culated from the difference between wet weight and that found after drying the whole organ in an oven at 120°C. In the case of muscle, fat and skin representative samples of 20 to 30 g were dried. No water content estimations were made on the alkali-treated organs.

Results are given in Table I.

Summary. The data show that percentages of organ water due to trapped blood are generally small using this method of sacrifice and except in cases of spleen and thymus are satisfactorily constant. Variability in spleen values is probably due to variable blood discharge during hemorrhage. Comparison of spleen weights in Table I with those of spleens from unbled rabbits(4) suggests blood discharge volumes of about 2 ml. Profound vasoconstriction presumably explains the smaller percentages of blood in muscle and skin(3), the latter normally comprising one of the largest blood reservoirs in the living animal.

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In vivo Inhibition of Rat Blood Serum Xanthine Dehydrogenase by Semicarbazide.* (28102)

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It is known that xanthine oxidase (XO) from milk and from rat liver is inhibited *in vitro* by phenylhydrazine and semicarbazide, this inhibition being completely reversed by 2-methyl-1,4-naphthoquinone(1). Semicarbazide (SC) also inhibits 3 pyridoxal phosphate catalyzed enzymatic systems, *i.e.*, l-cysteine desulfhydrase, glutamic-aspartic transaminase and l-glutamic acid decarboxylase which is competitively reversed by pyridoxal phosphate(2). Further, it has also been shown that SC is able to inhibit the xanthine dehydrogenase activity (XD) of rat serum(3).

Semicarbazide is of great pharmacological and biochemical interest since it produces in animals seizures which simulate *grand mal* epilepsy. These seizures are preceded by a latent period after administration of the drug (4).

It is shown here that XD activity from rat serum is strongly inhibited *in vivo* by SC and that this inhibition could be reversed by propanone (a compound having a free carbonyl group) or by NaBr, a compound which increases the latent period preceding the seizures.

Methods. Male Wistar white rats weighing 100-150 g kept on a well balanced diet were used throughout the experiments. All drugs were injected intraperitoneally in aqueous solutions except for menadione (2-methyl-1,4-naphthoquinone) which was dissolved in propylene glycol. Doses used for 100 g body weight were as follows: semicarbazide, 50 mg; pentamethylenetetrazol, 10 mg; NaBr, 20 mg; acetone (2-propanone), 40 mg; and menadione, 2 mg. At the dosage used, SC produced seizures which appeared 45-60 minutes after injection of the drug. Blood samples were collected by heart puncture under light ether anesthesia and the clear serum

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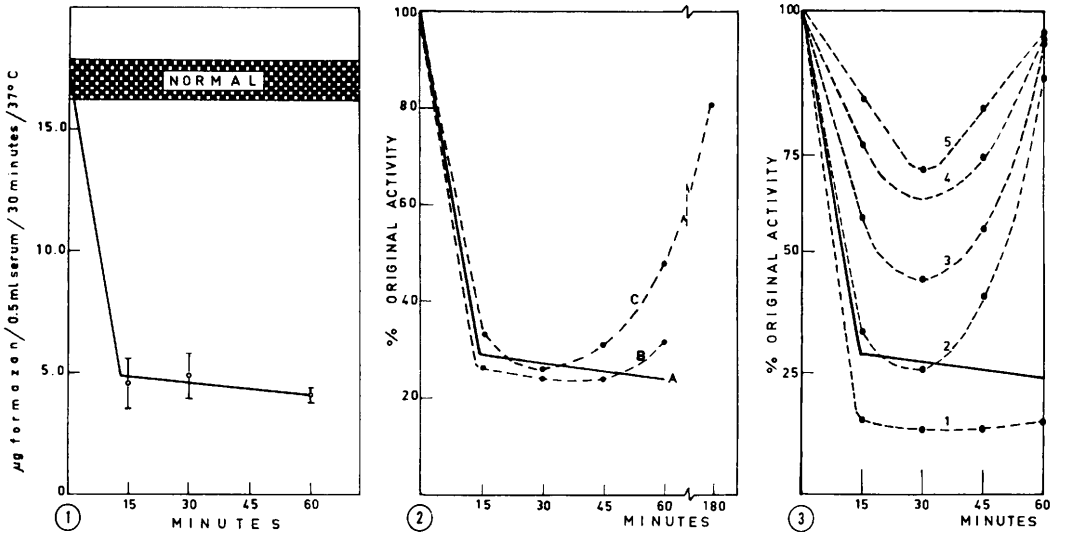

FIG. 1. Inhibition of xanthine dehydrogenase from rat blood serum by semicarbazide (50 mg/100 g body wt). Normal range represents mean ± standard deviation of 16 normal determinations.

FIG. 2. Protective effect of NaBr *in vivo* on inhibition of xanthine dehydrogenase by semicarbazide. (A) Enzyme activity in rats injected with semicarbazide. (B) Activity in rats receiving 20 mg (per 100 g body wt) NaBr before injection of semicarbazide. (C) Activity in rats receiving 60 mg NaBr (per 100 g body wt) before injection of semicarbazide.

FIG. 3. Protective effect of acetone on inhibition of xanthine dehydrogenase by semicarbazide. ——— Activity in rats injected with semicarbazide. – – – – Activity in rats given acetone before injecting semicarbazide. Numbers in graph represent amount of doses of 40 mg of acetone (per 100 g body wt) given to animals.

separated. The XD activity was determined colorimetrically by measuring the formazan produced by reduction of triphenyltetrazolium chloride (TTC) in evacuated Thunberg tubes (5). Each tube contained: 0.5 ml of serum, 2.5 ml of pyrophosphate buffer 0.015 M, pH 8.6 and, in the side arm, 0.1 ml of a 0.05 M solution of hypoxanthine and 0.3 ml of a 0.1% solution of TTC. After evacuating the tubes, they were placed in a water bath at 37°C for 10 minutes, then the substrate and the electrons acceptor were tipped in. The results were expressed as μg of formazan produced by 0.5 ml of serum in 30 minutes at 37°C.

