REVIEW ARTICLE

INFLAMMATION AND THE INFLAMMATORY MECHANISMS*

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During the past decade research concerning the mechanism of inflammation was focused mainly on the so-called mediators of the inflammatory tissue reaction. Numerous studies have been carried out, especially on rats, in which oedema-producing agents were employed, and the aetiology as well as the pharmacology of the resultant inflammation has been extensively investigated. Mainly as a result of these experiments it is generally believed that under the influence of inflammatory agents, vasoactive substances like histamine, 5-hydroxytryptamine (5-HT) or polypeptides are released or produced in the tissues and that actually it is these substances that induce the vascular inflammatory reaction. Recently published reviews of Feldberg (1956), Paton (1957) and Spector (1958) reflect impressively the progress in the elucidation of inflammatory processes achieved through this line of research.

In the present review, however, I wish to discuss new points of view concerning this topic. In our experiments made in the past few years we detected close relations existing between inflammatory processes and blood coagulation. On the basis of the results obtained we are convinced that some kind of a clotting process is involved in the mechanism of inflammation, and plays a decisive rôle in the causation of the inflammatory symptoms.

Before I begin to describe our own findings and the new concept based on them I should like to describe the method used.

We have developed, and described (Jancsó, 1960) a novel method for the visualisation of inflammatory reactions in the tissues of rats. Immediately before or after application of inflammatory stimuli, colloidal silver is injected into the blood stream, whereby the parts of the body involved in the inflammation process turn brown. We employed colloidal silver (Pharmacopoea Hung. V) in 1 per cent solution rendered isotonic with 5 per cent glucose. In most experiments 10 ml./kg. of this solution was injected into the tail vein. Whereas, Evans blue and other dyes used in inflammation research produce only a diffuse patch of colour, with colloidal silver a brilliant microscopic picture can be obtained which reveals the finest details of localisation.

Let us first consider one or two examples which illustrate how the silver introduced into the circulation is deposited in the inflamed tissues. Two different types of localisation can be observed. They may be clearly distinguished in Fig. 1 which shows a part of the mesenterium. Before the silver was injected 0·4 ml. of a 6 per cent kaolin suspension was injected into the peritoneal cavity to induce a violent inflammatory

* Based upon a Special University of London lecture given at King's College, London, on May 18, 1961.

reaction. After the injection of the silver colloid a large quantity of silver is seen to be bound by the tissue which forms brown coloured coatings on the internal surface of the minute and intermediate veins. At other sites, for instance in the subepidermal layer of the ear the silver

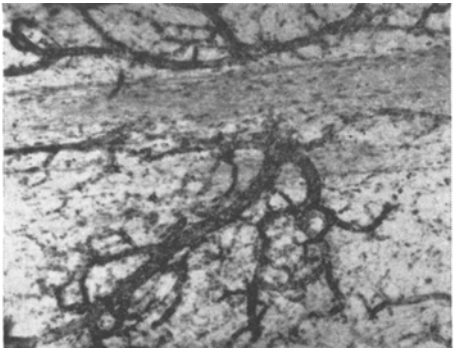


FIG. 1. Deposition of colloidal silver in the mesenterium of the rat subsequent to an intraperitoneal injection of 0·4 ml. of a 6 per cent kaolin suspension. Unstained stretch preparation.

coating may extend to the capillary walls, however, the site on which it mostly forms is the wall of the small venules. In the area of inflammation such coatings may develop within a few minutes.

In addition another form of localisation may also be distinguished on the photomicrograph. All histiocytes of the connective tissue are

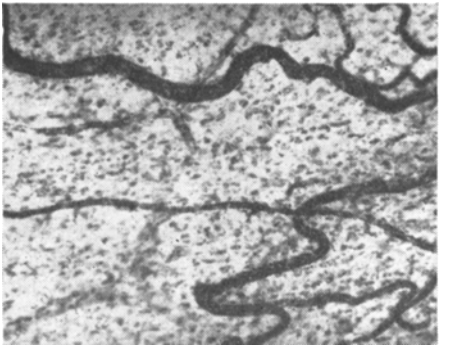


FIG. 2. Fixation of intravenous injected colloidal silver in the plantar skin of the rat paw after subplantar injection of 6 μg. of 5-HT. "Angiotaxis" and extravascular storage in histiocytes is seen.

crowded with silver granules proving that, owing to the inflammation, the vessel walls have become pervious to large colloidal particles, of about 200 Å diameter. In all probability this escape of the colloidal substance may be explained by the assumption that the inflammatory process opens large pores in the vessel wall through which the colloidal particles are driven out towards the surrounding tissue by hydrostatic forces. At

first only a diffuse brown imbibition could be observed in the tissue. It may last for 1–2 hr. till the histiocytes engulf the silver.

An essentially similar picture is obtained with other inflammatory substances also. Fig. 2 demonstrates the vascular and cellular silver deposition in the skin of the rat paw after subplantar injection of 6 μg. of 5-HT. The same picture is obtainable after local application of 10–20 μg. of compound 48/80. Fig. 3 shows the effect of 6 μg. of the venom of *Vipera aspis* in the plantar skin.

