Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

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Early Days of Gel Electrophoresis

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I suppose that few experimental scientists refuse an invitation to remember and to write about an exciting time in their life in front of the bench. I certainly did not when JIM CROW suggested that the fortieth anniversary of starch-gel electrophoresis was an appropriate occasion on which to reminisce.

At the present time, about 0.2 microgram of protein is quite sufficient for performing a high-resolution gel electrophoresis experiment, when the gel is silver stained. In the early 1950s, when I was a postdoctoral fellow in JACK WILLIAMS' laboratory in the Department of Physical Chemistry at the University of Wisconsin, we needed about 1,000,000 times more protein (0.2 gram) for electrophoresis in the department's (only) Tiselius moving boundary electrophoresis apparatus—itself a complex and elegant piece of equipment over five meters in length. Nor were the results particularly convincing when it came to assessing protein purity; anomalies in moving boundary electrophoresis caused large quantitative changes in the analysis to the extent that "it would . . . be impractical to use the method to follow further purification" (SMITHIES 1954).

When I moved to the Connaught Laboratories at the University of Toronto shortly thereafter, my immediate superior and later friend, DAVID A. SCOTT, allowed me to choose any project I wished, provided that it was related to insulin, which was of course discovered in Toronto. I decided to look for a precursor to insulin (I never found it!). My Wisconsin experiences had shown me that the quantity of material required for moving boundary electrophoresis, together with its potential anomalies, precluded my using it as an assay, so I turned to the literature and found that the newly invented procedure of zone electrophoresis had many advantages over the moving boundary method. The chief distinction between the two methods lies in the fact that, starting with a complex mixture of proteins, the boundary method gives *overlapping* boundary separations of the type

$$[A + B + C] \rightarrow [A + B + C] \cdots B + C] \cdots C]$$

whereas zone electrophoresis gives *discrete* zone separations,

$$[A + B + C] \rightarrow [A] + [B] + [C].$$

Furthermore, zone electrophoresis on filter paper required 100 times less material. Thus, one could hope to take a complex mixture of proteins, separate them by filter paper electrophoresis, stain a strip of the paper, and test the biological activity of the separated proteins on the remaining portion of the paper. But insulin was recalcitrant: it absorbed to filter paper under all the conditions that I could devise, giving results rather like a carpet unrolling—the length of the resulting smear was proportional to the amount of protein, not to its electrophoretic mobility.

Then on Saturday, January 23rd, 1954, I visited the laboratory of ANDREW SASS-KORTSAK at the Hospital for Sick Children, Toronto, to see a new method of zone electrophoresis that he was using (KUNKEL and SLATER 1952). The method used starch *grains* as a support for the electrophoresis. It was rather like carrying out electrophoresis in a wet bed of sand, with migration occurring through the buffer in the spaces *between* the grains. I noted with envy that the starch grains were gloriously free from absorption problems, and I thought that for this reason starch might solve my problems with insulin. Unfortunately, in order to detect the protein zones after starch grain electrophoresis, it was necessary to carry out Folin chemical assays for protein on about 40 transverse slices of the moist starch bed. This I could not manage to do, for I had no technical help of any type, not even a dishwasher. Fortunately, however, my childhood memories are strong, and I recalled one day when I was about 12 years old helping my mother with the laundry and observing that the starch she used for my father's shirts was liquid when hot but turned to a jelly when cold. Remembering this, I thought that if I cooked the starch and allowed it to cool, then the proteins could migrate *through* the resulting jelly, and could subsequently be detected by

