the amount of ^{14}C-testosterone converted to estradiol by slices of ovaries from anestrus dogs. Whether the testosterone → estrogen conversion is also subject to FSH control in rat ovaries, or whether an earlier step in steroid metabolism is involved, requires further study.

Although our results have been expressed in terms of estradiol equivalents, the identity of the estrogenic substance(s) produced has not been established. However, when rat ovary slices were incubated with androstenedione-4-^{14}C, and the phenolic fraction chromatographed in a ligroin-propylene glycol-methanol system, one of the 7 radioactive peaks detected corresponded in position to estradiol-17-β.

Summary. Slices of ovaries from rats treated with chorionic gonadotropin produced biologically detectable amounts of estrogen when incubated under the proper conditions *in vitro*. FSH, TPN and Δ^4-androstene, 3,17-dione, in various combinations, enhanced estrogen production. In the presence of maximally effective concentrations of androstenedione and FSH, one gram of ovarian slices formed the biological equivalent of about 1.2 μg of estradiol-17-β in 3 hours.

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Recovery and Behavior of Hepatitis Virus from Swiss Mice Injected with Ascites Tumor.* (28528)

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Weekly passage of an ascites tumor has been made in our laboratory in both Princeton (Pr) and Swiss mice as an index of contamination with murine hepatitis virus (MHV). Presence of the virus was indicated by an abnormal tumor response and appearance of necrotic lesions in the liver. Findings with Swiss mice are presented here as a supplement to an earlier report on Pr mice(1).

Materials and methods. The random-bred colony of Swiss mice at the Rockefeller Institute was begun in 1926 and operated for 32 years by conventional methods. In 1958 the breeders showed such a high rate of chronic respiratory disease (CRD) and were so heavily infested with mange mites and pinworms that a specific pathogen-free (SPF) sub-line was started as a replacement(2). The founding breeders were cesarian-derived and reared by foster mothers from our CRD-free colony of Pr mice.

Unless otherwise noted the experimental mice from the 2 Swiss and the Pr colonies were weanlings of either sex, 3 to 4 weeks old, (10-15 g), and were used in groups of 5. Intraperitoneal injections of both livers (ground) and ascitic fluids were usually made with .1 ml of approximately 10% suspensions in saline. The inoculum in nurslings and by other routes was .02 to .05 ml. Survivors were generally killed with ether on the 7th day and autopsied. Livers were examined with a dissecting microscope and ascitic fluids in a fresh state by phase contrast. Appearance of the fluid on the 7th day after injection was a valuable diagnostic aid in Swiss mice. The color was commonly blood-red in the absence of MHV and white or pale yellow in its presence. In the combined series ascitic fluid was injected first on one side of the abdomen and liver suspension

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after a short interval on the opposite side. Subsequent passages were made with fluid alone diluted 1:10 with saline. All of the tumor transfers in Swiss mice were begun with ascitic fluid from infection-free Pr mice.

Results. Earlier examination of several hundred Swiss mice from the conventional colony had revealed liver lesions in only 2 weanlings from a group injected with *Eperythrozoon coccoides*. In 1959, however, a fluctuating but persistent hepatic infection was indicated by the ascites tumor test. Liver involvement, pathognomonic of murine hepatitis, appeared in one or more mice from each of 6 different passage series after 6, 2, 5, 4, 5, and 11 tumor transfers.

It is of interest that several mice of a later series showed reduced amounts of ascitic fluid in the absence of hepatic lesions but accompanied by a white film over the liver. This reaction was unexplained then but is now known to be characteristic of the oncolytic virus (reovirus 3) later described by Tarnowski and the writer(3).

In the fall of 1959 a passage series with ascites tumor was begun in Swiss weanlings from the SPF colony. The Swiss mice used in all of the following experiments were from this colony. The tumor was transferred weekly in 520 mice over a 2-year interval with no evidence of contamination with MHV. In the 105th passage, however, reovirus 3 appeared and the series was discontinued. A second one was then started and has been maintained to date in 370 mice without appearance of either virus.

