isolated ileum. However, exploration of the peritoneal cavity at autopsy showed a considerable amount of unabsorbed cortisone acetate.

Table II summarizes the *in vitro* effect of cortisone acetate on the anaphylactic and histamine responses. Two sections of ileum from the same animal were run in parallel; one was controlled by the addition of ethyl alcohol. The results of cortisone acetate and ethyl alcohol in all concentrations were classified into two categories, reactors and nonreactors, arranged in a 2 x 2 contingency table and a χ^2 calculated, including the Yates correction for continuity. The χ^2, 3.74, was not significant at the $p = .05$ level of significance. Any inhibition of the anaphylactic response was undoubtedly due to the presence of ethyl alcohol and not cortisone acetate. This was further confirmed in six paired trials by the demonstration of an inhibitory effect

TABLE I. Effect of Cortisone Acetate Treatment on Arthus Reaction, Histamine and Anaphylactic Response of Isolated Guinea Pig Ileum.

	Cortisone			Saline			Control		
Group	Histamine, mm	Horse serum, %	Arthus	Histamine, mm	Horse serum, %	Arthus	Histamine, mm	Horse serum, %	Arthus
1	43, 45	34	+	29, 33	40	+	32, 36	45	+
	28, 33	41		38, 32	30		31, 33	51	
2	34, 39	61	+	30, 35	60	+	34, 40	46	+
	29, 30	58		36, 37	45		35, 30	75	
3	29, 40	47	+	28, 33	55	+	35, 32	54	+
	35, 40	53		31, 36	45		40, 40	40	
4	36, 40	68	+	35, 33	49	+	33, 35	71	+
	32, 37	60		34, 24	55		40, 30	60	
Wt gain, g		61			93			88	

Analysis of variance

Source of variation	D.F.*	Anaphylactic response F ratio	Anaphylactic response At. P	Histamine reaction F ratio	Histamine reaction At. P
Between treatments	2	2.03	P = .05, 3.6	1.33	P = .05, 3.6
" groups	3	7.90	P = .01, 5.1	I/F = 14.28	P = .05, 8.6
Interaction of groups × treatments	6	I/F = 1.92	P = .05, 4	I/F = 2.27	P = .05, 4.
Duplicate strips	12				

* D.F. = Degrees of freedom.
Interaction of groups × treatments, and duplicate strips pooled for estimate of error.

TABLE II. Effect of Cortisone Acetate *In Vitro* (Guinea Pigs).

Guinea Pig No.	Cortisone, γ	Alcohol, %	Equilibration period, min	Anaphylactic response Cortisone	Anaphylactic response Alcohol	Histamine response Cortisone	Histamine response Alcohol
19	560	.8	10	0	+	+	+
19	560	.8	10	0	+	+	+
19	560	.8	10	0	+	+	+
19	560	.8	10	0	0	+	+
29	560	.8	10	0	0	+	+
19	400	.8	10	+	+	+	+
19	280	.4	10	+	+	+	+
30	200	.28	5	+	+	+	+
30	100	.14	10	+	+	+	+
	No. of reactors			4	7	9	9

0 = No response. $\chi^2 = 3.74$. Not significant At. P = .05.

of 0.8% ethyl alcohol on the anaphylactic response of 4 out of 6 strips. All 6 control strips showed the typical response when challenged by horse serum. Neither cortisone acetate nor ethyl alcohol affected the histamine response. It was also found that those alcohol treated strips which did not respond to the first addition of horse serum, failed to respond to a second addition of horse serum even after the chamber was washed out three times with Tyrode's solution. Furthermore, the inhibitory effect of ethyl alcohol was not due to any effect on the horse serum *per se* since a solution in 0.8% ethyl alcohol elicited the typical anaphylactic response 5 minutes and 3½ hours after preparation.

