activity of the serotonin released by ADP, of that remaining in the platelets and that of the total serotonin of the untreated platelets are almost identical. 3 similar experiments gave the same result.

It can be concluded that the serotonin taken up by the platelets is mixed homogeneously with the serotonin already present in the platelets, and that the percentage of release, obtained from counting the radioactivity of the supernatant of an aggregated platelets suspension, is a true figure for the proportion of serotonin released during the treatment.

In this respect serotonin differs from the adenine nucleotides. IRELAND[16] and HOLMSEN[17] have shown that platelets labelled with adenosine-^{14}C or phosphate-^{32}P release very little radioactivity in the release reaction and concluded that the nucleotides to be released are stored in a pool not readily labelled. This pool is possibly localized in the granules which also store serotonin[18,19].

Zusammenfassung. Menschliche Blutplättchen in Zitratplasma wurden mit Serotonin-^{14}C inkubiert und danach mit ADP zur Aggregation gebracht. Die spezifische Aktivität des freigesetzten Serotonins war gleich der des in den Plättchen verbliebenen Serotonins.

P. MASSINI

Theodor Kocher Institute, University of Berne, 3000 Bern (Switzerland), 25 August 1969.

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[17] H. HOLMSEN, Scand. J. Lab. Invest. *17*, 239 (1965).
[18] Professor H. AEBI kindly made available his facilities for spectrofluorometry and for liquid scintillation counting; Miss M. SCHNEIDER gave competent technical assistance.
[19] L. M. ALEDORT, H. GILBERT and E. PUSZKIN, Blood *34*, 535 (1969) recently confirmed STACEY's[10] earlier findings (see text); they furthermore concluded that serotonin taken up in vitro does not equilibrate with endogenous serotonin. Whereas the possibility can not be excluded that preincubation with serotonin sensitizes the platelets for the release reaction by small amounts of ADP, the results reported by ALEDORT et al. do not necessarily imply that serotonin taken up in vitro does not equilibrate with its endogenous pool.

Application of the Electroprecipitin Test to the Detection of the Virus of Canine Distemper

In research facilities utilizing random source dogs, canine distemper or a distemper-like syndrome is frequently seen. Despite signs suggestive of canine distemper (i.e. fever, malaise, anorexia, catarrhal nasal and conjunctival exudate) it is impossible to accurately diagnose the disease without clinical laboratory evidence or seeing the clinical signs of paralysis, chorea, or convulsions.

Since available laboratory tests for canine distemper are unreliable or impractical in most research facilities utilizing large numbers of random source dogs, a rapid diagnostic test for canine distemper was sought. This is to report the results achieved by the production of an antibody to the virus in rabbits, and its subsequent use to detect the virus of canine distemper via the electroprecipitin test.

Materials and methods. Antigen and antiserum. A commercially available modified live virus canine distemper vaccine[1] was mixed with complete Freunds adjuvant[2] (1:1) and injected s.c. into 3 kg New Zealand rabbits[3]. After 3 weeks, blood was collected via saphenous venapuncture, the serum separated and stored frozen until tested. Dilutions of antigen were made in saline to be tested.

Electroprecipitin test. Canine distemper viral dilutions were prepared in saline and tested against the rabbit antiserum by the electroprecipitin test[4–6]. A veronal barbital buffer[7], pH 8.6, ionic strength 0.05, was used in all electroprecipitin tests. Cellulose acetate electrophoretic support strips[8] were pre-soaked for 30 min prior to using, then blotted and placed in the electrophoretic chamber. A 5 λ drop of antiserum was placed 1 inch from the cathode, and a current of 250 V, 5–7 ma, was passed for 30 min. The current was shut off, and the antigen(s) (approximately 5 λ) were applied with a fire-polished capillary tube (0.5–0.9 mm i.d.) as a straight line immediately behind (on the cathode side) the point of antiserum application (Figure 1). Current was restored for 20 min. At the end of that time, the strip was transferred to 0.85% saline solution and rinsed for 5–10 min, with occasional movement of the saline solution. The strip was then dried and placed in Ponceau S stain[9] for 5 min. The strip was then rinsed twice in 5% acetic acid and once in tap water. After drying, the strips were examined for precipitin bands by means of illumination from behind (Figure 2). Control tests consisted of pooled canine serum from dogs immune to canine distemper, and saline alone tested against the antiserum.

Gel diffusion tests. Double diffusion in one dimension[10] in tubes was used in all instances to confirm electroprecipitin test results. A 0.6% agar solution in 0.85% NaCl was employed as the interphase between antiserum and antigen.

Serum neutralization test. The rabbit antiserum prepared against the distemper virus was tested for serum neutralizing antibody, and the titer calculated by the Reed and Muench system[11]. Serum from 3 pre-immune rabbits, randomly selected, were also tested for neutralizing antibody.

