is metabolized rapidly by the microsomes seems to account for the need for repeated injections to prolong the prothrombin time and the need for relatively large amounts of puromycin, compared with studies *in vitro* involving inhibition of synthesis of protein by ribosomes.

Female rats of the Wistar strain showed a similar prolongation of prothrombin time, with the peak effect occurring at about 24 hr. However, male rats of this strain showed little or no prolongation on the same dosage schedule. Thus, in this strain a marked sex difference in response to puromycin was observed. As yet, higher doses and a greater number of injections have not been tried on these animals.

Puromycin does not appear to inhibit the clotting factors in the circulation. In separate experiments in which a single injection of puromycin was given by the intracardiac route, the prothrombin times of 12·5% plasma obtained 5 min after injection were the same as those of plasma prior to administration of the drug. In addition, the prothrombin time of plasma was not affected when puromycin was added directly to plasma in amounts up to 280 $\mu$g/ml, which would be somewhat greater than the amount present in plasma if the total amount injected into the experimental animals were uniformly distributed throughout the body water.

Although the coumarin anticoagulants and puromycin are similar in that both agents prolong the prothrombin time, it cannot be concluded on the basis of these studies that the coumarin anticoagulants act at the same site as puromycin. However, assumed that puromycin was acting by aborting protein synthesis in the liver at the ribosomal level as in other systems,[5] the present studies indicate that the ribosomes are susceptible to the action of drugs in the synthesis of the clotting factors and may be the site of action of the anticogulants. Shah *et al.* reported that puromycin antagonized the effect of vitamin K in vitamin K-deficient rats.[10] Further experiments are in progress to investigate the possibility that the microsomes are the site of action of the coumarin anticoagulants.

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## The long-term effects of a single dose of methyl prednisolone on $^{35}S$ uptake in ocular and nasal tissue*

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EFFECTS of steroid hormones on the steady-state dynamics of the connective tissue components of skin and cartilage—mucopolysaccharides (MPS) and collagen—are easily demonstrated.[1, 2] Suggestions have also been made relating steroid-induced reversible glaucoma with alterations in the MPS of the

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eye.[3, 4] Because of these suggestions, we decided to study the alterations of a component of ocular connective tissue (sulfated mucopolysaccharides) after treatment with a single dose of methyl prednisolone.

By using techniques similar to those described below, it has been shown that if inorganic $^{35}$S-sulfate is administered to an animal, about 93 per cent of the $^{35}$S incorporated in the cornea and about 83 per cent of the $^{35}$S incorporated in the sclera exist as sulfated mucopolysaccharides.[5] Most MPS in these tissues are sulfated;[6] therefore, the measurement of $^{35}$S incorporation would seem to be a fairly reliable index of the turnover of MPS in these tissues.

The experimental animals were male New Zealand white rabbits weighing between 3·25 and 3·75 kg. Half the animals were given a single subconjunctival injection of 8 mg of 6-α-methyl prednisolone 21-acetate (Depo-Medrol*) to the right eye; the other half received the steroid vehicle in the right eye. Twelve hours before death, all the animals were given an i.p. injection of 750 μc of $Na_2{}^{35}SO_4$. Six circular trephine samples of cornea, each 4 mm in diameter, and eight samples of sclera of similar diameter were obtained from each eye. Three samples (20 μl each) of whole blood and of aqueous humor were also obtained from each animal. Ocular and nasal mucosa tissue samples were first washed until free of unincorporated $^{35}$S. Nasal mucosa was air-dried (each sample was about 5 mg dry weight). The wet ocular tissues and the dried nasal mucosa were dissolved in concentrated formic acid. The $^{35}$S content of each sample was determined with a gas flow detector.

