blood and bone marrow. The patient expired in April 1976. The TALL-1 line was derived from bone marrow aspirated 1 week prior to death.

Cells from this culture line were serially transplanted for 5 passages into newborn hamsters treated with rabbit anti-hamster thymocyte serum, according to the method previously described[5]. I.p. implantation of $1-3 \times 10^7$ cells gave rise to progressive growth of tumors in all 15 recipients after 23-41 days. There were massive mesenteric and retroperitoneal tumors with or without ascites. Ocular lesions were observed in one or both eyes of 8 of them. TALL-1 cells infiltrated the ciliary body and choroid. In a few instances, the entire eyeballs were encircled by uveal infiltration with impingement on the optic nerve. Leukemic infiltrations were also present in the liver, gallbladder, kidneys, spleen, lymph nodes, thymus, bone marrow, brain, spinal cord and meninges. The majority of these animals showed leukemic cells in the peripheral blood, although leukocyte counts were not usually elevated.

T-cell ALL is characterized by massive leukemic infiltration and a poor prognosis as compared with a more common type of null-cell ALL[6]. It is interesting that even in heterologous hosts human leukemic T-cells behaved as in the donor patient and became widely disseminated in many organs including the eyes. Southam et al.[7] observed similar eye lesions in rats transplanted i.v. with cultured Burkitt's lymphoma cells. Our hamster system would be useful as a chemotherapeutic model of human T-cell leukemia-lymphoma.

1 Supported by a Cancer Research Grant from the Ministry of Health and Welfare of Japan.
2 A.R. Rosenthal, P.R. Egbert, J.R. Wilbur and J.C. Probert, J. pediat. Ophthal. *12*, 84 (1975).
3 K.H. Murray, F. Paolino, J.M. Goldman, D.A.G. Galton and C.F.J. Grindle, Lancet *1*, 829 (1977).
4 I. Miyoshi, S. Hiraki, T. Tsubota, I. Kubonishi, Y. Matsuda, T. Nakayama, H. Kishimoto, I. Kimura and H. Masuji, Nature *267*, 843 (1977).
5 I. Miyoshi, S. Hiraki, I. Kubonishi, Y. Matsuda, H. Kishimoto, T. Nakayama, T. Tanaka, H. Masuji and I. Kimura, Cancer *40*, 2999 (1977).
6 I. Tsukimoto, K.Y. Wong and B.C. Lampkin, New Engl. J. Med. *294*, 245 (1976).
7 C.M. Southam, J.H. Burchenal, B. Clarkson, A. Tanzi, R. Mackay and V. McComb, Cancer *23*, 281 (1969).

## Binding bovine immunoglobulins to anterior lobe cells of the hypophysis[1]

W.H. Boyd, A. Peters and G. Morris

*Biomedical Sciences, University of Guelph, Guelph, (Ontario, Canada), 16 February 1978*

*Summary.* Cells of the anterior lobe of the bovine hypophysis, show an affinity for immunoglobulins IgG, IgA and IgM. The phenomenon either provides a message to stimulate cellular secretion or to excite mechanisms associated with the synthesis and release of Ig's or their determinants.

Routine histological preparations (H&E, PAS, AF, orange G) of pituitary anterior lobe tissue show a compact arrangement of cells grouped into chromophils (basophils and acidophils) and chromophobes on the basis of their affinity or lack of affinity for various dyes. Viewed under white light, after fluorescent staining, useful criteria for identifying anterior lobe cells are the size and shape of the nucleus and the general cytological variations.

Because of the compact arrangement of anterior lobe cells, neither cryostat nor paraffin sections of glandular tissue proved suitable for study. We found that the best information was gained from isolated single and small groups of cells, secured from a 'cell drop technique' developed in our laboratory.

