PHYSIOLOGY

Protein and Ribonucleic Acid Synthesis after Exposure to Warmth

VARIOUS investigators[1,2] have demonstrated changes in metabolic and enzyme activity, and Hochachka[3] has demonstrated changes in isoenzymes in goldfish as a result of exposure to warmth. Precht discussed the possibility that the warm adjustment may be caused by changes in enzyme activity[4]. Since numerous other physiological changes also occur during this process, we decided to investigate whether or not new protein synthesis is a prerequisite for it, and to examine the nature of the regulation of protein synthesis if this is a causative factor.

McDonald *et al.*[5] showed that the velocity of nerve conduction in *Rana pipiens* increases with warm adjustment and decreases with cold adjustment when measured at 26° C. Conduction velocity was therefore used throughout this study as a convenient criterion for measuring the extent of adjustment to temperature.

The experiments consisted of taking small groups of frogs adjusted to 4° C, treating them with either actinomycin *D* (Merck, Sharp and Dohme Research Laboratory) or puromycin at various time intervals before and during a 6 h adjustment period at 26° C to inhibit RNA or protein synthesis. The average weight of the animals was 66·1 g. The 6 h warm adjusted animals were killed and the sciatic nerves removed and placed in frog Ringer's solution. The nerves were then transferred to a grid of platinum electrodes 1 cm apart and immersed in a bath of mineral oil at 26° C. The nerves were stimulated with repetitive supramaximal impulses at a rate of 10/sec. The monophasic compound action potential detected at two adjacent electrodes was displayed on a double beam oscilloscope. The time required for the action potential to traverse the distance between two electrodes (1 cm) was recorded and from this the velocity in m/sec was calculated. The drug dosage and the time sequence of administration are shown in Fig. 1. Comparable control animals were injected with Ringer solution instead of the antibiotic in each experiment. In some experiments the animals were treated with the drugs, but the temperature was kept constant in order to verify that the observed effects were on processes related to adaptation to changes of temperature, and not simply to effects of the drugs on the steady state metabolism.

The results of the experiments using actinomycin *D* showed that the drug had little or no significant effect on the normal increase (Fig. 1*A*) in conduction velocity brought about by adaptation to warmth when it was administered just before and during adaptation to a temperature of 26° C (Fig. 1*B*, *C* and *D*). However, when the drug was administered 14 h before and during adaptation at 26° C, the increase in the velocity of conduction resulting from adaptation to warmth was inhibited (Fig. 1*E*). The mean conduction velocity of the nerves of frogs adapted to cold before adaptation to warmth was found to be 28·4 m/sec (Fig. 1*A*). Fig. 1*F* shows that actinomycin *D* had no significant effect on the nerve conduction velocity of animals adapted to cold not undergoing adaptation to warmth.

The experiments using puromycin indicate that this drug inhibits the response to exposure to warmth when given 1 h before the beginning of exposure at 26° C (Fig. 1*G*). The drug appeared to have no effect on the conduction velocity of frogs not exposed to warmth and on animals previously exposed to warmth (Fig. 1*I*).

The experiments using puromycin seem to show that protein synthesis is required for adaptation to warmth (as measured by an increase in conduction velocity at 26° C). The experiments using actinomycin *D* suggest that new messenger RNA is not necessary for adjustment to warmth, but treatment with this drug 14 h beforehand probably depletes the pool of messenger RNA and consequently interferes with protein synthesis.

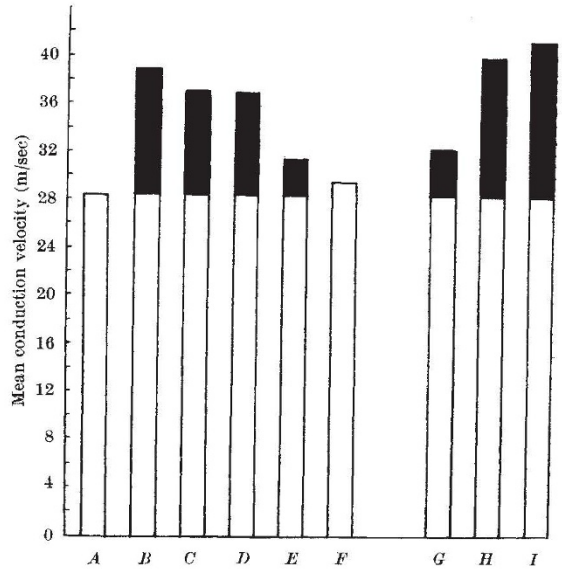

Fig. 1. *A*, Controls adjusted to 4° C, based on twenty-one animals giving a mean of 28·4 ± 4·7. *B*, Twenty-four control animals adjusted to warmth for 6 h having a mean of 38·8 ± 1·5. The shaded area represents the increase brought about by warm acclimation. *C*, Animals adjusted to warmth for 6 h treated with 12 μg of actinomycin *D* at the beginning of warm acclimation. Four animals had a mean of 36·6 ± 7·7, which did not differ significantly from the warm acclimated control (*B*) ($P > 0.8$). *D*, Animals treated with 600 μg of actinomycin *D*. 150 μg were given 1 h before adjustment to warmth, 300 μg at the beginning and 150 μg 3 h after the beginning of adjustment to warmth. The mean based on four animals is 36·5 ± 4·6, which does not differ significantly from the mean for control animals adjusted to warmth (*B*). *E*, Ten animals treated with 450 μg of actinomycin *D*. 300 μg were given 14 h before the beginning of exposure to warmth and 150 μg at the beginning of exposure. The mean, 31·6 ± 1·4, differs significantly from the control mean (*B*) ($P < 0.01$). *F*, Five animals adjusted to cold treated with two 300 μg doses of actinomycin *D*, 14 h and 0·5 h before death. The animals were not exposed to warmth. The mean, 29·7 ± 4·1, did not differ significantly from the untreated controls exposed to cold (*A*) ($P > 0.1$). *G*, Animals exposed to warmth for 6 h receiving 1·2 mg of puromycin 1 h before exposure and three 0·6 mg doses at 0, 1, and 3 h after the initiation of exposure to warmth. The mean of 32·4 ± 3·3 is based on ten animals and differs significantly from the control (*B*) ($P < 0.1$). *H*, Five control animals exposed at 26° C for 48 h receiving only Ringer solution. The mean is 39·9 ± 5·0. *I*, Animals fully adjusted to warmth receiving a total of 12 mg of puromycin in three equal doses 7, 5, and 3 h before killing. The mean based on five animals is 41·4 ± 4·4 and does not differ significantly from its control (*H*) ($P > 0.6$).

It would appear that the regulation is above the level of the gene and RNA synthesis, where actinomycin *D* is supposed to act[6]. It is likely that the site of regulation is at the ribosomes, where puromycin appears to exert its influence[7]. Since adaptation to temperature is a complex phenomenon, it is not known whether or not protein synthesis is required for other aspects of this adjustment. Our findings parallel those of Axelrod *et al.*[8] on another adaptive process, namely, changes in *o*-methyltransferase in the pineal gland on exposure to light. These investigators found that puromycin inhibits the increase of this enzyme, while actinomycin *D* appears to have no effect.

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