level. We are currently investigating the effects of HE on synthesis of *specific* protein hormones in an organ culture system.

Summary. Rat anterior pituitary glands cultured for periods up to four hours incorporate amino acid-^{14}C mixture or glutamic acid-^{14}C into pituitary protein in linear fashion. Addition of hypothalamic extracts to the culture system results in an increased incorporation and release of protein radioactivity as compared to glands incubated with cerebral cortex extracts. The stimulatory effect can be demonstrated at the level of the pituitary polyribosomes.

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Isolation of Human Enteroviruses from Beagle Dogs* (33038)

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Dogs are one of the most common household pets and have more intimate contact with man than other domestic animals. It is, therefore, not unexpected to find that agents such as mumps virus (1), reovirus (2,3), and ECHO virus (4) which typically infect man have been isolated from dogs. Such observations indicate the probability that other viruses causing disease in man may be found, at least as transients, in dogs. The studies reported here were initiated to determine whether these or other viruses could be recovered from beagle dogs maintained in an isolated colony for other experimental purposes. Several isolations of ECHO virus were again made, as well as multiple isolations of Coxsackie B viruses.

Materials and Methods. Animals. The beagle dogs used were reared and maintained in kennels which housed a total of approximately 800 animals during the course of this study. Compatible pairs of dogs were housed in runs with concrete floors and were separated from other dogs by a cinder-block wall and wire fence. In some instances dogs were housed individually in metabolism cages.

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The dogs were fed a commercial ration supplemented with ground beef. All dogs were immunized with anticanine–distemper–hepatitis–*Leptospira canicola* serum of canine origin followed by vaccination and boosters with these agents and rabies at appropriate intervals.

Specimen collection and preparation. Three groups of dogs were tested for virus. Group I, consisting of 16 dogs, was sampled for virus 16 times and Group II, consisting of 10 dogs, was sampled 17 times. Both groups were tested at regular intervals for 12 months. Group III, composed of 12 dogs, was sampled weekly for 5 weeks.

Plastic tubes containing swabs (Falcon Plastics, Los Angeles) designed to prevent contamination from the outer area of the anus were used to collect the rectal swabs. Throat and nasal swabs were collected with standard cotton swabs. Immediately after collection swabs were placed in individual tubes containing 2 ml of Hanks' balanced salt solution (BSS) containing 2000 units of penicillin G, 2000 μg of streptomycin sulfate, 2000 μg of neomycin sulfate and 50 μg of Fungizone/ml. Rectal swabs were incubated for 1 hour in a 37°C water bath then centrifuged at 10,000*g* for 20 min at 4°C. The supernatant from the rectal swabs was collected and stored in stoppered tubes at −70°C until cell cultures were available for inoculation.

Tissue culture systems. The tissue culture systems used for this study were primary rhesus monkey (MK) cells, (Shamrock Farms, Inc., Middletown, New York), HeLa cells, primary dog kidney (DK) and primary dog lung (DL). The DL and DK cell cultures were prepared according to the previously published procedures (5) from healthy beagle pups less than 1 month of age. The outgrowth media for the HeLa, DK, and DL cell cultures consisted of 10% calf serum in medium 199 with antibiotics (500 units of penicillin G sodium, 100 μg of streptomycin sulfate, 100 μg of neomycin sulfate, and 5 μg of Fungizone/ml of media). Confluent monolayers of HeLa, DK, and DL cell cultures were maintained on a medium consisting of 1% calf serum in Eagle's minimal essential medium (MEM) in Hanks' BSS with the same concentration of antibiotics as used in the outgrowth medium. The MK cell cultures were maintained on Eagle's MEM without serum.

Virus isolation and identification. Approximately 0.1 ml of the fluid from each swab was inoculated into each of 2 MK, HeLa and DK, or DL cell culture tubes. For economy of tubes during some of the observations of the Group I dogs, nasal and throat swabs were pooled for inoculation into the MK cell culture tubes. Inoculated tubes were examined for cytopathogenic effect (CPE) every other day for 7–10 days. Negative inoculated cell cultures were frozen at −70°C, thawed and inoculated into two fresh cell culture tubes. Three such passages were completed before a sample was discarded as CPE negative.

Positive CPE isolates were passed 2–3 times then identified by neutralization (NT) tests with known antisera (Microbiological Associates, Bethesda, Maryland) in the appropriate cell cultures. All of the CPE negative third passage MK and approximately half of the DK and DL cell cultures were tested for hemadsorption with guinea pig red blood cells (RBC) and for hemagglutination of human type O, guinea pig, chicken, and Beagle RBC at 4, 22, and 37°C. Antisera against three of the viruses isolated were prepared by the intravenous inoculation of rabbits with 1 ml of virus suspension at five successive weekly intervals following an initial intramuscular inoculation of 10 ml. These sera were used in NT tests with viruses of known types.

