and the slow growing states will now be evident. By recognizing and making use of the complications of growth and metabolism, conclusions can be derived that are unattainable if biochemical study is only upon systems which attempt to eliminate these complexities.

Much modern biochemistry is pre-occupied with cellular particles and particulate preparations. However, the study of metabolism in relation to growth, as here described, requires work on the whole cell to reveal the integrated operation of the whole system. Smuts's principle of "Holism"[25] applies here, for "The whole is not something additional to the parts : it is the parts in a definite structural arrangement and with mutual activities that constitute the whole" (loc. cit., p. 104).

In the use of radioactive substrates to reveal metabolic sequences it is therefore necessary to recognize the existence of separate phases or compartments in the cell and to recognize that in each of these, quite different reactions may involve simultaneously the same substrate ; but it is also encouraging that these complications can be surmounted. The means to do this is to determine, for all available tissue constituents, not merely the existence of radioactivity in a given compound, not merely the specific activity of the tissue metabolite at a given time, but, by the investigation of time sequences, to make an estimate of the average specific activity of the carbon which must have been involved in the metabolic change that occurred. From the relations which these calculated results bear to the known specific activity of the source supplied, it is possible to appreciate the kind of complications that occur within the cell. Since this enables the investigator to recognize clearly that reactions occur in different compartments of the cell and to recognize that the total complement of a given constituent is made up of at least two different metabolic pools which do not mingle, this adds a valuable technique to the carbon-14 tracer method.

Even a relatively simple experiment, when planned to deal with all these complications, becomes a formidable task. To solve these problems, teamwork becomes essential, and this was happily achieved in the present case by pooling resources of personnel, equipment, technique and even substances synthesized from three different laboratories in three different countries.

It is too soon and perhaps too optimistic to suggest that the results here described could be applied directly to the investigations of cancerous growth in animal organs. The possibility, however, remains that the characteristic properties of the cancerous cell, like those of carrot cells stimulated by coconut milk, may lie not so much in distinctive substances and reactions as in accentuated rate of 'turnover' of their metabolically active, labile constituents owing to enhanced synthesis and breakdown. Investigations of nitrogen metabolism[26] in relation to cancer seem not to have considered this possibility. In plants this cyclical process may in part control the release of usable energy via a phase of respiration which is peculiarly linked with the ability of the cell to grow by cell division and to utilize metabolic energy for assimilation in such processes as water intake and salt accumulation. If these results have wider application, this pioneer experiment will indeed have been justified, and this general account will have achieved its purpose.

The full details of the work will be published elsewhere in due course.

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A SPECIFIC CHEMICAL DIFFERENCE BETWEEN THE GLOBINS OF NORMAL HUMAN AND SICKLE-CELL ANÆMIA HÆMOGLOBIN

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A NEW and rapid technique of characterizing the chemical properties of a protein in considerable detail has been devised ; by its application a specific difference is found in the sequence of amino-acid residues of normal and sickle-cell hæmoglobin. This difference appears to be confined to one small section of one of the polypeptide chains.

Of all the abnormal human hæmoglobins, the one that has been most intensively studied is hæmoglobin *S* from patients with sickle-cell anæmia. In 1949 Pauling and his collaborators[1] demonstrated by electrophoretic experiments that at neutral *p*H the hæmoglobin *S* molecule has a net charge which is more positive by three units compared with the normal molecule, hæmoglobin *A*. It has since been suggested[2] that this difference is really due to hæmoglobin *S* having fewer free carboxyl groups than does hæmoglobin *A*. It is also known that in the reduced state the abnormal protein has a much lower solubility[3]. However, careful determinations of the amino-acid composition of the two proteins[4,5] did not show any significant differences between them within the accuracy of the methods employed. Comparison of the N-terminal[6] and C-terminal[7] amino-acids and of the sulphydryl groups[8] was equally disappointing. On this evidence alone, it is not possible to decide whether the difference between the proteins, which is in any event small, lies in the amino-acid

