Further investigation is necessary to clarify the intrinsic mechanisms of the inhibition in the double antibody immunoassay procedures.

Summary. An iodination procedure for human growth hormone using ^{125}I and the double-antibody immunoassay utilizing this labelled preparation as tracer are described. The mean specific activity was estimated to be 190 mC/mg. The growth hormone determinations were made on 0.1 ml of 1:3 diluted plasma. The results showed satisfactory recovery, accuracy and a sensitivity of 0.375 mμg/ml.

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Ribonucleic Acid Synthesis and Nuclear Ribonucleic Acid Polymerase Activity in Livers of Mice Infected With Mouse Hepatitis Virus (MHV-3).* (32461)

G. BUDILLON, C. DEL VECCHIO-BLANCO, M. CARRELLA, V. ZAPPIA AND M. COLTORTI (Introduced by Albert B. Sabin)
Istituto di Patologia Speciale Medica and Istituto di Chimica Biologica dell'Universita di Napoli, Naples, Italy

In the past few years we have been interested in the identification of early metabolic alterations in the livers of mice infected with the MHV-3 strain of murine hepatitis virus. A marked elevation in the blood plasma of many enzymes of hepatic origin was detected during the acute phase of the disease (1). At 36 hours after infection some mitochondrial alterations of liver cells were apparent, as shown by (a) uncoupling of oxidative phosphorylation and (b) release of some mitochondrial enzymes into the soluble phase of cytoplasm and from this to the bloodstream(2,3). In addition, recent work from this laboratory has shown that lysosomes are altered at an even earlier stage of infection (4). Very little is known, however, about the changes of nuclear structures and functions induced by hepatitis viruses. This paper deals with investigations on *in vivo* synthesis of ribonucleic acid (RNA) in liver tissue of mice infected with MHV-3, and on the *in vitro* activity of nuclear RNA polymerase.

Materials and methods. All experiments were performed with the Swiss strain of albino mice weighing 12-16 g. They were infected intraabdominally with 0.1 ml of a suspension of infected mouse liver containing about 10,000 LD_{50} of the Craig strain of MHV-3 virus. The viral titer, expressed as LD_{50}, was calculated according to Reed and Muench(5). The liver nuclear fraction was obtained as described by Barnabei *et al*(6). RNA polymerase activity was assayed in this fraction essentially according to Weiss(7,8). The composition of the incubating mixture is shown

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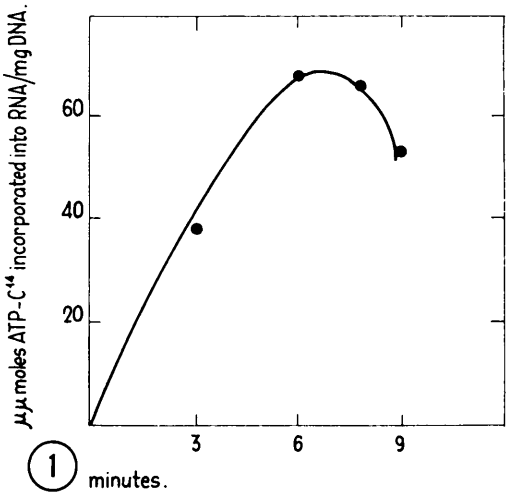
in Table I. The reaction was stopped with trichloracetic acid (5% final concentration), and the separation of RNA was obtained according to the method of Weiss, as reported by Barnabei(9). The RNA radioactivity was measured in a Packard Tricarb liquid scintillation spectrometer. The enzymatic activity was expressed as $\mu\mu$moles ATP-C^{14} incorporated into RNA per mg of deoxyribonucleic acid (DNA). The DNA content of the nuclear fraction was measured by the diphenylamine procedure(10).

RNA synthesis was measured as *in vivo* incorporation of radioactivity from orotate-6-C^{14} into liver RNA. At various times after infection mice were inoculated intraabdominally with orotic acid 6-C^{14} (The Radiochemical Centre, Amersham) (5 μc/100 g of body weight) dissolved in physiological saline (1 μc/0.2 ml). The animals were sacrificed by decapitation 20, 40 and 60 minutes after the injection. Nuclear and cytoplasmic fractions were obtained from pools of livers (3 g) and RNA was separated from these fractions according to Barnabei (9). The RNA radioactivity was measured as for experiments *in vitro* and the RNA concentration was estimated by the orcinol method(10).

