sealed flasks for deoxygenated samples, the reaction was stopped with 0.4 M of 0.6 N H_2SO_4.

Styrene oxide formation was evaluated according to a previously described method[17-19]. In this procedure at the end of incubation the styrene oxide formed is quantitatively chemically hydrated by overnight incubation with H_2SO_4 to the glycol, which is more suitable for gas chromatographic analysis. Styrene glycol is quantitatively determined by a sensitive gas chromatographic procedure using an electron capture detector[20].

Results and discussion. We have already shown that human erythrocytes are able to catalyze styrene oxidation to styrene oxide[13] and that this reaction was supported by methemoglobin and H_2O_2 (Cantoni et al.[21]). It is known that the oxygen in oxyhemoglobin is in a partially activated form[10,11] and that iron chelates in the presence of H_2O_2 can generate reactive oxygen intermediates[22,23]; the superoxide anion (O_2^-) can also be released in erythrocytes during autoxidation of hemoglobin[12]. The table shows that CO almost completely inhibited styrene oxidation, probably by O_2 displacement, suggesting an important role for hemoglobin in this reaction. Superoxide dismutase and catalase had no effect, indicating that O_2^- and H_2O_2 were not directly involved. With the styrene concentration used for this experiment (50 mM) a 100% cell lysis occurred in 5 min (data not shown) therefore these enzymes would be able to penetrate to the site where styrene oxidation occurs. Likewise, scavengers of hydroxyl radicals such as tryptophan[22,24], mannitol and dimethylsulfoxide[24,25] did not inhibit styrene oxidation to styrene oxide.

Figure 1 reports the time course of styrene oxidation with 2 different styrene concentrations in intact cells and in cell lysate. With the higher styrene concentration (50 mM), able to cause cell lysis, the time courses in both systems almost overlap (panel A). At the lower styrene concentration (0.8 mM) (panel B), which does not cause cell lysis, intact cells are more active than lysate probably because the oxyhemoglobin concentration is higher inside the cells than in the lysate, although the total amount of oxyhemoglobin was the same in both samples. It has been shown that O_2 release increases with the concentration of oxyhemoglobin[27].

Incubation of partially deoxygenated erythrocytes with styrene showed a linear relationship between styrene oxidation and the molar fraction of oxyhemoglobin contained in the red blood cells (fig. 2).

These findings seem to indicate that free reactive oxygen intermediates, able to oxidize organic molecules[28-30], are not directly involved in styrene oxidation in erythrocytes, but that this reaction is effected by oxyhemoglobin.

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Interference of azide in the estimation of total lipids by means of the sulphophosphovanillin method

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Summary. The commonly used bacteriostaticum sodium azide interacts with double bonds of lipids, causing an underestimation of lipid concentration when lipids are assayed in its presence. This can be circumvented by extraction of lipids prior to the assay.

Part of our research on reproduction physiology of the starfish *Asterias rubens* (L.) is concerned with the nature of materials transported from the storage organs (pyloric caeca) to the gonads. It was supposed earlier[2] that these materials are transported in a complex form as a glycolipoprotein.

The amounts of lipid and carbohydrate in fractions obtained by gel filtration of pyloric caeca and gonad homoge-

nates were determined with sulphophosphovanillin reagent (Zøllner and Kirsch[3]) and anthrone (Van Handel[4]), respectively. As usual, sodium azide was added to the elution buffer as a bacteriostaticum. It is known that this compound interferes with the anthrone reaction for the determination of carbohydrates[5]. Cholesterol estimation may also be affected by the presence of azide[6,7]. To test the reliability of the vanillin method for lipid estimation in the presence of sodium azide, which became questionable after the results of Vladescu et al.[8], a model study was undertaken with some lipid compounds in the presence of varying amounts of this bacteriostaticum.

