technically proficient to perform the test, future investigations utilizing antibody coated latex particles to detect virus via agglutination are planned. Investigations of nasal and conjunctival secretions and blood serum of newly arrived dogs are currently in progress.

Résumé. La production d'un anticorps contre le virus de l'encéphalite canine a été démontrée par électroprécipitation sur l'acétate de cellulose dans une dilution de 1:8. Une bande de précipitation bien définie a été obtenue dans un intervalle de temps trés court, ce qui indiquerait que le test pourrait servir à l'identification de l'antigène encéphalitique chez les chiens suspectés d'en être atteints.

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Intestinal IgA in the Pig

It is well recognized that the gastro-intestinal tract has every provision for generating immune reactions. Food residues and bacterial flora are present in abundance in the lumen and provide continuous sources of antigens. The production of antibodies by lymphoid tissues of the digestive tract upon local contact by specific antigens has been demonstrated in the rabbit[1,2]. Immunofluorescent studies of intestinal tissue in man demonstrate that IgA is the predominant immunoglobin contained in the lymphoid cells of the lamina propria[3-7]. IgA is the predominant immunoglobin in many external secretions[8] and it has been suggested that the IgA system is the important determinant of immune competence at all epithelial surfaces[9].

The IgA system has been well characterized in man and there is evidence that a similar system exists in the rabbit[10]. We have recently characterized IgA in the serum, milk, saliva and urine of the pig; in the investigations reported here IgA is demonstrated in the intestinal contents of the pig and localized by immunofluorescence in the intestinal mucosa of the duodenum, jejunum and ileum.

Materials and methods. The chromatographic techniques for the isolation of porcine immunoglobins IgG, IgA, IgM have already been described[11]. The specific rabbit anti-IgA serum used in the investigation was prepared against porcine colostral IgA and was absorbed with porcine IgG and IgM and precolostral piglet serum. When this antiserum was used in immunological double diffusion studies in agar against pig serum, only IgA was precipitated (Figures 1, a and b).

IgA was localized in the intestinal tissue at 3 levels of intestine (duodenum, jejunum and ileum), by the immunofluorescent antibody technique. The specific rabbit antiserum for colostral IgA was conjugated with fluorescein isothyocyanate (FITC)[11]. Blocks of tissue were snap-frozen in isopentane cooled to −196 °C and stored in liquid nitrogen till required. Replicate cryostat sections were fixed in either methanol, ethanol or acetone prior to incubation with the conjugated reagent.

The specificity of the reaction was controlled by (a) blocking with unconjugated antiserum prior to incubating with conjugated reagent, (b) absorbing the conjugated antiserum with colostral IgA before staining, (c) the use of non-immune rabbit serum. Details of microscopy and photographic techniques have already been given[11].

Results and discussion. Extracts of small intestinal contents from 9 weaned pigs varying in age from 3–8 weeks were examined by immunological double diffusion against specific antiserum to colostral IgA. The immunoglobin was demonstrable in all specimens.

Comparative studies with serum and colostral IgA are shown in Figure 1a; an extra precipitation line was apparent in all reactions against intestinal contents suggestive of free 'secretory piece' demonstrated in previous studies of human secretory IgA[12]. Absorption of the anti-

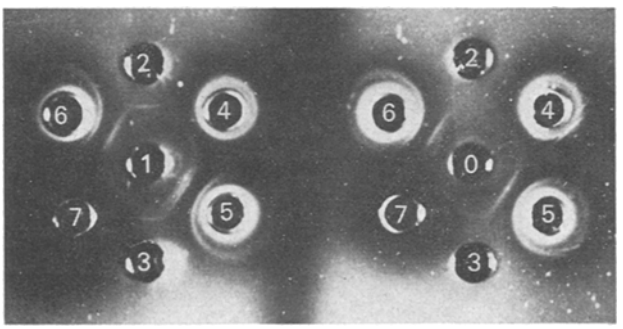

Fig. 1a. Comparative immunodiffusion studies in agar of serum IgA (2), colostral IgA (3) and intestinal contents (4, 5, 6, 7) using rabbit antiserum to colostral IgA (1). The same study using rabbit antiserum to colostral IgA absorbed with serum IgA (0).

