slightly increased, d) into the lecithin area is reduced, and e) into a new fraction in or slightly in advance of the cephalin and overlapping the cerebroside area is increased to the extent that it becomes the major fraction. In conjugated pteridine-deficiency, per cent ^{14}C-incorporation a) into the cardiolipin-like area is virtually normal, b) into the neutral lipid area is slightly reduced, c) into the sphingomyelin and especially lecithin area is reduced, and d) into a new fraction in the cerebroside area is increased to the extent that it becomes the major fraction. Thus in both unconjugated and conjugated pteridine-deficient cells a new major lipid fraction into which ^{14}C-acetate is incorporated is formed. The nature of this fraction is determined by the type of pteridine deficiency (Fig. 1).

Summary. We have shown by alteration in ^{14}C-acetate incorporation into lipid fractions during pteridine deficiencies that both unconjugated and conjugated pteridines affect lipid metabolism in *C. fasciculata.* The most obvious change appears as an alteration in the chromatographic profile of acetate incorporation into the lipid fractions concomitant with these deficiencies, *i.e.,* the disappearance of label from the cardiolipin fraction and appearance of label into new fractions whose nature depends upon whether the deficiency is of conjugated or unconjugated pteridines.

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Effect of Orally Administered Proteolytic Enzymes on Carbon Tetrachloride Induced Granuloma Pouch. (31628)

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The granuloma suppressing action of proteolytic enzymes has been adequately documented(1-4). In these studies the enzymes were administered parenterally and the decreased granuloma response was attributed to the anti-inflammatory properties of the proteolytic agents.

The purpose of the present study is to present data illustrating granuloma pouch and acute inflammatory responses to CCl_4 injection, and to evaluate the efficacy of orally administered proteolytic enzymes in modifying these host responses.

Material and methods. Sprague-Dawley female rats weighing 95-120 g were used. The backs of these animals were shaved from the upper neck area to the lower thorax. CCl_4 (0.2 ml) was injected subcutaneously in the interscapular area. Immediately following the CCl_4 injections the appropriate medication dissolved in saline was administered orally *via* stomach tube and/or, as in the case of epsilon aminocaproic acid, injected intraperitoneally. The substances examined for their effect on CCl_4 induced granuloma pouches were (1) crystalline trypsin, 3000 NF units/mg, (2) streptokinase-plasminogen (SK-P),* (3) inactivated crystalline

* Varizyme, Lederle.

