identical rate of Ca^{2+} efflux and rate of glycolysis to that observed for halothane nonreactors. With the exception of this single misclassified pig, all the Ca^{2+} efflux rates correlated very closely with the halothane test in that high Ca^{2+} efflux rates were only observed with halothane reactors, which also showed rapid glycolysis post-mortem. The values of the high and low Ca^{2+} efflux rates were also highly significantly different ($p < 0.001$)[19].

The rates of Ca^{2+} efflux correlate very closely with parameters associated with porcine stress and malignant hyperthermia syndromes. The figure illustrates the relationship of Ca^{2+} efflux rates to lactate (A) and to drip (B) produced postmortem; the correlation coefficients were 0.91 and 0.88 respectively. The Ca^{2+} efflux rates were also directly related to the rate of hydrolysis of creatine phosphate and of adenosine triphosphate (ATP). High Ca^{2+} efflux rates were only observed in LD muscles showing rapid rates of hydrolysis of both creatine phosphate and ATP, and low efflux rates with slow rates of creatine phosphate and ATP hydrolysis (unpublished data). Ca^{2+} efflux rates also correlated very closely with the quality of the carcasses in that PSE was only observed with LD muscles showing high efflux rates, and normal pork with muscles having low efflux rates.

Various methods have been reported for predicting porcine stress syndromes, based on either serum enzyme analysis[20,21], blood grouping[22,23], muscle biopsy analysis of metabolites[24] or halothane screening[24-27]. The reliability and sensitivity of Ca^{2+} efflux measurements with murexide was stringently tested and shown to correlate very closely with parameters associated with PSE and malignant hyperthermia syndromes. Measurement of Ca^{2+} efflux rates does not have the limitation of misclassification observed with halothane screening, though the latter method is less complicated than Ca^{2+} efflux measurements. However, the biochemical procedure for assessing porcine stress-susceptibility is in its infancy and no doubt could be simplified in the very near future. The results presented in this paper were carried out using post-mortem samples. Preliminary experiments showed that similar Ca^{2+} efflux rates were observed using biopsy and post-mortem samples. If the procedure for obtaining biopsy samples could be simplified, Ca^{2+} efflux measurements offer a very sensitive and reliable method for differentiating porcine stress-susceptibility and could probably be applied in breeding programmes to eliminate the undesirable porcine stress syndromes.

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Evidence for dissociation of ferrihemoglobin by poly-L-lysine

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Summary. Circular dichroism and absorption spectra of ferrihemoglobin were shown to be altered upon binding with poly-L-lysine at alkaline pH. When ferrihemoglobin immobilized to Sepharose gel was treated with poly-L-lysine, hemoglobin subunits were released from the gel. These results suggest that ferrihemoglobin was dissociated into subunits by poly-L-lysine.

Poly-L-lysine (PLL) is a polyelectrolyte which has often been used as a model compound for studies on interaction with proteins or enzymes. We have recently found that PLL inhibited the activity of α-ketoglutarate dehydrogenase complex purified from pig heart[3,4]. The inhibition seemed to be caused by interference with interaction between constituent enzymes[3] and by dissociation into them with an excess amount of PLL[4]. It is interesting to see whether this is a specific reaction occurring only with α-ketoglutarate dehydrogenase, since this seemed, to our knowledge, to be the first example of a polyaminoacid dissociated subunit enzyme. In the present study, another oligomeric protein, hemoglobin, was treated with PLL and it was found that PLL interacted also with hemoglobin at alkaline pH and dissociated it into subunits.

Materials and methods. Human oxyhemoglobin was pre-

pared from fresh blood as described by Tyuma et al.[5]. Ferrihemoglobin was obtained by the addition of solid potassium ferricyanide to the oxyhemoglobin and freed from the oxidant by passage through a Sephadex G-25 column. PLL-HBr with average mol. wt 30,000 was purchased from Sigma Chemical Co. Sedimentation analyses were carried out using a Hitachi model 282 ultracentrifuge equipped with a photoelectric absorption scanner[6,7]. The circular dichroism (CD) spectra measurements were made at 25 °C with a JASCO J-40C automatic recording spectropolarimeter. To obtain an immobilized ferrihemoglobin, about 55 mg of ferrihemoglobin were added to 1 g of CNBr-activated Sepharose 4B gel (Pharmacia Chemicals). The mixture was stirred gently at room temperature for 2 h, and then the gel was washed successively with 0.1 M ethanolamine-HCl (pH 7.0), 0.3 M NaCl and water.

