cells, recurrent collaterals of the Purkinje cells, granular cell axons and climbing fiber endings increase the synaptic volume more than in the outer part[10,11].

Since SDH in the animal brain appears to be most concentrated in the mitochondria, it is apparent that very strong SDH and DPN-D activity in the glomeruli of the granular layer and the deeper part of the molecular layer of the mature cerebellum, and in the stratum lacunosum and moleculare of the hippocampus is due to abundant mitochondria-containing synapses in these regions. Strong activity of these enzymes in the Purkinje cells of the mature brain may be explained by the numerous axon terminals of the basket cells surrounding the cell body terminating on the axon hillock[10,11].

The development of the synapse can thus be evaluated histochemically by demonstrating these enzymes in the developing brain[12].

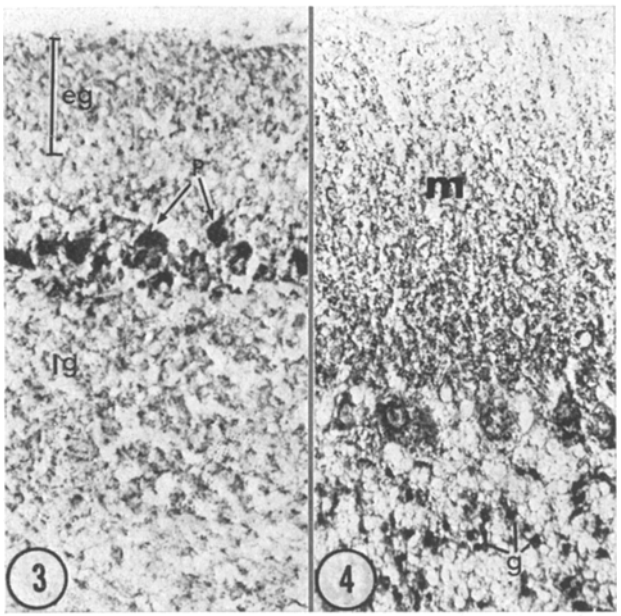

Fig. 3. Cerebellar cortex of a 10-day-old hamster. Immature Purkinje cells (p) show strong DPN-D activity. The external granular layer (eg) and the internal granular layer (ig) are weakly reactive. × 250.

Fig. 4. 20-day-old hamster. Perikaria of the Purkinje cell, deeper part of the molecular layer (m) and glomerulus (g) in the granular layer show intense DPN-D activity. × 250.

Riassunto. La deidrogenasi dell'acido succinico (SDH) e la diaforasi diposforpiridin-nucleotide (DPN-D) hanno dimonstrato attività intensa nei glomeruli delo strato granulare cerebellare, nella parte più profonda dello strato molecolare e nelle lamine lacunosa e molecolare dell'ippocampo. Tutte queste zone sono caratterizzate da numerose connessioni sinaptiche. L'attività degei enzimi ossidanti in queste zone aumenta rapidamente con la crescita degli animali.

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Increased Plasma Fibrinogen and the Release of a Fibrinogen Enhancing Factor in Tumour-Bearing Rats

It is a common observation that fibrinogen is increased in patients suffering from cancer[1,2]. This high level is said to be the cause of the elevated sedimentation rate of cancer patients[3]. Increased fibrinogen levels are also found in acute and chronic inflammatory diseases[4]. In experimental animals an elevated plasma fibrinogen has been described during the growth of a transplanted rat sarcoma[5] and the V2 carcinoma of the rabbit[6]. Systematic fibrinogen determinations in animals bearing autochtonous tumours have, to our knowledge, not yet been published.

In our experimental study in rats fibrinogen determinations were made according to the method of RATNOFF and MENZIE[7]. Blood samples were taken by aortic puncture. For experimental tumours we used the Yoshida sarcoma and the benzypyrene sarcoma.

Forty-five female rats (Wistar strain, 120–150 g) were injected with 10×10^6 carefully washed Yoshida ascites sarcoma cells into the upper thigh. 15 min and every 24 h after the tumour cell injection, 5 animals were bled and fibrinogen determinations were made over a period of 8 days. The results are shown in Figure 1. There is a continuous rise of the plasma fibrinogen level during the 8 days of tumour growth.

In 30 rats of both sexes (250–300 g) with benzpyrene sarcoma the plasma fibrinogen level was correlated with the tumour volume. The tumour volume was estimated by measuring the 2 major diameters of the tumour (d_1 and d_2) parallel to the surface of the animal and the greatest width (d_3) perpendicular to the surface with a Vernier calliper. Based on the assumption that the tumours were hemiellipsoids the volume was calculated

$$V = \frac{(4n/3) \times (d_1/2) \times (d_2/2) \times d_3}{2} = 0.5236 \times d_1 \times d_2 \times d_3.$$

Fibrinogen determinations were performed in tumours of a volume from 1,197 mm³ to 80,028 mm³. Figure 2 demonstrates the spread of the fibinogen values in correlation

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to the tumour volume. It is apparent that only small tumours up to a volume of 3000 mm³ had correspondingly low plasma fibrinogen levels. Above a volume of 3000 mm³ there was no correlation between tumour size and the fibrinogen level.

