ments must decide whether the "interference phenomenon" observed in the two classes of infectious agents is due to the same mechanism.

The "interference phenomenon" reported in this paper involving *D. andersoni* and small wild animals may have important implications in explaining the epidemiology of Rocky Mountain spotted fever. It may be involved in the explanation of the contention that in certain limited localities strains having high and low virulence persist year after year(3). Since a similar situation is found in scrub typhus(4), it is possible that such an "interference phenomenon" is important in the epidemiology of this rickettsial disease. However, much more work is necessary before final conclusions can be drawn, since many factors would be involved in determining the importance of such a phenomenon in nature.

It should be mentioned that with certain strains of lower virulence than the T strain, it has not been possible to infect cottontail rabbits, Columbian ground squirrels, field mice, or chipmunks, so that such animals will infect *D. andersoni*. These animals are among the main animal reservoirs for larvae and nymphs of *D. andersoni*. Multiplication of the rickettsiae of these strains of very low virulence could also not be demonstrated by repeated egg titrations of the organs and blood of the above animals. On the other hand, these strains grow about as well in *D. andersoni* and chick embryos as the virulent strains. It is possible that they are maintained by hereditary transmission. These low virulent strains, however, will still protect animals against highly virulent strains under the conditions described in Table I. The elucidation of the mechanism by which these strains are maintained in nature is of importance in understanding the epidemiology of not only Rocky Mountain spotted fever, but perhaps also of other rickettsial diseases.

Summary. 1. Eighty to 90% of the guinea pigs infected intraperitoneally with a strain of low virulence of *R. rickettsii* were protected against a simultaneous injection of a highly virulent strain of spotted fever, or boutonneuse fever, provided the low virulent strain was given in about 10 to 30 times the concentration of the virulent strain. 2. Infection with rickettsiae of Q fever, scrub typhus, endemic typhus, and epidemic typhus protected guinea pigs against a virulent strain of spotted fever under the above conditions. 3. Columbian ground squirrels, field mice (Microtus), and cottontail rabbits were infected by exposure to many *D. andersoni* containing a low virulent strain of Rocky Mountain spotted fever and a few *D. andersoni* containing a highly virulent strain. Uninfected ticks fed upon these animals were found to contain only the low virulent strain.

1. Price, W. H., *Science,* in press.

2. Bovarnick, M., Miller, J. C., and Snyder, J. C., *J. Bact.*, 1950, v59, 509.

3. Parker, R. R., *Bull. Montana State Board of Health,* 1923, No. 26, 33.

4. Philip, C. B., personal communication.

Received October 15, 1952. P.S.E.B.M., 1953, v82.

Role of Thioctic Acid in the Transfer of Acyl Groups.* (20061)

GERALD R. SEAMAN.

From the Carter Physiology Laboratory, University of Texas Medical Branch, Galveston, Texas.

Preliminary data(1) have suggested that in the oxidation of pyruvate thioctic acid has no role in the transfer of electrons over DPN,†

* This investigation was aided by grants from the National Institutes of Health, U. S. Federal Security Agency, and from the American Philosophical Society.

† The following abbreviations are used: Co A, coenzyme A; $DPN_{ox.}$ and $DPN_{red.}$, oxidized and reduced diphosphopyridine nucleotide; ATP, adenosine triphosphate.

but rather is essential for acetylation of Co A. The present investigation was undertaken to study in more detail the influence of thioctic acid in pyruvate oxidation, and in the analogous oxidation of α-ketoglutaric acid(2,3).

