dose of the test drug 48 hours after administration of antigen.

Where comparisons are possible, the results reported herein are in general agreement with those of Sterzl(12) although some minor differences with regard to the activity of specific compounds will be observed. In the present test to date, the activity with folic acid antagonists has been of moderate dimensions in contrast to the marked effects reported by others(4,12).

Perhaps the most important observation reported here is that the effects of the drug increase with increasing antigenic stimulus. The failure to employ an adequate antigenic stimulus may explain some of the failures to find suppression with 6-mercaptopurine. In the absence of a clear understanding of the processes involved in the inductive phase of antibody formation, the rôle of 6-mercaptopurine remains obscure. However, the present results suggest that maximal drug effects are obtainable only when these processes are stimulated maximally. It is noteworthy that all the substances found active to date also are active antitumor agents in tests with transplantable tumors in mice. The reverse, however, by no means follows.

Summary. A screening test is described for materials which suppress the immune response during its inductive phase. This test is based on the formation of hemagglutinins to sheep red blood cells in mice. 6-Mercaptopurine, 6-thioguanine and their S-imidazolyl derivatives, B.W. 57-322 and B.W. 57-323, show high activity.

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Effect of Stress on Response to Fludrocortisone Excess in the Rat.* (26759)

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We have reported that in rats treated with desoxycorticosterone acetate (DCA)(1,2) or STH(3), stress increases the hypertensive and/or nephrosclerotic action of the hormone, an effect not noted with cortisol(4). Selye(7) has reported that immature unilaterally nephrectomized rats given 200 μg/day of 9α fluoro-hydrocortisol (fludrocortisone) and 1% NaCl solution to drink, develop severe nephrosclerosis and ascites, but not cardiac lesions, encephalopathy or visceral periarteritis. Blood pressure showed an elevation to hypertensive levels in many animals, but this was not sustained, declining premortally.

It seemed of interest to determine the influence of simultaneous exposure to stress

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upon the patho-physiologic effects of fludrocortisone, since the steroid is potent both as a glucocorticoid and as a mineralocorticoid.

Materials and methods. Forty-two immature female rats of the Holtzman strain were used. Group 1 consisted of 10 animals exposed to stress for 12 consecutive hours daily. Group 2 consisted of 10 animals given 250 μg/day of an aqueous fludrocortisone acetate† suspension subcutaneously. Group 3 consisted of 12 animals given both of the preceding treatments and Group 4 consisted of 10 untreated controls. All animals were unilaterally nephrectomized on the day preceding inception of treatment and received 1% NaCl solution to drink throughout the experiment. Purina Laboratory Chow was fed.

Stress was imposed by the electrostressor (5) which delivered a brief electric shock to the animals at 2 minute intervals for 12 hours consecutively. Since food and water was unavailable during the imposition of stress, it was withheld from all animals during such periods.

Blood pressure was measured in unanesthetized animals by a tail plethysmograph, and pressures above 150 mm Hg were regarded as hypertensive. The animals were killed with ether on the 39th day and various tissues were excised for weight and/or histologic examination. Lesions in the heart and kidney, and in the mesenteric and pancreatic arteries, were evaluated microscopically.

Results. Stress alone did not cause hypertension. Fludrocortisone caused a definite increase in blood pressure to hypertensive levels in many of the animals. On the 33rd day of treatment hypertension was present in one animal under steroid treatment and by the 39th day 7 of the 9 survivors were hypertensive. In animals under dual treatment, 4 of the 10 survivors were hypertensive on the 33rd day, but by the 39th day 3 of these had died and only 2 of the group were hypertensive. The representative blood pressures are given in Table I. It was evident, particularly in the last 2 weeks of the experiment, that although stress alone caused but slight growth retardation it enhanced that caused by steroid. Mortality was also greater among those under combined treatment and half died before the experiment ended, although only one animal was lost from the group receiving only steroid. The 6 animals which succumbed to dual treatment had extensive gastro-intestinal ulcers and large, brown adrenals, indicating that stress had been much more severe in them.

