ICI 50172, MJ 1999 and methoxamine show almost similar β-adrenergic blocking potencies either in the papillary muscle contraction or in the sinoatrial pacemaker activity, while propranolol, H 56/28 and DCI show more potent blocking activity on the chronotropic effect of catecholamine than on the inotropic one.

Zusammenfassung. Nachweis, dass der β-Blocker LB 46 am blutperfundierten Papillarmuskel des Hundes eine grössere Aktivität aufweist als 6 andere bekannte β-Blocker.

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Specific Oedema-Inhibiting Property of a Natural Anti-inflammatory Factor Collected from Inflamed Tissue

While studying the effect of a series of copper-containing compounds (e.g. $Cu(OH)_2CuCO_3$) we found that the anti-inflammatory activity of most of them was regularly associated with marked tissue irritation at the site of injection, and that those compounds which did not provoke irritation were devoid of an anti-inflammatory effect[1]. We postulated that tissue irritation by the copper compounds may have released into the circulation a substance which inhibits inflammation at other sites in the organism. Several investigators[2–8] have noted that the administration of irritant substances inhibited experimental inflammation, but little is known about the specificity of this natural anti-inflammatory factor. In this communication we report the effects of a natural anti-inflammatory factor on different types of oedemas, which differ from each other in their mechanism and also in their responses to drugs[9,10].

Materials and methods. Male albino rats of a strain bred at TNO Animal Centre (Netherlands) were used throughout. Local tissue irritation was induced by i.p. injection of phenylquinone (0.03% solution, 1 ml/rat). Paw oedema was induced and measured according to methods described earlier[9]. Inflammatory pouches were produced by the method of Boris and Stevenson[11] and the exudate removed 4 days following the induction of the pouch. On average 1 animal yielded 9–10 ml of exudate which was then dialyzed and lyophilized. Gel filtration was carried out on Sephadex G-75, G-100 and G-150 columns (2.5 × 50 cm). Equilibration of the gel and elution were performed with 0.1 *N* acetic acid (pH 3.2).

Results and discussion. Table I shows that in rats which received an i.p. injection of phenylquinone, there was a marked inhibition of paw swelling by kaolin but not by serotonin or polyvinyl-pyrrolidone (PVP). These results indicated that tissue irritation caused by phenylquinone exerts a remote anti-inflammatory effect specifically towards those particular type of oedemas which are also inhibited by phenylbutazone.

In an attempt to transfer the postulated anti-inflammatory tissue factor into other rats, we demonstrated earlier that peritoneal exudate and paw oedema fluid collected from rats, inhibited the kaolin-induced paw swelling when injected into other rats[12]. In the present investigation we have examined the effect of exudates collected from inflammatory pouches. The water-soluble component of the exudate was found to contain the factor which inhibited the kaolin-induced rat paw oedema, and dialysis of the water-soluble portion revealed that the activity is due to a high molecular weight material, as most of the activity was found in the retentate. As seen in Table II, about 100 mg/kg of lyophilized retentate reduced the swelling of the rat paw to 50% when injected i.p., whereas 500 mg/kg dialysate reduces to only 32%.

We have further examined the activity of the water-soluble, non-dialyzable principles of the pouch material against the inflammation caused by kaolin, carrageenin, serotonin, histamine and PVP. We found that serotonin, histamine and PVP oedemas are not inhibited, whereas kaolin and carrageenin oedemas are definitely inhibited by this anti-inflammatory factor present in the pouch material (Table III). Inhibition of the kaolin-induced oedema was more pronounced than that of carrageenin oedema. We have regularly observed, however, that with our rat strain the carrageenin oedema is more resistant to inhibitory effects than the kaolin oedema. Previous work has shown that phenylbutazone counteracts the oedema

Table I. Hind-paw oedema. nhibition in peritoneally irritated and phenylbutazone treated rats

Pretreatment	Inhibition (%)		
	Kaolin[a]	Serotonin[a]	PVP[a]
Phenylquinone, i.p. irritation	49 (41–57)[b]	0	0
Phenylbutazone, orally 100 mg/kg	83 (74–92)	0	0

[a] Oedema inducer. [b] Figures are mean values of groups of 10 rats each. In brackets: 95% fiducial limits.

