did not observe pericentriolar bodies associated with the centriole of metaphase cells; he suggested that pericentriolar satellites apparent at interphase are not present at metaphase. Cilia associated with centrioles in the rat kidney appear to be highly infrequent in normal mammalian metanephros (*9*). Occasional cilia are observed in human mesonephros, but they are frequent in the pronephros; this suggests that cilia are an evolutionary remnant in the proximal tubules (*10*). Serial sections failed to indicate that the centrioles that we observed were associated with cilia, although the position of the centrioles close to the base of the microvilli and the presence of pericentriolar filaments resembling striated rootlets do not preclude this possibility. Cilia can be induced in interphase fibroblasts by the mitotic inhibitor colcemid (*11*). Because the centrioles in our study are apparently from cells arrested at interphase and occur in proximal tubule cells having a ciliary background, the pericentriolar filaments may have been induced by the combined stresses and could possibly differentiate into cilia.

The alterations of centrioles may also be related to their mitotic function. The frequency of centrioles was lowest in animals raised in air and receiving a chemically defined diet that contained adequate or greater quantities of vitamin E. Apparently, the quantity of vitamin A did not influence the number of centrioles. If it is assumed that centrioles will be observed only infrequently in normal mitosis, then the increased numbers of centrioles observed in animals depleted of vitamin E and exposed to oxygen suggest a dysfunction in the formation of the mitotic apparatus. Increased numbers of centrioles could result from an increased rate of mitosis or from inhibition of mitosis after centriole proliferation but before spindle formation. The accumulation of centrioles in animals exposed to air and depleted of vitamin E and in those exposed to oxygen suggests that an oxidative function is involved. Ample evidence of the antioxidant role of vitamin E exists (*12*). Sulfur amino acids are known to be important in the time sequence of the symptoms of muscular dystrophy associated with deficiency of vitamin E (*13*), and they are known to be easily oxidized by molecular oxygen or lipid peroxides (*12*). Molecular oxygen inactivates enzymes containing SH groups as well (*14*). Centrioles contain concentrations of SH groups; the maintenance of the reduced state of these SH groups appears necessary for the completion of mitosis (*15*). Soluble thiols (perhaps glutathione) decrease up to metaphase and then increase as cell division proceeds. Increased tensions of oxygen decrease (i) the concentrations of reduced glutathione in the blood (*16*), (ii) tissue protein SH groups, and (iii) nonprotein SH groups (*3*). The dysfunction of the centrioles may involve oxidation of cellular lipids which accumulate, or it may involve oxidation of SH groups. Vitamin E may function to protect SH groups directly or indirectly through an antioxidant chain. The pericentriolar bodies may be associated only with interphase, and the properties of vitamin E that alleviate sterility may be involved in the direct protection of the mitotic apparatus.

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Murine Hepatitis Virus: Effect on Liver RNA

Abstract. *After infection of mice with hepatitis virus MHV3, the RNA in the liver undergoes changes. The fraction extracted with phenol at 0°C does not alter. However, the fraction extracted with hot phenol at elevated* p*H (60°C,* p*H 8.3) shows a 16*S *peak on sucrose-density-gradient centrifugation. This fraction shows actinomycin D–resistant incorporation of* C^{14}*-orotic acid in infected but not in control livers—possible evidence of the RNA nature of MHV3.*

Direct evidence on the genetic material of hepatitis viruses has been difficult to obtain. In studies of murine hepatitis virus MHV3, elevation of serum enzymes (*1*), alteration in tissue culture morphology (*2*), changes in oxidative enzymes (*3*), lysosomes (*4*), and electron microscopic appearance (*5*) have been described. Actinomycin D and fluorodeoxyuridine have no effect on the course of the infection (*6*). From these findings, it may be tentatively concluded that MHV3 is an RNA virus, is independent of DNA-directed synthesis of RNA or DNA replication, and requires the induction of an RNA-RNA polymerase (*7*). More direct evidence of the RNA nature of the virus requires demonstration of alteration of RNA metabolism in infected livers, this alteration being insensitive to actinomycin D.

