Table III. Carbohydrates content of TCA precipitated proteins of mash seeds

Germination time (h)	Carbohydrates (%)[a]
0	3.54
24	3.48
48	3.44
72	3.50
96	3.34

[a] The results are expressed in terms of galactose.

Results and discussion. The data on soluble proteins, carbohydrates and insoluble proteins of mash seed germinated for different time intervals are given in Table I. These data indicate that the soluble proteins remained nearly constant, except for a slight increase at 48 and 72 h of germination. The soluble carbohydrates, on the other hand, decreased considerably during germination period. For the first 24 h of germination, a considerable decrease in the insoluble proteins with slight change in soluble carbohydrates and soluble proteins was observed. The loss of insoluble proteins during the first 24 h of germination was also observed by RACUSEN and FOOTE[12] in *Phaseolus vulgaris*. The observed decrease in the insoluble proteins content may be due to the biological solubilization of some unidentified nitrogen containing polymers possibly a nucleic acid.

The changes observed in the major glycoprotein fraction (fraction I, Figure 1) are presented in Table II. This fraction formed 37% of soluble protein at 0 h and 30% at 96 h of germination. On further purification by DEAE-cellulose column, the major fraction (fraction I, Figure 2) was found to be 12% of the soluble protein at 0 h and 9% at 96 h of germination. This fraction was found to be homogeneous when tested by starch gel electrophoresis and was found to possess arabinose and galactose as sugar moieties. A little decrease observed in soluble glycoprotein content during initial stages of germination may be due to the fact that these are utilized to a small extent at this stage.

The changes in the carbohydrates content in TCA precipitated proteins during germination are given in Table III. Up to 96 h of germination, a negligible change observed in the carbohydrates content of glycoproteins suggested that the carbohydrate moiety attached to protein is perhaps not as easily mobilized as the free soluble carbohydrates present in the seed.

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The effects of salicylate and aspirin on the activity of phosphorylase a in perfused hearts of rats

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Summary. Sodium salicylate and aspirin are known to have a glycogenolytic effect as judged by either the glycogen level or lactate production in perfused hearts of rats. In this work it was possible to demonstrate that phosphorylase a level was increased in the hearts subjected to the action of these drugs.

The well-known uncoupling effect of sodium salicylate on oxidative phosphorylation is accompanied both in experimental animals and in man by a compensatory increase in body catabolism. This includes an increase both in oxygen consumption and in the rate of glycogenolysis in liver and muscle[1]. Attempts to find an explanation for the salicylate glycogenolytic effect at the phosphorylase system level, have not been conclusive, since the enzymes of this system seem to be inhibited by the drug[2]. In the present paper, we show that both salicylate and aspirin actually increase the phosphorylase a content in perfused hearts of rats in situations where the lactic acid content is increased and the glycogen level decreased.

Heparinized adult rats were killed by decapitation and the hearts were rapidly removed and perfused for 10 min, without recirculation, with a Krebs-Henseleit bicarbonate buffer, gassed with a mixture of O_2:CO_2 (95:5) at 37 °C. After this time, either sodium salicylate or aspirin were added to the perfusion buffer to a final concentration of 5 mM. This is the concentration that usually causes maximum depletion of glycogen in rat liver[3]. Subsequently, the perfusion was carried out by a recirculation system. Aliquots of the perfusate were taken at half hour intervals and lactate production determined. At the end of perfusion, the hearts were frozen by using aluminium clamps cooled in liquid nitrogen. A sample of the frozen tissue was weighed and dumped into 30% KOH solution in the proportion of 5 ml/g and heated to 95 °C for 20 min for glycogen determination[4]. Lactate analysis was performed on aliquots of 0.1 ml of perfusate by the lactate dehydrogenase method[5]. Glycogen was determined on a sample of muscle after complete hydrolysis to glucose by the Nelson method[6]. The assay of phosphorylase activities in the frozen muscle was carried out as described previously[5].

The table shows the increased rate of glycogenolysis as measured by the decrease of muscle glycogen content and increase in lactate production in the perfusate during 30 min of salycilate treatment. The phosphorylase a level, as expressed by the ratio: (phosphorylase a/total phosphorylase) $\times 100$, is also found to increase, suggesting

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Effect of salicylate on perfused rat hearts. The percent of phosphorylase a was calculated after 10 min of perfusion. Glycogen content measured as μmoles of glucose (after hydrolysis) per g and lactate production was expressed as μmoles of lactate per h per g. The results are expressed on a fresh weight basis.

