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

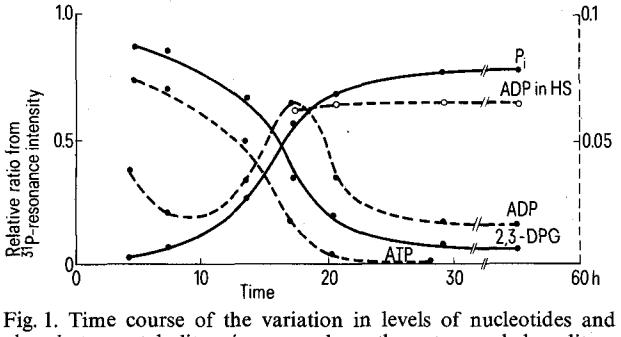

Fig. 1. Time course of the variation in levels of nucleotides and phosphate metabolites in normal erythrocytes and hereditary spherocytes (HS). The ordinate represents relative ratios of individual phosphoryl compounds as determined from ^{31}p resonance intensity. Data for ATP and ADP (dashed curves) 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.

decreased at 17 h after the blood was collected, although it increased to 7.15 afterward. On the other hand, the intracellular pH in hereditary spherocytes was slightly but distinctly lower than that in normal erythrocytes over a period from 20 to 60 h after the blood sample was drawn. These findings suggest that the lowering in intracellular pH may influence the metabolic fate of ADP, since Rapoport[3] reported that the metabolism of ATP and 2,3-diphosphoglycerate in normal erythrocytes was affected by changing the intracellular pH. Or it is also possible that disturbance in the utilization of ADP may be accompanied by lowering of intracellular pH. Thus, although it is not possible at the present time to derive any causal correlation between these findings: lower intracellular pH and high ADP level, this report may represent the first description of intracellular events in hereditary spherocytes using ^{31}p-NMR spectroscopy.

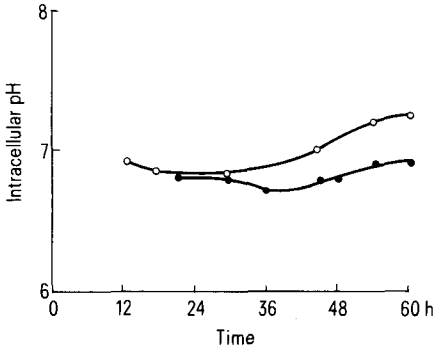

Fig. 2. Intracellular pH of normal erythrocytes (open circle) and hereditary spherocytes (closed circle) as obtained from chemical shift of Pi in ^{31}p-NMR-spectra.

1 T.O. Henderson, A.J.R. Costello and A. Omachi, Proc. nat. Acad. Sci. USA *71*, 2487 (1974).
2 R.J. Labotka, T. Glonek, M.A. Hruby and G.R. Honig, Biochem. Med. *15*, 311 (1976).
3 I. Rapoport, H. Berger, R. Elsner and S. Rapoport, Eur. J. Biochem. *73*, 421 (1977).
4 P.B. Garlick, G.K. Radda and P.J. Seeley, Biochem. biophys. Res. Commun. *74*, 1256 (1977).

Studies of autoimmune induction in the rat lacrimal gland

G.J. Mizejewski[1]

Department of Biology, University of South Carolina, Columbia (South Carolina, USA), 7 February 1978

Summary. Induction of autoimmunity in the rat lacrimal gland was presently assessed. Antibodies to lacrimal gland extract were detectable in 58% of the immunized rats. Skin tests were positive at 4 week postimmunization in 70% of the animals. Histological observations revealed the presence of mononuclear cell infiltrates surrounding the ductal and acinar epithelium at 2-6 weeks.

The lacrimal gland, termed the exorbital gland in the rat, is a compound tubuloalvealar secretory gland containing fat droplets and serous granules, presumably lysozyme. Little is known concerning the induction of autoimmunity in the lacrimal and associated exocrine glands of the head and neck. Animal models of exocrine autoimmune disease, especially in salivary and lacrimal glands, could aid in understanding the pathogenesis of Sjögren's syndrome in the human[2]. Sjögren's syndrome is characterized by sialoadenitis and dacryoadenitis, in association with keratoconjunctivitis sicca, xerostomia, and fibrosis[3]. The present study described an attempt at inducing autoimmunity in the inferior lacrimal glands of the laboratory rat. Autoimmune induction was assessed by the following criteria: 1. the humoral immune response; 2. the delayed hypersensitive response; and 3. histopathological lesions.

Material and methods. The present study employed a total of 114 male and female young adult rats of the Wistar strain. The lacrimal glands from 50 nontreated Wistar rats of both sexes were removed at sacrifice and prepared as a 1:5 tissue-saline extract following the method of Witebsky and Rose[4]. The tissue extract was prepared in phosphate buffered saline (pH 7.2) employing a motor driven ground glass tissue homogenizer. The tissue suspensions were shaken throughout a 3-day period (phenol as preservative) at 4 °C in 50 ml Erlenmeyer flasks. The suspensions were spun in a refrigerated centrifuge at 10,000 rpm for 1 h. Lipid which flocculated to the surface of the supernant was removed by skimming and the pellet was discarded. Protein determination of the supernant extract was determined by the Christian-Warburg method[5] following readings on a Cary recording spectrophotometer. The saline extract, determined as 30 mg protein/ml, displayed a protein: nucleoprotein ratio of 15:1. Rat thyroid saline extracts, prepared as above, were used to test for organ specificity.

The experimental design consisted of 4 groups, each comprising 16 animals. The rats were immunized with either 1.0 mg/ml (group I), 5.0 mg/ml (group II), or 10.0 mg/ml (group III); group IV consisted of age and sex-matched nontreated animals. Following immunization with the 3

The dose response levels of rats in the various treatment groups immunized with lacrimal gland extract is demonstrated

Interval tested	Antigen* dilution	Treatment groups (dose levels) Group I 1.0 mg/ml		Group II 5.0 mg/ml		Group III 10.0 mg/ml		Totals	(%)
A Serological response									
2 weeks	none	3/12	(25%)	4/12	(33%)	0/12	(0%)	7/12	(58%)
4 weeks	none	0/10	(0%)	2/10	(20%)	0/12	(0%)	2/10**	(20%)
B Skin test response									
4 weeks	1:10	3/10	(30%)	2/10	(20%)	2/10	(20%)	7/10**	(70%)
	1:100	1/10	(10%)	0/10	(0%)	2/10	(20%)	3/10**	(30%)

Percentages indicate numbers of animals responding by either (A) antibody production or (B) skin reaction. *Antigen = lacrimal gland saline extract. **2 animals of this group died.