The MHV3 virus obtained from the Rockefeller strain was inoculated into 21-day-old mice (white inbred Swiss strain). The strain was propagated by (i) homogenization of livers (10 percent weight to volume) infected for 48 hours, (ii) centrifugation at 2000*g*, and (iii) intraperitoneal injection of the supernatant. The supernatant stock was stored at −20°C. The course of the infection was determined by gross and microscopic examination of the liver and also by determination of serum lactate dehydrogenase and alanine aminotransferase (E.C. 2.6.1.2.). Seven hours before they were killed, infected and control mice received intraperitoneal injections of saline or of saline containing actinomycin D (100 μg per 100 g of body weight); 4 hours later, 20 μc per 100 g of body weight of C^{14}-orotic acid was also injected (specific activity, 5 mc per millimole). After the mice were killed, the liver was

Table 1. Alanine aminotransferase and lactate dehydrogenase activity in serum of infected mice with or without actinomycin D. Results are given in Sigma-Frankel (S.F.) and Berger-Broida (B.B.) units and represent the mean of ten individual determinations. In the control (no infection), the results represent the 30 determinations for serum alanine aminotransferase and the 25 for LDH. Actinomycin D (act. D) was administered 4 hours before the animals were killed.

Time after infection (hr)	Serum alanine aminotransferase (S.F. units)		LDH (B.B. units)	
	Without act. D	With act. D	Without act. D	With act. D
12	84.8 ± 5.6	78.3 ± 5.9	3459 ± 803	3923 ± 561
24	94.3 ± 9.1	74.8 ± 10.6	3265 ± 701	2825 ± 781
48	622.0 ± 112.1	362.3 ± 183.6	6417 ± 1208	5327 ± 845
60	1654.6 ± 344.7	855.0 ± 312.7	15037 ± 3113	7102 ± 1203
72	2492.1 ± 570.1	1807.8 ± 811.4	24394 ± 2651	15789 ± 6744
84	6463.3 ± 1013.4	5185.7 ± 1667.8	36323 ± 7540	21227 ± 5910
96	6059.1 ± 1442.0	4674.5 ± 1530.0	36977 ± 8863	25647 ± 8175
0	68.2 ± 2.9	59.8 ± 4.1	3051 ± 318	2611 ± 445

quickly removed, and the RNA was extracted. When DNA was to be extracted, C^{14}-thymidine was administered 24 hours before death, and no actinomycin D was given.

When RNA was to be extracted, the liver was homogenized (10 percent weight to volume) in 0.25*M* sucrose containing 0.05*M* tris-HCl at *p*H 7.6 and 0.003*M* $CaCl_2$ at 0°C in a Teflon-glass homogenizer. The S_1 fraction (first supernatant) was obtained after centrifugation at 450*g* for 10 minutes. Enough tris-HCl was added to the S_1 fraction to make it 0.1*M*, with respect to the tris (*p*H 7.6), and this was stirred with the same volume of water-saturated, freshly distilled phenol at 0°C for 40 minutes. After centrifugation (5000 rev/min for 2 minutes), the aqueous layer was used for precipitation of the RNA fraction at *p*H 7.6, and the residue was thoroughly mixed with an equal volume of cold distilled water in an all-glass homogenizer. An equal volume of 1*M* tris-HCl adjusted to *p*H 8.3 was added, and the solution was divided into two equal fractions; both were stirred for 40 minutes, one at 38°C and the other at 60°C. These two fractions were then used for preparation of two other classes of RNA, that obtained at 38°C and that obtained at 60°C, both at *p*H 8.3.

