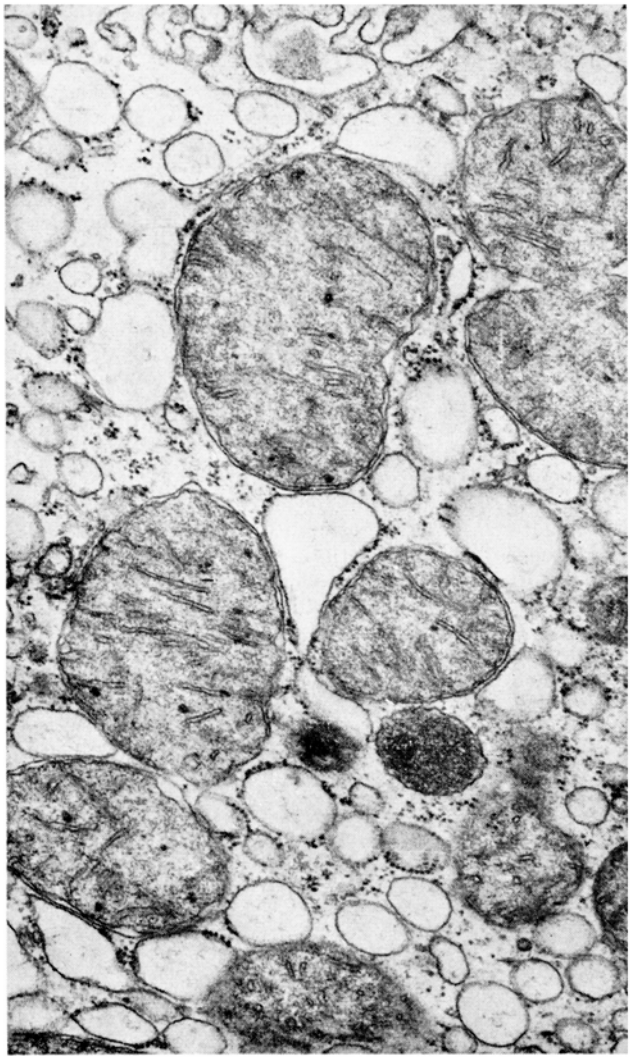

ascribed to the toxic action itself and seem to be linked to the reorganization of the cell. The behaviour of alkaline phosphatase is difficult to interpret: a cholostasis phenomenon does not seem a sufficient explanation.

These serial investigations point to some conclusions: in our experimental conditions the early evidence of structural damage from total extract of *A. phalloides* Fr. occurs in the nucleus and nucleolus and it is in these structures that the changes begin to regress; the signs of damage of the cytoplasm and its regression occur after nuclear changes. The reversibility of hepatic cell damage in the rat varies with the dose given, for there is gradual normalization of the intracellular organelles and enzymatic activity.

Riassunto. Nel ratto intossicato con estratto di *Amanita phalloide* le prime alterazioni strutturali dell'epatocita si realizzano a livello del nucleo e del nucleolo con formazioni di «nucleolar caps». Da queste stesse strutture iniziano i fenomeni di regressione delle alterazioni. Le manifestazioni sia del danno che della sua regressione a livello citoplasmatico risultano essere successive a quelle nucleari. Il comportamento del DNA e di alcuni enzimi del tessuto epatico è risultato nel complesso coerente con le alterazioni morfologiche. In rapporto con la dose usata queste alterazioni risultano reversibili poichè nelle cellule colpite entro 12 h cominciano i fenomeni di ristrutturazione.

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9 October 1967.

Fig. 4. 12 h after the poisoning. Depletion of glycogen granules. Disorganization of granular endoplasmic reticulum with dilatation of cisternae and loss of ribosomes. OsO_4 fixation. × 31.000.

Hypercoagulability and Thrombocytopenia After Platelet Factor 4 Infusion into Rabbits

Purified platelet factor 4 (PF_4) possesses several biological activities: it neutralizes heparin, it precipitates a solution of purified fibrinogen and it induces non-enzymatic clotting of soluble fibrin monomer complexes (paracoagulation). The latter reaction is strongly inhibited by citrate, oxalate and EDTA. PF_4 is rapidly released during platelet aggregation induced in platelet-rich plasma (PRP) by ADP, thrombin, adrenaline and collagen[1,2]. It is also released in vivo following thrombin infusion into rabbits[3]. PF_4 does not aggregate platelets in PRP, which is probably due to the inhibitory effect of citrate, and it only enhances platelet aggregation by ADP. For this reason it seemed of interest to study the effects of PF_4 in vivo.

PF_4 was isolated from pig platelets according to FARBISZEWSKI et al.[4]. The final product dissolved in 0.9% NaCl contained 160 μg protein/ml. In a dilution of 1:8 it shortened the heparin thrombin time of rabbit platelet-poor plasma from 75–26 sec. Purified PF_4 solution at a dose of 5 ml/rabbit was injected i.v. into the marginal ear vein of 5 rabbits (1.5–2 kg weight). Blood samples were obtained by heart puncture before and 5, 15 and 45 min after the injection. Plastic clotting time, platelet count, fibrinogen level, 'stypven' (Roussel viper venom, Borrough and Welcome, London) clotting time, and thrombin time were determined using routine laboratory methods. Platelet adhesiveness was estimated according to HELLEM[5]. Moreover, PF_4 was determined in platelet-poor plasma by

