4 °C and the hypotonic treatment was repeated twice. The cells were finally washed once then resuspended in sterile PBS to a cell population density of 1×10^8 cells per ml. This preparation of cells will subseqeuntly be referred to as 'ghosts', since the method used was similar to that used by DODGE et al.[8] for the preparation of erythrocyte ghosts.

Female CFLP mice were immunized by i.p. injection of a suspension of 'ghosts'. A challenge with viable tumour cells was made by i.p. injection 10 days after the last immunizing dose of 'ghosts'. The challenged mice were examined daily and those animals with palpable ascites tumours were recorded and sacrificed. The experiments were terminated after 30 days. Protein was measured by the method of LOWRY et al.[9].

Over 80% of the original cell numbers were recovered as 'ghosts', of which up to 20% were still viable by dye exclusion[10]. A loss of 25% of the TCA precipitable protein was found to occur during the preparation of 'ghosts' but a 100% recovery of the plasma membrane marker enzyme – $(Na^+ + K^+)$ ATPase and 90% recovery of the antigenic material – measured with ^{125}I-labelled IgG from 'ghost'-immunized mice, was found on a per cell basis (preliminary results).

Despite the high viability of the 'ghosts', determined by dye exclusion, the incidence of tumour development after 2 inoculations with 1×10^6 'ghosts', at an interval of ten days, was less than 5%.

The incidence of tumours in female CFLP mice immunized with a single dose of lysed cells and challenged with 10^5 EAT cells

Immunizing dose No. lysed cells	Incidence of tumours at 30 days
0	14/15[a]
10^1	13/15
10^2	14/15
10^3	15/15
10^4	14/15
10^5	10/15
10^6	5/15
10^7	2/10

[a] Ratio of the number of mice with tumours at 30 days to the total number of mice in the group.

Two i.p. injections of 1×10^6 'ghosts' at an interval of 10 days completely protected mice against a challenge with 5×10^5 viable EAT cells. The combined washings, obtained during the preparation of 'ghosts', when administered i.p., failed to protect mice against a challenge with 5×10^5 viable EAT cells. These inoculations were administered in an amount equivalent, on a protein basis, to 10^6 'ghosts'; approximately 250 µg protein. It is apparent therefore that the immunizing antigens are associated with the membrane systems of the EAT cell.

Mice immunized with a single dose of 'ghosts' were not protected against a challenge with 1×10^5 EAT cells until the immunizing dose of 'ghosts' was equivalent to or greater than the challenge dose of cells (Table).

It has been suggested that the immunogenicity of weak antigens of membrane origin is imapired if the integrity of the membrane is destroyed[11–13]. The simple expedient of hypotonic lysis offers a rapid method for the recovery of the membrane components of the EAT cells in an apparently intact form and high yield. Such a preparation is most suitable for the production of sera against membrane bound antigens without recourse to the use of adjuvants. The protection of mice which such a preparation provided was better than[4,5] or equivalent to[14] that reported by others.

Résumé. On décrit quelques caractéristiques biochimiques et immunologiques des «ghosts» de cellules de la tumeur ascitique d'Ehrlich, produits par une méthode facile de lyse hypotonique. Ces «ghosts» ne forment pas de tumeurs quand on les injecte aux souris par voie i.p. et ils protègent celles-ci contre les cellules EAT viables.

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Immunofluorescent Studies in Isoproterenol-Induced Myocardial Necrosis in Rats

The presence of circulating antibodies against human heart has been reported in the post-myocardial infarction and post-pericardiotomy syndromes, in acute ischaemic heart disease and in a variety of myocardial diseases. Circulating antibodies have been demonstrated by haemagglutination, complement fixation, antiglobulin consumption and immunofluorescent techniques[1–4]. It was possible to produce circulating anti-heart antibodies in experimental animals immunized by xenogenic and allogenic heart extracts. Studies on rats kept at intermittently reduced barometric pressure demonstrated the appearence of circulating anti-heart antibodies[5].

RÓNA et al.[6] described that isoproterenol (IPR) given s.c. once daily for 2 days caused infarct-like myocardial necrosis in rats. WEXLER and KITTINGER[7] considered the pathogenesis and repair of IPR-induced myocardial necrosis as similar to infarct in man. We supposed that the IPR-induced necrosis might liberate antigenic substances and provoke immune reaction. The purpose of the present study was to demonstrate anti-heart antibodies after IPR injections by immunofluorescence.

