were the generous gift of Dr Borgers, Janssen Pharmaceutica, Beerse, Belgium. Inhibitors were dissolved in the assay buffer and kept as 10^{-2} M stock solutions. For assay 0.2 ml of inhibitor solution replaced the equivalent volume of buffer.

Culture of bone rudiments. Chick embryonic femurs were cultured as described previously[3]. For cultures of rat bones, femurs from embryos of 17-day pregnant rats were used in a similar manner to chick explants.

Results. Chick bone alkaline phosphatase can be progressively inhibited with increasing concentrations of either levamisole or R8231 (figure 1). These 2 compounds are equally inhibitory for chick bone alkaline phosphatase and the 50% inhibition level is about 10^{-4} M. The other isomer of tetramisole (D-tetramisole, dexamisole) is without significant effect at concentrations up to 10^{-3} M.

Levamisole and R8231 are much more potent in inhibiting the alkaline phosphatase of adult rabbit bone, as compared with chick (figure 2). Additionally figure 2 demonstrates that R8231 is more than 10 times as potent as levamisole[11]; 50% inhibition by R8231 is achieved at 10^{-6} M. R8231 is a racemic mixture therefore it is likely that the active isomer is even more potent than data suggest. Doses of dexamisole above 10^{-4} M cause considerable inhibition.

Inhibition of avian and mammalian bone alkaline phosphatases by tetramisole, an analogue (R8231) and beryllium sulphate

Source of bone alkaline phosphatase	Percentage inhibition of bone alkaline phosphatase by dexamisole (D-tetramisole)	levamisole (L-tetramisole)	R8231	$BeSO_4$
Chick	5	10	11	79
Rabbit (adult)	5	36	84	72
Rabbit (embryonic)	0	33	75	74
Mouse	6	24	81	57

The values are averages of duplicate or triplicate estimations. All test substances were used at a concentration of 10^{-5} M.

The dose-response curves for levamisole and R8231 together with either embryonic rabbit, or mouse or embryonic rat bone alkaline phosphatase were essentially the same as for adult rabbit (figure 2). Beryllium sulphate[11] is more effective than either levamisole or R8231 as an inhibitor of chick bone alkaline phosphatase (table) and comparable when tested on mammalian enzymes. Because of the toxicity of beryllium salts we did not consider them further as a biological probe. When added to the culture media both isomers of tetramisole and R8231 caused more inhibition of growth of chick explants than rat, in terms of length and weight during 6 days in vitro. Above 10^{-4} M all these compounds were growth inhibitory for chick explants; since there is only 50% inhibition of alkaline phosphatase by either levamisole or R8231 at 10^{-4} M these inhibitors have limited usefulness for studies with chick explants. Much more importantly, with rat bone explants a dose of 10^{-4} M, which for R8231 blocks alkaline phosphatase activity almost completely, was without any growth inhibitory effects.

Discussion. These data fit well other results[7-11] that levamisole and R8231 are potent inhibitors of mammalian alkaline phosphatases. However, chick bone alkaline phosphatase is much less susceptible to inhibition by these compounds; doses needed to almost completely inhibit chick bone alkaline phosphatase were growth inhibitory when tested on bone explants in vitro. The difference between the inhibitory effects of levamisole and analogues on avian and mammalian alkaline phosphatase has also been noted for chick and pigeon liver and serum (M. Borgers and H. van Belle, personal communication), as compared with serum alkaline phosphatase from man, dog and rat. Majeska and Wuthier[12] also suggest that levamisole is not a potent inhibitor of chick alkaline phosphatase but showed that both the phosphatase and pyrophosphatase activities of the enzyme were equally blocked by levamisole.

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Effect of germination on the glycoprotein of mash (Phaseolus mungo) seeds

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Summary. The soluble carbohydrates and insoluble proteins of *Phaseolus mungo* seeds decreased considerably up to 96 h of germination, whereas the soluble proteins remained nearly constant. The carbohydrates content of glycoprotein also remained constant. This suggests that a negligible change took place in the glycoprotein during the initial period of mash seed germination.

Glycoproteins are ubiquitous in the plant kingdom. They are a rich source of many important substances such as hemagglutinins[2], toxins[3] and some enzymes[4,5]. Glycoproteins are also known to play an important physiological role in the transportation and storage[6] of carbohydrates. During germination the metabolic processes become active and the role played by glycoproteins in germinating seeds is still not fully known. Thus, in the present investigation, the changes in glycoprotein fraction in the germinating mash seeds have been reported.

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Table I. Analysis of the extracts obtained from 2 g mash seeds

Germination time (h)	Insoluble proteins (mg)	Soluble proteins (mg)	Soluble carbohydrates (mg)
0	142.2	310	108.5
24	103.8	317	102.5
48	119.8	331	89.0
72	113.2	329	81.4
96	86.4	306	46.8

Table II. Changes in the major glycoprotein fraction of mash seeds during germination

Germination time (h)	Major glycoprotein fraction as percentage of soluble protein		
	Sephadex column	DEAE-cellulose column	Carbohydrate (%)
0	37	12	3.9
48	36	nd[a]	nd[a]
96	30	9	3.8

[a] Not done.