*Materials and methods.* Anterior lobe tissue from 4 to 5 fresh bovine glands was harvested[2], placed in 5 ml of 0.005 M phosphate buffered saline (PBS pH 7.2) and minced with a scalpel into pieces 0.5 $mm^3$ or less. The mixture was poured through 3 layers of gauze into a centrifuge tube. The gauze was rinsed again with 2 ml of PBS and the mixture centrifuged for 5 min. Following this procedure, the supernatant was discarded, and the pellet with whitish appearing intermediate lobe cells on the top and dark brown red blood cells below, was fixed (5-10 min) by gently adding 2 ml of either cold Carnoy's or buffered formalin. The top or intermediate lobe cell layer was carefully pipeted off and placed in a test tube with the addition of a sufficient amount of fixative to secure the desired density.

After a period of refrigeration (1 h at $-4\,^{\circ}C$) a variable number of cells were resuspended with a pipet. A small drop of suspended cells was placed on precleaned microscopic slides and allowed to air dry. The type of anterior lobe cell was identified by staining directly with conjugated (FITC) rabbit anti-bovine IgG, IgA and IgM; indirectly (sandwich technique) with rabbit anti-bovine IgG, IgA, IgM and conjugated (FITC) goat as well as sheep anti-rabbit serum[3].

In the direct staining method, the controls were treated with rabbit anti-bovine IgG, IgA, IgM and incubated (1 h) in a moistened chamber at room temperature. In the indirect method, the controls were treated with a solution of equal parts, rabbit anti-bovine IgG, IgA and IgM and conjugated (FITC) rabbit anti-bovine IgG, IgA and IgM. Following the staining procedures, slides were gently rinsed ($\times 2$) with PBS, allowed to dry and mounted in PBS (10%) glycerin (90%) and covered.

Sections were examined under white and fluorescent light ($\times 300$) using the Wild microscope (M 20) mounted on the transmitted-light base 111. The Wild HBO-200 mercury vapour lamp proved suitable for blue light and FITC fluorescence with blue-light fluorescence exciting filter ø 32 mm (126 103 BG12); blue-light barrier filter ø 32 mm (202 202 OGlc); and barrier filters for UV-(GG 13c) and blue-light (OG 1c). The cells were photographed ($\times 480$) on Kodak Tri-X-135) film (ASA 400) and enlarged ($\times 6$).

*Results and discussion.* Microscopically, the 2 morphologically distinct basophils, (beta and delta)[4] usually recognized after routine staining, could be identified with limited clarity after fluorescent staining. Basophils, stained directly and indirectly with conjugated (FITC) rabbit anti-bovine IgG, IgA and IgM, showed many of the characteristics of the delta cell. One constant feature was the size of the nucleus (4.35 µm to 4.51 µm); well within the limits of similar cells stained routinely.

Stained with FITC/IgM and viewed under white light (figure 1, A), the basophilic cell appeared to be in a resting state i.e., there was no sharp cytoplasmic outline, the cytoplasmic granules seemed fused into homogeneity, and the nucleus was crenated and pycnotic. Under fluorescent

