weight there was no apparent correlation between recent weight changes and the efficiency of absorption. A number of animals were discarded because of tumors and others because of a chronic respiratory disease which was the most prevalent health problem.

Summary. Absorption of an oral dose of 120 μg of C^{14}-labelled thiamine by the rat has been studied as a function of age and the state of thiamine nutrition. Approximately 95% was absorbed up to an age of 19-20 months, when the efficiency declined to about 75% at 22-24 months. Absorption by thiamine-deficient animals was not different from that of rats receiving normal or excess quantities of thiamine in the diet. The proportion of absorbed radiothiamine found in esterified and unesterified forms in the livers of rats 22-24 months old was less than that in rats 5-8 months old.

1. Mills, C. A., Cottingham, E., Taylor, E., *Arch. Biochem.*, 1946, v9, 221.
2. Mills, C. A., *Am. J. Physiol.*, 1948, v153, 31.
3. Friedemann, T. E., Kmieciak, T. C., Keegan, P. K., Sheft, B. B., *Gastroenterology,* 1948, v11, 100.
4. Kirk, J. E., Chieffi, M., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 464.
5. Draper, H. H., Robbins, A. F., *ibid.,* 1956, v91, 174.
6. Westenbrink, H. G. K., Steyn-Parvé, E. P., *Intern. Rev. Vitamin Res.,* 1950, vXXI, 461.
7. Siliprandi, D., Siliprandi, N., *Biochim. et Biophys. Acta,* 1954, v14, 52.
8. Hanes, C., Isherwood, F., *Nature,* 1949, v164, 1107.
9. Rossi-Fanelli, A., Siliprandi, N., Fasella, P., *Science,* 1952, v116, 711.
10. Rossi-Fanelli, A., Siliprandi, N., Fasella, P., Siliprandi, D., Salvetti, M., *Experientia,* 1954, v73, 73.
11. Mameesh, M. S., Schendel, H. E., Norton, H. W., Johnson, B. C., *Brit. J. Nutr.,* 1956, v10, 23.
12. McCarthy, P. T., Cerecedo, L. R., Brown, E. V., *J. Biol. Chem.,* 1954, v209, 611.
13. Iacono, J. M., Johnson, B. C., *J. Am. Chem. Soc.,* 1957, v79, 6321.
14. Watanabe, T., *Vitamins* (Japan), 1951, v4, 353.
15. Magyar, I., Gábor, G., *Intern. Z. Vitaminforsch.,* 1949, v21, 1.

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Chlorpromazine Blockade of Water Imbibition by Frog Gastrocnemius. (23665)

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The imbibition of water by frog gastrocnemius muscle has been studied extensively by Halpern and Reuse(4) and by Courvoisier and Ducrot(1), in an attempt to determine the mechanism of action of various drugs, chiefly phenothiazine derivatives, in blocking water uptake by this tissue. These investigators were able to rule out antihistaminic and local anesthetic potency as actions capable of being correlated with water imbibition block. Van der Kloot(6) has measured rate of sodium extrusion from frog gastrocnemius in hyponatric media and has related this to cholinesterase activity, but has not correlated the phenomenon with water transport.

Since phenothiazine compounds possess other pharmacologic properties than those mentioned above, it has seemed of interest to us to examine selected structures of this type for correlation of these activities with water imbibition block. In the course of this work, a number of additional non-phenothiazine compounds were used for activity analogy in elucidation of the phenomenon.

Methods. The technic was essentially that of Halpern and Reuse(4), modified as follows: Gastrocnemius muscles were removed as rapidly as possible after pithing of the frogs, rinsed in distilled water, blotted and weighed. They were then assigned at random to beakers containing 45 ml of distilled water containing either no drug or the drug under examination. The drugs were used in a final concentration of 0.0016 M as employed by

TABLE I. Influence of Drugs on Water Imbibition and Ion Loss.

