was carried out at 37 °C with shaking. The reaction was stopped by the addition of 7.5 ml methylene dichloride-ethanol (5:1) to a 0.5 ml aliquot of the reaction mixture. Steroids were separated by thin layer chromatography on silica gel G with ethyl acetate-hexane (8:2). The bands were visualized with iodine vapor[17], scraped from the plates, and assayed for radioactivity by liquid scintillation spectrometry.

For lipid biosynthesis experiments, 0.5 g slices of liver were incubated at 37 °C for 3 h in 5 ml phosphate buffer (pH 7) containing 0.0006 *M* $MgCl_2$, 0.03 *M* nicotinamide, and 0.5 μCi (0.24 μ*M*) [1-^{14}C]acetate. The reaction was stopped by addition of 15% alcoholic KOH. Cholesterol was extracted from the saponification mixture and isolated as the digitonide[18]. The aqueous residue was acidified to pH 1 with strong mineral acid and fatty acids were extracted into ether. Cholesterol digitonides were dissolved in methanol[19] and assayed by liquid scintillation spectrometry. The fatty acids were counted directly.

All radioactive substrates were purchased from New England Nuclear Corporation, Boston, MA, and the cholesterol was purified by thin layer chromatography prior to use.

Results and discussion. The data are summarized in the Table. Even at a level of 0.3% of the diet, (22R)-22-aminocholesterol significantly affected weight gain. Although the livers of the rats fed the test diet were smaller than those of the controls, when calculated on the basis of g liver/100 g body weight, the livers were of similar proportionate size. The test compound did not significantly affect serum cholesterol or liver cholesterol levels. Liver cholesterol concentrations were identical and although the difference in serum cholesterol was not statistically significant, the cholesterol level of the test group was 24% higher than that of the control group. The average serum plus liver pool was higher in the control than in the test animals (30.1 vs 21.2 mg). The oxidation of [26-^{14}C]cholesterol to $^{14}CO_2$ by liver mitochondria was about 30% higher in the treated rats, but the difference was not statistically significant. The increased level of oxidation was observed both in the presence or absence of cytosol although oxidation in the absence of cytosol was reduced by almost 70%. This reduction of cholesterol oxidation in the absence of boiled supernatant (cytosol) has been observed consistently in our experiments.

The inital step in bile acid synthesis from cholesterol, 7α hydroxylation by liver microsomal preparations, was reduced by 43% in rats fed (22R)-22-aminocholesterol. We are not aware of any other pharmacologic agents which exert so drastic an effect on 7α hydroxylation of cholesterol. Livers of rats fed (22R)-22-aminocholesterol also showed greatly reduced lipogenesis. Liver slices from test rats converted significantly less [1-^{14}C]acetate to cholesterol than did slices from control rats. Conversion of [1-^{14}]acetate to fatty acids was even more severely restricted. Whereas cholesterol synthesis was inhibited by 27%, fatty acid synthesis was inhibited by 76% (15366 vs 3747 cpm/0.5 g of liver; $p < 0.05$).

The azasterols have all been shown to inhibit cholesterol synthesis at the hydrogenation of desmosterol, thus causing accumulation of desmosterol in blood and liver. Gas liquid chromatographic analysis of serum and liver extracts of rats fed (22R)-22-aminocholesterol showed no desmosterol or other sterols suggesting that inhibition of cholesterogenesis occurs at an early stage of the biosynthetic pathway.

The data indicate that (22R)-22-aminocholesterol fed for 7 days does not affect serum or liver cholesterol levels but significantly inhibits the activity of liver enzymes concerned with lipogenesis and cholesterol hydroxylation.

Zusammenfassung. Nachweis, dass das im Futter zugeführte (22R)-22-Aminocholesterin die Gewichtszunahme der Ratten hemmt, aber keinen Einfluss auf die Cholesterinkonzentration in der Leber und im Serum hat. In Leberpräparationen in vitro wird die Seitenketten-Oxidation von Cholesterin nicht beeinflusst, während die 7α-Hydroxylierung und Syntheserate vermindert wird.

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Carrageenin Hyperthermia in Rats

WINTER et al.[1] demonstrated that a subplantar injection of carrageenin produced edema in the rats hind paw. Many pharmacological laboratories adopted this procedure for screening anti-inflammatory agents. VINEGAR et al.[2] and DI ROSA et al.[3] investigated the phases of carrageenin edema. Carrageenin was also found to induce pleurisy[3,4] and an exudative inflammation of s.c. tissue[5]. Biochemical background of the carrageenin-induced inflammation was studied[3,5,6].

We report here that carrageenin also induces a potent hyperthermic response in rats.