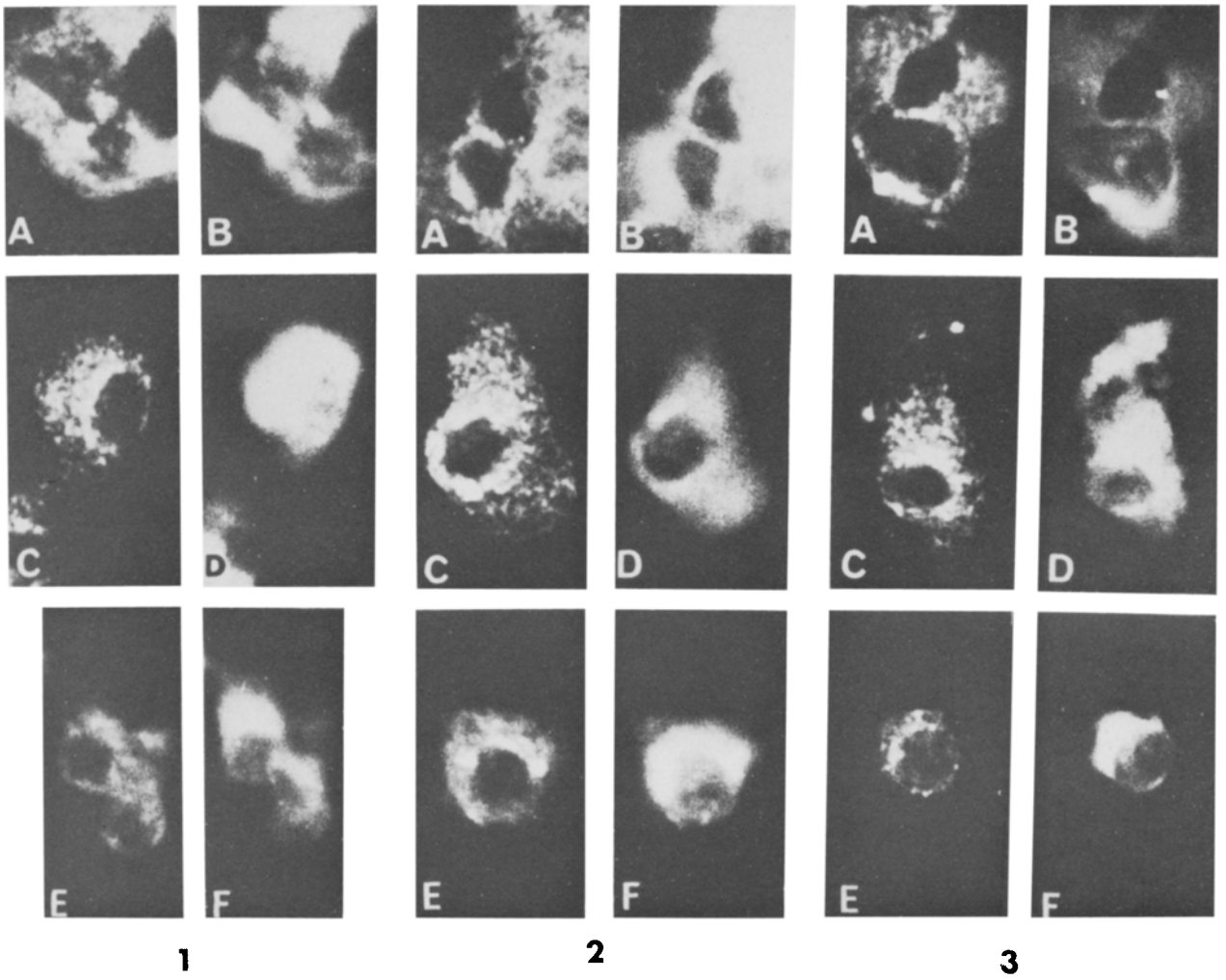


Fig. 1. *A* Basophil, *C* acidophil and *E* chromophobe cells stained with conjugated FITC/IgM and photographed under white light. The same cells *B*, *D* and *F*, photographed under fluorescent light. × 2880.

Fig. 2. *A* Basophil, *C* acidophil and *E* chromophobe cells stained with conjugated FITC/IgG and photographed under white light. The same cells *B*, *D* and *F*, photographed under fluorescent light. × 2880.

Fig. 3. *A* Basophil, *C* acidophil and *E* chromophobe cells stained with conjugated FITC/IgA and photographed under white light. The same cells *B*, *D* and *F*, photographed under white light. The same cells *B*, *D* and *F*, photographed under fluorescent light. × 2880.

light (figure 1, B) the cell showed a uniform smooth staining pattern involving the entire cytoplasm.

The basophilic cell, stained with FITC/IgG (figure 2, A) and IgA (figure 3, A), differed slightly from the FITC/IgM cell. The difference occurs in the nucleus, which is now fusiform or spherical in shape with a distinct nuclear membrane. The nuclear changes seen in this cell, typify a transitory period between the inactive and active state. The acidophilic cell, stained directly and indirectly with conjugated (FITC) rabbit anti-bovine IgM (figure 1, C and D), IgG (figure 2, C and D) and IgA (figure 3, C and D) is fully and discreetly granulated with a distinct cytoplasmic outline. The oval nucleus has a distinct membrane, and varies in size from 4.25 µm to 4.42 µm. The features exhibited by the acidophils shown in the figures, typify those observed in similar cells during the pre-secretory phase. The acidophils, oval in shape (figure 1, C and D) are usually arranged in cords, pseudoacini or clumps. Those columnar in shape (figures 2, C and D and 3, C and D) with an accentrically placed nucleus, are usually arranged around blood vessels or connective tissue septa. Under fluorescent light, acidophils (figures 2, 3, C and D) show a smooth, uniform staining pattern, involving the entire cytoplasm. With continued secretory activity[5], the granules in the acidophilic cell, are progressively depleted and the cells are thought to grade into chromophobes.