Compound	Salt	Molarity	Increment ratios 1 hr Wt	1 hr Na	1 hr K	2 hr Wt	2 hr Na	2 hr K
Chlorpromazine (17)	HCl	.0016	29.8	113.7	176.6	15.2	122.2	182.1
" + ATP (3)	HCl – phosphate	"	42.8		305.8	30.7		731.0
Promazine (3)	HCl	"	47.0	134.4	279.0	26.9	169.0	340.8
CPZ + epinephrine* (3)	HCl + bitartrate	.0008	50.5	105.3	188.3	34.1	137.5	237.8
Prochlorperazine (3)	Dimaleate	.0016	51.1	120.4	221.1	55.0	125.8	208.1
Chlorpromazine (6)	HCl	.0008	53.7	117.6	156.7	37.7	132.8	174.8
Promethazine HCl (5)	Dihydrochloride	.0016	55.4	129.0	177.0	36.0	186.0	216.0
Ethopropazine (3)	"		62.6	122.6	197.9	37.9	116.0	218.7
CPZ + Marsilid + (5) epinephrine*	HCl + phosphate + bitartrate	.0008	64.4	129.3	153.8	53.2	157.7	187.2
Methscopolamine bromide (5)	Bromide	.0016	65.9	113.0	97.0	70.8	129.0	111.0
Phentolamine HCl (5)	HCl	"	66.2	117.0	124.0	55.2	144.0	153.0
CPZ + Marsilid (6)	HCl + phosphate	.0008	66.6	109.0	145.6	62.9	117.5	179.1
Perphenazine (5)	HCl	.0016	69.8	123.0	171.0	85.1	140.0	233.0
Chlorpheniramine (5)	Maleate	"	73.8	88.0	109.0	66.5	149.0	135.0
Dinitrophenol (3)		"	80.1	97.7	131.0	73.4	97.3	152.3
Phenoxybenzamine (2)	HCl	"	81.1	101.0	123.0	115.1	107.0	152.0
Azapetine (3)	Phosphate	"	85.2	100.0	91.0	83.8	102.0	121.0
Marsilid (6)	"	.0008	85.7	107.8	107.5	83.9	110.1	102.3
Saline (5)		.0016	87.0		103.0	97.8		116.0
Marsilid + epinephrine* (6)	Phosphate + bitartrate	"	89.1	77.6	99.5	100.0	114.9	116.9
Aminopyrine (5)		"	88.2	108.0	105.0	93.0	147.0	126.0
Marsilid (5)	Phosphate	"	90.0	117.3	113.3	104.0	123.5	113.5
Prednisolone (3)	Hemisuccinate	"	92.5		108.8	90.6		107.2
Phenoxybenzamine (3)	HCl	.0008	93.9	76.0	116.5	97.3	78.8	100.9
Epinephrine (9)	Bitartrate	10^{-7}	96.2	109.3	107.4	100.0	109.7	116.0
Acetazolamide (5)		.0016	102.1	102.3	120.4	91.5	84.9	101.7
Phenylbutazone (5)	Na salt	"	104.0		98.0	107.2		109.0
Adenosine triphosphate (3)	Phosphate	"	106.8		128.9	99.4		149.9
Physostigmine (3)	Sulphate	"	108.7	92.3	98.6	107.9	85.0	89.9
Amphetamine (3)	"	"	111.0	95.0	77.0	111.5	93.0	118.0
Phenoxybenzamine (3)	HCl	.0032	114.3	128.8	181.2	133.3	111.5	161.7

No. in parentheses indicate No. of experiments. CPZ = Chlorpromazine.
* Molarity of epinephrine = 10^{-7}.

