previously vaccinated with group-specific antigen also demonstrated a rise in antibody titer. In contrast, no further increases in antibody titer occurred in any of the volunteers who had been vaccinated with type-specific antigen. It is noteworthy that the vaccine-induced neutralizing antibody titers were comparable to those which developed in the antibody-negative volunteers following challenge with infectious adenovirus type 1.

Summary. Vaccination with non-infectious, type-specific and group-specific soluble antigens of adenovirus type 1 induced neutralizing antibody in adult volunteers. As compared with non-immunized controls, ocular challenge of volunteers immunized with both preparations was followed by a significant reduction in throat and rectal virus shedding. There was almost complete suppression of illness in volunteers receiving type-specific antigen. It is difficult to interpret the illness pattern in the man who was given group-specific antigen. These findings suggest that soluble antigens of adenovirus type 1 may provide effective immunization in this disease.

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Received June 11, 1964. P.S.E.B.M., 1964, v117.

A New Inhibitor of *in vitro* Cholesterol Biosynthesis. (29532)

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Cholesterol biosynthesis, a normal body function, can be inhibited by a number of chemical entities. Included in the compounds which have been studied extensively in this regard are triparanol(1); benzmalecene(2); β-diethylaminoethyl diphenylpropylacetate hydrochloride, SKF-525A(3); fluoromevalonic acid(4); an azasterol, 20α-(2-dimethylaminoethyl)amino-5α-pregnan-3β-01 dihydrochloride(5); and vanadyl sulfate(6). Cholesterol itself also regulates its own formation through a feedback mechanism which acts prior to the mevalonate stage and probably on the conversion of hydroxymethyl glutaryl-coenzyme A (HMG-CoA) to mevalonic acid (7).

Benzyl N-benzyl carbethoxyhydroxamate (W-398) which is effective in reducing hypercholesteremia and plaque formation in animals has recently been described by Berger *et al*(8). This compound has now been investigated for its effect on the *in vitro* biosynthesis of cholesterol and has been found to inhibit this conversion prior to mevalonic acid. These findings are presented here.

Experimental procedure. The rat liver homogenate of Bucher(9) was employed as an enzyme source for biosynthesis of cholesterol.

TABLE I. Effect of W-398 on *in vitro* Incorporation of Acetate-1-C^{14} and Mevalonate-2-C^{14} into Rat Liver Steroid.

Run No.	Substrate	No. of replicates	Radioactivity incorporated into rat liver steroid (cpm) Untreated control	With 7×10^{-4} M W-398	t	p
1	Acetate-1-C^{14}	3	4700 ± 136	2300 ± 85	8.7	<.001
2	Acetate-1-C^{14}	3	2460 ± 65	1240 ± 88	13.8	<.001
3	Mevalonate-2-C^{14}	6	47,900 ±1780	49,700 ±1160	1.1	n.s.

TABLE II. Effect of W-398 on Incorporation of Acetate-1-C^{14} into Rat Liver Homogenate Fractions.

Fraction	No. of replicates	Radioactivity incorporated (cpm) Untreated control	With 7×10^{-4} M W-398	t	p
Fatty acid	3	7830 ± 384	8880 ± 290	2.8	n.s.
Acetone bodies	3	6900 ± 360	7700 ±1100	.68	n.s.

Sprague-Dawley rats, weighing 80-150 g, were used. The preparations consisted of 2.0 ml of rat liver homogenate supernatant in a final volume of 2.5 ml. Each reaction flask contained 0.028 M nicotinamide, 0.007 M magnesium chloride, 0.044 M phosphate buffer pH 7.3 to 7.4, 1.0 mg of ATP, 1.0 mg DPN, the compound being tested, and either 0.2 mg of sodium acetate-1-C^{14}, 2.33 μc, or 0.04 mg mevalonic acid-2-C^{14} dibenzylethylene diamine salt, 0.085 μc. The flasks were incubated at 37°C for 2 hours under oxygen atmosphere and cholesterol was isolated as the digitonide. The latter was plated and counted in a gas flow counter.* In the mevalonate experiment the nonsaponifiables were extracted into hexane, the solvent evaporated and the residue taken up in a counting solution† and the radioactivity measured.‡

The incorporation of radioactivity into fatty acids and acetone bodies was determined as described by Siperstein and Guest (7).

Results are given with standard error while the probability was calculated using Student's T Test.

Results and discussion. Table I shows results obtained when W-398 was incubated with rat liver homogenate and acetate-1-C^{14}. The data indicate that W-398 in a concentration of 7×10^{-4} reduced the conversion of acetate to cholesterol by about 50%. This inhibition was found to be dependent on

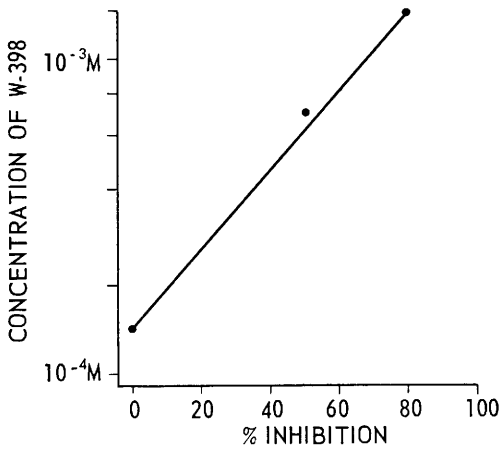

FIG. 1. Effect of various concentrations of W-398 on cholesterol biosynthesis.

W-398 concentration as shown in Fig. 1 where higher levels produced a greater blockage of C^{14} incorporation into sterol and lower levels had no effect.

The effect of W-398 on incorporation of mevalonic acid into cholesterol was evaluated in a similar fashion by incubating mevalonate-2-C^{14} with and without test compound in liver homogenate. No effect on incorporation of C^{14} into sterol (Table I) was found

* Nuclear Chicago Thin Window Geiger-Muller Counter.

† (0.05%) 1,4-bis-3-(4-methyl-5-phenyloxazoyl)-benzene plus (0.5%) 2,5-diphenyloxazole in toluene.

‡ Packard Tricarb Liquid Scintillation Spectrometer.

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 μc/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 μg 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.