from a 1/1 to 1/6 dilution agglutinated both sucrose washed normal erythrocytes and sucrose washed infected erythocytes whereas haptoglobin antiserum did not agglutinate normal erythrocytes but did agglutinate infected erythrocytes from a 1/1 to 1/4 dilution.

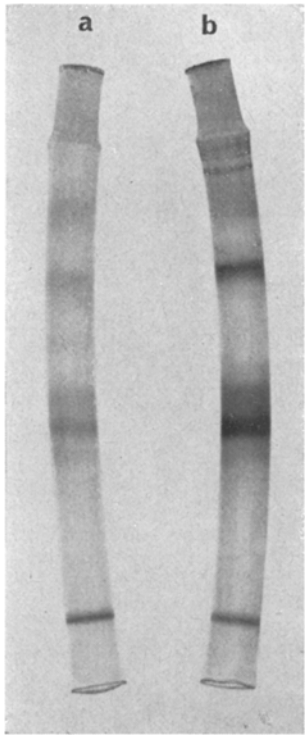

Fig. 2. Disc electrophoresis in 5% acrylamide gel of the saline eluate of sucrose washed normal bovine erythrocyte (a) and the saline eluate of sucrose washed bovine erythrocytes infected with *B. argentina* (b). The series of high molecular weight polymers in (b) was due to haptoglobin. The fast migrating band in both samples was due to a bovine albumin-amido black 10B marker.

These results are in accord with those of FIDALGO et al.[1] and indicate that sucrose washed erythrocytes appear to be coated with immunoglobulin. Whereas the human immunoglobulin is a distinct physiochemical fraction of IgG, the bovine counterpart appears to be solely IgG_2 as judged by its slow mobility and IgG reactivity. Like its human counterpart it probably aids in maintaining erythrocyte shape[8].

The presence of fibrinogen in the eluate of sucrose washed bovine erythrocytes requires some comment. It did not originate in platelets[9] which were removed during preparation of the erythrocytes. In addition, the haemagglutination of sucrose washed erythrocytes with fibrinogen antiserum indicated that the fibrinogen was complexed with erythrocytes. This finding agrees with the hypothesis that there is a basic relationship between erythrocytes and fibrinogen[10] and may explain why repeatedly washed stroma always contains this protein[11,12].

The presence of bovine haptoglobin in the eluate from sucrose washed erythrocytes infected with *B. argentina* is also of interest. Bovine haptoglobin differs markedly in polymerization sequence and chemical structure from its human counterparts and is not normally present in serum, but appears in times of stress[6]. Its presence in the eluate is difficult to explain but because of its specific and avid binding capacity for haemoglobin, it may act as a biological plug to prevent haemoglobin leakage from erythrocytes whose membranes have been damaged by the growth of the intracellular parasite.

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Autoantibody Formation Against Spleen in Rats 'Chemically Splenectomized' by Ethyl Palmitate

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3 November 1975.

Summary. The follow-up experiments with rats revealed auto-antibodies against spleens damaged by an i.v. ethyl palmitate injection. The antibodies could be shown in some animals only.

The work of STUART[1] and PROSNITZ et al.[2] report on a single i.v. injection of ethyl palmitate (EP) causing acute spleen destruction in some laboratory animals, a so-called 'chemical splenectomy'. We found a similar pharmaceutical effect of EP in a model experiment with Wistar rats (JIRÁSEK and ŠEBESTIK[3], ŠEBESTÍK et al.[4]). The administration of EP caused an initial storing of this drug chiefly in cells of spleen sinuses; after 24–48 h most animals developed a segmental or even total necrosis of the spleen. On day 5 to 7, following application of EP, some spleens revealed signs of recovery proportional to the extent of damage. The histological effect of EP, especially at the initial stage following injection of EP, was followed by a pronounced metabolic alteration of proteins and nucleic acids in the spleen (KUŽELA and ŠEBESTÍK[5]). Thus the damaged organ, as known from human pathology, may act in some diseases as an alien antigen and induce antibody formation. Since some research workers experiment with the possible application of EP which may abolish non-surgically, partially or completely, the function of the spleen, this finding is expected to become of actual importance.