Courvoisier and Ducrot(1) except in a few cases wherein more concentrated or dilute solutions were examined. The 0.0016 Molar (0.05% approx.) concentration was found by these workers to be the most effective of those tried. Each beaker contained 2 muscles from different frogs. Samples of the medium (5 ml) were removed at zero time for sodium and potassium determinations by the use of a Beckman DU spectrophotometer equipped with flame photometry attachment. The filled beakers were then shaken at room temperature in air in a Dubnoff apparatus for 45 minutes after which time samples of medium were taken for ion determinations and the muscles were blotted and weighed in random sequence. They were then returned to the shaker and the weighing and sampling procedure repeated after an additional 45 minutes of shaking. The data were treated as follows: The weights of the 2 muscles on any treatment were averaged and the percentage increment during the first and second hours calculated. This average percent increment was divided by that obtained from the control muscles in water alone and the result expressed as the following ratio: $\frac{\Delta\ \%\text{ with drug}}{\Delta\ \%\text{ without drug}} \times 100$. It will be seen that absence of drug effect will result in a ratio close to 100. Since the initial ion concentrations were negligible, similar ratios for ion accumulation in the medium were calculated directly using the increments expressed in milliequivalents/liter rather than percentage increments. Thus the ratio becomes: $\frac{\Delta\text{ meq with drug}}{\Delta\text{ meq without drug}} \times 100$.

Results. All of these results were ranked in order of increasing weight ratios in Table I.

Halpern has shown a lack of correlation of block of water imbibition with antihistaminic activity. This has been corroborated, since of the 3 antihistamines tested, chlorpromazine, promethazine, and chlorpheniramine, the latter although most potent in antihistaminic action, is least active in blocking water uptake in this preparation.

It will be seen that the least water imbibition occurred in muscles treated with chlorpromazine. Aside from the antihistaminic effect of this compound, among its most important actions are those of tranquilization, and adrenergic blockade. It would not appear that tranquilization is of import, since prochlorperazine and perphenazine are more effective as tranquilizers than is chlorpromazine [Irwin and Govier(5) Vischer(7)] but much less effective as blockers of water imbibition.

It would appear that if the blockade of water imbibition be concerned with adrenergic blockade, then epinephrine should be able to lessen the degree of block. However, addition of epinephrine (1×10^{-7} M) to beakers containing chlorpromazine (0.0008 M) did not reduce the block. Likewise, increase of epinephrine concentration to 0.0008 M was without result. In addition, as pointed out above, adrenergic blockers other than chlorpromazine are not impressive in their ability to inhibit water imbibition. It would appear that epinephrine should have been reasonably stable in this system, since we have been unable to demonstrate monamine oxidase activity in this preparation. Although the tissue was not examined for catacholase activity, the enzyme is apparently not often found in muscle, if at all.

Physostigmine at 0.0016 M did not alter water imbibition significantly.

Compounds characterized by carbonic anhydrase inhibition (acetazolamide), anticholinergic activity (methscopolamine bromide, ethopropazine), adrenergic blockade (promethazine, phentolamine, phenoxybenzamine, azapetine), analgetic-antiinflammatory activity (aminopyrine, phenylbutazone, prednisolone) adrenergic activity (amphetamine, epinephrine), monamine oxidase inhibition (Marsilid), cholinesterase inhibition (physostigmine), metabolic uncoupling (dinitrophenol) and high energy phosphate activity (adenosine triphosphate) do not produce outstanding inhibition of water uptake.

TABLE II. Correlation of Weight Gain with Ion Loss.

	1st hr	2nd hr
Sodium	−.45	−.58
Potassium	−.66	−.54

In an effort to determine whether or not pH of the solutions was correlated with water uptake, pH was determined on most of the media used. These values varied between pH 3.2 and pH 6.85 and showed no correlation with drug effect. Additionally, when muscles were soaked in phosphate buffers covering part of this range with extension to the alkaline side, (pH 5.5 to pH 8.05) all at 1.6×10^{-3} molar, no significant differences were seen in water uptake as the pH was varied.

In order to determine whether or not a correlation exists between weight changes and increase of sodium or potassium in the medium, correlation coefficients were calculated for weight increase versus sodium and potassium loss. These are recorded in Table II.