Methods. Pure cultures of the ciliate *Tetrahymena pyriformis* S‡ were grown in 5 gallon pyrex carboys containing 10 liters of 0.5% bacteriological peptone, 0.1% yeast extract and 8 p.p.m. of Dow Corning Anti-foam A. The carboys were well aerated on a vacuum line at room temperature (24-28°C). After 72 hours of growth the cells were harvested with the aid of a Sharples supercentrifuge and were washed 3 times with 5 volumes of distilled water. The washed cell paste was ground with ½ weight of powdered quartz in a chilled mortar and the mixture was brought into solution at pH 7.4. The quartz was removed by gentle centrifugation and the supernatant fractionated by isoelectric precipitation according to the procedure of Green *et al.*(5). The fraction which precipitated at pH 5.4 showed high pyruvic and α-ketoglutaric oxidase activity. In early experiments(1), the preparations were heated for 10 min at 54°C to destroy phosphotransacetylase activity. Later it was found by testing in the arsenolysis reaction(6) that *Tetrahymena* does not contain this enzyme. This is consistent with the previous observation that acetyl ~ phosphate is not formed in the course of acetate metabolism by this organism(7). However, heating coagulates many inactive proteins and enzymes which are present in the preparation after isoelectric precipitation. Therefore, this procedure was retained as the final step in the purification. Sodium pyruvate, α-ketoglutarate, ATP, and cocarboxylase were obtained commercially. Samples of highly purified Co A were obtained from Dr. F. M. Strong and from the Pabst Laboratories. Thanks are also extended to Dr. Helmut Beinert for a supply of acetyl ~ Co A. Thioctic acid was generously provided by Dr. E. L. R. Stokstad. *Oxidative activity* of pyruvic and α-ketoglutaric oxidase was measured spectrophotometrically following the rate of reduction of the dye, 2,6-dichlorophenolindophenol at 600 mμ. Optical density (O.D.) readings were taken at intervals for 3 min following addition of the enzyme and the values plotted against time. Oxidative activity is expressed as the decrease in O.D./min. A decrease in O.D. of 0.01/min corresponds approximately to the oxidation of 0.1 μM of substrate/hr. *Acyl groups* were measured by the hydroxamic acid method(8) and sulfanilamide by the colorimetric method of Bratton and Marshall(9). Acetate was determined enzymatically(10). Diacetyl and acetoin were determined by the method described by White *et al.*(11).

‡ This organism had previously been classified as *T. geleii* S. However Corliss(4) has indicated that *T. pyriformis* S conforms with correct nomenclature.

TABLE I.

Reaction mixture	Indophenol reduction/min. Pyruvic oxidase, O.D.	α-ketoglutaric oxidase, O.D.
Complete system	.057	.048
No ATP	.059	.052
" cocarboxylase	.048	.050
" DPN	.053	.057
" Co A	.051	.053
" $MgCl_2$	.002	.003
" substrate	.006	.002

Requirements for oxidative activity of pyruvic oxidase and of α-ketoglutaric oxidase. The complete system contained 20 μM of $MgCl_2$, 200 μg cocarboxylase, 10 μM ATP, 50 μg DPN, 60 units of Co A, 200 μM phosphate (pH 6.8), 20 μM of pyruvate or α-ketoglutarate, .10 ml of enzyme for pyruvic oxidase or .13 ml for α-ketoglutaric oxidase, and 2,6-dichlorophenolindophenol in 3 ml. The initial optical density was .410.

TABLE II.

Reaction mixture	Acylhydroxamic acid formed. Pyruvic oxidase, μM	α-ketoglutaric oxidase, μM
Complete system	.53	.58
No ATP	.58	.72
" cocarboxylase	.72	.64
" DPN	.64	.67
" Co A	.08	.06
" $MgCl_2$	.02	.09
" cysteine	.06	.06
" substrate	.04	.07

Requirements for acylation component of pyruvic oxidase and of α-ketoglutaric oxidase. The complete system contained 70 μg of cocarboxylase, 10 μM of ATP, 20 units Co A, 10 μM $MgCl_2$, 100 μg DPN, 10 μM of cysteine, 100 μM $NaHCO_3$, 10 μM of pyruvate or α-ketoglutarate, 3 μM hydroxylamine hydrochloride, .20 ml enzyme for pyruvate oxidation or .26 ml for α-ketoglutaric oxidation in 1 ml. Incubated 60 min. at 25°C.

TABLE III.