This coating of the internal vessel walls with colloidal material is identical with the phenomenon which I described years ago under the name "endothelial activation" (Jancsó, 1941, 1947, 1955). At the time I reported that after local or systemic administration of histamine the

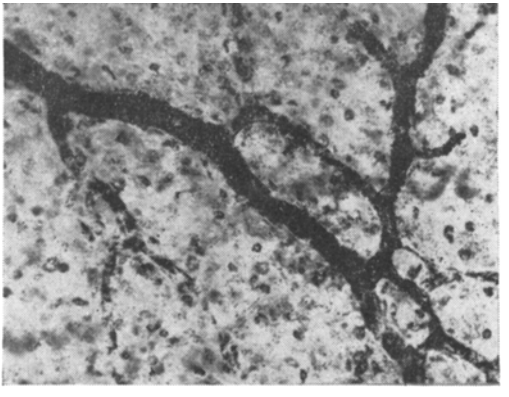


FIG. 3. Deposition of intravenous injected colloidal silver in the plantar skin of a rat after subplantar injection of 6 μg. of *Vipera aspis* venom.

vessels undergo a characteristic change which manifests itself by the deposition of the circulating Indian ink on the internal surface of the small veins. I also established (Jancsó, 1947) that this phenomenon can be inhibited with antihistamines, furthermore, that it also appears after the application of different histamine liberators. In addition, I also established that the endothelial cells soon engulf the carbon particles adsorbed on their surface and store them in granular form, that is to say, in this instance the endothelial cells fulfil a function which otherwise is a privilege of cells belonging to the reticulo-endothelial system. Some hours or days later the engulfed material appears in the surrounding tissue stored in phagocytic cells; by the intervention of some mechanism as yet incompletely understood the carbon particles emerge from the blood-vessels.

After my original report numerous authors interested themselves in this phenomenon (Benacerraf, McCluskey and Patras, 1959; Biozzi, Menè and Óváry, 1948; Gözsy and Kátó, 1957; Maltoltsy and Maltoltsy, 1951; Selye, Lemire and Cantin, 1959; Törö, 1942; Zemplényi, Fodor and Lojda, 1960). Besides the term "endothelial activation" the designations "endothelial colloidopexis" and "angiotaxis" were suggested.

This latter name proposed by Selye and others (1959) is perhaps the most adequate term. Till now attention has been focused chiefly on the phagocytic activity of the endothelium and on its possible rôle in the defence against infections (Gözsy and Kátó, 1957) or in the localisation of disease in the body (Benacerraf and others, 1959), respectively. In other papers the inhibitory action of antihistamines, phenothiazine derivatives and cortisone on this phenomenon was studied. Alksne (1959) confirmed with the electron-microscope that histamine prompts endothelial cells to take up colloidal particles from the blood stream.

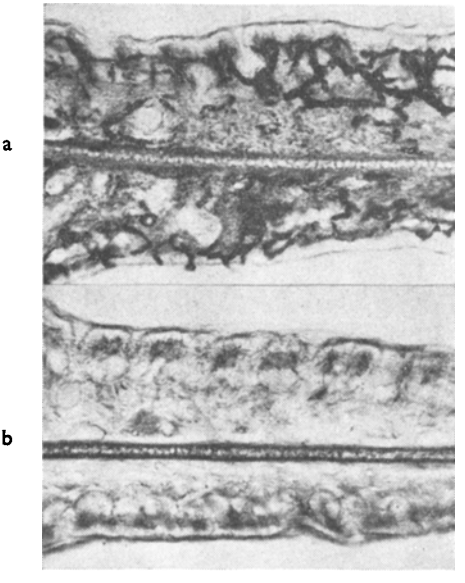


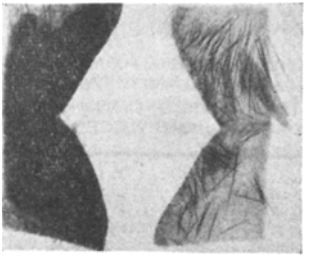
FIG. 4. a. Fixation of colloidal silver in the ear of a normal rat after painting with xylol; b. ear of a rat pretreated with Thrombodym 250 mg./kg. intravenously. No silver deposition after the application of the inflammatory stimulus.

At the moment, however, almost nothing is known about the mechanism of the angiotaxis phenomenon. Why do colloidal particles attach themselves to the vessel wall? What is the real cause of this conspicuous change in the properties of the vascular wall? In my book *Speicherung* published in 1955 (Jancsó, 1955) I put forward the hypothesis that angiotaxis is caused by a clotting process taking place in the vascular wall. The monomeric or low polymeric soluble fibrin produced during this coagulation process reacts immediately with the circulating colloidal particles forming a coloured precipitate on the internal surface of the vessels. Thus, in terms of this hypothesis, the coating on the inflamed vessel walls consists of silver and fibrin. (See also Jancsó and Jancsó-Gábor, 1960; Jancsó, 1960.)

It was tempting to consider such an explanation because an outstanding property of fibrinogen is that it adsorbs colloidal particles intensively in

the course of its coagulation (Jancsó, 1955). It is easy to show that coloured clots are formed if, after the injection of colloidal silver or carbon, an intravascular coagulation is induced by thrombin or any other coagulant. The microscopic picture of angiotaxis is also consistent with the coagulation-hypothesis since, besides typical parietal coatings, coloured precipitates and plugs can be detected in the inflamed vessels. Such findings suggest that some kind of precipitation takes place in the inflamed vessels.

To test our conception we made experiments with various anticoagulants. Surprisingly, heparin proved to be ineffective, but with several other anticoagulants important positive results were obtained (Jancsó and Jancsó-Gábor, 1960; Jancsó, 1960). Thus the expected effect



a b

FIG. 5. Silver deposition in the conjunctiva after instillation of a 0·5 per cent capsaicin solution. Transparent eyelid preparations; (a) from a control rat, (b) from a rat pretreated with Thrombodym, 220 mg./kg. intravenously.

could be achieved with compounds containing rare earth metals, as well as with sodium polyanetholesulphonate (Liquoid) and also suramin. These compounds were found in our experiments to exert a primary and specific inhibitory effect upon the process of angiotaxis. Moreover, these anticoagulants are able to inhibit the gross increase of vascular permeability caused by inflammatory agents. In this way, they prevent the escape of the colloidal silver particles from the terminal vascular bed and also much diminish the extent of oedema formation. Rare earth metals such as lanthanum, cerium, neodymium, praseodymium and samarium proved to be effective even in the form of their inorganic salts. In most experiments, however, we used the commercial preparations Helodym 88 (the didymium salt of β-acetylpropionic acid) or Thrombodym (the neodymium salt of sulpho-isonicotinic acid). Fig. 4 shows transverse sections of xylol painted ears of rats illustrating the characteristic effect of rare earths. Colloidal silver, 100 mg./kg., was injected into a rat and its ear was painted immediately afterwards for 10 sec. with xylol. Another animal was treated before this procedure with 250 mg./kg. of Thrombodym. The animals were killed 3 hr. later. Whereas in the ear of the control an intense accumulation of silver can be seen in the vessels and in histocytes, in the ear of the pretreated animal, silver deposits are absent. Likewise, the silver deposition in the conjunctiva which follows

the instillation of a strong, 0·5 per cent, capsaicin solution into the eye could be also totally prevented by a high dose of Thrombodym or Helodym 88 (Fig. 5).