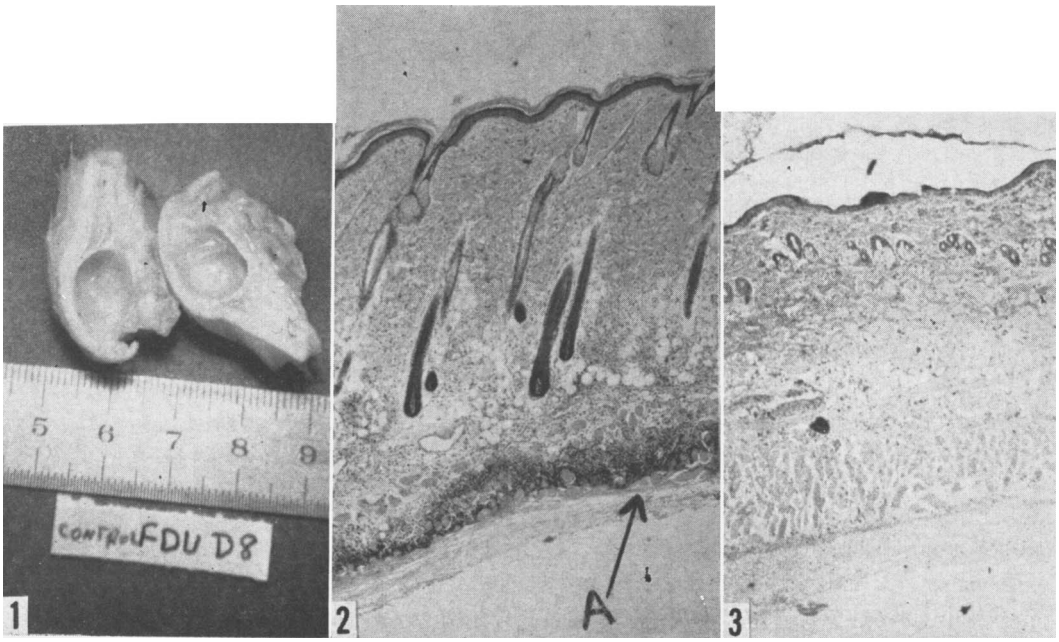


FIG. 1. Sagittal section of a granuloma pouch in CCl_4 treated rat.
FIG. 2. Histologic section through granuloma pouch of CCl_4 treated rat. (A) Diffuse, band-like arrangement of leucocytes in subcutaneous muscle.
FIG. 3. Histologic section through CCl injected area of SK-P treated rat reveals absence of the leucocytic band.

trypsin,† (4) epsilon aminocaproic acid (EACA), (5) bromelain, (6) papain, (7) chymotrypsin, (8) inactivated SK-P, (9) protease from *Aspergillus oryzae*.‡ Twenty-four hours following CCl_4 administration the shaved area was examined for pouch formation. A tense ballooned pouch (Fig. 1) constituted a positive response. Animals not exhibiting this reaction were regarded as "protected" animals in an experimental group. Histological studies were performed on specimens obtained from pouch tissue and overlying skin. Specimens from "protected" animals were taken from the area of injection previously marked with India ink. White blood counts were performed on 10 control, 10 trypsin treated and 10 SK-P treated rats, 24 hours after CCl_4 administration.

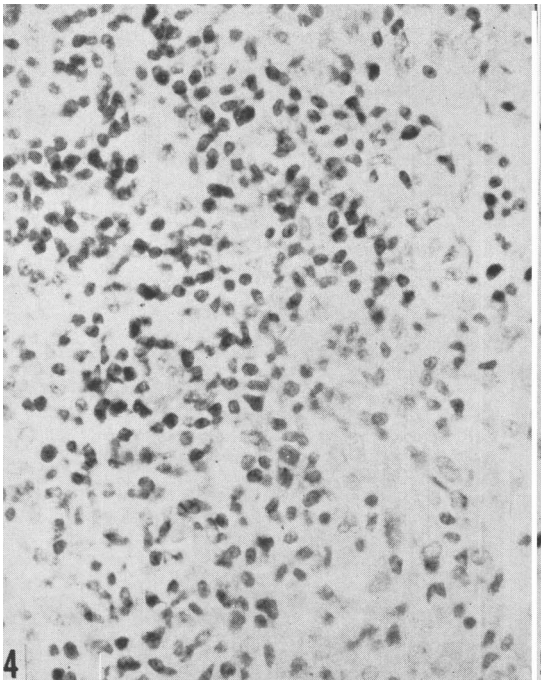
† Solution (5 mg/ml) adjusted to pH 1.0 with 1 N HCl and autoclaved at 15 lb pressure for 10 min. Esterolytic and proteolytic action could not be detected after treatment nor after standing for 24 hr in the cold room. SK-P was inactivated in similar manner.

‡ Asperkinase, Smith, Kline & French Labs.

Results. The data representing a composite of several experiments are presented in Table I. Orally administered trypsin and SK-P are strikingly effective in inhibiting CCl_4 induced granuloma pouch formation and acute inflammatory responses. Inactivated trypsin or SK-P is ineffective, as is trypsin or SK-P administered to animals pre-treated with epsilon aminocaproic acid. Bromelain, papain, chymotrypsin and Aspergillus protease are ineffective.

Histologic changes following CCl_4 in untreated animals are presented in Fig. 2 and 4. Specimens were taken from the peripheral walls of the pouch at the point of contact with the skin. The inflammatory response is "severe" and is characterized (Fig. 2) by dense infiltration of cutaneous muscle or granuloma tissue with leucocytes compressed in a tightly compartmented, band-like arrangement. The granuloma connective tissue appears edematous and densely infiltrated with leucocytes (Fig. 4). In "protected" rats no significant change is noted in the subcutaneous tissue obtained from the site of CCl_4

injection (Fig. 5). A "mild" reaction, found in "protected" rats, is characterized by sparse leucocytic invasion of cutaneous muscle or connective tissue, without any definitive band-like arrangement (Fig. 3). White blood counts are given in Table II. There is no evidence