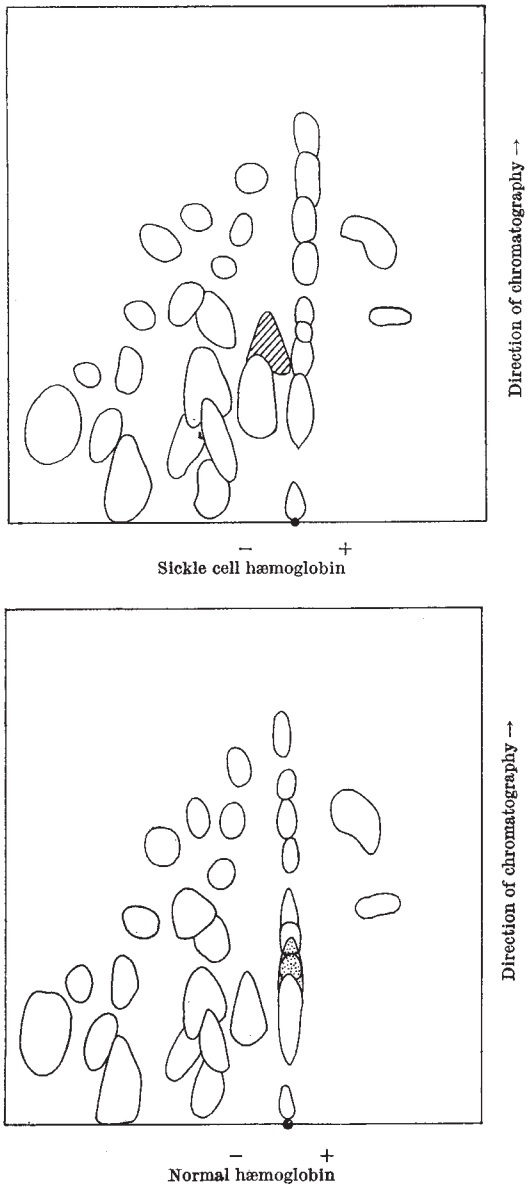

Fig. 1. 'Finger prints' of human normal and sickle-cell hæmoglobins. Electrophoresis at *p*H 6·4, chromatography with *n*-butyl alcohol/acetic acid/water (3 : 1 : 1). The shaded and the stippled spots are those belonging to the peptide showing the difference

sequences of the polypeptide chains or whether it lies in the folding of these chains leading to a masking of some amino-acid side-chains.

Hæmoglobin is still too large a molecule for detailed analysis of amino-acid sequence. However, it was thought that if a rapid method could be found of characterizing the chemical properties of the peptides in a tryptic digest, then perhaps a replacement of even a single residue for another might be detected without elaborate analysis.

The action of trypsin on proteins is at present the most reliable way of splitting a peptide chain at specific peptide bonds. The enzyme attacks only those bonds which are derived from the carboxyl group of the amino-acids lysine and arginine. There are about sixty of these in the hæmoglobin *A* and *S* molecules[5], but since it is expected[9] that each molecule is composed of two identical half-molecules, the number of peptides obtained by the action of trypsin should be about thirty, with an average chain-length of ten amino-acids. Small differences in the two proteins will result in small changes in one or more of these peptides. These should be detectable when the mixture is examined by a two-dimensional combination of paper electrophoresis and paper chromatography. It was decided to call the resulting chromatogram the 'finger print' of the protein.

To prepare such a 'finger print', samples of purified[10] hæmoglobins *A* and *S* were denatured by heat at 90° C. for 4 min. and digested with trypsin (2 per cent by weight) at *p*H 8 and 37° C. for 43 hr. Aliquots of these digests (equivalent to 3 mgm. of protein) were placed on large sheets of Whatman No. 3 *MM* paper between glass plates and subjected to electrophoresis[11] in pyridine/acetic acid at *p*H 6·4 for 150 min. at 16 V./cm. The paper was then dried. Ascending chromatography with *n*-butyl alcohol/acetic acid/water (3 : 1 : 1) and development of the peptide spots with ninhydrin produced the chromatograms shown in the tracings of Fig. 1. When the papers were run strictly in parallel, reproducibility was often good enough to superimpose the spots belonging to similar peptides in the two chromatograms.

The 'finger prints' show approximately thirty peptide spots, as was expected from the amino-acid composition. This confirms the view that the human hæmoglobin molecule consists of two identical half-molecules and not of four identical sub-units each carrying one of the four hæme groups. The same number of peptides was found for both hæmoglobins, as was to be expected from their containing the same number of lysine and arginine residues. Most of the peptides are well resolved, and appear to be similar in the two proteins. However, there is one peptide spot clearly visible in the digest of hæmoglobin *S* which is not obvious in the hæmoglobin *A* 'finger print'. In the tracing it has been marked by shading. Apart from its position, this peptide is characterized by its orange-colour reaction with

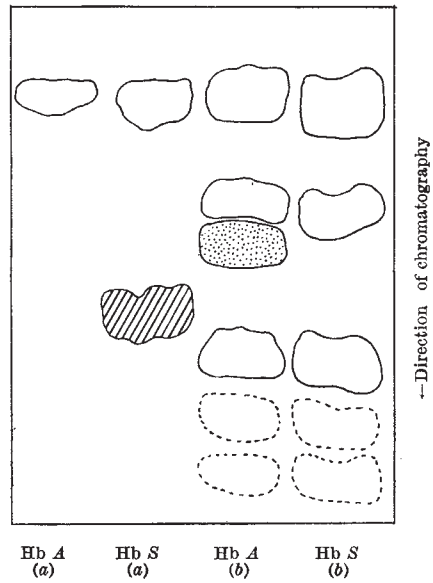

Fig. 2. Chromatogram of fractions of hæmoglobin *A* and *S* digests obtained by large-scale paper electrophoresis. (*a*) Slowest moving positively charged fractions; (*b*) neutral fractions

ninhydrin. Close inspection of the 'finger print' of hæmoglobin *A* shows the outline of an orange peptide spot in the 'back-bone' of neutral peptides which are not separated by electrophoresis. This spot, which has been stippled in the tracing, is not present in the neutral fraction of the digested hæmoglobin *S*. However, the neutral peptides are not really well separated by this two-dimensional technique.