A similar preventive effect could be established in inflammations induced by subplantar injection of various other agents, for example, compound 48/80, 5-HT, dextran, kallikrein, staphylococcus toxin, bee venom and diverse snake venoms. In these experiments, some plugs could often be detected in the minute vessels, but no typical angiotaxis or extravascular storage occurred.

In further experiments it could be shown that these anticoagulants are also able to inhibit the development of oedematous swelling to a great extent. Table I shows the effect of seven different oedema-producing agents on the rat paw and their inhibition by rare earths and Liquoid.

TABLE I

INHIBITION BY ANTICOAGULANTS OF OEDEMA FORMATION IN THE HINDPAW OF THE RAT INDUCED BY VARIOUS INFLAMMATORY AGENTS. AMPUTATION AND WEIGHING OF THE PAWS 1 HR. AFTER APPLICATION OF THE INFLAMMATORY AGENT. ALL FIGURES ARE MEANS FOR 10 ANIMALS WEIGHING 120–150 G.

Inflammatory agent	Dose subplantar in 0·1 ml.	Wt. increase of oedema-leg controls per cent.	Inhibition of oedema formation (per cent.)		
			Helodym 88* 9 ml./kg. i.v.	Thrombodym † 2 × 6 ml./kg. i.v.	Liquoid 50 mg./kg. i.v.
Comp. 48/80	10 μg.	51·6	69·0	90·7	80·7
5-Hydroxytryptamine ..	5 μg.	71·6	61·8	—	—
Peptone (Witte)	5 mg.	60·0	79·2	—	80·4
Dextran	0·5 mg.	56·8	—	70·0	—
Kallikrein (Padutin) ..	2 U	57·7	72·5	—	64·1
Saliva (human)	0·1 ml.	67·2	78·2	93·2	68·5
Staphylococcus culture filtrate	0·1 ml.	51·8	76·5	—	69·0

* 1 ml. = 9 mg. didymium metal. † 2·2 per cent solution.

In these experiments, in addition to compound 48/40, dextran, 5-HT and kallikrein, other substances were also used, the oedema-producing effect of which was detected in the course of our experiments. These substances were human saliva, staphylococcus toxin and peptone (Witte). The effect of these agents was much inhibited, even to an extent of 90 per cent, by anticoagulant substances.

According to our investigations the venom of different snakes in a dose of 4–20 μg. induces in the rat paw huge oedemas lasting for hours. Bee venom has a similar effect. Table II shows that these oedemas may be also strongly inhibited by anticoagulants.

These results are in complete agreement with the experiments made with colloidal silver which proved that anticoagulants prevent the escape of silver particles from the vessels. All these experiments provide evidence that anticoagulant agents are capable of preventing the extreme increase of vascular permeability which is the most characteristic symptom of inflammation.

It is important to note that, in contrast to the symptoms just discussed, the hyperaemic response is not influenced by anticoagulants. The ear

of rats pretreated with anticoagulants and painted with xylol becomes vivid red and warm although no oedematous swelling occurs. In the same way, after instillation of a strong, 0·1–0·5 per cent, capsaicin solution into the eye the usual conjunctival oedema does not develop, but the dilatation of the vessels is well observed. Probably, inflammatory hyperaemia is produced by vasoactive substances liberated from the tissues and neither the liberation nor the action of these substances is affected by anticoagulant agents.

This persistence of hyperaemia may account for the fact that the oedematous swelling of the rat paw cannot be prevented completely by anticoagulants. The dilatation of the terminal vessels may lead, through raising the intravascular hydrostatic pressure, to increased filtration and moderate accumulation of fluid in the tissue.

TABLE II

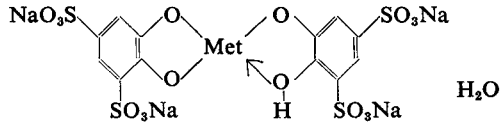
OEDEMA-PRODUCING EFFECT OF ANIMAL VENOMS AND ITS INHIBITION BY ANTICOAGULANT AGENTS. AMPUTATION AND WEIGHING OF THE HIND PAWS 1 HR. AFTER SUBPLANTAR APPLICATION OF THE TOXIN. ALL FIGURES MEANS FOR 8 ANIMALS WEIGHING 110–140 G.

Venom	Dose subplantar in 0·1 ml.	Wt. increase of oedema-leg controls per cent	Inhibition of oedema formation per cent	
			Helodym 88 9 ml./kg. i.v.	Liquoid 50 mg./kg. i.v.
Apis mellifera	Venom content of 1 bee	63·4	74·2	83·0
Naja naja	1 mouse U	64·1	66·0	79·5
"	0·5 "	46·3	71·0	—
Ancistrodon piscivorus ..	10 µg	56·7	64·1	73·9
Crotalus durissus	20 µg.	60·0	55·9	—
Sistrurus miliaris	10 µg.	58·0	78·5	84·4
Vipera ammodytes	12 µg.	58·8	64·8	68·6
Vipera aspis	5 µg.	62·4	79·0	75·2
Vipera berus	5 µg.	59·7	61·0	—

From all these observations we drew the conclusion that some clotting process must be involved in the mechanism of acute inflammation taking place in the wall and internal surface of the terminal vessels. Since the formation of fibrin must, in consequence of its pronounced adsorptive properties, result in a fixation of the circulating colloidal particles to the internal vascular wall, this conception provides a plausible explanation for the phenomenon of angiotaxis. At the same time it explains in an acceptable way the prevention of angiotaxis by anticoagulant agents. Moreover, our findings suggest that the gross increase of vascular permeability is also in some way connected with this coagulation process in the vascular structure.

