terone for 15 min under air in a total volume of 0.25 ml. After incubation, steroids were extracted with chloroform and ring A reduction (Δ^4-hydrogenase activity) was evaluated using the *p*-nitrophenyl hydrazine reaction [16] and u.v. absorption at 240 nm. The *in vivo* plasma half-life of corticosterone was determined using plasma samples obtained 5, 10, 15 and 20 min after the intravenous injection of 1.0 μCi[1,2-^{3}H]corticosterone (sp. act. 50 Ci/m-mole), as described previously [17]. Radioactivity present in 100-μl aliquots of plasma was determined using a Packard Tri Carb liquid scintillation spectrometer.

RESULTS AND DISCUSSION

Enzyme activities in Tables 1 and 2 are expressed as nmoles product formed or substrate metabolized per min/per g of liver. However, since indomethacin did not significantly affect microsomal protein concentration, the effects observed are equally valid when expressed per mg of microsomal protein. In female rats, within 24 hr after starting indomethacin treatment, the rates of ethylmorphine demethylation and aniline hydroxylation were reduced significantly (Table 1). With continued administration of the drug, enzyme activities were decreased further. The decline in drug metabolism was accompanied by a progressive fall in the hepatic microsomal concentration of cytochrome P-450, the terminal oxidase for ethylmorphine and aniline metabolism. However, enzyme activities decreased proportionally to a greater extent than cytochrome P-450 content. In male rats (Table 2), the effects of indomethacin on drug metabolism were qualitatively similar to those in females, but were considerably smaller in magnitude. Reinicke and Klinger [18] previously reported that a slightly smaller dose of indomethacin (6 mg per kg per day for 3 days), when given to phenobarbital-treated male rats, prolonged hexobarbital sleeping time, suggesting inhibition of hepatic hexobarbital metabolism. However, neither cytochrome P-450 concentrations nor microsomal metabolism of hexobarbital was determined in those studies.

Indomethacin treatment also produced a significant decrease in the activity of hepatic steroid Δ^4-hydrogenase (Tables 1 and 2), the rate-limiting step in corticosteroid metabolism [19, 20]. Accordingly, the rate of disappearance of corticosterone from plasma decreased with increasing duration of indomethacin administration. The effects of indomethacin on steroid metabolism were also greater in females than males. These observations indicate that doses of indomethacin used occasionally in studying prostaglandin actions can have profound effects on hepatic drug- and steroid-metabolizing enzymes. Such effects may alter the biological activity of other drugs being administered simultaneously and may modify plasma steroid concentrations independently of changes in endocrine secretion. Interpretation of apparent sex differences in drug or hormone action is further complicated by the greater potency of indomethacin in female rats than in males. Changes in hepatic function, therefore, must be considered when evaluating the effects of indomethacin administration of experimental animals for even relatively short periods of time.

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Influence of calcium ethylenediaminetetraacetate on the metabolism of collagen and noncollagen proteins of carrageenin granuloma in rats

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A number of agents, including steroids, have been used in attempts to alter inflammatory responses. Granulation tissue formed in the course of chronic inflammation contains a large amount of collagen in its extracellular space. Thus, agents affecting protein metabolism may be important in regulating the formation and resorption of

granulation tissue. Recently, Rogerson and Aronson[1] showed that, after parenteral administration of calcium ethylenediaminetetraacetate (CaEDTA), urinary excretion of hydroxyproline was increased markedly as a result of increased degradation of both mature and immature collagens. They[2] also showed that lysosomal stabilizers and labilizers attenuated and accentuated, respectively, the effect of CaEDTA in enhancing the degradation of collagen. Otsuka and Mori[3] demonstrated that the administration of CaEDTA daily for 3 or 4 days resulted in the resorption of pre-existing granulation tissue and significantly decreased the content of glycosaminoglycans and glycoproteins. The present study was designed to investigate the effect of CaEDTA on the metabolism of collagen and noncollagen proteins in order to study the mode of action of CaEDTA on the resorption of pre-existing granulation tissue.

