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SHORT COMMUNICATIONS

The effect of puromycin on the prothrombin time of rats

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THE COUMARIN anticoagulants were found to uncouple mitochondrial oxidative phosphorylation *in vitro*.[1] In view of this, it was suggested that their action as anticoagulants *in vivo* is a result of uncoupling. If this were the case, it would be expected that the ATP levels in liver of treated animals would be altered by these drugs. However, in previous studies *in vivo*, Dicumarol (bishydroxycoumarin) did not alter either the level or rate of turnover of ATP, as would be expected if uncoupling had taken place.[2] Because of the inconsistency between the results *in vitro* with mitochondria and the results *in vivo* on the ATP levels of liver, other possible sites of action than the mitochondria were considered.

The possibility that the anticoagulants inhibited protein synthesis directly was also studied by measuring the incorporation of ^{14}C-leucine into protein of rat liver slices and cell-free systems.[2] When the anticoagulants were added to rat liver slices, incorporation of isotope was depressed, but the incorporation of isotope into the protein of slices from Dicumarol-treated animals was no different from control samples. Dicumarol is tightly bound to liver proteins;[3] thus the difference between the results *in vitro* and *in vivo* does not seem to be due to washout of the drug during preparation of the tissue slices. Furthermore, Dicumarol and Warfarin had no measurable effect on the incorporation of ^{14}C-leucine into s-RNA, microsomal, or ribosomal protein. Since serum proteins are synthesized rapidly by the liver,[4] it seemed possible that the effects of the anticoagulants on ^{14}C-leucine incorporation might be masked by the synthesis of other proteins than the clotting factors.

In recent years certain antibiotics have been found to interfere with protein synthesis. In the case of puromycin, the interference has been determined to be at the level of the s-RNA ribosomal complex by aborting the completion of the polypeptide chain.[5] The blood-clotting factors, synthesized in the liver, have a relatively rapid turnover,[6, 7] which suggests that agents interfering with synthesis of proteins would rapidly depress the level of the circulating clotting factors. Since puromycin interferes with protein synthesis at the level of the s-RNA ribosomal complex, it seemed likely that this agent also should interfere with the synthesis of the clotting factors, resulting in a prolongation of the prothrombin time. This was found to be the case in these studies. The present report is concerned with the prolongation of the prothrombin time of rats by puromycin.

Puromycin (Nutritional Biochemicals Corp.), was dissolved in 0·9% NaCl for injection. Female Sprague–Dawley rats, weighing 200–275 g, were given puromycin by the i.p. route in six hourly injections of 25 mg/kg each. Control animals were given the same volume of solvent at the same intervals. The time of the first injection was designated zero time for the experiments to be described.

Blood samples for prothrombin times were obtained from animals lightly anesthetized with ether. Blood (0·45 ml) was drawn from the heart into a syringe containing 0·05 ml of 0·1 M sodium oxalate. The prothrombin times were made on 12·5% plasma as described by Campbell *et al.*, because this provided a more sensitive assay than with undiluted plasma.[8] Duplicate determination were made by the one-stage procedure, with Simplastin (Warner Chilcott, Morris Plains, N.J.) to start the reaction.

For consistent prolongations of prothrombin time, repeated doses of puromycin (25 mg/kg) were found to be more effective than either a single or a few doses. The time course of effect of puromycin on the prothrombin time of a female rat of the Sprague–Dawley strain is presented in Table 1. The prothrombin times for the animals presented in Table 2 were also made at the same time periods and followed until the prothrombin times had returned to normal levels. In all of these animals, a prolongation over the preinjection level (avg. 25·4 sec) was noted as early as 4 hr after the first injection of puromycin, and in most cases reached a peak prolongation at 8 hr. At this time, the control animals injected with saline had an average prothrombin time of 24·6 sec. Generally, the prothrombin times were nearly back to normal at about 24 hr. Animal 2 (Table 2) was more sensitive to puromycin than the others, with a greater prolongation than usual, which was not back to normal even at 48 hr. The

response presented in Table 2 is consistent with other experiments at different time periods. Male rats of the Sprague–Dawley strain also showed a prolongation of prothrombin time on this dosage regimen but, as will be pointed out later, male rats of the Wistar strain did not show a prolongation of the prothrombin time.

TABLE 1. TIME COURSE OF EFFECT OF PUROMYCIN ON PROTHROMBIN TIME OF A FEMALE RAT

Time (hr)	Prothrombin time (sec)
Preinjection	26
4	37
8	58
12	47
24	42
48	28
72	30

Puromycin was administered as described. Prothrombin times were determined on 12·5% plasma at the indicated times after the initial injection.

