that treatment of 2-aminobicyclo(2.2.2.)octane with nitrous acid also gives the same alcohol[2].

It seems, therefore, that the formation of a non-classical ion which leads to rearrangement is unique to systems which are strained. Since the geometry of both systems is essentially identical, the only difference being that (I) is strained and (III) is strainless, the driving force for the formation of the non-classical ion would seem to be mainly due to the relief of strain in going to the ion.

Although NEWMAN[3] in the hydration of (IV) obtained the rearranged alcohol, bicyclo(1.2.3.)octan-2-ol, and DOERING[4] the bicyclo(1.2.3.)octan-2-bromide by treating (III) (X = Br) with silver bromide, it should be pointed out that in the cases reported here, the ion has a short lifetime. Apparently, when the ion has a longer lifetime, as in the cases of NEWMAN and DOERING, rearrangement will occur. However, under the identical solvolysis conditions that were employed here, the norbornyl derivatives underwent rearrangement[5].

The rate of solvolysis of bicyclo(2.2.2.)octyl-2-p-bromobenzenesulfonate, and its comparison with cyclohexyl p-bromobenzenesulfonate, will give quantitative measure of the driving force and determine the amount, if any, of carbon participation. These experiments will shortly be undertaken in these laboratories.

H. M. WALBORSKY[6]

Department of Chemistry of the Florida State University Tallahassee, Fla., March 16, 1953.

Zusammenfassung

Es wird vorgeschlagen, dass die Bildung nicht-klassischer Ionen ein Merkmal gespannter Systeme darstellt.

Nach dieser Auffassung stellt die Entspannung, welche die Bildung des Ions begleitet, die treibende Kraft seiner Bildung dar.

[2] K. ALDER and G. STEIN, Ann. Chem. *514*, 211 (1934).

[3] The low m.p. is probably due to a slight impurity, since these substances have large cryoscopic constants. See: M. S. NEWMAN and Y. T. YU, J. Amer. Chem. Soc. *74*, 507 (1952).

[4] W. VON DOERING and M. FARBER, J. Amer. Chem. Soc. *71*, 1514 (1949).

[5] J. D. ROBERTS *et al.*, loc. cit.

[6] Present address: Institute for organic Chemistry, University of Basle.

Isolated Chromosomes and Casual Contaminants in Electron Microscopy[1]

Chromosomes, bacterial cells, and fungal filaments may often display similar size and shape and banded or helical internal structure. Careful controls are therefore needed to assure their clear discrimination in the electron microscope. This need has recently been emphasized by the publication of some micrographs[2] identified as isolated chromosomes but resembling certain casual contaminants.

Such internal structure in a contaminant is beautifully illustrated by the object encountered several years ago by ROBLEY C. WILLIAMS (Fig. 1), determined to be a bacterial contaminant of distilled water. This measures 1/6 by 3·8 μ and shows a helical pitch of 1/6 μ per gyre. Rather similar and smaller structures have been reported for some soaps and greases[1].

Against this background, one of us (A.R.T.D.), interested in the identity of isolated chromosomes[2], was concerned by the cited figures reportedly representing whole isolated chromosomes of the turtle *Clemmys japonica* (Fig. 1–4 of reference[3]), of carp (Fig. 7 *f* of reference[4]), and, subsequently, of carp, triton, and rabbit[5]. Moreover, a stalked «isolated chromosome» in this last paper[5] reminded one of *Caulobacter* as figured by HOUWINK and VAN ITERSON[6]. Such objects had not been encountered by this worker as chromosomal isolates from other materials. However, we had encountered some generally similar forms as casual contaminants on rare occasions (Fig. 2); on the basis of form and size these were presumed to be bacterial. The illustrated contaminant measures $^3/_4 \times 6{\cdot}8$ μ, the periods *ca.* 0·8 μ.

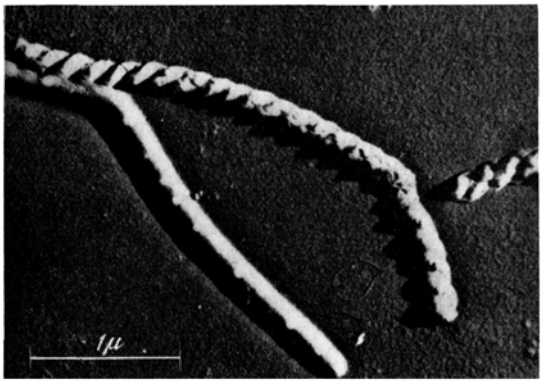

Fig. 1.—Helically organized bacterial contaminant of distilled water. Reproduced with the kind permission of Professor ROBLEY C. WILLIAMS, now of the Virus Laboratory, the University of California.

