Thus our present and previous results show that stimulation of cardiac adrenoceptors by pure α- and β-stimulants induces myocardial focal necroses which, although morphologically similar, can be distinguished by the use of theophylline and by selective α- and β-adrenoceptor blockers.

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Phosphatase activity of guinea-pig tissues on creatinol *O*-phosphate *in vitro*

Creatinol *O*-phosphate (COP; Aplodan) is a new drug synthesized by Ferrari & Casagrande (1965). It has a positive inotropic effect on the isolated rabbit atrium, and an ability to antagonize the toxic action of digitalis on the isolated atrium of rabbits previously treated with EDTA (Ferrini, unpublished observations). COP also increased the contractile force in the rat isolated heart, in the rabbit isolated and hypoxic heart and in rabbit hearts with experimental infarction *in situ* (Marchetti, Merlo & Noseda, 1971). Musso, De Ambroggi & Taccardi (1971) obtained a decrease in the duration of atrio-ventricular block and a rapid return to normal of reduced coronary flow in guinea-pig isolated hearts treated with high doses of (−)-adrenaline. These responses are the result of the action of whole COP molecule. In man, COP achieved good results in congestive heart failure, in atrio-ventricular conduction disturbances, in improving tolerance to digitalis (Melloni, Camerini & others, 1968; Bianchi, Guzalian & others, 1970) and in coronary insufficiency (Natale, 1968).

We have investigated *in vitro* the kinetics of dephosphorylation of COP incubated with blood and some tissue extracts. In effect, the first catabolic stage of COP is its dephosphorylation to give creatinol, a reaction catalysed by phosphatases.

When COP was incubated with alkaline phosphatase, the rate of COP dephosphorylation was about 10 times higher than with acid phosphatase. In addition, experiments on COP dephosphorylation at pH 4, 5·6, 8·1, 9·3, 10·5, 11·3 demonstrated that the optimum pH in this reaction is 10·5. All our experiments were therefore made at pH 10·5 in 0·1 M glycine buffer.

Skeletal muscle, heart muscle, liver, the small intestine and kidney were homogenized in glycine buffer (100 mg of tissue/ml of buffer). The homogenate was centrifuged and the supernatant incubated at 37° with 7 μmol/ml of COP. The phosphate released was determined quantitatively (*a*) (Fiske & Subbarow, 1925) at 30 min, 1, 2 and 3 h. The same determinations of phosphate released were made on

Table 1. *Dephosphorylation of COP incubated with skeletal muscle, heart muscle, liver. small intestine, kidney extracts at pH 10·5 and blood at physiological pH,* Mean values of 4 experimental findings in % COP incubated ± s.e.

Tissue 100 mg/ml	Substrate transformed into % 30 min	1 h	2 h	3 h
Skeletal muscle	1·1±1·15	0·2±0·22	0·9±0·68	5·2±3·46
Heart muscle	0·5±0·54	1·2±0·89	3·1±1·02	5·3±1·52
Liver	1·9±0·85	4·6±1·20	10·1±2·30	15·1±3·69
Small intestine wall	13·1±2·03	22·7±3·10	42·6±5·28	48·5±4·23
Kidney	50·9±3·64	67·0±7·27	76·6±3·54	82·3±2·29
Blood at physiological pH ..	0·0±0·06	1·1±0·20	1·8±0·23	2·4±0·35

tissue extracts without COP (*b*) and on COP in buffer without tissue extracts (*c*). The phosphate released in (*b*) and (*c*) was subtracted from that released in (*a*). The tests on blood were made at physiological pH without buffer and at a blood concentration 5 times higher than that of other tissues because of there being little phosphatase activity in the blood. The blood results are nevertheless reported in Table 1 as a concentration of 100 mg/ml (1 mg = 1 ml) to allow comparison with the other data.

When COP was incubated without enzymes in 0·1 M citrate buffer at pH 4·0 for 3 h, the amount of phosphate released was negligible. When COP was incubated without enzymes in 0·1 M glycine buffer at pH 10·5 at 37° for 3 h, dephosphorylation was only 3%.

Table 1 shows the phosphatase activity of blood and tissue extracts incubated in 0·1 M glycine buffer at pH 10·5 for 30 min, 1, 2 and 3 h. The kidney extract dephosphorylated 82·3% of incubated COP in 3 h, the small intestine 48·5%, the liver 15·1%, the skeletal muscle and heart muscle about 5% and the blood 2·4%.

Musso & others (1971) have shown that the pharmacological effects of COP are due to the whole molecule. The results we have obtained, which indicate that COP is only slowly dephosphorylated by blood and skeletal and heart muscle, therefore allow us to conclude that COP may remain for a considerable length of time in these tissues in a form enabling it to exert its pharmacological action. These findings satisfactorily complement those from a previous investigation on the absorption, distribution and excretion of [^{14}C]COP in the guinea-pig (Marzo, Ghirardi & others, 1971).

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