Table 1. Atomic coordinates for azulene.

Atom	x/a	y/b	z/c
C_1	0.033_5	0.09	0.760
C_2	0.134_5	0.29	0.819
C_3	0.153	0.45	0.700
C_4	0.039	0.47	0.387
C_5	-0.060	0.41	0.212
C_6	-0.136	0.21	0.186
C_7	-0.171_5	0.03	0.281
C_8	-0.106	0.02	0.462
C_9	-0.009_5	0.15	0.581
C_{10}	0.060	0.35	0.530

to the (010) plane. The general scheme of the crystal structure is similar to that of naphthalene (*4*). The shortest distance between atoms in neighboring molecules is 3.6_1 A. Details of the molecular configuration require a three-dimensional analysis. This is in progress.

Y. TAKEUCHI*
R. PEPINSKY
X-Ray and Crystal Analysis Laboratory, Department of Physics, Pennsylvania State University, University Park

References and Notes

1. H. Günthard, Pl. A. Plattner, E. Brandenberger, *Structure Reports* 11, 713 (1948).
2. H. Günthard, *ibid.* 13, 562 (1950).
3. This analysis is supported by grant A-228 from the National Institutes of Health. X-RAC and S-FAC calculations are supported by contract No. N6-onr-269, T. O. 16 with the Office of Naval Research. We are indebted to Pl. A. Plattner of Hoffman-La Roche in Basel for introducing us to the problem and furnishing crystalline material, to V. Vand for helpful discussions, and to Mrs. J. W. Turley for IBM computation of structure factors.
4. J. M. Robertson, *Proc. Roy. Soc.* (*London*) *A* 140, 79 (1933) and 142, 674 (1933).
* On leave from Mineralogical Institute, Department of Science, University of Tokyo, Hongo, Tokyo, Japan.

9 February 1956

International Relations in Science and Problems of Visas

In this brief report I wish to relate factually the events of the failure to bring to the United States a distinguished French astronomer for a conference 3–5 April 1956 that was sponsored by the National Science Foundation and the Leander McCormick Observatory of the University of Virginia.

On 3 May 1955, the steering committee of the conference drew up a list of twenty specialists in the field of cosmic distance determination. Daniel Chalonge of the Institut d'Astrophysique, Paris, France, eminently qualified in this field, was included in the list. Since Chalonge on a previous occasion had had difficulties in obtaining a visa, it was thought advisable to make inquiries with the State Department before inviting him. This was done through the Office of the Division of International Relations of the National Academy–National Research Council, the channel through which scientists handle matters of this type. In a letter dated 21 June 1955 to the division, I stated, "Before inviting Dr. Chalonge to take part in this conference I am anxious to learn the attitude of the State Department in view of the past history of the case. I do not wish to embarass him, our government nor ourselves. I understand the difficulties of the situation. We may be told that the case cannot be considered until he applies for a visa but I am afraid this will not solve the problem." The letter also included a long paragraph relating to the history of the previous failure as far as known to me. Early in August 1955, while I was in Oslo attending a meeting of the International Council of Scientific Unions, I was informed verbally by a member of the staff of the Science Advisor to the State Department, and in the presence of the other members of the American delegation, that on the basis of information he had received, I should proceed to invite Chalonge. With this assurance we extended the invitation late in August 1955, during the meeting of the International Astronomical Union in Dublin, Ireland.

I know that Chalonge spent much time during the succeeding months preparing for his visit to the United States, which was to include colloquium lectures at eight astronomical centers in the eastern United States following the Virginia conference. I understand that Madame Chalonge visited the U.S. Consulate in December 1955 and that she was informed that they had plenty of time for application for the visa. In January 1956 they applied and planned to sail on 22 March. Since by the middle of February they had received no reply, I wrote to the office of the Science Advisor on 22 February. The reply stated "Apparently Dr. Chalonge applied for his visa only recently. If he had followed the suggestion that I passed along to you, and you to him, last August of applying for his visa promptly, he probably would not have had any current worries." Here some misunderstanding must have occurred, for I do not recall being instructed of "prompt application," and the Paris Consulate did not indicate its need. On 2 March, I telephoned the Office of International Relations of the Academy and after they had consulted the State Department, they informed me that there were no complications and that the visa would be issued. On 12 March I telephoned again and I was told that the matter was being taken up with the Attorney General and that it would take a week or at the most 10 days for processing the case but I could rest assured that the action would be favorable. I advised Chalonge accordingly and suggested that he change the time of his departure to 29 March which I knew beforehand it was possible to do.

On the evening of 28 March, the day before his planned sailing, the U.S. Consulate informed him that since he is a member of the French-USSR Cultural Society, which under the American law is considered a communistic group, his visa could not be issued for more than three days, the duration of the conference. Under these conditions Chalonge refused the visa and wrote as follows.