In the figure is plotted the extinction at 536 nm, the optimal wavelength for the vanillin test, vs the amount of oleic acid in the presence of increasing quantities of sodium azide. It turns out that sodium azide decreases the extinction to an extent dependent on its concentration. This implies that the calculated lipid values will be underestimated, which confirms the results of Vladescu et al.[8]. With lecithin and lipoprotein (rat β-lipoprotein) a similar picture was obtained to that with oleic acid; a less pronounced curve was obtained with cholesterol (figures not depicted).

When lipids are extracted from the eluent before estimation there is no interference by azide. This was also found for other quantitation methods[7]. When azide is added after the formation of the alkenyl cation[9] by H_2SO_4 there is no effect either. Calculation shows that the underestimated amount of oleic acid is about equimolar with the amount of azide. We therefore think that the interference is caused by a competition for the double bonds between azide and H_2SO_4, and not by competition between azide and vanillin as was suggested by Vladescu et al.[8].

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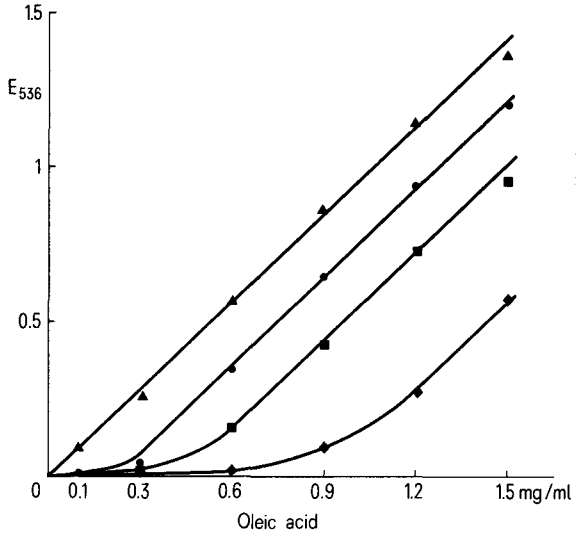

Plot of E_{536} vs the concentration of oleic acid in the presence of increasing concentrations of sodium azide. ▲—▲, 0% azide; ●—●, 0.005% azide; ■—■, 0.01% azide; ◆—◆, 0.02% azide.

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The tooth replacement phenomenon and growth in the green iguana, *Iguana iguana*

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Summary. A long term study of the relationship between growth and the increase in tooth width and number of teeth was conducted. The results indicate that the tooth replacement phenomenon provides a mechanism for increasing tooth size in relation to increasing body size.

A continuous succession of teeth is characteristic of the dentitions of non-mammalian vertebrates. Each new tooth is initiated superficially inside the jaw. As it grows it enters the oral cavity where it functions for a few months before being replaced from the side and below. The pattern of replacement is precise and results in a condition known as the wave replacement of alternate teeth[1,2]. The teeth are replaced in waves which generally sweep through alternate tooth positions from back to the front of the jaw[3]. The maintenance of a dentition in animals who use their teeth to grasp and hold prey is of great importance. The replacement of alternate teeth ensures that no toothless gaps occur[4]. The replacement of teeth may allow for the growth of the teeth in order to maintain their relative size to the growing dentary and maxilla. Records of body weight, body length, the number of teeth, and tooth width for 10 young green iguanas, *Iguana iguana,* were kept over a period of 2.7 years to examine the relationship between tooth size and the number of teeth and growth. This period of time is considerably longer than a typical 8 month period[5].

Methods. Ten young green iguanas, of approximately the same age, were maintained in a large cage well supplied with water and fed a varied vegetable diet which was supplemented with dog food and a nutritional supplement, SA-37 (Rogar/STB) for 2.7 years. The animals were kept at a constant temperature of 32 ± 1 °C, the mean daily temperature of Panama, their place of origin[6]. The photoperiod was maintained on a 12-h cycle. The fluorescent tubes used provided illumination having a spectrum similar to sunlight, including an UV component. In addition, 2 spot lamps were provided for basking.