The effect of sodium salicylate on the anaphylactic response is summarized in Table III. Successive additions of horse serum, 1:2000, were added to the chamber, each followed by washing with Tyrode's solution. Control strips from the same guinea pig were run in parallel. The response to the first addition of horse serum is considered 100% and rated 4+.

Treatment with sodium salicylate appears to prevent complete desensitization. The data were classified into reactors and non-reactors to the second addition of antigen and a χ^2 of 2.62 calculated by means of a 2 x 2 contin-

TABLE III. Effect of Sodium Salicylate *In Vitro* (Guinea Pigs).

Guinea Pig No.	Sodium salicylate, mg	Equilibration period, min	Reaction to successive addition of antigen 1	2	3	4	(Control) Reaction to successive addition of antigen 1	2	3	4
7	25	10	4+	1+	—	—	4+	0	—	—
7	50	10	4+	2+	+	0	4+	0	—	—
17	10	10	4+	2+	0	—	4+	0	—	—
17	20	5	4+	1+	+	0	4+	+	0	—
27	20	5	4+	0	—	—	4+	0	—	—
27	10	10	4+	0	—	—	4+	0	—	—
20	100	10	4+	4+	0	—	4+	0	—	—
	No. of reactors		7	5	2	0	7	1	0	—

4+ = 100%. $\chi^2 = 2.62$, P = .10.

gency table, with a P = .10. It is possible that in a more extensive series, the difference might be significant at the P = .05 level of significance. The histamine response was not affected. There did not appear to be any clear relationship between the dose of salicylate and the response to the second addition of antigen.

Discussion. Prolonged treatment of guinea pigs with cortisone acetate did not abolish the Arthus reaction or the anaphylactic response of the isolated guinea pig ileum. If the amount of fixed tissue antibody is a true measure of antibody production and release, the process was apparently not affected by prolonged cortisone treatment. It has been questioned whether the level of tissue antibody reflects the level of circulating antibody(8). The literature is replete with contradictory statements in regard to the effect of cortisone on antibody production(9). Since the level of circulating antibody was not measured in this investigation, no definite statement can be made. It can, however, be definitely stated that prolonged cortisone acetate treatment had no effect on the level of tissue antibody, as measured by the anaphylactic response of the isolated guinea pig ileum. Hyperadrenocorticalism of endogenous origin, indicated by gross adrenal hypertrophy, induced by the injection of a stressing agent for 27 days likewise had no effect on guinea pig anaphylaxis (10). The ineffectiveness of cortisone acetate *in vitro* indicates that it had no effect on the antigen-antibody reaction in the Arthus type of hypersensitivity in the guinea pig. This is in agreement with one unpublished report (11). The reactivity of the isolated ileum to histamine was not affected by cortisone treatment *in vivo* or *in vitro*.

The abolition of the anaphylactic response by low concentrations of ethyl alcohol was not due to any effect on the antigen or the reactivity of the tissue as measured by the histamine response. It was not due to a "blockade" of the antigen-antibody reaction since repeated washing did not restore the anaphylactic response. Possibly some interference with antigen-antibody reaction or effect on tissue antibody is involved.

It has been stated that salicylates block antigen-antibody reactions(12). The incomplete desensitization of the isolated ileum after treatment with sodium salicylate would indicate some blockade of the antigen-antibody reaction. However, the effect was minimal and a large series of trials is needed to definitely establish it as fact.

Summary. Treatment of guinea pigs during the period of sensitization to horse serum with 5 mg/kg of cortisone acetate daily for 20 days did not abolish the Arthus reaction or affect the level of tissue antibody as measured by the anaphylactic response of the isolated ileum. Injection of 25 mg intraperitoneally 4½ and 6½ hours before sacrifice likewise had no effect. Cortisone acetate *in vitro* had no effect on the antigen-antibody reaction of the Arthus type. Ethyl alcohol, at a concentration of 0.8%, usually abolished the anaphylactic response. Some blockade of the antigen-antibody reaction by sodium salicylate was indicated. The histamine sensitiv-

ity of the isolated ileum was not affected by cortisone acetate treatment *in vivo* or *in vitro*.