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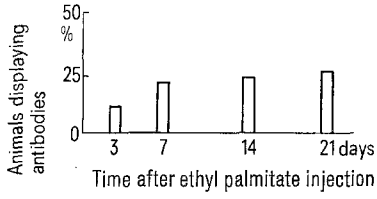
Material and methods. For the experiments, male Wistar rats (Velaz) of 220 ± 20 g of weight were used. The EP-emulsion in concentration of 0.5 g EP/ml was prepared as follows: The mixture of EP (Eastman, Rochester N.Y.) and 1% of Tween 20 in a 5% solution of glucose was first homogenized for 10 min in a glass homogenizer and then exposed to sonication for 5 min (ŠEBESTÍK et al.[6]). The particle size oscillated about 1 μm. This emulsion was injected to 4 groups of 10 animals each, into the tail vein, in amounts of 0.4 g EP per 100 g weight of the recipient. 2 days prior to injection of EP, 1.5 ml blood samples were collected from the incized tail vein of all animals for examination of antibody titers. After injection of EP, the second withdrawal for the group I, II, III and IV was performed on day 3, 7, 14 or 21 respectively. The antibodies against spleen were examined by the complement consumption test (CHUDOMEL et al.[7]). As antigen, the titrated rat spleen was used; thus less than 2% variances of biological results could be obtained.



Occurrence of auto-antibodies against spleen damaged by EP.

Results and discussion. Our experiments with rats demonstrate that auto-antibodies against spleen damaged by a single i.v. injection of EP are formed only in a limited number of animals, as shown in the graph. Where they do occur these autoantibodies are massive (semiquantitative evaluation).

The explanation why antibodies against spleens damaged by EP were formed in some animals only is difficult. We assume that a single injection of EP does not always induce antigenic changes massive enough to be normally detectable. Lastly, even the histological effects of EP have not always been of the same extent, and at a later stage after an injection of EP, signs of recovery in some spleens could be observed[3,4]. It may be useful to apply an immunization pattern using repeated smaller doses in order to establish suitable conditions for a permanent antigenic stimulus.

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Induction of Immunological Memory in Mice by RNA Extract

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Summary. RNA extract isolated from spleens of mice immunized with lipopolysaccharide from *E. coli* induced an immunological memory in normal mice. Application of small amounts of corresponding antigen provoked a specific secondary immune response in RNA primed mice.

RNA extracts have been shown to transfer immunological activity to non-sensitive lymphoid cells in certain in vivo and in vitro systems[1]. Recently MITSUHASHI and his collaborators have demonstrated that RNA extracted from mice or guinea-pigs, immunized with either diphtheria toxoid[2], sheep red blood cells (SRBC)[3], or *Salmonella flagella*[4], was capable of inducing immunological memory. RNA-primed animals are capable of responding to a small amount of corresponding antigen with a characteristic secondary immune response.

The following experiments were undertaken to ascertain whether it is possible to induce an immunological memory by RNA extract isolated from spleens of mice immunized with lipopolysaccharide (LPS) from *Escherichia coli*.

Materials and methods. Female mice of the AB strain, weighting 16 to 18 g, fed on a standard diet, were immunized with 10 μg LPS given in a single i.p. injection. LPS for immunization was extracted from *Escherichi coli* O 111:K 58 by the method of BOIVIN[5]. 5 days after antigen application, the mice were sacrificed and the spleens were pooled. RNA was isolated according to a modification of the method of ABRAMOFF[6] only with the exception of homogenizing the spleens in 0.05 *M* K-acetate buffer pH 5.8 containing 0.1% Na-dodecylsulphate and 0.001 *M* EDTA. Spleens of non-immunized mice were used for preparation of normal RNA (n-RNA). All procedures were carried out at 4 °C. RNA extracts were stored as pellets under ethanol at −20 °C until used in experiments. Spectrophotometric analysis of the RNA samples consistently showed an OD 260/280 of 1.98 to 2.10.

RNA extraction procedure provided a relatively pure preparation in which contaminations were limited to 1.5 to 2.5 μg protein and 0.5 to 1.5 μg DNA per mg RNA. Protein and DNA were measured by the method of LOWRY[7], using bovine serum albumine as standard, and the Indol test[8] respectively.

Gel chromatography of RNA samples on Sephadex G-100 (1.5 × 90 cm) in phosphate-buffered saline at pH 7.2 (PBS) yielded 3 peaks with the K_{av}-values 0.04, 0.38 and 1.00 respectively. Serum antibodies were determined by the indirect hemagglutination using the microtechni-

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