for their advice and assistance in the electrochromatographic and acid-evaporation technics. We are also indebted to S. A. Tyler for advice on statistical treatment of the data.

1. Causey, G., and Harris, E. J., *Biochem. J.*, 1951, v49, 176.

2. Rouser, G., and Neuman, W. F., *Fed. Proc.*, 1952, v11, 278.

3. Sato, T. R., Kisieleski, W. E., Norris, W. P., and Strain, H. H., *Anal. Chem.*, 1953, v25, 438.

4. Sato, T. R., Kisieleski, W. E., Norris, W. P., Smetana, F., and Strain, H. H., manuscript in preparation.

Received December 15, 1955. P.S.E.B.M., 1956, v92.

## A Strain of Unclassified Cells Isolated from Lymph Node of Patient with "Reticulo-Endotheliosis".* (22380)

SAMUEL D. BELL, JR. AND ROBERT E. JOHNSON. (Introduced by John C. Snyder.)
(With the technical assistance of Barbara Neuman.)
*Department of Microbiology, Harvard School of Public Health, Boston, Mass.*

Strains of human cells *in vitro* have been found useful in cytologic and virus investigations; interest has centered mainly on epithelial-like cells either of carcinomatous origin(1,2) or from normal tissues(3), although it is well known that human fibroblasts can be subcultured for long periods of time(4). The present report describes a strain of cells derived from a lymph node biopsy of a patient with the clinical diagnosis of "reticulo-endotheliosis" and now subcultivated for 20 passages (7 months).

*Clinical Data.* The patient from whom this strain of cells was isolated, a 50-year-old male, noted in 1943 the gradual onset of a generalized reddish-brown, maculo-papular rash which eventually covered his entire body, except his face, and which has persisted until the present time. In May 1951, he developed symptoms of epigastric distress and weight loss; at this time positive physical findings included enlarged axillary lymph nodes and splenomegaly to the umbilicus. Peripheral blood counts were normal and biopsies of a skin lesion and lymph node revealed "nonspecific inflammatory changes." X-ray therapy (225 r) to the spleen at this time produced no change in the patient's condition. Three years later the patient noted persistent diarrhea and, in Dec. 1954, presented the typical picture of decompensated cirrhosis with muscle wasting, marked hepato-splenomegaly and ascites. Laparotomy revealed marked mesenteric lymphadenopathy with areas of infiltration into the bowel wall, liver and spleen. Pathologic studies of a mesenteric node, liver, bone marrow and skin revealed infiltration by clumps of cells which were interpreted as reticulo-endothelial cells. Since May 1955, the patient has shown a dramatic response to ACTH therapy including cessation of diarrhea, clearing of ascites, marked reduction in the size of the liver and spleen, improvement in skin lesions and disappearance of the generalized lymphadenopathy.

*Materials and methods.* Tissue culture methods employed were those now in wide use(5,6). A cervical lymph node biopsy was performed 6 days after start of daily ACTH therapy. Half of the node was placed in fixative for pathologic examination and half placed in synthetic medium(7), hereafter referred to as MS, for transport to the laboratory. The node was minced and placed in roller tubes both in a plasma clot and directly on the glass. The nutrient medium consisted of MS 74%, horse serum 20%, human serum 5%, chick embryo extract 1% and antibiotics, either penicillin 50 units per ml and streptomycin 50 μg per ml or tetracycline 25 μg per ml. For virus work the human se-

* This work was supported in part by research grants from the National Foundation of Infantile Paralysis and from the Research Laboratories of Chas. Pfizer Co.

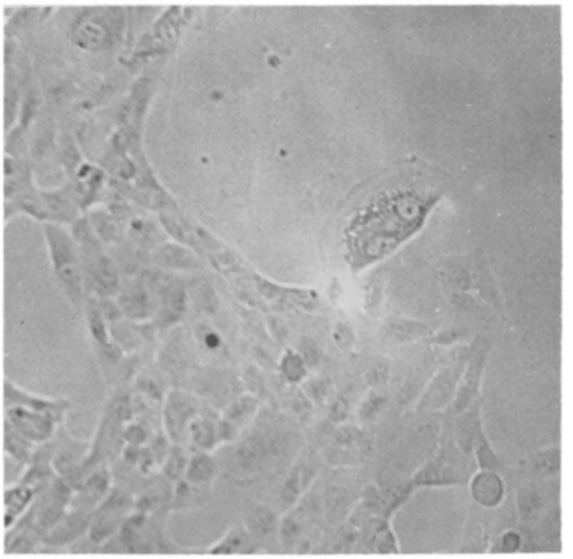

FIG. 1. Roller tube culture of LN cells. Unstained; 63×.

rum was omitted. Sera were inactivated at 56°C for 30 minutes and came from human and equine sources. Acid phosphatase activity was detected by the method of Gomori(8) and the periodic acid-Schiff procedure as described in his textbook(9).†

*Initial Isolation.* Fibroblastic outgrowths were noted in all tubes by 4th day and in some cultures groups of cells, probably macrophages, were seen. In certain cultures the latter appeared eventually to predominate and many unsuccessful attempts were made to separate them from the fibroblasts. On 29th day of cultivation, outgrowth in 3 tubes was pooled and subcultured. One of these subcultures showed marked proliferation of macrophages and thereafter frequent partial transfers were made from this source. In one such attempt, performed after this culture had been maintained for 81 days, a different cell type was observed. By scraping from the glass and transplanting, these cells, designated the LN strain, were eventually obtained in independent cultures.

