between 9 and 11. A titer of 6 was observed when anti-vesicle serum was tested against ganglion. However, the anti-ganglion serum did not produce positive reactions with synaptic vesicles. These results would imply that the central nervous system shares some antigens with the peripheral nervous system[13,14].

The immunofluorescence analysis revealed different histological lacolizations of antibodies from anti-ganglion and anti-vesicle sera. Namely, the anti-ganglion serum reacted almost exclusively with cell membranes and the thick network of nerve fibres of the ganglion (Figure A), whereas the anti-vesicle serum combined preferentially with antigens situated within the neuron (Figure B). Large, round to oval nuclei, usually excentrically placed, remained unstained in all examined sections. The staining of ganglion sections was inhibited by absorption of antisera with corresponding antigens, thus pointing to the presence of organ-specific antigens in the preparations used for immunization. No staining was observed in control tests. A number of ganglion sections treated with anti-vesicle serum exhibited a faint fluorescence of inter-neuronal particulates other than blood vessels. This was probably due to the contamination of the synaptic vesicle preparation with other antigens[4,15]. Besides, antibodies other than those reacting with synaptic vesicles, e.g. anti-tubulin antibodies[16], might be contributing to the staining. This aspect of cross-reactivity between different neuronal components is now under investigation. Relevant to the problem of antibody specificity is the observation that a rabbit anti-nerve ending membrane serum affects the axoplasm and synaptic vesicles[17]. The present experiment does not establish evidence with respect to the antigenic similarity between brain synaptic vesicles and cervical ganglion antigens, and a variety of rat brain specific antigens[18–20].

In summing up, the immunofluorescence analysis described here provides some basic information about the localizing properties of antibodies from anti-cervical ganglion and anti-brain synaptic vesicle sera, and thus justifies the use of those immune reagents in a functional study of the rat superior cervical ganglion[21].

Résumé. On a démontré que les anticorps contre les vésicules synaptiques du cerveau du rat se fixent de préférence dans le cytoplasme des neurones, tandis que les anticorps contre les ganglions sympathiques cervicaux supérieurs réagissent presqu'exclusivement avec les membranes des neurones du ganglion.

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Ovarian Cyst Fluid Specific Antigens

The presence of ovarian cyst specific antigens have been detected earlier[1,2]. A preliminary report of our preparation and partial characterization of the antigens have shown that they are glycoprotein containing 12.3% fucose (FRGP)[2]. Furthermore, anti-FRGP antibody reacted with an extract of human spleen. Several reports have presented evidence of antigens with organ specificity for human heart[3,4], liver[5], and basement membrane[6,7]. Renal glomerular basement membrane glycoproteins shared their antigenecities with basement-membrane-rich organs such as lung, placenta and urinary glycoprotein[7]. Limited studies of ovarian cyst content were performed using immunological procedures. The present communication describes the chemical and immunological properties of FRGP of ovarian cyst origin.

Materials and methods. Approximately 200 l of ovarian cyst fluid from a patient with ovarian cystoma were collected and filtered. After 10 l of the fluid were mixed with 3.62 kg of solid ammonium sulfate and 30 ml of concentrated hydrochloric acid to pH 4.3, the mixture was allowed to stabilize for 20 h at 4 °C. The centrifuged supernate was acidified with concentrated hydrochloric acid to pH 3.7, and then 2.31 kg of ammonium sulfate were added for 100% saturation. After a 20 h period at 4 °C, the precipitated crude glycoprotein (CGP) was recovered by centrifugation, dialyzed and lyophilized. Ethanol was added to a 2% solution of CGP in stepwise fashion to give concentrations of 38%, 66% and 80% (v/v) at 4 °C. A glycoprotein fraction obtained at 38–66% (v/v) ethanol concentration (first stage) was refractionated using the ethanol procedure described above. The precipitate formed at 38–66% (v/v) ethanol concentrations was centrifuged at 24,000 *g* for 80 min at 4 °C. The supernatant (FRGP) was lyophilized. Frozen post-mortem tissues from pulmonary carcinoma patients were thawed and homogenized in cold distilled water. Soluble constituents were collected by centrifugation and lyophilized. Approximately 100 mg of the distilled water extract were dissolved in 30 ml of distilled water and subjected to 'first stage' ethanol fractionation. Pseudomucins were a gift of Dr. Y. Nagai, Fukushima Medical College. Rabbit antisera against FRGP were prepared by repeated injections of the antigen with Freund's complete adjuvant (Difco). The antisera were absorbed with lyophilized pooled normal human serum[8]. Immunological analyses