Enzyme treatment	Indophenol reduction/min.		Acylhydroxamic acid formed	
	Pyruvic oxidase, O.D.	α-ketoglutaric oxidase, O.D.	Pyruvic oxidase, μM	α-ketoglutaric oxidase, μM
0	.053	.051	.63	.58
0 + 20 units/ml thioctic acid	.052	.050	.59	.61
Alumina	.048	.042	.03	.06
Alumina + 20 units/ml thioctic acid	.054	.048	.61	.64

Effect of thioctic acid on acyl formation and on oxidative activity. The incubation mixtures were as indicated in the legends of Tables I and II except that ATP was omitted from both incubation mixtures and that Co A was omitted from the oxidative mixture.

Pyruvate was estimated as the dinitrophenylhydrazone(12) and acetaldehyde was determined according to Stotz(13). Ferricyanide was measured by treating samples with $ZnSO_4$, HCl, and KI, and then titrating the liberated iodine with dilute thiosulfate(14).

Results. The enzyme which precipitates between pH 5.4 and 6.3 contains high activity for the acylative and oxidative components of pyruvic and α-ketoglutaric oxidases. The activities of the two oxidases, however, are not identical. It was found that activity toward α-ketoglutarate was only approximately 77% of that toward pyruvate. In order to better compare the properties of the two oxidations, the amount of enzyme added to incubation mixtures when α-ketoglutaric oxidase was studied was therefore increased to 1.3 times the amount used when pyruvic oxidase was examined.

Neither cocarboxylase, DPN, nor ATP are necessary for optimal activity of either component of the respective oxidations (Table I). Sufficient cocarboxylase and hydrogen acceptor, which would be expected to be requirements of the system, are apparently retained in the enzyme preparation. Similar non-requirement for these cofactors has been found in studies with partially purified mammalian oxidases(2,5). Cocarboxylase and DPN were nonetheless routinely added to incubation mixtures containing these enzymes. The acylative and oxidative steps of both enzymes have absolute requirements for Mg^{++}. In addition, Co A and cysteine are essential for acylative activity of both pyruvic and α-ketoglutaric oxidases (Table II).

The *Tetrahymena* preparation contains rather high amounts of thioctic acid. This factor however can be readily removed by merely adding a small amount of alumina.§ The mixture is then gently shaken for a few seconds, and the alumina removed by gentle centrifugation. The supernatant is free of thioctic acid when assayed for acetate replacing ability in the growth of *Lactobacillus casei*(15) and as a cofactor for acylative reactions carried out by living *Tetrahymena.* The *Tetrahymena* assay detects total protogen, bound as well as free forms. Whereas the rate of oxidation of pyruvate and α-ketoglutarate by the supernatant remains unchanged following alumina treatment, such thioctic acid-free preparations do not form acyl groups in the course of the respective oxidations (Table III). The addition of pure thioctic acid completely restores acylative activity to the level of activity prior to alumina treatment. The optimal concentration of thioctic acid required for acylative activity is in the region of 20 units/ml (Fig. 1). Addition of thioctic acid in amounts of 20 units/ml, to both untreated and alumina treated enzyme did not significantly alter the rate of dye reduction when either pyruvate or α-ketoglutarate served as substrate.

The effect of thioctic acid on the acetylation of sulfanilamide from acetyl ~ Co A was studied to ascertain whether the transfer of acyl groups *from* Co A as well as the transfer *to* Co A is dependent upon the factor. Table IV shows that alumina treatment of duck liver acetylase (prepared by the method of Kaplan and Lipmann)(16) is without effect on the rate of acetylation of sulfanilamide when active Co A serves as the acetyl donor.

§ Fisher "Adsorption alumina, 80-200 mm"

