a few cc. of the mixture to be analyzed, and passing the vapor over hot copper wire; the resulting CuO^{18} was then converted to water and Cu by reaction with hydrogen gas.

Since the O^{18} data yielded essentially self-diffusion coefficients, these are plotted in the figure as points (reproducibility, ± 2%). The protium diffusion data were treated as giving *integral* diffusion coefficients, and the values are given as lines drawn between the average compositions of the upper and lower compartments of the diffusion cell (reproducibility, ± 4%).

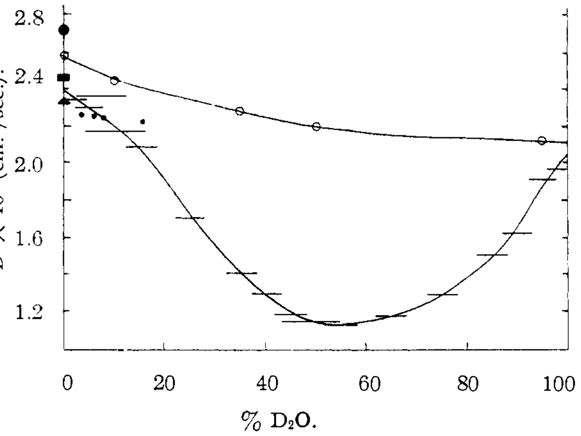

Fig. 1.—Variation of the diffusion coefficient with deuterium enrichment in water mixtures: present work, ○ O^{18} as tracer, — H_2O–D_2O; Longsworth, ●, H_2O–D_2O; Wang, *et al.*, ● O^{18} as tracer, ▲ H_2O–D_2O, ■ H^3 as tracer.

Considering first the region of low D_2O content, there has been a considerable fluctuation in literature values, but our results are in agreement with those of Wang, *et al.*,[4] and of Longsworth,[5] within experimental error. The point of interest, however, is that while the O^{18} diffusion coefficients varied with composition almost exactly as the inverse of the viscosity, the protium diffusion coefficients showed a marked minimum which, if corrected for the viscosity effect, fell at 50% D_2O content.

The explanation for these findings must be that somewhat different mechanisms are available for oxygen than for protium diffusion. For the former, there is the possibility of oxygen exchange between clusters, and for the latter, there is a variety of ways for a similar exchange to occur, since either long or short protium bonds and either a four or a five coördinated transition state may be involved. In any event, the results cannot be explained in terms of the usual assumption that diffusion in water occurs only by motion of individual molecules as units.

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(5) L. G. Longsworth, *J. Phys. Chem.*, **58**, 770 (1954).

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RECEIVED MARCH 23, 1957

SITE OF VANADIUM INHIBITION OF CHOLESTEROL BIOSYNTHESIS

Sir:

Vanadium compounds have been shown to inhibit the incorporation of radioacetate into cholesterol by rat and rabbit liver *in vitro* and *in vivo*.[1,2]

The following compounds have been generally accepted as intermediates in the biosynthesis of cholesterol from acetate: acetoacetate,[3] β-hydroxy-β-methyl glutarate (HMG),[4] β-methyl crotonate (BMC),[5] squalene,[6] lanosterol[7] and zymosterol.[8] Recently β,δ-dihydroxy-β-methyl valeric acid (mevalonic acid)[9] has been proposed as an intermediate because of its extremely efficient rate of conversion to cholesterol. This communication demonstrates that the inhibition by vanadium occurs between the six and five carbon intermediates.

Rat liver slices were incubated for two hours in phosphate buffer[1] containing 10 mg. of sodium acetate-1-C^{14} (1.0 mc./mM.) or 1 mg. of mevalonic acid-2-C^{14} (0.005 mc./mM.).[10] Aliquots of the same batch of slices were used for each experiment. Vanadium in a final concentration of 10^{-3} *M* was added to one flask as diammonium oxytartratovanadate.[10] An equimolar amount of tartrate was added to the control flask. Following incubation of the flasks containing acetate substrate, 30 mg. HMG, 40 mg. BMC, 35 mg. squalene, and 2 mg. of cholesterol were added to each flask as carrier. After saponification in 70% ethanol and 5% potassium hydroxide under nitrogen for one hour, the non-saponifiable fraction was extracted with petroleum ether and from it squalene and cholesterol were isolated by means of an alumina column.[6] The radioactivity of squalene was determined as the hexahydrochloride and cholesterol as the digitonide. The saponifiable fraction was acidified to *p*H 2 with concentrated hydrochloric acid and extracted continuously with ether for 24 hours. The ether was evaporated to dryness and HMG[4] and BMC[5] isolated and their radioactivity determined. Derivatives of each compound retained the calculated amount of radioactivity.