A granuloma pouch was induced in male rats of the Donryu strain, weighing 130–160 g, by subcutaneous injection of a 2% solution of Seakem 202 carrageenin according to a procedure described previously[4]. On day 9 after carrageenin injection, a dose of 50 μCi/kg of body weight of L[^{3}H]proline (generally labeled, 63 Ci/mmole) was injected into the granuloma pouch. Seven rats assigned for the initial control were killed on day 10 (10-day control). The treated group was injected with CaEDTA (300 mg/kg of body weight) into the granuloma pouch every 12 hr from day 10 to day 13, while the control rats (14-day control) were injected with 0.9% NaCl. Both the CaEDTA-treated and control rats were killed on day 14, and the pouch of granulation tissue was harvested. The collagen of the pouch was extracted twice as gelatin by autoclaving the tissue, and the resulting collagen-free residue was referred to as the noncollagen protein fraction. The amounts and radioactivities of collagen and noncollagen proteins were measured according to a procedure described previously[5].

The results are summarized in Table 1. The body weight of CaEDTA-treated rats was significantly lower than that of control rats. It seems likely that this body weight loss involves some changes in protein metabolism of rats treated with CaEDTA. However, the protein metabolism of granulation tissue may be only slightly influenced by the growth retardation, because Fukuhara and Tsurufuji[4] have reported that a dietary limitation, which induced body weight loss, had little effect on the formation and maintenance of carrageenin granulomas.

The amount and the radioactivity of collagen hydroxyproline in the whole granulation tissue of the CaEDTA-treated group were similar to those of the 14-day control (Table 1). The results suggest that CaEDTA has no effect on the collagen breakdown of granulation tissue. Rogerson and Aronson[1] reported that the urinary excretion of hydroxyproline was markedly increased after parenteral administration of CaEDTA. We also found that CaEDTA significantly increased urinary hydroxyproline excretion at day 14; values for the control group were 582.8 ± 81.2 and for the CaEDTA-treated group 973.4 ± 136.1 μg hydroxyproline/18 hr (mean ± S. E. of eight rats). Because of the lack of effect of CaEDTA on the collagen metabolism of the granulation tissue, the increase in urinary excretion of hydroxyproline may be due to enhanced collagen breakdown in other tissues.

On the other hand, the amount and the radioactivity of noncollagen protein in the whole granulation tissue of the CaEDTA-treated group were significantly lower than those of the 14-day control (Table 1). These results suggest that CaEDTA enhances the degradation of noncollagen protein. Rogerson and Aronson[2] reported that CaEDTA caused a labilization of lysosomes *in vivo*, as evidenced by a redistribution of lysosomal acid hydrolases in tissues and their appearance in blood plasma in increased amounts. This activation of lysosomes by CaEDTA may contribute to enhancing the degradation of noncollagen protein in granulation tissue of rats treated with CaEDTA.

The effect of CaEDTA on the syntheses of collagen and noncollagen proteins was studied by treatment with CaEDTA (300 mg/kg of body weight) every 12 hr from day 10 to day 13 as described above. On day 14 an additional injection of CaEDTA was made 12 hr after the last injection of CaEDTA on day 13, and 4 hr later the rats were killed. The granulation tissues were rapidly removed and minced into 1–2 mm pieces in an ice-cold Petri dish. The minced granulation tissue (1.0 g) was incubated under an atmosphere of 95% O_2–5% CO_2 at 37° for 2 hr in 10 ml of Krebs' saline serum substitute[6] containing penicillin (1 mg), dihydrostreptomycin (1 mg) and 5 μCi L[^{3}H]proline. At the end of the incubation, 50% trichloroacetic acid was added, to give a 10% concentration. Collagen and noncollagen proteins were isolated, and their radioactivities were measured according to a procedure described previously[5]. The amount of DNA in the granulation tissue was determined by the procedure described in a previous paper[7]. Synthesizing activities of collagen and noncollagen proteins by granulation tissue cells were expressed as the radioactivity of each protein/μg of DNA. As shown in Table 2. CaEDTA did not affect the syntheses of both the proteins, though the wet weight of granulation tissue and the amount of noncollagen protein were decreased markedly on day 14 by CaEDTA treatment.

