in the lymph than in the plasma from 4 h until one day after operation (Figure 3). Later on, the antiplasmin levels in lymph and plasma do not differ significantly.

The results of our investigations reveal a high fibrinolytic activity in thoracic duct lymph of rats. The variations of this activity in the course of the cannulation allow 2 kinds of interpretation: 1. The first peak in activity is due to the stress of operation. It is well known[9–11] that stress enhances the activation of fibrinolysis in various mammals. After a phase of depletion, there is a second stress-induced peak caused by the confinement in the narrow cages and the manipulations during the sampling periods. 2. Following a stress-induced activation of the fibrinolytic system in lymph and plasma, there is a phase of depletion. On day 6 and 7 after operation the fibrinolytic activity returns to normal values. Our results in these days therefore indicate an activation of the lymphatic fibrinolytic system even in the preoperative state.

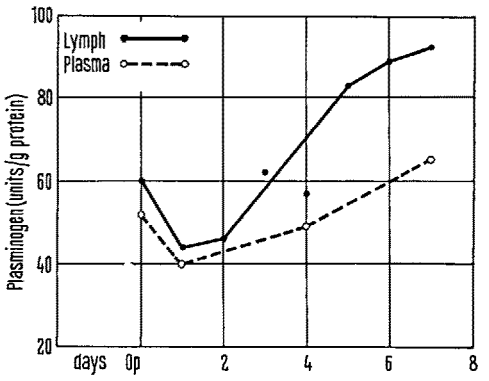

Fig. 2. Plasminogen levels in lymph and plasma of rats during 7 days of thoracic duct cannulation.

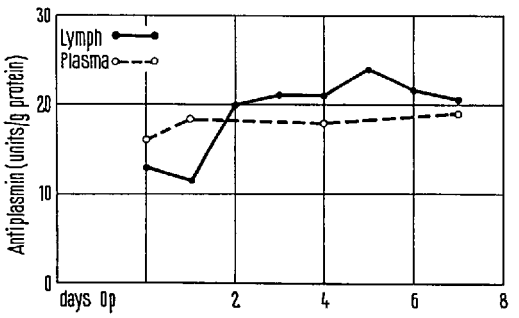

Fig. 3. Antiplasmin levels in lymph and plasma of rats during 7 days of thoracic duct cannulation.

Whatever kind of interpretation may be applied, the results reveal that, at least at some times, the fibrinolytic activity in rat lymph exceeds that of plasma. These findings are in part explained by the higher plasminogen-antiplasmin ratio in lymph, compared with that of plasma. A high plasminogen-antiplasmin ratio in lymph will enhance the fibrinolysis, but plasmin will be present only if an activation of plasminogen has taken place. BEARD et al.[10] stated that after stress the lysosomes in the rat liver release plasminogen activators into the body fluids. One could suggest that the activator reaches the blood stream not directly, but by the way of the lymphatic system. Following this hypothesis, the lymphatic system of rats would play a part in the activation of plasmatic fibrinolysis after stress.

The statement of a high fibrinolytic activity in lymph is in contrast to other investigations performed on dogs[3] and humans[1]. In these reports, however, no data are given as to what time has elapsed between cannulation and lymph sampling. It is possible that the lymph has been sampled in the phase of depletion where the fibrinolytic activity is low. The fibrinolytic system of rats, on the other hand, may behave differently to that of dogs and humans[12].

Zusammenfassung. Ratten zeigen regelmässig zu bestimmten Zeiten nach Ductus-Thoracicus-Kannulierung eine hohe, unter Umständen durch Stress induzierte fibrinolytische Aktivität in der Lymphe; diese Aktivität braucht nicht von einer plasmatischen Fibrinolyse begleitet zu sein.

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[12] Acknowledgments. We want to thank Miss M. KÜBLER for the technical assistence. This work was supported by grant of the 'Landesverband Württemberg zur Erforschung und Bekämpfung des Krebses'.

Immunological Reactivity of a Synthetic Polymannan

In recent years, the water-soluble polysaccharides (galactomannans, mannan, and glucans) of a number of dermatophytes have been studied in this laboratory[1]. Each species investigated contains a galactomannan I, galactomannan II and a glucan, except *Trichophyton rubrum*, which yielded a mannan instead of a galactomannan I. The molecular weights of these polysaccharides are about 10,000.

A synthetic polymannan, free of nitrogen, was obtained from Dr. C. SCHUERCH. It consists of α1→6 linked D-mannopyranose units resembling the basic chain of the dermatophyte galactomannans I, but has a molecular weight of 40,000[2]. It was, therefore, of interest to study the ability of this synthetic polymannan to induce cutaneous hypersensitivity and humoral antibody formation.

The polymannan was dissolved in sterile saline and emulsified in an equal volume of Freund's complete adjuvant for immunization of guinea-pigs and mice.

Hartley strain guinea-pigs were injected with 2.0 mg (Group I) or 4.0 mg (Group II) of synthetic polymannan in Freund's complete adjuvant. A total volume of 1.0 cm^3, 0.5 cm^3 s.c. in the nape of the neck and 0.5 cm^3 in the hind foot pads was injected. 21 days later, guinea-

pigs were tested for dermal reactivity. Guinea-pig skin was shaved and depilated on the day prior to testing. For skin tests, 0.1 cm³ of a 2.0 mg/ml solution of the polymannan in sterile saline was injected intradermally.

