Tibia	Days							
	4	11	18	33	46	60	74	102
Irradiated: control	> 0.05	> 0.05	> 0.05	< 0.05	> 0.05	< 0.05	< 0.05	> 0.05
Opposite: control	> 0.05	> 0.05	> 0.05	< 0.05	< 0.05	< 0.01	< 0.05	> 0.05

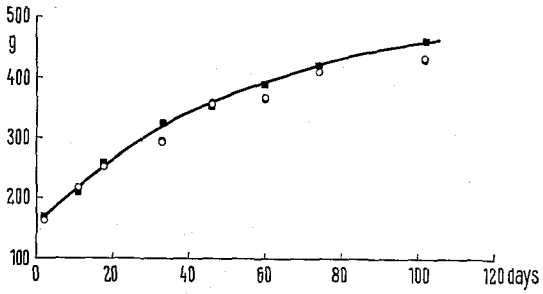

Fig. 1. Weights of dried bone substances from the whole tibia in healthy controls (■), in irradiated legs (○) and in opposite legs (●) of irradiated animals.

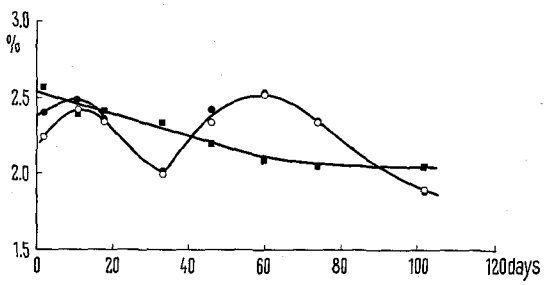

Fig. 2. The 48 h ^{45}Ca-uptake in the whole tibia expressed in per cent of the ^{45}Ca-dosis, given to the animals.

tion and that there were no gross macroscopic pathological findings in the bones. The onset of this metabolic aberration was relatively late.

From this experiment some evidence was gained about metabolic mineral disturbances in the bones, caused by even such small intensities of laser pulses, as used here. With respect to increasing uses of laser in various therapeutic interventions, the possible harmful effects on the bones are not neglectible and some further investigations about the permissible levels of energies used in clinical practice are justified.

Zusammenfassung. Bei männlichen Wistarratten wurden nach 3 Laserpulsen (Energie 9 J) deutliche metabolische Abweichungen vom 48-h-^{45}Ca-Empfang in den Knochen festgestellt, die einige Monate andauerten. Das makroskopische Aussehen der Knochen war dabei normal. Eine Bestimmung der zulässigen Energien der Laserstrahlen scheint für die klinische Praxis notwendig zu sein.

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The Influence of Sodium Salicylate on the Formation of Inorganic Phosphate in Human and Rabbit Erythrocytes in vitro

The rate of erythrocyte glycolysis is markedly influenced by the composition of the suspending medium[1]. Furthermore, it has been shown[2] that great differences occur in the rate of glucose consumption and lactate production of aliquots of the same erythrocyte sample suspended in different media. The rate of glycolysis of erythrocytes suspended in $0.1\,M$ potassium phosphate buffer pH 7.4 containing $0.01\,M$ glucose was 2–3 times that of cells suspended in Tyrode-Locke's solution containing $0.01\,M$ glucose. Inorganic phosphate is an important factor in regulating the rate of erythrocyte glycolysis[3–6] and by increasing its concentration in the incubation medium corresponding increases were seen in erythrocyte glucose consumption and lactate production[7].

Sodium salicylate in concentrations ranging from 1 to $5 \times 10^{-3}\,M$ increased glucose consumption and lactate production by both human and rabbit erythrocytes washed and suspended in Tyrode-Locke's solution containing $0.01\,M$ glucose[2]. The present investigation concerns the effect of sodium salicylate on inorganic phosphate formation in erythrocytes.

Human venous blood was obtained by venipuncture of the antecubital vein while rabbit blood was withdrawn

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by direct cardiac puncture of animals anaesthetized with pentobarbitone sodium (30 mg/kg i.v.). All blood was collected in heparinised plastic tubes (Staynes Laboratories Ltd.). Blood samples were immediately centrifuged at 3000 rpm for 10 min, the supernatant plasma and buffy layer being removed prior to washing 3 times in the required incubation medium. After the final wash the cells were adjusted to a haematocrit of 25% and incubated in a water bath at 37 °C for a few minutes before distribution into tubes containing 1/20th final volume of either 0.9% sodium chloride as a control or isotonic solutions of sodium salicylate of desired final concentration. Initial levels of inorganic phosphate were determined by deproteinising a control tube immediately with 9 volumes of 10% w/v trichloracetic acid. The remaining samples were incubated for exactly 1 h at 37 °C in a constant temperature water bath with constant agitation prior to deproteinisation with 10% trichloracetic acid as described above. The samples were immediately centrifuged at 1000 rpm for 5 min and the inorganic phosphate contents of the supernatant was determined as described by KING and WOOTON[8] using a Unicam SP. 1300 colorimeter.