Two strains of hepatitis from the tumor series in conventional Swiss mice were studied in detail. Liver suspensions, supernatants, and filtrates (Coors No. 3 filters) produced typical hepatic lesions on intraperitoneal injection in Swiss weanlings. The respective viruses were designated MHV(SR)1 and 3 to distinguish them from MHV(S) recently described by Rowe *et al.*(4). Both viruses were serially transmissible by injection of liver suspensions at weekly intervals. Ascites tumor cells were not detectable after the first passage. Pathologic reaction was limited to the liver and manifested by focal or semidiffuse necrosis with or without petechial hemorrhage. Intracerebral injection was also followed by hepatic involvement but was not attended by paralysis.

MHV(SR)1. The morbidity rate was 98% and mortality rate 17% in 100 Swiss weanlings during the first 20 passages. Deaths were sporadic and occurred on the 5th-7th day after injection. Survivors were generally underweight but otherwise normal in outward appearance. They showed considerable variation in the degree of hepatic involvement at autopsy. Active virus was demonstrable in the liver 2 weeks after injection but not after 3 weeks. Recovery was usually complete at this time and the livers normal but neutralization of the virus by blood serum was negligible.

Behavior of the virus in Pr mice was of particular interest. Twenty weanlings, injected with 4 different suspensions, were alive on the 7th day and showed no liver involvement. Survival of the virus in mice of the initial passage was indicated by appearance of liver lesions on transfer to susceptible Swiss mice. It was not demonstrable thereafter and could not be maintained by serial transfer.

Five-day old Pr nurslings were nearly as resistant as weanlings. One-day old infants were susceptible, however, and the virus was maintained in them for 5 intraperitoneal passages at weekly intervals. Only one of the 35 nurslings, in this series, failed to show either clinical or postmortem signs of hepatitis. Seventeen deaths occurred on the 5th-7th day after injection. Pathologic findings were again limited to the liver and characterized by necrotic lesions.

An unexpected result was obtained on testing the activity of the passaged virus in weanlings. The suspension from the 5th nursling transfer produced liver lesions not only in Swiss weanlings from which the virus was originally obtained but also in Pr weanlings in which it had formerly been inactive. The dual pathogenicity of the virus was maintained during 10 subsequent passages in the 2 mouse strains.

A combined series with both tumor and virus was begun in Swiss weanlings and continued for 4 weekly passages with fluid alone.

Twelve of the 25 mice were dead by the 7th day. The mortality rate was much higher than that with either agent alone, 48% as compared with 17% for the virus and less than 10% for the tumor. The 13 survivors showed distended abdomens at this time and yielded appreciable amounts of white or pale yellow ascitic fluid. Normal tumor cells were demonstrable in the fluids but were reduced in number. Carriage of the virus in the fluids was indicated by the presence of well developed necrotic lesions in all of the livers.

A combined series with both agents was also established in Pr mice and continued for 10 passages. Only 2 of the 50 mice died. Abnormal ascitic fluid was regularly obtained from the survivors and unexpectedly 43 of them showed hepatic involvement. The virus also produced liver lesions in the absence of the tumor and was maintained for 5 passages in both Swiss and Pr weanlings.

MHV(SR)3. The morbidity and mortality rates were 96 and 37% respectively in 75 Swiss weanlings during the first 15 passages. Aside from the greater number of deaths with strain 3 and minor differences in the histopathology of the liver, not dealt with here, the behavior of the 2 strains was much the same in Swiss mice.

Pr weanlings of comparable weight and age were resistant. Fifteen mice injected with 3 different liver suspensions showed neither hepatic involvement nor survival of the virus. Pr nurslings were also resistant. Twelve 5-day old (2 litters) and 20 one-day old infants (3 litters) injected with different lots of virus were normal when finally brought to autopsy. Active virus in low concentration was demonstrable in the pooled liver suspension from the 3rd group of one-day old mice but could not be maintained on passage.

The results of the combined series in Swiss weanlings were similar to those with MHV(SR)1. In the presence of both tumor and virus the mortality rate with 25 mice in 5 passages was 56%. Eleven survivors showed appreciable amounts of abnormal ascitic fluid and liver lesions. There was a striking difference, however, in the behavior of the 2 viruses in Pr weanlings. MHV(SR)3 was completely inactive on injection with the tumor and could not be recovered from either the fluid or the liver. Thirty-three mice in 2 series of 3 passages showed only a normal response to the tumor.