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Material and methods. Female Wistar rats, 120–180 g, were kept for 24 h at 25 °C ± 0.5 °C, and then rectal temperature was measured using ELAB termistor. The rat paw volume was measured according to the method of WINTER et al.[1]. Carrageenin (Marine Colloids, Viscarin, Lot No 45602) was sterilized under UV-lamp for 20 min and suspended in germ- and pyrogen-free saline. In some experiments carrageenin was heated to 130 °C for 16 h[2]. Carrageenin suspension was injected in a volume of 0.1 ml into subplantar area of the hind paw (s.p.)[1] or i.p. in a volume 0.5–1 ml. For the s.p. injections carrageenin was used at concentrations 0.001–2% and for the i.p. injections at a 1% concentration. Control animals were injected with saline. Rectal temperature and the paw volume were measured every 0.5–1 h after the injection of carrageenin. Anti-inflammatory and antipyretic drugs: aspirin (100 mg/kg), phenylbutazone (60 mg/kg) and indomethacin (6 mg/kg) were administered orally in the form of a 2%. suspension. Hydrocortisone (10 mg/kg) was injected i.m.

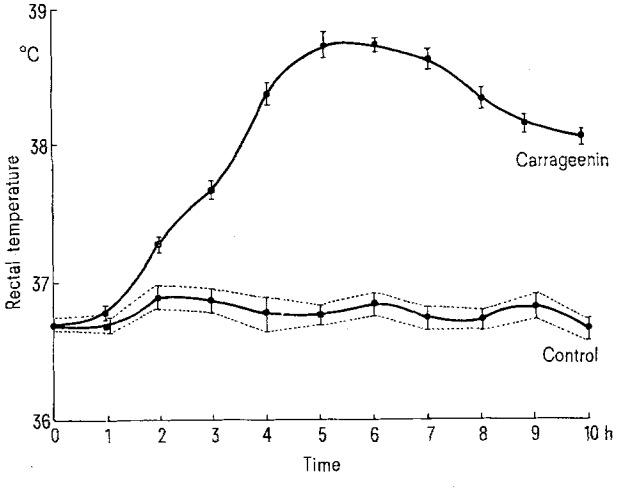

Fig. 1. Increase in rectal temperature of rats injected with 1 mg of carrageenin s.p. Control rats were injected with 0.1 ml of saline. Each point represents the mean ± S.E.M. of pooled measurements taken in 10 groups, each consisting of 10 rats.

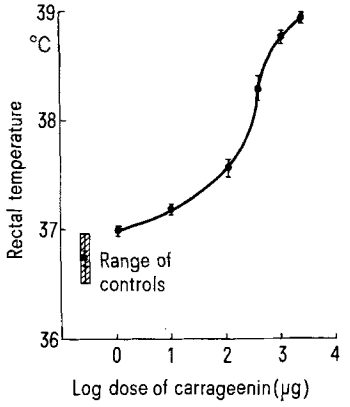

Fig. 2. Dose-dependent increase in rectal temperature 5 h after a s.p. injection of rats with carrageenin at doses from 1 µg to 2000 µg (logarithm scale). Each point represents the mean temperature in 10 animals ± S.E.M. Shaded field covers a range of rectal temperature recorded in 126 control rats.

The drugs were given 1 h before the injection of carrageenin (preventive treatment) or at the peak of the carrageenin-induced hyperthermia (curative treatment). Student's *t*-test was used for statistical analysis

Results. Subplantar injection of carrageenin at a dose 1 mg induces a long lasting hyperthermic response with its maximum +2.1 °C at the fifth h (Figure 1). Hyperthermic effect of carrageenin is dose-dependent at a range of doses from 0.2 to 2 mg (Figure 2). Pyresis due to an i.p. injection of a 10 mg of carrageenin is preceeded by an immediate but transistent decrease in rectal temperature (−0.5 °C), and therefore the peak hyperthermic effect (+2.0 °C) occurs only after 6 h.

Figure 3 demonstrates the difference between hyperthermic and inflammatory activity of native and heated carrageenin. Heated carrageenin has little hyperthermic effect and at the same time it is unable to produce the second phase of inflammation, which is so distinctly developed by native carrageenin.

Aspirin-like drugs administered at active anti-inflammatory doses reduce or delay the carrageenin hyperthermia. Phenylbutazone (60 mg/kg), when used as the preventive antipyretic agent, completely abolishes the carrageenin hyperthermia, while aspirin (100 mg/kg) and indomethacin (6 mg/kg) give a less complete protection. The curative treatment of the carrageenin pyresis with all 3 anti-inflammatory drugs results in a marked decrease in hyperthermia, as soon as 1 h after an injection of drugs. Hydrocortisone at a dose of 10 mg/kg i.m. has no influence on the pyretic response to carrageenin.

Discussion. We have demonstrated that algal polysaccharide, carrageenin, is not only a local irritant, resulting in a circumscripted inflammatory response, pain and local rise in the skin temperature[2], but it is also a potent pyretic agent in rats. The generalized hyperthermia develops between the 2nd and 3rd h and it reaches its maximum at the 5th h from the moment of an injection of carrageenin into the subplantar area. The site of the injection of carrageenin seems to be important for the pyretic effect of carrageenin. 10 times higher doses of carrageenin should be used when the polysaccharide is injected i.p.

The dose-dependence of the hyperthermic effect of carrageenin, our precautions to keep carrageenin germ-free, and the natural resistance of rats against pyresis, leave little doubt that the carrageenin hyperthermia is of a specific nature. The carrageenin hyperthermia resembles pyresis induced in rats by bakers yeast[7], but the carrageenin hyperthermia develops much faster.