Although we have not been able to observe the grading of acidophils into chromophobes, we do find that chromophobes tend to occur in clumps and are much smaller (3.50 µm) than the typical acidophil (figures 1, 2, 3 E and F), or the basophil. The typical angular or elongated chromophobe, contains a rather small amount of sparsely granulated cytoplasm, and the small crenated nucleus is surrounded by a poorly defined membrane. Under fluorescent light, these cells, like the others, show a smooth, uniform staining pattern, involving the entire cytoplasm.

Correlating cytology with function[6-15], it is generally accepted that anterior lobe cells are involved in the secretion of a series of hormones; GH, FSH, LH, ACTH, TSH and prolactin. The basophils are thought to be involved in the production of TSH, LH, FSH and ACTH; the acidophils in the production of LH, GH and prolactin; while the chro-

mophobes are said to be involved in the production of GH and prolactin.

With these data, we looked for further support of our findings by investigating (employing gel diffusion and immunoelectrophoresis) the relationship of immunoglobulins to bovine pituitary hormones[15]. We found that FSH, GH, LH and TSH demonstrated antigenicity and 'gamma' mobility. The phenomenon could not be demonstrated with either MSH or ACTH.

Precipitin lines of identity were found between LH, FSH, GH, TSH and IgG determinants, between GH, TSH and IgA determinants, and partial identity between LH and IgA determinants. These studies suggest that antigens of the 'gamma' class are conceivably hormones. In a similar view, recent studies by others[16], demonstrated that ACTH secreting cells of the human anterior pituitary, show an affinity for human immunoglobulins resembling that of normal mast cells for IgE.

We report the finding that chromophil and chromophobe cells of the anterior lobe of the bovine hypophysis, show an affinity for bovine immunoglobulins IgG, IgA and IgM. This phenomenon may have a dual significance; that of providing the appropriate 'message' to stimulate the cells into hormonal secretion plus an involvement in the synthesis and release of immunoglobulins or their determinants to the intact molecule[16,17]. Indeed, these findings have great physiological significance and clearly show the need for a continuing indepth investigation of the various pituitary mechanisms.

1 This study was supported by a grant from the National Research Council of Canada.
2 W.H. Boyd, Archivum histol. jap. *34*, 1 (1972).
3 M. Goldman, Int. Rev. trop. Med. *1*, 215 (1961).
4 N.S. Halmi, Endocrinology *47*, 289 (1950).
5 K.V. Jubb, Cornell Vet. *45*, 593 (1955).
6 A.G. Pearse, J. Path. Bact. *64*, 791 (1952).
7 K. Benda, Arch. Anat. Physiol. *314*, 373 (1900).
8 E.J. Kraus, Archs. Path. *40*, 19 (1945).
9 H.M. Evans and J.H. Long, Anat. Rec. *21*, 62 (1921).
10 P.E. Smith and L.P. Smith, Anat. Rec. *25*, 150 (1923).
11 M. Herlant, Archs. Biol., Paris *54*, 225 (1943).
12 L.M. Rinaldini, J. Endocr. *6*, 54 (1949).
13 W. Hohlweg, Klin. Wschr. *13*, 92 (1934).
14 H.R. Friedgood and A.B. Dawson, Endocrinology *26*, 252 (1942).
15 W.H. Boyd, and A. Peters, Endocr. exp. *12*, 59 (1978).
16 A. Pouplard, G.F. Bottazzo, D. Doniach and I.M. Riott, Nature *261*, 142 (1976).
17 D.R. Stanworth, Haematologia *8*, 299 (1974).

