Particulate-Bound Pigment of *Serratia marcescens* (*Chromobacterium prodigiosum*)

The red pigments produced by the microorganism, *Serratia marcescens*, are usually regarded as being cellular and water insoluble[1]. However, some reports have stated that the pigments can diffuse from the cells, and thus might be regarded as being water soluble[2]. MONK[3] reported spectral studies carried out on a pigmented extract obtained from *S. marcescens* cells disrupted in a Mickle disintegrator. Similar preparations were made by LINNANE and STILL[4] employing a high-speed mechanical disintegrator. The latter investigators reported that the pigment was isolated as a protein complex. However, no details concerning the complex were given in their report. The present communication extends the observations of MONK[3] and of LINNANE and STILL[4], and presents evidence that the red pigment obtained from mechanically disrupted cells is bound to small particles.

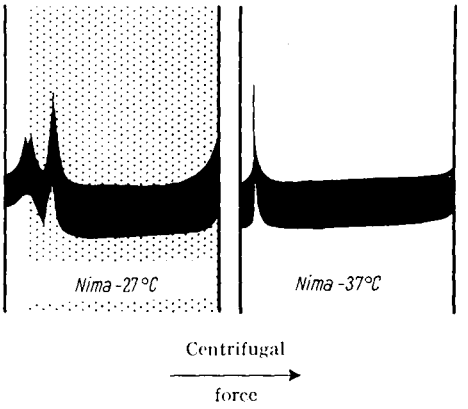
S. marcescens strain Nima was grown for 5 days on an agar medium[5]. The organisms were suspended in distilled water, and disrupted in a high-speed mechanical shaker[6]. Clarification of the resultant turbid material was carried out by step-wise centrifugation. The sediment remaining after each centrifugation was pigmented, but the supernatant remaining after treatment at $50\,000 \times g$ for 1 h was still a deeply pigmented, red color. This material showed no visible turbidity. However, when it was centrifuged at $105\,000 \times g$ for 2 h all of the pigment sedimented as a sharply circumscribed, red pellet. The supernatant was completely devoid of pigment.

The red pellet was washed, resuspended in distilled water, and analyzed in the spectrophotometer. In the visible region, the spectrum was identical to that reported by MONK[3], and showed maximum optical density at 495 mμ. A shoulder was present at 530 mμ, and the minimum was at 425 mμ. MONK[3] did not report the ultraviolet spectrum of his material. Our preparation exhibited a maximum peak in the ultraviolet region at 260–270 mμ. This absorbance probably represents protein and nucleic acid material present in the suspension.

Chemical tests carried out on the material established the presence of protein, lipid, carbohydrate, and nucleic acid substances. The pigment could not be dialyzed from the suspension, nor could it be separated from the sediment by paper electrophoresis. The latter procedure did not move the sediment.

In order to demonstrate the association of pigment with the sedimenting material, the organism was grown at 37°C. At this temperature no pigment was produced, and centrifugation at $105\,000 \times g$ of material prepared as outlined above produced a viscous, straw-colored sediment that did not show a sharp separation from the clear supernatant.

Washed preparations of material obtained from pigmented and colorless cells were examined in an analytical ultracentrifuge. The results are presented in the Figure. Pigmented material demonstrated three fractions, and the peak of one component coincided exactly with the pigment front. A second component sedimented more slowly than the pigment fraction while the third fraction was present within the pigmented area. The preparation obtained from colorless organisms exhibited only one component which appeared to be a slow-moving fraction when compared to the sedimentation pattern of the pigmented material. However, exact comparison between the two samples could not be carried out because the presence of pigment probably altered the sedimentation constants of the 27°C components.



Tracings of analytical ultracentrifuge patterns of $105{,}000 \times g$ preparations obtained from *Serratia marcescens* organisms grown at 27°C (pigmented) and at 37°C (colorless). The pictures were taken after the apparatus had reached a speed of 50,740 r.p.m. The stippled area represents the red pigment. Sedimentation proceeds to the right

Electron microscopic examination of washed material prepared from the sediment of pigmented organisms showed the preparation to be heterogenous and to consist of several sizes and shapes of particles. Similar material obtained from colorless organisms was homogeneous, and consisted of small, round particles.

Centrifugal studies have been carried out on cellular material obtained from several other *Serratia* strains which produced pigments bound to the cells. In all cases, an apparently clear red material prepared by mechanical disruption of the cells yielded a red pellet and colorless supernatant when centrifuged at $105\,000 \times g$ for 2 h.