"Dans ces conditions, je n'ai pas cru pouvoir accepter le visa car il était un peu humiliant pour moi d'être ainsi sous le contrôle de la police, comme un malfaiteur.

"J'ai pris cette décision avec beaucoup de peine en pensant aux efforts qu'ont fait mes amis américains pour me faire venir."

It is unfortunate that Chalonge felt that his limited visa implied police control. To be notified only at the very last moment of departure and be told that he is permitted to stay in the United States for 3 days only seems most unreasonable.

All this is, of course, regrettable for all parties concerned, including the State Department and the Attorney General's office.

In conclusion, I wish to emphasize that the aim of this report is to give all the facts as I know them with the hope that they might contribute in remedying a situation which is detrimental to science and our international relations.

Last August at the International Astronomical Union Meeting in Dublin, the American Delegation was authorized to extend, on behalf of the U.S. Government, an invitation to the union to hold its 1961 General Assembly in the United States. If such a meeting takes place, some 400 foreign astronomers may be coming. It seems obvious that under existing conditions careful consideration of this problem is needed, and before our General Assembly in 1958.

J. J. NASSAU
Warner and Swasey Observatory, Case Institute of Technology

22 June 1956

Neoplastic Changes Developing in Epithelial Cell Lines Derived from Normal Persons

That animal fibroblasts grown continuously in tissue culture can develop the ability to produce tumors considered histopathologically as sarcomas has been demonstrated on numerous occasions (*1*). The present report concerns the acquisition of a similar ability by four strains of human epithelial cells which

have been developed by R. S. Chang (*2*) from normal liver, kidney, and conjunctiva. Two of the cell lines, liver and conjunctiva, had been carried in our laboratory in a medium containing bovine amniotic fluid and human serum, and two, kidney and conjunctiva, had been carried in the laboratories of Coriell and McAllister at the South Jersey Medical Research Foundation in Eagle's synthetic medium to which horse serum had been added. The latter two cell lines were used only for implantation experiments in irradiated and cortisone-treated rats and human volunteers.

Our reasons for stating that the liver, kidney, and conjunctiva cell lines, after more than 50 passages in tissue culture, have developed characteristics which are commonly associated with neoplasia (here defined as the ability to produce a new growth) are as follows.

1) *Cytological observations.* Stained preparations when compared with the thirteenth passage from Chang, which he kindly provided to us, showed the following differences. The pavementlike characteristic arrangement of epithelium was disrupted, and although the cells continued to grow in sheets, the cell edges were less closely approximated. The cells varied in size and shape, the nuclei were often large, and the cytoplasm stained unevenly. There was great variety in the size, shape, and number of nucleoli, which were often drawn out into odd shapes. The centrosome, which was evident in our cell lines, seemed more prominent in the conjunctival cultures where it often indented the nucleus. There were occasional multinucleated cells and tripolar mitoses (Fig. 1) (*3*).

2) *Chromosome studies.* Examination of the chromosomes by Albert Levan of the Institute of Genetics, Lund, Sweden, revealed unequivocal abnormalities. Although tissue-culture preparations of normal adult human fibroblasts from our laboratory showed the usual 48 chromosomes and the idiogram was similar to one described by Hsu (*4*) for human cells (presumably fibroblasts), the findings in the conjunctiva and liver lines were quite different. Both had high chromosome numbers (72 to 80), and the majority of the cells were hypertriploid and had idiograms which differed from the normal in the distribution of chromosome types.

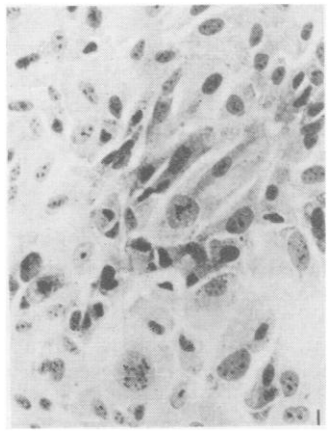

Fig. 1. Tissue culture preparation from the 62nd passage of liver cells (Chang). May-Grünwald stain. × 100.

3) *Inoculation into prepared rats.* It has been amply proved by immunological, chromosomal, and human implantation experiments that it is possible to grow human tissues, both tumors and embryonic tissues, in irradiated and cortisone-treated weanling rats. When 1 to 5 million cells of either the liver, conjunctiva, or kidney (including the strains from Coriell and McAllister's laboratory) were inoculated subcutaneously into the prepared rats, nodules became palpable in 1 week and continued to increase in size until removed for histological sections 2 weeks after implantation. Tumors diagnosed microscopically as malignant appeared in 12 of the 19 animals inoculated. In two instances, the tumor cells were regrown in tissue culture and studies of the chromosomes verified their human origin. Cells inoculated into comparable nonirradiated, untreated rats showed only reaction tissue. Human adult fibroblasts grown in tissue culture from many different types of tissue, both cancerous and normal, did not produce tumors when they were inoculated into prepared rats on 24 different occasions. Human embryonic fibroblasts inoculated three times similarly failed to produce tumors.

