vulsant activity of SC is not related to the XD inhibition. XD activity approaches control values either by addition of NaBr (which increases the latent period) or by administration of compounds containing free carbonyl groups, such as acetone, without increasing the latent period. Sulfite oxidation by the serum enzyme is also inhibited *in vitro* both aerobically and anaerobically.

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Response of Serum Haptoglobin to Inflammation in Adrenalectomized Rat.* (28103)

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Serum haptoglobin (Hp), an *α-2* glycoprotein characterized by its ability to bind hemoglobin stoichiometrically and irreversibly, undergoes characteristic alterations in disease. It is decreased or absent in hemolytic states, in hepatocellular disease, and after estrogen administration. Elevations of serum levels of this protein are found in acute and chronic infections, neoplastic disease, rheumatoid arthritis and other collagen diseases, obstructive biliary disease, and a variety of inflammatory states(1). In conditions causing its elevation, haptoglobin behaves as an acute phase reactant; its serum levels reflect the activity of the disease process(2). Thus, changes in serum Hp are analogous to other, presumably non-specific, changes in serum glycoproteins and seromucoids in disease(2, 3,4). Tissue injury is common to all the pathologic states resulting in elevated Hp levels. Vital to the defense of the animal against a variety of pathologic stresses are the adrenal glands. The suggestion has been made that the prime mediator of various acute phase serum protein alterations is the adrenal cortex(3,4,5). It appeared worthwhile, therefore, to attempt to define the role of the adrenal gland in the acute phase response of serum Hp. Accordingly, the effect of adrenalectomy, and of replacement corticosteroid therapy on the response of serum Hp to a standard inflammatory stimulus has been studied.

Methods. In the initial series of experiments, male Sprague-Dawley rats weighing between 200 and 300 g were used. Adrenalectomy was performed through a midline abdominal incision, closed in 2 layers with gut and silk respectively. The animals were maintained on 1% saline. In the second series of experiments, investigating the effect of replacement corticoid therapy, Sprague-Dawley rats weighing 75-125 g were used, and adrenalectomy was performed by a posterior approach. Preliminary experiments had indicated that the responses of both groups of animals were comparable. Blood for Hp determination was obtained by retro-orbital plexus puncture with capillary tubes. Hp was determined in a Coleman Jr. Spectrophotometer by the peroxidase method of Owen *et al.*(6) and was expressed as mg Hgb-binding capacity per 100 ml serum. The inflam-

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TABLE I. Alterations in Rat Serum Haptoglobin Following Induction of a Turpentine Granuloma.

	Intact (10)*	Sham adrenal-ectomized (8)	Adrenal-ectomized (10)	Adrenalec-tomized + cortisone (8)	Adrenalecto-mized + cor-ticosterone (9)	Sham + cortisone (4)	Sham + cor-ticosterone (3)
Control sera #1	60 ± 5†	51 ± 4	53 ± 6	53 ± 7	58 ± 5	37 ± 5	45 ± 4
Control sera #2 (10 days post-op.)	—	87 ± 8	67 ± 7	41 ± 7	49 ± 7	47 ± 12	44 ± 9
48 hr post-turp. injection	254 ± 17	261 ± 26	158 ± 18	148 ± 20	168 ± 9	75 ± 9‡	70 ± 5‡

* No. of animals.
† Hp, mg/100 ml, mean and standard error.
‡ No turpentine injection given.

matory stimulus consisted of injection of 1 ml of commercial grade steam-distilled wood turpentine into a pouch on the back formed by the subcutaneous injection of 15 ml of air, the so-called "granuloma pouch" technic(7).

Results. In the first series of experiments, the animals were divided into 3 groups. Group I, consisting of 10 intact rats, was injected with turpentine after control sera had been obtained. Group II consisted of 10 rats subjected to bilateral adrenalectomy. These animals were maintained on 1% saline. Two weeks after operation a second control serum was drawn. This was necessary since the operative procedure itself would tend to provoke a rise in serum Hp. The animals were then given turpentine injections. A third group of 8 rats was subjected to a sham operation, identical to that performed in the adrenalectomized animals except that the adrenals were left intact. Blood was drawn for Hp determinations 48 hours after turpentine injection, a time when peak Hp levels are observed(8).

