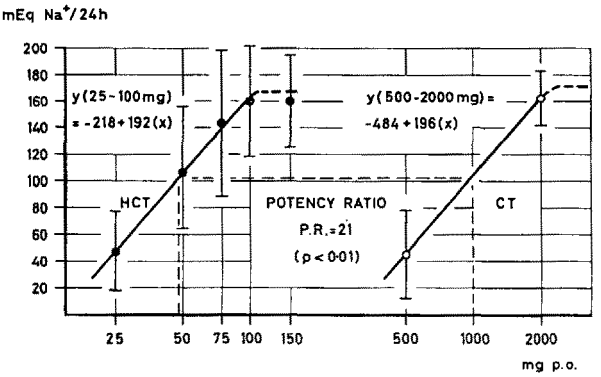
Natriuretic Potency of Hydrochlorothiazide[1] in Humans

Due to marked species differences, the therapeutic effectiveness of new diuretic agents can be estimated by clinical trial only. To eliminate subjective errors methods for human bioassay have been developed using a diuretic of known potency as a reference and body weight decrease in 24 h[2] or increase in daily sodium excretion as an index of diuretic potency[3]. If the dose of chlorothiazide[4], a non mercurial diuretic[5] is plotted on a logarithmic scale, a straight dose-response curve is obtained from 500 to 2000 mg[3]. Recently a new diuretic sulfonamide with similar structure[6], hydrochlorothiazide, has become available. Acute experiments in dogs and rats have shown it to be 4 to 16 times as potent as chlorothiazide[7]. Preliminary to clinical trials it was necessary to establish the potency of this new drug in humans.



Dose-response curves for hydrochlorothiazide (HCT) and chlorothiazide (CT) in humans (mean and standard deviation)

Ten patients suffering from mild right ventricular failure due to hypertensive or coronary heart disease, who needed at least intermittent diuretic therapy, were put on a diet with a constant sodium intake between 80 and 110 mEq/day. Sodium excretion during the control period varied between 70 and 105 mEq/day averageing 97 mEq/24 h. At 3 to 5 day intervals these patients received single doses of 25 (9 = number of assays), 50 (9), 75 (9), 100 (5), and 150 (5) mg of hydrochlorothiazide or 500 (5), 1000 (5), and 2000 (5) mg of chlorothiazide at 7 a.m. 24-h urines were collected and sodium excretion determined by flame photometry. Natriuretic potency was estimated by subtracting the average daily sodium excretion (97 mEq/day) from the individual responses.

The average increment in sodium excretion (mean and standard deviation) at different dose levels is shown in the Figure. If the doses are plotted on a logarithmic scale a straight dose-response curve is obtained between 25 and 75 (or 100) mg of hydrochlorothiazide and 500 and 2000 mg of chlorothiazide. 75 to 100 mg of hydrochlorothiazide induce a maximal response which cannot be further increased by additional amounts of the drug. For chlorothiazide the maximal response has been shown to occur with 2000 mg and, similar to hydrochlorothiazide, no further increase in natriuresis was noted with larger doses[3]. The potency ratio of hydrochlorothiazide to chlorothiazide as evaluated graphically (---) in the Figure is 21. Using the method of LITCHFIELD and WILCOXON[8] and 163 mEq increase in sodium excretion as a 100% response, it can be shown that the potency ratio for a 19/20 probability is 16 with confidence limits from 8 to 31. A detailed report of these experiments will be presented elsewhere[9].

From a physiological viewpoint it is interesting that the maximal response obtained with any dose of chlorothiazide and hydrochlorothiazide is quantitatively and qualitatively (electrolyte pattern in acute experiments in humans[9]) identical though hydrochlorothiazide is about 20 times as active as chlorothiazide. This probably means that both drugs inhibit sodium reabsorption at the same site and by the same mechanism of action. Enzymatic conversion of chlorothiazide to hydrochlorothiazide *in vivo*, similar to the hydrogenation of some steroid hormones might explain the discrepancy in diuretic effectiveness.

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Medizinische Universitätsklinik, Basel, 7. November 1958.

