Results of the antiglobulin consumption tests performed on 6 of the 8 sets of dog tissues substantiated the immunofluorescent observations. All 6 malignant tissues were found to adsorb anti-canine globulin; i.e., completely remove it from the supernatant fluid except in the case of 1 mammary tumor (the same one which gave the weak fluorescent reaction) which reduced the titer in the supernatant fluid from 32 to 8. Normal tissue counterparts of malignant tissues lacked the ability to adsorb anti-canine globulin; i.e., the titer in the supernatant fluids was either identical to, or within one dilution of, the titer of unadsorbed anti-canine globulin. Furthermore, normal tissues did not release any antiglobulin in the elution procedure as did the malignant tissues.

Although one cannot say with certainty that the globulins found on the tumor cells by the indirect fluorescent technique and by antiglobulin consumption tests are specific for tumor antigens on the surface of malignant cells (they could be normal globulins nonspecifically associated with the cell membrane), their correlation with the specific 7 S immunoglobulin in the sera leads one to believe that they are specific. These results are in direct contrast to those of STIRLING et al.[9] who observed no specific fluorescence of tumor cells from 24 human breast carcinomas when treated with an FITC-tagged anti-human globulin. However, they have been substantiated in our laboratory by CONGDON[10] who noted specific staining in 4 malignant canine tumors and KRAMER[11] who found immunochemical differences between the plasma membrane fractions of canine tumor tissues and their normal counterparts.

Zusammenfassung. Durch Immunofluoreszenz wird eine spezifische Bindung autologer γ-Globuline an neoplastische Zellen des Hundes nachgewiesen.

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Autoantibodies in Canine Neoplasms. II. Tumor Tissue Specificity and Lack of Cross-Reactivity with Embryonic Antigens

The cross-reactivity of antibodies produced in response to tumor antigens is variable. In man, tissue specific tumor antibodies[1], systems-specific tumor antibodies[2], and universal tumor antibodies have been described. Antibodies to some tumor antigens also react with embryonic antigens[2–4] whereas others do not[5].

An earlier study of 8 canine malignancies showed that canines react to their tumor tissues by producing a 7 S γ-globulin specific for the tumor tissue but not the corresponding normal tissue[6]. The degree of cross-reactivity of this globulin with other canine tumors of the same type, with canine tumors of various other types, and with embryonic canine tissues was examined and is reported here.

Dogs were bled prior to surgery and the globulin fraction of their sera separated by passage through a G-100 Sephadex gel column. Malignant tissues and normal counterparts from each animal were quick-frozen immediately upon surgical removal and stored at $-20\,^{\circ}C$. Tumors studied were: 5 mammary carcinomas, 3 mastocytomas, and 2 squamous cell carcinomas. Embryos in the second and third trimesters of gestation were obtained by Caesarean section and longitudinal sections of them were quick-frozen immediately upon delivery.

The methods used to conjugate the globulin fractions of each dog's serum to fluorescein isothiocyanate (FITC), to purify the conjugate, and to treat tissues with these immunofluorescent stains were described previously[6]. The FITC-tagged globulin fraction of each dog's serum was reacted with cryostat-cut sections of the dog's own

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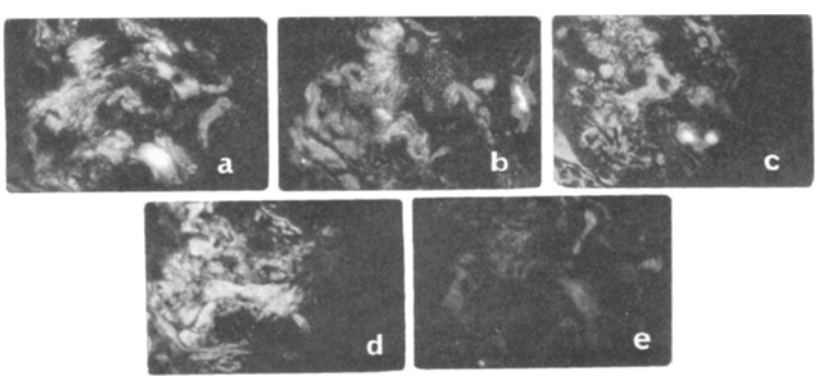

Tumor group specificity of fluorescent reaction. Serial sections of mammary carcinoma M_1 stained with: (a) FITC-labeled sera from M_1; (b) FITC-labeled sera from M_2; (c) FITC-labeled sera from M_5; (d) FITC-labeled sera from M_4; (e) unlabeled serum from M_1 + FITC-labeled serum from M_1. Note positive fluorescence in (a) through (d) and the lack of fluorescence in (e). × 400.

