suggesting other sites of action, possibly the spinal cord. Further work has to be performed to localize this effect.

Résumé. La destruction extensive de l'aire dépressive bulbaire provoque chez le chat une chute de la pression artérielle, du rythme cardiaque et une diminution de l'activité électrique du nerf splanchnique. La clonidine ne provoque alors qu'une augmentation de la pression artérielle, sans hypotension secondaire, ni diminution des potentiels splanchniques. La perte des influences sympatho-inhibitrices de la clonidine est encore plus nette chez le chien, car la destruction extensive de cette aire ne provoque qu'une diminution légère de la pression artérielle et de l'activité électrique du nerf splanchnique.

H. Schmitt, Hélène Schmitt and Simone Fénard

Faculté de Médecine Paris, Broussais Hôtel Dieu
15, rue de l'Ecole de Médecine
F–75 Paris 6ème (France), 30 April 1973.

Choline Activation of Lithium Transport

The therapeutic usage of lithium to treat manic depressives is well established[1–14]. Lithium carbonate is currently accepted as the standard treatment and typically is administered in daily amounts of from 1.5 to 3.6 g total. The dosages are usually given thrice daily[6] in 500 to 1200 mg amounts and result in a serum concentration of from 0.6 to 1.5 meq lithium/l.

While the general response to lithium treatment has been positive, a number of side effects have been reported. These include essential tremor, anorexia, vomiting and diarrhea[6]. Investigators have reported dermatitis[7], increases in thyroid size and iodide metabolism[8–11], EKG changes[6], and an increase in intracellular and extracellular water content[12]. Several reports[13,14] indicate that propanolol (Inderal) may be used to treat essential tremor unless the lithium is given concurrently with tricyclic anti-depressants[14]. Propanolol is known to inhibit cholinesterase activity and is used with this in mind.

In view of the aforementioned, it would seem beneficial to devise a system in which lithium transport was accelerated with the possibility of achieving similar therapeutic effects through lower dosage levels. Such a system is one in which choline facilitates the initial flow of lithium across bovine erythrocyte membranes as reported in this preliminary communication, the full details of which will be published elsewhere[15].

Material and method. Heparinized bovine blood, obtained by jugular puncture, was collected by centrifugation, washed twice with Normal Ringers buffer, and resuspended in 9 ml of buffer containing dextrose (0.2%) and choline iodide where appropriate. Suspensions were incubated at 37°C and 1 ml of lithium sulfate was added to initiate the reaction. At convenient intervals, 1 ml samples were withdrawn, centrifuged, and the supernatant separated. The pellets were treated with 1 ml of hemolyzing solution[16]. The resulting solution and the supernatant were analyzed separately for lithium content as before[16]. Efflux measurements were also performed as reported previously.

Results and discussion. A number of influx and efflux studies were completed at 37°C as a function of hematocrit. The data was clearly first order within a period of 30 min or less. The pseudo first-order rate constants were independent of hematocrit with k_e (efflux, 0.045 $\pm$ 0.001 min^{-1}) greater than k_i (influx, 0.035 $\pm$ 0.004 min^{-1}).

A cell volume study[15] in which choline was seen to shrink the average size of a bovine erythrocyte led to an investigation of the effect of choline on lithium influx, in which the total concentration of lithium and choline was kept at 4 mM in the initial supernatant. The influx rate constant increased from 0.052 $\pm$ to 0.064 $\pm$ 0.008 as the choline concentration was increased from 0.8 to 3.2 meq/l. When the lithium concentration is held constant (4 mM) and the choline concentration is increased from 4 to 12 mM, the value of k_i increased from 0.043 $\pm$ 0.005 to 0.075 $\pm$ 0.010. Specifically, $\log_e k_i = -3.37(\pm\ 0.07) + 0.068(\pm\ 0.008)$ [choline], where $\pm$ values indicate standard deviations. In addition, k_i was independent of the hematocrit (0.13–0.32) for a given choline concentration.

Previous workers[17–19] have studied the transport of choline across erythrocyte membranes. Askari[17] has compared choline uptake with potassium by means of Michaelis kinetics. His results include a larger V for potassium and a smaller K_m for choline which were interpreted as indicating fewer binding sites for choline and a higher site affinity for choline.

