Table III. Effect of 6-ANA (35 mg/kg body wt.) on blood glucose levels and body wt. in fed and starved mice compared to untreated starved controls

Treatment	Body weight (g)		Blood glucose (mmoles/l)		Hypoxic survival rates[b]
	1[a]	2	1	2	
Controls, starved (8)	29.9[c] ± 0.7	27,9 ± 1.0	8.87[c] ± 0.45	7.46 ± 0.68	–
6-ANA starved (8)	30.5[c] ± 1.3	27.9 ± 1.2	9.59[c] ± 1.22	7.13 ± 0.89	6
6-ANA, fed (8)	31.1[d] ± 1.0	30.3 ± 1.1	9.50[d] ± 0.94	13.40 ± 2.41	6

[a] Measurements were made prior to (1) and 6 h after the injection of 6-ANA (2). [b] Number of animals alive after 30 min of hypoxic hypoxia; the untreated controls were not subjected to hypoxia. [c] The values of body weight and blood glucose of untreated starved controls and starved treated animals were submitted to a two-way analysis of variance. There was a significant effect of time on weight and blood glucose in both groups ($p < 0.01$), differences between groups were not significant, however. [d] Differences were significant ($p < 0.01$) according to Students t-test for paired observations.

tolerance of 6-ANA-treated mice was seen with an injection of D-glucose, even though blood glucose levels were more than twice as high, compared with controls (Table II). Likewise the combined effects of 6-ANA and hyperglycemia failed to enhance the tolerance to comlpete anoxia.

Finally another attempt was made to ascertain the implications of blood glucose levels in the hypoxic tolerance of 6 ANA-treated mice (Table III). When mice given the usual dose of 6-ANA were starved for 6 h, blood glucose levels were similarly reduced to those of starved untreated controls. Thus 6-ANA-induced hyperglycemia, which is primarily brought about by concomitant rises in serum corticosterone and adrenaline[11], may be partly due to an impaired alimentary glucose tolerance. Even more important, however, is the identical hypoxic tolerance in both 6-ANA-treated groups.

Little or no effect on the hypoxic survival times was anticipated to arise from the blocking of the hexose monophosphate shunt in view of its minor contribution to the overall metabolism of glucose[2–5]. Indeed, rather than impairing the hypoxic tolerance, 6-ANA was found to effect the opposite, indicating that even hypoxia does not challenge this alternative glycolytic pathway. Furthermore, the results demonstrate that the enhanced hypoxic tolerance is not related to systemic effects of 6-ANA like hyperglycemia or hypothermia. Although an overall depression of cerebral metabolic activity similar to the one observed with anaesthetics[15] cannot be ruled by the present study, the data may also be consistent with the assumption that anaerobic glycolytic flux is increased by 6-ANA. During brain ischemia, lactate levels of 6-ANA-treated animals rise more rapidly and to higher levels than in control animals[16], possibly as a result of enhanced glycolysis. Whether such a mechanism actually applies during hypoxia remains open at present. That anaerobic utilization of glucose by brain may be effectively stimulated by pharmacological agents, is nevertheless an intriguing possibility, deserving close attention, also in view of possible implications in human medicine[17].

Zusammenfassung. Eine einmalige Injektion von 6-Aminonicotinamid (6-ANA) steigerte die Sauerstoffmangelresistenz von Mäusen in einer Atmosphäre mit 5% O_2 und 95% N_2. Hyperglykämie und Hypothermie, systemische Wirkungen von 6-ANA, konnten als Ursachen der gesteigerten Resistenz ausgeschlossen werden. Es wird deswegen vermutet, dass 6-ANA die anaerobe Utilisation von Glucose im Gehirn fördert.

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The Effect of Salicylates on Plasma Fibrinolytic Activity in the Rat

Circulating antiplasmins have been postulated to be in a dynamic equilibrium with plasmin (fibrinolysin) and to compete with fibrin for its active site[1]. Sodium salicylate was reported to inhibit the breakdown of fibrin by plasmin in vitro[2,3]. On the contrary several synthetic antiinflammatory agents have been shown to induce fibrinolysis in vitro[4,5]. In this paper the effect of administering sodium salicylate i.p. or acetylsalicylic acid orally on the plasma fibrinolytic activity of rats is examined.

Methods. Male Wistar rats weighing 180–250 g were injected i.p. with sodium salicylate dissolved in phosphate buffer 0.12 *M*, pH 7.4 at doses of 50 mg, 100 mg and 150 mg/kg. Acetylsalicylic acid was given orally in the same doses after mixing with tragacanth powder in the ratio 2:1 to make a gummy suspension. Control rats were given 0.9% sodium chloride solution i.p. or orally. After

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Table I. Effect of sodium salicylate on the rat fibrinolytic system