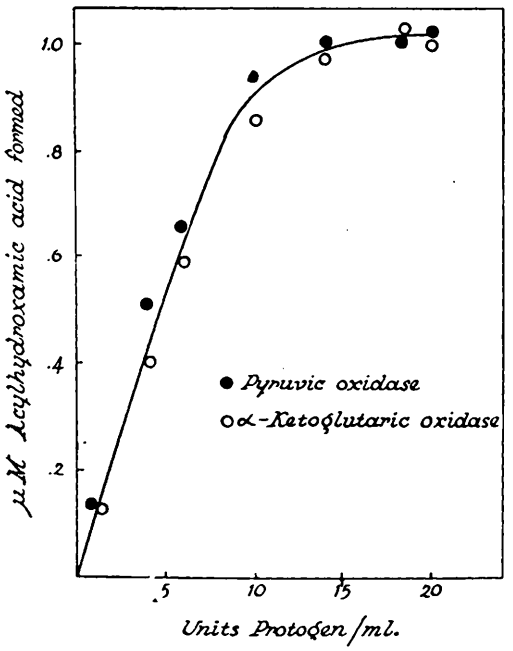

FIG. 1. Requirement of thioctic acid for formation of acyl ~ Co A from pyruvate and α-ketoglutarate. Incubation mixture was as described in the legend of Table II with the exceptions that ATP was omitted and that .4 ml of pyruvic oxidase and .62 ml of α-ketoglutaric oxidase were used. The mixture was incubated 60 min. at 25°C. Enzymes were treated with alumina.

It thus appears that transferase activity(17) with the resulting formation of acetyl ~ Co A is dependent upon thioctic acid, but that acylokinase activity(17) between the acyl carrier and the acyl acceptor is independent of protogen.

In the absence of thioctic acid the main product of pyruvate oxidation is acetate (Table V). Acetaldehyde is also formed in the course of the reaction. Diacetyl or acetoin could not be detected at any time in the incubation mixture. This is in contrast to the formation of these compounds by purified mammalian pyruvic oxidase(5,18), but is in agreement with previous investigations on *Tetrahymena* enzymes(7). As the concentration of thioctic acid in the incubation mixtures is increased, the rate of acylation is increased and the rate of acetate formation is decreased (Table VI); the rate of pyruvate disappearance is also increased. In the above mentioned experiments ferricyanide replaced the indophenol dye as hydrogen acceptor. It was thus possible to add relatively large amounts of this soluble acceptor and to allow the reactions to proceed for relatively long periods of time when measuring rates of hydrogen transfer. The increased rate of hydrogen transfer in the presence of increased amounts of thioctic acid is not interpreted as being at variance with the data presented in Table I. In the presence of necessary cofactors for both components of the oxidation, an additional pathway of utilization is made available, which is reflected as the increased rate of removal of substrate and must, of course, be accompanied by a corresponding increase in hydrogen transfer.

Discussion. The finding that thioctic acid is necessary for acyl transfer to Co A is consistent with growth studies carried out with this factor. Since acyl ~ Co A serves as the carrier for such acetyl acceptor systems as citrate(19), acetylcholine(20), and other acetylated amines(21), as well as for succinyl acceptor systems(22), it is not to be expected that acetate alone would serve completely to

TABLE IV.

Enzyme treatment	Sulfanilamide removed, μM
0	.14
0 + 20 units/ml thioctic acid	.16
Alumina	.11
Alumina + 20 units/ml thioctic acid	.11

Acetyl ~ Co A as acetyl donor in acetylation of sulfanilamide. The complete system contained 100 μM phosphate (pH 7.4), 20 μM $MgCl_2$, 10 μM cysteine, .2 μM acetyl ~ Co A, .2 ml of sulfanilamide acetylase in a vol of 1 ml. Incubation was for 30 min. at 32°C.

TABLE V.

	Pyruvate disappearance, μM	Ferricyanide reduction, μM	Acetate formed, μM	Acetaldehyde formed, μM
Observed	1.36	2.54	1.24	.13
Theory		2.72	1.36	
% theory		94	91	

Pyruvate oxidation balance. Reaction mixture contained 10 μM of pyruvate, 20 μM of $MgCl_2$, 50 μg of DPN, 200 μg of cocarboxylase, 200 μM of phosphate (pH 6.8), 20 μM potassium ferricyanide, and .4 ml of enzyme in a final vol of 2 ml. Incubation time was 60 min. at 25°C. Enzyme treated with alumina.

TABLE VI.

Thioctic acid conc., units/ml	Pyruvate disappearance, μM	Ferricyanide reduction, μM	Acetate formed, μM	Sulfanilamide disappearance, μM
20	1.12	2.03	.42	.68
10	1.06	2.08	.47	.54
5	.87	1.69	.56	.30
1	.79	1.52	.72	.08
0	.74	1.26	.68	.02
Blank*	.00	.03	.04	.00

* (20 units thioctic acid but 3 mg crystalline egg albumen in place of enzyme.)