TABLE 2. VARIATION IN PROTHROMBIN TIMES AFTER ADMINISTRATION OF PUROMYCIN

Animal	Prothrombin time (sec)
1	58
2	173
3	52
4	46
5	37

Puromycin was administered as described. Prothrombin times were determined on 12·5% plasma. The prothrombin times of the experimental animals 0·5 hr before the initial injection of puromycin averaged 25·4 sec. The prothrombin times 8 hr after initial injection of puromycin are presented above. Control animals were injected with saline at the same intervals as the experimental animals. The prothrombin time for the controls ($n = 4$) at 8 hr was $24·6 \pm 1·0$ sec (S.E.M.).

Considerable variation in response to puromycin was observed. Table 2 shows the variability of five animals in response to this treatment 8 hr after the initial injection. Control animals injected with saline had an average prothrombin time of 2·46 sec, which may be compared with the preinjection prothrombin times of the experimentals, which averaged 25·4 sec.

In view of the report that puromycin is rapidly demethylated by rat liver microsomes, and the demethylation is inhibited by SKF 525-A (2-diethylaminoethyl diphenylpropylacetate),[9] experiments were carried out with both SKF 525-A and puromycin in an attempt to retard the metabolism of puromycin. SKF 525-A (50 mg/kg) was given i.p. 1 hr before the initial dose of puromycin. However, the combination of the two drugs *in vivo* produced a high rate of mortality after 18 hr or more, which was not noted with either of the drugs administered separately. Because of the high mortality under these conditions, the combined use of the two drugs was not pursued further. The fact that puromycin

is metabolized rapidly by the microsomes seems to account for the need for repeated injections to prolong the prothrombin time and the need for relatively large amounts of puromycin, compared with studies *in vitro* involving inhibition of synthesis of protein by ribosomes.

Female rats of the Wistar strain showed a similar prolongation of prothrombin time, with the peak effect occurring at about 24 hr. However, male rats of this strain showed little or no prolongation on the same dosage schedule. Thus, in this strain a marked sex difference in response to puromycin was observed. As yet, higher doses and a greater number of injections have not been tried on these animals.

Puromycin does not appear to inhibit the clotting factors in the circulation. In separate experiments in which a single injection of puromycin was given by the intracardiac route, the prothrombin times of 12·5% plasma obtained 5 min after injection were the same as those of plasma prior to administration of the drug. In addition, the prothrombin time of plasma was not affected when puromycin was added directly to plasma in amounts up to 280 μg/ml, which would be somewhat greater than the amount present in plasma if the total amount injected into the experimental animals were uniformly distributed throughout the body water.

Although the coumarin anticoagulants and puromycin are similar in that both agents prolong the prothrombin time, it cannot be concluded on the basis of these studies that the coumarin anticoagulants act at the same site as puromycin. However, assumed that puromycin was acting by aborting protein synthesis in the liver at the ribosomal level as in other systems,[5] the present studies indicate that the ribosomes are susceptible to the action of drugs in the synthesis of the clotting factors and may be the site of action of the anticogulants. Shah *et al.* reported that puromycin antagonized the effect of vitamin K in vitamin K-deficient rats.[10] Further experiments are in progress to investigate the possibility that the microsomes are the site of action of the coumarin anticoagulants.

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REFERENCES

1. C. MARTIUS and D. NITZ-LITZOW, *Biochim. biophys. Acta* **12**, 134 (1953).
2. D. COURI and W. D. WOSILAIT, *Biochem. Pharmac.* **15**, 1349 (1966).
3. W. D. WOSILAIT, *J. Pharmac. exp. Ther.* **132**, 212 (1961).
4. L. L. MILLER and W. F. BALE, *J. exp. Med.* **99**, 125 (1954).
5. M. A. DARKEN, *Pharmac. Rev.* **16**, 223 (1964).
6. J. HELLEMANS, R. DEVREKER, M. VORLAT and M. VERSTRAETE, *Acta haemat.* **30**, 35 (1963).
7. K. PYORALA, *Annls. Med. exp. Biol. Fenn, suppl.* 3, **43** (1965).
8. H. A. CAMPBELL, W. K. SMITH, W. L. ROBERTS and K. P. LINK, *J. biol. Chem.* **138**, 1 (1941).
9. P. MAXEL, A. KERZA-KWIATECKI and J. SIMANIS, *Biochim. biophys. Acta* **114**, 72 (1966).
10. D. V. SHAH, V. K. SHAH, R. B. HILL, G. FORSYTH and B. CONNOR JOHNSON, *Fedn Proc.* **25**, 542 (1966).

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The long-term effects of a single dose of methyl prednisolone on ^{35}S uptake in ocular and nasal tissue*

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EFFECTS of steroid hormones on the steady-state dynamics of the connective tissue components of skin and cartilage—mucopolysaccharides (MPS) and collagen—are easily demonstrated.[1, 2] Suggestions have also been made relating steroid-induced reversible glaucoma with alterations in the MPS of the

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