one half of the unchecked production of PG respectively. I thus proved to be the strongest inhibitor of PG synthesis in vitro.

In the case of the partial destruction of membrane structures in the liver which occurs following organic diseases or intoxications, increased quantities of C20 fatty acids are released by lipolysis, which leads, amongst other things, to the increased activity of PG synthetase. Silymarin can counteract this deleterious process. The suppression of the (pathological) decomposition of membrane lipoids together with the inhibition of PG formation could provide a plausible explanation for the way in which this hepatotropic complex functions.

1 F. Fiebrich, Thesis, University of Vienna 1977.
2 H. Koch, Dt. ApothZtg *118*, 1844 (1978); reported in part at the Colloquium Pharmaceuticum in Münster, 21st November 1978.
3 G. Hahn, H.D. Lehmann, M. Kürten, H. Uebel and G. Vogel, Arzneimittel-Forsch. *18*, 698 (1968). - G. Vogel, W. Trost, R. Braatz, K.P. Odenthal, G. Brüsewitz, H. Antweiler and R. Seeger, Arzneimittel-Forsch. *25*, 82, 179 (1975). - R. Braatz and C.C. Schneider, eds: Symposium on the Pharmacodynamics of Silymarin. Urban & Schwarzenberg, München-Berlin-Wien 1976.
4 H. Wagner, P. Diesel and M. Seitz, Arzneimittel-Forsch. *24*, 466 (1974).
5 H.M. Rauen, H. Schriewer, U. Tegtbauer and J.F. Lasana, Experientia *29*, 1372 (1973); Arzneimittel-Forsch. *23*, 145 (1973). - H. Schriewer and H.M. Rauen, Arzneimittel-Forsch. *26*, 235 (1976).
6 A. Bindoli, L. Cavallini and N. Siliprandi, Epatologia *23*, 219 (1977); Biochem. Pharmac. *26*, 2405 (1977); Pharmac. Res. Commun. *10*, 133 (1978).
7 E. Castigli, I. Mezzasoma, D. Marini and G. Porcellati, Annali Fac. Med. Chir. Univ. Perugia *67*, 15 (1976). - E. Castigli, I. Montanini, R. Robert and G. Porcellati, Pharmac. Res. Commun. *9*, 59 (1977). - I. Montanini, E. Castigli, U.G. Arienti and G. Porcellati, Farmaco, ed. sci. *32*, 141 (1977).
7a F. Fiebrich and H. Koch, Experientia *35*, 1548 (1979).
8 A. Greimel and H. Koch, Experientia *33*, 1417, 1568 (1977); Planta medica *32*, 323, 331 (1977); Biochem. Physiol. Pflanzen *173*, 91 (1978).
9 E.D. Wills, Biochem. J. *99*, 667 (1966).
10 J. Robak and B. Sobanska, Biochem. Pharmac. *25*, 2233 (1976).
11 M. Hamberg and B. Samuelsson, J. biol. Chem. *242*, 5336, 5344 (1967); Proc. natl Acad. Sci. USA *70*, 899 (1973), *71*, 3400 (1974). - M. Hamberg, J. Svensson, T. Wakabayashi and B. Samuelsson, Proc. natl Acad. Sci. USA *71*, 345 (1974). - M. Hamberg, J. Svensson and B. Samuelsson, Proc. natl Acad. Sci. USA *71*, 3824 (1974), *72*, 2994 (1975). - C. Malmsten, M. Hamberg, J. Svensson and B. Samuelsson, Proc. natl Acad. Sci. USA *72*, 1446 (1975).
12 H. Bekemeier, A.J. Giessler and R. Hirschelmann, Pharmazie *29*, 671 (1974).
13 G.P. Lewis and P.J. Piper, Nature *254*, 308 (1975).
14 F.A. Kuehl, J.L. Humes, R.W. Egan, E.A. Ham, G.C. Beveridge and C.G. Van Arman, Nature *265*, 170 (1977).
15 J.R. Vane, Nature New Biol. *231*, 232 (1971). - H.J. Robinson and J.R. Vane, eds: Prostaglandin Synthetase Inhibitors. Raven Press, New York 1976.
16 J.R. Vane, Adv. Prostaglandin Thromboxane Res. *2*, 791 (1976).
17 L.H. Rome and W.E.M. Lands, Prostaglandins *10*, 813 (1975). - M. Hemler, W.E.M. Lands and W.L. Smith, J. biol. Chem. *251*, 5575 (1976).
18 J.E. Shaw and P.W. Ramwell, Meth. biochem. Anal. *17*, 325 (1969).

