The parallelism observed between RFC stimulation and lymphoblast transformation suggest that the rapid and simple RFC test could substitute the more laborious investigation of the mitogen-induced lymphoblast transformation for some purposes.

Zusammenfassung. Nachweis, dass Phytohaemagglutinin und Concanavalin A die spontane Rosettenbildung der menschlichen Lymphozyten anregt. Die Stimulation der Rosettenbildung und Lymphoblast-Transformation ergibt parallele Ergebnisse.

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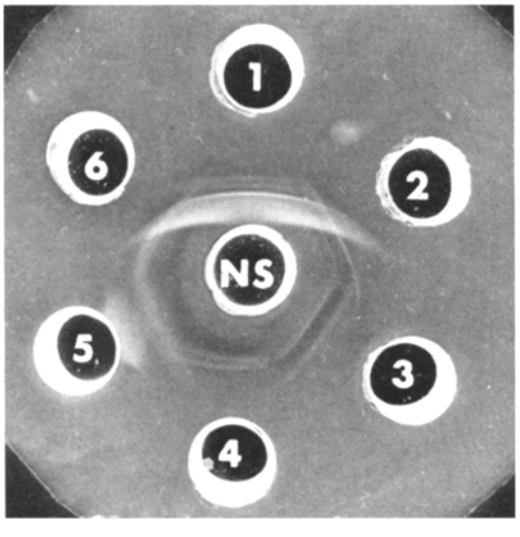
Tissue- and Species-Specific Antigens of the Rat Submandibular Gland

The presence of tissue-specific antigens have been detected in submandibular glands of rabbit[1], cattle[2], and man[3,4]. The object of the present study was to investigate the tissue- and species-specificity of the rat submandibular gland as a part of an effort to evaluate the antigenic constituents of normal salivary glands and their tumors.

Method. The submandibular glands of adult male Long-Evans rats were separated, and homogenized in saline. The homogenate was centrifuged at 10,000 rpm for 10 min and the supernatant was collected. Extracts of other rat tissues as well as submandibular glands of other species were prepared in the same manner. The protein concentration of the saline extracts was determined by the method of Lowry et al.[5]. Antisera were prepared by injecting saline extracts of rat submandibular gland subcutaneously into rabbits. The extract was incorporated into Freund's complete adjuvant for the first injection and into incomplete adjuvant for subsequent injections. Antiserum was absorbed with lyophilized rat serum in a concentration of 80 mg/ml, which was found to be a proper concentration for neutralization. When necessary, further absorption was carried out using lyophilized extracts of other rat tissues.

Two-dimensional immunodiffusion was performed according to the method of Ouchterlony[6]. Immunoelectrophoresis was carried out according to the method of Grabar and Burtin[7] as modified by Scheidegger[8] using 1% agarose in 0.05 *M* barbital buffer at pH 8.6. Cryostat sections of quick frozen rat submandibular gland were fixed in acetone and stained directly with fluorescein isothiocyanate conjugate of antiserum globulin according to the technique of Coons and Kaplan[9].



Immunodiffusion in agar gel. Antiserum to rat submandibular gland extract absorbed with rat serum and kidney extract (NS). Rat glandular extracts: 1. submandibular; 2. parotid; 3. extraorbital lacrimal; 4. intraorbital lacrimal; 5. pancreas; 6. sublingual.

Results and discussion. Immunodiffusion studies of antisera to submandibular gland exhibited several lines of precipitation with extracts of rat submandibular gland and other rat tissues, as well as with rat serum. Following absorption of the antisera with lyophilized rat serum, some precipitation lines with extracts of several rat tissues still remained. Further absorption of the antisera with extract of rat kidney abolished the reaction with the majority of rat tissue extracts and demonstrated that rat submandibular gland contained at least 5 antigenic components as well as other antigens shared with blood serum and tissues of the rat. Cross-reaction was still present with extracts of related exocrine glands: parotid, sublingual, pancreas, extraorbital and intraorbital lacrimal (Figure). Further absorption by these glands revealed that 2–3 of these antigens were specific for the submandibular gland and the remaining antigens were shared to varying degrees with the other glands. The immunologic relationship between these glands is substantiated by previous studies using antiserum to rat parotid gland[10].

It was also noticed that, of the 5 lines of precipitation exhibited on reacting the antisera with extracts of submandibular glands of male rats, only 4 lines could be detected with glands of adult female rats. It is interesting that this antigenic sexual dimorphism of the rat submandibular gland is associated with the presence of morphological differences between the glands of male and female[11].

