on key *A* are useless when the patch is dark. After most rewards, the shutter opens, and the lighted patch reappears. Continued darkness follows one reward in five; in this case, a peck on key *B* brings food a second time. These double rewards train the bird to attend to the stimulus patch after eating; without them, the bird would always peck key *A* after eating, regardless of the condition of the stimulus patch.

In the next stage of training, several pecks in a row, rather than a single peck, are required on key *A* to close the shutter and on key *B* to obtain food. The number of pecks required is varied randomly between one and eight. This increases the time between rewards and prevents the bird from getting a reward simply by pecking the two keys alternately, without attending to the stimulus patch. The time between rewards is further increased by introducing an interval after each reward during which no amount of pecking can close the shutter. The duration of this interval varies randomly about a mean of 7 sec.

When training is nearly complete, a final feature is added to the procedure: the luminance of the stimulus patch is put under the control of the bird's responses during the intervals between rewards. Each peck on key *A* reduces the luminance of the patch by a small amount, while each peck on key *B* increases the luminance of the patch. A pen continuously records these luminance changes. When the bird has learned to perform consistently under these conditions, the collection of threshold data can begin. Experimentation continues indefinitely without further alteration of procedure.

An account of a typical experimental session will serve to illustrate how the bird's threshold is traced. At first, the stimulus patch is brightly lighted, and the trained bird pecks only key *A*. The bird continues to peck key *A* until the patch becomes so dim that it falls below the bird's absolute threshold. Because the pigeon cannot distinguish this "dim-out" of the patch from the true "black-out" caused by the closing shutter, it begins to peck key *B*. But pecking key *B* increases the luminance of the patch, so in a short time the patch again becomes visible to the bird.

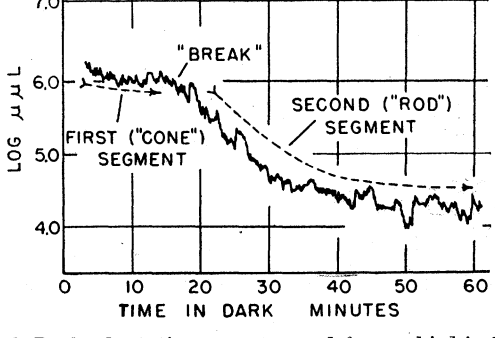

Fig. 2. Dark-adaptation curve secured from a bird in 1 hr. The luminance of the stimulus patch, in log micromicrolamberts, is on the ordinate.

When this happens, the bird switches its pecking back to key *A*, causing the stimulus to dim and to disappear as before. This process continues indefinitely; the bird alternately pecks keys *A* and *B*, and the stimulus fluctuates up and down across the bird's absolute threshold. The continuous record of the stimulus luminance traces the bird's absolute threshold through time. The randomly spaced rewards, when pecks on key *A* close the shutter and pecks on key *B* bring food, interrupt the continuity of this threshold record frequently but for only a few seconds.

During the first portion of an experimental session, the recording pen traces the pigeon's dark-adaptation curve. A reproduction of such a curve in a 1-hr session is shown on appropriate coordinates in Fig. 2. Before this particular session, the bird had spent 1 hr in darkness, followed by 10 min in a box with white walls at a luminance of 22 millilamberts.

References and Notes

1. B. F. Skinner, *Am. Psychologist* **8**, 69 (1953).
2. G. v. Békésy, *Acta Oto-Laryngol.* **35**, 411 (1947).

3. This research was supported in part by contract N5ori-07663 (project NR140-072) between Harvard University and the Office of Naval Research, directed by Floyd Ratliff. It represents part of a thesis submitted to the Department of Psychology, Harvard University, in partial fulfillment of the requirements for the Ph.D. degree. I am indebted to Ratliff for his constant interest and helpful advice. Present address: National Institute of Mental Health, Bethesda, Md.

4. C. B. Ferster, *Psychological Bull.* **50**, 263 (1953).

19 January 1955.

Chromatographic Separation of Polybromo Fatty Esters

David R. Howton

Atomic Energy Project, School of Medicine, University of California, Los Angeles

It was observed some time ago in connection with the preparation of certain carboxy-labeled fatty acids (*1*) that 1-bromoheptadecane is rapidly eluted from alumina by petroleum ether while 1,8,9,11,12-pentabromoheptadecane is not. An order of magnitude of affinity between vicinal pairs of bromine substituents and the hydrogen-bond-contributing type of adsorbent (such as alumina or silica) that had not been appreciated previously was thus revealed; it occurred to us that this observation might be the basis for a new approach to the problem of separating fatty acids differing in degree of unsaturation (*2*).

Recent studies (*3*) have shown clearly that the ease with which fatty acid esters are eluted from alumina or silica columns is an inverse function of the number of carbon-carbon double bonds that they contain; but the separations achieved are at best fair and the yields of pure constituents recovered are poor, reflecting a small contribution of the olefinic centers to the overall adsorption affinity of such substances. (In these studies, as well as in our own, the strongly polar carboxyl group of the free fatty acids is esterified in

order to make the contribution of the centers of unsaturation—actual or potential—as dominant as possible.)