Material and methods. Male Wistar rats with initial weights in the range of 180–200 g were used in 3 groups: 1. Animals were injected s.c. once daily for 2 days with 400 mg/kg IPR. 2. Animals were treated once daily for 7 days with i.p. injections of 5 mg/kg IPR. Sera from animals of group 1. and 2. were drawn weekly for 6 weeks for indirect immunofluorescence (sandwich technique). 3. Animals were treated as the first group, they were killed weekly, their hearts were examined by direct immunofluorescence. Control animals were treated with isotonic saline solution. Circulating antibodies were demonstrated

Anti-heart antibodies bound to rat myocardium following 400 mg/kg isoproterenol-induced necrosis

Weeks after 2 × 400 mg/kg Isoproterenol treatment	Intensity of the fluorescence					No. of hearts
1st week	−	−	−	+	−	5
2nd week	−	++	++	+	++	5
3rd week	++	++	++	++	+++	5
5th week	+++	++	+++	+++	+++	5
6th week	+	−	+	++	+++	5

Fluorescence was graded as follows; −, no fluorescence; +, weak; ++, strong; +++, bright fluorescence.

by indirect immunofluorescence. 8 μm cryostat sections of normal rat heart were used as substrate. The sections were treated with appropriate serum dilutions, then with fluorescein isothiocyanate labeled goat anti-rat γ-globulin (Hyland). Bound antibodies were demonstrated by direct immunofluorescence. The sections were stained with 1:32 diluted conjugate.

Results. Specific fluorescence was seen in the sarcolemmal, subsarcolemmal sites of myofibers in all positive sections prepared by direct or indirect technique. Anti-heart antibodies were present in 90% of rats treated with 400 mg/kg IPR once daily for 2 days.

Circulating antibodies were investigated at various intervals after IPR necrosis (Figure). 1:10 or higher dilutions of sera of control animals did not show fluorescence. Anti-heart antibodies appeared in the blood 2 weeks after IPR treatment. Titres of antibodies gradually increased, their maximum was seen on the 5th week and no further increase could be observed.

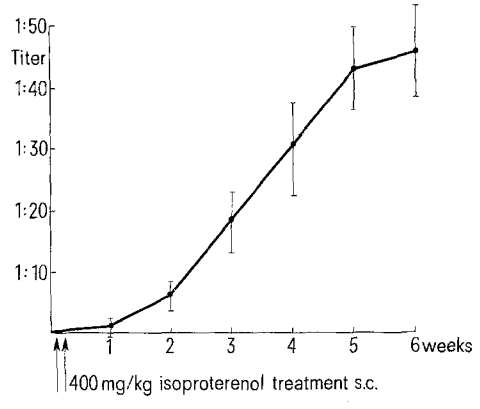
Anti-heart antibodies bound to heart was found from the second week after IPR treatment (Table). Bright fluorescence was observed in all sections in the 3rd and 5th week after 2 × 400 mg/kg IPR.

Macroscopic lesions could be seen in the hearts of treated animals. These were well demarcated pale areas involving the apex, the adjoining left ventricle and the apical portion of right ventricle.

Chronic IPR treatment of rats did not evoke unequivocal results. Appearence and slight increase of circulating anti-heart antibodies could be detected in 1 case while no detectable amount of antibodies could be observed in the other 9 cases.

Discussion. IPR induces hypotension accompanied by increased cardiac work and enhanced myocardial oxygen demand leading to infarct-like lesions[8]. Necrosis may cause antigenic changes in heart tissue which initiate specific immune reactions. In our experiments we have found that myocardial necrosis induced by 400 mg/kg IPR evoked the production of specific circulating anti-heart antibodies in rats. Titres of antibodies gradually increased and their maximum was seen in the 5th week after IPR treatment. Appearence of antibodies demonstrated in patients after myocardial infraction or commissurotomy accounts for changes in the special structure of myocardial proteins. Significance of anti-heart antibodies is not yet clear. Probably they have no pathogenic role, though it is possible that the high level and persistence of anti-heart antibodies is connected with complications of post-infarction syndrome[3]. Circulating anti-heart antibodies produced after IPR necrosis are able to bind in vivo to heart tissue. We could demonstrate antibodies bound to heart from the second week after IPR injections. Small doses of IPR given chronically cause hypertrophy and micronecrosis in rats[9], but in our experiments did not activate the production of antibodies. Circulating anti-heart antibodies could not be demonstrated after 120 mg/kg IPR treatment by haemagglutination methods in young rats[10].

It would be important to study the immunological processes after experimental infarction because the nature of anti-heart antibodies is unknown and their possible role in the pathogenesis of the progressive myocardial damage is not yet clear.



Titres of circulating anti-heart antibodies following isoproterenol-induced myocardial necrosis in rats. Points represent averages ± SE of 10 animals.

Zusammenfassung. Immunofluoreszenz-Nachweis von Myokard-Antikörpern nach Isoproterenol-induzierter Myokardnekrose bei der Ratte in 90% der Tiere.

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