Organ weights indicated that whereas fludrocortisone caused considerable adrenal atrophy in non-stressed animals ($P<.001$), it did not do so in stressed animals. Among the latter the adrenals were actually slightly larger proportional to body weight than in animals exposed only to stress, indicating that fludrocortisone-induced adrenal atrophy was blocked. Stress neither caused involution of the thymus and spleen, nor augmented that caused by steroid. Both renal and cardiac hypertrophy were evident in steroid-treated rats, and again this was not significantly increased by stress. The data are given in Table I.

Microscopic examination revealed no instance of visceral periarteritis in any of the animals and but a single example of focal myocarditis, the latter in an animal receiving only steroid. The kidneys of animals receiving hormone and which had developed elevated blood pressure, showed extensive change. Glomerular sclerosis or hyalinization of capillary loops was frequently noted. Occasionally fibrinoid necrosis of the wall of the afferent glomerular arteriole had taken place, although curiously intrarenal arteries and arterioles other than at this site were not involved. Hyaline casts occupied many of the tubular lumens. The incidence and severity of lesions seemed to be slightly greater in steroid-treated rats exposed also to stress, but the difference in severity was only slight and it seemed inadvisable to attempt quantitation of severity from examination of a single section from each kidney. Interstitial nephritis and/or fibrosis was noted in 3 animals exposed only to stress. Renal lesions were absent from controls.

† The steroid was generously supplied by E. R. Squibb and Sons.

TABLE I. Body and Organ Weight and Incidence of Lesions in Rats Exposed to Stress and Fludrocortisone Treatment Singly and Together.

		Stress	Fludrocortisone	Fludrocortisone + stress	Controls
Mortality, %		0	10	50	0
Body wt, g	Initial	59 ± 1*	58 ± 1	58 ± 1	57 ± 2
	Final	162 ± 2	109 ± 3	90 ± 5	184 ± 5
Blood pressure, mm Hg	Day 22	127 ± 4	129 ± 5	129 ± 1	120 ± 1
	" 33	127 ± 3	130 ± 5	148 ± 8	124 ± 2
	" 39	125 ± 4	166 ± 6	156 ± 9	122 ± 1
Organ wt, mg/100 g	Adrenals	39.8± 1.4	23.6± 1.2	41.4± 3.9	33.8± 0.7
	Thymus	212 ± 15	39 ± 5	35 ± 12	229 ± 9
	Heart	345 ± 9	431 ± 9	479 ± 22	318 ± 6
	Spleen	390 ± 28	235 ± 9	240 ± 12	328 ± 10
	Kidney	1059 ± 33	1600 ± 53	1511 ± 46	1019 ± 19
Lesions (% incidence)	Myocarditis	0	11	0	0
	Periarteritis	0	0	0	0
	Kidney	33.3†	66.6‡	100	0

* Mean ± S.E. of mean. † Interstitial nephritis. ‡ Glomerulosclerosis.

The kidney lesions in steroid-treated rats were similar to those resulting from desoxycorticosterone treatment, but differed in several details. Hyaline casts were not particularly abundant and there was little interstitial inflammation, perhaps reflecting the anti-inflammatory effect of fludrocortisone. The absence of involvement of renal arterioles, excepting those at the vascular pole of the glomerular tuft in the present circumstances, also contrasts with the necrosis, sclerosis and sub-intimal hyalinization so common in the vessels of DCA-treated hypertensive rats. It should be noted however that the degree of hypertension attained by animals in the present experiment was only moderate.