Our experimental work was made difficult by the fact that intravenous injection of both Thrombodym and Helodym is not well tolerated by rats. Unless the injection is administered extremely slowly respiratory, arrest, convulsions and death occur. Recently I have succeeded in synthetising new compounds of rare earth metals having anticoagulant properties and which are free from such acute toxic effects. These compounds are rare earth complexes of pyrocatechol sodium disulphonate

in which the metal atom is attached to the oxygen atoms of two phenolic residues (I)



(I)

Such complexes of lanthanum, neodymium, praseodymium and samarium do not cause shock-like symptoms in rats and they are exceptionally well tolerated by rabbits too. These new compounds proved to be also very effective in counteracting inflammatory reactions in rats induced by subplantar injection of bee venom, cobra venom,

TABLE III

OEDEMA-PRODUCING EFFECT OF VARIOUS COAGULANTS ON THE HIND PAW OF THE RAT AND ITS INHIBITION BY ANTICOAGULANTS. WEIGHING OF THE PAWS 1 HR. AFTER SUBPLANTAR INJECTION OF THE CLOTTING AGENT. ALL FIGURES MEANS FOR 8 ANIMALS, WEIGHING 110–140 G.

Coagulant	Dose subplantar in 0·1 ml.	Wt. increase of oedema-leg controls per cent	Inhibition of oedema formation per cent		
			Helodym 88 9 ml./kg. i.v.	Thrombodym 2 × 6 ml./kg. i.v.	Liquoid 50 mg./kg. i.v.
Thrombin	20 U	51·3	56·0	87·4	79·6
Thromboplastin (rat brain)	0·1 ml.	67·3	76·7	86·5	87·0
Cephalin (pig brain) ..	5 mg.	52·8	72·5	—	69·1
Russell's viper venom ..	4 μg.	53·0	—	60·0	69·0
Ninhydrin	0·8 mg.	62·3	82·9	—	66·7
Sodium 1,2-naphthoquinone-4-sulphonate ..	2 mg.	46·3	—	—	62·2

compound 48/80 or dextran. With high doses of 250–350 mg./kg. the inhibition of the oedematous swelling may even exceed 80 per cent.

Since in the light of our results the development of inflammatory symptoms is intimately connected with a local coagulation phenomenon it was logical to assume that agents possessing clotting activity will exert a definite inflammatory effect if injected into the tissues of rats. To test this assumption we injected various coagulants into the foot pad of rats (Table III). As expected, we found that bovine thrombin, thromboplastin from rat brain, and unpurified cephalin from pig or rabbit brain are very effective in inducing oedematous swelling. In the same way, Russell's viper venom which is a recognised powerful thromboplastic substance caused much oedema even in a dose of 4 μg. Furthermore, it could be established that anticoagulants also exert in these experiments a considerable inhibitory action. The oedema induced by Russell's viper venom proved to be relatively resistant, but with high doses of Liquoid it could be almost completely prevented. Similar results could be obtained with ninhydrin and sodium 1,2-naphthoquinone-4-sulphonate which, according to Chargaff and Ziff (1941), are direct coagulants of fibrinogen. All these oedemas are associated with pronounced angiotaxis. Fig. 6 shows the action of thromboplastin in the plantar skin,

and Fig. 7 the action of 25 units of thrombin in the subcutis of the dorsal region. This effect of thrombin suggests very impressively that thrombin gains access to the blood thus causing parietal clotting and that the coatings are formed by a fibrinous precipitate with adsorbed silver.

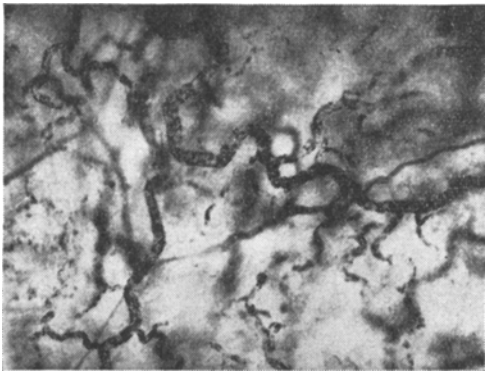


FIG. 6. Silver deposition in the plantar skin of a rat after subplantar injection of rat brain thromboplastin. Intense angiotaxis. The distended lympthatic network also contains colloidal silver.

Pretreatment with rare earths or Liquoid inhibits angiotaxis in the usual manner.

In the course of further experiments we attempted to prove the correctness of our conception in a direct way. Our experimental plan was to induce an afibrinogenaemia in rats and then to observe how these animals

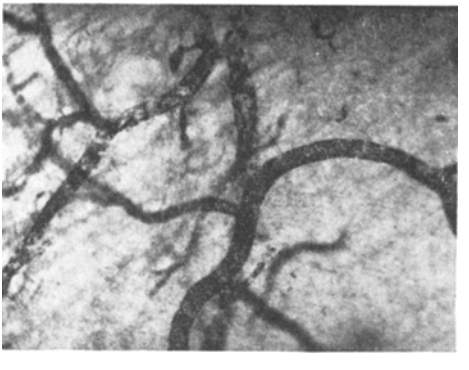


FIG. 7. Angiotaxis in the blood vessels of the dorsal skin region of a rat evoked by the subcutaneous injection of 25 units of bovine thrombin.

reacted to inflammatory stimuli. If our working hypothesis is really correct then the main symptoms of inflammation will fail to occur in such animals because the possibility of coagulation is precluded.