Investigation disclosed that helical structures had also been encountered by others. Some unidentified

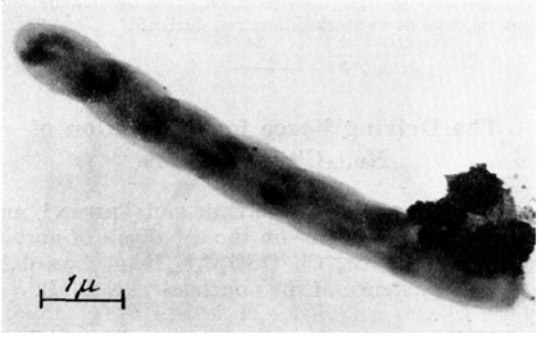

Fig. 2.—Relatively rare, structured casual contaminant from distilled water controls.

[1] This work by an Alfred P. Sloan Foundation Fellow (A.R.T.D.) was supported by grants from the National Cancer Institute of the National Institutes of Health, Public Health Service, from the Damon Runyon Memorial Fund, and from the American Cancer Society.

[2] G. YASUZUMI, Chromosoma *4*, 222 (1951). – G. YASUZUMI, G. MIYAO, Y. YAMAMOTO, and J. YOKOYAMA, Chromosoma *4*, 359 (1951). – G. YASUZUMI, T. YAMANAKA, S. MORITA, Y. YAMAMOTO, and J. YOKOYAMA, Exper. *8*, 218 (1952).

[1] A. Y. MOTTLAU, J. Appl. Phys. *20*, 1055 (1949). – S. G. ELLIS, Electron Microscopy, II. Oil Industry Uses: Petroleum Refiner *27*, 487 (September 1948).

[2] A. R. T. DENUES, Exp. Cell Res. *3*, 540 (1952); in press, *ibidem*.

[3] G. YASUZUMI, G. MIYAO, Y. YAMAMOTO, and J. YOKOYAMA, Chromosoma *4*, 359 (1951).

[4] G. YASUZUMI, Chromosoma *4*, 222 (1951).

[5] G. YASUZUMI, T. YAMANAKA, S. MORITA, Y. YAMAMOTO, and J. YOKOYAMA, Exper. *8*, 218 (1952).

[6] A. L. HOUWINK and W. VAN ITERSON, Biochim. Biophys. Acta *5*, 10 (1950) (fig. 16, p. 31).

contaminants found by one of us (C.A.S.) resemble strikingly the cited micrographs of the Japanese workers (Fig. 3 and 4). Such contaminants measure from 0·3 to 0·5 by 2 to 3 and up to 10 μ, the periods being 0·3 to 0·5 μ. Some generally similar micro-organisms have also been encountered by E. DeRobertis at Montevideo (Fig. 5) and, very recently, by David B. Slautterback at the Sloan-Kettering Institute. Mold mycelia have also shown such internal structure; the widths and periods here are both *ca.* ½ μ. Such forms were recently

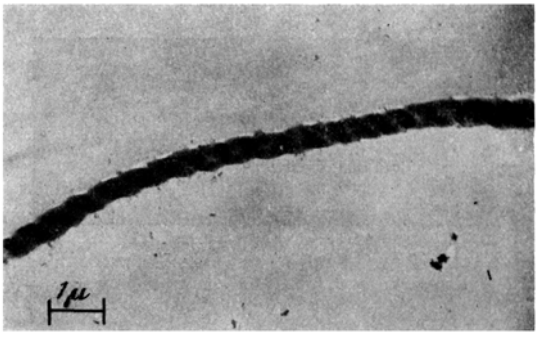

Fig. 3.—Unidentified structured casual contaminant.

encountered in controls by Miss Ruth E. Berger; these measure *ca.* ½ × 5 to 10 μ with a period of *ca.* ½ μ. Another banded contaminant measuring *ca.* 0·3 × over 20 μ, with a period of *ca.* ½ μ, was also recently encountered at SKI.

Seeking more direct evidence on the identification of the cited micrographs of «isolated chromosomes», three preparations were made by the technique of Mirsky and Ris as detailed by Yasuzumi *et al.*[1] employing the red cells of two turtles congeneric with the Japanese material, viz., *C. guttata* and *C. insculpta*. The results were disappointing in that the isolated chromosomes were found not similar to the beautiful objects figured by Yasuzumi *et al.*, either in fresh wetmounts in the phase microscope or in unfixed or acetocarmine-fixed preparations in the electron microscope. The isolated threads appeared in the phase work to include some bandings, but electron microscopy at 50 and at 100 kV revealed characteristic filaments of high electron opacity, relatively devoid of internal structure. No interesting growths were obtained in cultures of the blood of these animals.