Determinations*	Control	Salicylate
Ph. a (per cent)	7.2 ± 3**	23 ± 7
Glycogen content	83.3 ± 8	10.1 ± 4
Lactate production	25 ± 3.2	112.5 ± 14

*6 hearts were used as control and 6 for treatment with sodium salicylate. **S. E. M.

that the enzymes participate in activation of glycogenolysis caused by salicylate.

Experiments to determine the phosphorylase level in the hearts treated by aspirin, which is also a glycogenolytic agent[7], revealed that in fact the phosphorylase a level was increased about 3fold, i.e. from 7.2 ± 3 in the controls to 27 ± 4 in the hearts subjected to the drug. This effect of either sodium salicylate or aspirin on heart phosphorylase might be expected, since the high rate of glycogenolysis found in muscle subjected to the action of these drugs requires the maintenance of the enzyme in the activated form.

Our finding that salicylate increases phosphorylase a activity might be explained through a mechanism involving the uncoupling of oxidative phosphorylation. For example, previous work from our laboratory[8] has shown that DNP results in elevated levels of phosphorylase a of heart and skeletal muscle. This could be explained by a decrease in the ATP/ADP ratio which would increase phosphofructokinase activity and thereby maintain glucose-6-phosphate at a low level in the cell. Since glucose-6-phosphate is known to inhibit phosphorylase b to a conversion, this inhibition would be reduced in the presence of the uncoupling agent resulting in higher phosphorylase a levels. In support of this idea, other experiments with salicylic acid[3] have shown that in the isolated rat diaphragm the content of inorganic phosphate is increased, whereas creatine phosphate and ATP are severely reduced in the presence of the drug.

The results of Segal and Blair[2] of phosphorylase a inhibition in isolated hemidiaphragms is found only 30 min after salicylate administration, a time which, according to the authors, glycogenolysis proceeds at a much slower rate even though glycogen is still present in relatively high amounts.

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Interaction of N-(DL-seryl)N′-(2,3,4-trihydroxybenzyl)-hydrazine with L-Dopa decarboxylase from pig kidney

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Summary. Interaction of seryl trihydroxy-benzyl-hydrazine with a highly purified preparation of Dopa decarboxylase from pig kidney has been studied. This compound was found not to be a powerful inhibitor in vitro. Kinetic and spectral data suggest some possibilities on the binding nature of the inhibitor and substrates.

The N-(DL-seryl)N′-(2,3,4-trihydroxybenzyl)-hydrazine (Ro 4-4602) is a product used, in addition to L-Dopa, in the treatment of Parkinson's disease. This compound is an inhibitor of peripheral Dopa decarboxylase and does not appear to pass the blood-brain barrier at therapeutic doses in Parkinsonism. Its effect on cellular metabolism lies in potentiating the biochemical behaviour of peripherally administred L-Dopa. This pharmacological action, largely investigated from a clinical point of view, reflects the interaction of this compound at the molecular level with peripheral L-Dopa decarboxylase. Such interaction has been studied in vitro measuring the inhibition on crude homogenate of hog or rat kidney[1,2], not yet investigated on a highly purified enzymic preparation.

The present communication deals with the binding of Ro 4-4602 to an homogenous Dopa decarboxylase from pig kidney. The inhibition constants and the effect on the absorption peaks of the enzyme-bound pyridoxal-P give some information on the interaction of this compound with Dopa decarboxylase and may also provide more detailed insights of its active site.

Materials and methods. The enzyme, purified according to Borri Voltattorni et al.[3], appeared to be homogenous in polyacrylamide gel electrophoresis and in the ultracentrifuge. Its coenzyme content was in good agreement with that reported by Christenson et al.[4].

L-m-tyrosine and L-o-tyrosine were synthetized from corresponding racemic forms by the action of D-amino acid oxidase and catalase in a 0.05 M pyrophosphate buffer, pH 8.3 under O_2 stream. The enzymatic degradation of the D-amino acid form was followed measuring at time intervals the formation of the keto acid form with 2,4-dinitrophenyl hydrazine. The end-point of the reaction was achieved when, even after further additions of enzymes, no more keto acid was detected. Then the reaction mixture, brought to pH 3, was poured on a 50 × 8 Dowex column. After elution of the keto acid with 0.2 M pyridine buffer, pH 3.1, the L-amino acid was eluted with 2 M pyridine buffer, pH 5. Ether was added

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