Effect of thioctic acid on pyruvate balance. Mixture contained 20 μM $MgCl_2$, 200 μg cocarboxylase, 50 μg DPN, 60 units of Co A, 200 μM phosphate (pH 7.2), 10 μM of pyruvate, 25 μM of cysteine, 3 μM of sulfanilamide, 20 μM of potassium ferricyanide, .2 ml *Tetrahymena* oxidase and .2 ml of sulfanilamide acetylase in a vol of 3 ml. Incubation time was 60 min. at 25°C. Enzymes were treated with alumina.

replace the factor in the growth of *Tetrahymena*. It would also be expected that, as shown by Dewey *et al.*(23), growth of *Tetrahymena* in protogen-deficient medium results in the accumulation of pyruvate and of α-ketoglutarate in the medium, in the same manner that thiamin deficient cultures accumulate pyruvate(24).

Since protogen is apparently essential for acylative activity, but not the hydrogen transfer mechanism, it is obvious that the formation of the primary product of pyruvate and α-ketoglutarate oxidation is independent of a protogen requirement. This is in substantial agreement with the results of Schweet, *et al.*(18) which indicate that the first product of the oxidation is an activated C_2 fragment (or in the case of α-ketoglutarate oxidation, an activated C_4 fragment). This intermediate then either undergoes oxidation to acetate or succinate, or reacts with Co A to form acyl ~ Co A. Since the intermediate is an acyl compound, it appears that protogen is concerned only with the transfer, and not the formation of acyl groups.

Summary. 1. When examined in a purified oxidase preparation from the ciliate *Tetrahymena pyriformis* S, thioctic acid is required for acyl transfer from pyruvate and α-ketoglutarate, but not for oxidative activity as measured by reduction of 2,6-dichlorophenolindophenol. 2. The end product of the oxidative phase of pyruvate metabolism is acetate. However, a small amount of acetaldehyde is formed from pyruvate via a side reaction. It is probable that the product of α-ketoglutarate oxidation is succinate and that succinic semialdehyde is formed as a side reaction of this oxidation. 3. Thioctic acid is not required for the acetylation of sulfanilamide from acetyl~Co A.

1. Seaman, G. R., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 308.

2. Sanadi, D. R., and Littlefield, J. W., *J. Biol. Chem.*, 1951, v193, 683, and *Science*, 1952, v116, 327.

3. Kaufman, S. in McElroy, W. D., and Glass, B., *Phosphorus Metabolism*, Baltimore, 1951, v1, 370.

4. Corliss, J. O., *Trans. Am. Micro. Soc.*, 1952, v71, 159.

5. Green, D. E., Stumpf, P. K., and Zarundraya, K., *J. Biol. Chem.*, 1947, v167, 811.

6. Stadtman, E. R., and Barker, H. A., *J. Biol. Chem.*, 1950, v184, 769.

7. Seaman, G. R., *J. Biol. Chem.*, 1950, v186, 97.

8. Lipmann, F., and Tuttle, L. C., *J. Biol. Chem.*, 1945, v158, 505.

9. Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, v128, 537.

10. Soodak, M., and Lipmann, F., *Fed. Proc.*, 1948, v7, 190.

11. White, A. G. C., Krampitz, L. O., and Werkman, C. H., *Arch. Biochem.*, 1945, v9, 229.

12. Friedman, T. C., and Hangen, G. E., *J. Biol. Chem.*, 1943, v147, 415.

13. Stotz, E., *J. Biol. Chem.*, 1943, v148, 585.

14. Kolthoff, I. M., and Menzel, I. H., trans. by Furman, N. H., *Volumetric Analysis*, New York, 1929, v2, 426.

15. Guirard, B. M., Snell, E. E., and Williams, R. I., *Arch. Biochem.*, 1946, v9, 381.

16. Kaplan, N. O., and Lipmann, F., *Fed. Proc.*, 1947, v6, 266.

17. Chou, T. C., and Lipmann, F., *J. Biol. Chem.*, 1952, v196, 98.

18. Schweet, R. S., Freed, M., Cheslock, K., and Paul, M. A., in McElroy, W. D., and Glass, B., *Phosphorus Metabolism*, Baltimore, 1951, v1, 246.