All of these coefficients are significant at the 0.05 level. It would thus appear that water imbibition is related to ion transport in these experiments.

The observation that physostigmine did not block water imbibition, nor did it influence sodium or potassium loss, suggests that the frog gastrocnemius behaves differently in distilled water with regard to response to cholinesterase inhibition than in hyponatric Ringer as used by Van der Kloot. Determinations in our laboratories of the ability of chlorpromazine to inhibit specific (methacholine) and non-specific (butyryl choline) cholinesterase of frog gastrocnemius have shown the compound to be ineffective against the specific enzyme, but to produce 52.5% inhibition of non-specific cholinesterase at 1.6×10^{-3} M when substrate was used at 1×10^{-2} M.

Greig & Holland(2) and Greig & Gibbons (3) have reported that the presence and integrity of cholinesterase on cell membranes is necessary for the maintenance of membrane integrity and that chlorpromazine is able to

prevent removal of cholinesterase from red blood cells by lysolecithin. In an attempt to determine whether frog gastrocnemius loses cholinesterase on soaking in distilled water and whether chlorpromazine can block this loss, cholinesterase activity was determined manometrically on lyophilized, reconstituted water medium in which frog muscles had been immersed with and without chlorpromazine. The results demonstrated that if cholinesterase be leached out of the muscle, the amount is so small that it is impossible of determination by these technics. It was also found in characterizing the cholinesterase of frog muscle, that the absolute content of enzyme is exceedingly small.

Thus it would appear that no ready explanation is at hand for the ability of chlorpromazine and to a lesser extent certain other phenothiazines to inhibit water imbibition under these conditions, although correlation exists with increased cell membrane permeability to potassium and sodium.

No attempt has been made to determine the extent to which the drugs used are able to penetrate the frog muscle cell although differences in activity between such compounds as phentolamine, phenoxybenzamine, and azapetine suggest that permeability effects may have influenced these results to some extent.

Summary. 1. The chlorpromazine-induced inhibition of water imbibition by frog gastrocnemius is unrelated to tranquilizing ability, adrenergic blockade, and antihistaminic activity. 2. Selected compounds exhibiting carbonic anhydrase inhibition, anticholinergic activity, analgetic and antiinflammatory activity, adrenergic activity, monamine oxidase inhibition, metabolic uncoupling, cholinesterase inhibition and high energy phosphate activity produce only minor or no inhibition of water uptake. 3. Correlation exists between block of water imbibition and loss of sodium and potassium from the muscle.

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1. Courvoisier, S., Ducrot, R., *Arch. Internat. Pharmacodyn.*, 1955, v102, 33.

2. Greig, M. E., Holland, W., *Am. J. Physiol.*, 1951, v164, 423.

3. Greig, M. E., Gibbons, A. J., *Arch. Biochem. and Biophys.*, 1956, v61, 335.

4. Halpern, B. N., Reuse, J., *Compt. Rend.* 1949, v228, 514.

5. Irwin, S., Govier, W., *J. Pharmacol. Exp. Therap.*, 1957, v119, 154.

6. Van der Kloot, W., *Nature*, 1956, v178, 366.

7. Vischer, T. J., *New England J. Med.*, 1956, v256, 26.

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Effects of Chemotherapeutic Agents on Metabolism of Human Acute Leukemia Cells *in vitro*. (23666)

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Cancer chemotherapy is currently directed chiefly towards chemical synthesis, animal screening, and clinical trial of antimetabolites. Some of these antimetabolites are designed to act primarily against specific biosynthetic reactions with the thought that they might function as anti-tumor agents by interfering with nucleic acid or coenzyme production in neoplasms. None of the chemotherapeutic drugs employed has universal applicability to patients with cancer. Even within a single cancer type such as acute lymphatic leukemia, response of a particular patient to a given antimetabolite is unpredictable. This unpredictability is particularly serious in those patients with fulminating neoplasms for whom prompt selection of the most effective agent offers the only hope of remission.

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