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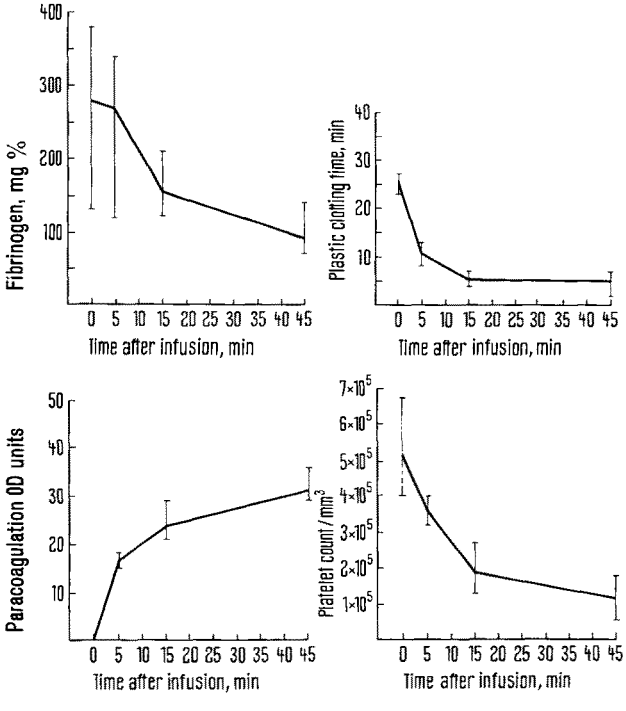
measuring heparin thrombin time[6] and by the so-called 'paracoagulation' method[1]. This method consists of recording the optical density (OD) increase of a solution of fibrin monomer complexes after mixing with the tested plasma which had previously been heated and dialysed.

The Figure shows that there is a rapid drop in platelet count, a considerable shortening of the plastic clotting time and a decrease of fibrinogen level after PF_4 injection into rabbits. At the same time OD increases in the 'paracoagulation' test, thus indicating the presence of platelet factor 4 in the circulating plasma. The Table shows a shortening of the heparin-thrombin and stypven times and an elevation of factor V activity, the thrombin time remaining unchanged. At the same time the adhesive platelet count increases. Four rabbits, injected with PF_4, died within $2^1/_2$ h after the infusion, one rabbit survived 20 h.

Contrariwise, the i.v. injection of Protamine sulphate (Roche) – a well-known antiheparin and paracoagulating agent – in a dose of 25 mg/rabbit caused prolongation of the plastic clotting time and did not affect platelet count and fibrinogen level in 5 animals investigated. All the rabbits survived. This observation emphasizes a specifity of PF_4 action in vivo. The infusion of saline into 2 rabbits was without any effect.



Effect of platelet factor 4 infusion in vivo. Mean values from 5 rabbits.

Effect of platelet factor 4 infusion in vivo

Time after infusion, min	Thrombin time, sec	Heparin-thrombin time, sec	Stypven time, sec	Factor V sec	Adhesive platelets, %
0	15 (14–15)	45 (42–47)	95 (80–105)	24 (20–25)	27 (18–38)
5	14 (14–15)	30 (27–35)	47 (35–54)	20 (16–23)	33 (18–47)
15	14 (14–15)	29 (32–22)	45 (29–55)	22 (15–23)	41 (28–60)
45	14 (14–15)	30 (37–27)	48 (35–61)	18 (16–19)	44 (29–62)

Mean values from 5 rabbits. Ranges of variations are indicated in brackets.

PF_4 added to citrate plasma did not cause any significant modification of the clotting tests except a slight shortening of the stypven clotting time.

It can be postulated that the infusion of PF_4 causes an intravascular aggregation of platelets followed by an increased availability of platelet factor 3 (PF_3) on the cell surface. This is indicated by the acceleration of the stypven clotting time[7]. Damage to the platelets and increased reactivity of PF_3 may trigger intravascular coagulation reflected by the activation of factor V and shortening of the plastic clotting time. A decrease of fibrinogen level may be due to the intravascular formation of fibrin or to a direct effect of PF_4 on fibrinogen. The level of PF_4 in blood plasma as tested by the 'paracoagulation' method continuously increases over a 45 min period. It is probable that the new portions of this factor are released from aggregated platelets, thus leading to the potentiation of the described effects.

Degranulation and release reactions may occur in vitro during platelet aggregation as well as in vivo in various pathological conditions related to intravascular blood clotting. THOMAS et al.[8] demonstrated that the release of serotonin from platelets is responsible for many harmful and even fatal effects in the course of pulmonary embolism. According to other authors[9] release of PF_3 by *E. coli* endotoxin is observed in the SANARELLI-SCHWARTZMAN phenomenon. It can be suggested that the release of PF_4 in vivo represents an important step in the pathogenesis of the defibrination syndrome or of diffuse intravascular clotting.

It seems of interest to compare the effects of ADP and of PF_4. The former substance, a highly specific agent aggregating platelets in vitro, produces an inconspicuous and temporary drop in platelets if infused i.v.[10,11]. Very large non-physiological doses of ADP are needed to produce thrombosis in experimental animals[12]. On the other hand, it is difficult to reveal the true effects of PF_4 in vitro, possibly due to the inhibitory effects of citrate. However, this substance is a potent and specific agent triggering platelet aggregation and blood clotting in vivo.

Résumé. Le facteur plaquettaire 4 injecté aux lapins par la voie i.v. cause un abaissement rapide de taux de fibrinogène et de nombre de plaquettes, le racourcissement de temps de coagulation et l'augmentation de plaquettes adhésives.

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