The mechanism of the carrageenin hyperthermia may be related to a release of prostaglandins at the site of an injection of carrageenin. Using VINEGAR'S procedure[8], we have found that heated carrageenin does not induce the 2nd phase of inflammation (Figure 3) and at the same time it has little pyretic effect in rats. On the other hand, the appearance of hyperthermic effect of native carrageenin overlaps the appearance of the 2nd phase of its inflammatory action. The 2nd phase of inflammatory action of carrageenin is attributed to the local generation of prostaglandins[3, 5, 6]. Moreover, non-steroidal anti-inflammatory drugs, which are known to inhibit bio-

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synthesis of prostaglandins[9] also abolish the carrageenin hyperthermia, while hydrocortisone is inactive in both respects. It has been reported that prostaglandin E_2 increases body temperature in rats when it is administered into lateral cerebral ventricles[10], but when it is injected i.p., a dose-dependent decrease of body temperature occurs. Therefore, if prostaglandins play any role in the carrageenin hyperthermia, an additional explanation is needed except for local generation of prostaglandins. In unpublished experiments we have found that healthy rats injected i.v. with the exudate (0.1 ml) from the carrageenin foot edema immediately develop a pronounced hyperthermia. A search for a hyperthermic principle in the carrageenin exudate seems to be promising.

We propose to use the carrageenin-induced hyperthermia for screening of antipyretic properties of potential aspirin-like drugs. Carrageenin produces in rats all 3 measurable defensive reactions: inflammation, pain and pyresis. All 3 reactions are abolished by anti-defensive[11] aspirin-like drugs, which inhibit prostaglandin biosynthesis[9].

Zusammenfassung. Nachweis, dass über subplantare Injektionen von Carrageenin eine allgemeine Hyperthermie bei Ratten zu erhalten ist und die pyretische Wirkung des Carrageenins durch nicht-steroidische Antiphlogistica gehemmt werden kann.

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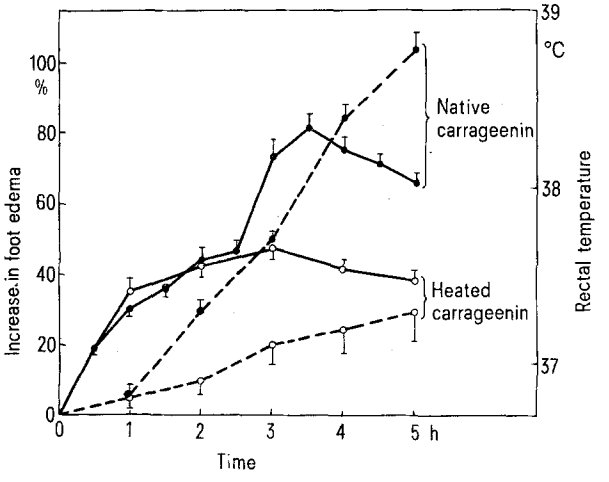

Fig. 3. Carrageenin paw edema (—) and carrageenin hyperthermia (---) induced by native carrageenin (●) or by heated carrageenin (○) injected at a dose of 1 mg s.p. Each point represents the mean of 16 experiments ± S.E.M. except for the group (○---○) consisting of 100 rats.

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5-Hydroxytryptamine in the Blood Platelets of Cirrhotic and Hypertensive Patients

There is evidence of disturbed metabolism and storage of monoamines in the cirrhosis of the liver[1], as well as in various experimental and human hypertensive diseases[2]. The binding and uptake of 5-hydroxytryptamine (5HT) by blood platelets is similar to that of other monoamine storing cells[3,4]. Therefore the blood platelets should reflect the changes occurring in the monoamine storing cells of a diseased organism. This paper reports the concentration and uptake of 5HT by the platelets of patients with cirrhosis of the liver as well as that of patients with essential hypertension.

Cirrhotic patients all had typical signs and abnormal liver function tests. The patients with untreated essential hypertension were divided into 2 subgroups (Table). All hypertensive patients had normal liver- and renal functions. Healthy volunteers served as controls.

About 30 ml of blood was drawn from the antecubital vein with a siliconized needle into polypropylene tubes and mixed immediately with $^1/_{10}$ volume of 3.8% sodium citrate. Platelet-rich plasma was separated by centrifuga-

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Endogenous platelet 5HT content, and platelet count in control, cirrhotic and hypertensive subjects

Group (number of subjects)	Platelet 5HT content (nmol/10^{11} platelets ± S.E.)	Platelet count (per ml × 10^8 ± S.E.)
Control (4♂, 4♀)	300 ± 45	3.4 ± 0.6
Cirrhotic (6♂, 1♀)	129 ± 22[a]	2.2 ± 0.5
Hypertensive Subgroup A (2♂, 3♀)	346 ± 83	3.9 ± 0.2
Hypertensive Subgroup B (1♂, 4♀)	231 ± 51	4.1 ± 1.1

Subgroup A, diastolic blood pressure between 100–109 mmHg; subgroup B, diastolic blood pressure between 110–150 mmHg.
[a] Compared to control $p < 0.01$.