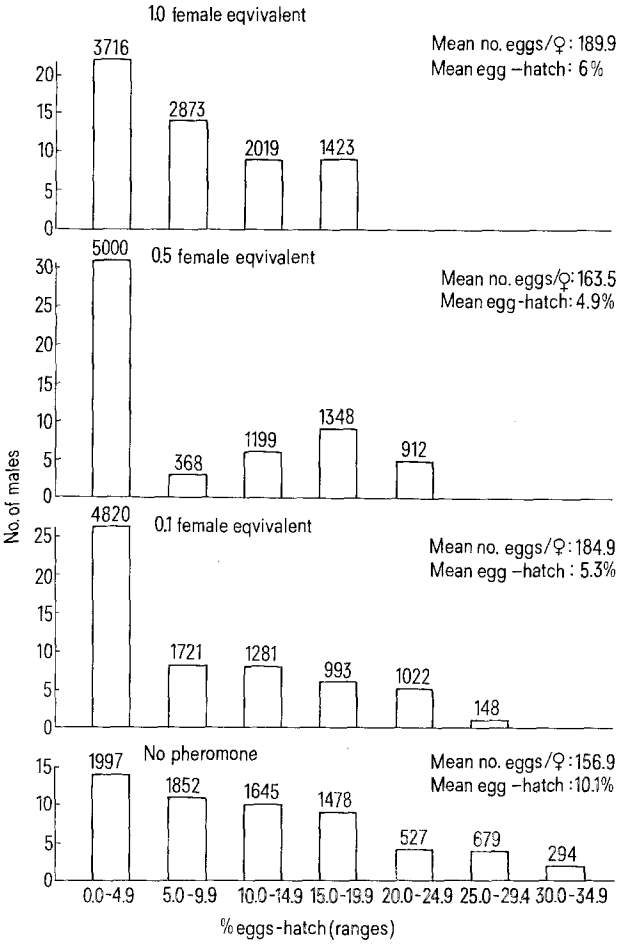

Hatch of eggs laid by *C. cautella* females mated to males irradiated with 40 krad in the presence of the female pheromone. (Total No. of eggs laid in each fertility group is indicated above the columns).

Filter papers treated with the solvent alone were introduced into the control group containers. Excitation and increased activity of the insects was observed in cages containing pheromone within seconds of its application. Subsequently, after irradiation, the treated males were paired individually with virgin females, 0–24 h old, and kept at constant conditions (26±1 °C, 60–70% RH). Oviposition and egg hatch were recorded for 54 pairs from each treatment, in which mating took place (spermatophore observed in each female's bursa copulatrix).

Tests of significance, based on normality criteria, are inappropriate because of the skewness of the data. However, it is clear, from the histogram (Figure 1) that the proportion of low percentage hatch increases with increased pheromone presence. The difference between absence and presence of pheromone is most marked, whereas this is not apparent between the different pheromone doses.

The above results are evidence of the value of this approach of preconditioning to irradiation, especially when induction of sterility is the objective.

Résumé. L'irradiation des mâles de la teigne *Cadra cautella* (Lepidoptera, Pyralidae) en présence de phéromone sexuel féminin a provoqué une sterilité plus élevée que l'irradiation sans phéromone. Il est à noter que cette méthode de préconditionnement peut permettre d'obtenir des insectes stériles à l'aide de doses de radiation diminuées.

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Inflamed-Tissue Factor(s): An Autoregulatory Mechanism of some Acute Inflammatory Responses

The term 'counter-irritation' indicates the phenomenon that irritation leading to local inflammation will counteract the inflammatory response to subsequent irritation in the same organism. The postulation that irritation may result in discharge of some anti-inflammatory substance(s) obtained support when it was shown that administration of the extracted inflamed-tissue factor results in suppression of experimental inflammation[1–9]. The recognition of the role of complement in acute non-immune inflammations led to the concept that fixation at the irritated site may leave insufficient complement available to trigger inflammatory responses of subsequent irritation[10,11]. The present work was aimed to investigate the underlying anti-inflammatory mechanism of inflamed-tissue factor (ITF), and an attempt is made to explain how this mechanism might be interrelated with the counter-irritant principle.

