experiment has been shown by Olcott *et al.*[6] to be essentially nontoxic for rats when given over a period of 18 days. This dose is 10 times the minimal effective dose for the treatment of acute arsenical poisoning in cats. Since the mechanism of cobalt-induced polycythemia is not known, little can be said concerning the apparent ineffectiveness of BAL to prevent it in rats under the conditions of this experiment. The toxicity of BAL seems to be relatively minimal since administration over a period of 3½ months produced little effect other than slightly retarding weight gain. This experiment indicates that a comparative study of the effect of radioarsenic (As^{76}) on cobalt-induced polycythemia in rats with or without BAL injection is feasible.

Conclusions. BAL in a dose of 0.2 mM/kg given 3 times weekly fails to prevent, cure, or materially alter cobalt-induced polycythemia in rats. The general toxicity of BAL appears minimal when given in this dosage over a 3½-month period.

6 Olcott, C. T., and Riker, W. F., *Science*, 1947, **105**, 67.

16624 P

A Fibrillar Structure in Rat Fibroblasts as Seen by Electron Microscopy.

FREDERIK B. BANG AND GEORGE O. GEY.

*From the Departments of Medicine and Surgery, Johns Hopkins University School of Medicine, Baltimore, Md.**

During the course of comparative tissue culture studies of a strain of normal rat fibroblasts and its malignant cell derivative,[1] we obtained preparations of both of these cell types which under the electron microscope† showed an unmistakable fibrillar structure in the thinly spread cytoplasm. The fibrils, of an estimated thickness of 10 to 100 $m\mu$, may converge and diverge in fan-like formations of great regularity (Fig. 1) but are more often seen gathered together in long bands of varying widths (Fig. 2). The composite bands are seen clearly in phase microscope movies of the cells,[3] but it is only by the increased resolution available with the electron microscope that the nature of the bands becomes clear. They are apparently composed of long thin converging fibrils which in general radiate from the dense central area. That the fibrils are not produced by a wrinkling of the formvar membrane on which the cell is stretched is indicated by their uniformity, by their sharp change in direction in certain areas of the periphery, and by their fine converging and diverging structure. Besides this, no wrinkles were seen in the membrane outside of the cellular area where clear visualization is possible.

Of interest are the relations of these bands to the general structure. The mitochondria, often long and thin, seem frequently to be associated with individual fat droplets. Some of the elongated mitochondria are found in areas which show many microsomes, with the

* Supported in part by a grant-in-aid from the U. S. Public Health Service.

† Our technic of study of tissue cultured cells is essentially that of Porter[2] with the exception that fixation and staining has been limited to 10 minutes exposure to osmic acid vapors.

We are indebted to Warren A. Hovis for constant careful technical assistance in the preparation of the slides and photographs.

1 Gey, G. O., *Cancer Research*, 1941, **1**, 737 (abs.); Firor, W. M., and Gey, G. O., *Ann. Surg.*, 1945, **121**, 700.

