[16] Shechter, E., J. P. Carver, and E. R. Blout, these PROCEEDINGS, **51,** 1029 (1964).

[17] Urnes, P., private communication. An alternate graphical solution of equation (10) is to plot $[m'] (\lambda^2/\lambda_{193}^2 - 1)$ against $(\lambda^2/\lambda_{193}^2 - 1)/(\lambda^2/\lambda_{225}^2 - 1)$, which directly yields A_{225} as the slope and A_{193} as the intercept.

[18] Rosenfeld, L., *Z. Physik,* **52,** 161 (1928).

[19] Iizuka, E., and J. T. Yang, *Biochemistry,* **3,** 1519 (1964).

[20] Katzin, L. I., and E. Gulyas, *J. Am. Chem. Soc.,* **86,** 1655 (1964).

[21] Yang, J. T., in *Polyamino Acids, Polypeptides, and Proteins,* ed. M. A. Stahmann (Madison, Wisconsin: University of Wisconsin Press, 1962), p. 225.

[22] Cassim, J., and E. W. Taylor, *Abstracts,* 5th Annual Meeting, Biophysical Society, Chicago, 1964, p. TC5.

[23] Doty, P., A. M. Holtzer, J. H. Bradbury, and E. R. Blout, *J. Am. Chem. Soc.,* **76,** 4493 (1954).

[24] Yang, J. T., and T. Samejima, *J. Biol. Chem.,* **238,** 3262 (1963).

[25] Yang, J. T., and W. J. McCabe, to be published.

[26] Yang, J. T., *Federation Proc.,* **21,** 406 (1962).

[27] Doty, P., and R. D. Lundberg, these PROCEEDINGS, **43,** 213 (1957).

[28] Yang, J. T., *Tetrahedron,* **13,** 143 (1961).

[29] Blout, E. R., presented orally at the 6th International Congress of Biochemistry, New York, 1964.

[30] Sogami, M., W. J. Leonard, Jr., and J. F. Foster, *Arch. Biochem. Biophys.,* **100,** 260 (1963).

---

*ELECTRON MICROSCOPY WITH HIGH-FIELD SUPERCONDUCTING SOLENOID LENSES**

BY HUMBERTO FERNÁNDEZ-MORÁN

DEPARTMENT OF BIOPHYSICS, UNIVERSITY OF CHICAGO

Communicated by W. Bloom, December 21, 1964

The resolving power of the electron microscope has extended the range of direct visualization to structural details of the order of a few angstroms. This corresponds to the size of small molecules and to the atomic spacing in crystalline lattices.[1-3, 9] However, although the wavelength of electrons in standard microscopes is 100,000 times shorter than the wavelength of light, the best electromagnetic and electrostatic lenses available have usable apertures limited, by aberrations, to semiangles of the order of $^1/_{100}$ radian as compared with the numerical apertures of 1.5 of the best light microscope lenses. Considering the numerous complex instrumental and preparative factors involved,[1-3, 9] the major steps which have to be taken for attainment of the ultimate theoretical resolution are correction of lens aberrations (mainly spherical and chromatic aberrations), stabilization of the lens excitation current, and accelerating voltage. Thus, the degree of stability required for very high resolving powers (in the range of 4 Å) is of the order of 1 to 2 parts per million, since the focal length of a magnetic lens is dependent on the electron energy as well as on the lens excitation current. In addition, if the present limitations of the strength and configuration of the axially symmetrical field formed by iron pole pieces could be overcome, "stronger lenses" of shorter focal length could be designed with correspondingly reduced aberrations.

With the introduction and availability of new high-field superconducting solenoids of alloys of niobium-zirconium and niobium-tin,[4-7] it is now possible to obtain magnetic fields in excess of 60 kilogauss over relatively large volumes when operating at liquid helium temperatures, without the continual expenditure of vast amounts of electrical power. It has been demonstrated[7] that operation of a superconducting solenoid, short-circuited, or in "the persistent current mode," yields large uniform magnetic fields which are highly homogeneous to better than one part in 10^6 to 10^7, and are highly stable and noise-free under appropriately controlled conditions.