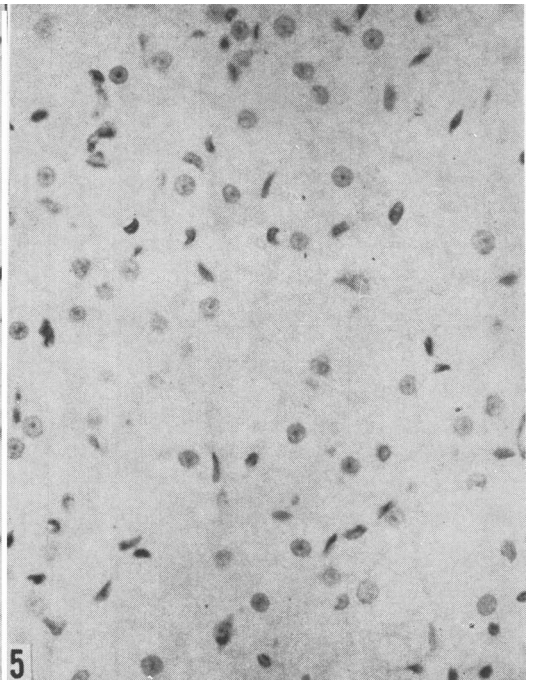


FIG. 4. Histologic section through subcutaneous connective tissue of a CCl_4 injected area. Note dense leucocytic infiltration consisting chiefly of lymphocytes and monocytes. 250 ×.

FIG. 5. Histologic section through subcutaneous connective tissues of a CCl_4 injected are a in an SK-P treated rat. The mesothelial interscrapular elements in the "brown fat" appear normal. 250 ×.

TABLE I. Effect of Orally Administered Proteolytic Enzymes and Epsilon Aminocaproic Acid on CCl_4 Induced Granuloma Pouch and Acute Inflammation.

Treatment	Dose/animal	No. of animals	No. "protected"	% "protection"
Saline	—	74	1	1.4
Trypsin	5 mg	93	88	95
Inactivated trypsin	5 mg	14	1	7.1
Trypsin + EACA (i.p.)	5 mg 100 mg	25	1	4.0
EACA (i.p.)	100 mg	25	0	0
SK-plasminogen	1,000 u	42	31	74
SK-plasminogen (inactivated)	1,000 u	15	0	0
SK-plasminogen + EACA (i.p.)	1,000 u 100 mg	15	0	0
Papain	5 mg	15	2	13
Bromelain	5 mg	15	0	0
Chymotrypsin	5 mg	40	2	5.0
Chymotrypsin	15 mg	10	0	0
Chymotrypsin (s.c.)	5 mg	10	0	0
Aspergillus protease	25 mg	15	0	0

i.p. = intraperitoneal administration; s.c. = subcutaneous administration.

TABLE II. Effect of Oral Enzyme Therapy on White Blood Count.

No. of rats	Treatment	W.B.C. (mean of 3 determinations)	W.B.C. range
10	CCl_4; saline	21,450	17,500-25,000
10	CCl_4; trypsin	15,500	14,200-17,600
10	CCl_4; SK-P	14,650	13,400-16,100

of white blood cell suppression in enzyme treated animals.

Discussion. Plasminogen and fibrinolytic activation have been implicated in anti-inflammatory activity. Astrup(5) has stated that plasminogen activation and fibrinolytic processes are basic repair mechanisms operative throughout the organism. Fearnley(6) has reported that pharmacological enhancement of endogenous fibrinolysis is attended by anti-inflammatory effects. Sherry(7) has related plasminogen activation to "all acute inflammatory reactions." Innerfield(8) has reviewed a number of clinical syndromes characterized by inflammation and defective fibrinolysis.

Trypsin and SK-P, enzymes effective in this study, activate plasminogen and are inhibited by EACA. Enzymes which have not been shown to activate plasminogen, *i.e.*, bromelain, papain, chymotrypsin, although salutary in other parameters of the inflammatory response(9-11), are ineffective in this study. These findings suggest a relationship between specific enzymatic activation of rat plasminogen and the anti-granuloma, anti-inflammatory effects observed herein. Indeed, there is evidence that the ultimate enzyme absorbed from the G.I. tract, and active in evoking anti-inflammatory effects, is not the orally given enzyme *per se*, but activated rat plasminogen (plasminogen activator). A prior study(12) demonstrated that SK catalyzes the formation of plasminogen activator from a zymogen present in gastric juice. In a later study, tissue plasminogen activator activity was significantly augmented following oral doses of streptokinase(13). These observations support the view that orally given enzymes need not be absorbed *per se* provided that they participate in the endogenous formation and absorption of plasminogen activator derived from plasminogen pro-activator in rat gastric and intestinal juices.