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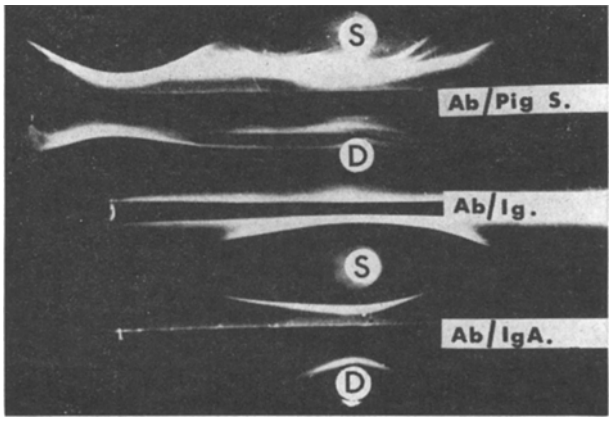

Fig. 1b. Immunoelectrophoretic studies of pig serum and intestinal contents demonstrating the presence of serum derived antigens in intestinal contents. The electrophoretograms of serum (S) and intestinal contents (D) are precipitated with rabbit antisera prepared against serum proteins, immunoglobulins, and colostral IgA.

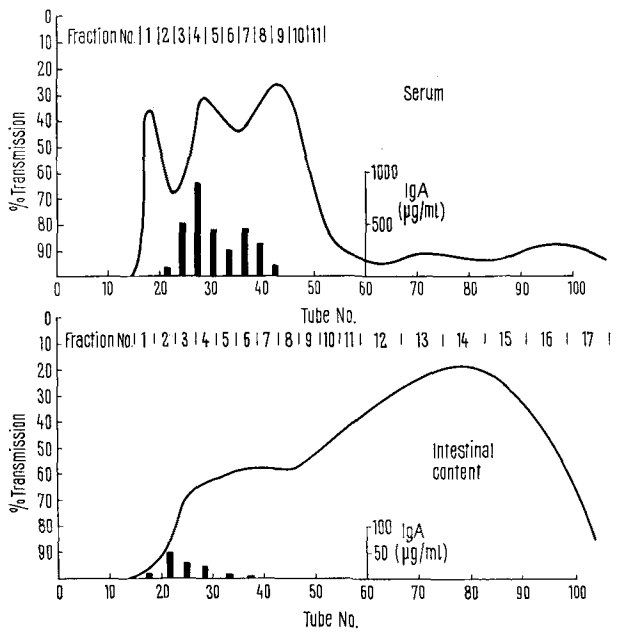

Fig. 2. Gel filtration of serum and intestinal contents on Sephadex G200, giving pooling data of selected fractions and elution of IgA assayed in fractions by radial immunodiffusion using specific rabbit anti-colostral IgA.

serum to porcine colostral IgA with serum IgA resulted in the removal of all reactivity with serum IgA, but a precipitin line remained for colostral IgA which showed a reaction of identity with a reactive compound in intestinal contents (Figure 1a).

Immunoelectrophoretic studies of intestinal contents consistently demonstrated that the main serum derived antigens were albumin, one or two α-globulins and IgA. Comparative studies of serum and intestinal contents were carried out by gel filtration on Sephadex G200; IgA was identified in selected fractions and assayed by quantitative radial immunodiffusion. Intestinal IgA appeared predominantly in earlier eluates than serum IgA, indicating a higher molecular size (Figure 2). The column fractions containing intestinal IgA were studied by immunodiffusion against the antiserum to colostral IgA absorbed with serum IgA. Precipitation reactions were evident in all fractions of intestinal contents in which IgA had been identified, substantiating the thesis that an additional antigen (secretory piece) was associated with the immunoglobin. The reaction was not demonstrable in serum or concentrated serum fractions containing IgA.