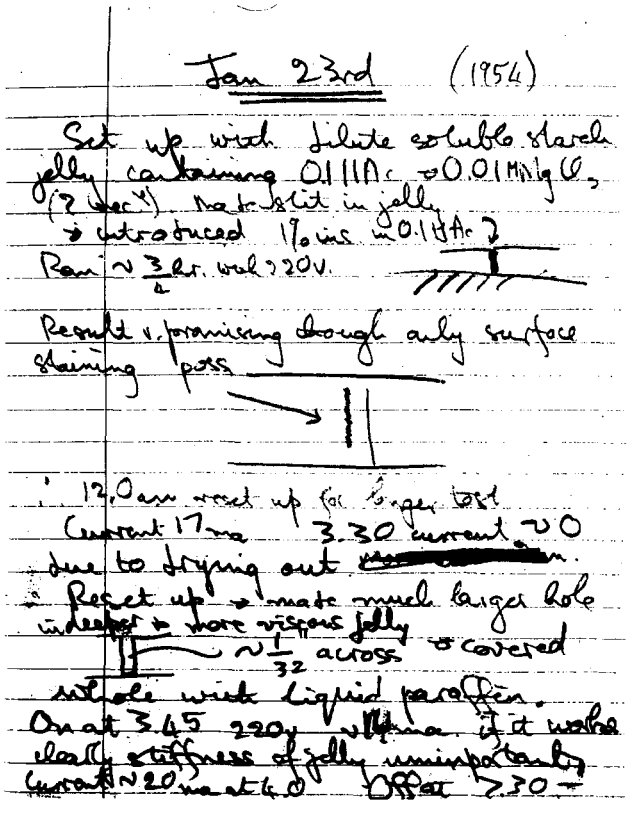

FIGURE 1.—A page from laboratory notebook I, January 23, 1954.

staining, in the way that worked with filter paper electrophoresis.

Previous nightly forays had made me cognizant of the whereabouts of all the chemical storerooms in Connaught Labs, and in one of them I found a bottle of "Starch according to Lintner." I tried cooking this Lintner starch with my electrophoretic medium (0.1 M acetic acid and 0.01 M $MgCl_2$) and found that at a concentration of about 15 g per 100 ml it gave a gooey liquid that set into a nice gel when cold. The first starch gel electrophoresis experiment was promptly performed in the afternoon of that same Saturday with purified insulin, using 220 V across the electrodes for $^3/_4$ hour. (In 1954, the University of Toronto still had 220-V *direct* current in some electric outlets, so that power supplies were not needed.) Figure 1 shows my lab notebook page of that day with a sketch of the resulting gel (no Polaroids!) and the happy comment, "Result v. promising."

About two months later, on Tuesday, March 23rd at 10:30 p.m. (with a bachelor's disregard for the time of day and night), out of curiosity I tried a short run with serum, "just for rough test." It looked good, and at midnight I had set up my first real test of starch gel electrophoresis of serum. On the following day my notebook contains the comment "Total ~11 components!" (Figure 2). Since at that time we always talked about five serum proteins (albumin and α_1, α_2, β, and γ globulins), it was with neither qualms nor regrets that I forever left my search for the precursor of insulin and concentrated on serum. (DAVID SCOTT was a tolerant boss, and we weren't dependent on grants.)

Then followed a busy seven months tuning up the method. In doing this I used serum from myself and from my two graduate-student friends, GORDON H. DIXON, now a distinguished professor at the University of Calgary, and GEORGE E. CONNELL, now a highly regarded past president of the University of Toronto. The results were very similar with these various sera (Figure 3) and I was about ready to publish when, on October 26th, I ran a gel on the serum of BETH WADE, CHARLIE HANES' technician. Figure 4 shows the result. "Most odd—many extra components."

At first, I thought the difference was sex determined, to the surprise of all and sundry. But after a week or so of running daily tests on serum from a male and from a female, my M and F patterns reversed, with a notebook comment, "must have muddled sera." But I had not, and I began to suspect the existence of genetic differences (later shown to be in the plasma protein haptoglobin), as indicated by the brief comment, "Hereditary factors may determine the serum groups" in my paper describing the starch gel procedure and the results obtained with it (SMITHIES 1955).

To investigate this possibility, I joined forces with NORMA FORD WALKER, who was Head of the Department of Genetics at the Hospital for Sick Children. She was to a large degree my first real, albeit informal teacher of genetics. In truth, we just learned together about what eventually became the new field of protein polymorphisms (SMITHIES and WALKER 1955). We also learned the hard way that the haptoglobin levels of infants are usually very low, and that nonpaternity in a family will challenge (but eventually confirm) a good genetic hypothesis. Serum haptoglobin types are still used as a common polymorphic trait in forensic situations, and their molecular biology has proved almost inexhaustibly interesting (MAEDA and SMITHIES 1986).

