in the presence of added W-398. Thus, the action of W-398 appears to take place at a step prior to mevalonate and after acetate.

Investigation of the effect of W-398 on the production of acetone bodies and fatty acids showed (Table II) that the compound did not influence the incorporation of acetate into these metabolic pathways.

*Summary.* Benzyl N-benzyl carbethoxy-hydroxamate (W-398) was found to inhibit *in vitro* cholesterol biosynthesis. Evidence has been obtained to indicate that the stage of inhibition is prior to the formation of mevalonic acid.

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Received June 12, 1964. P.S.E.B.M., 1964, v117.

## Demonstration of Rapidly Labelled RNA Fractions in Virus-Infected HeLa Cells.* (29533)

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The participation of RNA in the production of DNA-containing bacteriophages is well known(1). The phage DNA directs the formation of informational RNA which is then responsible for specific protein synthesis or for duplication of DNA. Accumulated evidence on the biosynthesis of vaccinia virus has strongly indicated that multiplication of this cytoplasmic DNA-containing virus is dependent upon the essential participation of RNA during the early stages of the infectious sequence(2,3,4). Thus, it seemed of interest to examine the different RNA fractions present in HeLa cell cultures infected with vaccinia virus. This report describes the demonstration of rapidly labelled RNA fractions in HeLa cell cultures infected with vaccinia virus through the use of sucrose gradient analysis.

*Materials and methods.* HeLa cells were originally obtained from Difco Laboratories, and routinely grown in Eagle's basal medium (EBM) containing 10% calf serum. To facilitate detection of any changes in the RNA fractions during infection, the monolayer cell cultures were maintained in a minimal state of metabolic activity. This was accomplished by changing the medium to a low-phosphate ($10^{-5}$ M)-containing EBM plus 2% calf serum for 1 hour before exposure to vaccinia virus at a multiplicity of about 10-15. Uninfected cultures inoculated with Hanks' balanced salt solution served as controls. At various periods after infection, $P^{32}$ orthophosphate (Oak Ridge) was added at a concentration of 3.3 $\mu$c/ml for the last 80 minutes of any infectious period examined. At the indicated times, the cells were scraped off, washed with ice-cold saline, resuspended in cold 0.01 M acetate buffer (pH 5.1), and sodium dodecyl sulfate and polyvinyl sulfate were then added to a final concentration of 0.5% and 2 $\mu$g per ml, respectively. RNA was extracted by the hot phenol method(5). Most of the DNA (>95%) remained either

* This investigation was supported by a U.S.P.H.S. grant AI 04685 from Nat. Inst. of Allergy & Inct. Dis., Contribution No. 024 from Pacific Biomedical Research Center, Univ. of Hawaii.