2 Porter, K. R., Claude, A., and Fullam, E. F., *J. Exp. Med.*, 1945, **81**, 233.

3 Gey, G. O., Gey, M. K., Firor, W. M., and Self, W. O., *Acta Union Internationale Contre le Cancer*, 1948, in press.

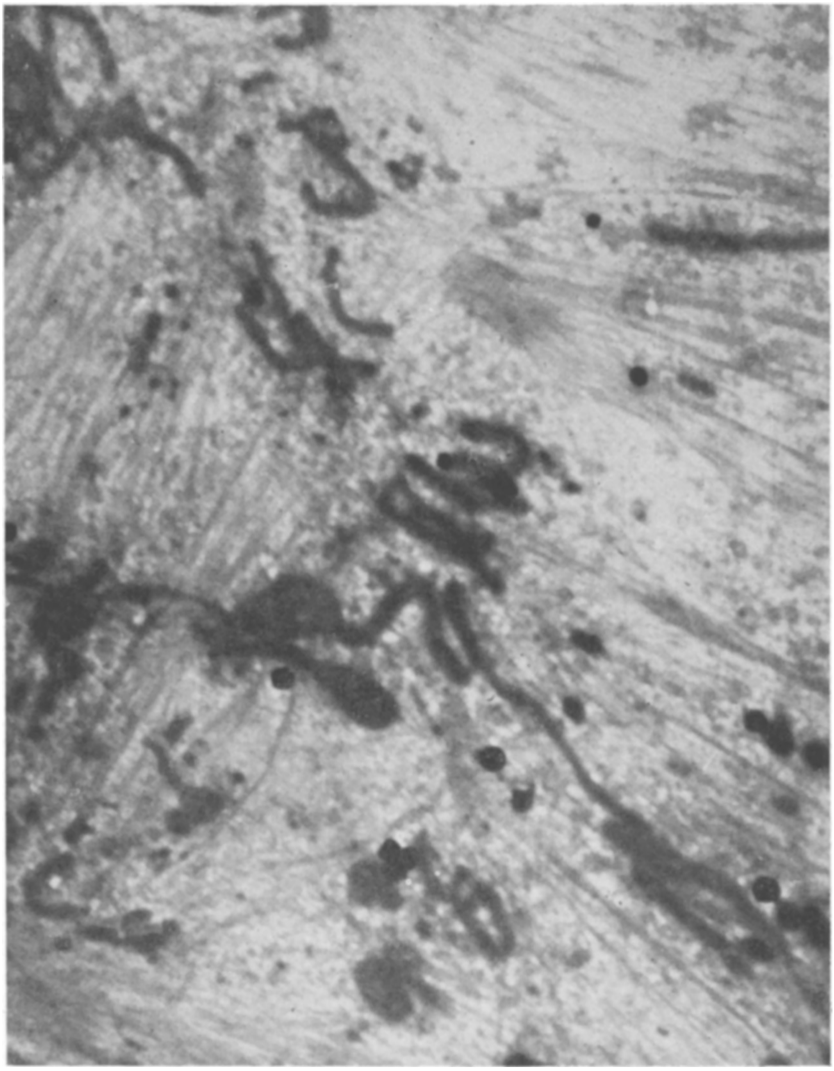

FIG. 1 × 6525.
Large masses of sausage shaped mitochondria are scattered over entire field. Smaller black round bodies are fat droplets. Fibrous structure may be seen criss-crossing throughout picture.

whole area bounded by parallel fibrous bands. This appears to be a static representation of the streams of material which can be seen in the movies of these cells. In other areas, the great masses of mitochondria may be seen crossing those fibrous bands.

This fibrillar structure when present is clear and definite but is not present in every cell and thus may well represent a phase in either the growth or migration of the cells. Such structures have not previously been described in electron microscope studies of similar cells[4,5‡] and many types of cells will have to be studied before their general significance can be established.

Although the above findings cannot in

4 Porter, K. R., and Thomson, H. P., *Cancer Research*, 1947, **7**, 431.

5 Claude, A., Porter, K. R., and Pickels, E. G., *Cancer Research*, 1947, **7**, 421.

‡ Porter has since stated that the "cytoplasm may show a number of fine fibrils which seem to be differentiations of an otherwise homogeneous cytoplasm," *Anat. Rec.*, 1948, **100**, 72.

