rated into I more rapidly than into cholesterol by preputial gland tumor slices incubated *in vitro* (unpublished).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.

rates of 10^4, 7×10^4 and 10^6 rads./h. A similar irradiation was carried out with 2 Mev. Van de Graaff electrons at a current of 1 microampere (a dose rate in the irradiation zone of 7×10^8 rads./h.). After irradiation the samples were examined gas chromatographically with attention focussed on the products in the C_{10} to C_{12} region (*cf.* Table I).

TABLE I

EFFECT OF DOSE RATE ON THE RADIOLYSIS OF CYCLOPENTANE-CYCLOHEXANE MIXTURES[a]

Source	Dose rate[b]	Relative yields[c] $C_{10}H_{18}$[e]	$C_{11}H_{20}$[f]	$C_{12}H_{22}$[g]	R[d]
Co^{60} γ-rays	0.01	1.65	1.75	0.60	1.71
Co^{60} γ-rays	0.07	1.51	1.83	0.66	1.54
Co^{60} γ-rays	1.0	1.29	1.95	0.76	1.30
2 mev. electrons	700	0.70	2.04	1.26	0.75

[a] Mixtures 0.46 mole fraction in cyclohexane. [b] Megarads per hour in irradiation zone. [c] Normalized to a total relative yield of 4. [d] R, the ratio of twice the C_{10} component + the C_{11} component to the C_{11} component + twice the C_{12} component. [e,f,g] Respectively, cyclopentyl cyclopentane, cyclopentyl cyclohexane and cyclohexyl cyclohexane.

It is seen that a very pronounced increase in the relative yield of cyclopentylcyclopentane and a corresponding decrease in cyclohexylcyclohexane accompanies the decrease in intensity from the fast electron to the γ-ray experiments. We have taken as a quantitative measure of this change the ratio (R) of the total C_5 component to the C_6 component represented in the C_{10} to C_{12} region. Irradiation of a similar (*i.e.*, 50/50) cyclopentane–cyclohexane mixture containing 2 mM. iodine with γ-rays (at 70,000 rads./h.) indicated that the ratio of cyclopentyl to cyclohexyl radicals initially produced was 0.70 in good agreement with the ratio R observed in the fast electron experiments. It is concluded therefore that at the high intensities represented by the Van de Graaff irradiations (10^8 rads./h. and above) secondary reactions are not product determining. The observed products therefore represent reactions of radicals initially produced by the radiation. At lower intensities secondary reactions occur here and may be expected to be of general importance in the radiolysis of pure liquid hydrocarbons.

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HYDROGEN MIGRATION IN GASEOUS ORGANIC CATIONS

Sir:

Many ionic dissociations induced by electron impact involve migration of a hydrogen atom from one part of a molecule to another. In compounds containing oxygen, a favorably oriented hydrogen usually is pictured as migrating to the oxygen atom, which is considered trivalent by virtue of localization of the charge in a non-bonding orbital.[1] In hydrocarbon ions, on the other hand, hydrogen-migration processes are generally thought to be rather non-specific. However, one such process, the formation of $C_7H_8^+$ ion from 1-phenylbutane, now has been found to show a high degree of specificity.

1-Phenylpropane, to a slight extent, and higher 1-phenylalkanes, to a great extent, give rise not only to the expected $C_7H_7^+$ ion of mass 91, but also to $C_7H_8^+$ of mass 92.[2] This ion must contain a hydrogen atom from the side chain in addition to the elements of the benzyl group. We suspected that the hydrogen comes from the gamma carbon, because this migration would lead directly to the formation of a stable 1-olefin as the neutral product of the dissociation.

To determine the origin of the migrating hydrogen atom, we made use of two independent approaches. The first consisted of labeling the molecule with deuterium in known positions, and examining the fragment ions for deuterium retention.[3] The second was blocking with methyl groups the positions from which the migrating hydrogen atom was thought to come.

Table I shows partial mass spectra of unlabeled 1-phenylbutane and 1-phenylbutane-3-*d* and -4-*d*. The 3-*d* species gives rise to $C_7H_8^+$ ions that are 95.2/196 or 48.6% labeled, close to the 50% expected on the assumption that the migrating atom comes solely from the gamma carbon and that no isotope effect is involved. The discrepancy, though small, is shown to be real by the 4-*d* spectrum, in which 4.5/196 or 2.3% of the $C_7H_8^+$ ions are labeled. Thus, about 95% of the migrating hydrogen atoms originates on the gamma carbon.

TABLE I

PARTIAL MASS SPECTRA OF DEUTERATED 1-PHENYLBUTANES

Corrected for naturally occurring C^{13}

m/e	Unlabeled	-3-*d*	-4-*d*
135		100.0	100.0
134	100.0	0.2	0.6
93		95.2	4.5
92	196	119.4	200
91	438	420	432

Table II shows partial mass spectra of 1-phenylbutane, 3-methyl-1-phenylbutane and 3,3-dimethyl-1-phenylbutane. The first two show large yields

TABLE II

PARTIAL MASS SPECTRA OF METHYLATED 1-PHENYLBUTANES

Corrected for naturally occurring C^{13}

m/e	Unsubstituted	3-Methyl-	3,3-Dimethyl-
Parent	22.8	24.0	28.4
106	0.3	0.3	30.0
105	8.2	13.3	23.6
92	44.8	132.4	5.0
91	100.0	100.0	100.0

of $C_7H_8^+$. Moreover, the mass-92 intensities shown define only lower limits for these yields; a pronounced metastable peak 90.0 $(92^+) \rightarrow (91^+) + 1$ in both spectra indicates that the $C_7H_7^+$ ion intensities at mass 91 are due, at least in part, to further dissociation of $C_7H_8^+$ ions. In sharp contrast, the spectrum of the 3,3-dimethyl compound shows but little $C_7H_8^+$ and no more than a suggestion of a metastable peak at 90.0. The process leading to

(1) See, for example, F. W. McLafferty, *Anal. Chem.*, **31**, 82 (1959).

(2) S. Meyerson, *Appl. Spectroscopy*, **9**, 120 (1955).

(3) S. Meyerson and P. N. Rylander, *J. Phys. Chem.*, **62**, 2 (1958).