Material and methods. The source of ITF was exudate from inflamed air-carrageenin pouches of rats produced by the method of Boris and Stevenson[12]. Six days following the induction of the pouch, the rats were sacrified to collect exudates which were filtrated, centrifuged, dialyzed and lyophilized. Previously we have shown that the anti-inflammatory factor was mostly retained within the dialysis sac[9] and at present only the retentate was used. For testing anti-inflammatory activity, the hind-paw inflammations of rats and mice were evaluated by measuring the diameter of the paws. Complement was measured in serum of blood obtained by cardiac puncture of rats, and the total complement was titrated by establishing the volume of serum giving 50% hemolysis of sheep erythrocytes to which antiserum of rabbits was added[13]. Complement activity was expressed as CH50/ml serum. For complement activity by ITF retentate in vitro, a range of different concentrations of the retentate was used and the concentration causing 50% inhibition was interpolated. Significance was calculated by Student's *t*-test.

Results and discussion. Anti-inflammatory effect of i.p. administered ITF retentate was observed on the rat-hind paw inflammation induced by carrageenin or kaolin, but no significant effect was fonnd on histamine- or serotonin-induced inflammation. The reproducibility of this preference to selective inhibition is indicated by the great similarity of 2 independent experimental series, as shown in Table I. Not shown in the Table are the data to dem-

onstrate that ITF retentate failed to inhibit the inflammatory dermal eryhtema induced by UV-irradiation (BHARGAVA and DE VOS, personal communication), which is also unaffected by counter-irritation[8]. This similarity caused us to consider that administration of the ITF material may have acted as a counter-irritant. ATKINSON et al.[14] suggested that the apparent anti-inflammatory action of an undialyzed ITF preparation was largely due to counter-irritant mechanism rather than to the presence of a specific anti-inflammatory material. However, we found that the anti-inflammatory effect was demonstrable after i.v. administration of 100 mg/kg ITF retentate, which in 2 independent experiments was proved to inhibit the kaolin hind-paw swelling to 46.8 and 25.5% respectively, while in the same series i.p. administration caused 35 and 47.5% inhibition. The chance of tissue irritation is obviously much smaller with the i.v. than with the i.p. injection. Further we compared the anti-inflammatory and irritant properties of 2 ITF batches obtained from different series of pouch-bearing rats. The anti-inflammatory effect was equi-effective, because the preparation coded as B-retentate causes 40.7% inhibition with 100 mg/kg, while the N_4-retentate in the same dose produced 37% inhibition of the kaolin hind-paw swelling. But after local injection into the hind-paw, the inflammatory effect of B-retentate was more pronounced and longer lasting than that of N_4-retentate (Figure). Dialysis of the B-retentate was 200 ml against 1250 ml, while with the N_4-retentate 50 ml was dialyzed against 2000 ml. Thus the more improved the process of dialysis the greater is the chance of eliminating irritant impurities while fully retaining the anti-inflammatory factor. It is plausible to assume that this explains the different conclusions of ATKINSON[14] and ourselves.

Adrenalectomy of the test animals did not reduce the anti-inflammatory effect of ITF (Table II), herewith ruling out the discharge of corticosteroids as a conceivable mechanism.

Table I. Anti-inflammatory effect (%) of carrageenin pouch-retentate on different hind-paw inflammations in rat

Hind-paw irritant	Retentate 100 mg/kg i.p.[a]		
	1st	2nd experiment	$N = 10$
Carrageenin	21[b]	38[b]	
Kaolin	63[b]	41[b]	
Histamine	0	4	$P < 0.05$[b]
Serotonin	0	11	
Polyvinylpyrrolidon	0	—	

[a] Data from first experiment are quoted from BONTA et al.[9]. This experiment was performed 2 years earlier than the second experiment. Note the reproducibility of the selective effect of ITF retentate.

Table II. Influence of adrenalectomy on effect of inflamed-tissue factor in kaolin hind-paw inflammation

Donor-condition	Test-condition	Inhibition (%)		N
		Sham operated	Adrenal-ectomized	
Rat carrageenin-pouch retentate	rats 100 mg/kg i.p. mice[a]	41	43	10
	200 mg/kg i.p.	57	89	5

[a] Observe that the ITF from rat source is also effective in mice.

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Table III. Effect of local irritation on complement activity in rat serum

Irritant	Dose	Site of injection	Time interval (min)	CH50/ml	N
Saline	0.2 ml	hind-paw	240	33.0 ± 1.7	8
Carrageenin	2 mg	hind-paw	240	4.3 ± 2.2[b]	8
Saline	10 ml/kg	intraperitoneal	240	31.6 ± 8.0	5
Carrageenin	5 mg/kg	intraperitoneal	240	10.6 ± 3.8[b]	5
Saline	0.2 ml	hind-paw	240	32.5 ± 3.5	5
Kaolin	10 mg	hind-paw	240	33.5 ± 0.5	5
Saline	0.2 ml	hind-paw	30	38.0 ± 3.6	5
5-HT	2 µg	hind-paw	30	32.5 ± 3.5	5
48/80	3 µg	hind-paw	30	27.5 ± 1.5[a]	5
Prostaglandin E_1	1 µg	hind-paw	30	29.0 ± 7.0	5

[a] $P < 0.05$. [b] $P < 0.001$.