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caused by kaolin, but fails to inhibit the oedema produced by serotonin, histamine or PVP[9]. It appears thus that the specificity of the natural anti-inflammatory activity is similar to that of phenylbutazone in these type of oedemas.

ROBINSON et al.[7,8,13] have described an anti-inflammatory factor from exudates of animals subjected to irritation by implanting polyester sponges. Our material showed some similarity to theirs, since on gel filtration both substances behave as macromolecules. ROBINSON et al.[7,8,13] investigated the effect of their natural anti-inflammatory factor only on carrageenin oedema, so it is still debatable whether these 2 factors are similar or identical in their pharmacological properties. The hypothesis that the natural anti-inflammatory factor is synthesized in the liver[14] and transported to the diseased site during inflammation[8], requires further investigation. However, our material possesses a higher molecular weight than the anti-inflammatory factor isolated by HUBER et al.[15] from bovine liver. Further pharmacological characterization of the anti-inflammatory material obtained from pouch is in progress.

Table II. Anti-inflammatory activity of retentate and dialysate of water-soluble components of pouch material

Components tested	Dose[c] (mg/kg)	Inhibition of oedema[a] (%)
Retentate	50	29 (11–44)[b]
	100	50 (34–65)
	200	49 (33–64)
	400	64 (48–78)
	800	87 (73–101)
Dialysate	500	32 (10–50)

[a] Oedema was induced by kaolin. [b] In brackets: 95% fiducial limits. [c] Test material was dissolved in physiological saline and a volume of 1 ml/100 g body weight of rat was administered i.p.

Table III. Effect of the natural anti-inflammatory factor of pouch material on different types of oedema

Oedema inducer	% inhibition caused by retentate[a] (100 mg/kg)
Carrageenin	21 (11–31)
Kaolin	63 (49–77)
Histamine	0
Serotonin	0
Polyvinylpyrrolidon	0

[a] Obtained by dialysis of water-soluble pouch material.

Zusammenfassung. Eine hochmolekulare Verbindung wurde aus Exudaten von Granulombeuteln von Ratten gereinigt. Diese Substanz, i.p. an Ratten verabreicht, hemmt das durch Kaolin oder durch Carrageenin erzeugte Pfotenoedem, nicht dagegen das durch Histamin, Serotonin oder Polyvinylpyrrolidon erzeugte Oedem.

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A Role for Glutathione in Muscle Contraction

The development of rather specific agents for the intracellular oxidation of glutathione, GSH, to GSSG has permitted the investigation of the function of GSH in many biological systems[1–6]. We now report a heretofore unsuspected linkage of GSH to muscle contraction.

The thiol-oxidizing agent used in the present experiments is diamide, $(CH_3)_2NCON{=}NCON(CH_3)_2$, which reacts with GSH according to the equation[2]:

$$2GSH + (CH_3)_2NCON{=}NCON(CH_3)_2 \rightarrow GSSG + (CH_3)_2NCONHNHCON(CH_3)_2$$

The half-life for the reaction of diamide with intracellular GSH under the conditions used would be 1 sec or less (W. CORREA, unpublished observations).

Primary cultures of muscle cells (*Rattus rattus norvegicus*) in medium 199 were supplied by Dr. DAVID YAFFE (Department of Cell Biology)[7]. 2-week-old cultures exhibited strongly contracting fibres. Experiments were begun by adding a small volume of diamide solution in isotonic saline to the culture medium (25 λ: 3 ml) followed by rapid swirling. Observations were made under a dissecting microscope. After 80–120 sec, the medium was aspirated from the plate and replaced with fresh 199 medium. The plates were examined immediately after medium change and then periodically after storage in an incubator at 37 °C.

Treatment of actively contracting fibres with a $5\times 10^{-4}M$ diamide solution led to complete cessation of acti-

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