Results and discussion. In a first group of experiments nuclear RNA polymerase activity of liver from healthy mice was studied as a function of incubation time and of enzyme concentration in the reaction mixture. Fig. 1 shows that the reaction rate is constant only for a few minutes. The results in Fig. 2 indicate that the incorporation of ATP-C^{14} into RNA, referred to as $\mu\mu$moles per mg DNA, decreases as the nuclear fraction in the reaction mixture is increased from 0.5 to 2 mg DNA. Therefore, in all further experiments the reaction was stopped after 5 minutes of incubation and the nuclear fraction in the incubating mixture was kept in the range of 0.45-0.6 mg DNA.

Table I summarizes the results of experiments in which nuclear RNA polymerase activity of livers from virus-infected animals was measured at various stages of infection. A 23% decrease of enzyme activity was detectable in the livers of animals infected



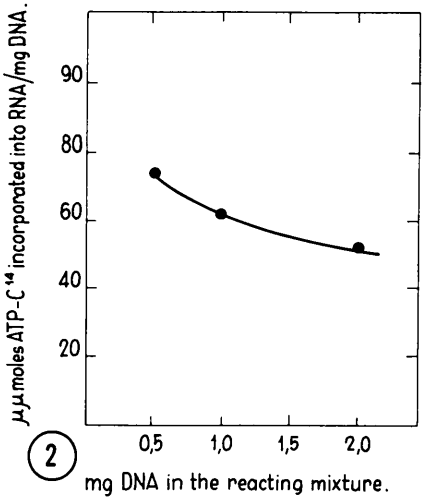


FIG. 1. Incorporation of ATP-C^{14} into RNA by liver nuclear fraction as function of incubation time. Each point on the curve represents the mean value of 3 experiments.

FIG. 2. Effect of different amounts of liver nuclear fraction on the incorporation of ATP-C^{14} into RNA. Each point on the curve represents the mean value of 3 experiments.

24 hours before, as compared with the uninfected controls; the decrease of enzyme activity reached 50% at 36 hours after virus infection.

The omission in the reaction mixture of UTP, GTP and CTP resulted in a marked decrease of ATP-C^{14} incorporation into RNA by liver nuclei of healthy mice, and in an almost complete lack of incorporation by liver nuclei of animals infected for 36 hours. This

TABLE I. Ribonucleic Acid Polymerase Activity of Nuclear Fraction from Livers of Healthy and Infected Mice.*

Hr after infection	$\mu\mu$moles ATP-C^{14} incorporated into RNA/mg DNA†	
	Complete system	No UTP, GTP, CTP
0	74.2 ± 12.3	17.8 ± 9.8
24	57.4 ± 8.0	7.6 ± 3.4
36	36.3 ± 7.3	1.9 ± 1.3

* Two ml of incubation mixture contained: nuclear fraction 0.25 ml, corresponding to 0.45-0.6 mg DNA; 0.1 μmol ATP-C^{14} (The Radiochemical Centre, Amersham) corresponding to 1.77 μc; 0.2 μmol each of UTP, GTP and CTP (Calbiochem); 10 μmol of L-cysteine; 5 μmol $MgCl_2$; 100 μmol KCl; 50 μmol NaF; 75 μmol Tris-HCl, pH 8.0. Incubation was for 5 min at 37°C in the Dubnoff shaking incubator.

† Mean values of 4 experiments ± standard deviation.

Abbreviations:

ATP-C^{14}—5′-triphosphate, sodium-8-C-14.

UTP—uridine-5′-triphosphate (disodium hexahydrate).

GTP—guanosine-5′-triphosphate (trisodium, pentahydrate).

CTP—cyti line-5′-triphosphate (disodium hexahydrate).

might be related to a lower level of free nucleotides in nuclei of infected mice as compared with normal mice.

The results of further experiments dealing with the *in vivo* incorporation of radioactivity from orotate-6-C^{14} in hepatic RNA, as shown in Fig. 3 indicate that nuclear RNA is labeled much more than cytoplasmic RNA, in healthy as well as in infected mice. Furthermore, the incorporation of orotate radioactivity into liver RNA of infected mice does not undergo an appreciable change during the first 24 hours after infection. At 36 hours after infection there is a significant increase of nuclear RNA radioactivity, but almost no difference between healthy and infected mice for the cytoplasmic RNA.

A comparison of the change in RNA synthesis with the extent of viral multiplication (Table II) indicates that the increased incorporation of radioactivity in nuclear RNA of infected livers cannot be related to viral multiplication since the viral titer reached the peak level at 24 hours at a time when no increase in nuclear RNA synthesis was as yet evident.