Zusammenfassung

Hydrochlorothiazide[1], ein oral verabreichbares Diuretikum, führt beim Menschen in Dosen von 25 bis 75 mg zu einer exponentiellen Zunahme der Natriumausscheidung. Eine maximale Diurese wird mit 75–100 mg erreicht. Das neue Pharmakon ist 20mal aktiver als Chlorothiazide[4].

[1] Hydrochlorothiazide is the generic name of a new diuretic agent manufactured by CIBA under the trade mark ESIDREX.

[2] T. GREINER and H. GOLD, J. Amer. med. Ass. *152*, 1130 (1953).

[3] R. V. FORD, J. H. MOYER, and C. L. SPURR, Arch. int. Med. *100*, 582 (1957).

[4] Chlorothiazide is the generic name of a diuretic agent manufactured by Merck, Sharp & Dohme under the trade marks CHLOTRIDE, DIURIL and CLOTRIDE.

[5] R. RICHTERICH, Schweiz. med. Wschr. *88*, 906, 931 (1958).

[6] G. DE STEVENS, L. H. WERNER, A. HALAMANDARIS and S. RICCA, JR., Exper. *14*, 463 (1958).

[7] To be published.

[8] J. T. LITCHFIELD and F. WILCOXON, J. Pharmacol. exp. Ther. *96*, 99 (1949).

[9] R. RICHTERICH, Klin. Wschr., in preparation.

Depolarizing Action of K-Strophantine and K-Strophantoside on Isolated Frog Skin

In past years many authors have worked on the potential in isolated frog skin using various drugs.

The action on the skin potential and water transport was studied with pitressin (CAPRARO and BERNINI[1], SAWYER[2], CAPRARO and TIENGO[3], BRAUN[4], KOEFOED-JOHNSEN and USSING[5]), epinephrine (USSING[6], CAPRARO and TIENGO[3], CAPRARO and FRANCESCHINI[7]), and some enzymatic inhibitors (i.e. acetazolamide: FUHRMAN[8], HUF *et al.*[9]), and more recently with serotonin (PICKLES[10]), Mersalyl, and theophylline (HUF *et al.*[9]).

[1] V. CAPRARO and G. BERNINI, R. C. Accad. Lincei Cl. Sci. fis. mat. e nat. [8] *11*, 385 (1951).

[2] W. H. SAWYER, Amer. J. Physiol. *164*, 44 (1951).

[3] V. CAPRARO and M. TIENGO, Arch. Sci. biol. *36*, 308 (1952).

[4] R. BRAUN, Naturwissenschaften *39*, 273 (1952).

[5] V. KOEFOED-JOHNSEN and H. H. USSING, Acta physiol. scand. *28*, 60 (1953).

[6] H. H. USSING, Acta physiol. scand. *19*, 1, 194 (1949a); *19*, 43 (1949b).

[7] V. CAPRARO and J. FRANCESCHINI, Exper. *8*, 142 (1952).

[8] F. A. FUHRMANN, Amer. J. Physiol. *171*, 266 (1952).

[9] E. G. HUF, N. S. DOSS, and J. P. WILLS, J. gen. Physiol. *41*, 397 (1957). – E. G. HUF, J. P. WILLS, and F. M. ARRIGHI, J. gen. Physiol. *38*, 867 (1955). – E. G. HUF and J. P. WILLS, J. gen. Physiol. *36*, 473 (1953).

[10] V. R. PICKLES, J. Physiol. *138*, 495 (1957).

According to these authors, the action of pitressin on the skin potential was not clear, while the active water transport seems increased; epinephrine depolarizes, and the active water transport is decreased or inverted.

Furthermore, the carbonic anhydrase inhibitors and serotonin respectively increase and decrease the potential. Mersalyl and theophylline, in low concentrations, stimulated active ion transport without leading to changes in the maintenance of electrolyte equilibrium.

From the literature, it is well known that the action of digitalis drugs is based mainly on the exchange of K^+ and Na^+ through the cellular membranes of muscular tissue (especially of myocardial tissue) (ROTHLIN and TAESCHLER[11]).