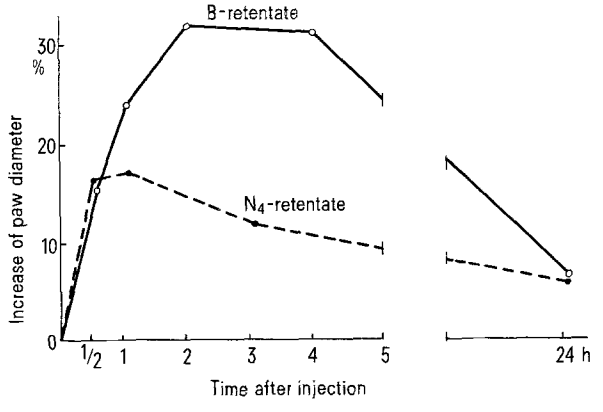
Table IV. Effect of different ITF-retentate batches on kaolin hind-paw inflammation and serum complement of rats

Administration of retentate	Parameter	Time after kaolin (h)	Batch		
			N8	*N9*	*N10*
Intraperitoneal (100 mg/kg)	Paw diameter % reduction	4 h	16[a]	41[b]	35[b]
Intraperitoneal (100 mg/kg)	CH50/ml % reduction	4 h	0	34.7	n.t.[d]
In vitro to rat serum	Dose for 50% [c]		260	95	60
	inhib. of compl. ID 50 µg		160	21	21

[a] $P = 0.05$. [b] $P < 0.001$. [c] The determinations of the ID 50 were performed in 2 experiments in triplicate. The scatter of the values obtained in each experiment was negligible. [d] Not tested because amount of material was insufficient.

In view of the fact that the ITF retentate was proved to suppress the hind-paw inflammations caused by carrageenin or kaolin, it was plausible to search for its mode of action in mechanism(s) mutually involved in the two inflammations. The middle phase of carrageenin inflammation is suppressed by pre-treatment with cellulose sulphate or ellagic acid, while the delayed phase was inhibited by pre-treatment with carrageenin[15]. A similar result was observed by us recently with inflammation induced by kaolin. Cellulose sulphate, similarly as ellagic acid, is known to deplete kininogen ,but carrageenin is shown to mostly suppress complement titres[16–18].

Since complement might be involved in the phase of inflammation suppressed by counter-irritation or by administration of ITF retentate, rats were submitted to a variety of irritant stimuli, and at the peak time of the inflammatory response serum was obtained for complement assay. Table III shows that carrageenin caused depletion of complement, while no such reduction was observed when kaolin, serotonin, the histamine releaser 48/80 or prostaglandin E_1 served as irritants. Since kaolin is insoluble and remains at the site of injection, it is conceivable that complement fixation was limited locally to the hind paws and insufficient to be reflected in blood collected from a remote site. The sodium salt of carrageenin, which we used, can easily enter the blood-stream to act on complement throughout the whole organism.



Comparison of the irritant effect of 2 pouch-extract retentates as measured on the rat hind-paw swelling following the subplantar injection of 1 mg each. Values represent the average of groups of 10 rats. Dialysis of B-retentate (○—○) consisted of 200 ml against 1250 ml, while with the N_4-retentate (●---●) 50 ml was dialyzed against 2000 ml. Note that the better the conditions of dialysis, the less pronounced is the irritant effect.

We had access to batches of ITF retentates collected from 3 independent series of donor rats. We knew from earlier experiments that 1 of the batches (N8) showed only a feeble anti-inflammatory effect, but the 2 other batches (N9 and N10) inhibited the inflammation induced by kaolin. The results in Table IV seem to show a correlation between the anti-inflammatory effect and reduction of serum complement. Batch N8 which had little anti-inflammatory effect did not reduce complement in vivo and its complement-reducing effect in vitro was also feeble. Both batches, which displayed a pronounced anti-inflammatory effect, inhibited serum complement in a low concentration in vitro and batch N9 also proved to reduce complement when administered in vivo. Though the evidence is circumstantial, we suggest that suppression of complement is associated with the anti-inflammatory effect of ITF-retentate. Activation of complement has been implicated in the release of kinin-like inflammatory mediators from plasma substrates[11]. Present findings might explain how ITF-retentate, though incapable of directly inhibiting kinin release (Bhargava, personal communication), is still capable of suppressing the carrageenin or kaolin induced inflammations, in which besides complement, kinin release has also been suggested to play a role[11,15,19]. It appears that the anti-inflammatory mechanism of ITF is different from that of counter-irritation. The latter was considered to cause tissue fixation of complement, rendering it not available to trigger the inflammatory reaction subsequent to irritation at a remote site[10]. The effect of ITF on complement was not longer lasting than 4 h, which makes inhibition more likely than depletion.