19 We acknowledge the gift of the test compounds by Dr K. Görler from Dr. Madaus & Co., Cologne.

20 H.C. Kiefer, C.R. Johnson and K.L. Arora, Analyt. Biochem. *68*, 336 (1975); Z. analyt. Chem. *279*, 336 (1976).

Causes of artificially high blood glucose values in experiments with diabetic rats and mice

M. Schreiber and F.H. Schmidt

Research Laboratories, Boehringer Mannheim GmbH, Sandhofer Strasse 116, D-6800 Mannheim (Federal Republic of Germany), 20 March 1979

Summary. Unusually high blood sugar values may be caused by the slightest contamination of the blood obtained after puncture of the tail vein by diabetic urine, that has dried onto the tails of the animals. This can be avoided by collecting blood that is allowed to drip from the severed tip of the tail or by carefully cleaning the tail before puncturing the tail vein.

During the course of a long-term study with streptozotocin-diabetic rats we noticed occasional blood sugar values that were very much higher than the usual blood sugar values for such animals and which also were not in accordance with the corresponding glucose excretion in the 24-h urine. In occasional cases, the values measured were more than 1200 mg/dl and thus markedly higher than the values given in the literature for very severe streptozotocin diabetes in the rat[1-4].

The hexokinase technique which was used for the glucose determinations is regarded as the scientific reference method and cannot be considered as the cause of the high blood sugar values because of its very low variability[5-10]. The 'Capiletten' (Labora Mannheim, Mannheim) that are used in our laboratory have an accuracy of ± 1-2% and can therefore also be excluded as a possible cause. Other explanations, such as the chance coincidence of changed eating habits, stress during blood collection, and obtaining blood from different vascular areas, can be excluded because of the size of the deviations. The only remaining possibility was a volume displacement of the plasma space, and this was the subject of our 1st investigations.

Materials and method. In experiment I, a series of blood sugar values and haematocrit values were determined in the same animals (Sprague-Dawley rats siv 50 SPF, Iwanova, Kisslegg). The blood collection was carried out on the one hand by means of puncture of the tail vein with a steel cannula followed by pipetting of 10 µl from the drops of blood that developed on the tail, and on the other by the almost simultaneous cutting off of the extreme tip of the tail (0.5-1 mm). The blood sugar determination was carried out by means of the hexokinase G-6-PDH method (Glucoquant®, Boehringer) in a haemolysate (Hämolyse-Reagenz, Boehringer). The scatter of the blood sugar values was determined in each group and was expressed as the coefficient of variation (table 1).

In order to clarify the results obtained, streptozotocin-diabetic rats were randomised into 2 groups of 10 in a 2nd experiment (II). Before removing blood, the tails of the animals in group A were carefully washed in water at body

Table 1. Coefficient of variation (in percent) of the blood sugar values from blood samples from different collection areas and the corresponding haematocrit values (in vol.-%). A mean of 5(3–8) blood sugar determinations were carried out for each animal and each type of collection. The mean blood sugar value was 500 mg/dl.

Animal	A	B	C	D	E	F	G	H	I	$\bar{X}$
Venous puncture	59.0	41.8	47.5	25.1	24.5	19.8	31.3	36.4	41.6	36.3%
Tail tip	21.0	14.4	15.6	7.0	10.7	8.7	18.4	4.7	12.0	12.5%
Haematocrit	42	45	45	46	45	45	47	46	33	43.8%

Table 2. Mean coefficient of variation (in percent) of blood sugar values after collection of blood from washed and unwashed tails in relation to the source of bleeding and their distance from the site of actual blood collection (a: collected directly at the site of puncture, b: about 1 cm from puncture, c: more than 1 cm from puncture site). An average of n = 6 (3–10) blood collections per animal were undertaken on 5–10 animals.

