(Table I). Although the data hardly lend themselves to precise quantification, crude ED_{50} values were calculated and potency comparisons made (Table II). Of the series under study, the three synthetic gonanes (relatives of 19-nortestosterone) were the most potent; 17α-ethynyl-19-nortestosterone was about equipotent with progesterone; and the acetoxyprogesterone derivative was intermediate. Comparison of these results with Clauberg test data (Table II) shows that the activities parallel each other; the most apparent discrepancy is 6α-methyl-17α-acetoxyprogesterone, which is the most potent compound in the series in its endometrial effects, but is only slightly more potent than progesterone in delaying parturition.

Summary. Various progestagenic steroids were studied for their effects in delaying parturition in pregnant female rats. 17α-Ethynyl-19-nortestosterone was about as potent as progesterone, the standard. 6α-Methyl-17α-acetoxyprogesterone was 3 times more potent than progesterone, while 13β-ethyl-17α-ethynyl-17β-hydroxygon-4-en-3-one and 13β-17α-diethyl-17β-hydroxygon-4-en-3-one were both about 10 times the standard. 17α-Chloroethynyl-13β-ethyl-17β-hydroxygon-4-en-3-one was the most potent compound of the series, being about 30 times more potent than progesterone.

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Lipid Variations Caused by Pteridine Deficiencies in *Crithidia fasciculata** (31627)

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The first unconjugated pteridine growth factor was identified by Nathan and Cowperthwaite(1) in the course of defining the nutritional requirements of the trypanosomatid flagellate, *Crithidia fasciculata*. This organism temporarily became a biochemical oddity when it was shown(2) that it required for growth both a conjugated and unconjugated pteridine. It is now apparent that the double pteridine requirement is a characteristic of every member of the family Trypanosomatidae in which the nature of the pteridine requirement has been investigated (reviewed in Guttman(3)).

By the nutritional bypass method in which supplying the product of the metabolic chain obviated requirement for the intermediate, it was shown that thymine is the growth-limiting product of conjugated pteridine (folic acid) metabolism in these organisms(2) and that a supplementary, non-limiting factor (a lipid?) is present in human serum(4).

Identification of products of unconjugated pteridine metabolism has been more difficult. Amount and kind of pteridine supplied to *C. fasciculata* alter the production of vitamin B_{12} and its congeners(5) but supplementation of the culture medium with vit B_{12} neither bypassed nor spared the unconjugated pteridine requirement. Similarly tyrosine, the product of an unconjugated pteridine-catalyzed hydroxylation of phenylalanine(6) neither bypassed nor spared the unconjugated pteridine requirement. Both phenylalanine and tyrosine are obligate nutritional requirements.

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Guttman(3) suggested that lipids may be the growth-limiting products of pteridine metabolism in *Crithidia*. This idea gained support with the publication of two abstracts (7,8) which noted that oxidation of glyceryl ethers to fatty acids required an unconjugated pteridine coenzyme(7) and that sphingosine spares the unconjugated pteridine requirement of *C. fasciculata*(8).

We report here on the reduction of total lipids as a result of deficiency of unconjugated (but not, however, conjugated) pteridines and on changes in acetate-^{14}C incorporation into various lipid fractions by either conjugated or unconjugated pteridine-deficient *Crithidia fasciculata*.

Materials and methods. *Crithidia fasciculata* (*Anopheles* strain) ATCC 11745 was used in these studies. Three different types of mass culture were grown in one liter batches in 2800 ml Fernbach flasks. The defined medium [Table IIIa in Guttman(3)] contained either a) folic acid 0.1 μg% + biopterin 0.1 μg% (normal), b) folic acid 0.001 μg% + biopterin 0.1 μg% (conjugated pteridine-deficient), c) folic acid 0.1 μg% + biopterin 0.001 μg% (unconjugated pteridine-deficient). Inoculum for each of the 3 types of mass culture consisted of the cell crop from 10 ml of log-phase culture grown under condition a) (normal). Sodium acetate -1-^{14}C (5.0 μc) was added to each mass culture along with the inoculum. Cultures were harvested in mid-log phase and stored lyophilized until lipids were extracted and analyzed.

Lipids were extracted with 16-20 volumes of $CHCl_3$:MeOH (2:1 v/v) and the residue removed by centrifugation. The supernatant was evaporated to dryness under reduced pressure and weighed. Samples (50-200 μg) were spotted on silica gel G TLC plates according to the method of Wagner *et al*(9) but without removing neutral lipids, and developed with $CHCl_3$:MeOH:H_2O (65:25:4 v/v/v). Sequential one cm fractions were scraped off the developed TLC plates, mixed with scintillation solution as suggested by Snyder (10), and the activity of each on cm fraction counted.

Results. Only unconjugated pteridine-deficient cells showed a reduction (10%) in total lipids. Incorporation of ^{14}C-acetate (as cpm/mg lipid) was reduced by 49% with unconjugated (biopterin) and by 58% with conjugated pteridine (folic acid) deficiency.

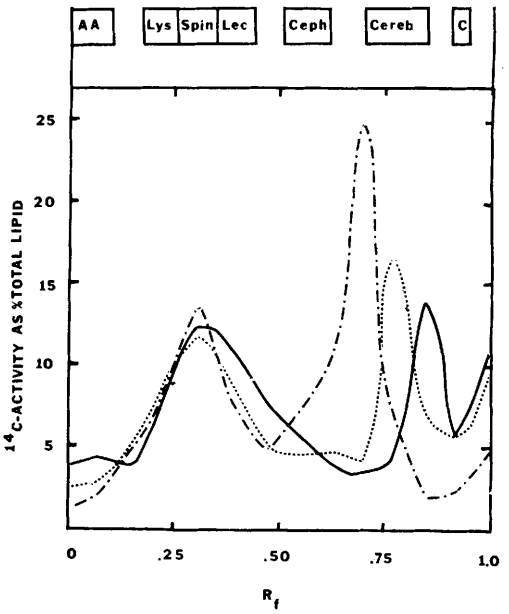

FIG. 1. Relative acetate-1-^{14}C incorporation expressed as percent ^{14}C incorporation into total lipid. Normal cells, 100% ^{14}C incorporated (———); unconjugated pteridine-deficient cells, 51% ^{14}C incorporated (—— - ——); conjugated pteridine-deficient cells, 42% ^{14}C incorporated (- - - -). R_fs of known compounds are given as reference: AA = amino acids; Lys = lysolecithin; Sphin = sphingomyelin; Lec = lecithin; Ceph = cephalin; Cereb = cerebrosides; C = cardiolipins.

In normal cells, virtually all the acetate incorporated into lipids is accounted for in 3 chromatographic fractions which are (Fig. 1), in descending order of amount of ^{14}C incorporated: 1. a broad band between R_f .24-.5 (the lysolecithin-sphingomyelin-lecithin area), 2. a well defined band, R_f .8-.9 (just behind the cardiolipin area), and 3. a neutral lipid fraction which travels with the solvent front. With either conjugated or unconjugated pteridine deficiency, there is a reduction in total amount of acetate incorporated and changes in distribution of ^{14}C. In unconjugated pteridine-deficiency, per cent ^{14}C-incorporation a) into the cardiolipin-like area is reduced, b) into the neutral lipid area is reduced, c) into the sphingomyelin area is

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.