Discussion. The findings with Swiss mice from the conventional colony indicated that at least 10% of the weanlings were carriers of MHV. Emergence of the virus during passage of ascites tumor was attended by appearance of liver lesions. The pathologic findings were essentially similar to those reported in our earlier work on lymphocytic leukemia in Pr mice(5). It is possible that MHV was carried to the liver from some other site by the respective neoplastic cells. It seems more probable, however, that the virus was already present in the liver in a non-infective state and was activated by the tumor in some unexplained way. There is no evidence to support the suggestion by Rowe *et al.* that the occasional appearance of MHV in the liver during passage of leukemic cells and also *Eperythrozoon coccoides* might result from fecal contamination of the inoculating needle or skin(4). Neither active nor inactive virus have ever been demonstrated in intestinal washings of normal mice from our colonies.

It may be of some significance that the high rate of viral carriage in the conventionally reared Swiss weanlings coincided with a high rate of infestation with ecto and endoparasites. These parasites were also present in mice from the Pr colony during the period when MHV was occasionally detected in leukemic weanlings. Virus has not appeared, however, in mice from either colony since mange mites and pinworms were virtually eliminated by appropriate procedures.

The 2 strains of virus, MHV(SR)1 and 3, which were recovered from Swiss mice not only differed from MHV(Pr) but also differed from each other. The alteration in host specificity of MHV(SR)1 by passage in one-day old Pr nurslings and by combination with ascites tumor in Pr weanlings was similar to that earlier reported for MHV(Pr) by passage in Swiss weanlings(6). The host specificity of MHV(SR)3 was unaffected by these procedures. It is the only related virus in our experience which has failed to multiply

in weanlings of a diverse mouse strain on combination with ascites tumor.

Summary. Abnormal response to ascites tumor on passage in weanlings from a conventionally maintained colony of Swiss mice indicated a high rate of infection with murine hepatitis virus (MHV). It was not encountered, however, during weekly transfer of the tumor over a 2-year period in weanlings from a specific pathogen-free colony of the same Swiss line. Two strains of the virus, MHV(SR)1 and 3, were differentiated by their behavior in Swiss and Princeton (Pr) mice. The host specificity of strain 1 was altered by passage in one-day old Pr nurslings and in presence of the tumor but that of strain 3 was unaffected.

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Autoradiography with the Agents of Psittacosis and Trachoma.* (28529)

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Microorganisms associated with psittacosis, lymphogranuloma venereum, and trachoma have been conveniently and perhaps appropriately designated as PLT agents(1). However, phylogeny remains an issue(2). Group associations are based on morphological(3,4), serological(5), and biochemical(6) criteria. In addition comparison of developmental cycles by fluorescence microscopy of acridine orange-stained preparations showed unique cytochemical patterns(7) which were not observed with *Rickettsia burnetti,* vaccinia, or adenovirus. Recently, Pelc and Crocker(8) noted a "low utilization" of thymidine by an unidentified strain of psittacosis. Pollard and Starr(9) reported that thymidine did not reverse the inhibitory effect of aminopterin on the TT strain of psittacosis in contrast to its reversal effect on the host cell. It was stated (10) on the basis of inhibition studies that the psittacosis agent cannot utilize preformed thymidine. Bernkopf *et al.*(11) reported incorporation of tritiated thymidine by the T'ang strain of trachoma during a late stage of development. Thymidine utilization by the agents of psittacosis and of trachoma has been reexamined by autoradiographic technics. Both agents were tested under similar experimental conditions.

Methods and materials. The McCoy cell line(12), derived from human synovial tissue, was grown on coverslips in Leighton tubes. Medium consisted of mixture 199 (Microbiological Associates) plus 10% heat-inactivated calf serum, 0.5% lactalbumin hydrolysate, and 0.1 mg streptomycin per ml. Psittacosis agent (TT strain)(13) was propagated in chorioallantoic fluid of 7-day old embryonated chicken eggs, pooled, ampouled, and stored at –40°. Trachoma agent (T'ang strain)(14) was adapted to McCoy cells(15). Stock was obtained from pooled sonicates of infected cells, ampouled, and stored at –40°.

Stocks were titrated by a modification(16) of the infected cell count technic described by Weiss and Huang(17). Inocula were prepared by dilution of stock in nutrient medium to insure infection of 60 to 80% of the cells. Both agents were treated alike in parallel experiments. In one series of tests, McCoy cultures were inoculated and after a 2-hour absorption period at 37° medium

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