## $^{31}$P-NMR study on nucleotides and intracellular pH of hereditary spherocytes

T. Kagimoto, F. Hayashi, M. Yamasaki, Y. Morino, K. Akasaka and S. Kishimoto

*The Second Department of Internal Medicine and the Second Department of Biochemistry, Kumamoto University Medical School, Kumamoto 860, and the Department of Chemistry, Faculty of Science, Kyoto University, Kyoto 606 (Japan), 2 December 1977*

*Summary.* As determined by $^{31}$p-NMR spectroscopy, intracellular pH of hereditary spherocytes was lower (pH 6.7–6.9) than that of normal red cells. The level of adenosine diphosphate in hereditary spherocytes was found to be persistently high. The metabolism of nucleotides and other phosphoryl compounds in human red blood cells have been studied in detail by $^{31}$p-NMR spectroscopy[1-3]. However, to our knowledge, there seems to be no report describing the result of $^{31}$p-NMR spectroscopy on red blood cells from hereditary spherocytosis.

*Materials and methods.* The heparinized whole blood from normal donors and the splenectomized patient of hereditary spherocytosis were centrifuged in the cold (2–5 °C) at approximately 1000 × g for 15 min. The sedimented red blood cells were collected for NMR measurement. During the preparation of samples, precaution was taken to keep them aseptic.

The NMR measurement was initiated at 25 h after the patient's blood was drawn and the red cells were collected. The blood sample from one of the authors (T.K.) was employed as a control and its measurement was initiated at 4 h after the blood was drawn. The $^{31}$p-NMR-spectra were recorded at 40.48 MHz on a JEOL-PFT-100 NMR spectrometer at about 25 °C. Proton noise decoupling was obtained under nonselective proton irradiation at 100 MHz. An acquisition time of 1.2 sec was used by the 45° single pulse sequence. 6000–10,000 repetitive scans were accumulated in the frequency domain to obtain a single Fourier transformed spectrum. The $^{31}$p-chemical shifts of nucleotides in ppm were measured from a 85% $H_3PO_4$ external reference. Intracellular pH was obtained from chemical shifts of inorganic phosphate. The accumulation of nucleotides and of phosphate metabolites were done as described in[4].

*Results and discussion.* Figure 1 shows the change with time of nucleotides and phosphate metabolites in normal red blood cells and hereditary spherocytes. The metabolic pattern in hereditary spherocytes was similar to that in normal erythrocytes except for that of ADP. ADP in normal erythrocytes reached a maximum level at 17 h and then leveled off. In contrast to this, ADP in hereditary spherocytes continued to be kept at a high level. Figure 2 shows the change of intracellular pH of normal erythrocytes and hereditary spherocytes with time. The initial pH of normal erythrocytes was at pH 6.9 and it slightly

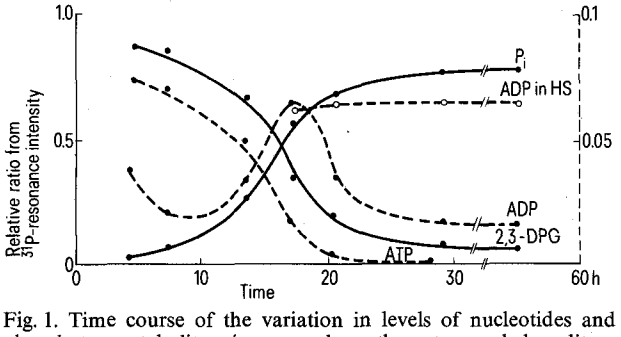


Fig. 1. Time course of the variation in levels of nucleotides and phosphate metabolites in normal erythrocytes and hereditary spherocytes (HS). The ordinate represents relative ratios of individual phosphoryl compounds as determined from $^{31}$p resonance intensity. Data for ATP and ADP (dashed curves) were plotted on a scale $^1/_{10}$ that for inorganic phosphate (Pi) and 2,3-diphosphoglycerate (2,3-DPG, solid curves). Open circles, data for normal erythrocytes; closed circles, data for hereditary spherocytes.