that this recovery function is expecially good at a load of about 25% to 30% of the preload.

Comparing diagram Figure 1 with diagram Figure 2, we noticed a much better recovery function in the second test. It is caused by the high preload of 32 kg. In both of these two tests we went down to the starting level for the recovery which was 25% of the preload, and in both cases the speed going down was 2 mm/min.

In the 3rd experiment, which is shown in diagram Figure 2b, we now went down with a rate of 8 mm/min, whereas the other conditions of the test were the same as in the 2nd test. Now the recovery function is better than in the 2nd test.

These examples are taken from a large series of experiments and can be regarded as representative. The experiments were performed on the large toe extensor tendon, which was extirpated from the right foot of a 70-year old woman.

Conclusion and discussion. The tests Nos. 1, 2 and 3 allow us to conclude: 1. The shape of the recovery function written after we allowed the tendon to relax to about 25% of the preload is better, the higher the preload was. 2. The faster the tension is lowered, the more significant is the form of the recovery function.

The mechanical recovery function of the collagen fibers, which represent a viscoelastic, biohighpolymer body, are regarded by us as an essential property of them. Because this property always occurs when the tension is partly reduced, the collagen fiber, though it is not endowed with the capacity for active contraction, is able to raise its load and hold it at a certain level. In this way the consequences of the permanent use are, apart from the biological restitution, also mechanically compensated.

We made a number of biomechanical tests on the tendon of the M. extensor hallucis longus, which mainly consists of collagen fibers. It shows the property of an increase of load after the tension has partly been taken away, although the length is held constant. The mechanical recovery function depends on the speedrate with which the tension is reduced, and on the other hand, it depends on the absolute quantity of the preload. The recovery function is an essential property of collagen fibers and is important in reference to tendons, ligaments, bones, vessels and peripheral nerves – nevertheless not much attention has been paid to it.

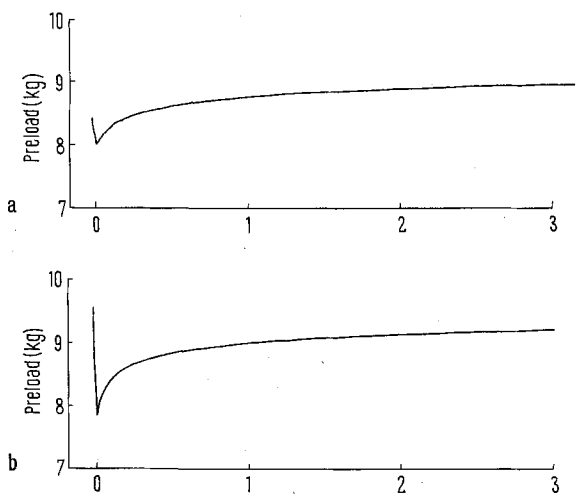

Fig. 2a (time recording). Here the preload reached 32 kg and we went down with a speed of 2 mm/min to the starting-point for the recovery. This starting-point – again 25% of the preload – was now located at about 8 kg.

Fig. 2b (time recording). The conditions were the same as in diagram Figure 2a, only the rate of oging down was now 8 mm/min.

Zusammenfassung. Es wird gezeigt, dass eine isolierte Sehne des M. extensor hallucis longus vom Menschen nach einer bestimmten Vorspannung, wenn diese auf $^1/_4$ reduziert wurde, bei gleichbleibender Länge eine Spannungszunahme aufweist.

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Effects of Lithium Chloride on Normal and Neoplastic Cells in vitro

The capacity of lithium ions to inhibit cell proliferation has been described for several cell types: fungi, bacteria, plants and mammals (for review see SCHOU[1]). Confirming these early works, DUBINI and BOLLOLI[2] observed recently the antimitotic action of LiCl on human leukocytes in vitro. Furthermore, GENEST and VILLENEUVE[3] reported a highly significant decrease of the mitotic index in manic-depressive patients treated with lithium. These data emphasize the need for a systematic re-evaluation of the antimitotic effect of lithium as this ion is now increasingly used as an effective drug in mania.

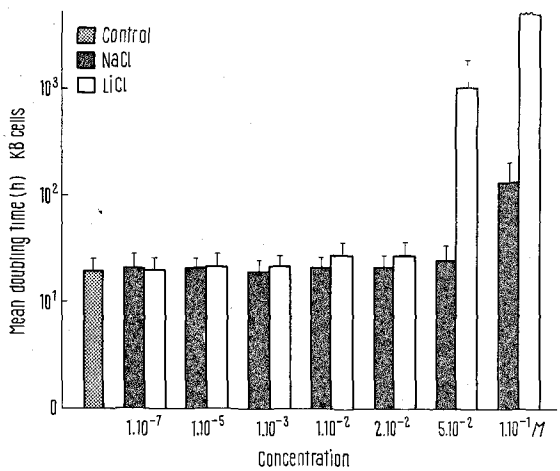
We report here preliminary results concerning the effects of increasing concentrations of LiCl ($1.10^{-7}M$–$1.10^{-1}M$) on the proliferation of normal Rhesus monkey kidney fibroblasts (RMK) and of neoplastic epidermoid KB cells cultivated in Eagle-Earle medium according to techniques described elsewhere[4]. The proliferation has been evaluated by enumeration of isolated cell nuclei and determination of mitotic index. The selective and differential fluorescence of both types of nucleic acids by acridine-orange has been used to investigate the possible action of LiCl on DNA and RNA. In order to control the eventual intervention of the anion (Cl-) and of the osmotic disturbance, the effects of LiCl were compared to those of equimolecular concentrations of NaCl ($1.10^{-7}M$–$1.10^{-1}M$) which were also additionally added to the medium. Complementary studies are in progress where the isotonic conditions are maintained by using original medium deficient in NaCl to which different mixing concentrations of NaCl and of LiCl are added to isotonicity.