It has now been demonstrated that the addition of bromine to unsaturated fatty acid esters enhances the adsorption-affinity contribution of each unsaturation site to such an extent that the resulting esters, differing in the number of pairs of vicinal bromine substituents that they contain, are easily and cleanly separated by gradient elution from alumina chromatographic columns. The original unsaturated fatty acids may then be regenerated from the separated polybromo fatty esters by conventional debromination and saponification procedures.

Resolution of an artificial mixture of methyl stearate (MeS), methyl *threo*-9,10-dibromostearate ($MeSBr_2$), and methyl *threo,threo*-9,10,12,13-tetrabromostearate (α-$MeSBr_4$, mp 56°C) is illustrated in Fig. 1. (Methyl esters are used because of their facile, quantitative preparability via diazomethane; the size or shape of the esterifying group does not appreciably affect the chromatographic behavior of a fatty acid ester.) Since MeS and α-$MeSB_4$ are crystalline substances and $MeSB_2$ is an oil, the efficacy of the separation of such a mixture is dramatically evident in practice.

Columns employed for the chromatography of about 500 mg of mixed polybromo fatty esters measure about 32 (diameter) by 100 mm and are packed in the dry state; the adsorbent is Harshaw alumina (AL-0109-P) reground to pass a No. 200 sieve and mixed 40 : 7 by weight (about 2 : 1 by volume) with vacuum-dried celite 535.

Chromatograms of artificial mixtures (see Fig. 1, in which 200 mg of each ester was placed on the column) show that recoveries are not quantitative, presumably as a consequence of saponification by basic impurities in the alumina employed. Attempts to avoid such losses by the neutralization of the adsorbent have been unsuccessful. Silica columns are much more inert in this respect, but the resolving power of this adsorbent is inferior; resolution of a mixture of MeS and α-$MeSBr_4$ on silica (eluting throughout with 2 percent ether in pentane) was appreciable but far from complete.

Application of the new technique to material derived from a naturally occurring mixture of fatty

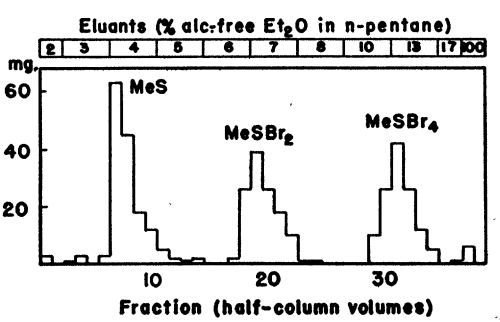

Fig. 1. Chromatogram of an artificial mixture of MeS, $MeSBr_2$, and α-$MeSBr_4$.

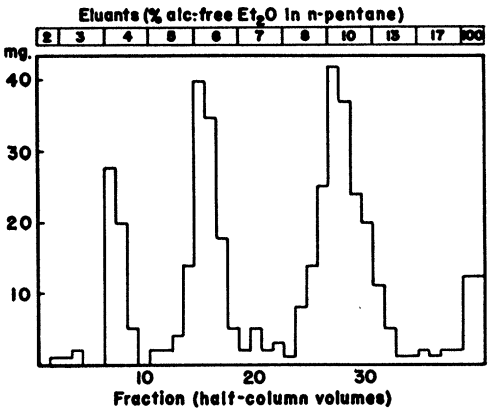

Fig. 2. Chromatogram of brominated poppy seed oil fatty acid methyl esters.

acids (poppy seed oil fatty acids from which most of the saturated and the 115°-9,10,12,13-tetrabromostearic acids had been removed) resulted in the chromatogram shown in Fig. 2. The three major peaks represent, as confirmed by ultimate analyses, methyl esters of bromine-free, dibromo, and tetrabomo fatty acids in order of elution. The tetrabromo fraction is presumed to consist almost entirely of the lower-melting (previously unknown in this state of purity) diastereoisomeric methyl *threo,threo*-9,10,12,13-tetrabromostearate (β-$MeSBr_4$).

Chromatography of materials derived from natural fatty acid mixtures containing linoleic acid has consistently given tetrabromide peaks, the fractions in the leading half of which are crystalline, while those emerging later are oils. A slight difference in adsorption affinity of the two esters (α- and β-$MeSBr_4$) expected to result on addition of bromine to methyl linoleate is thus indicated, and this difference has been confirmed by studies of the behavior of artificial mixtures of α- and β-$MeSBr_4$. The difference is not, however, of sufficient magnitude to result in overlap of the complex $MeSBr_4$ peak with those of $MeSBr_2$ or $MeSBr_6$ on either side. Thus, inasmuch as all the 2^{n-1} diastereoisomeric racemates expected to result from saturation with bromine of a fatty acid ester containing n isolated carbon-carbon double bonds will be expected to emerge from the chromatographic column as a discrete band, the present procedure has obvious advantages over the classical method of isolating small amounts of polyunsaturated fatty acids. The latter method, which depends upon solvent fractionation of the crystalline brominated fatty acids, promises recovery of no more than a fraction (2^{1-n}) of the individual unsaturated fatty acid originally present in the mixture.

