(36.8 ± 4.9% cleavage in 20 min) was several times more efficient than guinea-pig liver (8.1 ± 1.1%) in this respect. The more rapid hepatic metabolism in the rat undoubtedly accounts for the shorter half-life of the hydrazone in this species. Although the degree to which the 2 compounds react in the gastrointestinal tract remains to be determined, the possibility that hydrazone formation may interfere with the therapeutic effect of norethindrone, in some species, is suggested by the findings in the guinea-pig. Failure of contraceptive treatment has been noted in human subjects undergoing antituberculous therapy. The failures have usually been attributed to an effect of rifampicin. However, in many of the reported cases, isoniazid was given in combination with rifampicin[2]. The present report suggests that the former drug may also have been a contributing factor in the noted failures. Whether, and to what degree, human liver is able to metabolize the hydrazone, will be the subject of a future study.

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Preparation and oxygen binding properties of soluble covalent hemoglobin-dextran conjugates

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Summary. Stroma-free hemoglobin solutions present some drawbacks when used as blood substitutes, essentially because the hemoprotein has a low vascular retention, due to its small hydrodynamic volume. Covalent coupling of the protein with dextran derivatives artificially increases its size and affords polymeric conjugates whose oxygen-binding properties (Barcroft's curve, Hill coefficient) depend on the molecular weight.

For nearly a century, scientists have been tantalized by the possibility of transfusing stroma-free hemoglobin solutions to transport oxygen in man. However it is known that, because of its low molecular weight and the poor viscosity of its solutions, this hemoprotein is characterized by a short in vivo half-life in plasma[2] and that it is rapidly cleared by the kidneys and through other metabolic routes[3].

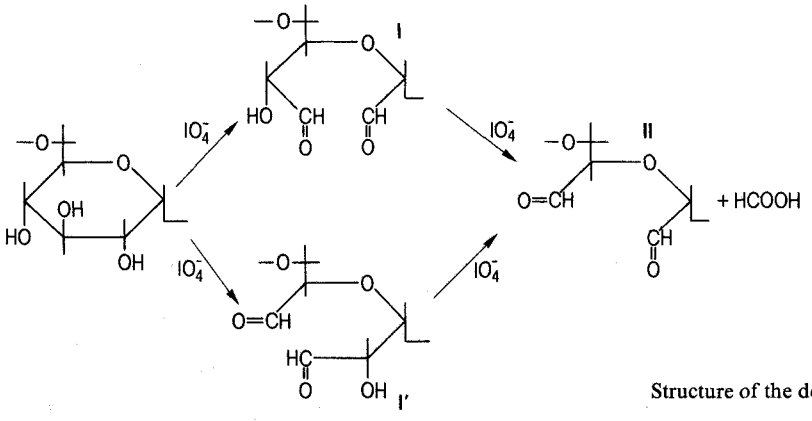
In order to increase the hydrodynamic volume of the hemoglobin molecule and hence the viscosity of its solutions, this protein has been polymerized[4], or cross-linked with albumin by means of glutaraldehyde and other polyfunctional reagents[5], or coupled to activated dextran[6]. Here some results are reported on the covalent coupling of hemoglobin with low molecular weight dextran oxidized with sodium periodate, and on the oxygen-binding properties of the conjugates thus obtained.

Materials and methods. Dextran T 40 ($\bar{M}_w = 40{,}000$) was obtained from Pharmacia (Uppsala, Sweden). Stroma-free hemoglobin was prepared according to the usual method from outdated human blood; the red blood cells are washed twice in saline solution, hemolyzed with demineralized water, and centrifuged twice at 30,000 × g to eliminate the stromata. The clear homoglobin solution is then dialyzed against demineralized water for 15 h at 4 °C and centrifuged once more, at 10,000 × g.

Oxidation of dextran was performed in water by 0.05 M sodium periodate according to the classical procedure[7]. The aldehyde groups formed during the oxidation was quantitated by measuring formic acid evolved as well as the amount of iodate and periodate remaining in solution at the end of the reaction. The dialdehyde dextran was extensively dialyzed against distilled water, lyophilized and then coupled with the hemoglobin molecule (6 °C, 0.3 M boric acid/sodium hydroxide buffer, pH 9.7, 1 mole dextran for 1 mole hemoglobin).