The RNA was precipitated by the addition of 0.1 volume of 10 percent NaCl and 2.5 volumes of 96 percent ethanol to the aqueous extracts and stored for 2 hours at −10°C. The precipitate was dissolved in 2.0 ml of cold twice-distilled water, and NaCl was added to a final concentration of 10 percent. After the precipitate had been left in the cold overnight, it was collected by centrifugation at 20,000*g* for 10 minutes and washed in 66 percent ethanol. The RNA was dissolved in 0.1*M* NaCl in 0.01*M* sodium acetate buffer, *p*H 5.1, and analyzed by ultracentrifugation in a linear sucrose gradient (5 to 20 percent) also containing 0.1*M* NaCl in 0.01*M* sodium acetate buffer at *p*H 5.1. The analysis was performed in the Spinco L2 with an SW39 rotor at 35,000 rev/min for 240 minutes at 0°C. Consecutive three-drop samples from the bottoms of the tubes were collected at the end of the run and diluted to 3.0 ml; the optical density was measured in a Beckman DU2 spectrophotometer at 260 and 280 nm, and the radioactivity was determined in a Nuclear-Chicago scintillation counter. The DNA was prepared according to Marmur's method.

Heparinized blood samples were obtained by exsanguination after neck dislocation. Alanine aminotransferase was assayed colorimetrically, and results were expressed as Sigma-Frankel units per milliliter (*8*). Lactate dehydrogenase (LDH) was estimated by the colorimetric determination of unchanged pyruvate after incubation. Results were expressed as Berger-Broida units per milliliter (*8*).

Table 2. Each result, given in disintegrations per minute per optical density unit, is the mean for two experiments.

Extraction temp. (°C)	RNA specific activity (dpm/O.D.)			
	28*S*	18*S*	16*S*	4*S*
	Control			
0°	200	305		714
38°	720	820	1700	
60°			1150	
	Control and actinomycin D			
0°	45	102		1248
38°	305	420	830	
60°			350	
	Infected (48 hours)			
0°	351	641		1163
38°	620	1000	1400	
60°			1010	
	Infected and actinomycin D			
0°	38	136		944
38°	280	195	1600	
60°			1080	

At 48 and 72 hours after injection of 0.1 ml of a supernatant centrifuged at 2000*g* [from a homogenate of liver (10 percent weight per volume)] into 25 mice, roughening of the fur was observed. Three mice died at 96 hours, 13 at 120 hours, and four at 144 hours; the overall mortality was 80 percent. No histological evidence of infection was observed until small necrotic foci became evident at 48 hours. These were comprised of necrobiotic or hyalinized parenchymal cells and contained acidophilic bodies. These foci increased in size and number until the whole liver was affected. Hence a 48-hour period was considered suitable for our studies of nucleic acid alteration.

The alanine aminotransferase began to rise (Table 1) as early as 12 hours after infection, but there was little further change at 24 hours. There was a progressive rise from 48 to 84 hours as measured at 12 hourly intervals, and after 84 hours there was no further rise. Serum LDH rose from 48 to 96 hours and appeared to have reached a plateau at that time. In both cases actinomycin D appeared to inhibit the increase in serum enzymes; this effect may be due either to direct inhibition occurring in the assay system, or to an effect on the synthesis or release of enzymes from the liver. Although direct effects could be demonstrated on the assay system for LDH when actinomycin D was added at a concentration 20 times greater than that used in the injection procedure, these effects were insufficient to account for the observed inhibition in vivo.

When DNA extracted from mouse liver was centrifuged through CsCl density gradients, one major peak corresponding to a density of 1.71 was observed. On occasion (about 30 percent of the time), there was a small satellite band of density 1.68. The incorporation rates of C^{14}-thymidine were approximately equal into both bands. This satellite band may be either mitochondrial DNA or DNA from another cell type appearing in liver after infection.