It could be foreseen that an *in vivo* defibrination of rats would not be an easy task. There are numerous data in the literature proving that

cautious intravenous infusion of coagulants in dogs or rabbits may cause a transitory total disappearance of fibrinogen. Such an effect could be for instance achieved with snake venoms (Mellanby, 1909; Rocha e Silva, 1955), thrombin (Basinger and Allen, 1951; Brayton and Zucker, 1957; de Nicola and Rosti, 1949; Jürgens and Studer, 1948; Quick, Hussey, Harris and Peters, 1959; Warner, Brinkhous, Seegers and Smith, 1939) or thromboplastin (Basinger and Allen, 1951). In our work we used thrombin. We succeeded only after long experimentation to elaborate a procedure with which fairly permanent defibrination could be achieved in rats. First 7–8 ml./kg. of carbon tetrachloride was injected subcutaneously into rats weighing 200–250 g. The defibrination was effected 40–48 hr. later by administering intravenously 120, 240 and finally 300 units of thrombin at intervals of 15 min. The inflammatory

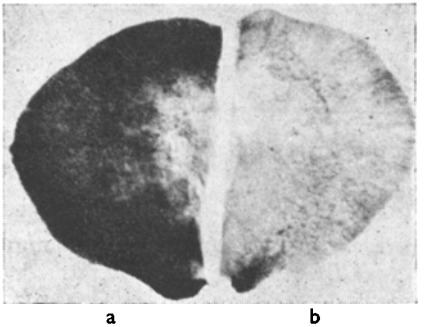


FIG. 8. Silver deposition in the xylol-painted ear; (a) ear of the control rat, (b) ear of the "defibrinated" rat.

agents were applied 5–15 min. after the last thrombin injection. Colloid silver was given, also, immediately afterwards in most of the experiments.

In recent experiments, before the thrombin injections, 300–400 units of heparin were first injected intravenously, to be followed at intervals of 15 min. by three or four injections each of 600 units of thrombin. After heparin-pretreatment the animals tolerate defibrination better; this may be because the fibrin is formed in the circulation in less coarse aggregates. Pretreatment with carbon tetrachloride is probably advantageous because it inhibits the replacement of fibrinogen through impairment of the liver. Others have shown that fibrinogen restituition fails to occur after elimination of the liver (Meek, 1912; Drury 1919).

The blood of rats treated in these ways becomes totally incoagulable; even 60–100 min. after the last thrombin injection the blood does not clot if thrombin is added, or only a small fragile clot appears. Normal blood clots in a coherent column even at a dilution of 200 times.

The inflammatory experiments made on the defibrinated animals led to results which fulfilled our expectations. It became evident that absence of fibrinogen favourably influences the inflammatory reactions in the same way as do anticoagulant agents. But the hyperaemic reaction is not modified; for instance, painting the ear with xylol produces a

vivid redness. Oedema, however, fails to develop. Likewise, instillation of a capsaicin solution into the eye causes only vasodilatation without conjunctival oedema. If colloidal silver is injected into the blood stream, the fixation of the metal in the affected area fails to occur, or only minute deposits are observed. Pictures of transparent preparations clearly show the diametrically opposed behaviour of normal and defibrinated animals (Fig. 8).

Similarly no oedema and no conspicuous silver deposition could be detected after subplantar or subcutaneous injection of compound 48/80, kallikrein, staphyococcus toxin, various snake venoms or thrombin. Microscopic examinations revealed that both angiotaxis and storage in the extravascular histiocytes is either totally absent or quite insignificant.

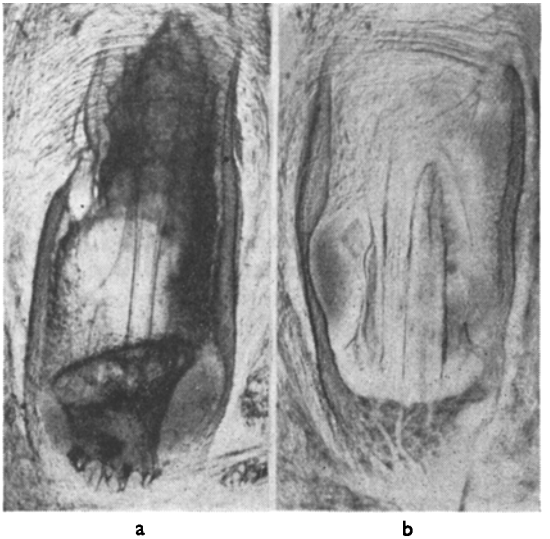


FIG. 9. Silver deposition in the blood sinus of sinus hairs after the intravenous administration of dextran, 0·4 ml. of a 6 per cent solution of "Macrodex" before the silver injection; (a) sinus hair of a *normal* rat; silver stained fibrin net in the blood sinus; (b) sinus hair of a *defibrinated* rat. Total absence of silver in the blood sinus.

A particularly interesting picture appears in the lacunae of the sinus hairs after an intravenous injection of dextran which is followed by much oedema of the nasal region (Fig. 9). In the non-defibrinated rat every blood sinus contains a brown coloured typical fibrin net evidencing clearly that because of the inflammation in the cavity fibrin is formed which adsorbs much colloidal silver. The snout of the defibrinated rat, on the other hand, was not swollen and in the sinuses no silver fixation can be observed.

These findings suggest that fibrinogen indeed plays a central rôle in the mechanism of the acute inflammatory reaction. They render very probable the conception that the cause of the angiotaxis-phenomenon

arises in the conversion of fibrinogen to fibrin on the inflamed vessel walls. This fibrin in turn forms a precipitate with the circulating colloidal particles. Similarly, the findings are consistent with the assumption that a gross increase of vascular permeability is also in close causal connection with this coagulation process. Apparently fibrin formation is able to increase greatly the number of large pores in the vessel wall.