Martin[18] noted that choline transport obeyed first-order kinetics for periods of 10 min or more at low hematocrit values (0.02–0.03). He also established that there is no apparent adsorption of choline to the cell membrane and that ouabain (5×10^{-5} M) had no effect on choline transport. Using the data from Table I and Figure 7 from[18] it is possible to calculate values for k_e (0.035) and k_i (0.023) in min^{-1} for choline.

1 N. Diding, J. O. Ottoson and M. Schou, Acta Psychiat. scand., Suppl. *207*, p. 49 (1969).
2 N. S. Kline, in *Modern Problems of Pharmacopsychiatry* (Eds. F. A. Freyhan, N. Petrilowitsch and P. Pichot, Karger, Basel 1969), vol. 3, p. 1.
3 P. C. Baastrup, J. C. Poulsen, M. Schou, K. Thomsen and A. Amidisen, Lancet *2*, 326 (1970).
4 A. Coppen, R. Noguera, J. Bailey, B. H. Burns, M. S. Swani, E. H. Hare, R. Gardner and R. Maags, Lancet *2*, 275 (1971).
5 R. P. Hullin, R. McDonald and M. N. E. Allsopp, Lancet *1*, 1044 (1972).
6 C. Larson, M. S. Kochar and R. I. H. Wang, J. clin. Pharm. *12*, 459 (1972).
7 R. E. Posey, J. Am. med. Ass. *221*, 1517 (1972).
8 J. H. Lazarus and E. H. Bennie, Acta endocr. *70*, 266 (1972).
9 M. Schou, A. Amdisen, S. E. Jensen and R. Olsen, Br. med. J. *3*, 710 (1968).
10 G. Sedvall, B. Jonsson, U. Pettersson and K. Levin, Life Sci. *7*, 1257 (1968).
11 T. B. Cooper and G. M. Simpson, Curr. Ther. Res. *2*, 603 (1969).
12 A. Coppen and D. M. Shaw, Lancet *2*, 805 (1967).
13 H. Pakkenberg, Lancet *1*, 633 (1972).
14 L. Kirk, P. C. Baastrup and M. Schou, Lancet *1*, 839 (1972).
15 W. R. Carper, D. D. Stoddard and D. F. Martin, to be published elsewhere.
16 D. F. Martin, M. G. Heyl and M. T. Doig, III, Life Sci. *12*, 241 (1973).
17 A. Askari, J. gen. Physiol. *49*, 1147 (1966).
18 K. Martin, J. gen. Physiol *51*, 497 (1968).
19 K. Martin, J. Physiol. *224*, 207 (1972).

In a recent study, MARTIN[19] reported the effects of various cations on choline influx, noting that the net transfer of choline obeyed first-order kinetics during the first h. Using the data in Table I from [19], we note that the presence of lithium increases the pseudo first-order rate constant of choline to 0.042 min^{-1} and that this value is independent of choline concentration over the range studied.

In a final series of duplicate efflux studies, we obtained a k_e of 0.085 min^{-1} wherein the supernatant contained a choline concentration of 12 meq/l. Thus we note that choline increases lithium's normal transfer rate for both influx and efflux[20].

Zusammenfassung. Nachweis einer Erhöhung des Li-Austausches in Rinder-Erythrozyten bei Anwesenheit von Cholinchlorid im Extrazellularraum.

W. R. CARPER[21], D. D. STODDARD and D. F. MARTIN

Department of Chemistry, University of South Florida, Tampa, (Florida 33620 USA), 11 May 1973.

[20] This work was supported in part by a grant from the National Institutes of Health, a National Institutes of Mental Health Special Fellowship to W.R.C., and a Public Health Service Career Development Award to D.F.M.

[21] Permanent address: Department of Chemistry, Wichita State University Wichita, Kansas 67208, USA.

A Comparative Study of Some Anti-Inflammatory Drugs in Wound Healing of the Rat

Anti-inflammatory drugs are widely used in the treatment of various inflammatory skin disorders[1,2]. The rate of wound healing is invariably depressed by these agents[3,4]. A comparative study between steroid and non-steroid anti-inflammatory drugs in wound healing is not yet available.

This study reports the effect on wound healing of phenylbutazone, dexamethasone and a new non-steroid anti-inflammatory drug: 10 undecen-1-thiopseudourea iodide, AHR-1911[5]. This latter drug has been recently reported to be useful in the treatment of several inflammatory skin diseases[6,7].