Results and discussion. The addition of PLL to ferri- and oxyhemoglobin at pH 8.8 resulted in a decrease in $S_{20,w}$ of hemoglobin from 4.37 to about 2.8, suggesting the formation of a complex. At neutral pH and at high concentration of salt, the sedimentation velocity of hemoglobin was scarcely affected. These facts suggest that the binding is mainly due to electrostatic interaction. Under the same conditions, the effect of PLL on CD and absorption spectra in the visible region of ferrihemoglobin was investigated. The most interesting features induced in CD-spectrum by PLL were the conversion of band at around 395 nm from the negative absorption to the positive one and a decrease in absorbance at Soret band (figure 1). The difference spectrum of ferrihemoglobin between the absence and the presence of PLL showed positive bands at 405, 500 and 635 nm (figure 2). The difference was again small at neutral pH. These changes in the CD and absorption spectra caused by mere electrostatic binding seem to suggest that PLL dissociated ferrihemoglobin into subunits, because they were also observed when hemoglobin was dissociated into α and β chains by p-mercuribenzoate treatment[8] or into $\alpha\beta$ dimers by the formation of hemoglobin-haptoglobin complex[9]. As to the mechanism for the dissociation, the following possibility may be considered; an electrostatic interaction between the basic polymer and the protein might neutralize electric charge on PLL molecule and thereby lead to a structural change in the polymer, which subsequently could weaken a noncovalent bonds between the subunits of the protein and eventually dissociate the protein. A structural change in PLL after binding to protein[10], alkylsulfates[11] and heparin[12] has been reported.

To obtain a more direct evidence for dissociation, hemoglobin was coupled to a Sepharose gel and it was tested whether hemoglobin subunits were released into the supernatant. Figure 3 shows clearly that hemoglobin subunits dissociated from the gel by the incubation of immobilized ferrihemoglobin with PLL. The rate of release was, however, rather slow. This might be due to a conformational change in the hemoglobin molecules caused by their binding, through 1- or multi-point, to the Sepharose gel, which

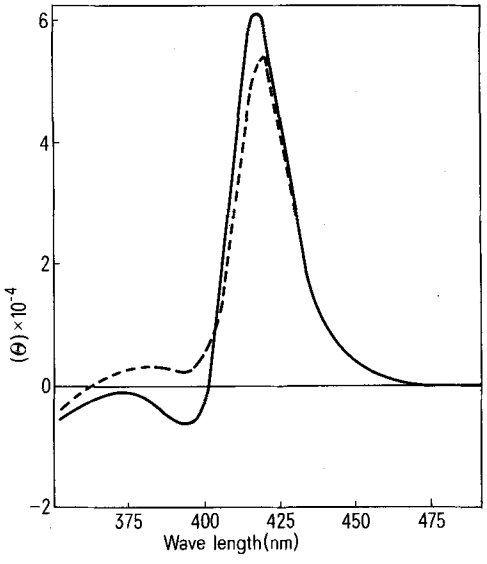

Fig. 1. Effect of PLL on the CD-spectra of ferrihemoglobin in the visible region. The CD-spectra were measured at a ferrihemoglobin concentration of 0.3 mg/ml in 50 mM Tris-HCl (pH 8.8) in the absence (straight line) and the presence of 3.0 mg/ml of PLL (dotted line).

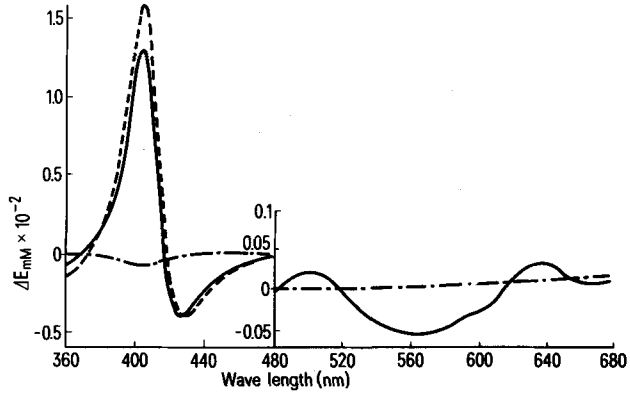

Fig. 2. PLL induced difference spectra of ferrihemoglobin. The sample and reference cuvettes both contained 1.81×10^{-5} M in heme) ferrihemoglobin in 50 mM Tris-HCl (pH 8.8). Difference spectra were obtained after the addition of 0.5 mg/ml of PLL to the reference cuvette (——). (- - -) spectrum after 60 min; (— · — · —) base line.