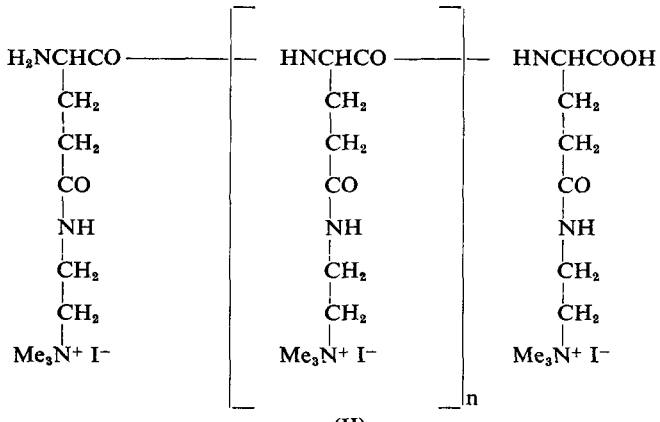
I must, however, confess that owing to haemodynamic reasons the experiments should be interpreted cautiously. Although the animals showing conspicuous prostration after the defibrination were discarded, in such experiments one must always take into account that the blood pressure falls and the blood supply of the peripery becomes deficient. We also established this deficiency in our defibrinated rats by a method once recommended by Rous and Gilding (1929) for control of the blood supply. We injected Patent Blue V into the blood stream of the defibrinated rats and observed the speed and depth of staining of the tissues by the highly diffusible dye. The experiments showed that the blood supply to the hind paws is strongly diminished; but, the circulatory supply to the fore legs and nasal region is fairly well maintained. In the staining of the ears there was no marked delay and the blood supply was appreciably enhanced if the ears were painted with xylol. Therefore, it is important that the effect of defibrination on the inflammatory reaction can be well demonstrated in the parts of the body just mentioned, the blood supply of which is not much impaired. It is even more important that the characteristic effect could also be clearly demonstrated in the diaphragm which may be considered as a permanently active muscle. Even after a severe haemorrhage Rous and Gilding (1929) found the diaphragm to be well supplied with blood. If 50 μg. of compound 48/80 was injected i.p. into rats pronounced angiotaxis could be provoked in the vessels of the diaphragm and the surrounding histiocytes were filled with colloidal silver. In defibrinated animals all this could be detected only in traces.

From all these findings it may be inferred that these defibrination experiments may after all be accepted and strongly support the view that fibrinogen is needed for the development of inflammatory reactions.

Yet we must admit that the assumed rôle of fibrinogen could be proved in a truly convincing manner only if these experimental results could be reproduced by a more adequate method of defibrination, and one which does not impair the circulation. Whilst we were searching for such a method, we discovered the interesting action of polyanthinium compounds on blood coagulation. It was soon revealed that with the aid of these compounds the desired aim can be achieved.

The compounds in question were prepared by Kovács and Kótai (1959) in the Institute of Organic Chemistry of the University of Budapest. Not long ago they synthethised from α-poly-L-glutaminic acid and ethylenediamine a soluble basic polypeptide derivative which was named "polyanthin". Kovács and Kótai recently synthethised some new derivatives in which one of the amino groups of the ethylenediamine is linked to the polypeptide chain, while the other one is quaternised with

alkyl groups. Most of our experiments were carried out with trimethyl-polyanthinium iodide. (II).



(II)

Trimethyl-polyanthinium iodide

The following compounds were also used: dimethylethyl-polyanthinium iodide and -methylsulphate and dimethyloxyethyl-polyanthinium chloride. The molecular weight of these compounds is approximately 15,000.

Theoretical considerations prompted me to investigate the effect of these interesting new compounds on blood coagulation. It was tempting to consider the possibility that not only polymers carrying electronegative groups such as heparin or heparinoids, but also macromolecular

TABLE IV

DEFIBRINATION OF THE CIRCULATING BLOOD BY POLYANTHINIUM COMPOUNDS

Species	Compound dose i.v.	Fibrin titre observed after hr.											
		0	1	2	3	4	5	6	10	20	24	30	48
Rabbit	A 70 mg./kg.	200		o	sm	sm	2	2		20			200
Rabbit	B 25 mg./kg.	250	o	o				2	20				250
Cat	A 65 mg./kg.	200			o			sm		20	50	100	200
Dog	A 65 mg./kg.	200		o		o		o		100			200
Rat	A 100 mg./kg.	200	o	o				sm			100		200
Rat	C 75 mg./kg.	200	o	o	o			sm			100		200

o = blood totally incoagulable. sm = small clot in undiluted blood.

A = trimethyl-polyanthinium-iodide; B = dimethylethyl-polyanthinium-iodide; C = dimethyloxyethyl-polyanthinium-chloride.

compounds of polycation character may interact with clotting factors and exert an anticoagulant activity. Animal experiments revealed that these compounds really possess a considerable anticoagulant and antithrombic activity. Their mode of action is quite different to that of recognised anticoagulant substances. Injected intravenously they induce a rapid fall of the fibrinogen content of the blood and in higher doses an

afibrinogenaemia lasting for several hours. After 24–48 hr. the fibrinogen level again becomes normal.

As Table IV shows, this peculiar effect could be equally well demonstrated on rats, rabbits, cats and dogs. In these experiments the fibrin titre was determined by the method of Schneider (1952) with minor modifications. Citrated blood is diluted and the highest dilution in which visible coagulation occurs when thrombin is added is determined. As can be seen, before the experiment is made, clot formation is detectable in a dilution of 200 or 250, whereas, at the peak of the effect, even the undiluted blood does not clot or only a small clot is formed. Thrombin was added as a powder to the undiluted blood.

TABLE V

INHIBITION BY POLYANTHINIUM COMPOUNDS OF THE INFLAMMATORY OEDEMA OF THE HIND PAW OF RATS. ALL FIGURES MEANS FOR 6 ANIMALS WEIGHING 160–200 G.