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Yield and chemical composition of glycoproteins

Fractionation stage	Yield (% of amount present in the original ovarian cyst content)	Chemical composition (%)[a]				
		Fucose	N-acetyl neuraminic acid	Galactose	Hexosamine[b]	Protein
Ammonium sulfate fractionation (crude glycoprotein)	21.3	5.3	1.6	9.3	12.0	63.0
Ethanol fractionation (fucose-rich glycoprotein)	0.7	12.3	2.8	17.2	16.4	58.5

[a] Values expressed as dry weight basis. [b] Determined after hydrolysis of samples in 2 *N* HCl for 4 h at 100 °C[16].

were carried out using the Ouchterlony double diffusion procedure[9] and also the immunoelectrophoretic method of SCHEIDEGGER[10]. Measurement of hexose, fucose, and sialic acid constituents was accomplished by means of the orcinol[11], GIBBONS[12] and WARREN[13] procedures, respectively. The method of LOWRY et al.[14] was used for protein analysis, with bovine serum albumin as standard.

Results and discussion. Recovery and composition data for the 2 glycoprotein fractions are shown in the Table. The initial yield of FRGP was 0.61 g from 10 l of ovarian cyst fluid. Galactose and fucose were identified by gas liquid chromatography[15] as the only neutral sugar components present; mannose was not detected. Glucosamine and galactosamine were detected[15,16]. The total carbohydrate content of the CGP was 28.2% while that of FRGP was 48.7%. Results of the carbazole-sulfuric acid test[17] were negative. The anti-FRGP serum absorbed with normal human serum (Ab-NHS) used in this work did not produce precitin lines when tested with pooled normal serum or serum obtained from the patient whose ovarian cyst fluid was used in this study. Immunoelectrophoresis of FRGP against antiserum produced 4 or 5 broad and less symmetrical precipitin lines, in the α_2- to γ-globulin region. 5 precipitin lines were elicited using the Ouchterlony method. Three of 711 pathological sera proved to have an antigenic substance which reacted with Ab-NHS. No cross-reaction of the antiserum with 28 ascitic and 2 pleural fluids was observed. Post-mortem tissue extracts, of liver, kidney, spleen, large intestine, abdominal muscle, great amentum, and lung were tested with Ab-NHS by the Ouchterlony procedure. Of these, 2 spleen samples (4-Sp and 8-Sp) reacted with the antiserum to form a precipitin line of complete identity with each other and showed immunological identity with one of the precipitin lines formed by FRGP against the antiserum (Figure 1). 4 pseudomucines were tested, and one of them was found to react with the antiserum by forming 2 precipitin lines. When the antiserum was absorbed with the spleen sample (4-Sp), the absorbed antibody failed to form the precipitin line against 4-Sp and 8-Sp (Figure 2); however, precipitin lines still formed between pseudomucin and the antibody with 4-Sp. The preceding data indicate the presence of at least 5 antigens in FRGP. Of the 5 antigens, 2 antigens are common to pseudomucine (1 out of 4 tested) and 1 antigen is attributable to spleen. Additional antigens exhibit the ovarian cyst content specificity. FRGP exhibited both A and H blood activities by the hemagglutination inhibition technique. Our study showed that the carbohydrate composition of FRGP is

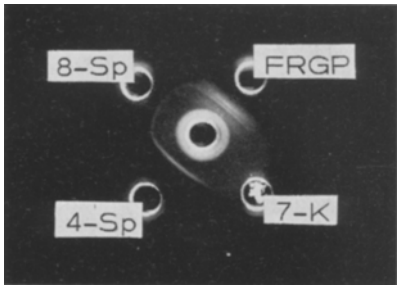

Fig. 1. Ouchterlony results showing the antigenic relationship of fucose-rich glycoprotein (FRGP), 4-Sp, 8-Sp and kidney (7-K). Central well contains anti-fucose-rich glycoprotein serum absorbed with normal human serum.