Discussion. The response of animals to fludrocortisone on the one hand, and the modification due to simultaneous exposure to stress were both distinctive. Steroid treatment caused certain changes that stress failed either to cause or modify; among these were enlargement of the heart and kidney and atrophy of the spleen and thymus. On the other hand, inhibition of body weight, presumably a reflection of the catabolic potency of the steroid, was greatest in animals under dual treatment, although stress alone showed some effect. Finally the steroid caused alterations opposite to those of stress, *e.g.*, effect upon adrenal size; and, under the circumstances of this experiment, stress predominated when both influences were imposed. The marked growth impairment, adrenal hypertrophy, prevalence of gastrointestinal ulcers and high mortality noted in the group under dual treatment left little doubt that the combination was much more severely injurious than either influence singly. The fact that the adrenals in these animals were, in proportion to body weight, if anything even larger than among rats exposed to stress alone, conformed with the high mortality observed. The amount of hormone, while sufficient to block resting ACTH secretion, as indicated by adrenal involution in the group given only steroid, clearly did not block the pituitary of animals under combined treatment. Insofar as hypertension and cardiovascular disease were concerned the results were less clear-cut. At the second blood pressure reading, on the 33rd day, the percentage of hypertensives among the group under dual treatment was much greater than in the group receiving steroid alone; hence the average pressure of the group as a whole was higher. Unfortunately the subsequent high mortality among hypertensives in the latter group reduced both average blood pressure and incidence of hypertension. Furthermore even the animals surviving dual treatment were emaciated and listless, and it is not surprising that the blood pressure proved to be somewhat unstable. It would thus appear that hypertensive disease was too great an additional burden for animals exposed to

both stress and the catabolic action of fludrocortisone to withstand.

Since cortisone enhances the renal damage caused by DCA(6), the great augmentation of renal enlargement and renal pathology by stress in rats receiving large doses of that steroid(2) could be construed as indicating that stress caused the adrenals to secrete a cortisone-like glucocorticoid. The present experiment seems to weaken the force of this hypothesis since stress did not as dramatically enhance the nephrotoxic action of fludrocortisone, which possesses both mineralocorticoid and glucocorticoid activity in high degree. It must be emphasized however. that stress enhances the pathologic effects of DCA on the vasculature much more markedly at high dosage of hormone than at low(2). and perhaps the results might have been more striking had a higher dosage of fludrocortisone been employed. The finding of either interstitial nephritis or fibrosis in 3 of the stressed animals is interesting. Such lesions were absent from controls, and animals receiving only steroid. Seemingly therefore the lesions were not of natural occurrence in the strain and may have been caused by stress, but suppressed by the anti-inflammatory effect of fludrocortisone in the steroid-treated stressed group. The slight incidence, however, precluded definite conclusions as to etiology and pathogenesis.

Summary. The influence of stress upon the response to fludrocortisone was assessed. The steroid caused atrophy of the spleen and thymus and hypertrophy of the heart and kidney, changes which were neither induced nor modified by stress. Body growth was slightly retarded by stress and markedly impaired by fludrocortisone, but the effect of combined treatment was greatest. Although the steroid alone caused adrenal involution, adrenal hypertrophy was found in animals subjected to stress or to stress and steroid treatment together. Mortality was insignificant from steroid alone, but great among animals subjected also to stress. There was some indication that stress enhanced the hypertensive and nephropathic response to steroid treatment, but precise assessment of this effect was rendered difficult by high mortality in the group exposed to dual treatment and the prevalence of emaciation and lowered vitality among survivors.

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Antithyroid Effect of Barbarin (Phenylthiooxazolidone), A Naturally-Occurring Compound from *Barbarea.** (26760)

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The capacity of certain crucifers to produce goiter has been known for some time (1). A potent antithyroid compound, goitrin (vinylthiooxazolidone), has been isolated from the edible parts of some members of this family(2,3,4,11) but seems to be contained in significant concentration only in turnip and rutabaga. It is present as an inactive thioglycoside, progoitrin(5), from which goitrin is released by enzymatic hydrolysis. Goitrin has an antithyroid potency 33% greater than propylthiouracil (PTU) in man and 2% that of PTU in the rat(2).

Recently a related compound, barbarin (phenylthiooxazolidone), has been isolated

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