TABLE 1

METHIONINE EFFECTS ON LIVER AND KIDNEY CATALASE ACTIVITY

Diet after 2 weeks protein-free diet	No. days on diet	Liver catalase activity*	Kidney catalase activity*	Kidney wt (%)
10% Gelatin, 5% methionine	2	222	23	0.73
" " " "	4	173	19	0.87
" " " "	9	87	9	0.95
25% " 1% "	9	418	68	0.80
25% " 3% "	9	276	47	0.78
25% " 5% "	9	386	29	0.83
25% Casein, 5% "	9	485	12	1.07
Control rats on normal diet	—	550 ± 50	36 ± 4	0.72 ± .04
" " " protein-free diet	—	265 ± 30	39 ± 5	0.68 ± .04

* Catalase unit = ml of O_2/sec/100 g body wt from 1 *N* hydrogen peroxide at 0° C.

methionine and protein suggest that there are different effects of methionine toxicity upon the liver and kidney. The present data and previous observations on the effect of excess dietary protein in increasing kidney catalase activity (*4*) indicate that catalase may be involved in some aspect of protein metabolism.

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Comments and Communications

Inactivation of Circulin by Lipase[1]

PETERSON and Reineke showed (*J. Biol. Chem.*, **181**, 95 [1949]) that circulin, a mixture of basic peptides produced by *Bacillus circulans* Q19, loses its antibiotic activity against *Escherichia coli* ATCC 26 when incubated with a lipase preparation that was free of proteolytic activity as tested by the Mett method (F. C. Koch. *Practical Methods of Biochemistry* [1934]). This observation prompted them to suggest that 6-methyloctanoic acid, which circulin is thought to contain in addition to L-threonine, D-Leucine, and L-α, γ-diaminobutyric acid (DABA), is joined to the peptide through threonine by an ester linkage. To avoid premature acceptance of such a view, we wish to stress the fact that some of the assumptions on the basis of which the existence of this linkage was suggested have not yet been proved. For example, Peterson and Reineke believed circulin to be a *cyclic* polypeptide, taking the following into consideration: (1) amino acid composition (threonine, leucine, and DABA seem to be present in a ratio of 1:1:5); (2) the fact that approximately one half of its amino nitrogen is uncombined (the amino nitrogen before hydrolysis was 7.5%; after hydrolysis, 15.8%); (3) the absence of free carboxyl groups, as shown by titration curves and a negative ninhydrin-CO_2 (Van Slyke) test; and (4) evidence that the amino groups of DABA were the only free amino groups in circulin. The fourth line of evidence is subject to some question and is being reinvestigated. It was based on the observation that the 2,4-dinitrophenyl (DNP) derivative of circulin, when hydrolyzed with HCl, apparently yielded no other products than DABA, α-amino-γ-(2,4-dinitroanilino)-butyric acid, threonine, and leucine, as observed by paper chromatography. However, since DNP derivatives of mono-amino acids do not react with ninhydrin, which was used to indicate the position of the various components on the chromatogram, and are visible only because of their yellow color, small quantities of such derivatives may have escaped detection. Moreover, the fact that not all the DABA appeared as its DNP derivative in the chromatogram makes one wonder whether (1) DABA is formed from its DNP derivative by acid hydrolysis, (2) the dinitrophenylation was not carried to completion, or (3) some DABA is combined in the intact molecule. The first hypothesis can be experimentally disproved (R. G. Shepherd, personal communication) and can therefore be excluded as an explanation for the occurrence of free DABA on the chromatogram. The second alternative is not unlikely, inasmuch as the dinitrophenylation was performed in the absence of alcohol, a condition under which the reaction is thought not to go to completion. This, however, does not exclude the third possibility, especially since the data on the combining weight of circulin were inconsistent. If circulin has a combining weight of approximately 300, as claimed, and actually has five free amino groups, its molecular weight should be 1500. However, calculations from the weight of the constituents that circulin is thought to contain indicate a molecular weight

[1] We are grateful to R. G. Shepherd, of the American Cyanamid Company, Stamford, Conn., for his stimulating comments on this problem.

close to 900. Accordingly, it would seem that either the determinations for the combining weight were inaccurate, or fewer than five amino groups are free.