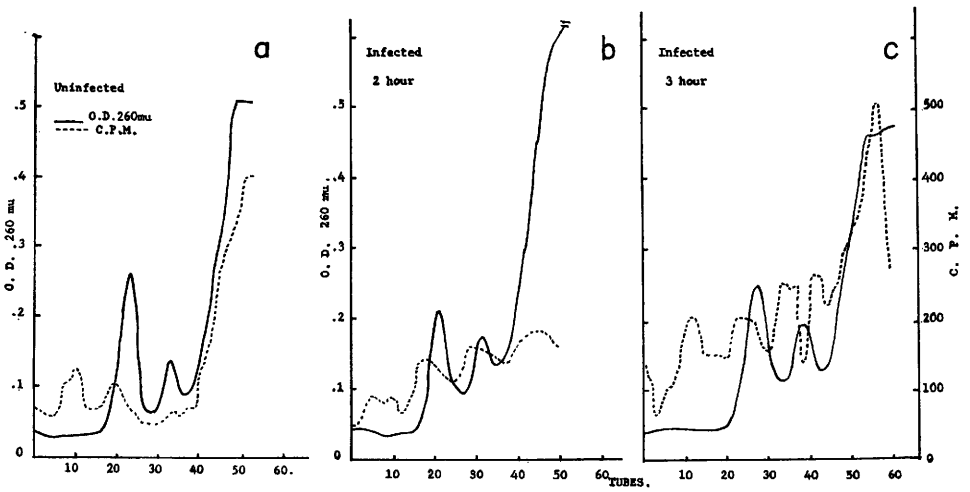


FIG. 1. Sucrose gradient analysis of RNA from normal and vaccinia virus-infected HeLa cells. Monolayer cultures of HeLa cells were pre-exposed for 1 hr to a low-$PO_4$-containing ($5 \times 10^{-5}$ M) Eagle's basal medium plus 2% calf serum. Infected cultures were exposed for 1 hr to approximately 15 PFU of centrifugally-purified vaccinia virus per cell. Control cultures were inoculated with Hanks' BSS and treated as above. Control cultures were labelled with 3.3 $\mu$c/ml of $P^{32}$ from 40-120 min (a). Infected cultures were labelled with 3.3 $\mu$c/ml of $P^{32}$ from 40-120 min (2 hr) (b), and 100-180 min (3 hr) (c). The cells were harvested, washed once with Hanks' BSS, and total RNA was extracted and analyzed by sucrose gradient as described in text.

in the interface or in the phenol layer under these conditions. The RNA was precipitated from the aqueous layer by adding NaCl and ethanol to a final concentration of 0.1 M and 75%, respectively(6), and redissolved in acetate buffer containing 0.05 M NaCl and $10^{-4}$ M $Mg^{++}$ (pH 5.1). The solution of RNA was layered onto 25 ml linear sucrose gradients of from 5-20% (w/v) concentration and centrifuged for 8 hours at 25,000 rpm in the SW25 head of the model L Spinco ultracentrifuge(7). After centrifugation, fractions of 5 drops each were collected and analyzed for RNA by measuring for U.V. absorption at 260 m$\mu$ and for the $P^{32}$ activity of the acid-precipitated fractions. Actinomycin D was generously supplied by Merck, Sharpe & Dohme.

*Results and discussion.* As previously found by others(7,8), the bulk of the RNA extracted from whole cells was contained in 3 optical density (O.D. 260 m$\mu$) peaks of the centrifuge patterns, which corresponds to the 28 and 16S ribosomal RNA fractions and the 4S transfer RNA (Fig. 1a). Incubation of the cells in a medium containing $P^{32}$ for 80 minutes revealed 2 additional fractions of RNA which are rapidly labelled in the areas of the 45 and 35S types described by Scherrer and Darnell(7). Except for the 4S RNA, very little or no labelling was observed in the ribosomal RNA fractions. Radioactivity was observed only in these fractions after prolonged exposure to the isotope.

Virus infection made relatively little impact on the sedimentation pattern of the different classes of RNA until 2 and 3 hours post-infection. The patterns of RNA extracted at these periods after 80 minutes of exposure to $P^{32}$ are shown in Fig. 1b and c. Incorporation of the label at 2 hours after infection revealed, in addition to the 45 and 35S fractions, the appearance of a labelled RNA fraction near the 16S ribosomal peak. The change in the pattern of labelling became more significant at 3 hours post-infection, when several new rapidly labelled fractions appeared (23 and 12S) between the 28 and 4S RNA fractions. There was essentially no change in the sedimentation pattern of the 45 and 35S fractions and in the O.D. pattern of the ribosomal RNA fractions at these two periods.

The polypeptide antibiotic actinomycin D has been found to inhibit the synthesis of DNA-dependent RNA in bacterial and ani-

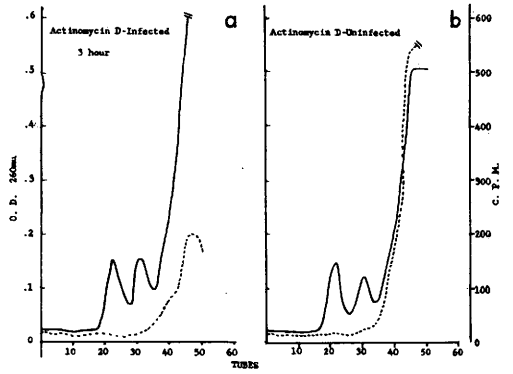


FIG. 2. Effect of Actinomycin on $P^{32}$ incorporation into RNA by normal and vaccinia virus-infected HeLa cells. Monolayer cultures of HeLa cells were pre-treated with Actinomycin D (5 μg/ml) for 1 hr before infection with vaccinia virus. Control cultures were inoculated with Hanks' BSS. Both infected (a) and control (b) cultures were labelled with 3.3 μc/ml of $P^{32}$ from 100-180 min (3 hr). RNA was extracted and examined in a sucrose gradient as described in text.

mal cell systems(9,10), and DNA-containing vaccinia virus(11). It was therefore of interest to ascertain whether the new rapidly labelled fractions formed in infected cells are sensitive to the action of this antibiotic.