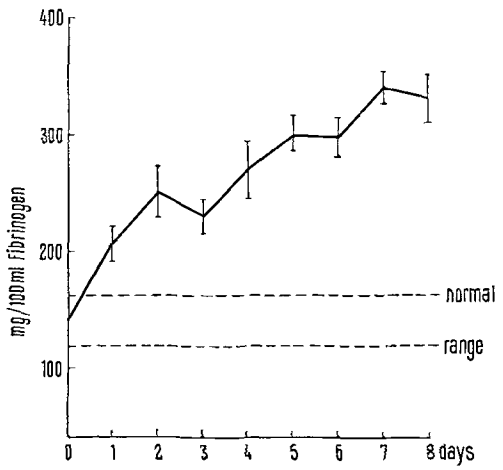

Fig. 1. Plasma fibrinogen during 8 days after transplantation of 10×10^6 Yoshida sarcoma cells.

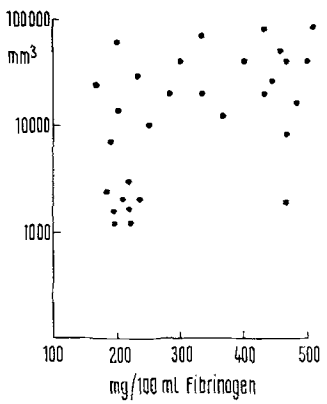

Fig. 2. Fibrinogen levels in benzpyrene sarcoma bearing rats in correlation to the tumour volume.

Table I. Plasma fibrinogen in rats 18 h after i.p. injection of various tissue extracts

	Mean fibrinogen level	Standard deviation
NaCl (control)	148	18
Abdominal muscle	210	25
Croton oil necrosis	394	43
Yoshida sarcoma	366	41
Benzpyrene sarcoma	305	39

Table II. Plasma fibrinogen in rats after a double injection of NaCl, normal rat serum and necrosis serum

	Mean fibrinogen level	Standard deviation
NaCl (control)	148	18
Necrosis serum	340	22
Normal serum	195	19

The intramuscular injection of 0.5 ml croton oil causes a severe necrosis after 2 days at the site of injection, whereas 0.5 ml of a 1:200 croton oil dilution only induces the typical signs of acute inflammation such as edema and flush. Fibrinogen determinations were made 48 h after injection of pure and diluted (1:200) croton oil in 10 animals in each group. The mean plasma fibrinogen level in the first group was 565 mg/100 ml (S.D. 43 mg/100 ml) and in the second group 342 mg/100 ml (S.D. 62 mg/100 ml).

Tissue extracts were made from croton oil necrosis, Yoshida sarcoma, benzpyrene sarcoma and normal rat muscle (abdominal muscle) by homogenating 10 g of tissue with 20 ml 0.9% saline. After centrifugation and filtration 1 ml of the supernatant was injected i.p. into 10 female rats (Wistar strain, 120–150 g). 18 h later a plasma fibrinogen determination was made. The results are shown in Table I. The injection of the necrotic tissue extract caused the highest fibrinogen level.

In another experimental arrangement 2 ml of serum of rats with croton oil necrosis and of normal rats was injected i.p. into 10 female rats (Wistar strain, 120–150 g). Since a single injection did not lead to a significant increase of fibrinogen, 36 h later the same injection was repeated. 72 h after the first injection fibrinogen determinations were made. A control group received 0.9 saline in the same amount. The results are shown in Table II. The elevation of the fibrinogen level reached a maximum in the rats treated with necrotic tissue extract; the difference is statistically significant ($p < 0.01$).

Our results confirm earlier investigations that an increase of plasma fibrinogen takes place during the growth of experimental rat tumours. In Yoshida sarcoma-bearing rats the plasma fibrinogen level is directly proportional to the tumour age. A correlation between the tumour volume and the plasma fibrinogen in benzpyrene sarcoma-bearing rats could only be found in the very small tumours. It could be shown that this phenomenon is not tumour-specific and is rather dependent upon the extent of necrosis present. The fact that extracts of necrotic tissue provoke an imminent rise in plasma fibrinogen when injected i.p. suggests the existence of a fibrinogen enhancing factor in the necrotic tissue. This factor must also be present in the blood, as shown by the i.p. injection of serum from animals bearing a croton oil necrosis. WINKERT et al.[8] have shown that normal rat serum is able to produce a slight elevation of the plasma fibrinogen when injected into normal healthy rats. These data could also be confirmed by our experiments. Whether the fibrinogen enhancing factor released from necrotic tissue is the same as that present – in a smaller concentration – in normal serum, or if it is another serum component, remains unknown[9].

Zusammenfassung. Die Freisetzung eines die Fibrinogensynthese stimulierenden Faktors aus nekrotischem Gewebe in die Blutbahn ist für den Anstieg des Plasmafibrinogens bei experimentellen Rattentumoren verantwortlich.

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