All these groups achieved a significant rise in mean Hp levels 48 hours after application of the inflammatory stimulus (Table I). However, the increase among the adrenalectomized rats was significantly less than that of the untreated or the sham-operated controls. Serum Hp among the sham-operated animals rose to a mean and standard error of 261 ± 26 mg% after turpentine injection. In contrast, serum Hp in the adrenalectomized animals rose to only 158 ± 18 mg%. The difference between these responses is highly significant ($p<0.01$).

These results indicated that a distinct but sub-maximal Hp response could occur in the absence of the adrenals. The next series of experiments was designed to determine if replacement corticosteroid therapy at physiologic doses(9) would restore the response to normal. Two compounds were tested, cortisone and corticosterone; the latter is the chief natural product of the rat adrenal cortex. "Pharmacologic" doses of steroids were avoided, since these may of themselves increase the serum Hp to abnormal levels(1). Adrenalectomized rats were divided into 2 groups and given, respectively, 0.25 mg of corticosterone and 0.25 mg cortisone as aqueous suspensions subcutaneously on 4 successive days, turpentine being injected on day 3.

The Hp response of the adrenalectomized animals maintained on these corticoids was not restored to normal. In terms of their Hp responses, both groups of these animals behaved as did the saline-maintained rats. However, there was a striking difference in survival after the stress of the turpentine injection: while mortality among the saline-maintained adrenalectomized animals was 75% after turpentine injections, there were no deaths among the corticoid-maintained adrenalectomized rats.

The sham-operated animals given corticoids alone, without turpentine, maintained normal Hp levels.

Discussion. A potent inflammatory stimulus evoked a marked elevation of Hp within 48 hours among intact and sham-operated animals; this response was essentially independent of the animals' age and weight. Adrenalectomized rats, after the same stimulus, also responded with an elevation of serum

Hp. Adrenal glands, therefore, are not essential for the development of this response. Levels achieved in the adrenalectomized rats, however, were significantly lower ($p<0.01$) than those of the control group. It is of interest that similar findings have been reported in studies of the response of fibrinogen levels to trauma in adrenalectomized rats(5).

When replacement steroid therapy was undertaken, a dissociation of corticoid effects was seen. Adrenalectomized animals on corticoids received full protection from the lethal effects of the turpentine abscess, but their Hp responses were not affected. That the doses of corticoids used were adequate is demonstrated by the fact that all treated rats survived this rather intense stress. It may be speculated that higher doses of steroids might have induced a normal Hp response. The intact animal subjected to inflammatory stress probably increases his steroid output above the basal levels necessary for maintenance of life, and it is impossible to say whether the replacement doses used in these experiments were fully equivalent to the endogenous secretion of corticoids among the animals with intact adrenals. However, the fact that Hp values among the steroid-treated rats were *no* higher than those among the untreated adrenalectomized rats suggests that the replacement dose was not the determining factor. If it were, one would expect at least a value intermediate between that of the adrenalectomized group and the normal group, with steroid doses high enough to assure 100% survival. It is concluded that the role of the adrenals is permissive; in their absence an Hp response to tissue injury occurs but is significantly impaired.

Summary. The response of serum haptoglobin to turpentine-induced inflammation in intact and adrenalectomized rats was studied. Adrenalectomized rats maintained on saline developed a rise in serum Hp, but one which was significantly less than that of the control animals. Replacement corticoid therapy in adrenalectomized rats improved survival markedly, but failed to restore the response of serum Hp to normal.

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Antibodies to Rabbit γ-Globulin After Immunizing with Various Preparations of Autologous γ-Globulin.* (28104)

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Current evidence indicates that circulating autologous protein injected into animals does not produce antibody unless it has first been treated by methods which produce some degree of denaturation or alteration, however slight. Ample proof of the antigenicity of slightly altered autologous γ-globulin has been presented by Milgrom and Witebsky (1) and by McCluskey, Miller and Benacer-

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