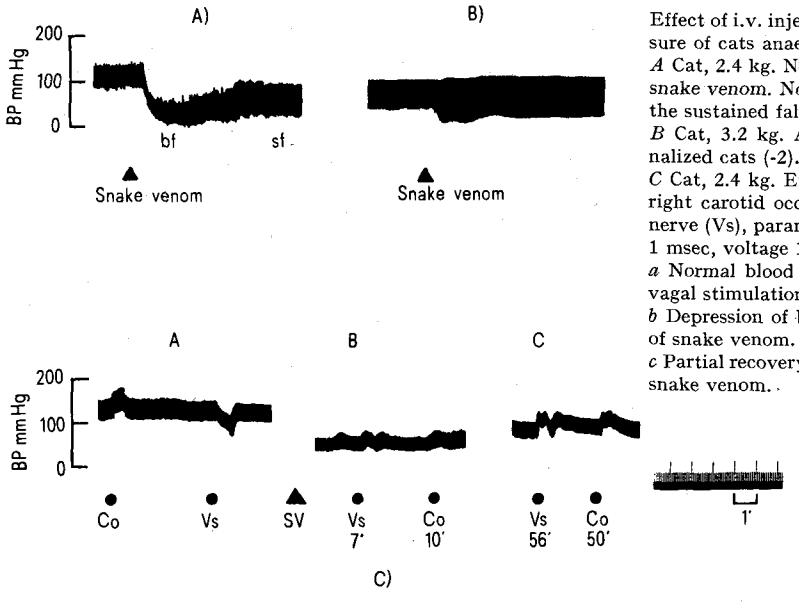

Effect of i.v. injection of snake venom (300 µg/kg) on the blood pressure of cats anaesthetized with ether-chloralose (80 mg/kg).
A Cat, 2.4 kg. Normal record of blood pressure after i.v. injection of snake venom. Note the initial brief fall (bf) the partial recovery and the sustained fall (sf).
B Cat, 3.2 kg. Absence of biphasic vasodepressor response in spinalized cats (-2).
C Cat, 2.4 kg. Effect of snake venom on blood pressure response to right carotid occlusion (Co) and central stimulation of right vagus nerve (Vs), parameters of stimulation: frequency 10 Hz, pulse width 1 msec, voltage 10 V.
a Normal blood pressure response to carotid occlusion and central vagal stimulation. Duration of stimulation in both were 30 sec.
b Depression of both responses (7–10 min) after i.v. administration of snake venom.
c Partial recovery of the response (56–59 min) after administration of snake venom.

blocked (figure B) in all the cats but the effect of the venom on the initial brief fall was not consistent, and in 3 preparations the initial brief fall in blood pressure (56.7 ± 5.8 mm Hg) was noted. Similar initial brief hypotensive response was noted in bilateral cervical vagotomized (N=2) and spinalized cats (N = 4).
Pretreatment i.v. with atropine (2 mg/kg) and mepyramine (5 mg/kg) did not significantly alter the venom-induced hypotension. In cats (N = 4) pretreated with i.v. pentolinium (5 mg/kg), administration of whole venom resulted in an immediate steep fall in blood pressure and a fatal response in all cats. The snake venom has no effect on contraction of nictitating membrane or on vasopressor response to i.v. administration of DMPP. The influence of the venom on the peripheral vasculature was studied by its influence on vasodepressor response of i.v. administered sodium nitrite (5 mg/kg), the venom had no effect on this response. The results of experiments (N = 2) on the effect of whole venom (300 µg/kg) on central vasomotor responses (figure C) indicated that these responses to central vagal stimulation and carotid occlusion respectively were depressed with partial recovery within 59 min.