Based on previous work in low-temperature electron microscopy,[8-10] the author has pointed out the unique advantages to be derived from the use of superconducting electromagnetic lenses operating at liquid helium temperatures, with regard to both instrumentation and specimen preservation. As part of a comprehensive program under way in the Department of Biophysics at the University of Chicago, preliminary experiments have been successfully carried out with a simple electron microscope which can be used for transmission electron microscopy and electron diffraction, using high-field superconducting solenoid lenses in an open-air-core, liquid-helium Dewar, preferably operating in the persistent current mode. In a series of controlled, reproducible experiments, electron microscopic images have been recorded of test specimens with accelerating potentials of 4–8 kV, using a niobium-zirconium solenoid without pole pieces, operating at 32.2 kilogauss in the persistent current mode. These preliminary experiments have demonstrated over a period of 4–8 hr of continuous operation the exceptional stability of the images, and also their relatively high quality at magnifications of 50 to 100 ×. The results obtained with this experimental approach, as well as some interesting observations bearing on imaging phenomena with superconducting solenoids, are described in this report.

Experimental.—As shown in Figure 1*A* and *B*, the system consists of a vertical-air-core Dewar, provided by Westinghouse Cryogenics Systems Division. This Dewar is a nitrogen-shielded, stainless steel vessel consisting of six concentric cylinders providing the required cryogenic environment, and with an inner vessel containing 6–7 liters of liquid helium in which a superconducting solenoid is immersed. An important feature of this Dewar is that it has an open air core of 1.5 inches in diameter for ready access of specimens at room temperature. The superconducting magnet coil used for these experiments is a niobium-zirconium solenoid, which can be operated in the persistent current mode at a field of 32,200 gauss; rated field uniformity is 6% in a $^1/_2$ inch diameter sphere; rated current is 15.3 amp; working volume: 2.000 inch diameter × 2.427 inch long cylinder. Auxiliary equipment provided by Westinghouse Cryogenics Systems Division includes a transistorized power supply, model 503, a gaussmeter, liquid helium level gauge, and accessories.

The electron microscope (Fig. 1*B*) is a simple, miniaturized instrument consisting of a stainless steel, nonmagnetic tube with suitably scaled electron gun (of the re-entry cathode shield type) which can be used with standard or pointed filaments.[9] The filament was operated at 2.3 volts, 3-amp heating current with a variable bias of 180–200 volts. The high voltage in these initial experiments was 4–8 kV, which can be highly stabilized by using a special motor-generator set with Transistat type TRA-11, Westinghouse solid-state regulator. This system is sealed and evacuated to a vacuum of 10^{-5} to 10^{-6} mm Hg. The test specimen, a 200-mesh copper grid (hole diameter: 136 μ) can be placed at a convenient position within the field of the superconducting solenoid and can be moved during the experiment. The image is focused on the fluorescent screen and photographed from the outside of the glass chamber by using Polaroid film (types 42 and 47), 35-mm Plus-X or Royal-X Pan Kodak film, and also movie film (16-mm Royal-X Pan). As shown in Figures 3 and 4, photography from the outside results in a characteristic distorted perspective of the original image. Adequate provisions were taken for ensuring a high degree of mechanical stability and for shielding from magnetic and electrical perturbations.

The system is actually a very flexible electron optical bench which can be taken out and dis-

