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

D'AMICO[16] and P. P. FOÀ

Department of Physiology and Pharmacology, The Chicago Medical School, Chicago 12 (Illinois), December 5, 1958.

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.

D'AMICO[6] and P. P. FOÀ

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.

[5] H. NELSON, J. biol. Chem. *153*, 375 (1944).

[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-

[1] G. CHEN, C. R. ENSOR, and B. BOHNER, Proc. Soc. exp. Biol. Med. *86*, 507 (1954).

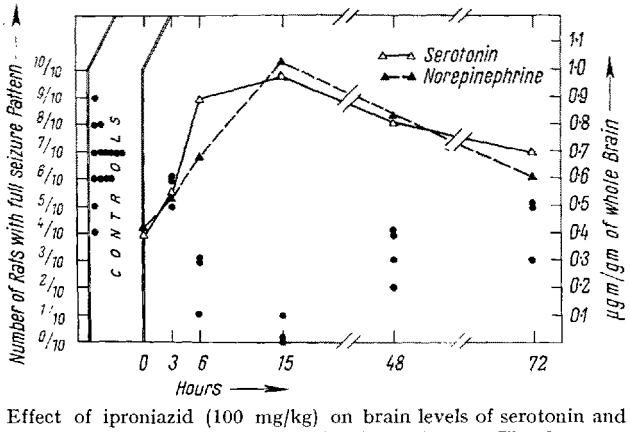
[2] B. B. BRODIE, J. S. OLIN, R. G. KUNTZMAN, and P. A. SHORE, Science *125*, 1293 (1957).

Effect of various drugs on electroshock seizures

	No. of rats with full seizure pattern	Mean latent period for tonic extension
	per group of 10	seconds
Controls	6·7 ± 1·23 S. D. (15 groups)	4·0 ± 1·19 S. D.
Iproniazid 100 mg/kg	1/10, 0/10, 0/10	—
JB 516 10 mg/kg	0/10, 0/10, 0/10	—
JB 807 50 mg/kg	0/10, 0/10, 0/10	—
Diphenylhydantoin 25 mg/kg	0/10, 0/10, 0/10	—
Reserpine 5 mg/kg	10/10, 10/10, 10/10	1·1 ± 0·05 S. D.
Isoniazid 25 mg/kg	5/10, 7/10	4·3 ± 1·32 S. D.
Isoniazid 100 mg/kg	10/10, 10/10, 7/10	2·5 ± 1·06 S. D.

shock convulsions[3]. Since the brain content of the amines is increased by monoamine oxidase inhibitors[4], it occurred to us that these drugs might antagonize evoked seizures.

Three irreversible monoamine oxidase inhibitors were studied: Iproniazid (1-isonicotinyl-2-isopropylhydrazine), JB 516 ([phenylisopropyl]-hydrazine), and JB 807 (1-[phenylisopropyl]-2-isopropylhydrazine). Rats received the drugs intraperitoneally and seizures were induced by supramaximal electroshock[5]. An anticonvulsant effect was defined as suppression of tonic extension of the hind legs, a criterion widely used in screening for antiepileptic drugs.



Effect of iproniazid (100 mg/kg) on brain levels of serotonin and norepinephrine and on electroshock seizures in rats. The drug was injected intraperitoneally into female Sprague-Dawley rats weighing 125–150 g. At various times thereafter, a 60 cycle AC current of 300 mA was applied through corneal electrodes for 0·3 s (2 to 3 times maximal shock). Each solid circle represents the number of animals per group of 10 showing tonic extension of the hind limbs. No animal was shocked more than once. Amine levels in whole brain were measured by previously described methods [6]. Each triangle represents the average value of six animals.

Iproniazid (100 mg/kg) suppressed the tonic extensor phase and raised levels of brain amine two to threefold. Both effects were maximal in 15 h and persisted for about two days (Fig.). JB 516 (10 mg/kg), a more potent and faster acting inhibitor[7], in 6 h also prevented the tonic extensor phase and produced a similar rise in brain amines. The anticonvulsant effect and elevation of brain amines gradually declined over two days. JB 516 had a cumulative action; for example, a single dose of 5 mg/kg showed little activity, but given daily for seven days exerted a pronounced effect. JB 807 (50 mg/kg) after 6 h was also anticonvulsant (Table) and had raised the brain amines. Isoniazid (isonicotinylhydrazine), a congener of iproniazid, did not elevate brain amines[4] and had no anticonvulsant effect 2 or 15 h after doses of 25 mg/kg. In doses of 100 mg/kg, the drug facilitated electroshock seizures, presumably due to its anti-pyridoxal action[8].