Dose of sodium salicylate	Plasma salicylate concentration (mg/100 ml)	Euglobulin clot lysis time (min)	Plasma antiplasmin index	Plasminogen activator activity (mm^2)
Control	0.00 ± 0.00 (3)	112.42 ± 2.98 (14)	1.62 ± 0.01 (10)	17.88 ± 1.25 (18)
50 mg/kg	11.56 ± 0.06 (3)	127.14 ± 4.46 (7) [a]	1.71 ± 0.03 (5)	17.88 ± 2.63 (9)
100 mg/kg	18.35 ± 0.02 (3)	104.85 ± 2.54 (7)	1.28 ± 0.02 (5) [b]	19.44 ± 2.69 (9)
150 mg/kg	25.15 ± 0.02 (3)	67.33 ± 1.70 (7) [b]	1.19 ± 0.03 (5) [b]	17.33 ± 2.50 (9)

Number of observations in parenthesis. [a] Statistically significant compared with controls where $p < 0.05$; [b] Statistically significant compared with controls where $p < 0.005$.

Table II. Effect of acetylsalicylic acid on the rat fibrinolytic system

Dose of acetylsalicylic acid	Plasma salicylate concentration (mg/100 ml)	Euglobulin clot lysis time (min)	Plasma antiplasmin index	Plasminogen activator activity (mm^2)
Control	0.00 ± 0.00 (3)	117.14 ± 2.49 (7)	1.56 ± 0.01 (5)	8.37 ± 2.48 (8)
50 mg/kg	17.30 ± 0.00 (3)	114.28 ± 1.20 (7)	1.56 ± 0.00 (5)	9.62 ± 2.49 (8)
100 mg/kg	24.18 ± 0.01 (3)	112.42 ± 2.46 (7)	1.36 ± 0.00 (5) [b]	13.75 ± 2.65 (8)
150 mg/kg	31.10 ± 0.00 (3)	63.85 ± 1.31 (7) [b]	1.10 ± 0.01 (5) [b]	13.12 ± 3.24 (8)

Number of observations in parenthesis. [a] Statistically significant compared with controls where $p < 0.05$; [b] Statistically significant compared with controls where $p < 0.005$.

90 min to 100 min the animals were sacrificed and blood collected into ice cold polythene vials. The euglobulin fraction of plasma was prepared from fresh plasma according to BLIX[6]. Rat euglobulin (0.2 ml) was added to 1.6 ml phosphate buffer 0.12 *M*, pH 7.4 to which 0.1 ml 0.025 *M* calcium chloride solution was daded and the mixture clotted with 0.1 ml bovine thrombin solution (50 NIH U/ml). Calcium chloride was added to the euglobulin mixture to replace calcium lost during euglobulin precipitation from plasma, and to produce a firm opaque clot necessary for an accurate visual estimation of clot lysis times. All clots were incubated at 37 °C in a constant temperature incubator and the time for complete lysis recorded. Plasma antiplasmin activity was measured as described by CHATTOPADHYAY and CLIFTON[7] and expressed as an index[8]. Plasminogen activator activity was measured by incubating 0.03 ml aliquots of the unclotted euglobulin fraction on bovine fibrin plates prepared according to von KAULLA[9]. The plates were incubated in a constant temperature incubator for 20 h and the cross diameter of lysed zones in mm^2 taken as a measure of the activator activity. Plasma salicylate concentrations were measured according to TRINDER[10].

Results and conclusions. Though sodium salicylate caused a significant prolongation of rat plasma euglobulin clot lysis times at a dose of 50 mg/kg, there was no significant change at a dose of 100 mg/kg nor with acetylsalicylic acid at 50 and 100 mg/kg (Tables I and II). However there were significant reductions in the plasma antiplasmin activity at 100 mg and 150 mg/kg, with both drugs but only with the 150 mg/kg dose was there a sufficient reduction in antiplasmin activity to cause significant reductions in the plasma euglobulin clot lysis times. The euglobulin lysis was complete in about half the time obtained with control plasma samples. The overall plasminogen activator activity was unchanged from the controls with each of the 3 doses of sodium salicylate or acetylsalicylic acid. Considerable variation exists between control values of rat plasminogen activator activity for sodium salicylate (Table I) and for acetylsalicylic acid (Table II). This variation reflects batch differences in the fibrin plates used in these two studies, possibly differences in the nature of the fibrin gel between the two batches and the effectiveness of the 0.03 ml drops of the euglobulin solutions on each batch of fibrin plates. Plasma salicylate levels obtained with acetylsalicylic acid administered orally were substantially higher than those obtained with sodium salicylate given by intraperitoneal injection. It is concluded that sodium salicylate and acetylsalicylic acid can enhance plasma fibrinolytic activity in the rat in vivo only at a high dose. These results suggest that this increased fibrinolytic activity in the rat is not due to intrinsic plasminogen activation but rather to the inhibition of circulating levels of plasma antiplasmins by high doses of sodium salicylate or acetylsalicylic acid[11].

Zusammenfassung. Zufuhr von Kalium-Salicylat und Acetylsalicylsäure führte bei der Ratte zu einer Verminderung der Plasma-Antiplasmin-Aktivität (100 mg und 150 mg/kg). Somit erhöhen Salicylate in höheren Dosen die fibrinolytische Tätigkeit.

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