FIG. 2.
Parallel fibrous bands enclosing mitochondria and fat droplets in bed of ground substance.

themselves establish the presence of these fibrils in living cells, electron microscope studies have previously demonstrated fibrillar structure in specialized components of the cytoplasm such as the axons of nerve cells[6] and the tails of sperm cells.[7]

Furthermore, it has been supposed for some time that cytoplasm may have a basic fibrillar structure.[8] This is based on the demonstration of the elasticity of protoplasm (both to externally applied tensions and to internally displaced foreign bodies) and on the viscosity of the cytoplasm. Monné believes that the studies using the polarization microscope "prove that the ground cytoplasm of normal living cells has a fibrillar structure".[9]

The motility of *Amoeba proteus* has been

6 DeRobertis, E., and Schmitt, F. O., *J. Cell. and Comp. Phys.*, 1948, **38**, 1.

7 Schmitt, F. O., In Advances in Protein Chemistry I., New York, Academic Press, 1944.

8 Frey-Wyssing, A., Submicroscopic Morphology of Protoplasm and Its Derivatives, New York, Elsevier Publishing Co., 1948.

9 Monné, L., In Advances in Enzymology VIII., New York, Interscience Publishers, 1948.

formulated in terms of a continuously changing sol-gel system (Mast)[10] and this concept has been further developed and applied by Lewis[11] to rat fibroblasts. Since gels probably acquire their rigidity from a structure of interwoven fibers, this does not basically differ from the theory of the fibrillar structure of cytoplasm.

10 Mast, S. O., *Protoplasm*, 1931, **14**, 321.

11 Lewis, W. H., In the Structure of Protoplasm. A Monograph of the Am. Soc. of Plan Phys., Iowa State College Press, 1942.

16625 P

The Structure of Unstained Reticulocytes.

GEORGE BRECHER. (Introduced by K. M. Endicott.)

From the Pathology Laboratory, Experimental Biology and Medicine Institute, National Institute of Health, U. S. Public Health Service, Bethesda, Md.

Brilliant cresyl blue and certain other basic dyes, if applied to fluid blood, bring out a reticulum in young red blood cells. This reticulum fails to appear if brilliant cresyl blue or other basic dyes are applied to dried and alcohol fixed blood smears. Moreover, no reticular structure could hitherto be demonstrated in the young red blood cells by microscopic examination of unstained blood with direct or oblique illumination, dark field illumination or in ultraviolet light.[1]

While examining unstained wet blood films of albino mice with the phase microscope, we found that an occasional red blood cell showed a very delicate, hardly discernible reticulum. The cells showing this reticulum were fewer in number than the reticulocytes demonstrable by supravital staining with brilliant cresyl blue. However, when certain hypotonic salt solutions, preferably potassium oxalate 0.8% or ammonium oxalate 1.2% were mixed with the fresh blood, a larger number of cells, corresponding to the percentage of reticulocytes, showed distinct granules and short rods within their cytoplasm.

Most of these rods and granules showed prominent Brownian movement. In such preparation, varying number of cells showed marked loss of hemoglobin. Granules predominated in hemolyzed cells, while rods were more prominent in cells containing ample hemoglobin. The percentage of cells showing rods or granules was higher in those areas of the preparation in which hemolysis was marked. Smears, dried and fixed in alcohol, failed to show these cytoplasmic structures. However, dried but unfixed smears, if mounted in 10% formalin or 1.2% ammonium oxalate, (Fig. 1), showed rods and granules resembling those seen in the fresh preparations. Also, fixation of dried smears in 3% aqueous potassium dichromate did not interfere with the demonstration of the rods and granules. It is noteworthy that both formalin and dichromate applied to the dried smear hemolyzed the red blood cells. Formalin added to fresh blood

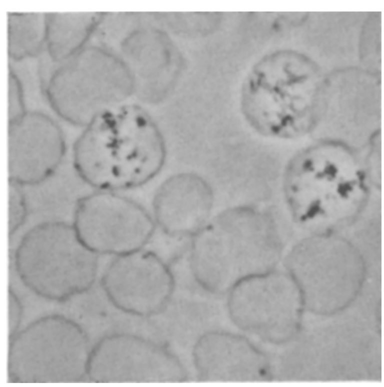

FIG. 1.
Phase illumination of dried film of mouse blood mounted in 1.2% ammonium oxalate.

1 Isaacs, R., in Downey, Handbook of Hematology (New York: Paul Hoeber), 1938, **1**, 16.