19. Stern, J. R., Shapiro, B., Stadtman, E. R., and Ochoa, S., *J. Biol. Chem.*, 1951, v193, 703.

20. Korkes, S., del Campillo, A., Korey, S. R., Stern, J. R., Nachmansohn, D., and Ochoa, S., *J. Biol. Chem.*, 1952, v198, 215.

21. Chou, T. C., and Soodak, M., *J. Biol. Chem.*, 1952, v196, 105.

22. Shewin, D., and Wittenberg, J., *J. Biol. Chem.*, 1951, v192, 315.
23. Dewey, V. C., and Kidder, C. W., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 302.
24. Tittler, I. A., Belsky, M. M., and Hutner, S. H., *J. Gen. Microbiol.*, 1952, v6, 85.

Received November 15, 1952. P.S.E.B.M., 1953, v82.

Effect of Some Steroids and of Corticotropin (ACTH) on Cellular Activity. (20062)

VALY MENKIN.

From the Agnes Barr Chase Foundation for Cancer Research, Temple University School of Medicine, Philadelphia, and the Marine Biological Laboratory, Woods Hole, Mass.

The writer pointed out that adrenal cortical extract suppresses the increased capillary permeability induced in inflammatory states by leukotaxine or by the unfractionated crude inflammatory exudate(1). The same type of inhibitory effect was found with Compound E or cortisone(2). Subsequently it was found that the suppressing capacity of cortisone is only effective against an alkaline exudate or leukotaxine, whereas in the case of an acid exudate the hormone was ineffective. Corticotropin (ACTH), was found capable of repressing another factor present in acid exudates also capable of increasing capillary permeability(3,4). In this way two factors were shown to exist in inflammatory exudates capable of increasing permeability, namely an initial substance, leukotaxine, and another factor termed exudin(3-5). Leukotaxine has the additional property of inducing leukocytic migration, but exudin merely causes increased capillary permeability in the later stages of an acute inflammation without essentially displaying any chemotactic effect towards leukocytes. Subsequent studies have indicated that cortisone and ACTH are capable of localizing from the circulating blood into an area of inflammation(6-8).

It is of importance to determine or at least attempt to throw some light on the mechanism of suppression by cortisone and ACTH in inflammation. The present report indicates that cortisone and corticotropin (ACTH), as well as some other hormones derived from the adrenal cortex, suppress cell activity. On the contrary, another steroid which is concerned in protein anabolic processes, testosterone, and a growth-promoting hormone of the pituitary, somatrofin (STH), have no such effect in inhibiting cellular activity. Cellular activity has been measured on Arbacia ova by their ability to segment following fertilization. If the ovum is adversely affected by a given steroid, the incidence of cleavage or developmental capacity is correspondingly reduced.

Method. The first series of experiments on sea urchin ova were performed by the addition of 2.5 mg of commercial cortisone acetate (Merck) suspended in about 0.1 cc of its vehicle in a Syracuse dish containing 10 cc of sea water. A batch of ova in approximately 0.5 cc of sea water was then added. An interval ranging between 15 to 20 minutes or so elapsed before the addition of 5 or 6 drops of sperm suspension. The interval prior to the addition of the sperms thus gave the ova sufficient time to come in contact with the cortisone suspension. For a period of one hour and a half to about 3 hours later, 50 ova taken at random were counted. The eggs which showed some degree of cleavage were recorded, and the eggs which failed to display any segmentation were also noted. In another Syracuse dish containing 10 cc of sea water but no cortisone, the same procedure was followed to control for the results obtained with cortisone. As a *control* for the vehicle in which cortisone is suspended, a few experiments were performed with the filtrate obtained when the cortisone suspension was filtered through ordinary filter paper (Whatman No. 5). Several experiments were also set up in which the ova were exposed to 0.1 cc of the commercial vehicle utilized in the prep-