RNA metabolism in mouse liver was

determined for infected and control groups, with and without actinomycin D treatment, by extraction carried out at 0°C and at *p*H 7.6. The data in Table 2 were obtained in mice infected for 48 hours, but essentially identical results were obtained at 24, 72, and 96 hours. Three major peaks of optical density and C^{14}-orotic acid incorporation were found, corresponding to 4*S*, 18*S*, and 28*S* fractions. Although there appeared to be a slightly higher specific activity in the infected livers (a result observed at other times also), the effect of actinomycin D was essentially the same in both groups, with suppression of incorporation of labeled orotic acid into all high-molecular-weight RNA and sparing of the 4*S* fraction.

With an extraction temperature of 38°C and a *p*H of 8.3, three peaks of optical density were found corresponding to 28*S*, 18*S*, and 16*S*. Orotic acid incorporation was observed in all three fractions and was sensitive to actinomycin D. In contrast, these three groups of RNA molecules in infected livers were selectively affected by actinomycin D in that residual incorporation of orotic acid-6-C^{14} was observed in the 16*S* fraction. Furthermore, as in control liver, there was considerable asymmetry between peaks of optical density and count incorporation.

With extraction at 60°C also at *p*H 8.3, a single major peak was seen in both groups, which corresponded to a 16*S* sedimentation value. With actinomycin D there was significant suppression of orotic acid incorporation into this fraction in control livers, but virtually no such suppression in infected mice (Fig. 1). Table 2 shows the effect of actinomycin D on the specific activity of the different RNA fractions obtained from control and infected livers. There was a lack of effect of antinomycin D on the 16*S* fraction in infected liver and sparing of the 4*S* fraction in control livers (Fig. 1).

One interpretation of these results is that infection in some way alters the response of the liver to actinomycin D. However, in both control and infected livers, the optimum dose of actinomycin D (100 μg per 100 g of body weight) reduced orotic acid incorporation into the 18*S* and 28*S* peaks. It is unlikely, therefore, that only the response of the 16*S* fraction is affected by infection, unless it is more directly related to the infectious process.

An alternative explanation is that the RNA extracted at 60°C, *p*H 8.3, from the infected livers sedimenting at 16*S* is directly related to the virus. In this case, viral infection established an RNA-dependent polymerase system as suggested by the resistance of the infection to actinomycin D. Although reovirus RNA shows a similar sedimentation value, the possibility in the 0°C group of degradation of viral