Results. A total of 164 isolations of CPE-producing viruses were made from 1602 rectal, nasal, and throat swabs collected at intervals from three groups of beagle dogs. One hundred and eighteen of the isolates were identified as Coxsackie virus B1, 4 were B3, 14 were B5 and 22 were ECHO virus 6 (Table I). Six unidentified virus isolates which produced CPE in MK but not in HeLa or DK cell culture and which hemagglutinated human but not guinea pig or chicken RBC, were isolated but could not be readily identified. These isolates were all neutralized with

TABLE I. Viruses Isolated from Beagle Dogs.[a]

Viruses isolated	Source of swabs				
	R	N	T	N+T	Total
Coxsackievirus B1	51	23	27	17	118
Coxsackievirus B3	3	0	1	0	4
Coxsackievirus B5	9	3	2	0	14
ECHO virus 6	9	5	1	7	22
Unknown	2	3	1	0	6
Total	74	34	38	18	164

[a] Abbrev.: R = rectal swabs, 534 tested; N = nasal swabs, 391 tested; T = throat swabs, 391 tested; N + T = pooled nasal and throat swabs, 143 tested.

antisera prepared by the inoculation of one of the isolates into rabbits. The identity of these six viruses is presently under investigation. Four additional CPE-producing viruses were recovered but could not be subcultured and identified after the original isolation. Antibody prepared against one of the Coxsackie virus B1 isolates, one of the B5 isolates and one of the ECHO virus 6 isolates neutralized known viruses of the same type and did not neutralize related viruses.

Coxsackie virus B1 was recovered repeatedly from all 16 dogs in study Group I for periods of up to 10 months after the initial isolation. Coxsackie B5 was recovered once from one dog and twice from samples collected 1 month apart from another dog. In this group of dogs ECHO virus 6 was isolated at least once from 10 of the 16 dogs being studied. When this virus was recovered more than once from any one dog the positive swabs were collected no more than 1 month apart.

Coxsackie viruses B1, B3, and B5 were recovered from the dogs in Group II only during the seventh month that they were sampled. At that time, B1 virus was recovered from one dog, B3 virus from 2 dogs and B5 virus from the remaining 7 dogs. ECHO virus 6 was recovered only once. The unidentified viruses were recovered from single swabs from two dogs and from swabs from another dog on 3 successive weekly intervals. Forty-two isolates of the myxovirus, SV-5, were made in the MK cell cultures. None were isolated in the DK or DL cell cultures.

The dogs in Group III were sampled only for a few weeks compared with the year long observations of the two previous groups. During this time, however, all of the viruses that had been isolated from the other two groups were recovered at least once from the dogs in this group. Coxsackie virus B1 was recovered once from one dog, B3 virus once from each of two dogs, B5 virus once from each of two dogs and one of the isolations of the unknown viruses was made once from one dog.

There was no consistency in isolations from any particular location, other than that there were more isolations from rectal than from nasal or throat swabs (Table I).

Sera collected from many of these dogs before and after they were sampled for viruses were tested for NT antibody against the virus isolated from the individual dog. Neutralizing antibody titers of 1:4 to 1:16 against Coxsackie virus B3 and B5 isolates were observed. Antibody against Coxsackie virus B1, ECHO virus 6 and one of the unknown viruses could not be demonstrated. There was no correlation between the time of isolation and the development of NT antibody against the viruses. In several instances NT antibody was present in the dogs several weeks prior to the recovery of the specific virus from the dog.

Coxsackie virus B1, B3 and B5 were isolated in the HeLa and MK cell cultures, whereas all of the ECHO virus 6 and the unidentified viruses were recoveed in MK cell cultures only. No viruses were isolated in the DK or DL cell cultures.

There was a tendency toward a seasonal occurrence of these viruses in beagle dogs. The Coxsackie viruses occurred most frequently in the summer and fall of 1964, winter of 1965, and spring of 1966. The ECHO viruses were most prevalent in the winter and summer of 1965 and winter and spring of 1966.

Discussion. In a previous investigation of the possible presence of viruses in the feces of dog using DK cell cultures, infectious canine hepatitis virus was the only agent recovered (6). Subsequent studies of naturally occurring viruses in dogs resulted in the recov-

ery of reovirus 1 in DK cell cultures (2) and also in African green monkey cell cultures (3). The recovery in MK cell cultures of ECHO virus type 6 from dogs has previously been reported from this laboratory.

In the present investigation no viruses were recovered in the DK or DL cell cultures although 164 virus isolations were made in HeLa and MK cells. This might be expected since the types of enteroviruses recovered will not multiply in primary DK cell cultures (7,8). If "canine" enteroviruses were present, no evidence of them was found in the cell cultures used. The recovery from dogs of enteroviruses apparently identical to those of human origin is not surprising since low NT antibody titers against human enteroviruses have been reported to occur in dogs (9). Titers of 1:8 to 1:32 against poliovirus types 1 and 3, Coxsackie virus types A9 and B2 and ECHO virus types 6, 7, 8, 9, and 12 were reported.