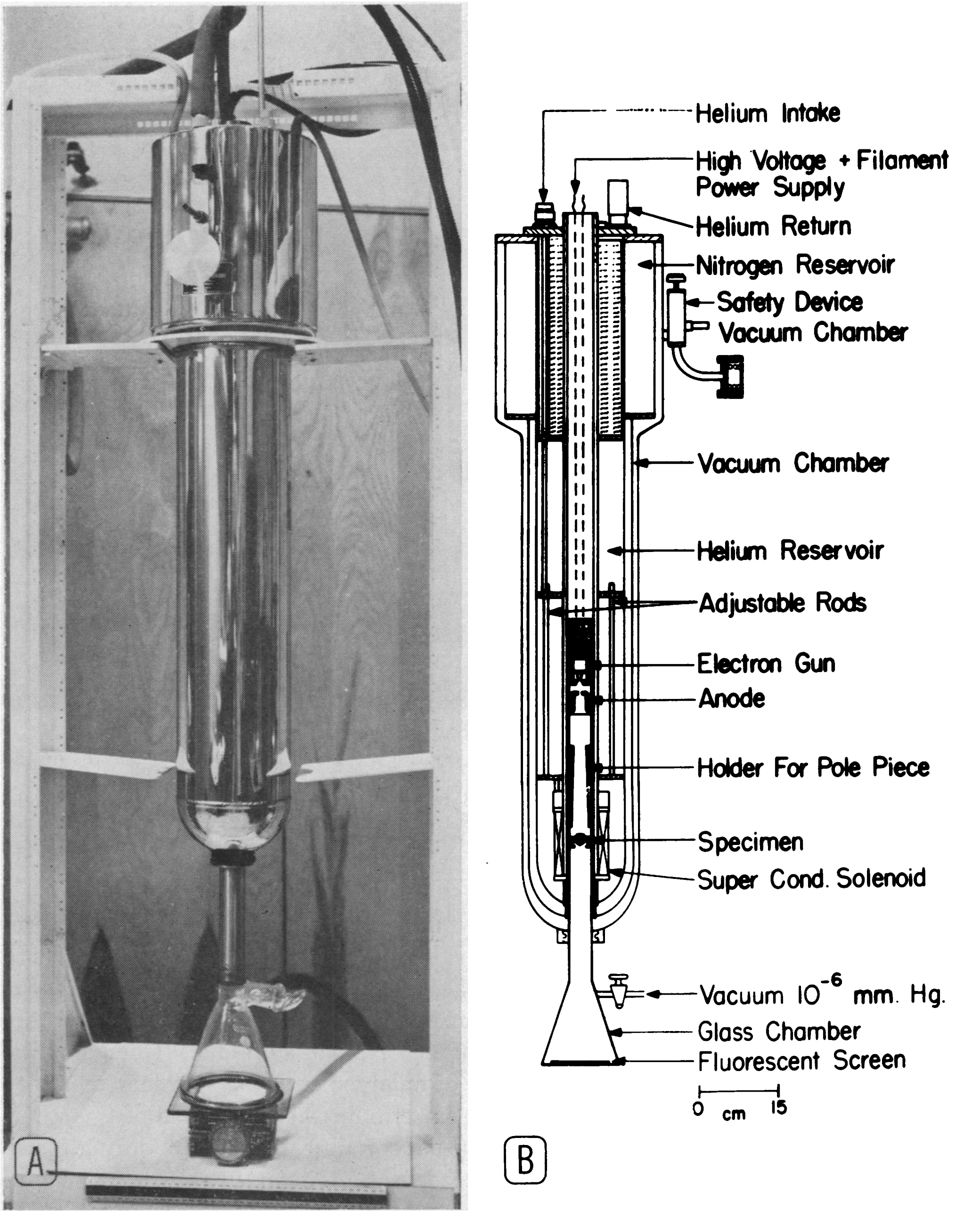

FIG. 1.—(*A*) Photograph, and (*B*) diagram of basic equipment for electron microscopy with high-field superconducting lens, comprising air-core liquid-helium Dewar with superconducting solenoid (operating at 32,200 gauss in persistent current mode), and inserted electron microscope.

mantled in a few minutes and permits a wide variety of experiments, ranging from straightforward shadow electron microscopy, transmission electron microscopy of thin specimens, and electron diffraction. It can also be adapted for use of iron pole pieces of different sizes and configurations.

Results.—The test specimen grid is shown in Figure 2 at 10 × enlargement. The cryo-electron microscope column, with the specimen grid in place, is inserted into the Dewar core, carefully positioned and photographed (Fig. 3) without turning on the