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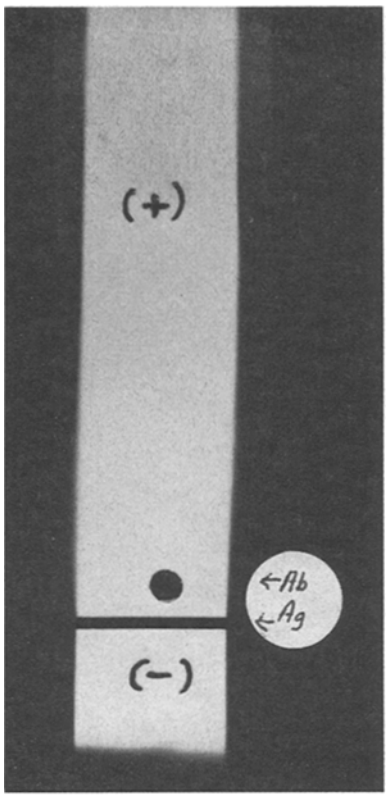

Fig. 1. One drop of antiserum (Ab) applied at the start. Antigens (Ag) applied as a straight line 30 min later.

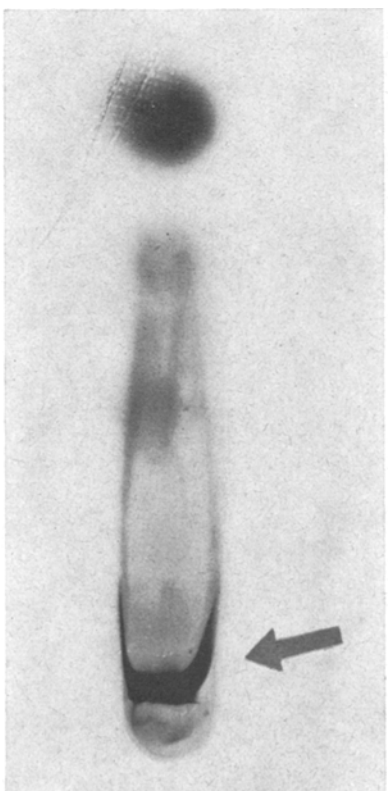

Fig. 2. Precipitin band depicting reaction between rabbit anti-canine distemper serum and canine distemper virus.

Results. When the rabbit anti-distemper antiserum was tested against distemper viral dilutions, multiple reactions were noted. Since the viral preparation used for immunization was of canine tissue culture origin, it was suspected that antibodies had also been produced against canine tissue and/or serum components. Therefore, the antiserum was tested against pooled canine serum. Multiple reactions were noted. After absorption of the rabbit anti-distemper antiserum with pooled canine serum, however, no reaction occurred to pooled canine serum, but a reaction persisted to a component present in the viral suspension as evidenced by a distinct electroprecipitin band (Figure 2). The reaction persisted to a viral dilution of 1:8. Saline alone did not react with the antiserum. Gelatin diffusion tests confirmed electroprecipitin test results.

Serum neutralization tests performed on the rabbit antiserum to the distemper virus revealed a neutralizing antibody titer of 1:300 as calculated by the Reed-Muench system. Pre-immune rabbit serum was not able to neutralize the distemper virus.

Discussion. Since there is reportedly one antigenic type of the canine distemper virus[12], it lends itself to a variety of tests. Serological tests such as complement fixation[13], gel diffusion[14] and serum neutralization[15,16] have been used successfully to measure antibody response to the distemper virus. Unfortunately in a research facility using large numbers of random source dogs, it is impractical to wait for a rising titer as an adjunct to diagnosis. It would be far more practical to demonstrate distemper antigen in the patient. The fluorescent antibody test has been reported accurate during the early catarrhal stage at demonstrating distemper antigen in conjunctival, genital or blood films[17], although the special equipment necessary and technical difficulty in performing the test has prevented its widespread usage.

There is a need in research facilities utilizing random source dogs for a rapid means of detecting newly arrived dogs viremic with distemper, to eliminate a costly medical treatment regime while in isolation. The production of a strong precipitating antibody in rabbits to the canine distemper virus can be used to demonstrate the virus via the rapid ($1^1/_2$ h) electroprecipitin test as indicated here. The gel diffusion test employed to confirm electroprecipitin test results does work well, but a minimum of 48 h is necessary to accurately determine a negative result.

Although rabbits are an unnatural host for canine distemper and to this authors knowledge the disease has never been identified in them, they do produce strong precipitating antibodies to most antigens and were selected for antibody production for that reason. Also, since all antibodies produced by rabbits are relatively slow moving gamma globulins electrophoretically, they are well suited for the electroprecipitin test.

Although the procedure described herein does require special electrophoretic equipment and an individual

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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-

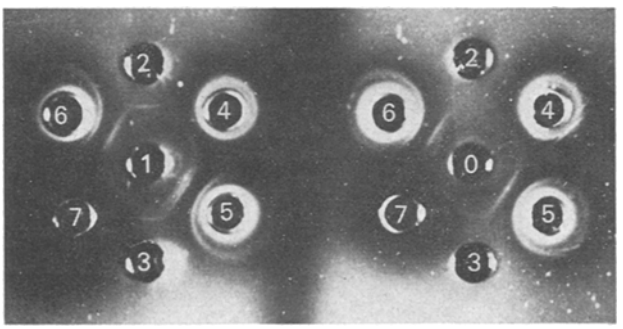

Fig. 1a. Comparative immunodiffusion studies in agar of serum IgA (2), colostral IgA (3) and intestinal contents (4, 5, 6, 7) using rabbit antiserum to colostral IgA (1). The same study using rabbit antiserum to colostral IgA absorbed with serum IgA (0).

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