	Puncture of tail veins			Tail tip cut off
	a	b	c	
Group A: tails washed	4.8 (6 animals with n = 4–7)	5.1 (10 animals with n = 4–9)	6.4 (7 animals with n = 7–10)	3.6 (10 animals with n = 5)
Group B: tails not washed	5.1 (5 animals with n = 3–5)	16.3 (8 animals with n = 4–10)	23.3 (10 animals with n = 3–10)	4.6 (10 animals with n = 5)

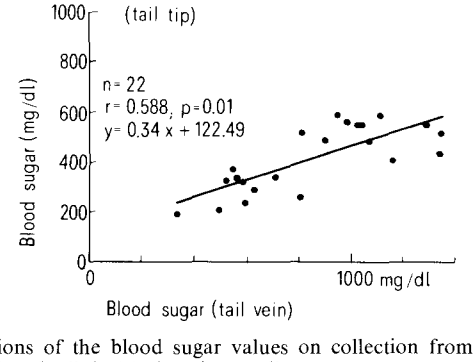
temperature until no further glucose could be detected in the wash water (Glukotest®, Boehringer). The tails of the animals in group B were left untreated. The blood collection itself took place on one occasion by puncture of the tail vein and on another by allowing the blood to drip from the severed tip of the tail. With the venous puncture, a careful note was made of whether the Capilette was placed directly on the site of the puncture (a) or whether the exuding blood had moved about 1 cm only (b) or more than 1 cm (c) before the Capilette was applied (table 2). All the animals, i.e. twice N = 10, were used for the experiments in which blood was collected from the severed tail tip; for the puncture of the tail vein, N was 5–10 with an average of 8. The respective number of blood samples are given in the table with n. The coefficients of variation given are then related to the mean of the respective number N (see also table 2).

Results. Our results (table 1) confirmed the previous observations. It was noticed that the blood sugar values obtained by puncture of the tail vein showed a greater scatter than those from the blood simultaneously obtained from the tail tip. No dependence on the haematocrit could be found; the haematocrit values determined do not show any essential differences from the situation in healthy animals[11]. It may be deduced from our 2nd experiment that the scatter in the blood sugar values found is the result of contamination with dried urine residues on the tails of the diabetic rats.

Discussion. Determinations of blood sugar from blood obtained from the tail veins of diabetic rats and mice, which are frequently carried out in research laboratories, can give implausibly high blood sugar values, as the data from the long-term study by Trapp and Siegel[12] show, too. Our investigations show that this is caused by contamination of the exuding blood by urinary glucose. The contamination can vary from slight to very marked depending on the time that the blood is in contact with the tail and the area covered. There is a correlation between the height of the artificially elevated blood sugar values and the degree of diabetes mellitus, i.e. the urinary sugar concentration or the blood sugar concentration (figure).

A rough calculation may serve to explain the importance of our observation. A contamination of 10 µl blood (blood sugar: 500 mg/dl) – corresponding to 50 µg dissolved glucose – by only 1 µl dried urine (urinary sugar: 16 g/24 h at a urinary volume of 200 ml) – corresponding to 80 µg dissolved glucose – gives a blood sugar value of 1300 mg/dl. In the 2nd experiment we found accordingly in group B (unwashed tails) coefficients of variation which rose from 5.1% to 4.5 times greater, whilst the scatter in group A, with washed tails, rose only slightly.

The following conclusion should be drawn for investigations in diabetic rats and mice. The collection of blood must take place either by venous puncture from tails that are always carefully washed and are free from urinary sugar, or by cutting off the tail tip. Both methods in our investigations gave good correlation of the absolute blood sugar values and a low scatter of the values in the series.



Deviations of the blood sugar values on collection from the tail vein (x) and tail tip (y) given in mg/dl.

1 A. Junod, A.E. Lambert, W. Stauffacher and A.E. Renold, J. clin. Invest. *48*, 2129 (1969).
2 B. Rudas, Arzneimittelforschung *22*, 830 (1972).
3 F.H. Schmidt, E. Siegel and V. Trapp, Diabetologia, in press (1979).
4 E. Siegel, V. Trapp and F.H. Schmidt, Diabetologia *12*, 419 (1976).
5 Mitt. Dt. Ges. klin. Chemie e.V. *1*, 11 (1978).
6 W. Frölke, A. Niggeschulze and H. Stötzer, Z. Versuchstierk. *15*, 342 (1973).
7 R.B. Passey, R.L. Gillum, J.B. Fuller, F.M. Urry and M.L. Giles, Sel. Meth. clin. Chem. *8*, 9 (1974).
8 F.H. Schmidt, in: Diabetes mellitus vol. 2. p. 913. Ed. Pfeiffer. J.F. Lehmanns, München 1971.
9 F.H. Schmidt, Internist *4*, 12, 554 (1963).
10 F.H. Schmidt, Klin. Wschr. *51*, 520 (1973).
11 P.K. Upton and D.J. Morgan, Lab. Anim. and *9*, 80 (1975).
12 V. Trapp and E. Siegel, Dissertation *17* (1975).