The reaction mixtures were analyzed by gel permeation chromatography on Ultrogel AcA 44 (fractionation range 10,000–130,000) in a 0.05 M sodium phosphate buffer, pH 7.2, at 6 °C.

Oxygen equilibrium curves were determined manually with a tonometer according to a spectrophotometric method first described by Benesch et al[8]. and modified by Labie and Byckova[9]. The optical density of the solutions (0.1 M sodium phosphate buffer, pH 7.2) at 25 °C (thermostatically controlled) was measured at 560 and 578 nm in a 320 Hitachi spectrophotometer.



Structure of the dextran units after oxidation by sodium periodate.

The amount of methemoglobin, spectrophotometrically determined at 630 nm according to Sunderman et al.[10], was found always to be less than 1%, before and after the coupling of hemoglobin with the dextran derivatives.

Results and discussion. Dextran is a polysaccharide consisting of D glucopyranose units joined by $\alpha(1\rightarrow 6)$linkages and slightly branched by a small number of $\alpha(1\rightarrow 3)$bonds. The oxidation of dextran by sodim periodate produces different species in a ratio depending on the amount of sodium periodate added to the dextran solution. The oxidation reaction initially yields I and I′ (scheme). This oxidative ring opening is subsequently followed by a second oxidation resulting in the liberation of formic acid[11,12] and the production of II.

The oxidation of dextran T 40 ($\bar{M}_w \simeq 40{,}000$) by aqueous periodate was investigated in order to determine the respective percentages of structures I and II obtained, as a function of the amount of sodim periodate consumed. The results are reported in figure 1. In order not to modify the intrinsic properties of the original polysaccharide, the dialdehydic dextran samples used in the coupling reactions with hemoglobin were prepared with low ratios of oxidized glucose units (< 10%).

Condensation of the hemoglobin molecule with a partially oxidized dextran (8.9% of oxidized glucose units: 5% I, 3.9% II) leads to conjugates the molecular weight of which depends on the reaction time. Figure 2 shows the different elution profiles corresponding to various condensation times. From these curves, it turns out that, after 6 h of reaction, hemoglobin is almost completely coupled, since there is no significant peak left at the elution volume of the free protein. One may assume from a preliminary calibration of the columns that the slowest eluting peak corresponds to a dimer conjugate (1 mole hemoglobin/1 mole dextran) while the fastest peaks account for higher molecular weight complexes (fig. 2b–d).

It is known that the affinity of hemoglobin for oxygen can be strongly altered by covalent linkages with organic molecules[12]. Therefore, some oxygen equilibrium experiments have been carried out on the dextran-hemoglobin conjugates to investigate the effect of reaction times longer than 6 h. Figure 3 shows that the affinity of the linked hemoglobin for oxygen increases with the reaction time or, in other words, as the cross-linking between dextran and hemoglo-