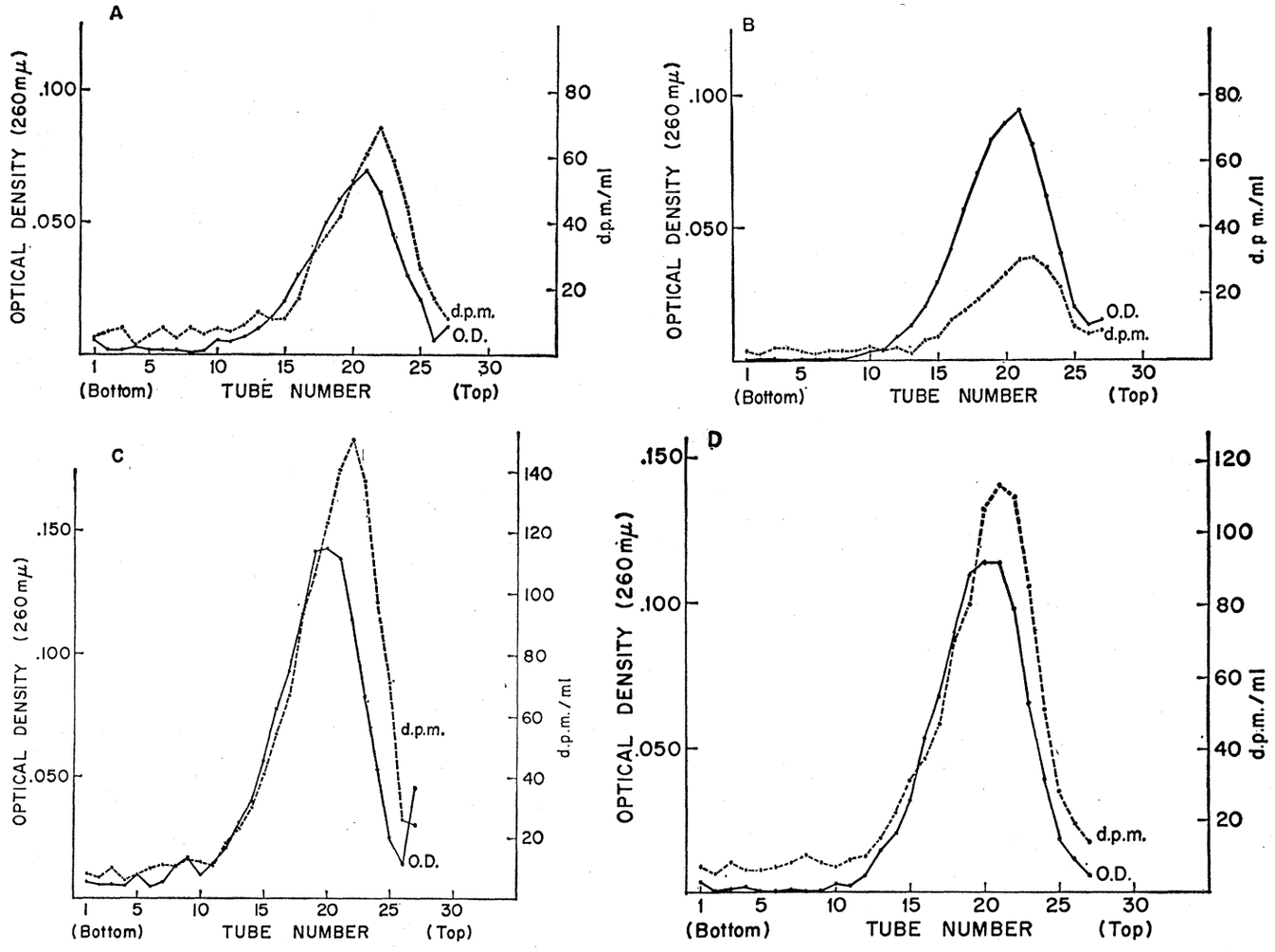

Fig. 1. Orotic acid-6-C^{14} incorporation into the mouse-liver RNA extracted at 60°C and *p*H 8.3. (A) Control, (B) control + actinomycin D, (C) infected, (D) infected + actinomycin D.

RNA with a higher *S* value at the phenol-water interface cannot be excluded, particularly since MHV3 is thermolabile.

Thus, after MHV3 infection, an actinomycin D–insensitive 16*S* RNA is extractable at 60°C from the phenol residue obtained at 0°C. This RNA may be related to the MHV3 virus, and may in fact be viral RNA.

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17β-Estradiol: Inducer of Uterine Hexokinase

Abstract. *Administration of estradiol to ovariectomized rats induced new synthesis of uterine hexokinase which was prevented by actinomycin D, 5-fluorouracil, cycloheximide, or ethionine. The estradiol-induced increase in uterine hexokinase activity was detectable as early as 4 hours. The increase in enzyme activity was dependent upon the dose of the hormone. The evidence indicates that the increased hexokinase activity induced by 17β-estradiol may entail an acceleration of the synthesis of certain RNA species.*

An understanding of the mechanisms underlying hormone action at the molecular level is facilitated by the concept that certain hormones act on whole "functional genic units" resulting in a synchronized increase or decrease in the biosynthesis of functionally related, rate-limiting enzymes governing one-way reactions (*1*). Earlier investigations supported this concept and provided evidence for the action of glucocorticoids as inducer and of insulin as both inducer and suppressor on well-defined functional genic units in hepatic carbohydrate metabolism (*2*). The three key enzymes of hepatic glycolysis—glucokinase, phosphofructokinase, and pyruvate kinase—exhibit relatively low activities and catalyze one-way reactions. It was suggested that this triad of key glycolytic enzymes in liver might occupy the same functional genome unit because of their strategic metabolic role and similarity in response to nutritional and hormonal influences (*2*, *3*). In accordance with the "functional-genome-unit" concept of hormone action, all three of these glycolytic enzymes were expected to be induced or suppressed in a synchronous manner by male and female sex hormones in their respective target organs.

