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Salicylate poisoning—Effect on 2, 3-diphosphoglycerate levels in the rat

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SALICYLATE is known to inhibit enzymes at several steps in erythrocyte glycolysis.[1–3] 2,3-Diphosphoglycerate (2,3-DPG) is a product of erythrocyte glycolysis found in many mammalian species, and its concentration in the erythrocyte has been found to have an important effect on the affinity of hemoglobin for oxygen.[4,5] Decreased levels of 2,3-DPG have been shown to increase this affinity, and this in turn may impede delivery of oxygen to tissues.[6] For this reason, the present study was designed to study the effect of large doses of salicylate on levels of erythrocyte 2,3-DPG.

Male Sprague–Dawley rats (mean body wt, 270 g) were given sodium salicylate (100 mg/ml) intraperitoneally in doses of either 400, 500 or 600 mg/kg. A fourth group run concurrently with the others was given an equivalent volume of isotonic saline. No anesthesia was used, and the animals were not disturbed between procedures. They had access to food and water *ad lib.* Blood was obtained by amputation of the tail tip just before and approximately 24 hr after the administration of the sodium salicylate. In three of the animals given 600 mg/kg of sodium salicylate, blood was also obtained at 4 hr. Analyses were performed for 2,3-DPG by a chromotropic acid method[7] as follows. Heparinized packed red blood cells were washed four times in iced isotonic saline and then mixed with 2 vol. of 6·7% trichloroacetic acid per volume of packed red cells. The samples were kept frozen for up to 7 days and after defrosting, mixing and centrifugation, 0·1 ml of the supernatant was mixed with 4 ml of a solution containing 10 mg of chromotropic acid (Eastman Organic Chemicals) per 100 ml of concentrated sulfuric acid and incubated in a boiling water bath for 135 min. A reagent blank of 0·1 ml H_2O and standards of 0·1 ml with a concentration of 1, 2 and 3 μmoles/ml of 2,3-diphosphoglyceric acid (Sigma Chemical Company) were run simultaneously with the unknown samples. Standards and the unknown samples were analyzed in duplicate. The optical densities at 695 nm were determined in a Coleman model 6D spectrophotometer, the standards graphed, and the unknown values were read from the resulting curve. The sample of whole blood was also analyzed for hemoglobin concentration and hematocrit, and the mean corpuscular hemoglobin concentration (MCHC) was calculated as previously described.[8] The concentration of 2,3-DPG in the unknown sample was multiplied by three to correct for dilution with the trichloroacetic acid and then divided by the MCHC and multiplied by 100 to give the concentration of DPG in micromoles per gram of hemoglobin. Salicylate concentration in serum was determined by Trinder's technique.[9] Two of the nine rats given 500 mg/kg and two of fourteen given 600 mg/kg of sodium salicylate died before the second blood sample was obtained.

The results are presented in Table 1. At 24 hr after the administration of sodium salicylate, there

TABLE 1. EFFECTS OF SPECIFIED DOSAGES OF SODIUM SALICYLATE ON CONCENTRATION OF SALICYLATE IN SERUM AND 2,3-DIPHOSPHOGLYCERATE (2,3-DPG) CONCENTRATION OF RED BLOOD CELLS*

Dose of sodium salicylate (mg/kg)	No. of rats in group	Salicylate in serum at 24 hr (mg/100 ml)	Initial 2,3-DPG (μmoles/g Hb)	2,3-DPG at 24 hr (μmoles/g Hb)
0	9		16·9 ± 0·56	16·5 ± 0·81
400	15	12·8 ± 1·06	15·0 ± 0·84	13·6 ± 0·62
500	7	20·4 ± 1·42	16·9 ± 0·28	13·7 ± 1·14
600	12	29·5 ± 2·93	16·8 ± 0·37	12·6 ± 0 58

* Values are means ±1 S.E.M.

was a decrease in red blood cell 2,3-DPG concentration related to the dosage and serum level of salicylate. At the 600 mg/kg dosage of sodium salicylate, there was a fall in average red blood cell 2,3-DPG concentration from 16·8 to 12·6 μmoles/g of hemoglobin (Hb) or a 25 per cent decrease. At the 500 mg/kg dosage, there was a 19 per cent decrease and at 400 mg/kg, a 9 per cent decrease. The lower initial 2,3-DPG concentration in the rats given 400 mg/kg of salicylate is unexplained. The rats given no salicylate had a 2 per cent fall in 2,3-DPG concentration. One-way analysis of covariance revealed $P < 0{\cdot}005$.

In three of the rats given 600 mg/kg of sodium salicylate, the measurements of 2,3-DPG that were made additionally at 4 hr after administration of the salicylate revealed intermediate reductions in 2,3-DPG levels in all three, with a mean of 16·5 μmoles/g of Hb initially, 13·9 μmoles/g of Hb at 4 hr and 10·2 μmoles/g of Hb at 24 hr.