Figure 1 shows that sodium salicylate in concentrations ranging from $2–20 \times 10^{-3} M$ causes significant increases in the formation of inorganic phosphate in rabbit erythrocytes washed and resuspended in either 0.9% sodium chloride or Tyrode-Locke's solution. Further experiments have shown that an increased formation of inorganic phosphate by rabbit erythrocytes is evident in the presence of concentrations of sodium salicylate as low as $5 \times 10^{-4} M$.

Sodium salicylate also increased the inorganic phosphate level in human erythrocytes which have been washed and resuspended in either 0.9% sodium chloride or Tyrode-Locke's solution (Table). However, the increased formation of inorganic phosphate by human erythrocytes incubated with sodium salicylate is not nearly as marked as that seen with rabbit erythrocytes as is shown in the Figure 2.

That there is a connection between the increased inorganic phosphate and the acceleration of erythrocyte glycolysis induced by sodium salicylate remains to be established; the work of ROSE and WARMS[7] which showed that the rate of erythrocyte glycolysis was related to the amount of inorganic phosphate in the incubation medium does, however, support such a possibility. This suggests that the primary effect of sodium salicylate is to increase the erythrocyte inorganic phosphate concentration which in turn stimulates erythrocyte glycolysis.

The site in the erythrocyte at which salicylate stimulates the release of inorganic phosphate remains to be determined. Stimulation of adenosinetriphosphatase activity would be expected to increase the release of inorganic phosphate and salicylate has been shown to stimulate such an enzyme derived from liver mitochondria[9–11].

The influence of sodium salicylate on the formation of inorganic phosphate by human erythrocytes suspended in either 0.9% sodium chloride or Tyrode-Locke's solution.

Concentration sodium salicylate ($\times 10^{-3} M$)	Inorganic phosphate (mg phosphorus/100 ml erythrocytes/h ± S.E. of mean)	
	Erythrocytes suspended in	
	0.9% Sodium chloride	Tyrode-Locke's solution
0	1.87 ± 0.23	1.38 ± 0.22
2	2.27 ± 0.28	1.86 ± 0.28
5	2.61 ± 0.26	2.16 ± 0.37
10	2.90 ± 0.27	2.33 ± 0.29
20	3.08 ± 0.29	2.50 ± 0.39

Each value represents the mean of 4 separate experiments.

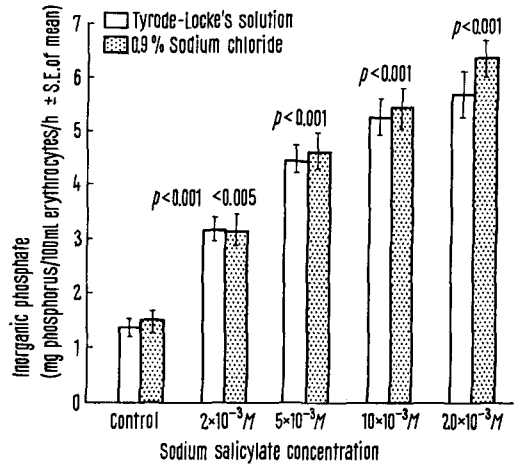

Fig. 1. The effect of sodium salicylate on the formation of inorganic phosphate by rabbit erythrocytes suspended in 0.9% sodium chloride or Tyrode-Locke's solution.

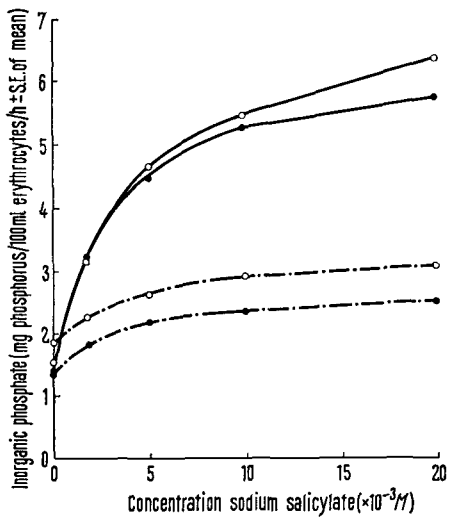

Fig. 2. Comparison of the effect of sodium salicylate on the formation of inorganic phosphate by human and rabbit erythrocytes suspended in Tyrode-Locke's solution or 0.9% sodium chloride. —○—, rabbit erythrocytes in sodium chloride; —●—, rabbit erythrocytes in Tyrode-Locke's solution; -·-○-·-, human erythrocytes in sodium chloride; -·-●-·-, human erythrocytes in Tyrode-Locke's solution.