Results. The XD activity for 16 normal control rats was 17.00 ± 0.78 μg of formazan/0.5 ml of serum/30 minutes.

After administration of SC the XD activity was greatly reduced, the inhibition being of the order of 75% (Fig. 1). It remained practically constant for about 45 minutes when seizures appeared and the animal died.

Sodium bromide increased the latent period preceding the seizures. A single dose of 20 mg/100 g body weight injected 15 minutes before administration of SC showed little effect. However, three doses of NaBr given each 24 hours increased the latent period from 60 to 180 minutes after administration of SC.

The inhibition of XD activity by SC in the rats treated with sodium bromide was much reduced. Sixty minutes after administration of SC, inhibition was 52.23% (75% for animals not previously injected with sodium bromide) and 180 minutes after injection of SC the inhibition was only 19.49%. These results, and values for controls receiving NaBr alone, are given in Fig. 2.

Since SC is a carbonyl trapping agent, acetone (a metabolite containing a free carbonyl group) was tested, to see whether it could protect *in vivo* the inhibition of XD by SC. Acetone was injected each 24 hours and SC 15 minutes after the last injection of acetone. In this case, seizures appeared 45 minutes after SC administration and animal died. Even 5 consecutive doses of acetone did not prevent the appearance of seizures and sub-

sequent death of the animal. However, serum XD activity was less inhibited than in the animals receiving SC alone. These results, and the original XD activity of rats injected with equivalent doses of acetone alone, are shown in Fig. 3.

Other drugs were tested to see if they were able to protect the inhibition caused by SC. Menadione was not effective although it lengthened the latent period to 80-100 minutes. Pentamethylenetetrazol which produces seizures identical to those caused by SC showed no effect on XD activity (average values: 22.77 μg formazan/0.5 ml serum/30 minutes) during the latent period.

Further experiments showed that SC (0.1 mg/0.5 ml serum) is also able to inhibit XD *in vitro*. This inhibition can be protected by sodium bromide (5-10 mg NaBr/0.5 ml serum) and by acetone (10-100 mg acetone/0.5 ml) to a lesser extent. Sulfite oxidation by the enzyme in presence of hypoxanthine was also inhibited *in vitro* by SC (0.1 mg/0.5 ml of serum) both aerobically and anaerobically, using TTC as electrons acceptor.

Pyridoxin (1-5 mg/0.5 ml serum) was also tested *in vitro* and the results showed that it does not protect inhibition of XD by SC.

Discussion. Xanthine dehydrogenase activity decreases a few minutes after intraperitoneal administration of SC indicating (as in the inhibition of glutamic acid carboxylase by larger doses of thiocarbohydrazide(6)) that XD inhibition by SC is not a slow reaction.

Protection of XD inhibition by SC *in vivo* by supplementing carbonyl groups suggested that an inhibition could be due to a reaction between SC and a carbonyl moiety of the enzyme molecule with formation of the corresponding semicarbazone which would be devoided of enzymatic activity. However, as *in vitro* experiments(1), a semicarbazone derivative would not be expected to form *in vivo*. The results of our experiments suggested that supplementation of propanone probably promotes formation of acetone semicarbazone, a compound which does not inhibit XD activity although it presents a greater convulsant activity than SC(4).

However, the possibility of a reaction between SC and a carbonyl group of the substrate could not be excluded. According to Fridovich and Handler(7), during the enzymatic sulfite oxidation hypoxanthine is first reduced to a derivative of unknown structure and then reoxidized. This reoxidized form of hypoxanthine or the reduced form have a carbonyl group which can bind HSO_3. Our results on inhibition of sulfite oxidation activity of XD by SC *in vitro* suggest that the carbonyl group of the substrate (which is able to bind sulfite) could first bind the SC, a carbonyl reagent.

Since XD activity may approach control values by administration of NaBr (which increases the latent period) it seems that the convulsant activity of SC is not related to the XD inhibition of the serum. Nevertheless, pentamethylenetetrazol produces seizures identical to those produced by SC without inhibition of serum XD activity.

The results obtained with NaBr *in vivo* suggested that NaBr only appeared to increase recovery rate and not reduce inhibition of XD by SC. However, the *in vitro* experiments (where no latent period or physiological mechanisms are involved) seem to show that NaBr does reduce inhibition of XD by SC.

As Westerfeld *et al.* pointed out (1), SC inhibits XO activity *in vitro* but has no effect on XD activity of milk and of the rat liver enzyme. The *in vivo* XD inhibition found in the present investigation suggests that SC exerts its effect on the dehydrogenase portion of the enzyme as well. Since we have found that XD activity of rat liver is also inhibited *in vivo* by SC an identity between the serum and liver enzyme becomes evident. This identity was previously suggested by Affonso *et al.*(5) in view of the activation of both enzymes by CCl_4 *in vitro* and *in vivo* and by the identical mobility rate shown by the serum and liver enzymes during paper electrophoresis experiments(8).

Summary. Xanthine dehydrogenase (XD) activity of rat serum was shown to be inhibited *in vitro* and *in vivo* by semicarbazide. The inhibition *in vivo* occurs during the latent period that precedes the seizures produced by the drug. It seems that the con-

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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