lowered resistance to multiplication of bacteria.

Summary and conclusions. Five series of rats were given either 1 or 2 intravenous injections of a broth culture of *S. mitis* or 2 injections of a culture of *Staph. aureus*. Each series was divided into a control group given bacteria alone, a group given 2 daily doses of 5 mg/kg epinephrine in oil subcutaneously beginning the day before the first bacterial injection, and a third group receiving, in addition, 2 doses of 50 mg/kg Dibenamine hydrochloride 1 and 2 days before the first dose of epinephrine in oil. The animals receiving bacteria and epinephrine in oil developed a significantly higher incidence of bacterial endocarditis than either controls or rats given Dibenamine prior to epinephrine in oil. The incidence of bacterial lesions involving the renal papilla was also higher in rats given epinephrine in oil than in controls. All groups had a high incidence of focal interstitial nephritis.

It is suggested that a relative and localized hypoxia induced by epinephrine in oil may be a major factor contributing to the increased susceptibility of epinephrine-treated rats to bacterial endocarditis and renal bacterial papillitis.

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Deoxyribonuclease I and II Activities in Liver and Spleen of Mice Experimentally Infected by Mouse Hepatitis Virus.* (30665)

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In recent years a great deal of work has been done on the metabolic changes that occur in animal tissues and in cell cultures after viral infection. We have been particularly interested in the biochemical changes in mice infected with the MHV-3 strain of murine hepatitis virus(1-3). This virus multiplies to the greatest extent in murine liver (4), but grows also in almost all other tissues including spleen, kidney, brain and myocardium(5,6). In liver, viral multiplication causes a severe necrotic hepatitis, but in other organs the damage is not very severe and is characterized by limited necrotic lesions.

Most of the biochemical changes observed during the course of experimental infection appear at the acute stage of the disease (36-48 hours after inoculation of the virus), when viral multiplication has already reached its maximum and the histological lesions are extensive. During the acute phase of the disease (48 hours after virus inoculation) many enzymatic activities are altered; an increase of phosphoglycomutase and arginase, but a decrease of adenylpyrophosphatase, succinic dehydrogenase, glutamic oxylacetic transaminase and glutamic pyruvate trans-

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aminase(1,2). However, other enzymatic activities do not show any change.

Also in the early phase of the disease (24-36 hours) a decrease in the nucleoside phosphate and ATP content has been noted. Also noted in this early phase are the following severe alterations of mitochondrial functions in liver cells: uncoupling of oxidative phosphorylation(7), increase of Mg^{++} stimulated ATPase and decrease of DNP-induced ATPase(8), and alterations of mitochondrial stability *in vitro* and mitochondrial permeability *in vivo*(9).

This paper concerns our investigations on DNAse I and II in hepatic and splenic tissue of mice infected with MHV-3 during the acute phase of the disease (48 hours after inoculation of the virus). As mentioned, in these 2 organs there is a rapid and intense viral multiplication which causes noticeably different histological lesions. In hepatic tissue after 48 hours there is an intense and diffuse necrosis with complete disintegration of the histological architecture of the organ, while in the spleen the necrotic lesions are slight and the histological structure is well-preserved. One observes only congestion and atrophy of the follicles(6).

The activity of DNAse I and II in liver of mice with acute CCl_4 poisoning has been studied to observe the behavior of the same enzyme activities during a non-viral hepatic necrosis.

We have also determined in all animals the serum glutamic pyruvate activity as a biochemical index of the extent of the hepatic necrosis and the DNAse I and II activities of the serum.

Materials and methods. White mice (strain N.M.R.I., Recordati breed) weighing 12-16 g were used. The MHV-3 virus (derived from the strain of Dr. A. W. Gledhill) was used. The animals were injected intraperitoneally with 10,000 LD_{50} of virus and were sacrificed after 48 hours.

The acute CCl_4 poisoning was induced by intraperitoneal injection of CCl_4 (0.001 ml per g body weight), dissolved in anhydrous olive oil. The animals were sacrificed 30 hours after injection.

Animals were decapitated. Blood was collected with a paraffin-lined funnel in small centrifuge tubes. Immediately after coagulation the serum was separated by centrifugation at 4°C. The liver and spleen were homogenized in 10 volumes of twice-distilled water at 0°C.

The DNAse I activity was determined on the liver and spleen homogenates and on the serum by the technique of Allfrey and Mirsky(10) based on the colorimetric determination with Dische's diphenylamine procedure of the acid soluble deoxypentose compounds released in the course of enzyme reaction, according to McDonald(11). The following reaction mixture was used:

DNA 0.2%	1.0 ml
Tris buffer 0.05 M pH 7.4	1.0 "
$MgSO_4$ 0.1 M	.5 "
Homogenate of liver or spleen, 10%, or serum diluted 1:4 in Tris buffer	.5 "

Incubated 2 hours at 37°C.

DNAse II activity was determined by Laskowski's modification(12) of the Allfrey and Mirsky method. The following reaction mixture was used:

DNA 0.15%	1.0 ml
Acetate buffer 0.2 M pH 4.8	1.0 "
Versene 4 mM	.5 "
Homogenate of liver or spleen, 10%, or serum undiluted	.5 "

Incubated 2 hours at 37°C.

Readings were followed in the Beckmann DU, model G-4700, at $\lambda = 600$ mμ and the optical densities obtained were converted to deoxypentose-P equivalents by comparison with those obtained from a standard solution of DNA.

The serum glutamic pyruvate activity was determined by the method of Tonhazy *et al* (13) modified by De Ritis *et al*(14).

Results. The results obtained are shown in Tables I and II. Both in the liver and in the spleen of mice in the acute phase of MHV-3 infection, there is an increase of DNAse I activity which statistically is very significant. There is also an increase in the serum of the same animals. However, in the liver of mice poisoned with CCl_4 the DNAse I activity does not vary significantly, although it increases notably in serum.