The present study was undertaken to determine the possible mechanism of digitalis drugs in other tissues than the muscular, by testing firstly the behaviour of frog skin potentials after treatment with K-Strophantine and K-Strophantoside.

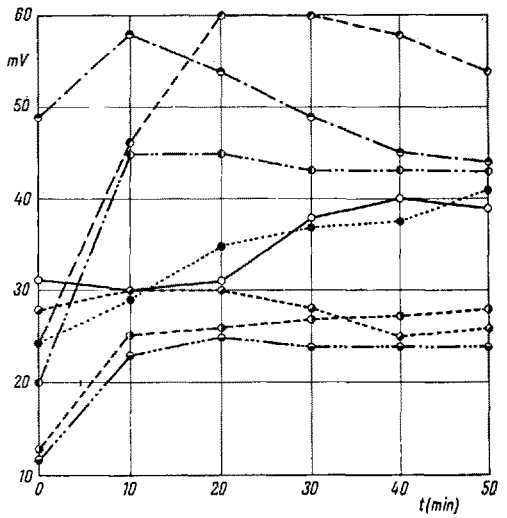

Fig. 1.—In this graph, the values of the potential of frog skin under normal conditions are reported. On the abscissae the time is shown and on ordinates the mV.

Method. All experiments were carried out on *Rana esculenta*. A portion of dorsal skin was employed for our purpose. The skin was mounted across a perspex chamber, which was divided into two sections. The edges were clamped between the two sections, separating the fluids outside and the inside the skin from each other.

Solutions. The outside bathing solution was represented by a NaCl solution of 6%; the inside was a normal Ringer solution for amphibians buffered to pH 7·38 with 10‰ of Sörensen buffer. The sections of the chamber was connected to electrodes by agar bridges. The potentials were measured by a Metrohm potentiometer at different times and at 28°C (the times and the potentials are reported in the graphs).

K-Strophantine and K-Strophantoside were added to the inside bathing solution after 20 min.

In our experiments, we used different doses of K-Strophantine and K-Strophantoside. The initial high doses (500–100 γ/ml) were gradually decreased in order to determine the minimum active doses (10 γ/ml of K-Strophantine and 5 γ/ml of K-Strophantoside).

[11] E. ROTHLIN and M. TAESCHLER, *Fortschritte der Kardiologie*, vol. 1 (S. Karger, Basel-New York 1956), p. 289.

Results. From the graphs, it can be seen that K-Strophantine, in 10 γ/ml concentration, and K-Strophantoside, in 5 γ/ml concentration, lead to an decrease of the skin potentials.

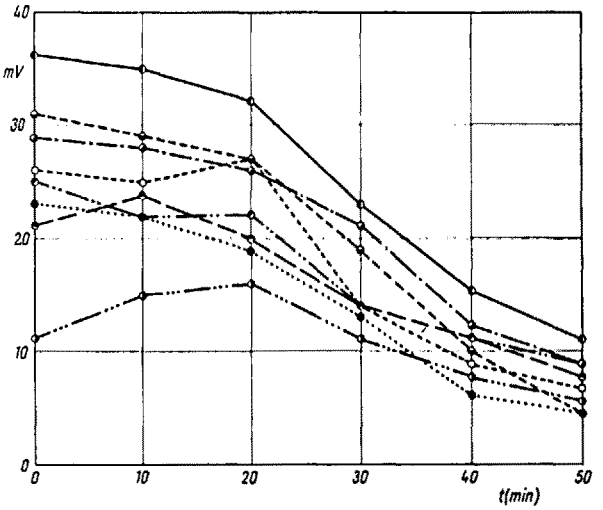

Fig. 2.—In this graph the values of potential after treatment at 20 min with 5 γ/ml of K-Strophantoside are reported.

It is well known that the chief cation of epithelial cells of frog skin is potassium. Outside the epithelial cells, the main cation is sodium.