Compound mg./kg. dose i.v.	Interval min.	Inflammatory agent subplantar	Killed after min.	Wt. increase of oedema-paw		Inhibition per cent
				pretreated per cent	control per cent	
A 100	60	comp. 48/80 10 μg.	60	14·3	45·4	68·6
A 125	60	dextran 600 μg.	80	14·8	58·0	74·5
A 100	60	kaolin 0·1 ml. 10 per cent	140	13·0	60·0	78·0
A 100	60	cephalin 5 mg.	60	13·0	46·6	71·8
C 65	60	cobra venom 1 mouse U	60	11·7	45·7	74·4

The animals tolerated large, fibrinogen-depleting doses well. In 3 cats, after administration of 65 mg./kg. of the trimethyl derivative, the blood pressure showed only minimal changes during the experiment. The blood pressure was measured without using anaesthesia by means of a polythene tube inserted into the aorta.

In view of the fibrinogen-depleting effect it could be expected that the polyanthinium compound will exert an antithrombotic action too. We investigated, in rats whether pretreatment with polyanthinium exhibits a protective action against lethal thrombosis caused by an intravenous injection of thrombin. The animals were given 100 mg./kg. of trimethylpolyanthinium iodide intravenously (LD50 = 160 mg./kg.). It could be established that even tremendous doses of thrombin, such as 2000 units, did not evoke thromboembolic death. In controls the injection of 400 units is nearly always fatal. For these experiments the standardised thrombin preparation Thrombofort-"Richter" was used.

As these findings show, with the aid of polyanthinium compounds, the aim which was always in our mind in the course of our work is easy to achieve. A single injection of polyanthinium brings about a lasting disappearance of fibrinogen, whilst the animal maintains its fit condition. Thus we possess a simple and reliable method with which it could be

unequivocally decided how inflammatory reactions develop in the absence of fibrinogen.

The inflammatory experiments on polyanthinium treated animals revealed important results which confirmed our previous conclusions in every respect. We found that on such animals oedematous inflammation is so reduced that it can be hardly detected by inspection and the local fixation of injected colloidal silver is totally absent.

Table V shows the far reaching inhibitory effect of polyanthinium compounds on the oedematous swelling of the paw of the rat due to

a b

FIG. 10. Prevention of the inflammatory fixation of intravenous injected colloidal silver by trimethyl-polyanthinium iodide, 100 mg./kg. intravenously 1 hr. before application of the inflammatory stimulus. Plantar skin of a normal (a) and of a polyanthinium-treated rat (b) after subplantar injection of 10 μg. of compound 48/80.

compound 48/80, dextran, kaolin and cobra toxin, respectively. The inflammatory agents were applied in these experiments 1 hr. after the polyanthinium compounds, at which time the blood is already free of fibrinogen.

The local hyperaemic reaction was not inhibited by the polyanthinium compounds; this is in accordance with our previous experiments which all showed that this symptom is independent of clotting factors. The xylol-painted ear of the pretreated animals becomes vividly red just like that of the normal animals, but, the oedematous swelling fails to occur. In the same way, if a capsaicin solution of 0·1 per cent is instilled into the eye vasodilatation is obvious, but the usual conjunctival oedema does not develop.

To study the silver fixation phenomenon rats were defibrinated with trimethyl-polyanthinium iodide and dimethylethyl-polyanthinium methyl-sulphate respectively. Of the former 100 mg./kg. of the latter 80 mg./kg.

was administered intravenously and then we waited for 1 hr. for the blood to become free of fibrinogen. Subsequently, different inflammatory substances were applied locally and ultimately 120 mg./kg. of colloidal silver was injected into the tail vein. The experiments demonstrated that the usual silver deposition does not take place. Neither angiotaxis nor granular accumulation in the histiocytes of connective tissue could be observed. Thus, no visible silver deposition was found in the xylol-painted ear and capsaicin-treated conjunctiva. The same negative result was obtained in the plantar skin and musculature after subplantar injection of compound 48/80, 10 and 20 μg., dextran, 600 μg., toxin of the rattle snake *Crotalus durissus*, 10 μg., and thrombin, 40 U., respectively. In the controls, as usual, ample deposits of silver could be

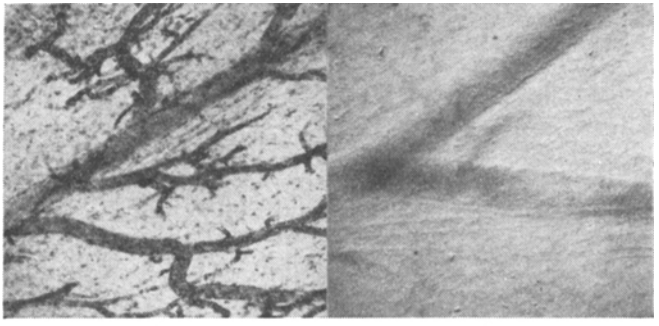


FIG. 11. Silver fixation in the omentum of the rat after the intraperitoneal injection of a suspension of fine glass particles; (a) normal rat; ample deposition of the intravenous injected colloidal silver on the internal surface of the vessels and in extravascular histiocytes, (b) "defibrinated" rat: no visible silver deposition. Defibrination was effected by the intravenous injection of 80 mg./kg. of dimethylethyl-polyanthinium methylsulphate 1 hr. before the application of the inflammatory stimulus.

observed in all inflammatory sites. Figs. 10 and 11 show in an impressive way, the difference in response of the depleted animals and of the controls. It should be noted that in the Kupffer-cells of the liver a fine and even storage of silver could be observed in the polyanthinium-treated animals too.

All these findings strongly support our view that in rats fibrinogen plays indeed a fundamental rôle in the mechanism of acute oedematous inflammation.