For quite some time after the starch gel technique was working, we did not know the correct identity of the protein bands that were resolved. Only when my friend and collaborator M. DAVID POULIK set out to compare the results of filter paper electrophoresis and starch gel electrophoresis were the true properties of the starch gels recognized (POULIK and SMITHIES 1958). We found that many proteins migrated through starch gels in an order different from their migration through the strictly aqueous buffer surrounding filter paper fibers. We realized that "the high degree of resolution obtained [in the starch gels] . . . appears to be

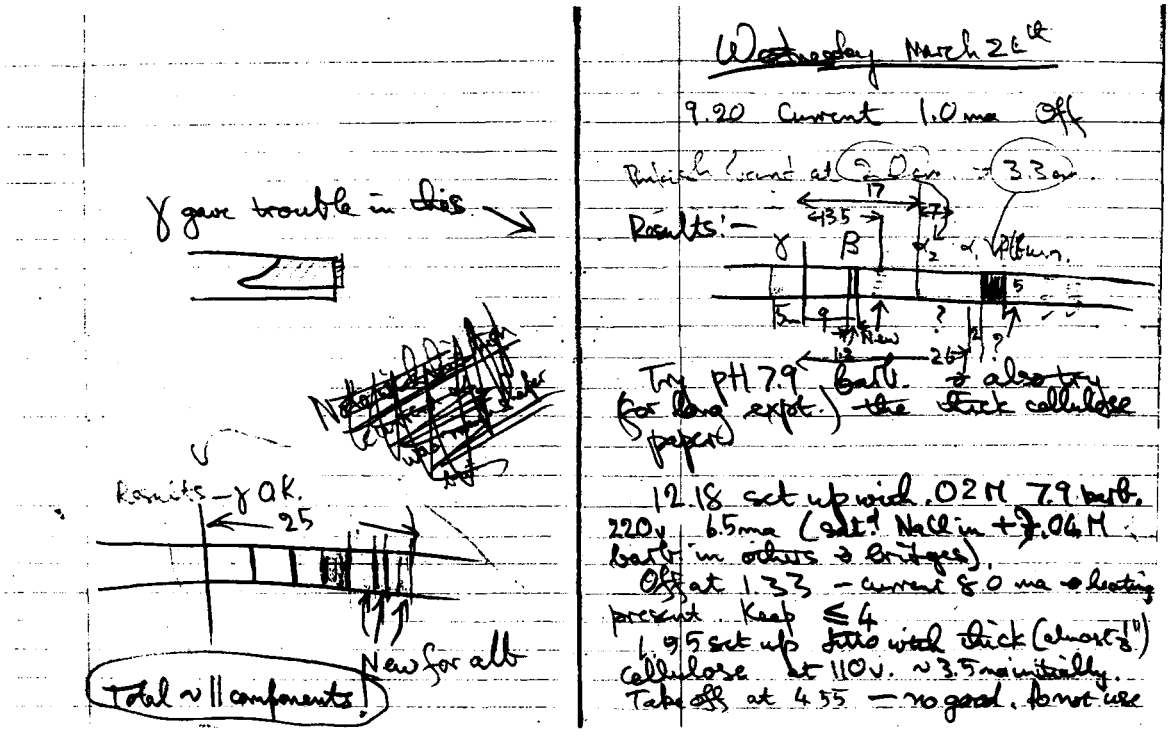

FIGURE 2.—Two pages from notebook II, March 24, 1954.

due to the use of a supporting medium the pore size of which approaches the molecular dimensions of some of the proteins." Two-dimensional electrophoresis was conceived and born as a result of this realization, with filter paper in one dimension and starch gel in the other (SMITHIES and POULIK 1956). With it we proudly resolved about 20 protein spots in serum. A measure of progress since those halcyon days is that two-dimensional electrophoresis of serum can now resolve more than 750 protein spots (ANDERSON and ANDERSON 1991).

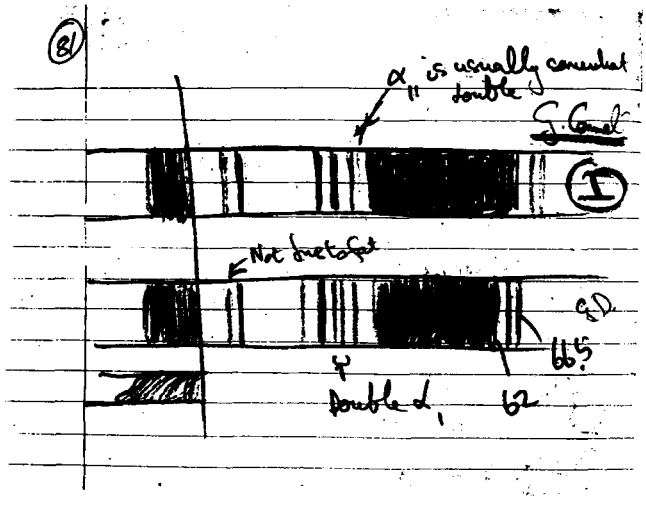

FIGURE 3.—A page from notebook IV, October 15, 1954.

