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-

[1] Aided by a grant from the Chicago Heart Association.
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[12] Gift of Abbott Laboratories.
[13] Gift of Sandoz Chemical Works, Inc.
[14] Gift of Eli Lilly and Co.
[15] J. B. KAHN, JR. and G. H. ACHESON, J. Pharmacol. exper. Therap. *115*, 305 (1955).

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	

bated normal human erythrocytes and that this blocking action of ouabain was not modified by insulin.

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Riassunto

La conservazione al freddo degli eritrociti umani e di pollo per 6 giorni causa una fuoruscita di potassio dai globuli. Parte del potassio rientra nelle cellule durante incubazione a 37°C.

La uabaina blocca questo fenomeno. L'insulina non ha effetto significativo nè sull'assunzione di potassio da parte del globulo rosso, nè sull'azione bloccante della uabaina.

[16] Trainee, Diabetes Teaching Grant No. 2A-5102, National Institute of Arthritis and Metabolic Diseases, Public Health Service.

Glucose and Potassium Transfer in the Isolated Heart of *Venus mercenaria*[1]

The measurement of glucose uptake by the isolated rat diaphragm has been used for the determination of plasma insulin activity, but has several disadvantages. Among these are the difficult dissection, the variability of uptake from sample to sample, the relatively flat slope of the dose-response curve and the high cost. The heart of *Venus mercenaria*, the common sea clam, is a thin walled organ, of remarkably uniform size, cheap and easily obtained. It can be excised with little manipulation and without injury and continues to beat for 1 h or more when immersed in artificial sea water. Purpose of this work was to study the effect of insulin on the uptake of glucose and potassium by the clam heart *in vitro*.

Table I

Effect of insulin on glucose and potassium uptake by clam hearts incubated at 20°C in artificial sea water containing glucose

	No. of experiments	Glucose uptake (mg/g/h ± S.D.)	Potassium uptake (mEq/g/h/ ± S.D.)
Controls . .	10	2·08 ± 0·54	2·39 ± 0·40
Insulin . . .	10	2·15 ± 0·73	2·80 ± 0·58

Clams were obtained from local commercial sources and kept in a wet burlap sack at about 4°C. Live animals, which would tighten their shell shut when gently tapped, were cracked with a hammer. The heart was excised by cutting the large blood vessels, the piece of intestine which passes through it was slipped out gently and the heart was allowed to contract in artificial sea water of VAN'T HOFF[2], prepared without KCl. After changing the liquid three times to allow complete rinsing of the heart cavities, 2 hearts were placed in a 20 ml beaker with 4 ml of sea water containing KCl (K = 5 mEq/l) and glucose (2 mg/ml). To some beakers glucagon-free insulin[3] (0·5 u/ml) was added. In other experiments the hearts were immersed in sea water containing ouabain[4] ($12{\cdot}5 \times 10^{-4}$ mg/ml) for 1 h, before transfer to the incubation medium. Incubation was carried out in a Dubnoff shaker at 20°C in an atmosphere of 95% O_2–5% CO_2 and moving at the rate of 50 oscillations per min. After 60 min the hearts were removed, blotted on filter paper, and weighed on a torsion balance. Duplicate samples of the incubation medium were analyzed for glucose, according to NELSON[5] and for potassium with a Coleman flame photometer.

[1] Aided by a grant from the Chicago Heart Association.

[2] W. J. V. OSTEROUT, Bot. Gaz. *42*, 127 (1906).

Table II

Effect of insulin on potassium loss in pre-digitalized clam hearts incubated in artificial sea water containing glucose

	No. of experiments	Potassium loss (mEq/g/h/ ± S.D.)
Controls	8	1·48 ± 0·60
Insulin	8	1·52 ± 0·79

Table I shows that the uptake of glucose and potassium by the heart of the sea clam is measurable and relatively uniform and that it is not modified by insulin added to *in vitro*. Table II shows that pre-digitalized hearts lose potassium into the incubation medium and that this loss is not influenced by insulin.

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Department of Physiology and Pharmacology, The Chicago Medical School, Chicago 12 (Illinois), December 29, 1958.

Riassunto

Il cuore di *Venus mercenaria* consuma glucosio durante incubazione in acqua di mare artificiale. L'assunzione di glucosio è accompagnata da una penetrazione di potassio nell'organo. Cuori pretrattati con uabaina diffondono potassio nel medium di incubazione. L'insulina non modifica né l'assunzione di glucosio e potassio dei cuori normali né la diffusione di potassio dai cuori digitalizzati.

[3] Gift of Eli Lilly & Co.

[4] Gift of Sandoz Chemical Works, Inc.

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[6] Trainee, Diabetes Teaching Grant No. 2A-5102, National Institute of Arthritis and Metabolic Diseases, Public Health Service.

An Anticonvulsant Effect of Monoamine Oxidase Inhibitors

CHEN *et al.*[1] observed that reserpine, though a sedative, lowers the threshold for electroshock and pentylenetetrazol (Metrazol) convulsions in mice. The time course of the effect coincides roughly with the lowering of brain serotonin (5-hydroxytryptamine) and norepinephrine[2]. In contrast, phenothiazine tranquilizers do not lower the brain amines and have no clear-cut influence on electro-

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