4) *Human implantation.* One to 5 million cells of all four cell lines have been implanted subcutaneously into the forearm of volunteers with far advanced cancer. In all there was an immediate transient erythema and swelling due to associated trauma which disappeared the following day. In eight of 11 implantations, a nodule became palpable in 7 days and was removed for section in 9 to 14 days. Two individuals who developed tumors in one forearm failed to develop a tumor in the opposite forearm which had been implanted with 1.5 million human embryonic fibroblasts. There have been no regrowths at the implantation site following excisional biopsy. In each instance, an equal number of similar cells was inoculated into prepared weanling rats and a similar result was produced.

5) *Histopathological diagnosis.* The tumors (Fig. 2) which arose in the treated rats and human beings are described as follows. Examination of the cells grown in rat and man revealed no specific histologic features which would enable one to distinguish the conjunctival from the hepatic cells or to determine the origin of either of these cell types. Furthermore, the patterns of growth in the rat and in man were essentially the same. The cells, whether conjunctival or hepatic, were generally rounded and uniform in appearance. Relatively slight variations in size and shape of nuclei were observed. However, the hyperchromatism and the irregular coarse chromatin distribution was of the type one associates with cancer cells. The cells were distributed haphazardly in the subcutaneous fibrous tissue and fat, in clumps and small sheets, or occasionally in rows. Inflammatory cells, which were sometimes noted, could easily be distinguished from the transplanted cells. The pattern of growth sometimes resembled that of human reticulum cell sarcoma. In areas where there were moderate amounts of fibrous tissue surrounding the cells, the pattern was strongly reminiscent of human breast cancer which has metastasized to skin.

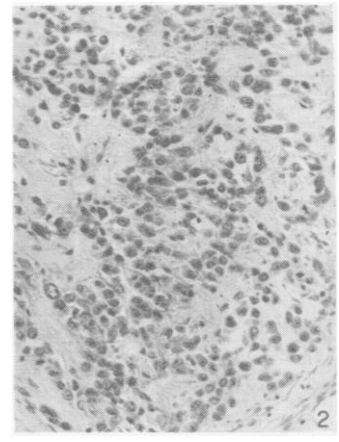

Fig. 2. Neoplasm which formed 9 days after the subcutaneous implantation into a human volunteer of liver cells transferred in tissue culture 63 times. Hematoxylin and eosin stain. × 90.

It must be emphasized that the changes enumerated here have not proved that these cells produce cancer in the clinical sense. It is not known whether they would continue to grow, whether metastasis would occur, or, indeed, whether they would grow in normal human beings. Our basis for saying that the cells are no longer normal rests on their ability to produce tumors in specially treated rats and in human beings, which, when examined microscopically, have the appearance of malignant tumors.

Addendum. Since the preparation of this report, other investigators have noted difficulty in distinguishing "normal" cell liver (Chang) from "tumor" cell lines. Fennell has noted no difference between them when they were examined by the exfoliative cytological technique used clinically, and Leighton, Kline *et al.* have pointed out that

Chang's conjunctiva and Henle's intestine 407, both derived from normal individuals, are indistinguishable cytologically from known tumor cell lines (*5*).

A. E. MOORE
C. M. SOUTHAM
S. S. STERNBERG

Division of Experimental Pathology, Sloan-Kettering Institute for Cancer Research, New York

References and Notes

1. K. K. Sanford, G. D. Likely, W. R. Earle, *J. Natl. Cancer Inst.* **15**, 215 (1954); G. O. Gey *et al., Acta Unio Intern. contra Cancrum* **6**, 706 (1948).
2. R. S. Chang, *Proc. Soc. Exptl Biol. Med.* **87**, 440 (1954).
3. We wish to thank John Biesele for his help in analyzing the cytological picture.
4. T. C. Hsu, *J. Heredity* **43**, 167 (1952).
5. These findings were presented by the authors named at the Tissue Culture Association Conference in Milwaukee, Wis., 3–4 Apr. 1956. Abstracts of the papers can be found in *Anat. Record* **124**, No. 2 (Feb. 1956).

15 March 1956

X-ray Microscopy of Veins of the Skull

The projection x-ray microscope at the University of Redlands is being successfully applied to the study of the distribution and communications of the veins of the diploë in the dog, an x-ray micrograph of which is shown in Fig. 1.