*The LN strain.* A small group of cells, epithelial-like in appearance, was first observed on 10th day following subculture from a tube containing predominantly macrophages. At first these cells formed a monolayer of polyhedral cells, but after subcultivation the cells tended to form small clumps several layers thick. Their characteristics have not apparently changed during 20 passages (7 months). If allowed to grow, these clumps quickly detached and occasionally began to grow elsewhere in the bottle or tube. The cells could be scraped off the surface with ease, usually coming off the glass in small clumps which could easily be broken up into very small groups of cells or into single cells by pipetting; occasionally the cells could be simply washed from the glass surface. Growth of these cells is rapid following subcultivation in medium containing human serum, somewhat slower without this ingredient. Although these cells can be subcultured by the use of trypsin(6), it was early found that they were easily and satisfactorily subcultivated by scraping and pipetting. When observed early in their growth period through glass or in stained preparations, the cells look not unlike the HeLa strain(1) or any of several epithelial-like strains derived from normal human tissues(3). (Fig. 1).

*Susceptibility to Cytopathogenic Agents.* Because of their morphologic appearance and source, it was thought likely that these cells

† These procedures were carried out in the Department of Anatomy, Harvard Medical School, through the courtesy of Dr. Leon P. Weiss.

TABLE I. Titration of Poliomyelitis Virus, Type I, in Human Liver and LN Cells.

| Virus log dilution | Cell strain | Time in hr 0 | 22 | 30 | 48 | 56 | 72 | 96 | 120 |
|---|---|---|---|---|---|---|---|---|---|
| -1 | L | — | 4+ | | | | | | |
| | LN | — | 4+ | | | | | | |
| -2 | L | — | 4+ | | | | | | |
| | LN | — | 3+ | 3+ | 4+ | | | | |
| -3 | L | — | 2+ | 4+ | | | | | |
| | LN | — | — | 1+ | 3+ | 3+ | 4+ | | |
| -4 | L | — | — | 2+ | 4+ | | | | |
| | LN | — | — | 1+ | 3+ | 3+ | 4+ | | |
| -5 | L | — | — | — | 2+ | 3+ | 4+ | | |
| | LN | — | — | — | 1+ | 2+ | 3+ | 4+ | |
| -6 | L | — | — | — | — | — | — | — | — |
| | LN | — | — | — | — | — | — | — | — |
| -2 + immune serum | L | — | — | — | — | — | — | — | — |
| | LN | — | — | — | — | — | — | — | — |

L = Liver epithelial cells(3). LN = LN strain from lymph node of patient with "Reticulo-endotheliosis."

— = No cytopathogenic effect, cells normal. 1+ = 1-5 groups of cells showing cytopathogenic effect. 2+ = 6-20 groups of cells showing cytopathogenic effect. 3+ = Most cells show cytopathogenic effect, but normal appearing cells still present. 4+ = Cytopathogenic effect complete, no normal cells remain.

would respond to poliomyelitis virus in a manner similar to other human cell lines; some differences have been observed. The LN cells are destroyed more slowly than the strains of normal epithelial-like cells maintained in this laboratory(3). Table I presents data from one of three experiments in which this time relationship has been consistent.

*Histochemical Procedures.* Since macrophages are known to exhibit considerable acid phosphatase activity(10) and since the LN strain appeared to originate from this type of tissue, parallel cultures of liver epithelium and LN cells were stained on coverslips by the calcium phosphate method at pH 4.6. The liver cells were well stained but even macroscopically it could be seen that the LN cells were far more heavily stained, indicating a high degree of acid phosphatase activity. Essentially similar findings were obtained when the two lines were stained by the periodic acid-Schiff procedure, indicating a high concentration of complex saccharide or lipid substances containing potential aldehydes. These studies are being continued.

*Summary.* A strain of human cells isolated from the lymph node of a patient under treatment with ACTH with the clinical and pathologic diagnosis of "reticulo-endotheliosis" is reported. This cell line can be subcultured readily after 20 passages (7 months) and, although morphologically similar to the human epithelial-like strains now established, differs from them with respect to its source, certain growth characteristics, and in its response to the cytopathogenic action of poliomyelitis virus. The cells show striking acid phosphatase activity and stain heavily by the periodic acid-Schiff procedure.

1. Gey, G. O., Coffman, W. D., and Kubicek, M. T., *Cancer Research,* 1952, v12, 264.

2. Berman, L., Stulberg, C. S., and Ruddle, F. H., *Blood,* 1955, v10, 896.

3. Chang, R. S., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 440.

4. Swim, H. E., and Parker, R. F., *ibid.,* 1955, v83, 577.

5. Weller, T. H., Enders, J. F., Robbins, F. C., and Stoddard, M. B., *J. Immun.,* 1952, v69, 645.

6. Scherer, W. F., Syverton, M. D., and Gey, G. O., *J. Exp. Med.,* 1953, v97, 695.

7. Scherer, W. F., *Am. J. Path.,* 1953, v29, 113.

8. Gomori, G., PROC. SOC. EXP. BIOL. AND MED., 1939, v42, 23.

9. ———, *Microscopic Histochemistry,* University of Chicago Press, Chicago, 1952.

10. ———, *Arch. Path.,* 1941, v32, 184.

Received December 16, 1955. P.S.E.B.M., 1956, v92.