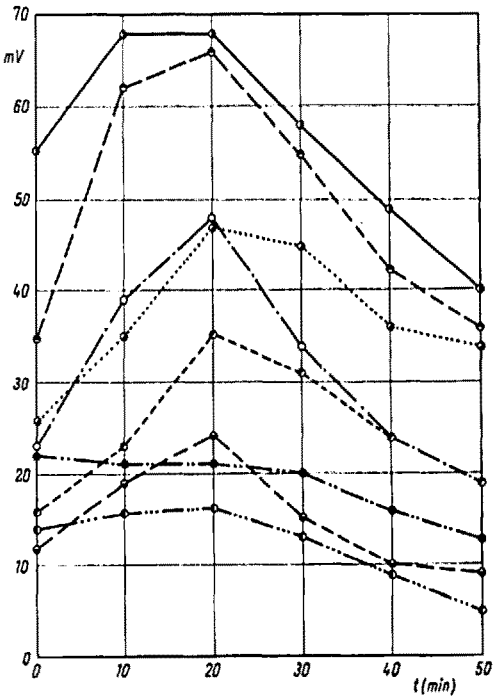

Fig. 3.—In this graph the values of potential after treatment with 10 γ/ml of K-Strophantine at 20 min are reported.

It is also known that, when the skin potential is low (e.g. mV 40), approximately one equivalent of K^+ appears at the epithelial side for each 85 equivalents of Na^+ that move towards the corion; in other words, there is no appreciable $K^+ \rightleftarrows Na^+$ exchange across the skin (low K^+ leakage). When the skin potential is high (e.g. mV 80) one equivalent of K^+ appears at the epithelial side for

each six equivalent of Na^+ that move towards the corion (high K^+ leakage) (HUF and WILLS[12]).

Such skin approaches the situation which prevails in nerve, muscle, and red cells in which metabolically coupled ion movements are dominated by a $K^+ \leftrightarrows Na^+$ exchange across the cell membrane (HARRIS[13], HODGKIN and KEYNES[14], KEYNES and ADRIAN[15], MAIZELS[16], SOLOMON[17], STEINBACH[18]).

The frog skin depolarization, which can be obtained under differents conditions, results from an active transport of Na^+ through the skin. We must argue, therefore, that K-Strophantine and K-Strophantoside act with the same mechanism as other drugs. Of course, to maintain the ionic equilibrium, the Na^+ entry must be compensated by an exit of the K^+ through cellular membrane, as occurs in the myocardial cells. Consequently the digitalis drugs act on the frog skin potentials by the same mechanism which works at the level of myocardial tissue. It suggests that this action of digitalis drugs must be fundamentally the same in all parts of the organism.

Hypothetically one might also think that, since the frog skin from the functional point of view is practically like the renal tubulus, the digitalis drugs act directly on the tubular epithelium. The hypothesis that digitalis drugs can act on the diuresis mechanism has always been rejected; on the basis of some recent results, howewer, this hypothesis can be re-accepted (FARBER *et al.*[19]).

It is very difficult, on the basis of our experiments, to confirm such a hypothesis; but we can certainly say that the striking depolarizating actions of the K-Strophantine and K-Strophantoside can be correlated to an exit of the K^+ ions and an active transport of Na^+ ions through the membrane of the epithelial cells of frog skin.

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Institute of General and Special Physiology of Domestic Animals and Biological Chemistry, University of Milan, Italy, July 29, 1958.

Résumé

Après une rapide revue de la littérature, les auteurs rendent compte des résultats de leur étude sur l'action dépolarisante de la strophantine K et du strophantoside K sur la peau isolée de la grenouille.

Les résultats démontrent, selon les auteurs, que la strophantine K et le strophantoside K ont la même action sur les potentiels de la peau de la grenouille que sur le myocarde. L'action dépolarisante peut être mise en corrélation avec la sortie des K^+ions et le transport actif des Na^+ ions dans la membrane des cellules épithéliales de la peau de la grenouille.