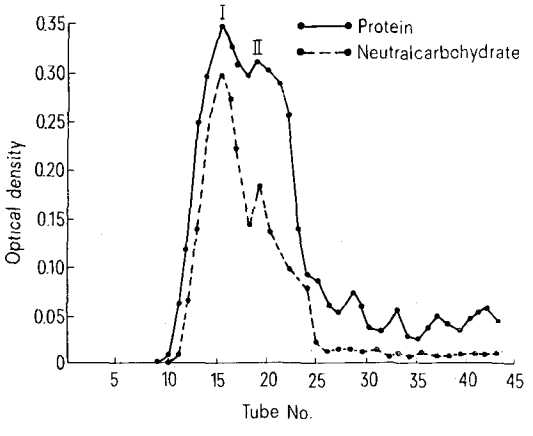

Fig. 1. Fractionation of soluble protein of mash seed on Sephadex G-150.

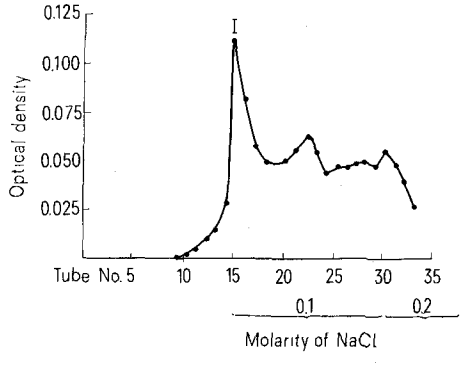

Fig. 2. Purification of the fraction I from Sephadex on DEAE-cellulose.

Materials and methods. Extraction of proteins. Mash (*Phaseolus mungo*) seeds were obtained from Pulse Breeder, Punjab Agricultural University, Ludhiana. Healthy seeds (2 g) were treated with 0.1% mercuric chloride solution for 1 min and rinsed with sterilized distilled water. The sterilized seeds were placed in a number of petridishes (each containing 2 g of seeds) lined with filter paper and allowed to germinate in dark for different time intervals (24, 48, 72 and 96 h). After removing the embryos, the seeds were ground in the ice-cold mortar and extracted with 19.0 ml boric acid (0.02 *M*) buffer pH 9.0. After centrifugation, the residue was again extracted with 19.0 ml of boric acid buffer. Both the supernatants were combined. For control study (0 h) mash flour, prepared by passing the dry seed through willey mill containing a 40 mesh sieve, was also extracted in a similar way with boric acid buffer.

The combined supernatants were analyzed for soluble proteins[7] and soluble carbohydrates[8]. The residue of the seed was analyzed for insoluble proteins by microkjeldahl method[9].

A part of the soluble proteins extract was precipitated with 15% trichloroacetic acid (TCA) solution and the resulting precipitate was first centrifuged and then washed with 7.5% TCA and then with ethanol and ether and finally dried under vacuum.

Purification procedure. The major glycoprotein fraction from the soluble proteins was isolated by sephadex G-150 column (2.2 × 50 cm) which was eluted with 0.025 *M* phosphate buffer (pH 8.0). Fractions of 6 ml each were collected in tubes and analyzed for protein content by the method of LOWRY et al.[7]. The optical density of each tube was plotted against the tube number. The tubes under each peak were combined. The major fraction was further fractionated by DEAE-cellulose column (1.5 × 35 cm) using 0.05 *M* phosphate buffer with stepwise increasing concentration of NaCl (0.1, 0.2, 0.3 and 0.4 *M*) in the buffer. Buffer concentration was changed after 80 ml of the previous content was collected. Each fraction of 6.0 ml was collected and analyzed for protein content. The fractions under major peak were collected, dialyzed and concentrated.

Electrophoresis. The concentrated protein fractions were tested for homogeneity by starch gel electrophoresis according to the procedure of POULIK[10].

Separation and determination of carbohydrate. The separation of carbohydrate from glycoprotein was done by the method of HANAFUSA et al.[11]. The protein was hydrolyzed with $2N$ H_2SO_4 at 100 °C for 2 h. The acid hydrolysate was then neutralized with 10% barium hydroxide and centrifuged. The precipitate was washed thrice with hot water. The supernatant was dried under vacuum, dissolved in 1–2 ml of water and transferred to a column (1 × 15 cm) containing Amberlite IR-120 of 100–200 mesh. The column was washed with 100 ml of water and the effluent which contained neutral sugars was dried in vacuum and determined according to the procedure of DUBOIS et al.[8]. The different neutral sugars in glycoprotein fraction were identified by paper partition chromatography using *n*-butanol-acetic acid-water (4:1:5 V/V) as solvent system.

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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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