It is assumed that dogs become infected by exposure to infected humans. The dogs apparently develop subclinical infections as indicated by the fact that throughout the present study all dogs remained in excellent health even when shedding virus. At the present time it is not known if these viruses can be passed from dog to dog or whether their sole source of infection is from humans. From our observations it is evident that dogs either shed these viruses for prolonged periods or they are reinfected frequently, even in the presence of low neutralizing antibody titers. In this laboratory beagle dogs have been observed to develop mild symptoms of infection after being experimentally fed ECHO virus 6 and that the virus could be recovered from the feces of some dogs for up to 35 days (10).

Until the problems of transmission of these viruses among dogs is resolved, the significance of the findings reported here related to the epidemiology of enteroviruses in humans will remain unknown. Their occurrence in beagle dogs most frequently during the fall, winter, and spring months when they are found less frequently in humans, warrants further investigation.

SV-5 agents isolated in MK cell cultures were assumed to be contaminants and not from the dogs being studied. The recent isolation of SV-5-like viruses from dogs with respiratory illness by others (11), might suggest that some of our isolates could have been from dogs. Our observations do not support this possibility. No similar viruses were recovered in the DK or DL cell cultures, which were both susceptible to infection with the SV-5 viruses. Such viruses were not isolated a second time from the original specimens and furthermore, there were no symptoms of respiratory infections among any of the dogs during this study.

Summary. One hundred and sixty-four isolations of Coxsackie viruses B1, B3, and B5 and ECHO virus 6 related if not identical to human viruses of the same types, were isolated from nose, throat, and rectal swabs collected from beagle dogs. Such isolations have not been previously reported except from the ECHO virus 6 which was again recovered. Six unidentified, apparently identical viruses were also isolated. Low NT antibody titers against Coxsackie virus B3 and B5 were present in some of the sera collected from the dogs. No titers were observed for Cosxackie virus B1, ECHO virus 6 or one of the unidentified viruses. There was no correlation between virus isolation and serum titers.

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Enhancement by Thymectomy of Tumor Formation by Oncogenic Adenoviruses* (33039)

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The role of the thymus in the development of immunological competence has been reviewed by Miller (1). Briefly, the removal of the thymus from perinatal mammals frequently results in an impairment of the production of specific immunoglobulins (antibodies), of the development of delayed hypersensitivity, and of the rejection of tissues from histoincompatible donors. The specific effects of thymectomy on the Golden hamster (*Mesocricetus auratus*) have been described by Sherman *et al.* (2).

A variety of animal neoplasms possess new antigens, functionally similar to minor histocompatibility antigens, that are capable of eliciting immune responses (3). It has been demonstrated that neonatal thymectomy reduces these immunological responses, frequently resulting in an enhanced susceptibility to tumor induction (4).

Yohn *et al.* (5) reported that thymectomy at 3 weeks following injection of adenovirus type 12 (A-12) into newborn Syrian hamsters enhanced susceptibility to tumor induction in male animals. Kirschstein *et al.* (6) found that A-12 produced tumors in BALB/c and C3H/HeN mice only if they had been thymectomized at birth. Allison *et al.* (7) observed a greater incidence of tumors in CBA mice inoculated with low doses of A-12 and either thymectomized or injected with antilymphocyte sera.

Previous reports describe the results of inoculation of 30 human adenoviruses into neonatal hamsters (8,9). Adenovirus type 12 (A-12) induced tumors in a high incidence and short latent period (8). Tumors were also observed with types 1, 7, 8, 14, 18, 21, and 24, but the incidence was generally low and the incubation period long (9). The present report gives the results of the inoculation of most of these latter viruses of suspected oncogenicity into thymectomized hamsters.

Methods. Syrian hamsters, of both sexes, less than 24 hours old were inoculated either subcutaneously (s.c.) or intraperitoneally (i.p.) with 0.1 ml of undiluted adenovirus types 1, 7, 8, 14, 21, or 24. The viruses were grown and titered in human embryonic kidney cells.[1]

For thymectomy, newborn or 1-week-old animals were secured with masking tape. Using a dissecting microscope for visualizing the surgical field, the skin and sternum were opened down to the second or third rib and the width of the incision was extended with forceps. A cotton tipped applicator stick was gently rotated about the thymus until both lobes became detached. The sternum and skin were then closed by the use of collodion. All inoculated animals were thymectomized on day 7 except those for adenovirus 21 which were done on day 0 and for adenovirus 7 which were done either on day 0 or day 7.

Results. The results of inoculation of adenovirus types 1, 7, 8, 14, 21, and 24 into thymectomized and nonthymectomized hamsters are shown in Table I. To date, tumors have been observed in thymectomized groups inoculated with types 7 and 21 (Table I). With adenovirus 7, 17% of the 76 (s.c. and i.p. routes combined) thymectomized animals

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