It should be emphasized that the length of the fatty acid chain is a factor of negligible importance in determining the elutability of a brominated fatty ester from an alumina column; thus, a mixture originally containing both oleic and palmitoleic acids would be expected to give rise to a single dibromo ester peak containing material derived from both parent sub-

stances. (Similarly, Fig. 2 illustrates the emergence of the methyl esters of stearic and palmitic acids as a single peak.)

Although our attention has been focused on applications of this chromatographic procedure to separation of mixtures of fatty acids, there would appear to be no reason to believe that it could not be applied to other classes of compounds that contain no functional groups of sufficient polarity to eclipse the absorption-affinity contribution of vicinal pairs of bromine substituents.

These preliminary observations are published in the belief that the technique holds considerable promise as a means of sampling saturated, mono-unsaturated, and di-unsaturated fatty acids obtained from quite small amounts of naturally occurring lipids. We are currently engaged in such applications and in studies of the chromatographic behavior of fatty acid esters obtained by bromination of more highly unsaturated substances and of those containing isolated single bromine substituents in addition to vicinal pairs of bromine substituents.

References and Notes

1. D. R. Howton, R. H. Davis, J. C. Nevenzel, *J. Am. Chem. Soc.* **76**, 4970 (1954).
2. This paper is based on work performed under contract AT-04-1-GEN-12 between the U.S. Atomic Energy Commission and the University of California at Los Angeles.
3. M. F. White and J. B. Brown, *J. Am. Chem. Soc.* **70**, 4269 (1948); S. F. Herb, L. P. Witnauer, R. W. Riemenschneider, *J. Am. Oil Chemists' Soc.* **28**, 505 (1951) and earlier publications.

10 January 1955.

Communications

More on Induction of Tooth Defects by Thermal Shock

The communication by D. G. and H. A. Pohl [*Science* **120**, 807 (1954)] is interesting, but it appears to be based on several assumptions that do not appear valid from either clinical or histological viewpoints.

The first generalized assumption of "changed eating habits" carries with it the statement of present-day exposure of the teeth to extremes of temperatures and consequent probable induction of small cracks. Clinical observations clearly demonstrate that the anterior teeth are shielded by the lips when food or beverage of extreme temperatures are placed in the mouth. Complaints by patients of thermal sensitivity usually indicate a latent period of 10 to 15 sec. Extensive experience in clinical dentistry has clearly shown me that cracks of the dental enamel, when present in children or young adults, are the result of direct trauma; in the aged, they are found in the anterior teeth when the occlusion is of an end-to-end relationship. In the latter, roentgenograms will also disclose narrowing of the pulp chambers and canals, which probably indicates diminishing metabolic activity.

The second assumption, that the tooth enamel can be compared to the physical properties of "vitreous materials," is not sound. A glass or bone china dish is of homogeneous structure, whereas the dental enamel is a complex organ. In youth, and throughout life, remnants of an outer protein covering (primary surface cuticle) ensheath the millions of enamel rods that are closely intertwined in the cuspal areas. Each rod is encased by an organic sheath [D. B. Scott *et al.*, *J. Dental Research* **31**, 74 (1952)] and cemented to the adjacent rods by dense interprismatic material. To suggest that there is an implied validity in equating the smashing action of a weight on a dried extracted tooth (it is not stated whether the specimens are single or multirooted teeth) lying horizontally on a concrete floor with the normal occlusal forces on cusps or incisal edges of the teeth suspended by an elastic periodontal membrane in an alveolar socket is open to criticism.

Since the basis for the entire experiment is the hypothesis that cracks induced by thermal shock "furnish entrance sites for bacterial decay," one should expect a greater incidence of dental decay on the labial surfaces of incisor teeth that are more susceptible to such thermal shocks. All statistical evidence relative to the incidence of dental decay points to the immunity of the lower anterior teeth and of the labial surfaces of the upper anterior teeth.

BENJAMIN B. KAMRIN
Department of Anatomy, College of Medicine at New York City, State University of New York, Brooklyn

9 March 1955.

Although we feel that our communication was only a brief preliminary to necessary extensive work, and was, therefore, undeserving of any extended analyses, we feel that certain misunderstandings evident in Kamrin's comments may require clarification.

We agree wholeheartedly with Kamrin that further and more conclusive work needs to be carried out to show the fallacy or true extent of the indication in the data that thermal shock can damage teeth. If the inference is proved true, then a cure or abatement of the damage needs to be found.

Kamrin appears to imply that direct blows are the only cause of cracks in dental enamel. Have other causes, such as thermal shock, been generally recognized?

In regard to his comments about comparison of the resistance of dental enamel and ceramic materials to thermal shock, we would recall that crystallinity generally plays a very minor role. Whether or not a hot