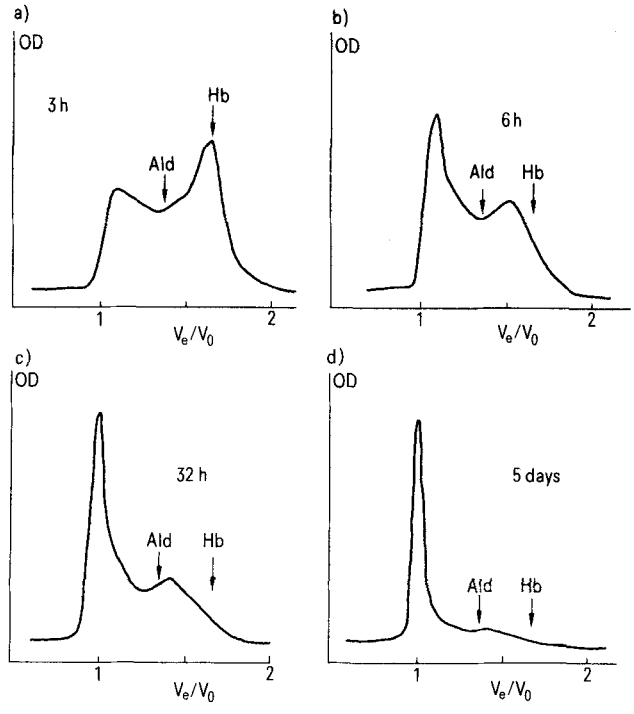


Figure 2. Gel filtration on Ultrogel Aca 44, of the reaction mixture containing dialdehyde dextran and hemoglobin after reaction during a: 3 h, b: 6 h, c: 32 h, d: 5 days. V_e is the elution volume; V_0 is the void volume of the column (300 ml bed volume; elution buffer, 0.05 M sodium phosphate, pH 7.2; injection 0.2–0.3 ml, flow rate: 20 ml/h). The arrows correspond to the normal elution volume of hemoglobin and aldolase (M = 158,000). For dextran T40 $V_e/V_0 = 1.7$; for proteins which mol. wts are higher than 200,000, $V_e/V_0 = 1$. Absorbance was measured at 280 nm.

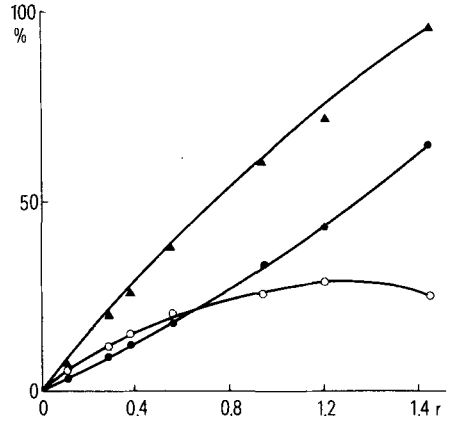


Figure 1. Oxidation of dextran T40 by sodium periodate. Influence of the amount of periodate consumed on the final structure of the dextran. ▲ = percentage of oxidized glucose units; ○ = percentage of I or I′ with respect to the total glucose units; ● = percentage of II with respect to the total glucose units; r = molar ratio of periodate consumed per glucose unit.

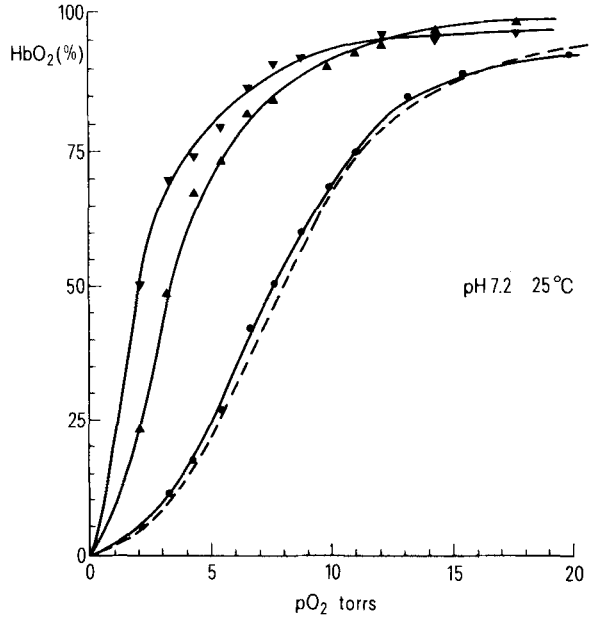


Figure 3. Oxygen-binding curves of the dextran-hemoglobin conjugates obtained after different reaction times. ● = 6 h, oxyhemoglobin; ▲ = 32 h, oxyhemoglobin; ▼ = 5 days, oxyhemoglobin; ----- = barcroft's curve for free hemoglobin.

bin develops. It is then likely that the formation of this multichain network favours the stabilization of the hemoprotein in the R conformation (R, 'relaxed', in the presence of oxygen). In the same manner, the Hill coefficient diminishes when the molecular weight of the conjugates is increased (respectively 2.70, 2.33 and 1.40 for 6 h, 32 h and 5 days reactio times) and this later observation reflects a lack of cooperativity in O_2-binding of the high molecular weight compounds.

