rated into I more rapidly than into cholesterol by preputial gland tumor slices incubated *in vitro* (unpublished). I is the probable precursor for the Δ^7-isomer (III), which has been found in rat feces[11] and in cactus,[8] but has not been identified in the tumor. The precursor of I is less obvious, since the pathway from lanosterol may lead through either dihydrolanosterol or through 14-norlanosterol.[14]

(14) F. Gautschi and K. Bloch, *J. Biol. Chem.*, **233**, 1343 (1958).

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PURIFICATION AND CHARACTERIZATION OF THE ANTIVIRAL AGENT HELENINE

Sir:

We wish to report a purification procedure for the antiviral agent helenine, and data which suggest that it is a ribonucleoprotein. It is of interest that this uniquely active agent is apparently of the same class of chemical compounds as are the viruses against which it acts.

Helenine is a product of the mold *Penicillium funiculosum*. It has been reported by Shope[1] to protect mice against Columbia SK encephalomyelitis and Semliki Forest viruses and to prevent development of poliomyelitis in monkeys.[2] The assay used to follow the fractionation was an *in vivo* test in mice, a modification by McClelland of that described by Shope.[1] To obtain more definitive assay values, a 24-hour pre-treatment dose was substituted for the 24-hour post-treatment dose described, the observation period was extended to 15 days, and survivors were evaluated statistically.

Helenine was extracted from the mycelium by homogenization in 0.005 M tris-(hydroxymethyl)-aminomethane or phosphate buffer, pH 7, containing 0.005 M Mg^{++}, precipitated by addition of one volume of acetone, and then taken up in more of the same buffer. About 20 g. of such material was obtained from an 80-gal. fermentation. All fractionation processes were performed near 0°.

The acetone-precipitated material was further purified by repeated ultracentrifugation for two hours at 110,000 × g. Helenine was sedimented completely. From every gram of acetone-precipitated material, 40 to 50 mg. of pellet was obtained, with a 25-fold increase in potency. The material was active at 50 to 100 μg. in mice. A well-defined 260-mμ absorption peak was noted with this fraction. It contained about 40% protein[3] and gave a pentose test with orcinol[4] and with sulfuric acid–cysteine.[5] No deoxyribose was detected. The perchloric acid hydrolysate[5] contained guanine, adenine, cytosine and uracil.[5] No hexose contamination was detected during the orcinol test, but approximately 1% non-pentose sugar was noted during the sulfuric acid–cysteine reaction.

The purified helenine was found to be unstable to lyophilization and to repeated freezing and thawing. Removal of Mg^{++} by dialysis caused a loss of activity. Helenine was more stable when stored in 0.25 M sucrose solution but, even under these conditions, inactivation occurred.

Our best preparations were heterogeneous when examined by electrophoresis and ultracentrifugation.[6] Three major components with sedimentation constants ranging from 43S to 100S usually were observed during ultracentrifugation. Electrophoresis showed one major peak (70%) and several minor ones. The mobility of the major component was -5.2×10^{-5} cm.^{2}/volt/sec. at pH 7. It seems likely that the inhomogeneity is caused by alteration of the native helenine and not by extraneous impurities in the usual sense of the word, since dissociation of ribonucleoproteins is well known.[7] Attempts at further purification were made using diethylaminoethyl-cellulose columns, but singly eluted peaks readily dissociated into smaller components. Because of this heterogeneity, final characterization of helenine cannot be made now. All the physical and chemical observations, however, including stability data, are consistent with the hypothesis that helenine is a ribonucleo-protein.

(1) R. E. Shope, *J. Exp. Med.*, **97**, 601 (1953).

(2) K. W. Cochran and T. Frances, Jr., *J. Pharmacol. Exp. Therap.*, **116**, 13 (1956).

(3) E. W. Sutherland C. F. Cori, R. Haynes and N. S. Olsen, *J. Biol. Chem.*, **180**, 825 (1949).

(4) Z. Dische in "The Nucleic Acids" edited by E. Chargaff and J. N. Davidson, Vol. I, Academic Press, Inc., New York, N. Y., 1955, p. 285.

(5) A. Bendich in "Methods in Enzymology" edited by S. P. Colowick and N. O. Kaplan, Vol III, Academic Press, Inc., New York, N. Y., 1957, p. 715.

(6) We wish to thank Dr. D. E. Williams for these studies.

(7) A. Tissieres and J. D. Watson, *Nature*, **182**, 778 (1958).

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ON THE EFFECT OF DOSE RATE ON THE RADIOLYSIS OF LIQUID HYDROCARBONS[1]

Sir:

Recent studies of the radiolysis of cyclopentane–cyclohexane mixtures with cobalt-60 γ-rays have indicated that secondary reactions between primary radicals and solvent play an important role in determining the path of this radiolysis.[2] Cyclohexyl radicals apparently preferentially abstract hydrogen atoms from cyclopentane and increase the relative yield of cyclopentyl radicals. From this one expects an effect of dose rate on the over-all radiolysis since the lifetime of the radicals and therefore the probability of abstraction decreases with increasing dose rate. Preliminary experiments were therefore undertaken to examine the dose rate dependence of the secondary reactions in this system and are briefly reported here.[3]

Degassed mixtures of equal volumes of Phillips research grade cyclopentane and cyclohexane were irradiated to a total dose of 5×10^6 rads. (3×10^{20} ev./g.) with cobalt-60 γ-rays at absorbed dose

(1) Supported in part by the U. S. Atomic Energy Commission.

(2) G. A. Muccini and R. H. Schuler, to be published.

(3) H. A. Dewhurst and E. H. Winslow, *J. Chem. Phys.*, **26**, 969 (1957), previously have compared the radiolysis of a simple hydrocarbon (*n*-hexane) by γ-rays and by fast electrons and have reported a difference in product ratios presumably due to the widely different dose rates involved in the comparison. *Cf.* also H. A. Dewhurst and R. H. Schuler, THIS JOURNAL, in press.