Ltd., Imperial Chemicals Ltd., K. W. Chemicals Ltd., A. H. Marks and Co. Ltd., and May and Baker Ltd.

Elsie Conway

Department of Botany,
University of Glasgow.
June 3.

J. A. Forrest*

West of Scotland College of Agriculture,
Glasgow.

* Present address : Baywood Chemicals Ltd.,

[1] Conway, E., *Scot. Agric.*, **31**, 1 (1952).
[2] Conway, E., and Stephens, R., *N.A.A.S. Quarterly Rev.*, No. 25, 1 (1954).
[3] McCreath, J. B., and Forrest, J. D., *West of Scotland Agric. Coll. Research Bull.*, No. 24 (1958).
[4] Braid, K. W., *Agri. Prog.*, **14**, 29 (1937).
[5] Braid, K. W., *J. Brit. Grasslands Soc.*, **2**, 181 (1947).
[6] Bates, G. H., *Brit. Rubber Publicity Assoc. Bull.*, **14**, 1 (1940).
[7] Conway, E., and Forrest, J. D., Proc. Third. Brit Weed Con. Conf., 255 (1956).
[8] Conway, E., and Forrest, J. D., Proc. Fourth Brit. Weed Con. Conf. (in the press).
[9] Conway, E., *Nature*, **177**, 1088 (1956).
[10] Fryer, J. D., Chancellor, R. J., and Evans, S. A., Proc. Fourth Brit. Weed Con. Conf. (in the press).
[11] Fryer, J. D., *Down to Earth*, **14**, No.4, 1 (1959).

HÆMATOLOGY

Two Hæmoglobins in Chum Salmon

Occurrence of multiple hæmoglobins has been reported recently in fish as well as in other vertebrates. In particular, Salmonidæ was found electrophoretically to contain fairly close amounts of two or more components[1,2]. Some physico-chemical properties of the two components of blood hæmoglobin in the salmon *Oncorhynchus keta*, which were prepared from hæmolyzate by starch zone electrophoresis, have already been reported[3]. The fast-migrating component in the physiological *p*H region (designated component *F*) was more labile to heat denaturation, less soluble in concentrated salt solution, and behaved less anionically in electrophoresis than another component (designated as component *S* in view of its slow migration). Their behaviour in column chromatographic and absorption spectra in the Soret region were also more or less different from each other.

The present communication deals with crystallization and several properties of the two components. Hæmolyzate from chum salmon blood saturated with carbon monoxide, was 0·35 saturated with ammonium sulphate at neutral *p*H and suctioned. The filtrate was 0·6 saturated with the salt and the resulting precipitates were collected, dissolved in distilled water, and the solution was made slightly turbid by adding ammonium sulphate at *p*H 6·8–6·9, and left standing overnight in a cold place, then the crystals of component *F* appeared in trapezoid or square-shaped form. From the filtrate of the solution, component *S* was crystallized by the similar procedure, but at slightly lower *p*H (about 6·3) and after slightly longer period of standing (usually 2–3 days). This component was obtained in clusters of needle-shaped crystals (Fig.1).

Both preparations, after recrystallized twice, were found to be practically homogeneous in moving boundary electrophoresis and then analyzed for sedimentation and diffusion constants in a phosphate buffer 0·1 *M*, *p*H 7·0. Molecular weight and frictional ratio were also calculated using these constants and assuming partial specific volume for this protein of 0·750. As seen in Table 1, there are appreciable differences in these values between the two components. The sedimentation and diffusion constants and the frictional ratio of component *F* are in a good agreement with those reported for vertebrate hæmoglobins[4], whereas those of component *S* are not. It is, however, a question whether the same relation holds for molecular weight or not, considering a fairly large variation in the values calculated by such means for mammalian hæmoglobins (63,000–76,000[5]). The axial ratio of component *S* is more than double that of component *F*, namely *S*10·2, *F*4·6 (as prolate) or *S*12·8, *F*4·8 (as oblate). Thus it was proved that both components are different in molecular shape as expected from the results obtained previously[3].

Table 1. Some physical properties of two components *F* and *S*

	Component *F*	Component *S*
Sedimentation constant, $s_{20,w}$ (*S*)	4·16	3·64
Diffusion constant, $D_{20,w}$ (cm.²/sec.)	$6·6\times10^{-7}$	$4·9\times10^{-7}$
Molecular weight, $M_{s,D}$	61,000	72,000
Frictional ratio, f/f_0	1·23	1·56

Another difference was observed also in the function as the respiratory pigment between them. Oxygen dissociation curve was measured in phosphate buffers of various *p*H's (7·75–6·6) for both preparations isolated from hæmolyzate by starch block zone-electrophoresis. The effect of *p*H on the oxygen dissociation curve was observed for component *F*; a decrease in *p*H of the solution from 7·75–6·6 resulted in such drastic lowering in oxygen affinity that most of the hæmoglobin remained reduced even in the atmospheric pressure. On the contrary, the oxygen affinity of component *S* was scarcely affected by such a change in *p*H. Details of this work will be published elsewhere.

We thank Prof. B. Maruo and Miss Y. Wadatsu for measuring the diffusion constants, and Dr. J. Koyama for carrying out the ultracentifuge analyses.

K. Hashimoto
F. Matsuura

Laboratory of Fisheries Chemistry,
Faculty of Agriculture,
University of Tokyo.
July 24.

[1] Hashimoto, K., and Matsuura, F., *Bull. Japan. Soc. Sci. Fish.*, **24**, 719 (1959).
[2] Buhler, D. R., and Shanks, W. E., *Science*, **129**, 899 (1959).
[3] Hashimoto, K., and Matsuura, F., *Bull. Japan. Soc. Sci. Fish.*, **24**, 724 (1959).
[4] Svedberg, T., and Pedersen, K. O., "The Ultracentrifuge", 357 (Oxford Univ. Press, Oxford, 1940).
[5] Neurath, H., and Bailey, K., "The Proteins", vol. 1, Pt. B., 634 (Academic Press, New York, 1953).

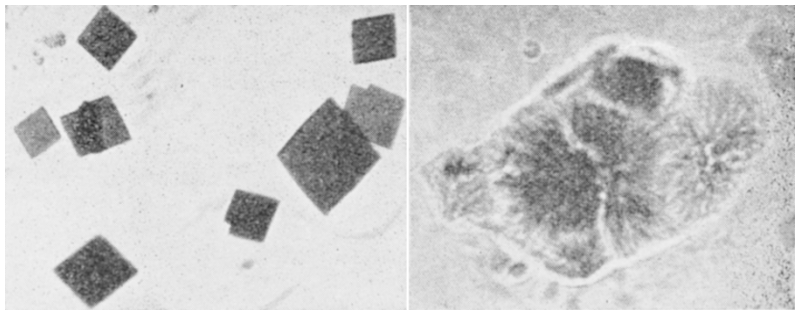

Fig. 1. Crystals of component *F* (left, ×75) and *S* (right, ×410) of salmon hæmoglobin

© 1959 Nature Publishing Group