We reported previously[8] that an anti-inflammatory

Table 1. Effect of CaEDTA locally injected every 12 hr from day 10 to day 13 on a pre-existing carrageenin granuloma labeled with [^{3}H]proline*

	10-Day control	14-Day control	CaEDTA treatment
Net body wt (g)	195 ± 3	201 ± 5	183 ± 6†
Exudate (g)	25.5 ± 2.2	31.7 ± 3.2	27.6 ± 2.7
Granulation tissue, wet wt (g)	3.54 ± 0.18	4.95 ± 0.43	3.66 ± 0.16†
Collagen			
Collagen hydroxyproline (mg) in the whole tissue	10.757 ± 0.506	15.111 ± 1.976	13.306 ± 1.223
Radioactivity of collagen hydroxyproline (dis./min × 10^{-5} in the whole tissue)	2.440 ± 0.141	1.506 ± 0.117	1.637 ± 0.152
Noncollagen protein			
Protein (mg) in the whole tissue	174.6 ± 7.3	218.1 ± 17.0	152.8 ± 7.8‡
Radioactivity of protein (dis./min × 10^{-6} in the whole tissue)	6.924 ± 0.364	2.526 ± 0.072	1.954 ± 0.106‡

* Data are shown as means ± S. E. There were seven rats in each group.
† Values are significantly different from 14-day control, $P < 0.05$.
‡ Values are significantly different from 14-day control, $P < 0.01$.

Table 2. Incorporation of [^{3}H]proline into collagen and noncollagen proteins during incubation *in vitro* of minced granulation tissue from rats treated with CaEDTA*

	14-Day control	CaEDTA treatment
Net body wt (g)	199 ± 5	172 ± 8†
Exudate (g)	31.9 ± 3.6	36.2 ± 5.0
Granulation tissue, wet wt (g)	5.87 ± 0.43	3.81 ± 0.26‡
Collagen hydroxyproline (mg) in the whole tissue	17.212 ± 1.288	16.158 ± 1.590
Noncollagen protein (mg) in the whole tissue	263.07 ± 21.17	153.19 ± 11.53‡
Incorporation of [^{3}H]proline		
Radioactivity of collagen hydroxyproline (dis./min/μg DNA)	3.396 ± 0.627	3.017 ± 0.367
Radioactivity of noncollagen protein (dis./min × 10^{-2}/μg DNA)	2.154 ± 0.243	2.127 ± 0.133

* Data are shown as means ± S. E. There were eight rats in each group.
† Values are significantly different from 14-day control, $P < 0.05$.
‡ Values are significantly different from 14-day control, $P < 0.01$.

steriod enhanced the resorption of pre-existing granulation tissue through a strong inhibitory action on protein synthesis without apparently affecting the degradation of noncollagen protein. CaEDTA also enhanced the resorption of pre-existing granulation tissue. However, the mode of action of CaEDTA is different from that of the steriod; CaEDTA enhances the resorption of pre-existing granulation tissue as a result of the increased degradation of noncollagen protein without apparently affecting the syntheses of collagen and noncollagen proteins.

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Inhibition of methyltransferases by some new analogs of *S*-adenosylhomocysteine

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S-adenosylmethionine (SAMe)-dependent methyltransferases are inhibited by the reaction product *S*-adenosylhomocysteine (SAH). Numerous synthetic derivatives of SAH have been prepared and studied as inhibitors of methyltransferases (see, for example, Ref. 1–3). We are reporting here the inhibition of three mammalian SAMe-dependent methyltransferases by a group of novel antibiotics of microbial origin that are chemically related to SAH. The enzymes studied were norepinephrine *N*-methyltransferase (EC 2.1.1.28), the epinephrine-forming enzyme; histamine *N*-methyltransferase (EC 2.1.1.8), which methylates histamine on the aromatic ring; and catechol *O*-methyltransferase (EC 2.1.1.6), which *O*-methylates catecholamines and other catechols. The inhibitors studied are shown in Fig. 1. Compound I (known as sinefungin[4]) differs from SAH only in having the sulfur replaced with an amino-substituted methylene unit. In compound II, an amide linkage between that amino group and the carboxyl group forms a six-membered ring. Compound III is like I except that it has a double bond at the 4′, 5′-position. The lactam IV has the same relationship to III as II does to I. These compounds are all metabolites of *Streptomyces griseolus* (the lactams II and IV may have been formed during the extraction and purification process) and were isolated because of the antifungal activity of compound I (sinefungin)[5]. Their structural similarity to SAH led us to study their effects on these methyltransferases.

Norepinephrine *N*-methyltransferase was purified from rabbit adrenal glands and assayed, with L-norepinephrine as substrate, by previously described methods[6]. Histamine *N*-methyltransferase was purified from guinea pig brain through the stage of ammonium sulfate fractionation and assayed with histamine as the methyl acceptor by the methods of Brown *et al.*[7]. Catechol *O*-methyltransferase was prepared from rat liver through the stage of ammonium sulfate fractionation and assayed with L-norepinephrine as the methyl