baboon kidney cell is being considered in our laboratory as a possible substrate for poliovirus vaccine production.

Summary. Baboon (*Papio doguera*) kidney cells demonstrate a susceptibility to human enteroviruses, in general, similar to that of the rhesus monkey. Its relative freedom from "native" viruses and ability to demonstrate the presence of SV_{40} virus suggests a more widespread application to virological studies and vaccine production.

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Hypoxanthine Dehydrogenase in the Developing Chick Embryonic Kidney. (27785)

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Although several investigations(1,2,3) have established the pattern of uric acid excretion in the allantoic fluid of chick embryos, no studies have been reported on the development of enzymes which accomplish the synthesis of uric acid in embryonic tissues. Morgan(4) showed that embryonic liver did not perform the reaction. Adult avian kidney was shown by Edson *et al.*(5) to be an active site of uric acid synthesis, and recently this reaction was found to be catalyzed by a DPN dependent hypoxanthine dehydrogenase(6). It, therefore, seemed of interest to study the changes in hypoxanthine dehydrogenase in renal tissue during the embryonic period.

At the 5th day of incubation of eggs, uric acid appears in the allantoic fluid. The mesonephric kidney of the embryo functions from the 5th to 12th day as the excretory organ(7). Between the 12th and 15th day the metanephric kidney, which becomes the adult avian kidney, rapidly develops and becomes functional. Both mesonephric and metanephric tissues thereafter perform secretory and excretory functions in the chick embryo, although involution of the mesonephros begins after the 15th day(8). In this paper the specific activity of hypoxanthine dehydrogenase in the mesonephric and metanephric renal tissue is reported for the period of incubation from the 5th to the 21st day.

Materials and methods. Diphosphopyridine nucleotide (DPN) was purchased from Pabst Laboratories and hypoxanthine from Nutritional Biochemical Co. Rhode Island Red eggs obtained from a local farm were incubated at 37.8° in a constant temperature wooden incubator whose humidity was kept at approximately 60%. To insure optimal growth conditions, the eggs were rotated every 24 hours. Morphological development was classified according to the stages proposed by Hamburger and Hamilton (9) for normal development of the chick embryo. The average incubation time quoted by these authors for each stage was used, so that the morphological development is expressed as "corrected incubation time." After varying periods of incubation, the eggs were candled

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and their shells marked on the surface to indicate the position of the embryo. Embryos with their extra-embryonic tissues were separated from the egg yolk and white, washed with ice cold Ringer solution and distilled water to rid the embryo of the extraneous yolk and blood.

Under observation with a dissecting microscope, mesonephric and metanephric renal tissues were separately removed from the embryos. The amount of tissue taken for homogenization varied between 50 and 200 mg. In the early incubation period 20 to 50 embryos were required, the number decreasing with advancing age of the embryo until by the 12th day, 6 embryos sufficed. The tissues were disrupted in 1.5 ml of 0.05 M phosphate buffer, pH 8.0, at 0° in glass homogenizers. Homogenates were centrifuged at 34,000 × *g* for 20 minutes at 0°. The supernatant fractions were removed and kept chilled in ice. Assays were performed within one hour after homogenizing the tissue.

Hypoxanthine dehydrogenase (H x DH). Enzyme activity was measured by the increase in absorption at 340 mμ due to the reduction of DPN during the production of uric acid from hypoxanthine(6). Hypoxanthine + 2 DPN ⟶ Uric Acid + 2 DPNH. The reaction mixture contained 2.0 μM of DPN and 1.0 μM of hypoxanthine in 1.0 ml of 0.1 M tris buffer, pH 8.0. To this solution 0.1 or 0.2 ml of the tissue supernatant fraction was added. These conditions were optimal with respect to pH and substrate concentration. The incubations were conducted in 1-ml silica cuvettes at room temperature in a Beckman spectrophotometer. The optical density of the incubation mixture was read against a blank from which enzyme was omitted. The optical density of the reaction mixture was then measured at zero time, 5 minutes, and thereafter at 10-minute intervals for a total period of 40 minutes.