Studies of glandular extracts of other species demonstrated high degree of species-specificity of the rat submandibular gland. Comparisons were made with extract preparations of submandibular glands of man, monkey, dog, guinea-pig and mouse and no cross-reaction was detected. Also, no cross-reaction was detected when the

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antisera were studied with extracts of several rabbits including the the ones used for antisera preparation, indicating that under the present experimental conditions heteroimmunization of rabbits with rat submandibular gland extract did not elicit the production of auto- or isoantibodies.

Immunoelectrophoretic studies confirmed these observations and demonstrated the presence of both serum albumin and globulins in addition to other antigenic components. The presence of serum constituents was confirmed by studying the gland extract with antiserum to rat serum. The specificity of the reaction of the antiserum was confirmed by immunofluorescent staining. When gamma globulin of absorbed antiserum to rat submandibular gland was used for direct immunofluorescence, specific bright green fluorescence was noticed in cytoplasm of the acinar, tubular and ductal cells.

Résumé. En évaluant par héteroimmunisation les caractères antigéniques de la glande submandibulaire du rat, nous avons découvert plusieurs antigènes spécifiques et autres mis en réaction avec le sérum sanguin et les tissus. Lorsque l'on compare les extraits glandulaires de diverses espèces, on constate que la glande submandibulaire du rat a des caractères hautement spécifiques.

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A Circulating Activator of Granulocytes in Liver Disease

Patients with chronic alcoholism and liver disease have been shown to be associated with increased morbidity and mortality from infections[1–3]. In an attempt to elucidate the cause of this increased susceptibility to infections, granulocyte function in patients with liver disease was investigated, as measured by their phagocytosis-stimulated glucose oxidation (PSGO). No intrinsic granulocyte defect was found, but plasma from these patients was shown to cause an increase in the resting glucose oxidative activity of granulocytes.

Methods. 15 patients with liver disease (10 with alcoholic cirrhosis, 5 with infectious hepatitis) were investigated. Normal blood specimens were obtained from healthy subjects. 20 ml of blood were collected into a heparinized plastic syringe; 10 ml were centrifuged and the plasma was collected. The remaining 10 ml were used for the separation of leukocytes by dextran sedimentation. The white cells were centrifuged and resuspended in either the patient's or normal plasma at a concentration of 1×10^7 to 2×10^7 leukocytes/2 ml plasma. Differential leukocyte counts were performed on these suspensions. The PSGO activity of granulocytes was measured continuously by an ionization chamber method[4]. Base line (resting) metabolism and maximal rate of metabolism after stimulation with 0.05 ml of a 10% suspension of latex particles (0.81 microns – Dow Chemical Co.) were measured for patient cells in patient plasma and in normal plasma, and for normal cells in patient plasma and in normal plasma. Studies were also carried out with normal cells in Krebs-Ringer-Bicarbonate buffer (pH 7.4) with 50 mg/100 ml glucose and either 2 g/100 ml or 4 g/100 ml bovine serum albumin (BSA). Bilirubin (B-grade, Calbiochem) was dissolved in 1 *N* NaOH and the pH was adjusted to 7.4 with 1 *N* HCl. Bilirubin was added in a concentration of 12.5 mg/100 ml or 25 mg/100 ml to appropriate leukocyte-buffer suspensions and PSGO measurements were performed. Glucose in plasma was measured by Glucostat (Worthington Biochemical Corp.).

Table I. Phagocytosis-stimulated glucose oxidation by granulocytes

Number studied		Resting[a] mean ± S.E.	Stimulated[a] mean ± S.E.
15	Normal cells in normal plasma	115 ± 16	414 ± 42
15	Normal cells in patient plasma	185[b] ± 27	364 ± 34
15	Patient cells in patient plasma	145 ± 22	369 ± 55
15	Patient cells in normal plasma	120 ± 21	438 ± 77

[a] Nanomoles CO_2/h per 10^7 granulocytes; [b] $p < 0.02$, compared to normal cells in normal plasma.

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Table II. Summary of bilirubin effect on granulocyte activity

No. of Experiments	Albumin (g/100 ml)	Bilirubin (mg/100 ml)	Resting[a] Mean ± S.E.	Stimulated[a] Mean ± S.E.	Stimulated/Resting Mean ± S.E.
6	4	0	62 ± 10	285 ± 27	5.0 ± 0.7
2	4	12.5	69 ± 12	342 ± 27	5.2 ± 1.2
3	4	25.0	72 ± 27	260 ± 14	5.7 ± 2.6
5	2	0	40 ± 7	255 ± 46	6.6 ± 0.7
6	2	25.0	89[b] ± 14	176 ± 22	2.1 ± 0.1

[a] Nanomoles CO_2/hr/10^7 granulocytes; [b] $p < 0.02$, as compared to 2 g/100 ml albumin and 0 mg/100 ml bilirubin