The Table shows the 24 h reactions of guinea-pigs injected intradermally with polymannan 21 and 120 days after immunization. Guinea-pigs of Group I did not show any visible reaction at 5 h whereas those of Group II started to develop an erythematous reaction. Both groups showed maximum erythema and induration at 24 h. Guinea-pigs still reacted to the polymannan 120 days after immunization. Both groups developed erythema and induration at 5 h which reached a maximum at 24 h. At this time, 5 guinea-pigs were bled by cardiac puncture. No antibodies were detected by complement fixation tests and only sera of Group II guinea-pigs reacted well by precipitin ring tests.

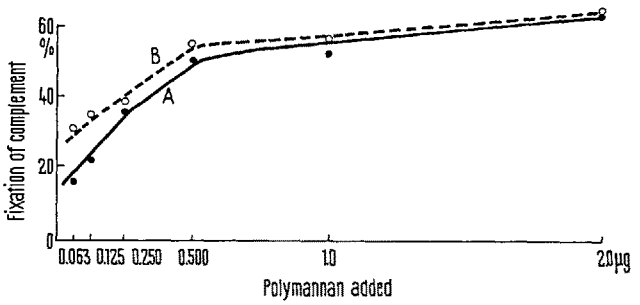
Group I guinea-pigs, 1 and 2, still had dermal reactivity to the polymannan 5 months after initial injection. These were skin tested with the galactomannans I from dermatophyte species *Microsporum quinckeanum*, *Trichophyton granulosum* and the mannan I from *Trichophyton rubrum*, each having molecular weights of about 10,000. Only the mannan from *T. rubrum* elicited a reaction when 0.1 cm³ of a 2.0 mg/ml solution was injected intradermally. There was induration and erythema of 11 and 13 mm diameter after 5 h. The reactions were diminished at 24 h.

Neither the galactomannans I nor the mannan isolated from dermatophytes elicited any cutaneous hypersensitivity reactions in normal guinea-pigs or in cutaneously infected guinea-pigs[3]. The synthetic polymannan induced an erythematous reaction in 2 guinea-pigs previously infected with *T. mentagrophytes*. The average diameters of the erythema were 10 mm and 12 mm after 5 h. These reactions disappeared by 24 h. Similar reactions were elicited in 2 normal guinea-pigs and in 2 guinea-pigs injected with Freund's adjuvant alone.

Cutaneous reactions of guinea-pigs immunized with α1→6 polymannan[a]

Group	21 days Average diameter 24 h	120 days Average diameter 24 h
I (1)	10 mm	13 mm
I (2)	18 mm	9 mm
I (3)	19 mm	15 mm
II (1)	14 mm	X[b]
II (2)	15 mm	14 mm
II (3)	12 mm	8 mm

[a] Reaction is given as the average diameter of the erythema. There was induration and erythema at the site of injection. [b] X = omitted.



Complement fixation curves of the reactivities of pooled sera from mice immunized with polymannan. 0.2 cm³ of a $^1/_{100}$ dilution of serum was used for analysis.

Polysaccharides free of nitrogen do not usually induce or elicit delayed hypersensitivity reactions in guinea-pigs. Rare instances of induction of the delayed response with polysaccharides have been reported using the antigen emulsified in Freund's complete adjuvant[4,5]. In these studies the polysaccharides were of microbial origin and still contained traces of nitrogen.

The ability of this synthetic polymannan to induce humoral antibody formation was subsequently investigated in strain ICR mice. Two groups of 10 mice were immunized with the synthetic polymannan in Freund's complete adjuvant. A total volume of 0.04 cm³ containing 25 µg (Group A) and 50 µg (Group B) was injected, 0.02 cm³ per hind foot pad. Sera were obtained by orbital bleedings 12 days after injection. Pooled sera from each group were analyzed by precipitin ring tests and complement fixation analysis. These sera reacted by precipitin ring tests. The Figure shows the complement-fixation reactions of pooled sera from both groups with the polymannan. Neither the galactomannans I nor the mannan from *T. rubrum* reacted with these mouse antisera by complement fixation analysis.

The synthetic polymannan did not fix complement with any of the normal mouse sera tested. However, it did react with normal sera from adults, including mice, rabbits, guinea-pigs and humans by precipitin ring tests. Although the nature of these reactions was not readily explainable, sera from 20 newborn babies did not react with the polymannan by precipitin ring tests. These results tend to rule out nonspecific interaction of this polymannan with serum proteins.

This preliminary investigation indicates that a nitrogen-free polysaccharide can induce a delayed-type of cutaneous reactivity in guinea-pigs as well as humoral antibodies in mice. In addition, this synthetic polysaccharide confers cutaneous reactivity to a dermatophyte polysaccharide. This phenomenon could be of great significance for diagnosis and protection and will be pursued[6].

Zusammenfassung. Ein Stickstoff-freies Polysaccharid bewirkt eine cutane Überempfindlichkeit beim Meerschweinchen sowie Komplement fixierende Antikörper bei Mäusen.

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[6] Acknowledgment. The synthesis of this polymannan was achieved at the State University College of Forestry at Syracuse University under research grant No. GM 06168 of the Division of General Medical Sciences, National Institutes of Health. This investigation was supported by a grant from the John A. Hartford Foundation, Inc., New York, N.Y. and by Public Health Service research grant No. 1 R01 A107705 from the National Institute of Allergy and Infectious Diseases.