CC/NUMBER 30
JULY 28, 1980

This Week's Citation Classic

Ashton G C & Braden A W H. Serum β-globulin polymorphism in mice. *Aust. J. Biol. Sci.* **14**:248-53, 1961.
[Div. Anim. Genet., CSIRO, Cattle Res. Lab., Rockhampton, Queensland, and Div. Anim. Physiol., CSIRO, I.C. Ross Anim. Res. Lab., Prospect, NSW, Australia]

The main purpose of this publication was to record for the first time that mouse β-globulins (transferrins) are polymorphic. However, interest in the paper was generated mainly as a result of the buffer and electrolyte system developed to effect the separation. [The *SCI*® indicates that this paper has been cited over 160 times since 1961.]

Geoffrey C. Ashton
Department of Genetics
John A. Burns School of Medicine
University of Hawaii at Manoa
Honolulu, HI 96822

January 18, 1980

"This paper has become known for the starch gel electrophoresis technique which it describes rather than for the topic covered (although the paper has been cited a few times for that, too).

"Smithies published his pioneering paper on starch gel electrophoresis in 1955[1] and as a geneticist I immediately became interested in this as a promising method for revealing further genetic variation. The major challenge was to prepare suitable hydrolyzed potato starch, and it was not long before my laboratory approached unforgettable dance-floor slipperiness due to starch spillage.

"In retrospect, my main goal in life at that time seems to have revolved around reproducing the tantalizingly ephemeral superb separations which occasionally occurred. Producing the 'best' starch, and working out the 'best' buffer systems became fascinating but time-consuming pursuits. I had tentatively experimented with mixed buffer systems and achieved some good separations, only to be chastened by a respected senior colleague who cautioned that I would be laughed at if I did not follow the established principles of physical chemistry. A short while later Poulik's publication on the separation of highly purified toxins in a discontinuous buffer system appeared.[2] This weakened my respect for my colleague but renewed my interest in mixed buffers, even though Poulik's system did not work particularly well for animal or human serum proteins.

"In 1958 I moved from England to Australia, and spent several months at CSIRO's Sheep Biology Laboratory, Prospect, NSW, in the laboratory next to K.A. Ferguson and A.L.C. Wallace. These workers were trying to separate anterior pituitary proteins on starch gel and had modified Poulik's discontinuous buffer system in two significant ways.[3] Firstly, they replaced NaOH in the electrolyte by LiOH, which reduced the production of Joule's heat in the gels. Secondly, they premixed a small volume of the LiOH/boric acid electrolyte with the tris/citric acid buffer. This modified the drop in potential behind the moving boundary which characterized Poulik's system and made it so useful. As an avid collector of buffer systems, I applied Ferguson and Wallace's system to sheep and cattle serum, but the initial results were disappointing. However, by adjusting reagent concentrations, and most importantly by adjusting the proportion of the electrolyte added to the gel buffer, I finally achieved a mixture which did what I wanted. That is, it sharpened the normally diffuse serum albumin zone and permitted detection of the previously hidden variation in the pre-albumin and post-albumins. Coincidentally, it also improved resolution of other constituents of the serum, including transferrins and haptoglobins.

"During earlier work with cattle transferrins I had noticed some aberrant segregation ratios and fertility effects. Alan Braden was working with the t-allele system in mice, and it seemed worth examining his mice to see if they showed transferrin polymorphism. They did, and later on in Honolulu I explored fertility effects associated with mouse transferrins."[4]

1. **Smithies O.** Zone electrophoresis in starch gels: group variations in the serum proteins of normal human adults. *Biochemical J.* **61**:629-41, 1955.
2. **Poulik M D.** Starch gel electrophoresis in a discontinuous system of buffers. *Nature* **180**:1477-9, 1957.
3. **Ferguson K A & Wallace A L** C. Starch gel electrophoresis of anterior pituitary hormones. *Nature* **190**:629-30, 1961.
4. **Ashton G C & Dennis M N.** Selection at the transferrin locus in mice. *Genetics* **67**:253-65, 1971.