[12] E. G. HUF and J. P. WILLS, J. gen. Physiol. *36*, 473 (1953).
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[14] A. L. HODGKIN and R. D. KEYNES, J. Physiol. *120*, 46P (1953).
[15] R. D. KEYNES and R. H. ADRIAN, Disc. Faraday Soc. *21*, 265 (1956).
[16] M. LAIZELS, Symp. Soc. exp. Biol. *8*, 202 (1954).
[17] A. K. SOLOMON, J. gen. Physiol. *36*, 57 (1952).
[18] H. B. STEINBACH, Amer. J. Physiol. *167*, 284 (1951); Proc. nat. Acad. Sci., Wash. *38*, 451 (1952).
[19] S. J. FARBER, J. D. ALEXANDER, E. D. PELLEGRINO, and D. P. EARLE, Circulation *4*, 378 (1951).

The Spasmolytic Effect of Patulin

Patulin was first isolated from filtrates of a strain of *Penicillium patulum* Bainier and characterized by BIRKINSHAW *et al.*[1]. The substance has been studied mostly on account of its bacteriostatic effects but pharmacological data are very scanty. The LD_{50} for mice is about 25 mg/kg bodyweight after intravenous administration. AMBACHE has studied the effect of different lactones on the contraction of isolated hamster colon induced by irin[2]. He found that patulin blocked the effect of irin on this preparation.

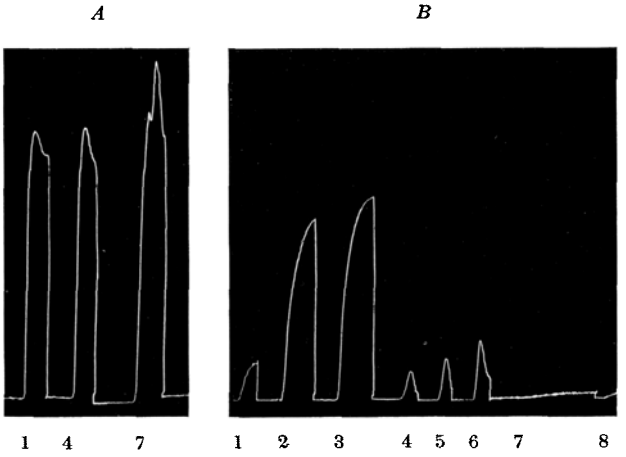

Fig. 1.—Isolated guinea-pig ileum. Bath volume 3 ml.
A Before patulin. *B* With 6·5 μg patulin per ml bath fluid.
Acetylcholine: 1 = 0·075 μg, 2 = 0·15 μg, and 3 = 0·20 μg.
5-HT: 4 = 5 μg, 5 = 10 μg, and 6 = 30 μg.
Prostaglandin: 7 = 0·04 units and 8 = 0·2 units

The modifying effect of patulin on the contractions of isolated guinea-pig ileum induced by different agents was tested in a 3 ml organ bath. The Tyrode solution was kept at 38°C and aerated with oxygen.

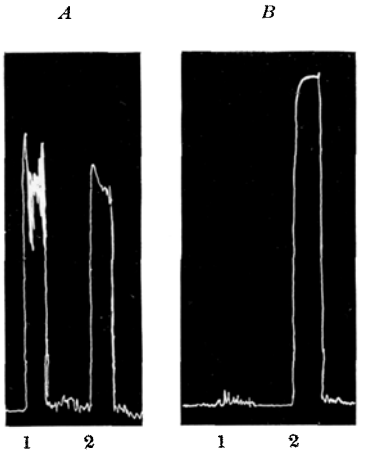

Fig. 2.—Isolated guinea-pig ileum. Bath volume 3 ml.
A Before patulin. *B* With 5 μg patulin per ml bath fluid.
Prostaglandin: 1 = 0·04 units; Substance P: 2 = 2 units

Patulin added to the bath fluid in concentrations of 3–10 μg/ml inhibited the contractions induced by nico-

[1] J. H. BIRKENSHAW, S. E. MICHAEL, A. BRACKEN, and H. RAISTRICK, Lancet *245*, 625 (1943).
[2] N. AMBACHE, J. Physiol. *140*, 24 P (1958).