[1] M. SCHOU, Pharmac. Rev. *9*, 17 (1957).
[2] F. DUBINI and A. BOLLOLI, Archo. ital. Patol. Clin. Tum. *12*, 79 (1969).
[3] P. GENEST and A. VILLENEUVE, Lancet *1*, 1132 (1971).
[4] J. HUOT, Gl. NOSAL and C. RADOUCO-THOMAS, Int. J. clin. Pharmac., *5*, 249 (1971).

The data presented in the Figure show the effects induced on the mean doubling time of neoplastic KB cells by equimolecular concentrations of LiCl and of NaCl. These are expressed by means of groups of 2 adjacent columns. The first column of each group presents the effects exerted by NaCl while the second shows comparatively those induced by an equiconcentration of LiCl. The osmotic pressure on the cultures is thus the same for 2 adjacent columns of the same group.

As compared to the control column (stippled), the results obtained show that increasing concentrations of LiCl added to the medium progressively reduce and inhibit the proliferation of the cells during the logarithmic phase of growth. The induced increase in the mean doubling time of treated cells is not significant for concentrations of LiCl $\leqslant 1.10^{-3}M$ but highly significant ($p < 0.001$; t-test) for $5.10^{-2}M$ LiCl. Concentations of LiCl $\geqslant 1.10^{-1}M$ are toxic even for the cells in interphase, causing their degeneration as shown here by the broken column. For NaCl, a significant effect is observed only at concentrations of $1.10^{-1}M$ (2.3 mg/ml Na^+). Furthermore, even at this concentration, the corresponding mean doubling time is still significantly lower ($P < 0.01$; t-test) than that observed with $5.10^{-2}M$ LiCl (0.346 mg/ml Li^+). This selectivity would be in accordance with the data of SAMOILOV[5] who has shown in vivo that the toxicity of lithium is 10 times higher than that of sodium.

In addition, for exposure higher than 24 h, LiCl $5.10^{-2}M$ would induce a decrease in cytoplasmic RNA as suggested by our cytochemical studies which showed that the intensity of the RNA characteristic reddish-orange fluorescence was reduced in LiCl-treated cells. This effect appears to be in general agreement with the recent results of DEWAR and READING[6].



Effects of LiCl and NaCl on the mean doubling time of neoplastic KB cells.

With the normal RMK cells the results are grossly similar to those obtained with KB cells. However, more studies are needed to evaluate quantitatively the differences between these two cell types with regard to their reactivity toward lithium.

The mechanism of the antimitotic action of lithium ions is still unknown. However, it could be related to some known effects of this cation (SCHOU[7]) such as extrusion of intracellular potassium or interference with oxydative phosphorylation and amino acid metabolism. An action through catecholamines should also be considered since lithium activates the desamination[7] of these mitogenic substances[8]. In addition, it is not impossible to assume an intranuclear accumulation of lithium and its eventual combination with the anionic chromatin, as suggested for high concentrations of sodium[9]. The observed effect could also be partially related to the lithium-induced decrease of cytoplasmic RNA we have noted, here or to its blocking effect on DNA polymerase[10]. Moreover, it would be of interest to investigate the effect of lithium on the mitotic spindle. Indeed, lithium is known to interfere with ciliary movements[7] while antimitotic agents such as colchicine which act by impeding spindle formation also block many kinds of ciliary motility processes.

Nevertheless, whatever is the mechanism, in agreement with the recent work of PEDERSON and ROBBINS[11], our results emphasize the important role of electrolytes in the physiological control of cell division and the need for further investigations concerning their effects at this crucial level.

Résumé. L'action du lithium (LiCl) a été étudiée comparativement sur des cellules néoplasiques humaines KB et normales de rein de singe. Les résultats obtenus démontrent que le LiCl en concentrations égales et supérieures à $5.10^{-2}M$ entraîne une inhibition significative de la prolifération des deux types cellulaires. D'autre part, pour des temps d'exposition supérieurs à 24 h, cet effet cytoinhibiteur s'accompagne d'une diminution de la quantité des RNA cytoplasmiques. Ces effets du LiCl apparaissent sélectifs par rapport à ceux du NaCl utilisés en concentrations équimoléculaires.

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27 September 1971.

[5] N. N. SAMOILOV, Russian Pharmac. Toxic. *33*, 266 (1970).
[6] A. J. DEWAR and E. A. READING, Psychol. Med. *1*, 254 (1971).
[7] M. SCHOU, Psychopharmac. Bull. *5*, 33 (1970).
[8] J. P. MACMANUS, J. P. WHITFIELD and T. YOUDALE, J. Cell. Physiol. *27*, 103 (1971).
[9] R. H. RIXON and J. F. WHITFIELD, Expl. Cell. Res. *26*, 591 (1962).
[10] C. C. BISHOP and J. E. GILL, Biochim. biophys. Acta *227*, 97 (1971).
[11] T. PEDERSON and E. ROBBINS, J. Cell. Biol. *47*, 734 (1970).

Natural Antibody Production in Human Tonsils

The tonsils, as lymphoepithelial organs, are known[1] to play an important role in the defence mechanism against invading bacteria and viruses. However, the actual function of tonsils has not been elucidated as yet. Recently[2,3], functional similarities between palatine tonsillar tissue and thymic tissue have been demonstrated. SURJAN and SURJAN[4] found antibody production in tonsils of parenterally immunized animals, showing a functional similarity between nonregional lymph nodes and tonsils.