The radioactivity (Table I) of BMC, squalene and cholesterol from acetate as substrate was depressed by vanadium in comparison to the control. The increased radioactivity of HMG in the vanadium inhibited reaction might be expected since it is known that inhibition at any step in a sequence will produce an accumulation of the intermediate just below the inhibition with increased trapping of the radioactive molecules. The radioactivity of cholesterol obtained with mevalonic acid as substrate was also depressed by vanadium. When biosynthetic C^{14}-labeled squalene was employed as

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(1) G. L. Curran, *J. Biol. Chem.*, **210**, 765 (1954).

(2) G. L. Curran and R. L. Costello, *J. Exp. Med.*, **103**, 49 (1956).

(3) G. L. Curran, *J. Biol. Chem.*, **191**, 775 (1951).

(4) J. L. Rabinowitz and S. Gurin, *ibid.*, **208**, 307 (1954).

(5) J. L. Rabinowitz and S. Gurin, THIS JOURNAL, **76**, 5168 (1954).

(6) R. G. Langdon and K. Bloch, *J. Biol. Chem.*, **200**, 129 (1953) and **200**, 135 (1953).

(7) R. B. Clayton and K. Bloch, *ibid.*, **218**, 319 (1956).

(8) J. D. Johnson and K. Bloch, THIS JOURNAL, **79**, 1145 (1957).

(9) P. A. Tavormina, M. H. Gibbs and J. W. Huff, *ibid.*, **78**, 4498 (1956).

(10) Generously supplied by Merck Sharp and Dohme.

TABLE I

EFFECT OF VANADIUM ON THE INCORPORATION OF ACETATE-1-C^{14} AND MEVALONIC ACID-2-C^{14} INTO CHOLESTEROL

Each flask contained 20 ml. phosphate buffer,[1] C^{14}-labeled substrate, 1 g. liver slices, and tartrate (control) or diammonium oxytartratovanadate (vanadium) in a final concentration of $10^{-3}M$. Gas phase was 95% O_2–5% CO_2.

Substrate	Compound isolated	Experiment	Radioactivity recovered[a] Control	Radioactivity recovered[a] Vanadium
10 mg. sodium acetate-1-C^{14} (1.0 mc./mM.)	β-Hydroxy-β-methyl glutaric acid	1 2	2,908 3,138	4,820 6,839
10 mg. sodium acetate-1-C^{14} (1.0 mc./mM.)	β-Methylcrotonic acid	1 2	3,510 3,920	482 986
10 mg. sodium acetate-1-C^{14} (1.0 mc./mM.)	Squalene	1 2	280 303	92 94
10 mg. sodium acetate-1-C^{14} (1.0 mc./mM.)	Cholesterol	1 2	41,248 51,500	9,446 11,575
1 mg. mevalonic acid-2-C^{14} (0.005 mc./mM.)	Cholesterol	1 2	340 388	52 26

[a] Recovered radioactivity is expressed as counts per minute per infinitely thick layer.

substrate,[11] no inhibition by vanadium was found between squalene and cholesterol.

These data are interpreted as demonstrating that vanadium inhibits cholesterol biosynthesis between HMG and BMC. Vanadium also inhibits the conversion of mevalonic acid to cholesterol.[12]

[11] F. Dituri, F. A. Cobey, J. V. B. Warms and S. Gurin, *J. Biol. Chem.*, **221**, 181 (1956).

[12] Supported in part by U.S.P.H.S. Grant H-1947C-2.

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RECEIVED MAY 6, 1957

TWO-DIMENSIONAL TRANSITIONS IN ADSORBED MONOLAYERS

Sir:

Because of the obvious interest[1,2] in theoretically-simple adsorption systems which exhibit transitions, we are moved to make this preliminary report on our measurements for krypton adsorbed on sodium bromide, Fig. 1.