The present investigation reveals that i.v. administration of *Naja mossambica pallida* venom causes a biphasic vasodepressor response, the initial brief fall in blood pressure which does not seem to be due to peripheral vasodilatation, as pretreatment with atropine mepyramine does not abolish it and also as the venom does not influence the sodium nitrite-induced hypotension. Spinal cord transection with or without bilateral cervical vagotomy does not consistently alter this response. In another study to be reported, the present authors[6] have shown the direct depressant effect of the Naja venom on myocardium, and it is quite likely that this may be responsible for the initial brief fall. The prolonged hypotensive response is probably due to depression of vasomotor centre since it is absent in spinalized cats and confirmed by abolition of central vasomotor response by venom. The fatality noted in pentolinium-pretreated cats may be due to combination of effects of loss of peripheral sympathetic tone by ganglion blockade and direct myocardial depressant effect of the venom.

6 R. K. Raina, J. N. Ng'ang'a and B. V. Telang, Agressologie, accepted for publication (1977).

Comparison of human adult and fetal hemoglobin: Aminophenol-induced methemoglobin formation[1]

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Summary. Human fetal hemoglobin was more susceptible to methemoglobin formation in the presence of aminophenols than was adult hemoglobin. This was due to the intrinsic properties of the proteins rather than the presence of methemoglobin reductases.

Newborn susceptibility to drug-induced methemoglobinemia, cannot be fully accounted for on the basis of a decrease in methemoglobin reductase activity in their red cells. Since fetal hemoglobin is both spontaneously and chemically transformed to methemoglobin more readily than adult hemoglobin[2,3], perhaps the changes observed are due to intrinsic properties of the proteins.

In this communication, we report our comparative studies on human fetal and adult hemoglobin. These studies reveal that fetal hemoglobin exhibits an enhancement in methemoglobin formation in the presence of aminophenols, when compared to adult hemoglobin. Aniline, which can be metabolized to p-aminophenol[4] produces significant methemoglobinemia in the newborn[5].

Materials and methods. m-, o-, and p-Aminophenol were obtained from Eastern Organic Chemicals and were recrystallized twice to remove any impurities. Human fetal hemoglobin was prepared from cord blood, collected in sodium citrate buffer at the time of delivery at New York University Hospital, by a modification[6] of the Zade-Oppen method[7] to allow preparation of larger quantities of methemoglobin-free hemoglobin. These preparations contained at least 98% fetal hemoglobin, as measured by alkali denaturation[8]. Human adult hemoglobin was obtained from blood samples of healthy adults which were collected in sodium citrate buffer. The lysates of the adult samples were prepared by the same procedure as for fetal hemoglobin. The concentration of hemoglobin F, hemoglobin A and the percentages of oxy, deoxy and methemoglobin were measured in a Cary 14 spectrophotometer[9].

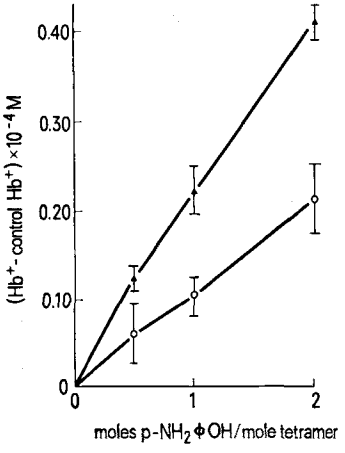

Fig. 1. Methemoglobin formation: p-aminophenol concentration study. This study was conducted in 0.01 M Bis-Tris buffer 0.9% NaCl at pH 6.6 and 25 °C in the presence of 0.5, 1 and 2 moles of p-aminophenol per mole of hemoglobin tetramer (5×10^{-4} M). The molar difference of methemoglobin formed over control values after a 60-min-incubation is shown for fetal (▲) and adult hemoglobin (○). Hb^+ on ordinate axis refers to methemoglobin concentration. Control Hb^+ was never more than 6×10^{-6} M.