Because of the known effect of salicylate on the pH of blood[10] and of pH on 2,3-DPG levels,[11] seven rats were injected intraperitoneally with 600 mg/kg of sodium salicylate and were studied in the same way as the others, except for the determination of pH on heparinized retro-orbital capillary blood drawn under light ether anesthesia before and at 4 hr, 12 hr and 24 hr after administration of the sodium salicylate. Two of the rats died. The initial mean pH was 7·319, at 4 hr the pH was 7·300, at 12 hr 7·332, and at 24 hr was 7·348, while the 2,3-DPG fell 12 per cent. It therefore seems unlikely that a fall in blood pH was responsible for the observed effect on 2,3-DPG. The smaller decrease in 2,3-DPG concentration in this group when compared to the other group given the same dosage of salicylate may have been due to repeated blood drawing and possible hypoxia during the ether anesthesia.

This study reveals a reduction in erythrocyte 2,3-DPG concentration related to the dosage of sodium salicylate and to levels of salicylate in serum. This is consistent with the observations that salicylate inhibits enzymes at a variety of steps in red cell glycolysis[1–3] and decreases levels of 2,3-DPG *in vitro*.[3] An increase in oxygen affinity of hemoglobin produced by lowered 2,3-DPG may impede oxygenation of tissues,[6,12] and may be partially responsible for the observation of a 10–20 per cent increase in venous oxygen saturation induced by high doses of salicylate in the rat and man.[13] This effect on 2,3-DPG levels may also account in part for the observation by Miller and Tenney[14] of a fall in tissue oxygen tension in rats given 200 mg/kg of sodium salicylate in divided doses. These studies suggest that impairment of oxygen delivery to tissues as a result of a reduction in the concentration of red blood cell 2,3-DPG may be an effect of salicylate.

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Department of Pediatrics,
Albert Einstein College of Medicine and
Montefiore Hospital and Medical Center,
New York, N.Y. 10467, U.S.A.

RICHARD E. KRAVATH
GÜNER ABEL
ANITA COLLI
HELEN MCNAMARA
MICHAEL I. COHEN

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Inhibition of hexose monophosphate shunt by ethanol—An experimental evaluation

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REALIZING the ubiquitous distribution of the enzymes of the hexose monophosphate shunt (HMP) and the importance of this pathway in the probable regulation of the synthesis of nucleotides, Beaconsfield and Reading[1] have attempted to use ethanol as an inhibitor of the HMP. By using ethanol and alcohol dehydrogenase (ADH) in the incubation medium, they have shown that the incorporation of ^{32}P-labeled phosphate into nucleotides of liver slices and mammary tissue was inhibited. The action of the ethanol–ADH system has been interpreted as due to the reduction of NADP and consequent deprivation of this cofactor for the operation of the shunt pathway. This assumption is based on the work of Kini and Cooper,[2] who have shown that NADP, in addition to NAD, is a cofactor for ADH. Selective inhibition of the pentose phosphate pathway by the ethanol–ADH system, if indeed substantiated, is of far reaching significance, since this is in contradiction to the majority of the experimental work which points out that ethanol exerts its inhibitions by reduction of NAD causing a low NAD–NADH ratio.[3,4] All the enzymatic reactions which are favored by a high NAD–NADH ratio are inhibited by ethanol. Paradoxically, Higgins[5] reports a stimulation rather than inhibition of the HMP pathway by ethanol in rat brain minces.

In view of these contradictions in the literature, investigations were undertaken to evaluate the ADH–ethanol system as an inhibitor of HMP using liver slices. Labeled CO_2 derived by catabolism of [1-^{14}C]glucose and [6-^{14}C]glucose in rat liver slices was measured in the presence of ethanol. Also, incorporation of [1-^{14}C]glycine into proteins of liver slices in the presence of ethanol was measured and the magnitude of inhibition by ethanol was compared with the inhibition of catabolism of [1-^{14}C]glucose and [6-^{14}C]glucose.

Wistar male rats (160–180 g) were sacrificed by decapitation, the liver slices were prepared in the cold using a Staddie–Riggs microtome, and incubated at 37° in Krebs–Ringer phosphate buffer containing various concentrations of ethanol, crystalline horse liver dehydrogenase (350 μg/ml) and glucose (5 mM) as [1-^{14}C] or [6-^{14}C]glucose (specific activity, 0·5 μc/3·0 ml of medium). The liver slices (100–120 mg) from 3 to 4 rats were weighed on a torsion balance and transferred into incubation medium. The incubations were carried out in Warburg vessels with KOH in the center wells and O_2 as the gas phase. Labeled CO_2 was collected by absorption into KOH, converted to $BaCO_3$ and assayed by counting on an end window gas flow counter.[6]

Incorporation of [1-^{14}C]glycine into protein was studied in liver slices (500 mg) using Krebs–Ringer bicarbonate medium (pH 7·4). The medium contained 2 mM glycine (417,000 counts/min/3·0 ml of medium), ADH (350 μg/ml) and ethanol (3%). After incubation for 2 hr, the liver slices were collected by precipitation with trichloroacetic acid (15%); a suspension of protein, free of nucleic acids and lipids,[7] was plated on aluminum planchets, counted on an end window gas flow counter and counts were corrected for infinite self-absorption.

Low concentrations of ethanol (3 mM) inhibited $^{14}CO_2$ yields from [1-^{14}C] and [6-^{14}C]glucose by 27 and 67 per cent respectively (Table 1). With 1 M ethanol this inhibition was markedly increased