Apart from detoxification by the alkylation of thiol groups, some degree of hydrolysis must occur, since neither IMS nor *iso*-propyl iodide are excreted unchanged, and the excretion of methanesulphonic acid from ^{35}S-IMS parallels that of ^{35}S-methanesulphonic acid itself (Table). The hydrolysis product, *iso*-propyl alcohol (VI), and its metabolite[8] acetone, were not detected from these compounds but as both *iso*-propyl alcohol and acetone are partially oxidised in vivo[9], it is probable that the expired ^{14}C-carbon dioxide (Table) represents this hydrolytic pathway.

As the excretion patterns of *iso*-propyl iodide and *iso*-propyl alcohol are almost identical, hydrolysis of the former probably represents the major detoxification route, alkylation reactions such as conjugation with cysteine (glutathione) representing only a minor pathway. The different pattern of excretion of radioactivity from IMS indicates that although in vivo hydrolysis is rapid (half-life at 37 °C is 13 min at pH 7)[1], the alkylation reaction is a major pathway (Figure 2). This can be interpreted as reaction of the compounds by 2 different mechanisms; bimolecular for *iso*-propyl iodide (and presumably the bromide) and unimolecular for IMS. The production of the highly reactive dimethylcarbonium ion (IV) from IMS by a unimolecular reaction is consistent with a rapid degree of alkylation both in the detoxification route and in the reaction of IMS with DNA in vitro[10].

Whereas the different biological actions of alkylating agents have been attributed to their mechanisms of alkylation[11], present and recent[12] studies indicate that, at least for methanesulphonate esters, this may not be true. IMS and dimethylmyleran react by different mechanisms yet possess similar biological activities suggesting that in some instances, the mechanism of alkylation may not be an important factor in their mode of action.

Zusammenfassung. Nachweis, dass die im Rattenharn auftretenden Metaboliten Isopropyljodid und Isopropylmethansulfonat für einen unterschiedlichen Wirkungsmechanismus der Substanzen in vivo sprechen. Aus Isopropylmethansulfonat entsteht durch einen monomolekularen Prozess das äusserst reactive Dimethylcarboniumion, während Isopropyljodid aufgrund einer bimolekularen Reaktion haupsächlich durch Hydrolyse entgiftet wird.

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Acceleration of Red Cell Glycolysis by Citrate due to Intracellular pH Enhancement

Since the introduction of sodium citrate for blood preservation[1], it has been used as an important ingredient for blood preservation media: acid dextrose citrate (ACD) and citrate phosphate dextrose (CPD) solutions. Citrate has been added to preservation media as an anticoagulant and the effect of the anion on red cells has not been thoroughly studied. Although citrate anion is known to be practically impermeable to red cell membrane[2], its high concentration is expected to exert some influence on red cell metabolism. Recently we have found that the intracellular pH (pH_i) of red blood cells stored in ACD medium is higher than the extracellular pH (pH_e) of the suspension[3]. This finding urged us to study the glycolysis of red cells in the presence of citrate.

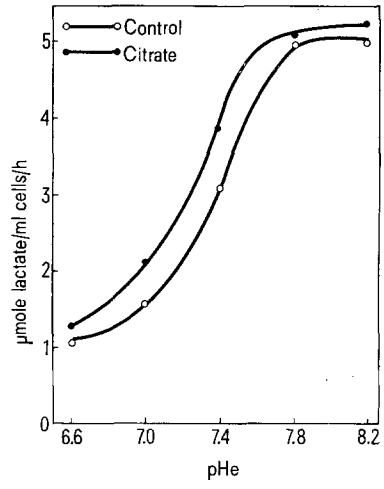

Fig. 1. The pH-curves of lactate formation in red cells in presence and absence of 33 m*M* citrate.

Methods. One-day-old ACD blood was obtained from a local blood bank and red cells were washed thoroughly with isotonic saline. The cells were suspended in a solution (120 m*M* NaCl, 5 m*M* KCl, 1 m*M* $MgCl_2$, 1 m*M* inorganic phosphate and 10 m*M* glucose) and incubated at 37°C. During incubation, the pH was kept constant by a pH-stat with the addition of 0.2 *M* NaOH. After 2 h preincubation, sodium citrate solution was added to a final concentration of 33 m*M* and incubated for further 2 h. Samples were taken out at intervals for analyses.