The specific activity of hypoxanthine dehydrogenase, expressed as μmole DPNH increase/min/mg protein, was calculated from the increment of 340 mμ absorption between the 5-min and 40-min reading. Under the conditions of the assay described, the increase in 340 mμ absorption was linear throughout this period for 0.1-ml or 0.2-ml aliquot of supernatant fraction. The particle fraction contained no hypoxanthine dehydrogenase activity.

Protein determination. The Folin-Ciocalteau method as modified by Sutherland *et al.* (10), was employed for determination of soluble protein. Bovine plasma albumin dissolved in 0.1 M phosphate buffer (pH 9.0) was used as the standard protein.

Results. The data of Fig. 1 show the rapid development of hypoxanthine dehydrogenase activity which takes place in the mesonephric kidney between the 5th and 7th days of incubation. Activity continued to increase in the mesonephros and reached a maximum at the 14th day. Reference to Fig. 2 shows that the sharp rise in hypoxanthine dehydrogenase activity which took place between the 5th and 7th day in this tissue was not accompanied by an increase of the ratio of supernatant (soluble) protein to the wet weight of tissue. The rise in hypoxanthine dehydrogenase activity between the 9th and 14th days was associated with a gradual rise in the proportion of soluble protein in the mesonephros. Following the 15th day in the mesonephric kidney, the specific activity of hypoxanthine dehydrogenase declined as did, to a lesser extent, the ratio of soluble protein to weight of the tissue. These changes are correlated with the involution of the mesonephric kidney which slowly takes place in the late embryonic period(8).

In the incubation period of 12-15 days the metanephric kidney undergoes rapid morphological and functional development(8). The specific activity of hypoxanthine dehydrogenase increased abruptly at this time (Fig. 1) but did not reach the maximum value found for mesonephric tissue. The ratio of soluble protein to weight of metanephric tissue showed a constant increase from day 8 to day 14, and a more abrupt rise at day 15 followed by a relatively stable maximum. The weight of the metanephric kidney rapidly increased in the late embryonic period but the principal change in hypoxanthine dehydrogenase and soluble protein pattern appeared to be established by the 16th day.

Extracts prepared from embryonic liver at

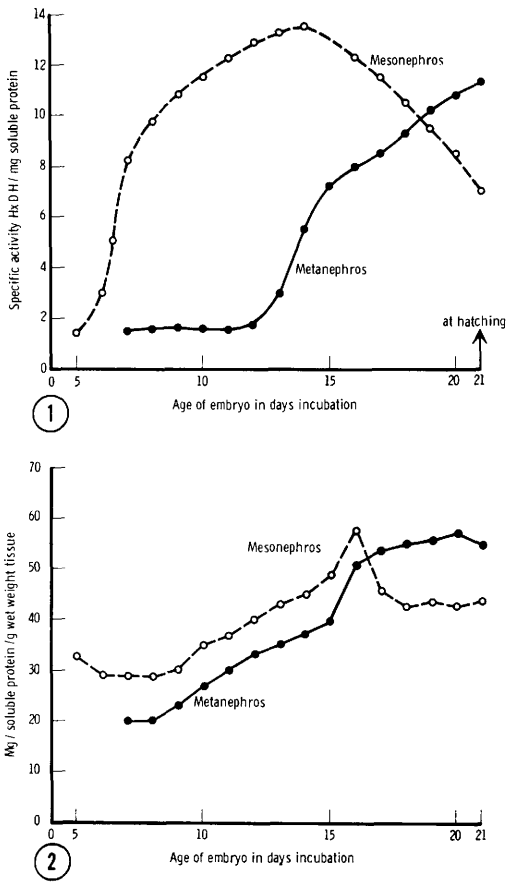

FIG. 1. Specific activity of hypoxanthine dehydrogenase (HxDH) in mesonephric and metanephric renal tissues of the chick embryo at various stages during incubation period. Each point represents specific activity of a pooled sample of tissue from several embryos (see *Methods* section). Two to 4 separate batches of eggs were harvested at each day to establish the points represented. In any individual assay experimental variation was less than 10%. Variation between different batches of eggs was not great except for periods of incubation characterized by changing levels of enzymatic activity.

FIG. 2. Amount of soluble protein (supernatant fraction) per g wet wt of mesonephric and metanephric renal tissues of chick embryo at various periods of incubation. Values are avg obtained from the experimental data represented in Fig. 1 and vary within $\pm$ 4 mg.

several stages of incubation were incapable of converting hypoxanthine to uric acid, a finding first reported by Morgan(4).