The immunofluorescent studies demonstrated that there was little variation in the location of IgA in all tissues examined regardless of the level of intestine or age of the animal. IgA was concentrated in the epithelial cells occupying the lower part of the crypt. It appeared to be confined to the apical cytoplasm of the cells (Figures 3, a and b). Similar observations have recently been reported for human intestine[6,7]. The lamina propria contained a number of brightly fluorescing plasma cells. These were mainly confined to the intercrypt stroma, though very occasionally an isolated cell was seen in the core of a villous. These cells were easily differentiated from the more numerous eosinophils which gave a whitish autofluorescence. The association of intestinal IgA with the plasma cell is well established in man[4–7]. In the lymphoid follicles of the ileal mucosa, the germinal centres stained strongly whilst the cells at the periphery remained unstained except for an occasional plasma cell.

In addition to these findings which have variously been recorded for human tissue, an extravascular reaction was observed in the region of active plasma cells in the lamina propria of several animals. This usually appeared as a

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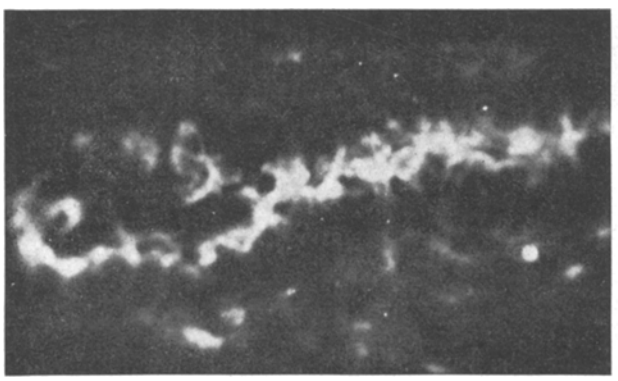

Fig. 3a. Normal pig jejunum stained with fluorescein conjugated antiserum to IgA showing fluorescence in the apical cytoplasm of the epithelium in a crypt. × 800.

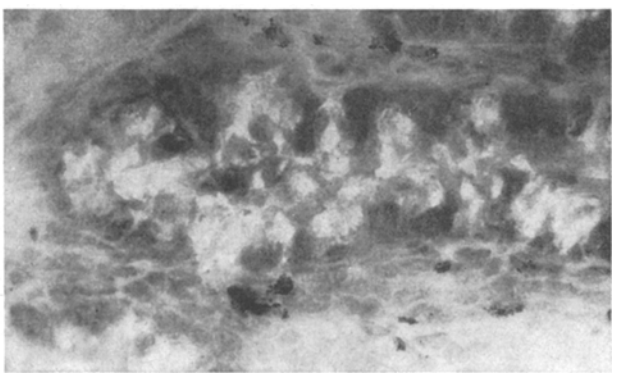

Fig. 3b. The field shown in (a) stained with haematoxylin and eosin. × 800.

dim homogeneous background stain in preparations fixed in methanol, whilst in acetone fixed sections of the same material it was seen as a finely dispersed amorphous deposit. We did not consider this to be an artefact since apart from its absence from the controls there was a marked degree of uniformity in the strength of reaction between sections of the same tissue subjected to the 2 treatments[13].

Zusammenfassung. Immunglobulin IgA wird beim Schwein immunochemisch im Darminhalt und immunofluoreszenzoptisch in der Mukose des gesamten Dünndarms nachgewiesen.

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Osseal Changes in Young Mice Undergoing Graft-versus-Host Reaction

The features of graft-versus-host (GVH) reaction are characterized by weight loss, lethargy, ruffled fur, hunched back posture, splenomegaly, the decrease of the absolute lymphocyte count and by impaired immunological reactivity with low level of circulating antibodies and immun globulins. Moreover the animals are generally retarded, and the progressive atrophic state is remarkable[1-8].