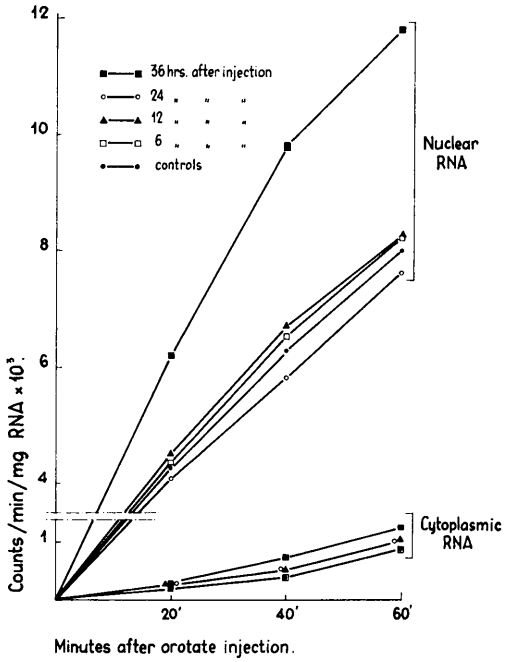


FIG. 3. Specific activity of nuclear and cytoplasmic RNA from livers of healthy and infected mice, sacrificed at different times after inoculation of orotate-6-C^{14}.

TABLE II. Concentration of Virus in Livers of Mice Infected Intraabdominally with 10,000 LD_{50} of MHV-3.

Hr after infection	Titer of virus* Log 10 TCD_{50}
1	0
3	0
6	4.3
12	6.7
24	8.0
48	8.0

* Dilution of hepatic tissue suspension in sterile broth corresponding to the virus concentration causing 50% mortality of mice inoculated with 0.1 ml of suspension (calculated from the experimental data according to Reed and Muench). Values represent the average of 2 experiments.

It is difficult to reconcile in an unambiguous interpretation the increase of RNA biosynthesis *in vivo* and the decrease of RNA polymerase activity *in vitro*. Both phenomena observed in livers of infected mice, however, could be attributed to the cellular disorganization which occurs in the later stages of infection. The reduction of nuclear RNA polymerase activity might be secondary to

the release of nucleases, that are latent *in vivo* and interfere with the *in vitro* measurement of the enzyme. In this respect it is interesting that a significant increase of DNAase 1 was demonstrated by Giusti *et al* in homogenates of livers from mice infected 48 hours previously with MHV-3(11). On the other hand the marked increase of RNA synthesis can be related to an alteration of the metabolic and structural factors regulating this pathway and occurring in the later stages of infection.

It may be inferred that RNA biosynthesis is not a metabolic function that is greatly altered in the early stages of this form of virus hepatitis. Therefore, the mechanism of the initiation and rapid development of the hepatocellular lesion does not appear to derive, initially at least, from an alteration of the RNA synthesis system.

Summary. RNA polymerase activity, measured *in vitro* in the nuclear fraction of livers from mice infected with MHV-3 virus, was decreased 23% and 50% at 24 and 36 hours respectively after inoculation of the virus. The *in vivo* incorporation of orotate radioactivity in the nuclear RNA of liver cells showed no change from the normal during the first 24 hours after infection when viral multiplication had already reached its peak level, but a significant increase was evident at 36 hours. The cytoplasmic RNA of liver cells showed no change in *in vivo* incorporation of orotate radioactivity even at 36 hours after infection when the hepatocellular lesions were already marked.

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A Toxic Factor in *Leptospira pomona*. (32462)

O. H. V. STALHEIM (Introduced by R. W. Dougherty)

National Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Ames, Iowa

The icterogenic character of leptospires prompted some investigators to credit them with toxic properties(1,2), but reports of toxins in cultures of leptospires(3,4,5) could not be confirmed by either the classical methods for bacterial endotoxins(6,7) or by the use of cultured mammalian cells. Although cytopathic effects were reported in cultured cells after the addition of leptospires(8,9,10), Miller *et al*(11) observed no cytopathology until after a week or more if the medium was adequate for the survival and growth of both the cells and virulent *Leptospira pomona*.

A new technique for *in vitro* demonstration of bacterial endotoxin(12) was used for the detection of leptospiral toxins. This report concerns a toxic factor(s) in both virulent and avirulent *L. pomona*.

Materials and methods. Strains. Cultures of virulent *L. pomona* strains Ohio and DM-2 were grown in Stuart's medium(13); the avirulent variants were selected and grown in modified Tween synthetic medium (MTSM) (14). The virulence of these strains for guinea pigs(14), hamsters(15), swine(16), and cattle(17) has been described.