LETTERS TO THE EDITORS

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Specificity of Creatine Phosphokinase

SOME of the properties of the enzyme creatine phosphokinase have recently been described¹; but no experiments were reported on the activity of the enzyme with substrates other than adenosine triphosphate, adenosine diphosphate, phosphocreatine and creatine. Such experiments are in progress and will be reported in due course. A recent publication by Armstrong², in which it was shown that the administration of N-ethylglycocyamine (negmine) was followed by the urinary excretion of N-ethylglycocyamidine (negmidine), suggested that a phosphorylation reaction may have been involved. It was therefore of interest to determine whether negmine could be phosphorylated by the creatine phosphokinase system.

Some experiments have been carried out with a system similar to that already described¹ for the reaction:

but differing in that negmine was substituted for creatine and the temperature was controlled at 30°. The course of the reaction was followed by removal of samples at various time-intervals after the addition of adenosine triphosphate to the system, and determinations of negmine were made by the same method as that used for creatine¹.

Fig. 1. Rate of phosphorylation of creatine (O—O) and negmine (□—□) by the creatine phosphokinase - adenosine triphosphate system

It will be noted that negmine was phosphorylated, but at a much slower rate than was creatine. On the other hand, the amounts of each which were phosphorylated in the maximum time-interval investigated (18 hr.) were not greatly dissimilar.

The product of the reaction, phosphonegmine, was not isolated; but its properties (Table 1) are similar to those of phosphocreatine in the ease with which it may be hydrolysed under mild conditions (9 min., 0.1 N hydrochloric acid, 65°). It will also be noted that, as with creatine, there is an almost quantitative recovery of negmine following hydrolysis.

The fact that negmine may be phosphorylated by the creatine phosphokinase - adenosine triphosphate system is of importance, for, since the administration of glycocyamine is not followed by the excretion of glycocyamidine, and since glycocyamine is not phos-

Table 1. RECOVERY OF CREATINE AND NEGMININE BY HYDROLYSIS FOR 9 MIN. AT 65° IN 0.1 N HYDROCHLORIC ACID AFTER REACTION WITH CREATINE PHOSPHOKINASE - ADENOSINE TRIPHOSPHATE FOR 18 HR.

	Initial	Final (18 hr.)	Final (after hydrolysis)
Creatine (μmoles)	20.0	7.2	19.6
Negmine (μmoles)	20.0	8.0	19.6

phorylated by this system³, it may be inferred that creatinine does arise from phosphocreatine^{4,5}. This does not, however, provide proof of the hypothesis⁶ that creatinine arises as a result of the non-enzymic breakdown of phosphocreatine. The results also confirm the belief² that the urinary excretion of negmidine followed the *in vivo* formation of phosphonegmine.

The complete results of this investigation will be reported in greater detail elsewhere.

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Sept. 23.

¹ Ennor, A. H., and Rosenberg, H., *Biochem. J.*, **57**, 203 (1954).

² Armstrong, M. D., *Fed. Proc.*, **12**, 170 (1953).

³ Ennor, A. H., and Rosenberg, H. (unpublished observations).

⁴ Lipmann, F., "Adv. in Enzymol.", **1**, 99 (1941).

⁵ Borsook, H., and Dubnoff, J. W., *J. Biol. Chem.*, **168**, 493 (1947).

Mode of Action of Tetanus Toxin

THOUGH the general effects produced by tetanus toxin on the nervous system have been reported by many investigators, there is still no precise evidence as to its mode of action¹. Sherrington² pointed out that tetanus toxin closely resembles strychnine in many of its effects, and postulated that both substances convert synaptic inhibition into excitation. Recently³, it has also been found that tetanus toxin resembles strychnine in that the monosynaptic tendon jerk is but little affected by moderate dosage. Since it has now been shown that strychnine acts specifically by diminishing all types of synaptic inhibitory action^{4,5}, it was of interest to investigate the action of tetanus toxin on synaptic inhibition.

The tetanus toxin was administered by injection within the sheath of the sciatic nerve in order that its effect on the spinal cord would initially be localized, and hence control responses would be provided by the other side. The procedure for investigating direct inhibition resembled that already described⁴, single maximum volleys in the large stretch afferents from quadriceps muscle being employed to inhibit the monosynaptic reflex discharges which were evoked from biceps-semitendinosus motoneurons at varying testing intervals thereafter. In Fig. 1 the inhibitory curves so constructed at 21-43 hr. after the injection of the toxin give a typical illustration of our experimental findings. The earliest test, at 21 hr., revealed that the inhibition amounted to 65 per cent, in