It took GEORGE CONNELL, GORDON DIXON and me about another three years to work out the molecular genetics of the serum haptoglobins at the protein level, by which time I was back in Wisconsin, now a molecular geneticist rather than a physical chemist. There are still many stories to tell about those days, but they must wait for another time—although I cannot close without recalling the day (after returning to Madison from a consultation in Toronto with GEORGE and GORDON) on which I asked JIM CROW, "Is it possible for two allelic genes on homologous chromosomes to join together and give a double-sized gene?" His response was to introduce me to the *Bar* locus in Drosophila, with its long and fascinating history of homologous and nonhomologous recombination and gene duplication (STURTEVANT 1925; BRIDGES 1936). This introduction served me well. With it as a clue, we solved the intricacies of the "many extra components" of haptoglobin (SMITHIES *et al.* 1962). Extensions of the ideas of homologous and nonhomologous recombination continue to be enthralling in these days of gene targeting. My most recent paper, as it happens, talks about using homologous recombination (gene targeting) to duplicate genes in order to analyze quantitative genetic traits in mice (SMITHIES and KIM 1994). We find that simply duplicating a gene at its normal chromosomal locus is sufficient to cause a modest increase in the amount of gene product without any obvious changes in gene regulation. I imagine that JIM CROW will enjoy this continuity of

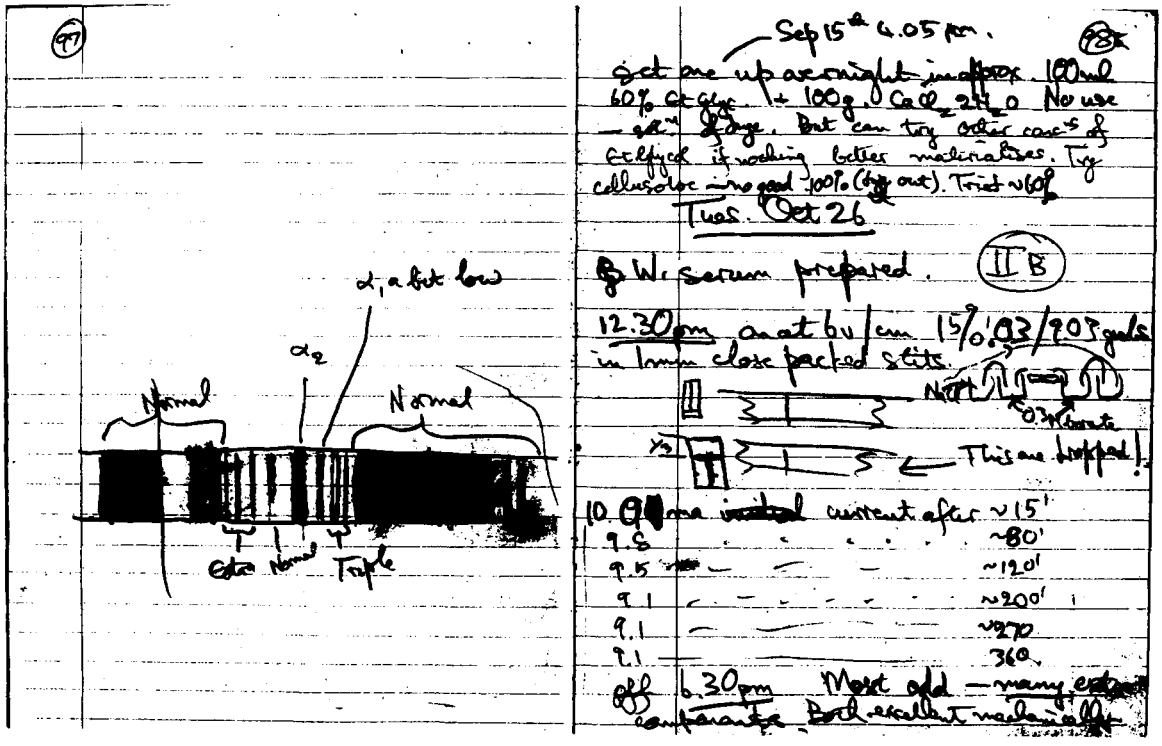

FIGURE 4.—Three days later, the first evidence of genetic differences.

thought, as I do, and as the mother of the 12-year-old boy would have done if she were still alive.

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