The $^{35}$S levels of all the tissues measured (cornea, sclera, aqueous humor, blood, and nasal mucosa) responded in much the same manner when treated with the steroid. For about 20–25 days the tissues from the steroid-treated animals had a lower level of $^{35}$S than had the tissue of the vehicle-treated animals. For the following 10–15 days, tissues from the steroid-treated animals had a higher level of $^{35}$S than the controls. Figure 1 represents data obtained from corneal tissue. Figure 2 indicates the

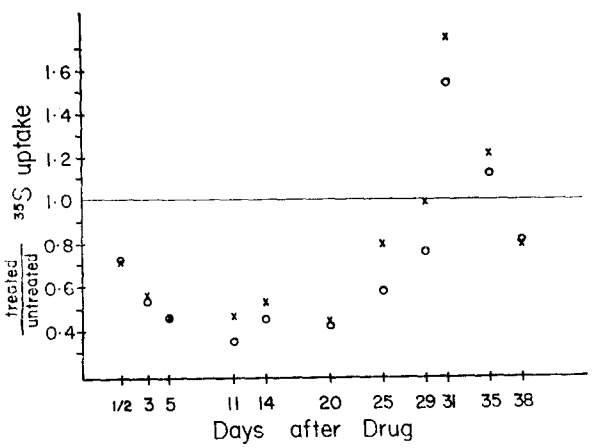


FIG. 1. Cornea. The ratio of $^{35}$S uptake in steroid-treated vs. untreated corneal tissue. Eight mg of 6-α-methyl prednisolone administered subconjunctivally to the right eye at zero time. The open circle represents the right eye, the × the left eye. Each point is the average of the values of all the buttons taken from three to six eyes of steroid-treated animals compared to the values from three to six eyes of untreated mates.

results obtained with blood and aqueous humor. The chronological pattern of turnover in the other tissues was similar; i.e. low initial uptake in steroid-treated tissue, followed by elevated uptake, followed by return to control level of uptake.

We have seen a biphasic alteration of the $^{35}$S level of the blood induced by a single injection of methyl prednisolone acetate. Presumably the $^{35}$S levels of the other tissues also displayed a biphasic pattern because of coming into equilibrium with the blood.

* Registered, Upjohn Co., Kalamazoo, Mich., U.S.A.

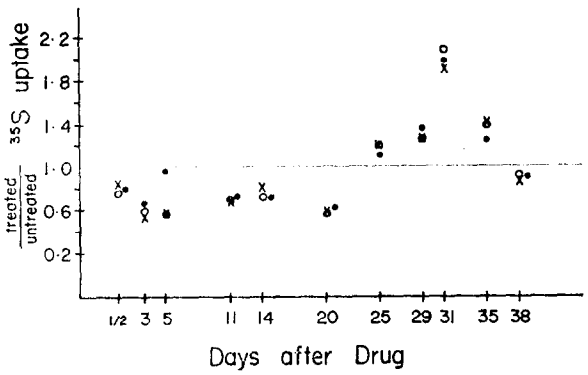


FIG. 2. Aqueous humor and blood. Solid circles, blood; open circles, right eye; ×, left eye.

Also, if one accepts as valid the premise that the $^{35}S$ levels measured here reflect, mainly, sulfated mucopolysaccharides, then a profound change is occurring in colour MPS. The changes that were measured in blood and nasal tissue would imply that this effect is not localized to the eye.

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## Effect of hypoglycemic agents on liver regeneration

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MANY oral hypoglycemic compounds have been synthesized and investigated in animals, but only a few with activity have survived clinical testing, because of their high toxicity, especially in provoking renal and hepatic reactions. Even with agents under use in man, such as carbutamide and metahexamide, to mention but two, severe liver damage has been reported.[1, 2] The current investigation was instituted with prominent therapeutic sulfonylurea and biguanide derivatives with the view of discerning hepatic changes as reflected in the extent of tissue weight restoration or the liver increment in partially hepatectomized rats, and to study the effect of prolonged systemic hypoglycemia, which must involve the liver directly or indirectly, on the regenerative process. In spite of numerous studies, there is little accord about the site and mechanism of action of the agents except that the hepatic glucose output is diminished. In this conjunction, carbutamide (N-sulfanilyl-N′-butylcarbamide) is claimed to cause a decrease in the rate of liver regeneration in operated rats force-fed one or four dosages, each at 2·0 g/kg and sacrificed 80 hr after surgery.[3, 4] The present communication also describes experiments with insulin NPH, glucagon, and the deproteinated extract Depropanex. The last, which is virtually free of insulin, has been employed clinically as a smooth-muscle antispasmodic and vasodilator.