According to current views the increase in vascular permeability is an outcome of the direct action of the so-called mediators of the inflammatory reaction such as histamine or 5-HT. It is generally believed that it is an intrinsic property of these substances to induce increased vascular permeability. I do not want to deny the important rôle played by these mediators in the elicitation of inflammatory responses. For instance, the experiments of Rowley and Benditt (1956), Parratt and West (1957, 1958), Doepfner and Cerletti (1957) and Stenger (1958) suggest very strongly that dextran- or eggwhite-oedema in rats is mainly caused by

the liberation of 5-HT or 5-HT plus histamine. There is, however, no proof that these mediators exert their effect by a simple direct action on the vascular wall. The whole affair seems to be far more complicated since our results suggest that the intervention of the coagulation system is a pre-requisite to typical inflammatory vascular reaction.

The assumed coagulation process in the vascular wall may be initiated by the inflammatory agent itself or by endogenous thromboplastic factors produced under the influence of these agents. Unfortunately, we are at present completely ignorant of the nature of such endogenous thromboplastic factors.

In retrospect it may be stated that a wealth of observations suggest that the blood clotting system plays an important rôle in the mechanism of inflammation in addition to its function in haemostasis and thrombus-formation. Apparently, a coagulation process is involved in the inflammatory tissue reaction taking place in the walls of the terminal vessels. In all probability it is this coagulation process which induces the most characteristic vascular inflammatory reactions, that is, the angiotaxis phenomenon and the excessive increase in permeability. Such a conception would then be in good agreement with the observation that neither angiotaxis nor gross oedema formation can be achieved in the absence of fibrinogen or if the coagulation is inhibited by an appropriate anticoagulant.

REFERENCES

Alksne, I. F. (1959). *Quart. J. exp. Physiol.*, **44**, 51–66.
Basinger, C. and Allen, J. G. (1951). *J. Lab. clin. Med.*, **38**, 784.
Benacerraf, B., McCluskey, R. T. and Patras, D. (1959). *Amer. J. Path.*, **35**, 75–91.
Biozzi, G., Menè, G. and Óváry, Z. (1948). *Clinica Nuova*, **6**, 393–402.
Biozzi, G., Menè, G. and Óváry, Z. (1948a). *Rev. d'Immunol.*, **12**, 320–334.
Biozzi, G., Menè, G. and Óváry, Z. (1949). *Lo Sperimentale*, **99**, 341–351; 352–362.
Brayton, R. G. and Zucker, M. B. (1957). *Proc. Soc. exp. Biol. N.Y.*, **96**, 418–421.
Chargaff, E. and Ziff, M. (1941). *J. biol. Chem.*, **138**, 787–788.
de Nicola, P. and Rosti, P. (1949). *Boll. Soc. ital. biol. sper.*, **25**, 1184.
Doepfner, W. and Cerletti, A. (1957). *Int. Arch. Allergy*, **10**, 348–354.
Feldberg, W. (1966). *Ibid.*, **8**, 15–31.
Gözsy, B. and Kátó, L. (1957). Studies on Phagocytic Stimulation. Institute of Microbiol. and Hyg., University of Montreal.
Jancsó, N. (1941). *Ber. Physiol.*, **126**, 475.
Jancsó, N. (1942). *Magyar Orvosi Archivum*, **43**, 367–368.
Jancsó, N. (1947). *Nature, Lond.*, **160**, 227–228.
Jancsó, N. (1955). Speicherung, Stoffanreicherung im Reticuloendothel und in der Niere, Akadémiai Kiadó, Publishing House of the Hung. Acad. Sci., Budapest.
Jancsó, N. (1960). *Klin. Wschr.*, **11**, 1067–1068.
Jancsó, N. (1960). *Bull. Millard Filmore Hosp.*, 1960, **7**, 53–77.
Jancsó, N. and Jancsó-Gábor, A. (1960). *Arch. exp. Path. Pharmak*, **238**, 83–84.
Jürgens, R. and Studer, A. (1948). *Helv. Physiol. Acta*, **6**, 130–149.
Kovács, K. and Kótai, A. (1959). *Acta Chim. Hung.*, **21**, 453–460.
Matoltsy, G. and Matoltsy, M. (1951). *J. Pharmacol.*, **102**, 237–249.
McMaster, 0. and Drury, 0. (1919). *Proc. Soc. exp. Biol., N.Y.*, **26**, 490. cited by Heinlein, H. (1943). *Z. exp. Med.*, **112**, 535–558.
Meek, 0. (1912). *Amer. J. Physiol.*, **30**, 161, cited by Heinlein, H. (1943). *Z. exp. Med.*, **112**, 535–558.
Mellanby, J. (1909). *J. Physiol.*, **38**, 28–40.
Parratt, J. R. and West, G. B. (1957). *J. Physiol.*, **139**, 27–41.
Parratt, J. R. and West, G. B. (1958). *Brit. J. Pharmacol.*, **13**, 65–70.
Paton, W. D. M. (1957). *Pharmacol. Rev.*, **9**, 269–328.
Quick, A. J., Hussey, C. V., Harris, J. and Peters, K. (1959). *Amer. J. Physiol.*, **197**, 791–794.

Roche e Silva, M. (1955). *Histamine*, Springfield: Thomas.
Rous, P. and Gilding, H. P. (1929). *J. exp. Med.*, **50**, 189–211.
Rowley, D. A. and Benditt, E. P. (1956). *Ibid.*, **103**, 399–411.
Selye, H., Lemire, Y. and Cantin, M. (1959). *Med. exp.*, **1**, 11–16.
Schneider, C. L. (1952). *Amer. J. Obstet. Gynec.*, **64**, 141–147.
Spector, W. G. (1958). *Pharmacol. Rev.*, **10**, 475–505.
Stenger, E. G. (1958). *Arzneimitt.-Forsch.*, **8**, 693–694.
Törö, I. (1942). *Z. mikrosk.-anat. Forsch.*, **52**, 552.
Warner, E. D., Brinkhous, K. M., Seegers, W. H. and Smith, H. P. (1939). *Proc. Soc. exp. Biol., N.Y.*, **41**, 655–657.
Zemplényi, T., Fodor, J. and Lojda, Z. (1960). *Quart. J. exp. Physiol.*, **45**, 50–54.