The data presented indicate that the pigment obtained by mechanical disruption of certain strains of *S. marcescens* is bound to cellular particles. Similar associations between bacterial pigments and subcellular particles have been reported for *Streptococcus pyogenes*[7], *Corynebacterium* species[8], and *Rhodospirillum rubrum*[9]. The particles of the latter organism apparently function in the photosynthetic mechanism of the organism, but the function of the substances in the other two organisms is unknown. As has been demonstrated in *Serratia*, colorless strains of *Corynebacterium* species or *R. rubrum* lack particles similar to those associated with pigmented organisms.

The origin of the particles demonstrated in *Serratia*, as well as their function, and the nature of the bound pig-

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ment must still be elucidated. MONK[3] speculated that the orange fraction of *Serratia* pigment[5] might be bound to intracellular particles and to be of importance in cellular metabolism.

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Résumé

Le broyage mécanique de *Serratia marcescens* pigmentés produit un liquide rouge clair. Après centrifugation à 105000 × *g*, le pigment se sédimente sous forme d'un agglomérat rouge et la phase liquide reste incolore. La courbe du matériel de l'agglomérat remis en suspension montre à l'ultracentrifuge un seul sommet coïncidant avec le pigment. *S. marcescens* non pigmentés ne produisent pas d'agglomérat après broyage et centrifugation et donnent une courbe différente à l'ultracentrifuge.

Effect of Insulin on Potassium Transfer in Human and Chicken Erythrocytes[1]

Several observations suggest that insulin may increase the permeability of a variety of cell types to a variety of substances. For example, insulin stimulates the penetration of glucose[2] and of amino acids[3,4] into muscle cells and the efflux of aldolase from skeletal muscle[5], and modifies the membrane potential of muscle fibers[6]. In addition, it has been shown that intracellular potassium increases in liver, muscle, erythrocytes, and brain during hypoglycemia[7–11]. The experiments described in this paper were carried out to study if insulin modifies the loss of potassium from erythrocytes during cold storage and its partial re-entry following incubation at 37°C.

Blood samples were obtained from the antecubital vein of 4 normal human subjects, 1 untreated patient in diabetic coma, and from the heart of 4 normal white Leghorn chickens. The needle was removed from the syringe and the blood transferred carefully to flasks containing powdered heparin[12] and glucose (10 mg/ml) and stored at about 4°C. Agitation was avoided to minimize hemolysis and aseptic technic was used throughout. After 6 days of cold storage, 5 or 6 5 ml aliquots of each sample of blood were incubated in a Dubnoff shaker at 37°C moving at the rate of 50 oscillations/min, in an atmosphere of 95% O_2 and 5% CO_2. Ouabain[13] ($7{\cdot}2 \times 10^{-6} M$ in 0·1 ml) and/or glucagon-free insulin[14] (1 u in 0·1 ml) were added to the samples as indicated. Potassium was measured in the plasma with a Coleman flame photometer before and after incubation and the potassium uptake by the cells calculated by difference, according to the method of KAHN and ACHESON[15]. The volume of cells was measured by means of hematocrit determinations before and after incubation, in duplicate.

Table II

Effect of insulin and ouabain on potassium uptake by incubated human erythrocytes previously stored at 4°C for 6 days. Incubation time: 3 h at 37°C

	No. of experiments	Potassium Uptake (mEq/l of cells ± S.D)
Controls	4	+3·1 ± 0·76
Ouabain	4	−0·6 ± 0·35
Ouabain + Insulin .	4	−1·3 ± 0·34

Table I shows that normal human erythrocytes removed measurable quantities of potassium from the incubation medium, that the potassium uptake of the erythrocytes of a patient in diabetic coma was appreciably smaller and that the uptake of the nucleated red cells of the chicken was appreciably greater than that of normal human red cells. Insulin had no measurable effect on potassium uptake under the conditions of these experiments. Table II shows that ouabain blocked potassium uptake by incu-

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Table I

Effect of insulin on potassium uptake at 37°C by erythrocytes previously stored at 4°C for 6 days

		No. of experiments	Potassium Uptake (mEq/l of cells ± S.D.) Incubation Time, min		
			15	60	180
Human, Normal	Controls	8		1·4 ± 0·15	3·3 ± 0·86
	Insulin	8		1·4 ± 0·15	3·1 ± 0·74
Human, Diabetic	Controls	1			0·8 ± 0·006
	Insulin	1			0·8 ± 0·006
Chicken, Normal	Controls	6	3·4 ± 0·14	5·2 ± 2·61	
	Insulin	6	3·1 ± 0·33	5·3 ± 2·78	