The purpose of this communication to discover the background of this general retardation of the growth and of the progressive atophic state, which develops in young mice undergoing GVH reaction.

Materials and methods. GVH reaction was produced in (C57B1 × A) F_1 hybrid mice, without regard of sex. The animals in experiment I were 2 weeks old, and in experiment II were 3 weeks old. The mice were injected i.p. with 150×10^6 spleen cells from adult C57B1 donors of both sexes (GVH groups). The mice of control groups received the same amount of isologuous spleen cells. In both experiments, 3 weeks later following the injections, the establishment of the GVH reaction was confirmed by the significant decrease of the absolute number of circulating lymphocytes, and the decline of the body weight.

Radiographs of 10-10 animals with GVH reaction and 10-10 control mice were taken 3 weeks later, following the spleen cells transfer in experiments I and II, simultaneously in pairs with controls by the same exposition time for radiomicrometric measurements[9]. The animals were then sacrificed and dissected. The splenomegaly with ascites and peripherial lymphonodular enlargement were confirmed macroscopically.

The distal end of femora were chosen for histological study; 4% formalin fixative, decalcification paraffin embedding and hematoxylin-eosin staining were used.

Results and discussion. The decrease of the absolute lymphocyte count and the decline of the body weight in experiments I and II are presented in Figure 1. In the radio-

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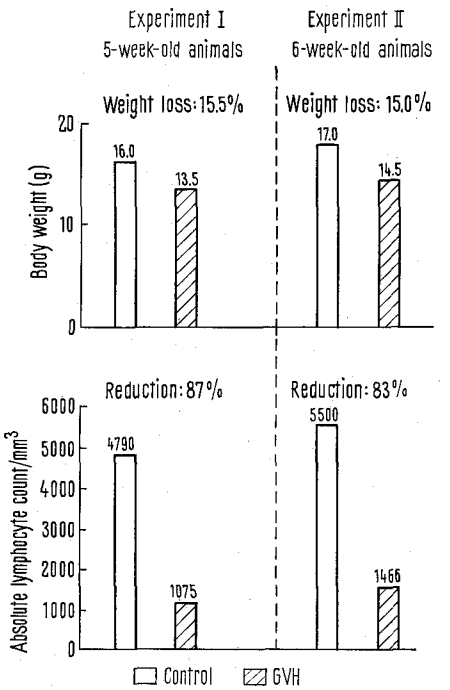

Fig. 1. The average body weight and absolute lymphocyte count in mice of GVH and of control groups.

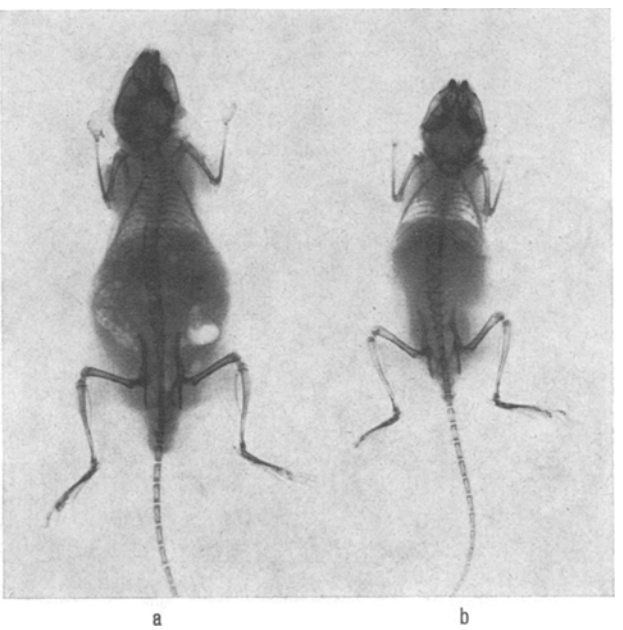

Fig. 2. Radiogram of a 6-week-old mouse undergoing GVH reaction (b) and a control littermate (a) from experiment II.