Injection of hypoxanthine in amounts 5, 10 or 20 μmole into the chick embryo at the 5th day of incubation did not lead to a specific activity of hypoxanthine dehydrogenase which was higher than normal in the kidney tissue assayed on the 8th day of incubation.

Discussion. Uric acid synthesis and excretion were found to be accomplished in the chick embryo by both the mesonephric and metanephric kidneys. The differentiation of these tissues with respect to hypoxanthine dehydrogenase activity is well correlated with previous findings of renal function in the embryo studied by secretion of dyes by the renal tubules(8). The studies reported here indicate that some aspects of protein synthesis and enzymatic differentiation might be experimentally approached through studies *in vitro* of the metanephric tissues of the 14-15-day embryo.

Summary. The activity of hypoxanthine dehydrogenase was determined in mesonephric and metanephric renal tissue of the chicken embryo and related to the amount of soluble protein in these tissues during successive stages of incubation. The period of most rapid increase of specific activity occurred at days 6 and 7 in the mesonephros and at days 13 to 15 in the metanephros. The highest specific activity was obtained on day 14 in the mesonephric tissue which in the subsequent period of incubation exhibited decreasing hypoxanthine dehydrogenase activity. The metanephric tissue, which becomes the functional kidney of the chicken, attained a plateau of hypoxanthine dehydrogenase activity by the 16th day.

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Cancer Induction in Hamsters by Human Type 12 Adenovirus. Effect of Age and of Virus Dose.* (27786)

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We have recently reported(1) that hamsters injected intrapulmonarily at birth with human type 12 adenovirus develop a high incidence of malignant sarcomas in the thorax and liver in from 1 to 3 months. The tumor-inducing activity is not lost by Selas 02 filtration or by tissue culture passage in HeLa cells. The induced tumors grew in and killed a high percentage of unconditioned young adult hamsters into which they were transplanted. Polyoma virus antibodies (HI) were not present in tumor bearing or control hamsters. Evidence was presented that the tumor inducing effect was not attributable to contamination with SV40 or other animal viruses, but was indeed associated with the human adenovirus. No such tumors occurred in hamsters injected with control tissue culture fluids or in control breeder hamsters. The present report deals with the effect of age at time of injection, and with the dose-response relationship.

Materials and methods are as previously reported(1) unless otherwise stated. Human type 12 adenovirus was propagated in human embryonic lung cells kindly supplied by Drs. Hayflick and Koprowski of the Wistar Institute, or in HeLa cells from Microbiological Associates, Inc. Virus titer was determined by cytopathic effect in tissue cultures of HeLa cells as previously described. The final reading was made on day 5. The titers given are therefore probably underestimates of the actual virus titer. Hamsters 8 days of age or younger were intrapulmonarily injected as previously reported. In hamsters of 2 weeks of age or older, the lung could not be seen through the chest wall and these were injected by inserting the needle 7 to 10 mm through the right ventral chest wall.

Results. In hamsters less than 24 hours old at time of injection 500 tissue culture infective doses (TCID) of virus produced tumors in 26 of 27 animals, and as little as 0.5 TCID produced a tumor in 1 of 5 animals. The 50% tumor dose lies somewhere between 0.5 and 50 $MTCID_{100}$ (Table I). Susceptibility to tumor induction decreased rapidly with increasing age at time of injection. After injection at 8 days of age 500 TCID induced tumors in only 2 of 9 animals. A dose of 1000 TCID produced one tumor in 8 hamsters injected at 2 weeks of age, but none in hamsters injected at 3 weeks of age or older. The tumors induced were, as previously reported(1), undifferentiated sarcomas at the site of injection, arising after a short latent period.

Discussion. The observed decrease in susceptibility with increasing age is in keeping with what is known of other tumor virus systems. It is possible that doses larger than 1000 TCID may result in tumor induction in hamsters older than 2 weeks at time of injection. It is even more probable that factors which depress immunological reactivity, such as whole body X-irradiation, may permit tumor induction following injection of virus into still older (irradiated) hamsters(2). If malignant transformation induced by adeno-

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