chloramine-T (oxidant) in 10 μl phosphate buffer; 48 μg sodium metabisulfite (reductant) in 10 μl phosphate buffer. All reagents were added with micropipettes, with thorough mixing by bubbling through the pipettes. Generally less than 15 to 20 seconds elapsed between addition of insulin and completion of the procedure. The I^{131} employed in all our experiments was produced at the Sterling Forest Research Center and supplied through Iso/Serve, Cambridge, Mass.

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6. Densitometric readings were taken only in the region of strict linearity with radiation dose, as determined from measurements made on a test spot of radioactivity exposed for increasing periods. Agreement was excellent regarding the relative intensities of spots obtained from films after different exposures to the same gel. Since the various spots differ in width and trailing of radioactivity produces spurious enrichment of radioactivity in the more cathodal spots, the relative *intensities* are not measures of the relative *amounts* of I^{131} in the spots. However, the same spot usually shows about the same width in all samples and the amount of trailing does not vary appreciably.
7. At 10 atoms of iodine per molecule and higher ratios, iodination efficiency decreases and substitution asymptotically approaches 8 atoms of iodine per molecule. Each insulin monomer contains 4 tyrosine residues, each with two available sites for iodine substitution.
8. Increase in anodal mobility with increase in iodination of tyrosyl residues would be expected in view of the lower *p*K of the phenoxy group of diiodotyrosine than of tyrosine [*Handbook of Chemistry and Physics* (Chemical Rubber Publishing, ed. 38, 1956–7), p. 1649].
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12. After prolonged storage at −15°C various mammalian insulins dissolved in 0.005*M* HCl develop anodally migrating components in the unlabeled state, probably as a consequence of slow deamidation. Therefore only freshly prepared insulin solutions should be used for these studies.

5 January 1966

Hemoglobins M: Identification of Iwate, Boston, and Saskatoon Variants

Abstract. *Hemoglobin M variants, M Iwate, M Boston, M Saskatoon are easily and accurately identified by electron spin resonance with small amounts of patients' blood. In hemoglobin M Iwate and M Boston the electron spin resonance of both fresh blood (unprocessed) and isolated pure ferrihemoglobin M revealed similar signal shapes; whereas that of hemoglobin M Saskatoon was doublet in fresh blood and triplet in pure ferrihemoglobin M.*

A methemoglobin with a unique absorption spectrum was found by Hörlein and Weber (*1*) in a family whose members exhibited cyanosis. This abnormal hemoglobin, designated by Singer (*2*) as hemoglobin M (Hb M), is a genetically dominant character. Several investigators have shown that Hb M is heterogeneous with respect to certain physicochemical properties (*3*), and characteristic abnormalities of its amino acid sequence occur in some variants (*4*). The primary structures of the variants are represented as $\alpha_2{}^{58Tyr}\beta_2{}^{A}$ for Hb M Boston; $\alpha_2{}^{A}\beta_2{}^{63Tyr}$ for Hb M Saskatoon, $\alpha_2{}^{A}\beta_2{}^{67Glu}$ for Hb M Milwaukee-I, and $\alpha_2{}^{87Tyr}\beta_2{}^{A}$ for Hb M Iwate (*5*), respectively. Some variants of Hb M have been identified as belonging to one of above types: for example, Hb M Osaka to Hb M Boston (*6*), Hb M Kurume to Hb M Saskatoon (*7*) and Hb M Kankakee to Hb M Iwate (*8*). The structures of other variants, such as Hb M Chicago (*9*), Hb M Oldenburg (*10*), Hb M Leipzig-I (*11*), and so on, remain unknown.

Usually the classification of these variants is based on the difference of absorption spectra in the visible region, on their electrophoretic properties, and sometimes on their reaction rate with cyanide (*3*). These procedures demand pure material and very skillful technique, yet often fail to give accurate information. The primary structures of the variants have been determined by amino acid analysis after two-dimensional or column chromatographic separation had been made.

We now report the use of electron spin resonance (ESR) as a simple and accurate means for identification of Hb M Iwate, M Boston (Osaka), and M Saskatoon (Kurume).