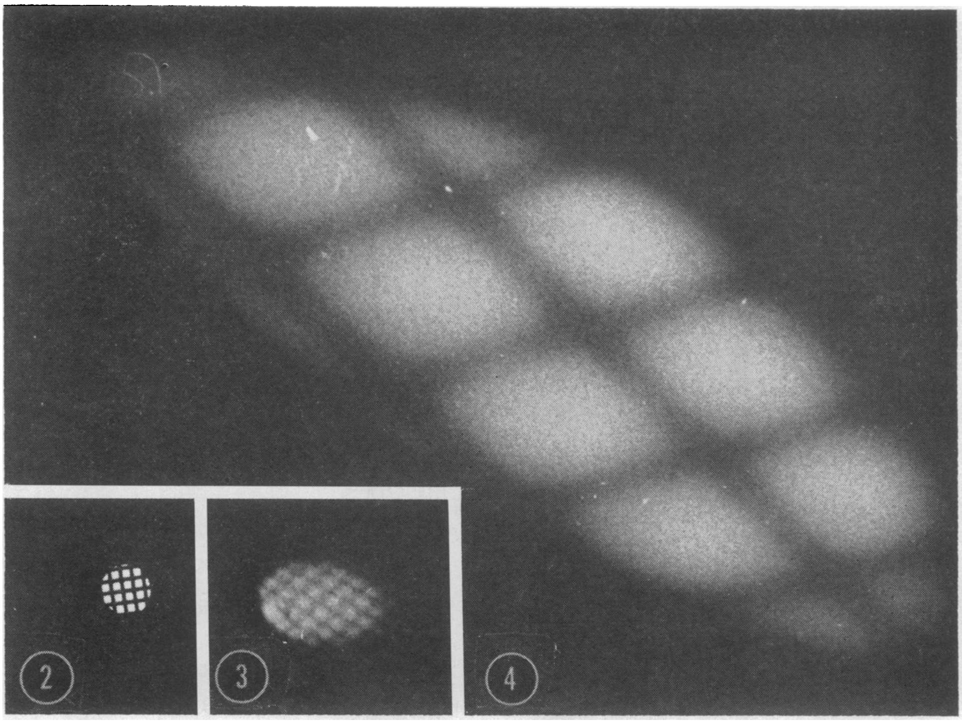

FIG. 2.—Micrograph of copper specimen grid (200-mesh) at 10 × magnification.

FIG. 3.—Same grid photographed externally from fluorescent screen in oblique position within the cryo-electron microscope.

FIG. 4.—Electron micrograph of specimen grid recorded from fluorescent screen with high-field superconducting lens (32,200 gauss in persistent current mode); 4-kv accelerating potential. Original electron optical magnification: 100 ×.

field. The solenoid is gradually energized manually according to standard procedure, and a remarkable series of changes in the image occurs. The image starts to spiral and is successively enlarged (about 5–10 × initially), and comes into focus fleetingly. As the field continues to build up, the image of the specimen grid is further enlarged, with progressive alternation of focusing and spiraling. One gains the impression that the alternating succession of enlargement and spiraling seems to take place by "steps" or "jumps." Movie-film frames were recorded of this interesting phenomenon, which differs from the usual spiraling that occurs during focusing of images in the standard electromagnetic lenses. The nature of these phenomena is being further investigated. They are subject, of course, to the rate of energizing of the coil current and to the field strength parameters. At any time during the process, the coil can be switched into the persistent current mode by short-circuiting the solenoid, and the desired magnification at the lower magnetic fields is obtained. There is an actual focusing control of the superconducting solenoid, essentially similar to that of standard lenses. Moreover, there is a possibility of adjusting the focus by moving the column and the specimen within the air core of the Dewar.

In two of our experiments, the field was driven to the maximum of 32,200 gauss and then held in the persistent current mode for 4–8 hr. Under these conditions, when there was therefore no connection between the magnet and the power supply, the grid squares could be clearly seen as observed with a 12 × binocular microscope in a slightly eccentric position (Fig. 4). The most noteworthy feature was that the individual squares of the grid appeared sharply delimited. The whole image was exceedingly stable with no fluctuations, either in size or position, as determined by visual observation and recorded by photographic exposures of 30 sec to 1 min (Fig. 4). The exposures, taken at 5-min intervals over a period of 3–4 hr, evidenced exceptional stability. This stability stands in contrast to the slight changes which would have occurred over such long periods with standard, highly regulated magnetic lenses at the recorded magnifications of 50–100 times. Longer periods of operation (12–40 hr) in the persistent current mode are also possible with this system. In order to establish the operating conditions of noise-free fields, experiments are being planned with improved high-voltage regulation and direct photographic recording of the electron image.

The instrument can also be used for transmission electron diffraction patterns. Exploratory experiments were carried out by inserting iron pole pieces of conventional design at the appropriate place. When operating at low fields (6–12 kilogauss), the imaging obtained was satisfactory. However, at higher fields, complex imaging effects were observed which remain to be analyzed.