Materials and methods. We have used albino rats, of either sex, weighing between 300 and 350 g. Under ether anesthesia, an incision 4 cm long was made with a scalpel through the skin of the back previously shaved. The incision was sutured with separate stitches about 0.5 cm apart. A group of rats were adrenalectomized and maintained on normal saline.

The tensile strength of the wound was determined 4 and 6 days after wounding with a tensiometer designed in our laboratory according to the principles already followed by other authors[8]. A brief report about this tensiometer has been previously published[9].

The amount of hydroxyproline was determined in unwounded skin and in wounds 6 and 12 days after operation. Preparation of skin samples was performed according to the method followed by SANDBERG and ZEDERFELDT[10]. Hydroxyproline concentration was measured by the method of MITOMA et al.[11]. Color measurement was determined in a Klett-Summerson photo-electric colorimeter using green filter. Our recoveries ranged between 94–102% for amounts of hydroxyproline added to samples in the range of concentrations studied. Statistical analysis was performed by the Student-*t*-test for non-paired groups[12].

Table I. Effect of phenylbutazone, dexamethasone and AHR-1911 on wound healing of the rat

Treatment	Tensile strength of the wound (g)[a]	
	4th day	6th day
Control	114 ± 3.09	216 ± 5.83
Phenylbutazone, 50 mg/kg	70 ± 2.90 (–39)	139 ± 8.70 (–36)
Dexamethasone, 1 mg/kg	84 ± 2.68 (–26)	155 ± 2.24 (–28)
AHR-1911, 50 mg/kg	92 ± 2.00 (–19)	177 ± 2.51 (–18)

[a] Mean ± S.E. for 5 to 6 experiments. Values for treated groups are different from controls, $P < 0.001$. Percent change of controls in parenthesis.

Table II. Effect of AHR-1911 on wound healing of adrenalectomized rats

Treatment	Tensile strength of the wound (g)[a]	
	4th day	6th day
Control	114 ± 3.09	216 ± 5.83
Adrenalectomized rats	135 ± 4.32[c] (+18)	250 ± 7.88[d] (+16)
AHR-1911, 50 mg/kg	92 ± 2.00[b] (–19)	177 ± 2.51[b] (–18)
AHR-1911, 50 mg/kg Adrenalectomized rats+	98 ± 5.32[d] (–14)	190 ± 7.30[d] (–12)

[a] Mean ± S.E. for 5 to 6 experiments. Values for treated groups are different from controls as follows, [b], $P < 0.001$; [c], $P < 0.01$ and [d], $P < 0.05$. Percent change of controls in parenthesis.

[1] E. W. ROSENBERG, Archs Derm. *104*, 632 (1971).
[2] C. A. SCHLAGEL and J. I. NORTHAM, Proc. Soc. exp. Biol. Med. *101*, 629 (1959).
[3] E. L. HOWES, C. M. PLOTZ, J. W. BLUNT and C. RAGAN, Surgery *28*, 177 (1950).
[4] H. P. EHRLICH and K. H. THOMAS, Ann. Surg. *167*, 324 (1968).
[5] N. ERCOLI, J. ARBONA and E. TABERNERO, Proc. Soc. exp. Biol. Med. *136*, 1328 (1971).
[6] C. J. RIOBUENO, Curr. ther. Res. *12*, 718 (1970).
[7] J. DI PRISCO, Curr. ther. Res. *11*, 634 (1969).
[8] P. SANDBLOM, P. PETERSEN and A. MUREN, Acta chir. scand. *105*, 252 (1953).
[9] M. VELASCO y E. ROMERO, Acta cient. venez. *21*, Suppl 1, 38 (1970).
[10] N. SANDBERG and B. ZEDERFELDT, Acta chir. scand. *126*, 182 (1963).
[11] C. MITOMA, T. E. SMITH, J. D. DAVIDSON, S. UDENFRIEND, F. M. DA COSTA and A. SJOERDSMA, J. clin. Lab. Med. *53*, 970 (1959).
[12] G. W. SNEDECOR and W. G. COCHRAN. *Statistical methods*, 6th edn. (Iowa State University Press, Iowa City 1971).