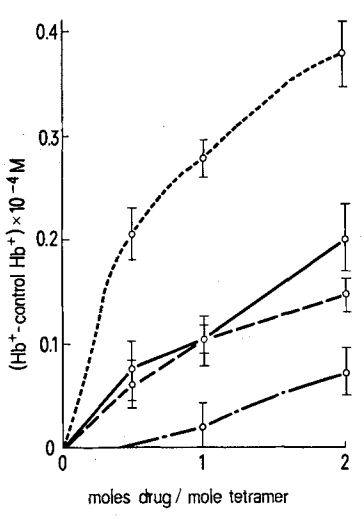

Fig. 2. Methemoglobin formation: A comparative study. Samples were incubated in 0.01 M Bis-Tris buffer 0.9% NaCl at pH 6.6 and 25 °C for 20 min in the presence of 0.5, 1 and 2 moles of m-, p- and o-aminophenol per mole of hemoglobin tetramer (5×10^{-4} M). The The molar difference of methemoglobin formed over control values for fetal and adult hemoglobin in the presence of m-aminophenol (.....), fetal (—) and adult (—.—) hemoglobin in the presence of p-aminophenol and fetal (– – –) and adult (— —) hemoglobin in the presence of o-aminophenol is shown. Hb^+ on ordinate refers to methemoglobin concentration. Control Hb^+ was never more than 6×10^{-6} M.

Results. Incubation of both human fetal and adult hemoglobins in the presence of 2, 1 or 0.5 moles of p-aminophenol per mole of hemoglobin tetramer (5×10^{-4} M) caused a significant ($p < 0.01$) increase in the fraction of methemoglobin formed as compared to the control values. Fetal hemoglobin was much more susceptible to methemoglobin formation by p-aminophenol than was adult hemoglobin at each drug level studied (figure 1). The effect of m-, p- and o-aminophenols on methemoglobin formation was compared. For each drug, fetal hemoglobin was more susceptible than adult hemoglobin (figure 2). m-Aminophenol caused no methemoglobin formation with either fetal or adult hemoglobin.

Studies at different pH values were run in the presence of 2 moles of the aminophenol per mole of hemoglobin (5×10^{-4} M). At each pH studied (6.35, 6.65 and 7.20) fetal hemoglobin was significantly ($p < 0.01$) converted to methemoglobin more readily than was adult hemoglobin by both o- and p-aminophenol. The extent of methemoglobin formation was greater for o-aminophenol than for p-aminophenol at each pH. m-Aminophenol did not cause methemoglobin formation in either fetal or adult samples at any pH studied.

Discussion. The results of experiments on hemoglobin susceptibility to methemoglobin formation demonstrated that both human fetal and adult hemoglobin were most susceptible to the action of o-aminophenol, less susceptible to p-aminophenol and resistant to the action of m-aminophenol. Fetal hemoglobin was much more susceptible to methemoglobin formation under any given condition, than was adult hemoglobin. This susceptibility was an intrinsic property of the hemoglobin protein, rather than a result of the presence or absence of methemoglobin reductases. This was demonstrated in this study by treating the hemolysate with DEAE-cellulose, which removes both the methemoglobin reductases and their cofactors[10]. No difference in methemoglobin formation was observed in DEAE-cellulose-treated and normal preparations of either adult or fetal hemoglobin.

The formation of methemoglobin in the fetal circulation can compromise fetal oxygenation because of its inability to carry oxygen. Since fetal hemoglobin is more sensitive to methemoglobin formation than adult hemoglobin, any drug which potentially causes methemoglobinemia should be used with great caution in pregnancy.

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2 W. Kunzer, A. Schutz and E. Schutz, Acta haemat. *16*, 137 (1956).
3 H. Versmold, B. Ulmer, K. Riegel and K. Betke, Klin. Wschr. *51*, 93 (1973).
4 B. B. Brodie and J. Axelrod, J. Pharmac. exp. Ther. *94*, 29 (1948).
5 A. C. Allison, Lancet *1*, 699 (1955).
6 M. Wind, Thesis, Department of Pharmacology, N.Y.U. School of Medicine (1973).
7 A. M. M. Zade-Oppen, Scand. J. clin. Lab. Invest. *15*, 491 (1963).
8 R. Benesch, G. MacDuff and R. E. Benesch, Analyt. Biochem. *11*, 81 (1965).
9 J. H. P. Jonxis and H. K. A. Visser, A. M. A. J. Dis. Child. *92* 588 (1956–1957).
10 M. A. Hennessey, A. M. Waltersdorph, F. M. Huennekens and B. W. Gabrio, J. clin. Invest. *41*, 1257 (1962).