The biological activities of the compounds herein described are compared with those of other anti-inflammatory steroids in an accompanying Communication.[17]

(17) G. E. Arth, J. Fried, D. B. R. Johnston, D. R. Hoff, L. H. Starett, R. H. Silber, H. C. Stoerk, C. A. Winter, THIS JOURNAL, **80**, 3161 (1958).

GLEN E. ARTH
FUNDAMENTAL RESEARCH DAVID B. R. JOHNSTON
MERCK SHARP & DOHME JOHN FRIED
LABORATORIES DIVISION WILLIAM W. SPOONCER
MERCK & CO., INC. DALE R. HOFF
RAHWAY, NEW JERSEY LEWIS H. SARETT

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REQUIREMENT OF CYTIDINE TRIPHOSPHATE FOR THE BIOSYNTHESIS OF PHOSPHOPANTETHEINE[1]

Sir:

The enzymes, present in cell-free extracts of *Proteus morganii*, which catalyze reactions 1, 2 and 3

pantothenic acid + ATP[2] ⟶
4′-phosphopantothenic acid + ADP + P_i (1)

4′-phosphopantothenic acid + cysteine + CTP ⟶
4′-phosphopantothenylcysteine + CDP[3] + P_i (2)

4′-phosphopantothenylcysteine ⟶
4′-phosphopantetheine + CO_2 (3)

have been named, respectively: (1) pantothenic acid kinase[4,5]; (2) phosphopantothenic acid–cysteine coupling enzyme[5]; and (3) phosphopantothenylcysteine decarboxylase. A crude extract, prepared from cells ruptured in a Hughes press, was treated with ammonium sulfate and calcium phosphate gel to yield a preparation of the coupling enzyme which was purified 20-fold and was free of the other two enzymes.

The substrates for the reaction catalyzed by the purified coupling enzyme were found to be 4′-phosphopantothenic acid and cysteine. The amount of product formed, 4′-phosphopantothenylcysteine, was determined by measurement of the disappearance of 4′-phosphopantothenic acid by determining the amount of pantothenic acid which could be regenerated by treatment with phosphatase. For this purpose, pantothenic acid assays were performed with *Saccharomyces carlsbergensis* 4228.[6] Unexpectedly, it was found that the purified coupling enzyme did not function unless a small amount of crude extract was also included in the reaction mixture. The activating factor in the crude extract was heat labile and appeared to be an enzyme. Of a large number of compounds which were tested only one, CTP, was able to replace the requirement for this extra enzyme. The activating effects of the crude extract and CTP are shown in Table I. Other nucleoside di- and triphosphates were inactive. Additional experiments have shown that only 0.2 μM of CTP is required to give maximal activity. It seems probable that the extra enzyme required in the absence of added CTP was nucleoside diphosphate kinase, whose function was to replenish the small amount of CTP which was present in the enzyme preparations.

TABLE I

REQUIREMENT OF CTP FOR SYNTHESIS OF 4′-PHOSPHOPANTOTHENYLCYSTEINE

The reaction mixture contained: 0.08 μM 4′-phosphopantothenic acid, 5 μM ATP, 10 μM $MgCl_2$, 80 μM tris-(hydroxymethyl)-aminomethane buffer at *p*H 7.4, 10 μM cysteine, 700 γ of purified coupling enzyme and additions as shown below in a total volume of 2 ml. Incubation was for 3 hr. at 37°, followed by heating for 5 min. at 100° and centrifugation to separate denatured protein. The supernatant solutions were analyzed as described in the text.

Addition	4′-Phosphopantothenylcysteine formed, $\mu M \times 10^2$
None	1.91
ATP, 5μM	1.25
ATP, 5μM + crude extract	4.38
ATP, 5μM + boiled extract	1.91
CTP, 5μM	6.92

Incubation of the purified enzyme with cysteine and CTP gave no detectable cytidine-containing, sulfur-containing compound. Thus it seems likely that the CTP requirement in the reaction is for the activation of the carboxyl group of 4′-phosphopantothenic acid in a manner similar to the way ATP functions in the synthesis of pantothenic acid from pantoic acid and β-alanine.[7]

(7) W. K. Maas, *Federation Proc.*, **15**, 305 (1956).

DIVISION OF BIOCHEMISTRY
DEPARTMENT OF BIOLOGY GENE M. BROWN
MASSACHUSETTS INSTITUTE OF TECHNOLOGY
CAMBRIDGE, MASSACHUSETTS

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16-METHYLATED STEROIDS. II. 16α-METHYL ANALOGS OF CORTISONE, A NEW GROUP OF ANTI-INFLAMMATORY STEROIDS. 9α-HALO DERIVATIVES

Sir:

Syntheses of the acetates of 16α-methylated analogs of cortisone, hydrocortisone and their 1,2-unsaturated derivatives, I and II, respectively, are reported in an accompanying communication.[1] The enchanced activity and freedom from salt retention characteristic of this group of compounds prompted extension to 9-halogenated analogs.

A dimethylformamide–pyridine solution of 16α-methylhydrocortisone acetate[1] (III) was treated with methanesulfonyl chloride,[2] affording 16α-methyl-4,9(11)-pregnadiene-17α, 21-diol-3,20-dione 21-acetate (IV), m.p. 205–208° λ_{max} 239 mμ, ε 17,300, [α] + 93°.[3] (*Anal.* Found: C, 71.96; H, 8.30) which was converted to 16α-methyl-9α-

(1) This investigation was supported by National Science Foundation Grant G4580.

(2) ATP, ADP, CTP and CDP are adenosine and cytidine tri- and diphosphates.

(3) Fragmentary evidence only indicates that CDP and inorganic phosphate (P_i) are products of this reaction.

(4) G. B. Ward, G. M. Brown and E. E. Snell, *J. Biol. Chem.*, **213**, 869 (1955).

(5) G. M. Brown, *Federation Proc.*, **17**, 197 (1958).

(6) L. Atkin, W. L. Williams, A. S. Shultz and C. N. Frey, *Ind. Eng. Chem., Anal. Ed.*, **16**, 67 (1944).

(1) G. E. Arth, D. B. R. Johnston, J. Fried, W. W. Spooncer, D. R. Hoff and L. H. Sarett, THIS JOURNAL, **80**, 3160 (1958).

(2) Modification of unpublished procedure of E. M. Chamberlain and J. M. Chemerda; *cf.* J. Fried, K. Florey, E. Sabo, J. Herz, A. Restivo, A. Borman and F. Singer, *ibid.*, **77**, 4181 (1955).

(3) Ultraviolet spectra are of methanolic solutions of the compounds. Rotations were determined in chloroform at 25°, concn. = 100 mg./10 ml. using the sodium-D line, unless otherwise noted.