It must be noted that the forces exerted at 32.2 kilogauss were so great that the whole mechanical assembly was affected: the pole pieces were jerked out of their sockets and holding pins, and propelled toward the anode. Also, severe strains were induced which led to cracking of the vacuum joints and consequent vacuum leaks. Although, undoubtedly, provision for greater mechanical strength and rigidity can be made, this would indicate that pole pieces of conventional size, involving rather large iron masses, are not as convenient to use as smaller pole pieces. In fact, this points clearly to the desirability of miniaturizing pole pieces and coils as originally suggested by the author.[9]

Discussion.—Demonstration of the high stability of the electron microscopic images obtained with high-field superconducting lenses is of considerable potential significance in connection with the other unique properties of superconducting magnets. Nuclear magnetic resonance experiments[7] have already clearly demonstrated that large, uniform magnetic fields can be maintained within a 2-cm sphere inside a superconducting solenoid at 47 kilogauss which is homogeneous to better than one part per million. This high degree of spatial homogeneity and time stability is of particular importance in electron microscope lens design, since attempts to obtain highly homogeneous fields in the short pole piece gaps above 10 kilogauss have thus far met with difficulties due to the saturation limit of the iron (about 20–25 kilogauss). With superconducting solenoids of suitable design, and operating in a persistent current mode under appropriate conditions (i.e., minimum external magnetic perturbations), it should be possible to obtain high spatial homogeneity and time stability of an unprecedented degree, at high fields of the order of 40–80 kilogauss. This in itself should allow the production of "stronger" electromagnetic lenses which would have a shorter focal length, a wider tolerance range for the voltage and current fluctuations, and improvement in the limiting aberration factors.[1–3] It is feasible to consider the design of miniature lenses using very fine niobium-zirconium or niobium-tin wire or even ultrathin films of these alloys evaporated on suitable substrates. Moreover, by using appropriately designed objective lens pole pieces of the rare earth metals dysprosium, holmium, or erbium[11] instead of the standard iron pole pieces, it should be possible to obtain stronger objective lenses of much shorter focal length and generally improved characteristics, if the miniature coil approach does not prove feasible. Dysprosium and the above-mentioned rare earth metals have a higher saturation value (about 40 kilogauss) at liquid helium temperatures than iron. Finally, there are numerous additional possibilities of forming and compensating asymmetries in the superconducting magnetic field by the use of shimming coils and related devices.

As described previously,[9] the desirability of examining specimens at liquid helium temperatures would represent in itself a major advantage in pursuing this experimental approach. Thus, by development of the concepts embodied in our low-temperature electron microscopy work,[8, 10] it is possible to design a new type of miniaturized high-resolution electron microscope totally immersed in liquid helium which makes use of these completely stable superconducting lenses, including single-crystal pointed filaments and other distinctive features. These "cryo-electron microscopes" operating at temperatures of 1–4°K would embody the following significant features: (*a*) highly stable superconducting electromagnetic lenses (20–60 kilogauss) with noise-free magnetic fields of a persistent current in the optimum case; (*b*) operation in the ultrahigh cryogenic vacuum at liquid helium temperatures resulting in decisive advantages of minimized specimen contamination, specimen damage, and thermal noise; (*c*) optimum conditions for both low-voltage (i.e., 1–10 kV) and high-voltage electron microscopy. In addition, the use of high-efficiency image viewing (single-crystal fluorescent screens), electronic image intensifiers, and recording devices which operate at optimum low temperatures would make it possible to use high-speed cinematography and stroboscopic recording (e.g., obtained through pulsed T-F emission from pointed filaments) for attainment of high temporal resolution combined with high spatial resolution. In principle, such a "cryo-electron microscope" would also be an ideal device for controlled application of electron microbeams (*ca.* 50–500 Å diameter) of precisely defined intensity and duration for ultraminiaturization, storage of information, and in general, for controlled observation, irradiation, and manipulation of hydrated biological systems at the molecular level under conditions of minimum perturbation. An instrument of this type is being developed at our laboratories in the University of Chicago, as part of a comprehensive research program in the field of low-temperature electron microscopy. The described simple microscope design is well-suited as an electron optical bench which would enable us to make the necessary preliminary tests using superconducting magnetic fields with a minimum of manipulations at room temperature. It is also a simple tool for basic research of the electron optical phenomena encountered in the new domain of superconducting high magnetic fields, providing striking visual demonstration of their properties. This work will be reported in greater detail in subsequent papers.