The cortisone acetate used in these experiments was kindly supplied by Dr. S. Bernstein, Lederle Laboratories Division, American Cyanamid Co.

1. Fischel, E. E., *Bull. N. Y. Acad. Med.*, 1950, v26, 255.
2. Dougherty, T. F., and Schneebeli, G. L., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 854.
3. Landau, S. W., Nelson, W. A., and Gay, L. N., *Bull. Johns Hopkins Hosp.*, 1951, v88, 395.
4. Malkiel, S., *J. Immunol.*, 1951, v66, 379.
5. Nelson, C. T., Fox, C. L., Jr., and Freeman, E. B., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 181.
6. Germuth, F. G., Jr., Oyama, J., and Ottinger, B., *J. Exp. Med.*, 1951, v94, 139.
7. Selye, H., *Canad. Med. Assn. J.*, 1949, v61, 553.
8. Roberts, S., and White, A., *Endocrinology*, 1951, v48, 741.
9. Kass, E. H., and Finland, M., *New England J. Med.*, 1951, v244, 464.
10. Gray, W. D., Unpublished observation.
11. Loewe, S., cf. ref. 2.
12. Coburn, A. F., and Kapp, E. M., *J. Exp. Med.*, 1943, v77, 173.

Received November 13, 1951. P.S.E.B.M., 1951, v78.

Nucleic Acid Changes in Tumor-bearing Mice.* (19181)

L. R. CERECEDO, D. V. N. REDDY, A. PIRCIO,† M. E. LOMBARDO, AND J. J. TRAVERS.
From the Department of Biochemistry, Fordham University, New York.

Reddy and Cerecedo(1) observed a marked rise in the nucleic acid content of the tissues of mice bearing transplanted Sarcoma 180, and of those with transplanted melanoma S-91. An increase in the liver desoxypentose nucleic acid (DNA) fraction of rats fed p-dimethylaminoazobenzene, long before any hepatoma developed, was found by Masayama and Yokoyama(2). These findings have been recently essentially confirmed by Price *et al.* (3). In this laboratory, similar changes were observed in the tissues of mice during gestation(4).‡

In this paper we report data showing a relationship between the rate of growth of the tumor and the changes in the nucleic acid content of certain organs in the host. We also present data on the nucleic acid, purine, and pyrimidine content of melanoma S-91 at different stages of growth.

Materials and methods. Two strains of mice, dba and the Swiss, were employed in this study. The tumors, Sarcoma 180 and malignant melanoma S-91 were obtained from The Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. They were transplanted subcutaneously into the right pectoral region of 5- to 6-week-old mice of mixed sexes. Groups of 4, 5 or more animals were killed by decapitation at given intervals. The pooled tissues were homogenized at 0°C, and the nucleic acids extracted with hot trichloroacetic acid(5). The pentose nucleic acid (RNA) was determined according to the method of von Euler and Hahn(6), and the DNA according to that of Stumpf(7). Guanine and adenine were determined according to the methods of Hitchings(8), and of Woodhouse(9), respectively, as used in the combined procedure of Lombardo and Cerecedo (10). Uracil and cytosine were prepared in this laboratory. Thymine was generously supplied by The Fleischmann Laboratories, New York, through the courtesy of Dr. F. J. DiCarlo. The pyrimidines were estimated by means of paper chromatography. Since the

* This investigation was supported in part by grants from the National Cancer Institute, of the National Institutes of Health, Public Health Service, and the Damon Runyon Memorial Fund for Cancer Research.

† Present address: Department of Pharmacology, University of Chicago.

‡ After preparing the manuscript of this paper, our attention was called to a publication of Kelly and Jones(19), who studied the turnover of desoxypentose nucleic acid in mice bearing transplants of Strong's mammary carcinoma. Their results are in harmony with our findings.