Heparinized blood from the patients was used without further treatment. The blood (0.2 ml) was placed in a 3-mm quartz tube and inserted into the resonant cavity of Varian ESR spectrometer V-4500 (100 kcy/sec magnetic field modulation). The ESR was measured at liquid-nitrogen temperature, and the signal was recorded on the *Y*-axis of an *X*-*Y* recorder. The output voltage of a Hall-effect element, which was proportional to magnetic field intensity, was measured on the *X*-axis. The microwave frequency was measured by cavity wave meter for the determination of *g*-value.

The ESR signals in the region $g \approx 6.0$ are important because the ESR of Hb M variants revealed remarkable abnormality in this region; in the amorphous state the detection of the ESR in the region $g \approx 2.0$ was difficult owing to the low intensity of the signal. Hemoglobin of normal human blood is in ferrous state, so its ESR was not observed. In Hb M of patients' blood the ESR signal was strong at about $g \approx 6.0$ (Fig. 1), an indication of ferric hemoglobin (ferri-Hb). There are distinct differences among the Hb M variants, and the shapes of the curves appear to be characteristic of each variant. The shapes of Hb M Boston (Osaka) and M (Saskatoon Kurume) signals are doublet. The shape of Hb M Iwate signal, though it closely resembles that of ferri-Hb A, is also

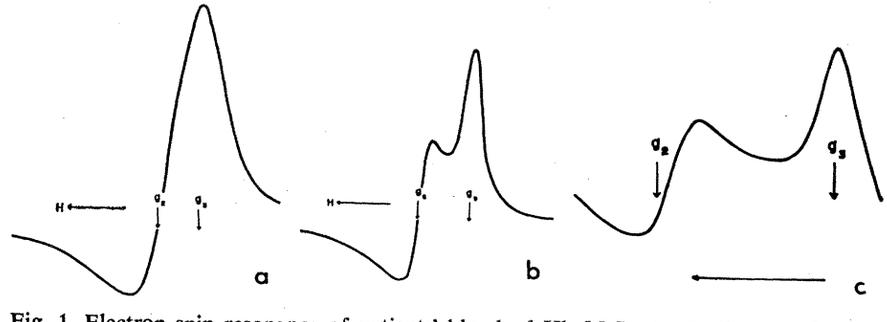

Fig. 1. Electron spin resonance of patients' blood of Hb M Iwate, M Boston (Osaka), and M Saskatoon (Kurume). Magnetic field modulation is 12 gauss and microwave power attenuation is 10 db. Horizontal arrow represents direction of magnetic field (110 gauss). Vertical arrows: left, g_2; right g_3. (*a*) Hb M Iwate, 9.1 × $10^{-3}M$, 160 times the sensitivity of the output of the ESR spectrometer. (*b*) Hb M Boston (Osaka), 9.0 × $10^{-3}M$; sensitivity 80 times. (*c*) Hb M Saskatoon (Kurume), 8.5 × $10^{-3}M$, sensitivity 400 times.

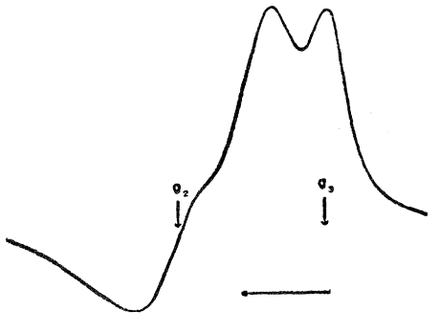

Fig. 2. Electron spin resonance of pure ferri-Hb M Saskatoon (Kurume). Magnetic field modulation is 12 gauss and microwave power attenuation is 10 db. Horizontal arrow represents direction of magnetic field. This material was examined at a hemoglobin concentration of $7.6 \times 10^{-3}M$ in $0.1M$ potassium phosphate buffer at *p*H 7.0 with 100 times the sensitivity of the output of ESR spectrometer.

considered a kind of doublet, because the peak of Hb M Iwate signal is shifted to the lower magnetic field than ferri-Hb A and a slight shoulder is at the higher side.