Summary.—In a series of experiments with a simple electron microscope without pole pieces, using high-field superconducting niobium-zirconium solenoid lenses in an open-air-core, liquid-helium Dewar, electron microscopic images of test specimens have been recorded while operating at 32,200 kilogauss in a persistent current mode, with accelerating potentials of 4–8 kV. These preliminary experiments have dem-

onstrated the exceptional stability of the images (both short-term and long-term) over a period of 4–8 hr, and the relatively high quality of the images at magnifications of 50–100 ×. The results obtained with this experimental approach and some interesting observations bearing on imaging phenomena with superconducting solenoids have been described here.

I gratefully acknowledge the expert technical assistance of Mr. Louis P. Ouwerkerk, Mr. R. J. Szara, and Dr. K. Yada in the course of these experiments. I also wish to thank Prof. L. Meyer and his colleagues of the Low-Temperature Laboratory, and Prof. W. Bloom of the University of Chicago for valuable suggestions. I am greatly indebted to Prof. W. H. Sweet, Massachusetts General Hospital, Dr. S. Autler, Prof. S. C. Collins, and Prof. F. O. Schmitt of Massachusetts Institute of Technology for their early interest and encouragement in this project since 1961. Sincere thanks are also due to Mr. C. L. Hough, Mr. R. Luftig, Miss L. LaDeur, Miss J. Richardson, Miss A. Hollinger, and other associates from our laboratory. I am also indebted to Mr. J. Hanáček, Mr. J. Costa, and other members of the workshop for their valuable assistance. Sincere appreciation is due Mr. F. Lins, Mr. C. L. Berrington, and other members of the Westinghouse Cryogenics Systems Department for their kind help.

* This work was in part supported by U.S. Atomic Energy Commission contract AT (30-1)-2278, by grants B-2460, C-3174, and NB-04267 from the National Institutes of Health, and by NASA grant NsG 441-63 from the National Aeronautics and Space Administration, and by the L. Block Fund of the University of Chicago.

[1] Ruska, E., in *Proceedings Fifth International Congress for Electron Microscopy*, ed. S. Breese (New York: Academic Press 1962), vol. 1, A-1.

[2] Leisegang, S., in *Encyclopedia of Physics* (Berlin: Springer, 1955), vol. 33, p. 396.

[3] Haine, M. E., and V. E. Cosslett, *The Electron Microscope* (New York: Interscience Publishers, Inc., 1961).

[4] Autler, S. H., in *High Magnetic Fields*, ed. H. Kolm *et al.* (M.I.T. Press and J. Wiley & Sons, Inc., 1962), p. 324.

[5] Kunzler, J. E., *Rev. Mod. Phys.*, **33**, 1 (1961).

[6] Hulm, J. K., M. J. Fraser, H. Riemersma, A. J. Venturiano, and R. E. Wien, in *High Magnetic Fields*, ed. H. Kolm *et al.* (M.I.T. Press and J. Wiley & Sons, Inc., 1962), p. 332.

[7] Marshall, H. L., and H. E. Weaver, *J. Appl. Phys.*, **34**, 3175 (1963).

[8] Fernández-Morán, H., *Ann. N. Y. Acad. Sci.*, **85**, 689 (1960).

[9] Fernández-Morán, H., *J. Roy. Microscop. Soc.*, **83**, 183 (1964).

[10] Fernández-Morán, H., *Circulation*, **26**, 1039 (1962).

[11] Henry, W. E., in *High Magnetic Fields*, ed. H. Kolm *et al.* (M.I.T. Press and J. Wiley & Sons, Inc., 1962), p. 324.

---

*ISOLATION AND CHARACTERIZATION OF RECOMBINATION-DEFICIENT MUTANTS OF ESCHERICHIA COLI K12**

BY ALVIN J. CLARK AND ANN DEE MARGULIES†

DEPARTMENT OF BACTERIOLOGY, UNIVERSITY OF CALIFORNIA, BERKELEY

Communicated by Michael Doudoroff, December 30, 1964

Certain features of the process of genetic recombination at the molecular level have recently become evident: (1) Recombination in bacteria and viruses involves the physical interaction of and subsequent inheritance by recombinant progeny of double-stranded elements of DNA derived from two parents.[1–5] (2) The unreplicated recombinant DNA may contain a double-stranded region in which