Accordingly, larger amounts of the digests from both hæmoglobins were run in one direction only, using the electrophoretic conditions described earlier. Many bands were obtained. Both the band of neutral peptides and the slowest of the positively charged bands were eluted in each case, but separately. The eluted peptide mixtures were subjected to descending chromatography with *n*-butyl alcohol/acetic acid/water (4 : 1 : 5). Fig. 2 is a tracing of the chromatogram obtained showing the behaviour of the 'extra' or 'sickle cell' peptide in the two digests. This confirms that there is a particular peptide in the neutral fraction of the hæmoglobin *A* digest, which in the hæmoglobin *S* digest is shifted to the slowest positively charged zone. In addition, the R_F value of the hæmoglobin *A* peptide in the chromatographic solvent is lower than that of the analogous peptide derived from hæmoglobin *S*.

Summarizing the results of the electrophoretic and chromatographic examination, it can be seen that there is one peptide among the thirty or so which in hæmoglobin *S* is positively charged, but which is uncharged at the same *p*H in digests of hæmoglobin *A*. This agrees with the higher net positive charge[1] of the parent protein, hæmoglobin *S*. There is also a small change in the chromatographic behaviour of the peptide. On the other hand, corresponding pairs of all the other peptides show similar behaviour in these separating systems and therefore probably have similar constitutions.

One can now answer at least partly the question put earlier, and say that there is a difference in the amino-acid sequence in one small part of one of the polypeptide chains. This is particularly interesting in view of the genetic evidence[12] that the formation of hæmoglobin *S* is due to a mutation in a single gene. It remains to be seen exactly how large a portion of the chains is affected and how the sequences differ.

Full details of these experiments will be reported elsewhere. I am indebted to Dr. A. C. Allison (Oxford) and Dr. G. Seaman (Cambridge) for supplying samples of hæmoglobin *S* and blood from patients with homozygous sickle-cell anæmia; and I acknowledge the encouragement and interest shown by Dr. M. F. Perutz, and the expert assistance of Mrs. Leslie Barnett and Miss Rita Prior.

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IDENTIFICATION OF A NEW NORMAL EMBRYONIC HÆMOGLOBIN

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DURING a current study of the process of formation and appearance of the two hitherto known types of normal hæmoglobin, and of the different serum protein fractions of the fœtal blood throughout the various stages of embryonic life, we were able to examine the blood of a ten-week old embryo, recovered from an intact extra-uterine pregnancy. To the best of our knowledge this is the smallest human embryo of which blood chemistry data have been reported in the literature.

Using the method described by Singer[1] we found that the hæmoglobin of the ten-week old embryo consisted of 83 per cent of the alkali-resistant type.

The electrophoretic examination of this hæmoglobin was carried out in a Thomas paper electrophoresis apparatus on Whatman No. 1 filter paper using a veronal buffer solution of 0·04 ionic strength at 8·6 *p*H. In a 20-hr. run at 150 volts and 1·5 m.amp. per in. of filter paper a 3 per cent hæmolysate of this embryonic hæmoglobin, prepared by Drabkin's[2] method, showed a definitely slower mobility compared with the electrophoretic mobility of adult hæmoglobin used as control as shown in Fig. 1.

Later on we discovered an embryonic hæmoglobin with an identically slow electrophoretic mobility in the blood of a 20-week old fœtus weighing 450 gm. The concentration of the alkali-resistant type of hæmoglobin in this blood was 52 per cent. The electrophoretic mobility of the embryonic hæmoglobin was compared both with that of hæmoglobin recovered from cordal blood of a full-term newly born with approximately the same concentration of alkali-resistant fraction and with adult hæmoglobin. The hæmoglobin of the 20-week old fœtus showed a distinctly slower mobility as compared with both the newly born's cordal blood and the adult hæmoglobin. A mixture of the embryonic and the newly born's cordal blood hæmoglobins showed an electrophoretic mobility between both, as shown in Figs. 2 and 3.

The fact that, on one hand, the hæmoglobin of the embryonic and of the newly born's cordal blood, containing the same percentage of the alkali-resistant type of hæmoglobin, moved with different mobilities, and on the other hand, the hæmoglobins of the two embryos (Fig. 3, *a* and *c*), although differing substan-