We have discussed the origin (*12*) and the theoretical basis (*13*) of the doublet signal in studying the chromatographically isolated ferri-Hb M Iwate and M Boston (Osaka). This doubtlet signal was attributed to splitting of a degenerated *g*-value of 6.0. The estimated values of g_2 and g_3 of Hb M variants calculated by the method of Kneubühl (*14*) were $g_2 = 5.80$ and $g_3 = 6.21$ in Hb M Iwate; $g_2 = 5.72$ and $g_3 = 6.26$ in Hb M Boston (Osaka); and $g_2 = 5.35$ and $g_3 = 6.53$ in Hb M Saskatoon (Kurume) respectively. Hence Hb M Iwate, M Boston (Osaka), and M Saskatoon (Kurume) can be easily and accurately identified by the ESR method.

In Hb M Iwate and M Boston (Osaka) ESR signals of both fresh blood and pure ferri-Hb M (*15*) were similar. However Hb M Saskatoon (Kurume) showed a doublet while the pure ferri-Hb M showed a triplet signal (Fig. 2). The signal between the doublet had the *g*-value of 6.0 as ferri-Hb A. This signal diminished by the addition of sodium azide at the concentration required for the formation of azide complex with the α-chain of ferri-Hb M Saskatoon (Kurume). It also diminished, like that of ferri-Hb A, above the temperature of liquid-nitrogen though the doublet signal showed no remarkable change (*16*). Therefore this signal is attributed to the heme, linked with the α-chain of Hb M Saskatoon (Kurume). In ferri-Hb M Iwate and M Boston (Osaka), the ESR signal due to heme linked with the β-chain should also have been revealed, but in fact it was not; and in this way the ESR of blood and of ferri-Hb M closely resembled each other. The absence of signals could result from the narrowness of the ESR signal of ferri-Hb M Iwate and M Boston (Osaka), especially that of abnormal heme of these hemoglobins: that is, when the absorption band is narrow, the peak height of ESR markedly increases, and the effect of the heme of β-chain becomes negligibly small. The recorded signal height of ferri-Hb M Iwate was about ten times larger than that of ferri-Hb A, and the intensity of ferri-Hb M Boston (Osaka) was about 20 times larger than that of ferri-Hb A (*12*).

Mention should be made of the ESR signal of Hb M Milwaukee-I, which was not available to us. The structure of this Hb M is represented as $\alpha_2{}^{A}\beta_2{}^{67Glu}$ and has similar physicochemical properties to Hb M Saskatoon (*17*). Therefore it is suspected that the shape of the Hb M Milwaukee-I signal might be similar to that of Hb M Saskatoon.

Furthermore it is expected that the ESR study of Hb M variants whose primary structures remain uncertain might furnish clues to their structures.

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7 February 1966

Bacteriophage T5 Chromosome Fractionation: Genetic Specificity of a DNA Fragment

Abstract. *The bacteriophage T5 DNA fragments retained by a population of blended early complexes, formed under conditions of limited viral DNA transfer to host cells, appear to include only one of six tested cistrons. When complexes harboring wild-type fragments are infected with appropriate amber mutants, recombination occurs but apparently is not needed for productive infection.*

When *Escherichia coli* is infected with bacteriophage T5 at high bacterial concentration (5×10^9 cells/ml), transfer of the phage DNA molecule to the host cell effectively stops after about 8 percent has been transferred (*1, 2*). Such arrested complexes are called FST (first-step transfer) complexes, and the transferred section of phage DNA, which is cut off and remains with the complexes upon vigorous stirring in a blendor, is called the FST section. Blended FST complexes are unable to form colonies and, except for rare survivors of blending, do not produce phage; but about 50 percent of the complexes can be superinfected (secondarily infected) productively with T5 (*3*). When unblended complexes are diluted (for example, to 10^8 cells per milliliter), the rest of the phage DNA is transferred after a short lag (*1*). Circumstantial evidence suggested that the FST section might contain structural genes for at least two proteins: one involved in breakdown of the host DNA (*4*), and the second needed to complete the transfer of the phage DNA molecule (*3*). We now report results of experiments that indicate that the FST section indeed carries at least