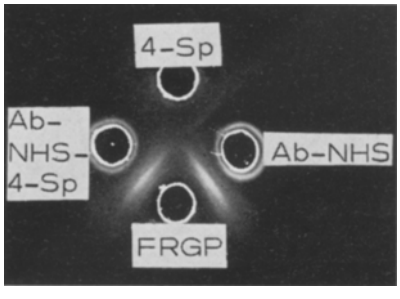

Fig. 2. 0.1 ml of the anti-fucose-rich glycoprotein serum absorbed with normal human serum (Ab-NHS) was absorbed with 1 mg of 4-Sp. The absorbed antibody (Ab-NHS-4-Sp) failed to form any precipitin line against the 4-Sp.

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different from FRGPs in urine and parotide saliva[18–20]. Substances of this type found in ovarian cyst material seem to be a kind of blood-group substance type[21]. It has been shown that this FRGP from ovarian cyst content shares a common antigenecity with spleen tissue.

Zusammenfassung. Fucose-reiches Glykoprotein wurde aus Eierstockpseudomucin isoliert. Mit Hilfe spezifischer Anti-Glykoprotein-Seren wurde die Antigenizität der Pseudomucine mit Immunoelektrophorese und die Ouchterlony Methode untersucht.

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Effect of Removing one Ovary and a Half on Ovulation Number in Cycling Rats

The Law of follicular constancy indicates that a given number of follicles mature during each estrous cycle regardless of the amount of ovarian tissue present. Whether this same generalization applies to the number of ova shed, remains to be answered. If it does, then the ovulation number for an animal should not change when ovarian tissue is removed or when extra-ovarian tissue is added. In agreement is the fact that unilateral ovariectomy in intact 4- and 5-day cycling rats resulted in the remaining ovary doubling the eggs shed[1]. However, conflicting with this idea is the finding that two extra-ovarian allografts in Fischer 344 rats caused a decrease in the number of eggs ovulated from the in situ ovaries (Chihal and Peppler, unpublished).

At 7–8 weeks post-operative in hemicastrated rats or those in which one ovary and a half had been removed, the remaining ovarian tissue contained the same number of large follicles[2]. However, hypertrophied half ovaries left in situ only contained 6 fresh corpora lutea 5 weeks after the removal of one ovary and a half[3]. Because of the implied discrepancy between these two reports, this investigation was performed to determine if removing one ovary and a half for 1 estrous cycle resulted in the normal number of eggs being ovulated (compensatory ovulation) at the next estrus.

Holtzman, female virgin rats were received at 50 days of age and divided into control ($N = 8$) and experimental ($N = 10$) groups. The animals were maintained in groups of 2 per cage with water and laboratory chow provided ad libitum. The lighting schedule was regulated for 14 h of illumination and 10 h of darkness. Daily vaginal smears were taken until 3 cycles were observed. Day 1 of the cycle refers to estrus.

Seventy-five percent of the ovarian tissue was removed on day 2 of the cycle by a dorsal-lateral approach. One ovary (18.5 ± 1.7 mg/100 g body weight) was completely removed and half (7.9 ± 1.5 mg/100 g body weight) of the other ovary was removed either by making a transverse cut across both the superior and inferior ends of the ovary or by making a lateral parasagittal cut. The ends of the ovarian bursa were pulled around the remaining ovarian fragment and pressed together.

Animals were killed 1 vaginal cycle later on day 2 (metestrus). Body weight and various organ weights were recorded. Each oviduct was dissected from the ovary (control group) or ovarian fragment (experimental group) and flushed with normal saline to determine ovulation number.

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Effect of removing one ovary and a half on ovulation number and various organ weights

Group	Body weight (g) ± S.E.	Eggs ovulated per rat ± S.E.	Organ weights (mg/100 g body wt. ± S.E.		
			Ovary[a]	Uterus	Adrenal[b]
Control ($N = 8$)	216.7 ± 2.8	9.5 ± 0.6	16.1 ± 0.8	117.5 ± 2.8	26.1 ± 1.2
One ovary and a half removed ($N = 10$)	212.3 ± 2.8	5.1 ± 0.6[c]	18.8 ± 1.4	114.3 ± 4.7	30.2 ± 1.0[c]

[a] Mean ovarian weight is one-half of the sum of both ovaries for control rats and the weight of the remaining ovarian fragment for the experimental group. [b] Mean adrenal weight is both adrenals for all rats. [c] $P < 0.025$ when compared to value for control rats.