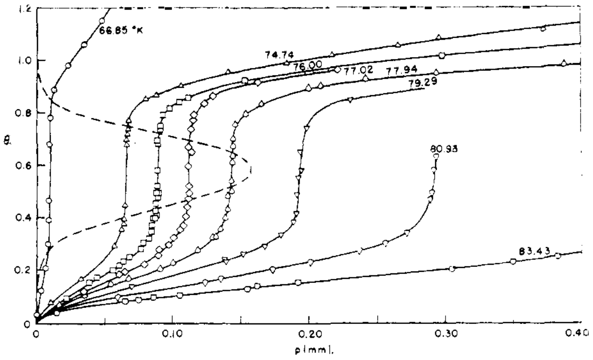

Fig. 1.—Adsorption isotherms of krypton on sodium bromide.

Localized adsorption with nearest-neighbor interactions which, in addition to their usual effects, alter the *lateral* frequency $\omega_{||}$ of the adsorbed molecule with increasing coverage θ according to the prescription $d \ln \omega^2_{||}/d \ln \theta = \gamma$ (= 1 for Kr) leads to the isotherm

$$\ln p(1 - \theta)/\theta^{1+\gamma} = -\beta(\chi - 2w\theta) + \text{const.} \quad (1)$$

where $\beta = 1/kT$, and χ and w are the energies of adsorption and of lateral interaction as employed by Fowler and Guggenheim.[3] As a result of important but compensating differences, our equation with $\gamma = 1$ is similar to that of Rushbrooke.[4] With $\gamma = 0$ it reduces to the Fowler "crude" theory,[3] and with $w = 0$ in addition, to the Langmuir result.

In Fig. 2 we show how the data of Fig. 1 are rectilinearized according to the prescription of Eq. (1). Evidently the intercepts and slopes may be used to evaluate χ and w, both of which are in reasonable agreement with independent theoretical calculations.

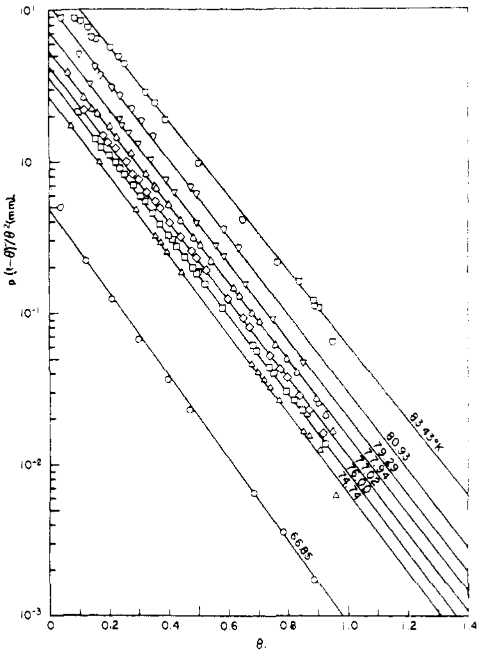

Fig. 2.—Adsorption isotherms of krypton on sodium bromide, plotted according to Eq. (1), $\gamma = 1$.

The authors wish to thank the Institute of Geophysics, University of California at Los Angeles,

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[1] S. Ross and G. E. Boyd, "New Observations on Two-Dimensional Condensation Phenomena," MDDC Report, 864 (1948); S. Ross, THIS JOURNAL **70**, 3830 (1948); S. Ross and H. Clark, *ibid.*, **75**, 6081 (1953); **76**, 4291, 4297 (1954); S. Ross and W. Winkler, *ibid.*, **76**, 2637 (1954); *J. Coll. Sci.*, **10**, 319; 330 (1955).

[2] H. Edelhoch and H. S. Taylor, *J. Phys. Chem.*, **58**, 344 (1954).

[3] R. H. Fowler and E. A. Guggenheim, "Statistical Thermodynamics," Cambridge University Press, 1939, p. 1007.

[4] G. S. Rushbrooke, *Proc. Camb. Phil. Soc.*, **34**, 424 (1938).