Billingham et al.[20] suggested that material from irritated tissue has no anti-inflammatory effect by itself, but triggers the liver to synthetize proteins with anti-

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inflammatory properties. Since reduction of complement results in suppression of acute inflammatory reactions, our in vitro experiments show that the involvement of a hepatic process is not required for the anti-inflammatory action of ITF. Further, there is uncertainty whether the tissue factor which we recovered is identical with that found by BILLINGHAM.

Zusammenfassung. Bei Ratten wurde aus entzündetem Gewebe ein Hemmer für das Komplementsystem extrahiert, welcher die durch Carrageenin oder Kaolin erzeugten Entzündungen zu unterdrücken vermag.

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The Migration of Lymphoid Cells in Malignant Disease

Circulating sensitized lymphoid cells do not attack malignant homografts. Their lack of cytotoxicity, in vivo, has been attributed to certain serum factors: antibodies[1], antigens[2] or antigen/antibody complexes[3]. These are thought to block lymphocyte/tumour target cell interaction. Tumour produces a soluble substance which inhibits cellular adhesion and pseudopodia formation[4] and impairs the migration of leucocytes[5]. It is suggested that this substance paralyses all manner of lymphocyte locomotion: diapedesis, directional migration and attachment to target cell and that it is this paralysis, and not blocking by antibody, which enhances tumour growth.

Materials and methods. White Wistar outbred rats, weanlings of 80–100 g and adults of 150–180 g were obtained from Messrs. Tuck & Son, Rayleigh.

Tumour: A transplantable tumour, originally induced in the rat uterus[6] was maintained by serial passage and used in these experiments.

Two plastic millipore discs, 25 mm in diameter, pore size 6 nm, from Messrs. Millipore, London, were heat-sealed to form a cell impermeable diffusion chamber. Accessible lymph nodes were excised, minced in an aliquot of saline and centrifuged until the supernatant was clear. The latter was discarded and the sediment only used.

Tumour fragments of 0.2–0.3 cm^3 were grafted into the left flank of an adult animal. About 12 days later, when the tumour had reached a diameter of 10–15 mm, a piece was excised and divided into 3 portions of 0.2 cm^3 each.

One portion was grafted into the right flank of the donor. The second fragment was sealed into a millipore chamber and the third portion was enclosed in a chamber together with 0.2 ml of packed lymphoid cells. The 2 chambers were placed into the abdominal cavity of the donor animals and left in situ for 7 days. At the end of this period, the chambers were again removed, the tumour fragments taken out and implanted s.c. into weanling rats.

In a control series a similar procedure was adopted using tumour free animals and homologuous tumour.

Liver fragments of 0.3 cm^3 were implanted s.c. and full thickness skin allografts of 10 mm diameter were sewn to the anterior chest wall of adult rats.

Grafts, their substrate and the draining lymph nodes were excised at various intervals after implantation, fixed in formol saline and stained with haematoxylin eosin or methyl green pyronin.

Results. Non-malignant homografts induced a progressive accumulation of mononuclear cells at the graft site, culminating in the rejection or destruction of the graft, usually by the 10th-13th day.

There was a similar accumulation of mononuclear cells at the implantation site of malignant homografts, and also a blastic response in the regional node. However, the peritumoral white cell reaction reached a peak on the 2nd post-implantation day and from then on gradually subsided to completely disappear by the 6th day.

The Table shows that tumour fragments in millipore chambers remained viable but lost their viability and transplantability when exposed to lymph node cells, particularly to lymphocytes from the tumour bearing host. Close contact with tumour for a period of 7 days also conferred a certain degree of cytotoxicity to non-sensitized lymphocytes.

Cytotoxicity was not affected by serum factors penetrating the pores of the chamber, even though the host carried 2 subcutaneous tumours, one of which 19 days old and about to cause the death of the animal.

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Growth of tumour fragments enclosed for 7 days in an intraperitoneal diffusion chamber and thence grafted into weanling rats

Content of Chamber			
Tumour and nonsensitized lymphoid cells	Tumour and sensitized lymphoid cells	Tumour only	Control Tumour autograft
Number of tumour bearing weanlings			Adults
6/12	1/14	26/26	14/14