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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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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Bio-Res., Orangeburg, New York); 2 ml/100 mg of aminoacid mixture by NEUMANN and McCOY in which L-lysine and L-isoleucine were omitted; ovalbumin, 2× crystall., salt free 0.5%; BME vitamin mixture 1 ml/100 mg; glucose 0.4%; Penicillin G 10,000 IU; these substances were added to 96 ml of Hank's solution. The pH of the medium prepared in this way was adjusted to 7.6 by sodium carbonate.

The cultures were incubated for 48 h at 37 °C in rolling tubes and then frozen at −25 °C for 2 h. After thawing at 37 °C and centrifugation at 16,000 rpm (18,000 *g*) for 20 min, the cell-free supernatant was removed and then dialyzed for 3 days at 4 °C against 0.015 *M* phosphate buffer pH 7.6. Every day the buffer was changed. After dialysis, the culture fluid was then concentrated by lyophilization and then dissolved in 0.1 ml of bi-distilled water. Then this fluid was placed in a little tube and conserved at −25 °C till radioimmunoelectrophoretic analysis was performed. The immunoelectrophoretic analysis was carried out by Scheidegger's method under standard conditions. The culture fluid (30 µl) was filled with normal human carrier serum in the well of agar slides; after electrophoretic separation, the anti-human protein and specific anti-immunoglobulin sera (Hyland Lab.) were placed in the central well. After diffusion and subsequent washing in phosphate buffer saline for 3 days, the slides were dried and stained with 0.5% amido black solution.

The immunoelectrophoresis slides were left in contact with Kodak RS Pan 650 ASA film for 21 days. The autoradiographic patterns can then be compared with the electrophoresis slides. Labelling of an immunoglobulin line indicates ex novo synthesis during the incubation in vitro period. The amount of immunoglobulin synthesis in the autoradiographic patterns was determined by comparison with a standard sample[2].

Results and discussion. The results of the autoradiographic analysis of the fluid cultures are given in the Table. The results show that all the mucous membranes examined synthetize immunoglobulins, as do other human tissues from the gastroenteric and respiratory tracts[3,4]. In addition, one notes that IgG and IgA are present in all fluid cultures (Figure), while IgM is scarce in nasal, conjunctival and rectal membranes, and is absent from oral and vaginal membranes. IgD was absent from all fluid cultures examined. IgG synthesis prevailed in conjunctival, vaginal and rectal tissues, while IgA synthesis prevailed in nasal and rectal tissues.

Immunoglobulin synthesis in nasal, conjunctival, oral, vaginal and rectal mucous membranes

Mucous membranes	No. of cultures	Autoradiography IgG	IgA	IgM	IgD
Nasal	7	+−	+	+−	−
Conjunctival	7	+	+	+−	−
Oral	7	+	+−	−	−
Vaginal	8	+	+−	−	−
Rectal	3	+−	+	+−	−

The intensity of the areas of the autoradiographic lines were interpreted as follows: −, absence of synthesis; +−, slightly visible, weakly positive synthesis; +, clearly visible, positive synthesis.

The data regarding nasal and rectal membranes agrees with findings from immunofluorescence studies performed by us[4] and other authors[5,6]. The prevalence of IgA could be related to the presence of glandular elements in this mucous membrane. A fact which strengthens this point of view is that IgA formation prevails in some tissues with a glandular structure[7,8]. In other tissues, however, with abundant lymphoid structure such as the ileum, colon and appendix, there is a predominant IgG synthesis[3,4]. In the other mucous membranes studied (conjunctival, oral and vaginal), in which lymphoid tissue is found in slight amounts, there occurs a production of IgG of medium intensity (+) which almost always prevails over that of IgA.

In conclusion, the synthesis of immunoglobulins by mucous membranes having a direct contact with an antigen surrounding, shows their ability to protect, not only structurally, but also by providing an immunological defense. In this respect the formation of immunoglobulins by these mucous membranes can be regarded as a part of a 'local defense mechanism'[9].

Riassunto. Gli autori hanno dimostrato che alcune mucose della specie umana (mucosa congiuntivale, gengivale, nasale, vaginale e rettale) a contatto con l'ambiente esterno, sono in grado in vitro di sintetizzare le immunoglobuline (IgG, IgA, IgM) in diversa proporzione. La formazione di queste immunoglobuline può rivestire il significato di un meccanismo di difesa locale di tipo immunitario.

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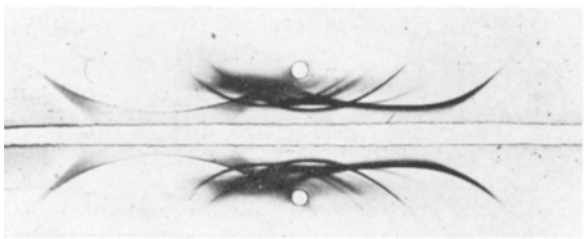
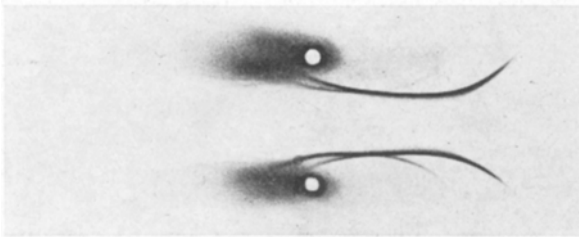


(a) Immunoelectrophoretic pattern of carrier serum and conjunctival culture (above), and of vaginal culture (below) developed with horse anti-human serum.



(b) Autoradiograph with labelling IgG++, IgA+ and IgM ± line (above) and IgG ± and IgA ± line (below).

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[9] The authors wish to express their sincere thanks to Miss L. GUIDI for her competent and devoted assistance.