While the precise mechanisms of the anti-inflammatory response observed in this study remain obscure, two striking observations emerge with respect to modification of the host response to CCl_4: (1) that an orally administered enzyme capable of activating rat plasminogen modifies the rat inflammatory response; (2) the enzymatic nature of this action, at least in regard to trypsin and SK-P, is supported by the fact that the "protective" action is vitiated by enzyme inactivation or by systemic EACA administration.

Summary. Two host responses developed in rats 24 hours after CCl_4 injections subcutaneously: (1) tense, ballooned-out granulomas; (2) dense leucocytic invasion, edema and necrosis of cutaneous muscle. These responses were inhibited, or significantly modified, in rats receiving SK-P or trypsin orally. Epsilon aminocaproic acid blocked the anti-inflammatory and granuloma-inhibiting effects of trypsin or SK-P. Papain, bromelain, chymotrypsin and Aspergillus protease were inactive. The important implications of the data are: (1) that orally administered trypsin or SK-P is active in modifying the inflammatory response to CCl_4, and (2) that the action of these substances is related to their enzymatic activity.

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Effects of Glucose, Pyruvate, Lactate and Starvation on Contractility of Isolated Rat Atria.* (31629)

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The utilization of substrates has been extensively studied in cardiac tissue slices(1-5), isolated hearts(6-11), and hearts *in situ*(12-14), and it has been demonstrated that glucose, pyruvate, lactate, acetoacetate, and fatty acids can be oxidized by the myocardium. However, the functional importance of the different substrates for cardiac contractility has not been fully clarified. Recent work on the regulation of glucose metabolism has shown that glycolysis can be inhibited by pyruvate, acetoacetate, and fatty acids(15-17), and that high concentrations of pyruvate suppress endogenous respiration(18) and glucose uptake(19) in the isolated heart. A reduction of pyruvate uptake in the presence of glucose and insulin was also observed. In addition, lactate and glucose compete for utilization by the rat heart(9). Current interpretations emphasize the phosphofructokinase reaction as an important regulatory step in glycolysis(16); it is proposed that during the accelerated oxidation of pyruvate, fatty acids, or ketone bodies this enzyme is inhibited, leading to the accumulation of hexose phosphates, which in turn decreases hexokinase activity and glucose uptake. These inhibitions may be mediated by elevated levels of citrate and ATP(20-24). More distal enzymes in the Embden-Meyerhof pathway, such as phosphoglyceraldehyde dehydrogenase or pyruvate kinase, may also be involved in this regulation(19).

Results obtained with rat atria(25) and rabbit atria(26) suggest that although the glycolytic energy yield is comparatively small, either the uptake of glucose or the operation of the glycolytic pathway are important for a fraction of the contractile activity, inasmuch as pyruvate is only partially effective in restoring the developed tension in the absence of glucose or during block with 2-deoxy-glucose. The present work examines the effects of glucose, pyruvate, and lactate under different conditions in order to evaluate their capacities for maintenance of atrial contractility.

Methods. Atria were removed from decapitated rats and suspended in a modified Krebs-Ringer bicarbonate medium with the following compositions: Na^+ 145 mM, K^+ 6 mM, Ca^{++} 1.22 mM, Mg^{++} 1.33 mM, Cl^- 126 mM, $SO^{=}_4$ 1.33 mM, bicarbonate 25.3 mM, phosphate 1.2 mM, and glucose 5.5 mM. The medium was gassed with 95% O_2-5% CO_2 at a rate of 200 ml/min and maintained at pH 7.4 and 30°C. Cryoscopic determination gave a value of 286 milliosmolar for this medium. A constant resting tension of 750 mg was exerted on the atria with a micrometer head, the developed tension was recorded through a strain gauge, and the atria were electrically stimulated at a rate of 200/min. An equilibration period of 60 minutes was

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