The Reaction of p-Aminophenol
with Hemoglobin and Oxygen in vivo and in vitro

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With 3 Figures in the Text

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p-Aminophenol was the first metabolic product of aniline discovered (Schmiedeberg; Jaffe and Hilbert; Müller). Its capacity to oxidize hemoglobin to hemiglobin (methemoglobin) was observed in dogs by Hinsberg and Treupe and was studied further by Heubner (1910; 1913; 1914), and Heubner and Meier. Although p-aminophenol was then considered to be the derivative of aniline that caused the formation of hemiglobin in aniline poisoning, only few attempts have since been made to ascertain its activity in various animals and in vitro.

After injecting subcutaneously 6 mg p-aminophenol per kg into cats, Schwedtke spectroscopically detected hemiglobin in their blood. von Isserkutz found that 15 mg p-aminophenol per kg injected subcutaneously into 4 cats oxidized about one third of the hemoglobin. In the experiments of Hauschild nearly 0.6 of the hemoglobin was oxidized following the subcutaneous injection of 50 mg p-aminophenol per kg into cats. Schaff gave intraperitoneally large doses of p-aminophenol (240–300 mg/kg) to rats. The blood taken 1½ to 3 hours later contained hemiglobin in concentrations amounting from 0.06 to 0.15 of the total blood pigment. In the experiments of Cox and Wendel the intravenous injection of 20 mg p-aminophenol per kg into dogs was followed by an increase in hemoglobin concentration to about 0.6 of the total blood pigment. Again, nearly 0.27 of the hemoglobin in dogs was oxidized by the intravenous injection of 10 mg p-aminophenol per kg (Spicer and Neal). Vandenberg, Pfeiffer, Kaiser and Sibert injected intravenously 1 mg p-aminophenol per kg into a dog. The hemoglobin concentration rose to 0.014 of the total blood pigment. Further, Greenberg and Lester gave 0.5 g p-aminophenol hydrochloride per os to two human subjects. Half an hour later they found that the concentration of free p-aminophenol in the blood was about 1 µg per ml, whereas hemoglobin could not be detected. The action of p-aminophenol in human blood in vitro was found to be weak. After 5 or 10 mg p-aminophenol per 100 ml of blood were added the concentration of hemoglobin only rose to 0.13 and 0.21 of the total blood pigment.

During an investigation of the reactions of aniline and some of its derivates in young and adult dogs we also studied the formation of hemiglobin after the intravenous injection of p-aminophenol (Baader et al.). The high rate of hemiglobin formation immediately after the intravenous injection of p-aminophenol and then its rapid disappearance
from the blood pointed to a high reactivity of p-aminophenol in the organism. Since p-aminophenol effected only a slow formation of hemoglobin in ox-blood in vitro, an activation of the p-aminophenol in vivo was assumed (Baader et al.). Later experiments showed a small “activating effect” of tissue homogenates. This was, however, much too small to explain the difference in the rate of reaction of p-aminophenol in dogs and in red ox-blood cells in vitro. Therefore further studies of the reactions of p-aminophenol in the red blood cells of dogs and in red ox-blood cells became necessary.

Methods

For the intravenous infusion of p-aminophenol dogs were anaesthetized by intravenously injecting 0.05 g chloralose and 0.5 g urethane per kg. A motor-driven syringe infused a solution of p-aminophenol hydrochloride into a vein in one of their hind legs. Samples of blood for analysis were taken from the veins in the other legs.

The red cells of dog’s blood were obtained from heparinized blood that had been used in a heart-lung machine for some hours. The ox-blood was defibrinated at the slaughterhouse or mixed with sodium citrate immediately after bleeding. Cats were bled in ether anaesthesia; the clotting of the blood was prevented by adding citrate. Red cells of human blood were obtained from fresh blood or from banked blood. The red cells were washed on the centrifuge twice with a 0.9% sodium chloride solution and once with a Krebs-Ringer phosphate solution. Then they were suspended in an equal volume of this solution. Neither glucose nor any other substrate for the enzymic reduction of hemoglobin was added. Solutions of hemoglobin were obtained by adding 4 volumes of water to the washed red cells. In order to remove the red cell membranes the solutions were centrifuged.

p-Aminophenol hydrochloride was dissolved under nitrogen in a 0.9% sodium chloride solution for infusing into dogs or red cell suspensions. It was dissolved in water for adding to hemoglobin solutions.

The method of Brodie and Axelrod was used for determining the p-aminophenol in the blood, the cell suspensions, and the hemoglobin solutions.

Hemoglobin was estimated by measuring the increase in extinction at 550 μm caused by adding cyanide to the solution of a blood sample. The sum of hemoglobin and hemiglobin was estimated after oxidizing the hemoglobin to hemiglobin by means of ferricyanide.

Results

1. Experiments on dogs

After some experiments with various rates of infusion, 0.5 mg p-aminophenol hydrochloride per kg and min were infused into 4 dogs. In 30 min the concentration of free p-aminophenol in the blood rose to 3.5 μg per ml and the hemoglobin concentration to 0.3 of the total blood pigment. When the concentration of free p-aminophenol became 2 μg per ml of blood the hemoglobin concentration was found to be 0.12 of the total blood pigment, while the rate of hemoglobin formation amounted to 0.01 of the total blood pigment per min. The same rate of hemoglobin formation was also observed in earlier experiments with a