Despite the foregoing indications it remains possible that objects such as those reported by the Japanese workers[1] might indeed be proved by fully documented, controlled studies to represent isolated chromosomes. However, from the evidence now available it is concluded that these objects could as well be micro-organisms as chromosomes, and that such an error in identification of the cited micrographs is very likely.

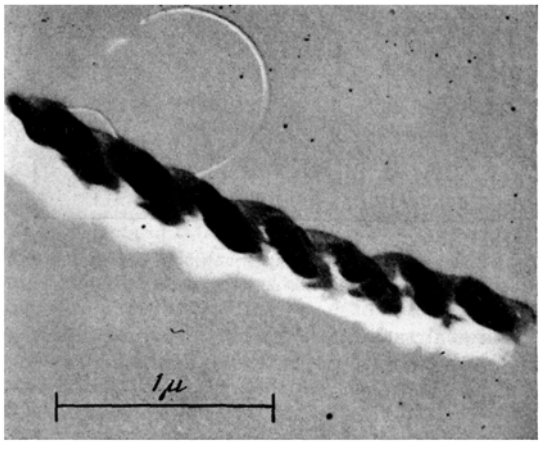

Fig. 4.—Unidentified structured casual contaminant.

[1] G. Yasuzumi, G. Miyao, Y. Yamamoto, and J. Yokoyama, Chromosoma *4*, 359 (1951).

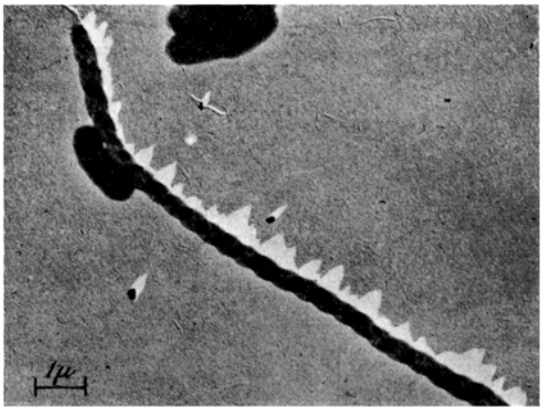

Fig. 5.—Helically organized bacterial contaminant, reproduced with the kind permission of Dr. E. DeRobertis of the Instituto de Investigación de Ciencias Biológicas, Montevideo.

Acknowledgments. We are indebted to many colleagues for discussions, especially to Dr. J. G. Gall, now at the University of Minnesota, and to Dr. R. C. Williams; to Dr. G. Yasuzumi for helpful correspondence; to Dr. R. C. Williams for figure 1, to Dr. E. De Robertis for figure 5; to Mr. E. Champaygne of the North American Philips Company for making possible the examination of our material at 100 kV; and to Theodor R. Marcus, Mark E. Gettner, and Clifford E. Grey for assistance with the preparations and microscopy at the Sloan-Kettering Institute.

A. R. T. Denues and C. A. Senseney

Sloan-Kettering Institute for Cancer Research, 444 East 68th Street, New York 21, N. Y., and Chemical Corps Biological Laboratories, Camp Detrick, Frederick, Md., 12 November 1952.

Addendum. After completion of this manuscript the informative note of Houwink appeared in Experientia *8*, 385 (1952). This identifies the stalked form as *Caulobacter* spec., as cited above, and calls attention to contaminating micro-organisms, apparently more common in the Netherlands, with double-stranded helical structure.

Addendum. Stalk bacteria as contaminants, including twisted forms from the genus *Gallionella*, were described by Julia M. Coffey in 1951 (Annual Report, Division of Laboratories and Research, New York State Department of Health, Albany *1951*, 25–26).

Zusammenfassung

Objekte, welche in jüngst veröffentlichten elektronenmikroskopischen Abbildungen als isolierte Chromosomen identifiziert wurden, sind nichtchromosomalen Beimischungen sehr ähnlich. Ein Irrtum in der Identifizierung dieser Objekte ist wahrscheinlich. Wir haben solche Strukturen in Chromosomen nicht gefunden.

[1] G. Yasuzumi, Chromosoma *4*, 222 (1951). – G. Yasuzumi, G. Miyao, Y. Yamamoto, and J. Yokoyama, Chromosoma *4*, 359 (1951). – G. Yasuzumi, T. Yamanaka, S. Morita, Y. Yamamoto, and J. Yokoyama, Exper. *8*, 218 (1952).