In contrast to the monoamine oxidase inhibitors, reserpine (5 mg/kg) in 2 h increased the response to electroshock and shortened the latent period for appearance of tonic extension (Table).

The converse effects of reserpine and monoamine oxidase inhibitors on electroshock convulsions suggest that their actions were not due to the drugs *per se* but were related to the opposite changes induced in brain amines. Further support for this view was obtained from experiments in which the combination of reserpine and iproniazid elicited opposite effects, depending on which drug was given first[9]. Rats were treated with reserpine 2 h before iproniazid. 2 h after the iproniazid administration the brain amines were depleted, and an enhanced response to electroshock typical of reserpine was noted. In other experiments animals were given reserpine 2 h after iproniazid. 2 h following the reserpine administration, an anticonvulsant action typical of iproniazid was observed and the brain amines showed little change from normal since they had been liberated when monoamine oxidase activity was blocked. Not only are these results in accordance with the view that reserpine and iproniazid do not act *per se* but they suggest that the level of free brain amines may be an important factor in the effects on electroshock.

Diphenylhydantoin (Dilantin) in doses of 100 mg/kg twice daily for four days has been reported to elevate brain serotonin levels in rats[10]. We observed that doses of 25–75 mg/kg blocked the tonic extension component of electroshock, but did not increase the brain amines. The anticonvulsant action of diphenylhydantoin in rats

[3] D. H. TEDESCHI, J. P. BENIGNI, C. J. ELDER, J. C. YEAGER, and J. V. FLANIGAN, J. Pharmacol. exp. Therap. *123*, 35 (1958).

[4] S. SPECTOR, D. J. PROCKOP, P. A. SHORE, and B. B. BRODIE, Science *127*, 704 (1958).

[5] J. E. P. TOMAN, E. A. SWINYARD, and L. S. GOODMAN, J. Neurophysiol. *9*, 231 (1946).

[6] D. F. BOGDANSKI, A. PLETSCHER, B. B. BRODIE, and S. UDENFRIEND, J. Pharmacol. exp. Therap. *117*, 82 (1956). – P. A. SHORE and J. S. OLIN, J. Pharmacol. exp. Therap. *122*, 295 (1958).

[7] A. HORITA, J. Pharmacol. exp. Therap. *122*, 176 (1958). – J. H. BIEL, A. E. DRUKKER, P. A. SHORE, S. SPECTOR, and B. B. BRODIE, J. Amer. chem. Soc. *80*, 1519 (1958).

[8] R. H. RIELLY, K. F. KILLIAM, E. H. JENNY, W. H. MARSHALL, T. TAUSIG, and N. S. APTER, J. Amer. med. Assoc. *152*, 1317 (1953).

[9] P. A. SHORE and B. B. BRODIE, Proc. Soc. exp. Biol. Med. *94*, 433 (1957).

[10] D. D. BONNYCASTLE, N. J. GIARMAN, and M. K. PAASONEN, Brit. J. Pharmacol. *12*, 228 (1957).

does not appear, therefore, to be associated with a measurable rise in brain amine levels.

The data presented here show that monoamine oxidase inhibitors elevate norepinephrine and serotonin brain levels and suppress the tonic extensor component of electroshock seizures in rats. In contrast, reserpine releases brain amines and facilitates electroshock seizures. The results do not permit the conclusion that the effects of the drugs are attributable to changes in serotonin or norepinephrine, but suggest the possibility that a substance released by reserpine and metabolized by monoamine oxidase is involved[11].

Further studies have demonstrated that JB 516 and JB 807 suppress the tonic extensor phase of convulsions evoked by intravenously administered pentylenetetrazol in mice. A detailed account of these results will be published elsenwhere.

The possible clinical usefulness of monoamine oxidase inhibitors in epilepsy is now under investigation.

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Laboratory of Chemical Pharmacology, National Heart Institute, U. S. Department of Health, Education, and Welfare, Bethesda (Maryland), December 11, 1958.

