An investigation of the interaction between isoniazid and the contraceptive steroid norethindrone in vivo

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Summary. Isonicotinic acid hydrazide (isoniazid) was shown to react readily with 17α-ethinyl-17β-hydroxyestr-4-en-3-one (norethindrone) to form the isonicotinyl hydrazone of the steroid under conditions likely to exist in the stomach. The hydrazone was detected in guinea-pig, but not rat, plasma following its oral administration. Rat liver tissue metabolized the compound more rapidly than guinea-pig liver in vitro which probably accounts for the failure to detect the hydrazone in rat plasma.

Isoniazid, a highly reactive molecule, is known to form hydrazones readily with Δ4-ketosteroids, as initially shown by Lederer[1]. Since the therapeutic dose for isoniazid is many times higher than for steroid drugs, the question arose as to the effect of co-administration on the disposition of exogenous steroids. The present report describes preliminary investigations on the absorption and disposition of the isonicotinyl hydrazone of a contraceptive steroid, norethindrone, in the guinea-pig and the rat.

Materials and methods. (G-^{3}H)Norethindrone (4.39 Ci/mmole, New England Nuclear Canada, Lachine, Que.) and (C^{24}-carboxyl)isoniazid (14.7 mCi/mmole, Amersham Corp., Oakville, Ont.) were more than 97% radiochemically pure prior to use. The hydrazone was synthesized by allowing a mixture of norethindrone and isoniazid, pH 2.5, to stand at room temperature for 30 min. The solution was then adjusted to pH 7.0 and extracted with dichloromethane. The product was purified either by crystallization or by TLC on Silica gel GF-254 (Brinkman Instruments Ltd) (chloroform:methanol, 9:1). Various biological fluids were analysed for radioactivity either directly or following extraction with water-washed dichloromethane. The hydrazone, norethindrone and several steroid metabolites were quantitatively removed into the organic phase. Isoniazid and acetyl isoniazid remained in the aqueous layer. The extracts were washed with water, dried and the residue made up in an appropriate volume of methanol. All radioactive samples were combusted to ^3H_2O and $^{14}CO_2$ (Packard Oxidizer, Model 306, Packard Instruments Co., Downer's Grove, Ill.) and assayed for radioactivity by liquid scintillation spectrometry.

Hepatic metabolism of the hydrazone was determined in vitro by incubating liver homogenates (57 mg liver/ml) with several concentrations of the doubly labelled hydrazone in phosphate buffer, pH 7.4, at 37 °C under 95% O_2+5% CO_2 for 20 min. A mixture containing no tissue, and one containing liver homogenate which had been inactivated by boiling, were included as controls. The reaction was stopped by adding methanol containing carrier hydrazone. The protein was removed by centrifugation and the supernatant dried, made up in water and extracted with dichloromethane. The extracts and the aqueous phase were analyzed for radioactivity as described above.