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However, studies on the sodium-potassium activated transport adenosinetriphosphatase of rabbit erythrocytes have suggested that this enzyme is not stimulated by sodium salicylate[2,12].

Studies are in progress to investigate further the site(s) of salicylate stimulated erythrocyte inorganic phosphate release, and to determine whether this phenomenon is related to the salicylate induced acceleration of erythrocyte glycolysis.

Zusammenfassung. Natriumsalizylat in Konzentrationen von $2–20 \times 10^{-3} M$ verursacht einen bemerkenswerten Anstieg an anorganischem Phosphat sowohl in Kaninchen- wie auch in Menschenerythrozyten, die in 0,9% Natriumchlorid oder Tyrode-Locke's Lösung mehrfach gewaschen und suspendiert wurden.

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2 December 1968.

[12] Acknowledgment. We wish to record our appreciation of the technical help given so readily by R. Weston.

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Respiratory Reflexes During Anaphylactic Bronchial Asthma in Guinea-Pigs

Reversible bronchial asthma is produced in guinea-pigs sensitized to egg albumen by inhalation of antigen aerosol. A series of investigations carried out on several hundred animals with the object of throwing further light on the neurophysiological factors that underlie anaphylactic bronchial asthma – and in particular the role played by the vagus, chemoreceptors and central nervous mechanisms – have led to the demonstration that characteristic structural changes occur in the lungs during an attack, and that these in turn give rise to respiratory and circulatory reactions[1–3].

At the onset of rising bronchial resistance, a marked inspiratory reaction, characterized by tachypnoea, occurs; the resultant increase in lung volume is augmented by air trapping. The inspiratory reaction, observed in anaesthetized animals as well as in animals decerebrated at mid-collicular level, is not due to stimulation of the chemoreceptors for it is not abolished by denervation of the carotid and aortic bodies. At later stages, however, hypoxia develops, and the chemoreceptors come into play. Depending on the strength and duration of a severe asthma attack, hypercapnia bringing about direct reinforcement of central respiratory mechanisms may in addition arise, to which extra-vagal proprioceptive reflexes should probably also be added.

The inspiratory reaction represents the dominant respiratory effect, and is mainly responsible for the circulatory changes that follow, namely, a reversible fall in mean arterial blood pressure and an increase in heart rate. The inspiratory reaction is not affected by atropine (Figure, signal a) and, as it is abolished following section of the vagus on both sides (signals b and c), is mediated by afferent fibers running in this nerve. It has been possible by means of reversible cold block, selective electrical stimulation, and recording of the neurogram from vagal filaments, to establish that the fibers specifically concerned with the reflex arise from deflation receptors. The latter become active during the expiratory phase of asthmatic breathing. Further studies have shown that these receptors respond to forced deflation (i.e., increased intrathoracic pressure) during shift of the intrapleural negative pressure to positive values. This increase in intrathoracic pressure in turn leads to increase in bronchial resistance. Compression of the chest wall augments the inspiratory reflex, whereas thoracic distension, during which the expiratory intrapleural pressure reverts to subatmospheric values, diminishes it. These results lend support to the conclusions of Wyss[4,5] that stimulation of the deflation receptors enhances inspiratory efforts, and thereby suppresses pulmonary collapse, or local effects such as atelectasis and pulmonary compression, but hampers the mechanisms subserving the self-regulation of respiration[6,7].

It has been shown by Paintal[8] that the deflation receptors in the cat are situated in the respiratory bronchioles, atria or alveoli. Our histological studies of lungs fixed in vivo during an asthma attack reveal that the anaphylactic narrowing or obstruction, which occurs predominantly in the bronchioles, leads to emphysema and microscopic atelectasis, conditions which imply a disturbance in the intrapulmonary distribution of air ventilating the alveoli, in particular, an increase in functional residual capacity. These findings strongly suggest that the afferent vagal fibers responsible for the inspiratory reaction arise in atelectatic areas dispersed throughout the overinflated lungs, whereas augmented pulmonary stretch receptor activity is correlated with emphysema. A chain of events would thus be set up which appears to develop in the following order: Structural pulmonary changes, i.e., anaphylactic obstruction of bronchioles resulting in emphysema and atelectasis; atelectasis, giving rise to the inspiratory reaction associated with tachypnoea and increase in functional residual capacity. These events – in which the Hering-Breuer reflexes are suppressed by deflation receptor activity – lead to ever-increasing disturbance of the lung mechanics described. The cycles succeed one another as long as reversible anaphylactic airway obstruction persists.

The inspiratory reaction is produced by inhalation of antigen aerosol particles that do not exceed 0.5–10 μ in

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