TABLE I. DNAse I and II Activity in Liver and Spleen of Mice Infected with Murine Hepatitis and in Mice with Acute CCl_4 Poisoning.

(Values are expressed in γ of deoxypentose-P liberated per 100 mg fresh tissue after 2 hr incubation at 37°C.)

	Liver			Spleen	
	Normal	Virus MHV-3 (48 hr)	CCl_4 (30 hr)	Normal	Virus MHV-3 (48 hr)
DNAse I—Activity					
Avg $\pm \sigma$	90.4 ± 10.9	196.7 ± 26.6	87.2 ± 15.5	65.0 ± 14.4	110.0 ± 18.9
P		<.001	>.10		<.001
DNAse II—Activity					
Avg $\pm \sigma$	48.3 ± 9.9	30.3 ± 6.5	38.5 ± 6.9	154.3 ± 39.9	114.9 ± 21.3
P		<.001	<.05		<.005

Avg of 12 determinations. Each determination was made on livers of different animals and a pool of spleen of 2 different animals.

Discussion. During the course of experimental infection by murine hepatitis virus, the increase of DNAse I activity occurs in spleen as well as in liver, even though in spleen the degenerative and regenerative processes due to viral multiplication are very slight as compared with those of the liver. In hepatic tissue of mice acutely poisoned with CCl_4, changes in DNAse I activity are not observed although regressive changes are of the same severity and extent as those caused by the hepatitis virus. The latter is shown by the levels of serum GPT activity.

Such observations suggest that the increases of DNAse I activity in the liver and spleen of mice in the acute phase of MHV-3 infection are a consequence of metabolic modifications connected with the multiplication of the virus itself and are independent of regressive changes that accompany viral disease.

From the data reported in the Tables it seems that DNAse II behaves differently. In fact it decreases slightly but significantly, in both the liver and the spleen of mice infected with MHV-3. It decreases also in the liver of mice poisoned with CCl_4. Under the same experimental conditions, modifications of DNAse II in serum have not occurred.

Increases of DNAse I and DNA polymerase have been noted(15) in BHK21 cells infected with the DNA-containing herpesvirus. The virus MHV-3 has not yet been purified and subjected to chemical analysis. There exist, however, data in the literature suggesting that it is an RNA virus. An increase of cytoplasmic RNA occurs in hepatic cells during the course of the disease(16,17). No increase of DNA content in the liver tissue extracts has been found(18). Finally, the multiplication of virus MHV-3 in macrophage culture of mice *in vitro* is not inhibited by addition to the culture medium of 5-fluorodeoxyuridine and actinomycin at concentrations sufficient to inhibit DNA virus(19).

Summary. The activities of DNAse I and II in liver and spleen of mice experimentally infected with MHV-3 virus and in liver of mice with acute CCl_4 poisoning have been measured. A statistically significant increase of DNAse I activity occurred in virus-infected mice but not in those poisoned with CCl_4. The DNAse II activity decreases slightly in

TABLE II. Deoxyribonuclease I and II and Glutamic Pyruvate Transaminase Activity of Serum of Mice Infected with Murine Hepatitis Virus and of Mice with Acute CCl_4 Poisoning.

(Values expressed in I.U.—according to the International Union of Biochemistry.)

DNAse I activity			DNAse II activity			GPT activity		
Normal	Virus MHV-3 (48 hr)	CCl_4 (30 hr)	Normal	Virus MHV-3 (48 hr)	CCl_4 (30 hr)	Normal	Virus MHV-3 (48 hr)	CCl_4 (30 hr)
30.1 ± 6.9	150.6 ± 17.3	143.1 ± 15.7	0	0	0	105 ± 11	4900 ± 670	3270 ± 425

Avg of 6 determinations, each determination on a pool of serum from 6 animals.

both liver and spleen of virus-infected animals and in liver of mice with CCl_4 poisoning.

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Characteristics of the Growth Cycles of Four Simian Enteroviruses (SV2, SV6, SV42, SV49).* (30666)

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During the course of our studies on the characteristics of simian enteroviruses it was found that they could be placed into 4 groups on the basis of their plaque morphology(1). Group A viruses produced hazy plaques with an indistinct border, 8-12 mm in diameter in 7 days; group B produced large, sharp plaques with islets of viable cells within their periphery, 10-20 mm in diameter; group C produced small, clear plaques with irregular borders, 5-8 mm in diameter; and group D produced large polio-like plaques, 20-25 mm in diameter. In order of their time of appearance, group D plaques were generally first, followed by B, A and lastly C.

While it was apparent that the plaque morphology was largely dependent on the type of cytopathology (CPE) observed in cell cultures under liquid media(1), we decided to study several aspects of the growth cycle of representatives of each plaque group to try to determine what role they might play in the size and rate of appearance of plaques. For this purpose rates of adsorption, penetration and multiplication in primary rhesus monkey kidney cell cultures (PMK) by SV6 (group A), SV42 (group B), SV2 (group C) and SV49 (group D) were determined.

Materials and methods. Viruses. SV2 was obtained from the American Type Culture Collection, Viral and Rickettsial Registry, and SV6 from Dr. R. N. Hull. SV42 and SV49 were isolated in this laboratory from rectal swabs of a cynomolgus monkey and a rhesus monkey, respectively(1). All 4 viruses were plaque purified in LLCMK2 cells and passed several times in PMK cells prior to their use in this study.

Tissue culture. One- and three-ounce bottle monolayer cultures of PMK cells were prepared as previously described(2).

Rate of adsorption of viruses to PMK cells. PMK monolayers in 3-ounce bottles

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