Reaction of FITC-labeled canine globulin with a variety of normal and malignant tissues and with embryonic tissues

	Tumor canine tissue										Embryonic canine tissue		Normal canine tissue								
Canine sera	M_1	M_2	M_3	M_4	M_5	T_1	T_2	T_3	S_1	S_2	35 days	25 days	M_1	M_2	M_3	M_4	M_5	T_1	T_2	T_3	S_1	S_2
M_1	+	+	+	+	+			±	±				−	−	−	−	−	−			−	
M_2	+	+	+	+	+						−	−		−								
M_3	+	+	+	+	+						−	−			−							
M_4	+	+	+	+	+											−						
M_5	+	+	+	+	+						−	−					−					
T_1	−					+	+	+	−		−		−					−	−	−	−	
T_2						+	+	+											−			
T_3						+	+	+			−	−								−		
S_1	−							−	+	+	−	−									−	−
S_2									+	+												−
N	−					−				−				−				−			−	

N, normal dog; M_1–M_5, 5 dogs with mammary carcinomas; T_1–T_3, 3 dogs with mastocytomas; S_1–S_2, 2 dogs with squamous cell carcinomas; +, positive fluorescence; −, negative fluorescence; ±, doubtful reaction.

normal and malignant tissue as well as with sections of the same tumor type from other dogs, with tumor sections of various other types of canine neoplasms, with a variety of normal tissues, and with canine embryonic tissues. Controls included normal and malignant tissue sections stained with pooled, normal canine serum which had been concentrated 3 times that of normal serum. Blocking reactions in which each unlabeled serum was added to a serial section of tissue prior to the addition of the labeled globulin were run in parallel to all specific staining reactions. No reaction was considered specific unless it was blocked in this manner.

The results of this study are shown in the Table. Each FITC-tagged globulin reacted with malignant cells of the same tumor type to which it had been stimulated (see Figure) but, except for serum *M* 1, did not cross-react with other tumor cell types nor with normal or embryonic tissues. Tagged, pooled and concentrated normal canine sera did not react specifically with either normal or tumor tissue. These findings indicate that the tumor antigens which stimulate antibody production in their canine hosts are tumor-type specific and that they are not similar to, or identical with, embryonic antigens[7].

Zusammenfassung. Durch Immunofluoreszenz wird eine Kreuzreaktion zwischen Tumoren des gleichen histologischen Typs, jedoch keine solche zwischen verschiedenartigen Tumoren des Hundes festgestellt.

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[7] The authors gratefully acknowledge Drs. G. P. Wilson III and M. Wyman, Department of Veterinary Surgery, The Ohio State University, Columbus (Ohio) for supplying the tissues used in this study.

[8] Supported, in part, by a National Science Foundation fellowship.

Synthesis in vitro of Immunoglobulins Produced by Different Human Mucous Membranes

Various authors have demonstrated the formation of immunoglobulins (IgG, IgA and IgM) by cultivating in vitro fragments of spleen, lymph nodes, bone marrow and lymphocytes from the peripheral blood of various animal and human donors[1,2].

Recent experiments show that in addition to lymphoid tissues, mucous membranes from the gastro-enteric and respiratory tracts are capable of forming immunoproteins in vitro[3,4]. This paper summarizes the results of our studies of the capacity of various mucous membranes which have a direct contact with antigen surroundings, to synthetize immunoglobulins (IgG, IgA, IgM and IgD).

Investigations were conducted on conjunctival, oral, nasal, vaginal and rectal membranes using the tissue culture technique and subsequent radioimmunoelectrophoretic analysis of the culture fluids[1].

Materials and methods. The tissues were removed from adult subjects by biopsy or during surgery. Macroscopic and microscopic examinations showed no trace of flogosis or neoplastic infiltration.

Tissue cultures and autoradiographs were prepared by the method described by Asofsky et al.[1]. Tissues were fragmentized in sterilized chambers by surgical blades in a petri dish containing Hank's solution. Then the fragments were placed by capillary pipettes against the wall of the rolling tubes. For each culture the weight varied from 70–100 mg. After removing the Hank's solution, 1 ml of Eagle medium was added to each tube prepared by us in the following way: 2 μc/ml of 2 radioactive aminoacids (^{14}C L-lysine and ^{14}C L-isoleucine; Schwarz,

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