This type of x-ray microscope uses a point source of x-rays less than 1 μ in diameter to cast an enlarged shadow image on a photographic plate. The small source of x-rays is produced by focusing a beam of electrons with two magnetic lenses so that the point source of electrons strikes a window target in the vacuum wall of the x-ray tube. The specimen is kept at atmospheric pressure and yet can be placed within a few microns of the source of x-rays, so that high x-ray magnification can be obtained with the photographic plate or fluorescent screen only a few millimeters away.

The penetrating power of x-rays produces contrast in the image from internal detail of thick specimens, both from natural density differences and from injected radio-opaque material. In addition, the shadow-projection method of x-ray image formation produces an image of all planes of the specimen in focus at once. This makes possible stereoscopic views of the internal detail of a specimen, whereby the orientation within the object is shown in a three-dimensional relationship. A recent review article on x-ray microscopy compares all methods used and lists 102 references to the literature on the subject (*1*). Specific details of design and operation of x-ray microscopes can be found therein.

The veins of the diploë are difficult to expose and to visualize by the usual methods of serial sectioning and graphic reconstruction. Projection x-ray microscopy (*2*) that has been recently developed by Nixon and Cosslett (*3*) over comes many of these difficulties.

To prepare the skull of each dog for study after the animals were sacrificed, the head was perfused through the carotid arteries with physiologic saline to flush the blood from all blood vessels. This was followed by perfusion with red vinyl plastic. To prevent confusion in observing arterioles and venuoles, blue vinyl plastic was injected into the veins by way of the external jugulars. The soft tissues were cut away from the skull, and the floor of the skull was opened to allow for removal of the brain. This procedure allowed the dura mater to remain intact and adherent to the brain case.

Fixation of the skull was achieved by placing it in 10-percent formalin. The skull was mapped into parts and cut, each part being about 1 in.^{2} The skull parts were decalcified by using 5-percent nitric acid and were dehydrated by being passed through 15-percent, 40-percent, and 75-percent isopropyl alcohol solutions. The parts were kept in 75-percent isopropyl alcohol until they were x-ray photographed.

Figure 1 shows the plastic-filled veins of the diploë of the dog in longitudinal parallel formation as they lie between the inner and outer tables of the skull. The x-ray micrograph demonstrates the communications of the smaller diploic veins as branches of the large diploic vein at the top of the micrograph as they approach the center of the skull and connect with the superior sagittal sinus of the dura mater (not shown). In the background, trabeculae, which make up the porous structure of the diploë, appear as light lines, whereas spaces caused by osteoclastic activity are dark.

Fig. 1. X-ray micrograph showing diploic veins of the dog skull. Five-minute exposure on Eastman lantern plate contrast emulsion; 12-kv, 25-μa beam current; University of Redlands x-ray microscope (× 3.5).

The normal methods of preparation for study and graphic representation of this tissue by light microscopy would have taken at least 25 hours, whereas the same information has been obtained with a single 5-minute x-ray micrograph. A detailed study of the entire calvarium of the dog by light microscopy would take several months, but the same information could be obtained in a few 5-minute exposures using the x-ray microscope.

Future studies are proposed, which will include x-ray micrographic representation of the veins of the diploë in skulls of man and monkey. From the observations made thus far, there appears to be a characteristic difference between the distribution of diploic veins in the skull of the dog and that of man.

Other applications of the x-ray microscope have been made with striking results. Engstrom, Bellman, and Engfeldt (*4*) have used contact microradiography on living tissues. Bohatirchuk (*5*) was successful in studying the aging of the vertebral column with this method of microradiography. X-ray micrographs have been made of the kidney by Nixon and studies of gallstones and kidney stones have been forecast.

C. GORDON HEWES
Department of Anatomy, College of Medical Evangelists, Loma Linda, California

W. C. NIXON
Cavendish Laboratory, Cambridge University, Cambridge, England

ALBERT V. BAEZ
Department of Physics, University of Redlands, Redlands, California

OTTO F. KAMPMEIER
Department of Anatomy, College of Medical Evangelists, Loma Linda, California

References and Notes

1. W. C. Nixon, *Research (London)* **8**, 473 (1955).
2. V. E. Cosslett and W. C. Nixon, *Nature* **168**, 24 (1951).
3. W. C. Nixon and V. E. Cosslett, *Brit. J. Radiol.* **28**, 532 (1955).
4. A. Engstrom, S. Bellman, B. Engfeldt, *ibid* **28**, 517 (1955).
5. F. Bohatirchuk, *ibid.* **28**, 389 (1955).
6. This work was done while W. C. Nixon was visiting research associate at the University of Redlands under a National Science Foundation grant.

3 April 1956

We must never forget that metaphysics divides people, and science unites them.
—PHILIPP FRANK.