Uterine phosphofructokinase decreased on castration and returned to the normal range by treatment with estradiol (*4*). Because the estradiol-induced rise in phosphofructokinase activity was blocked by a variety of inhibitors of protein biosynthesis, it was suggested that the observed increases in uterine phosphofructokinase activity represent enzyme synthesis *de novo*. Smith and Gorski (*5*) reported that uterine tissue, unlike liver, contains little or no glucokinase [enzyme with high Michaelis constant (K_m)] and that, in the uterus, glucose is phosphorylated by the hexokinase with low K_m. We now confirm their observations and show that estradiol induces new synthesis of hexokinase in the uterus; this synthesis can be prevented by injection of actinomycin, 5-fluorouracil, cycloheximide, or ethionine.

Table 1. Effect of varying doses of 17β-estradiol on uterine hexokinase. Means ± S.E. represent three determinations of enzyme activity. Each determination was carried out in uteri pooled from two to three rats. Groups of eight to ten ovariectomized rats were given 17β-estradiol and killed after 16 hours. Percentage of control represents the statistically significant difference as compared to the values of control rats ($P < .05$).

17β-Estradiol (μg/100 g)	Hexokinase activity	Percentage of control
Control	5.6 ± 0.6	100
1	10.4 ± 0.9	186
2	11.7 ± 0.6	209
5	12.8 ± 1.4	218
10	15.3 ± 1.2	273
20	13.5 ± 0.7	241
30	13.6 ± 1.1	241

Young female rats of the Wistar strain, weighing approximately 150 g, were used 2 weeks after bilateral ovariectomy. Uteri were quickly excised, and homogenates and supernatants were prepared (*4*). Hexokinase activity was assayed in the supernatant under linear kinetic conditions by a modification (*2*) of the method of DiPietro and Weinhouse (*6*). The rate of formation of reduced nicotinamide-adenine dinucleotide phosphate was measured at 340 nm (37°C) in a constant recording Unicam spectrophotometer, model SP-800. Enzyme activity is expressed in micromoles of substrate metabolized per hour per gram (fresh weight) of uterus times the weight of the organ (*4*, *7*). The data were evaluated statistically, and the significant differences between the means were calculated as *P* values.

We were not able to show, in supernatants from uteri of either castrated or normal rats, any significant amounts of glucokinase activity with high K_m. Our observations agree with those of Smith and Gorski (*5*) who showed that the uterus of the immature rat contains little or no glucokinase with high K_m. The absence of glucokinase and the presence of high activities of hexokinases in rat intestine, heart, and skeletal muscle have been reported by Katzen and Shimke (*8*).

To detect the earliest increase in uterine hexokinase activity after administration of estradiol, we followed the sequence of events for 24 hours. Groups of ovariectomized rats were injected intramuscularly with doses of 10 μg of estradiol per 100 g of body weight and killed after 2, 4, 8, 16, and 24 hours. Figure 1 shows that the earliest detectable increase (127 percent) in uterine enzyme activity was observed 4 hours after administration of the hormone. Hexokinase activity was further increased to 154, 294, and 280 percent of the control values at 8, 16, and 24 hours, respectively. The time course for induction of uterine hexokinase by estradiol appears to be qualitatively similar to that reported for phosphofructokinase and phosphohexose isomerase (*4*).

We performed studies to establish whether smaller doses of the hormone were also capable of inducing significant increases in the activity of this uterine enzyme. Groups of ovariecto-