Zusammenfassung

Inhibitoren der Monoaminoxydase haben eine antikonvulsive Wirkung, die mit einer Erhöhung des Serotonin- und Noradrenalingehaltes im Gehirn verbunden ist. Da anderseits Reserpin durch Freisetzung zu einer Verminderung dieser Amine im Gehirn führt und die Krämpfe verstärkt, ist es wahrscheinlich, dass gewisse physiologisch aktive Amine bei der Entstehung experimenteller Konvulsionen eine entscheidende Rolle spielen. Die Resultate stützen die Annahme, dass gewisse Formen von Epilepsie auf eine lokale Störung im Stoffwechsel der Gehirnamine zurückzuführen sind.

[11] In preliminary experiments, harmaline, a reversibly acting monoamine oxidase inhibitor [12] was found to raise the brain levels of amines and block the tonic extensor phase of electroshock seizures in rats.

[12] S. Udenfriend, B. Witkop, B. G. Redfield, and H. Weissbach, Biochem. Pharmacol., in press.

* Fellow, Life Insurance Medical Research Fund.

Regeneration of Cartilaginous Matrix from the Dissociated Chondrocytes *in vitro*

Recently, it has been well established that the constituent cells of a precartilaginous blastema can, after dissociation, reaggregate into a chaotic assemblage and finally differentiate into organised cartilage[1]. However, if the dissociation of the cells is done in the stages before the onset of visible differentiation of cartilage, the second process, that is, the transformation of the initial reaggregates into organised cartilage, can be considered as essentially the same process as the differentiation of cartilage from the intact precartilage[2]. This provides, therefore, little direct information as to the relative importance of the intercellular matrix and the constituent cells in the maintenance of the tissue architecture. It seemed to the present author that useful information on this point might be obtained by studying the process of tissue reconstitution from differentiated chondrocytes after freeing them from the already deposited matrix. In this paper, the results of an experiment along these lines will be described.

Experimental. Suspensions of chondrocytes were made from femora and tibiae of chick embryos after 8 days' incubation. In this material, a considerable amount of cartilaginous matrix, which gives strong metachromatic staining with toluidine blue, is deposited intercellularly. The demarcation of diaphysial and epiphysial zones is quite clear in femora, and hypertrophy of chondrocytes is conspicuous.

The procedure of making cell suspension by trypsin digestion was essentially the same as that established by Moscona[3] except for slight modification[2]. With the present material, however, much more prolonged treatment by trypsin than that applied to the precartilaginous material was necessary, in order to obtain a soft mass which could then be pipetted with a small-bore pipette or syringe to make a cell suspension. Approximately 30 min pretreatment in Ca- and Mg-free saline, and a further one hour's incubation in the trypsin solution, proved adequate. The periosteum, which was developed as a thin conical cylinder around the diaphysis of the femur, was so resistant to enzymatic digestion that it remained as a hard sheath, even after such prolonged treatment. The final suspension did not, therefore, contain any cells of this tissue.

The description of the sequence of events which will be given below is based on 34 specimens fixed in various steps of the experiment, together with some smeared preparations of the discrete cells of the suspension. For the histological examination, alternative sections were made, half being stained with haematoxyline (Meyer or Heidenhain) and the other half stained with toluidine blue for the detection of cartilaginous matrix.

The observation of the smeared preparations of the discrete chondrocytes showed only the simple spherical cells which are commonly found in cell suspensions of any kind of embryonic tissue, without showing any structural characteristics of the initial chondrocytes, i. e. hypertrophic and flattened cells (Fig. A). Mortality of cells in the suspension was much higher than in suspensions made from earlier embryonic stages. A higher mortality of discrete cells obtained from older tissue was also described for nervous tissue[4]. The fatal effect of prolonged trypsin treatment and trauma by vigorous pipetting are likely to be the reasons for the high percentages of damaged cells.

Reaggregation of the discrete cells was tried in two ways: (1) The cultivation of the very slimy soft mass obtained after centrigugation of the suspension, or (2) the cultivation of a dense suspension within a hollow ground slide or solid watch-glass filled with the liquid medium. A mixture of modified Tyrode[5], horse serum, and embryo extract in equal proportions served as the culture medium.

During 24 hours' cultivation within the liquid medium, the isolated cells assembled spontaneously into small reaggregates, whilst the slimy aggregates formed by centrifuging lost their stickness and became more or less firm, probably owing to the contraction of a very viscous substance which surrounds the intact cells and appears first after centrifuging. These initial aggregated masses

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[3] A. Moscona, Exp. Cell Res. *3*, 535 (1952).

[4] M. Cavanaugh, Exp. Cell Res. *9*, 42 (1955).

[5] M. Jones and S. L. Bonting, Exp. Cell Res. *10*, 631 (1956).