In conclusion, it has been found that the low molecular weight hemoglobin-dextran conjugates exhibit good Barcroft's curves, that the cooperativity in O_2-binding of linked hemoglobin is retained, and that solutions of conjugates do not present any hemagglutination phenomena nor toxicity when injected into rabbits. Therefore the possibility of their use as oxygen-carriers can be reasonably expected and some in vivo experiments are now being carried out to investigate this.

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Effect of acrylonitrile on trehalase, phosphorylase and acetylcholinesterase activities in *Tribolium castaneum* Herbst and *Trogoderma granarium* Everts

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Summary. Acrylonitrile inhibited trehalase and phosphorylases in larvae and adults of *Tribolium castaneum*. In *Trogoderma granarium* larvae phosphorylases alone were inhibited. Acetylcholinesterase was not affected.

Literature survey indicates that hitherto very little work has been done on the effect of acrylonitrile, a highly toxic fumigant, on enzyme systems of storage pests. Because of its extensive industrial usage, most of the biochemical studies of acrylonitrile are limited to mammals only. Trehalase is an important enzyme in the carbohydrate metabolism of insects as it regulates the level of trehalose – a circulating source of energy[2].

Phosphorylases (active and inactive) are another group of enzymes closely associated with the energy supply in insects[3]. The effect of fumigants on trehalase and phosphorylases has not been investigated so far. Acrylonitrile has been reported to increase acetylcholinesterase (AChE) activity in the brains of exposed rats[4]. In fact, the level of AChE (and catalase) activity has been used as a criterion for occupational chronic poisoning by acrylonitrile in industry[5]. In the present investigation, the effect of fumigation with acrylonitrile on the 3 enzymes mentioned was studied in *Tribolium castaneum* (larvae and adults) and *Trogoderma granarium* (larvae).

Materials and methods. 14-day-old larvae of *T. castaneum* and *T. granarium* and 2–3-week-old *T. castaneum* adults obtained from laboratory cultures were weighed into 300-mg batches. In the petri dishes containing *T. castaneum* adults, 3 folded filter paper-strips of 5×1 cm were placed to absorb their defensive secretions. The insects were

Effect of acrylonitrile fumigation at sublethal and LD50 levels on certain enzyme activities in *T. castaneum* and *T. granarium*

Species	Stage	Acrylonitrile dosage (mg/l)	Trehalase[a]	Phosphorylase[b] Active	Total	AChE[c]
T. castaneum	Adult	Nil	955.62 ± 43.89	0.28 ± 0.01	1.05 ± 0.05	0.36 ± 0.02
		0.53	714.61 ± 52.66[d]	0.09 ± 0.01[d]	0.61 ± 0.05[d]	0.35 ± 0.02
		1.05	553.44 ± 71.13[d]	N.D.	0.40 ± 0.03[d]	0.34 ± 0.02
	Larva	Nil	698.73 ± 24.24	1.54 ± 0.04	2.74 ± 0.26	0.69 ± 0.02
		0.40	618.56 ± 39.32	1.14 ± 0.08[d]	2.08 ± 0.02[d]	0.71 ± 0.01
		0.79	534.75 ± 17.51[d]	0.79 ± 0.05[d]	1.43 ± 0.12[d]	0.69 ± 0.02
T. granarium	Larva	Nil	470.12 ± 67.69	1.88 ± 0.23	3.04 ± 0.24	0.23 ± 0.01
		0.47	433.22 ± 49.36	1.32 ± 0.03[d]	2.29 ± 0.09	0.24 ± 0.01
		0.93	461.06 ± 67.60	0.49 ± 0.01[d]	2.24 ± 0.15[d]	0.22 ± 0.01

[a] µg glucose produced/mg protein/h. [b] µmoles inorganic phosphate released/mg protein/h. [c] µg acetylcholine (chloride) hydrolyzed/mg insect/h. All values are mean ± SE of 6 estimations. [d] Significantly inhibited ($p < 0.05$). N.D.: Not detected, below limits of detectability.