Results and discussion. That norethindrone reacts readily with isoniazid was shown initially by incubating the 2 compounds in vitro in the proportions expected in therapeutic usage (1:40). The reaction was almost complete in 6 min, and the pH for the maximum rate of formation was pH 2.5, which is close to that expected in the stomach content. To determine whether the hydrazone could be absorbed intact from the gastrointestinal tract, the radioactive products in plasma were analyzed following the oral administration of ^{3}H-norethindrone-^{14}C-isoniazid to guinea-pigs. 30 μg of the doubly labelled hydrazone (^{3}H cpm/^{14}C cpm = 0.71) was administered to each of 3 female Hartley Albino guinea-pigs (305-370 g, Canadian Breeding Farms, Constant-Laprairie, Que.) and blood samples withdrawn at 30 min by cardiac puncture. The ^{3}H cpm/^{14}C cpm ratio was followed throughout the work-up of the plasma samples as indicated in the table. There was a small enrichment of ^{3}H in plasma as compared to the administered compound. Dichloromethane extracted significantly greater amounts of ^{3}H than ^{14}C from the plasma. TLC ($CHCl_3$:MeOH, 95:5) of the extracts showed that although most of the ^{14}C was associated with the reference hydrazone standard, more than 50% of the ^{3}H had an R_f similar to that of the norethindrone standard. Separation of this 'norethindrone' spot, however, did not reduce the ^{3}H cpm/^{14}C cpm ratio of the 'hydrazone' spot (1.65) sufficiently to achieve the ratio of the injected hydrazone (0.71). The higher sample ratio suggested a possible contamination by metabolite(s) of the steroid or a contamination due to transfer of ^{3}H to other endogenous compounds with an R_f similar to the hydrazone. In a further experiment, separation of the contaminant(s) from the hydrazone was achieved by combined thin-layer and paper chromatography. 30 μg of either ^{3}H-norethindrone (control) or ^{3}H-norethindrone-isoniazid (experimental) were administered p.o. to each of 4 guinea-pigs per group and blood withdrawn at 30 min. The dichloromethane extracts of the plasma were pooled for each group and subjected to TLC (multiple runs, $CHCl_3$:MeOH, 98:2). 8% and 20% of the extracted ^{3}H were associated with the hydrazone standard in the control and experimental samples respectively. When the 'hydrazone' spots were eluted and further chromatographed on paper (formamide impregnated Whatman 3MM, chloroform:cyclohexane, 1:1), none of the radioactivity in the control sample travelled with the hydrazone standard. However, in the experimental sample, 64% of the ^{3}H was associated with the standard. The remainder of the radioactivity had an R_f similar to that of the control sample. Based on these results, it was concluded that the higher sample ratio in the previous experiment was largely attributable to contamination by a metabolite(s) of norethindrone and further that the hydrazone was indeed absorbed intact, to a significant extent, from the guinea-pig gastrointestinal tract. In contrast to the findings in the guinea-pig, the isonicotinyl hydrazone of norethindrone was not detected, using similar techniques, in rat plasma. Minimal quantities of the plasma ^{14}C was extractable with dichloromethane. A major portion of the ^{14}C (96%, ^{3}H cpm/^{14}C cpm = 0.08) was excreted in the urine within 6 h in a non-extractable form, suggesting efficient absorption and rapid metabolism of the hydrazone in vivo. In vitro experiments showed that liver homogenate from both species was able to metabolize the hydrazone. However, rat tissue

^{3}H cpm/^{14}C cpm ratios of plasma products following administration of ^{3}H-norethindrone-^{14}C-isoniazid

	^{3}H cpm/^{14}C cpm
Administered hydrazone	0.71
Plasma	0.94
Dichloromethane extract	2.72
'Hydrazone' spot (TLC)	1.65

(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].

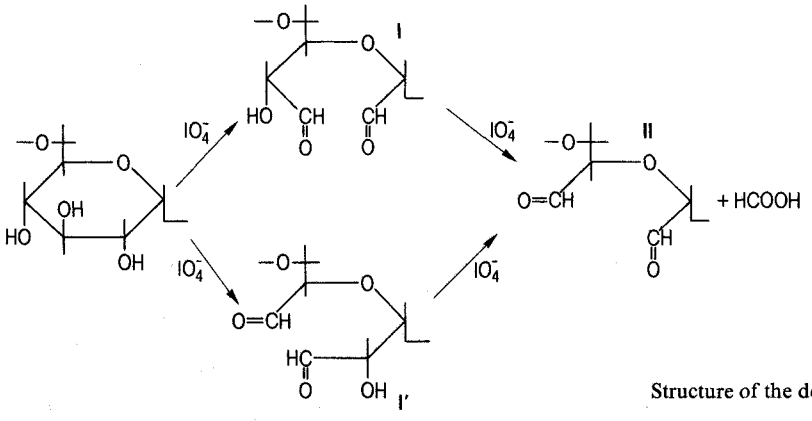
In order to increase the hydrodynamic volume of the hemoglobin molecule and hence the viscosity of its solutions, this protein has been polymerized[4], or cross-linked with albumin by means of glutaraldehyde and other polyfunctional reagents[5], or coupled to activated dextran[6]. Here some results are 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.