Results and discussion. More than 20% increase of the lactate formation was observed when citrate was added to the cell suspension at pH 7.4. The increase by the citrate addition depended on the pH of the suspension as shown in Figure 1. Shift of the pH curve was observed by the addition of citrate, which suggests the increase of the intracellular pH. The possibility of pH increase inside the cells was further supported by the changes of the glycolytic intermediates. Hexose monophosphates decreased

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and the intermediates below fructose diphosphate increased by the addition as shown in Figure 2. This pattern is similar to that observed when incubation pH is shifted to alkaline side[4]. The actual change of the pH_i was checked by the method of CALVEY[5] using 5,5'-dimethyloxazolidine-2,4-dione (DMO). C^{14}-DMO was added to the cell suspension and the incubation was carried out as indicated above. About 0.2 unit increase of the intracellular pH was observed when citrate was added to the suspension, though the extracellular pH remained constant, being in agreement with the previous observation on ACD blood at 4°C[3].

TSUBOI and FUKUNAGA[6] observed the acceleration of red cell glycolysis by replacing Ringer solution with isotonic solutions of membrane impermeable substances. They suggested that this phenomenon is due to some unknown membrane transport function utilizing ATP. However, since no decrease of ATP by the citrate addition was observed in the present experiment, the acceleration could not be ascribed to any ATP utilization. A plausible explanation is that citrate shifts intracellular pH of red cells to alkaline side and thereby accelerates glycolysis. The present experiment may serve as an example to stress the importance of intracellular or local pH changes for regulation of metabolism.

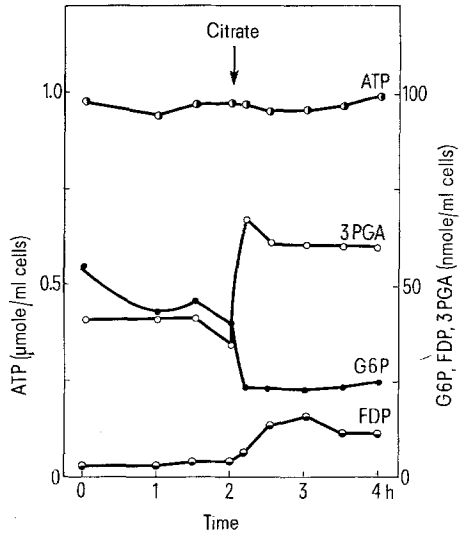

Fig. 2. Changes of glycolytic intermediates due to the addition of 33 m*M* citrate in red cells incubated at pH 7.4. Intermediates were assayed enzymatically[9]. G6P, glucose 6-phosphate; FDP, fructose 1,6-diphosphate; 3PGA, 3-phosphoglycerate.

Previously we showed that the pH_i at 4°C of red blood cells in ACD was 7.61 at the initiation of the storage and dropped to 7.12 after a month[3], indicating that the pH inside the cells of ACD blood is not so acidic as we usually expect from the observation of the pH_e at 37°C (7.0 to 6.6). It may be suggested that besides the role as an anticoagulant, citrate has another role in blood preservation media to keep the intracellular pH higher than the extracellular pH and that the use of acidified media (pH 5.0 for ACD and 5.65 for CPD) for the preservation is to keep the pH_i to physiological range, though the acidification was originally carried out to prevent caramelization of glucose during autoclaving[8].

Zusammenfassung. Nachweis, dass in Konsequenz der intra- und extrazellulären pH-Differenz, das Citrat die Erythrocyten Glycolyse beschleunigt.

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Effect of Stimulating a Central Giant Serotonin-Containing Neuron on Peripheral Muscles in the Snail *Helix pomatia*

The giant serotonin-containing neuron (GSC)[1,2] in each cerebral ganglion of *Helix pomatia* sends an axon branch into each cerebro-buccal connective, and a third axon branch into the external lip nerve[3]. The branches in the cerebro-buccal connectives form excitatory monosynaptic links with identifiable neurons in the buccal ganglia[4,5]. This report describes the results of experiments made to locate structures innervated by the axon of the GSC running in the external lip nerve.

Materials and methods. The cerebral ganglia, external lip nerves, and lips were dissected from *Helix pomatia*, and pinned to the base of a small perfusion chamber which contained 3 ml of saline[6]. A double-barrel microelectrode was inserted into one of the GSCs. The external lip nerve and its branches, and muscles located at the peripheral ends of the external lip nerves, were held in suction electrodes for stimulating and recording. Conventional electrophysiological recording methods were used.

Results and discussion. The small peripheral branches of the external lip nerve (Figure 1) contain axon branches of the GSC. The evidence is as follows: 1. Stimulation of any such nerve at its point of contact with the muscle triggered an antidromic action potential in the GSC. The antidromic action potential occurred in two steps indicating sequential invasion with an axonal potential (A spike) firing before the cell body (S spike). The axonal potential was made smaller and ultimately blocked by artificially hyperpolarizing the cell body. 2. When the GSC was stimulated directly a small spike could be recorded extracellularly from the nerves close to the lip muscles. The latency between the GSC spike and the recorded action potential was constant for any particular branch of the external lip nerve at about 30 msec., and

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