observations made in our previous work,[2] in which methionine was used as the inhibitor, to p-aminobenzoic acid as the inhibitor. Furthermore, using this inhibitor, 2 additional sulfonamides, sulfanilamide and sodium sulfathiazole, showed the same effect, whereas sodium sulfadiazine was the sulfonamide selected in the previous work.

The hydrogen ion concentration values determined at the time of plating, in general were found to increase with the amount of growth. This indicates that the urea effect may not be due to sulfonamide ionization. This point is being studied further. Fox and Rose,[3] Schmelkes, Wyss, *et al.*,[4] Cowles,[5] and others,[6, 7] claim that the bacteriostatic efficiency of sulfonamides is influenced by their degree of ionization.

The mode of action of urea is not as yet elucidated. It is not known whether or not urea acts similarly to azochloramid and certain purines such as adenine and hypoxanthine, which when used in conjunction with sulfonamides, were bacteriostatically effective in the presence of sulfonamide inhibitors.[8-11]

Summary. The concentrations of sulfanilamide, sodium sulfathiazole and sodium sulfadiazine which were required to exert bacteriostatic effect on *Escherichia coli* in a synthetic medium, were determined. Concentrations of para-aminobenzoic acid which caused a significant anti-sulfonamide activity were determined. Concentrations of urea which had no effect on bacterial growth were also determined.

Combinations of these concentrations of sulfonamides and urea, proved to possess bacteriostatic action even in the presence of the sulfonamide inhibitor.

3 Fox, C. L., Jr., and Rose, H. M., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 142.

4 Schmelkes, F. C., Wyss, O., Marks, H. C., Ludwig, B. J., and Strandskov, F. B., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 145.

5 Cowles, P. B., *Yale J. Biol. and Med.*, 1942, **14**, 599.

6 Tolstoouhov, A. V., *Proc. Am. Chem. Soc.*, Sept., 1942.

7 Roblin, R. O., Jr., and Bell, P. H., *Proc. Am. Chem. Soc.*, Sept., 1942.

8 Neter, E., *J. Bact.*, 1942, **44**, 261.

9 Neter, E., *Am. J. Surg.*, 1942, **58**, 69.

10 Schmelkes, F. C., and Wyss, O., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 263.

11 Harris, J. S., and Kohn, H. I., *J. Biol. Chem.*, 1941, **141**, 989.

13920

On the Adenosine Triphosphate-Myosin System.

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Recent data indicate that the chemical energy resulting from the breakdown of carbohydrate in muscle is converted to the mechanical energy required for muscle contraction by means of a reaction between adenosine triphosphate and myosin.[1] Engelhardt and Ljubimova have shown that this is an enzymatic reaction during which adenosine triphosphate is hydrolyzed to adenosine diphosphate and inorganic phosphate.[2] Needham *et al.* suggest that this reaction is associated with the stretching of the myosin fiber from the contracted state, to which it returns on nerve stimulation.[3] Since the adenosine triphosphate-myosin system is so intimately related to the contraction of the myosin fibrils,

1 Kalckar, H. M., *Chem. Rev.*, 1941, **28**, 71.

2 Engelhardt, W. A., and Ljubimova, M. N., *Nature*, 1939, **144**, 668.

3 Needham, J., *et al.*, *Nature*, 1941, **147**, 766.

TABLE I.
Hydrolysis of Adenosine Triphosphate by Myosin in Presence of Added Substances.

No.	System*	Mg inhibitor added	Total P liberated in γ	γ P liberated enzymatically	% enzymatic hydrolysis
1	A.T.P.	—	9	—	0
2	A.T.P. + Myosin	—	17	8	14
3	A.T.P. + Ca + Myosin	—	27	18	31
4	A.T.P. + Ca + Myosin + Acetylcholine Bromide	0.1	27	18	31
5	A.T.P. + Ca + Myosin + Ethoxycholine Bromide	0.1	27	18	31
6	A.T.P. + Ca + Myosin + Curare†	0.1	28	19	33
7	A.T.P. + Ca + Myosin + Zinc Chloride	1.0	9	0	0
8	A.T.P. + Ca + Myosin + Copper Sulfate	1.0	10	1	1.7

*1.2 mg of the barium adenosine triphosphate were added in each experiment as the sodium salt. The time of reaction was 12 minutes.

† The curare, Squibb Intocostrin, was obtained in solution from E. R. Squibb and Sons, New York City. Its concentration is based upon physiological assay.

it was considered of interest to study the effects of chemical mediators of nerve stimulation, acetylcholine and epinephrine, which influence muscle contraction, on the velocity of this reaction with a view toward elucidating the site of action of these substances.

It was observed that the liberation of inorganic phosphate was entirely uninfluenced by any of the following substances: acetylcholine, ethoxycholine, epinephrine, eserine, atropine, curare, nicotine, cysteine, heparin, fluoride, thiocyanate, and cyanide. Only when copper* and zinc salts were added was the reaction inhibited. It would appear, therefore, that the point of action of such muscle stimulators as acetylcholine, epinephrine, curare, etc. is not the adenosine-triphosphate system, at least as it was studied *in vitro*.