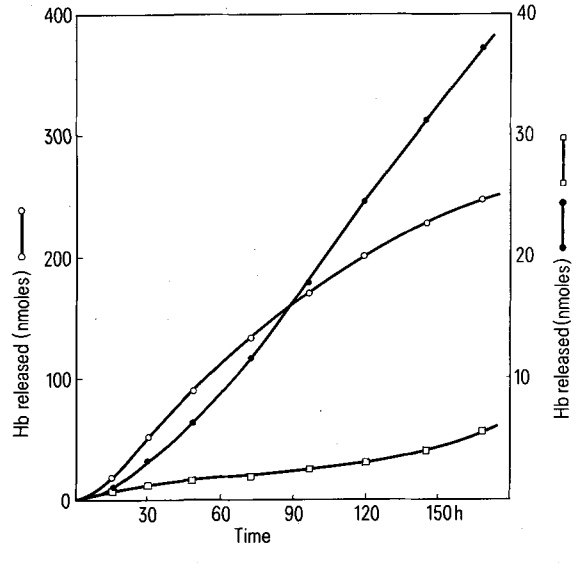

Fig. 3. Release of ferrihemoglobin subunits from Sepharose-ferrihemoglobin by PLL. Ferrihemoglobin was coupled to the Sepharose gel as described in the 'Materials and methods'. The coupled gel was divided into 3 portions in the test tubes and treated with 1. 0.1 M Tris-HCl (pH 8.8), (—□—), 2. PLL 10 mg/ml in 0.1 M Tris-HCl (pH 8.8) (—●—), 3. 0.2 M glycine buffer (pH 10.5) (—○—). At the time indicated in the figure, the supernatants were obtained by centrifugation at 1000 rpm and the hemoglobin contents were determined by the measurements of absorbancy at 405 nm.

in turn strengthened in some way associations among subunits. This inference was drawn from the fact that the release was also retarded when the immobilized ferrihemoglobin was treated with the buffer of pH 10.5, which is otherwise very efficient for the dissociation of native hemoglobin[13].

Recently Amiconi et al.[14] has reported that macromolecular polyanions, dextran sulfate and heparin, were strongly bound by hemoglobin and that their complexes existed as octamer. A primary interaction has been described to be electrostatic also in this case, but resulting complexes were quite different from those of hemoglobin-PLL. Thus it may be said that polyanion and polycation produce a distinct effect on molecular assembly of hemoglobin molecule.

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γ-Glutamyltranspeptidase (GGTP) and cytochrome P-450 after portacaval shunt in the rat

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Summary. The increased hepatic activity of γ-glutamyltranspeptidase after portacaval shunt is due to derepression of a fetal enzyme rather than to an induction mechanism.

End to side portacaval shunt (PCS) in the rat leads to an increase of GGTP activity in the liver; under normal conditions it is rather low[1]. High levels of hepatic GGTP activity, similar to those after PCS, have been observed during fetal development[2]. This increase has been interpreted as a sign of functional changes occurring in the liver and leading to a reappearance of the fetal enzyme[2]. It has been speculated that the underlying mechanism may be associated with the derepression of an enzyme normally present only in fetal and neonatal liver.

Since GGTP is located mainly in the microsomal fraction of the liver, it might be stimulated by different pharmacological agents[3]. It has never been shown, however, whether the increase of GGTP after PCS may not be due to enzyme stimulation or induction rather than to derepression of a fetal enzyme. We therefore also examined PCS rat livers for cytochrome P-450, an enzyme known to be induced by many pharmacological agents, as is GGTP. We wanted to see if the functional changes after the shunt operation might have an effect on cytochrome P-450 content similar to that on GGTP activity.

Material and methods. The PCS experiments were carried out on a strain of SPF Sprague-Dawley male rats bred in a closed colony. Free access to standard food (Altromin R 300) and water was allowed. Coprophagy was not prevented. The general conditions for maintenance of the animals and the techniques of portacaval shunting and of sham operation have been described earlier[4]. 10, 20 and 30 days after PCS the animals were killed by decapitation. The liver was removed and processed immediately. The

Activity of γ-glutamyltranspeptidase (GGTP) and amount of cytochrome P-450 in liver of control rats and in 3 groups of animals 10, 20 and 30 days after portacaval shunt (PCS) operation

Enzymes	Sham operated controls (n = 10)	Days after PCS 10 (n = 8)	20 (n = 4)	30 (n = 7)
GGTP				
nmoles/min/g liver	112 ± 29	329 ± 39	386 ± 118	613 ± 188
		p < 0.001	p < 0.001	p < 0.001
nmoles/min/mg microsomal protein	2.3 ± 0.6	6.4 ± 0.8	7.9 ± 2.4	14.3 ± 4.4
		p < 0.001	p < 0.001	p < 0.001
Cytochrome P-450				
nmoles/g liver	27 ± 11	11 ± 6	14 ± 2	13 ± 6
		p < 0.005	p < 0.05	p < 0.01
nmoles/mg microsomal protein	0.6 ± 0.2	0.2 ± 0.1	0.3 ± 0.06	0.3 ± 0.13
		p < 0.001	p < 0.01	p < 0.005

Each value represents the mean ± SD of the number of animals (n). p-Values indicate significance versus sham-operated controls.