Although it is possible that circulin has a cyclic structure, the contentions that all its free amino groups are furnished by DABA, and that all DABA side chains are unsubstituted, still remain to be proved. This proof is prerequisite to the claim that an O-acyl, rather than an N-acyl, linkage exists between 6-methyloctanoic acid and the rest of the molecule. Moreover, one needs to demonstrate that the lipase preparation used does not hydrolyze N-acyl, as well as O-acyl, linkages. Isolation of the fatty acid and the intact peptide after inactivation of circulin by lipase, and demonstration that a hydroxyl group rather than an amino group becomes liberated during inactivation, are also necessary before any concept on the manner in which the fatty acid is attached to the rest of the molecule can be accepted.

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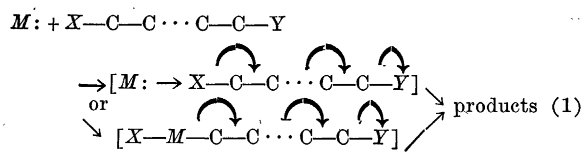
The Action of Metals on 1,4-Dihalides and Similar Compounds

A study is under way to determine the extent and mechanism of the general reaction



M is a metal such as magnesium, zinc, or sodium (other reducing agents, such as iodide ion, may also function in the reaction); *X* is halogen; and *Y* is an electronegative group—e.g., halogen. The dotted line represents a multiple bond.

Earlier examples of Equation (1) include the reaction of zinc or magnesium with 1,4-dibromo-2-butenes to yield 1,3-butadienes (*1, 2*), and the reaction of γ-phenoxycrotyl bromide with magnesium to yield 1,3-butadiene (*1*). More recently, the authors reported the formation of butatriene in high yield from 1,4-dibromobutyne-2 and zinc in diethylene glycol diethyl ether (*3*). With zinc in ethanol or water, butatriene was formed in only small yield, the principal product being butadiene. An extensive study of the properties of unsubstituted cumulenes such as butatriene has been undertaken.

Other examples of the general reaction (*1*) have now been found. Thus, magnesium in tetrahydrofuran reacted with γ-bromocrotonaldehyde diethylacetal to yield 1-ethoxy-1,3-butadiene in 78% yield, and with γ-bromocrotonaldehyde diacetate to yield 1-acetoxy-1,3-butadiene in 60% yield. Ethyl ortho-γ-bromocrotonate and magnesium yielded a material which largely polymerized in the tetrahydrofuran solvent. Treatment of the reaction mixture with hot aqueous HCl gave a small yield of crotonic acid. This could have resulted from the expected product of the reaction with magnesium, 1,1-diethoxy-1,3-butadiene, by reaction of this ketene acetal with water to yield ethyl crotonate (or ethyl vinylacetate) and then hydrolysis to the acid.

Iodide ion perhaps can be substituted for the metal in the above elimination reactions. For example, potassium iodide in aqueous methanol converted 1,4-dibromo-butene-2 to butadiene-1,3, and 3,6-dibromocyclohexene to cyclohexadiene-1,3. That some degree of unsaturation in the carbon chain is required is shown by the fact that 1,4-dibromobutane failed to yield any ethylene when treated with magnesium.

Other examples on which the elimination reaction (1) will be tried include γ-chlorocrotonylchloride, γ-chloropropiolic acid chloride, fumaryl and maleic acid chloride, and acetylenedicarboxylic acid chloride. Perhaps the reaction also can be extended to six carbon analogs, such as the conversion of 1,6-dibromohexadiyne-2,4 to hexapentaene.

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Three- and Four-Dimensional Plotting

The examination of experimental results by plotting a graph in two dimensions is almost universal, but little use is made of plots of variables in three dimensions. The lack of a suitable simple apparatus for plotting in three dimensions is one reason for the neglect of this potentially useful technique. In connection with a statistical analysis of data on acid, chloride, and volume for samples of gastric juice (R. B. Fisher and J. N. Hunt. *J. Physiol.*, **111**, 138 [1950]), a simple method of plotting in three, and even four, dimensions was worked out, and it may be of value to other workers with similar problems.

The apparatus shown in Fig. 1 consists of a cube of Lucite constructed of 20 numbered sheets of the same thickness locked together by two bolts and illuminated from below by a small electric bulb. The lowest sheet is ruled with a grid. To plot in 4 dimensions, two dimensions are represented by the position of points plotted in waterproof ink on the surface of the plates, the third dimension is represented by the number of the plate selected, and the fourth by the color of the ink used to plot the point. In practice,