Myosin was prepared by the method of Greenstein and Edsall.[5, †] The monobarium salt of adenosine triphosphate was prepared according to Kerr.[7] It had a total phosphorus content of 13.1%, an easily hydrolyzable phosphorus content of 8.6%, and was free of inorganic phosphate. Before each experiment it was converted to the sodium salt by treatment with sodium sulfate. The runs were carried out in borate buffer of pH 8.6. To 0.2 cc of a myosin solution containing 2.8 mg N per cc, 1.8 cc of borate buffer, 0.1 cc of a solution of the substance being tested, 0.05 cc of a 0.1 molar calcium chloride solution, and 0.01 cc of a solution containing the equivalent of 1 mg of the barium salt of adenosine triphosphate were added. This system was incubated at 37°. After a specific interval which varied from 12 to 25 minutes in different experiments, 2 cc of a 10% trichloracetic acid solution were added and the protein precipitate was removed by centrifugation. Inorganic phosphate was determined in the supernatant solution by the method of Fiske and Subbarow.[8] In some experiments the calcium chloride was omitted. Appropriate control experiments were carried out without myosin and without adenosine triphosphate.

In Table I are given results obtained in a typical experiment. It is apparent that the organic substances tested were inactive. When studied in the same manner, epinephrine

* The inhibiting effect of copper salts has recently been reported by Bailey.[4]

4 Bailey, K., *Biochem. J.*, 1942, **36**, 121.

5 Greenstein, J. P., and Edsall, J. T., *J. Biol. Chem.*, 1940, **133**, 397.

† It was considered desirable for the purpose of the present experiments to avoid more than one precipitation of the myosin. It may be expected, for this reason, that it contained significant amounts of adenosine diphosphatase.[4,6]

6 Ljubimova, M. N., and Pevsner, D., *Biochimia*, 1941, **6**, 178.

7 Kerr, S. E., *J. Biol. Chem.*, 1941, **139**, 121.

8 Fiske, C. H., and Subbarrow, Y., *J. Biol. Chem.*, 1925, **66**, 375.

hydrochloride, eserine sulfate, atropine sulfate, nicotine and sodium fluoride, in 100 γ quantities, and cysteine hydrochloride, potassium thiocyanate, potassium cyanide, and the sodium salt of heparin‡ in 1 mg quantities were also inactive.

Summary. Acetylcholine, epinephrine, a series of alkaloids, and several other substances were without influence on the hydrolysis of adenosine triphosphate. Copper and zinc salts inhibited the reaction.

‡ This was obtained from Hoffmann-La Roche and Co., Nutley, N.J.

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In vivo Activity of Streptothricin Against *Brucella abortus.**

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An antibiotic substance possessing rather strong bacteriostatic and bactericidal properties against many gram negative and certain gram positive bacteria has been isolated from a soil *Actinomyces* by Waksman and Woodruff[1] and designated as streptothricin. This substance has now been tested extensively against *Brucella abortus,* the causative agent of brucellosis, and was found to have a strong inhibitory action against this organism not only *in vitro,* but also *in vivo.*

Bactericidal tests of the action of streptothricin† were made by adding graduated doses of the crude preparation of this substance to 10 cc portions of a suspension of *Br. abortus* in nutrient broth and incubating 2 hr at 37°C. This incubated suspension was then plated on tryptose agar. No growth of the organism was obtained on plates inoculated from the preparations containing 0.1 mg or more of the crude streptothricin per 10 cc of broth.

The *in vivo* tests of the activity of streptothricin against *Br. abortus* were at first conducted with incubating eggs. Toxicity tests indicated that the chick embryo tolerated enough of the crude preparation of this substance to warrant testing its activity against this pathogen in such a living medium.

A series of groups of 15-day incubated eggs were inoculated on the chorioallantoic membrane with approximately 2,000 *Br. abortus* cells, using 48-hr-old cultures of Strain No. 19 grown on tryptose agar, suspended in saline solution. Cultures made from control groups of eggs 4 to 5 days after inoculation indicated this to be an infective dose. On the day following the inoculation of the eggs, those scheduled for treatment were given varying amounts of streptothricin, all of which were below the toxic level. The material was administered on the chorioallantoic membrane. These treated eggs were cultured on the 19th or 20th day of total incubation. The results showed that 10 mg of crude streptothricin, administered 24 hr after the inoculation of the eggs with *Br. abortus,* had been sufficient to bring about complete destruction of this organism in the living chick embryo.

These experiments with chick embryos were followed by other experiments employing guinea pigs. At first, an initial dose of 100 mg of crude streptothricin was administered simultaneously with the inoculation of the animal with a small infective dose of *Br. abortus.* The streptothricin was given intraperitoneally to one group and subcutaneously to another group of animals, whereas the controls were left untreated.

* Journal series paper, New Jersey Agricultural Experiment Station, Rutgers University, Departments of Dairy Husbandry and Soil Microbiology.

1 Waksman, S. A., and Woodruff, H. B., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 207.

† We are indebted to Merck and Company, Rahway, N.J., for supplying some of the streptothricin used in this work.