The effect of pre-treatment for one hour with actinomycin D (5 μg/ml), on the various classes of RNA in uninfected and vaccinia virus-infected cultures is shown in Fig. 2a and b, respectively. As was previously indicated(12), the antibiotic effectively inhibits the synthesis of the rapidly labelled 45 and 35S RNA fractions in uninfected cultures (cf. Fig. 1a). Labelling is found only in the 4S RNA fraction. Examination of the RNA fractions of the antibiotic-treated infected cultures reveals that the synthesis of these new rapidly labelled fractions (23 and 12S) is also effectively inhibited. Again, in such cultures labelling is found only in the 4S fractions (Fig. 1c).

The studies of the distribution of the RNA fractions of whole HeLa cells following pulse label with $P^{32}$ by the sucrose gradient method described here, and by others(7,8), have failed to show the presence of rapidly labelled RNA fractions of lower sedimentation coefficients other than the 45 and 35S species. It was also determined that these rapidly sedimenting fractions were confined only to the nucleus of the cell(8). However, studies of the cytoplasmic polyribosomes have yielded messenger-RNA fractions with sedimentation values ranging from 6 to 25S(13). Furthermore, polyribosomes of larger sedimentation values have been obtained from HeLa cells infected with vaccinia virus, and it was suggested that the wider distribution of the cytoplasmic polyribosomes may represent several species of messenger-RNA(14). The present evidence of new rapidly labelled RNA fractions appearing only in infected cultures and their sensitivity to the action of actinomycin D, strongly suggest that these fractions may represent cytoplasmic messenger-RNA(s) synthesized in response to infection with the DNA-containing vaccinia virus.

Preliminary investigation on the nature of these new RNA fractions has indicated that, whereas the 45S and 35S fractions were completely degraded to acid soluble products, a significant portion of the new fractions was resistant to ribonuclease. Although the significance of the resistant fractions is not clear, the existence of ribonuclease-resistant native hybrids of DNA-RNA complexes in T2 phage-infected *E. coli* has been reported (15). The resistance to digestion by the specific nuclease has been considered as a criterion of a true hybrid of DNA-RNA complexes(15). It should be noted that RNA alone apparently can exist in ribonuclease-resistant states under certain conditions(16, 17). Whether or not the ribonuclease-resistant fraction found here represents hybridization between vaccinial DNA and its complementary RNA remains to be determined. Further characterization of these fractions is in progress.

*Addendum.* Since the preparation of this manuscript two independent groups of investigators(18,19) have reported on the appearance of rapidly labelled RNA fractions in vaccinia virus-infected HeLa cells. These fractions were found to possess a base composition similar to vaccinial DNA and are capable of hybridization with the viral DNA. The investigators concluded that these fractions represent messenger RNA formed in consequence to infection.

*Summary.* The various classes of RNA in uninfected and vaccinia virus-infected HeLa cell cultures have been examined through sedimentation analysis in sucrose gradients. Infection with vaccinia virus results in the appearance at 3 hours post-infection of new rapidly labelled RNA fractions. These fractions are distinct from ribosomal RNA and are sensitive to the antibiotic Actinomycin D. The role of these new RNA fractions is discussed.

It is a great pleasure to acknowledge the expert technical assistance of Miss Marge Soergel.

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Received June 15, 1964. P.S.E.B.M., 1964, v117.

## The Serum Proteins in the Alcoholic and Mentally Ill Treated with Chlorpromazine.* (29534)

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The interrelationship of biochemical factors with mental and behavioral abnormalities is an incompletely understood area in spite of the very considerable investigation that it has received and a number of suggestive results. Thus, based in part on an electrophoretic procedure developed by Grunbaum *et al*(1), Fessel and Grunbaum(2) found statistically significant variations from normal of certain blood proteins in various groups of chronically psychotic patients confined in a California state hospital. These involved, especially, deviations in the gamma and alpha-2 bands, and suggested among other things, an elevated macroglobulin level.

In continuation of this type of investigation, a broader group of patients involving several types of psychotics and chronic alcoholics was subjected to a similar electrophoretic analysis for the blood protein fractions. In addition, the effect of chlorpromazine on the serum protein distribution of these patients was studied because of the beneficial effects of this drug on the mentally ill. The parameter for normal serum protein values was established by Grunbaum(3) on a population of 221 Caucasian males, the data serving as a basis of comparison in evaluating the proteins in the sera of the alcoholic and the mentally ill.

*Experimental.* The subjects for this inves-

* This investigation was supported in part by a grant from the Committee on Research, Univ. of California.

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