terol, phospholipids and triglycerides in lipid extracts of rat tissues occurred between 4 weeks and 8 months of age, with no further changes, except in skin, at 2 years of age. 2. Sex differences in lipid composition occurred only in skin.

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Inhibition of Release of Intracellular Potassium by Non-Narcotic Analgesic Agents. (25629)

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It has been shown that all types of pain-producing stimuli also release intracellular potassium (K) and that inhibition or reversal of K release is associated with decreased pain sensation(1). This new approach to pain might have some bearing on the action of analgesic agents. Therefore the present investigation is an attempt to determine whether non-narcotic analgesic agents inhibit release of intracellular K.

Two *methods* were used to produce a controlled release of intracellular K. The first one is distention of the intestine of guinea pigs. This procedure is well established as a painful stimulus(2,3). However K release under these conditions has not been shown previously. The other procedure used was exposure of washed erythrocytes to cold. In this case K release is a well established fact (4,5), but the relation to pain is purely by analogy. In the first procedure a guinea pig is killed by a knock on the head. The abdominal cavity is opened and 10 pieces of intestine, 2½ inches long, are cut. Intestinal chyme is gently pressed out. Each piece is washed in physiological saline and weighed (wet weight). One end of the strip is tied off with heavy nylon thread. The other end is tied to a glass rod. These rods have been prepared previously from glass tubing, external diameter 6.2 mm and internal diameter 5 mm. The rods are tapered and a small bulb is blown at the end to provide firm attachment for the strip (Fig. 1). The preparations are then transferred into 50 ml test tubes filled with 20 ml of K free Tyrode solution. Control experiments showed that there is a gradient of increasing K release down the intestinal tract (Table I). Therefore experimental preparations containing the analgesic agent are alternated with control tubes. The whole set of tubes is then transferred to an automatically stirred water bath kept at 37°C. After a control period of 30 minutes the intestine is distended with Tyrode solution (Fig. 1) until a steady fluid pressure of 2.5 cm is obtained. Samples are taken immediately after distention, one hour later and 2 hours

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TABLE I. Gradient of K Release along Intestinal Tract. (5 pieces taken from successively lower levels.)

	½ hr control	S.E.	1 hr after distention	S.E.	2 hr after distention	S.E.
1	269	29	742	35	903	19
2	312	29	803	31	934	37
3	331	29	859	42	995	33
4	384	37	888	45	988	39
5	418	40	929	61	1,082	42
Mean	343	22	844	31	980	27
Mean without distention	339	18	603	26	689	17

Values indicate K concentration of bathing fluid (microequivalents/g dry wt of intestine) at given time and represent mean of 10 experiments with distention, and 4 experiments without distention.

later. For each test 2 ml of external fluid are withdrawn and replaced by 2 ml of K free Tyrode solution. In calculations adjustment is made for the resulting dilution. Each sample is diluted with an equal volume of 1,600 p.p.m. lithium chloride and analyzed for K in a Perkin-Elmer flame photometer using indirect technic. At the end of experiment the strips are removed from the glass rods, cut open and washed in saline, then dried by exposure to an infra-red lamp at a distance of 10 inches for 2 hours. Dry weight is determined and the tissue is macerated and extracted in cold water (20 ml). Extraction fluid is filtered and analyzed for K.

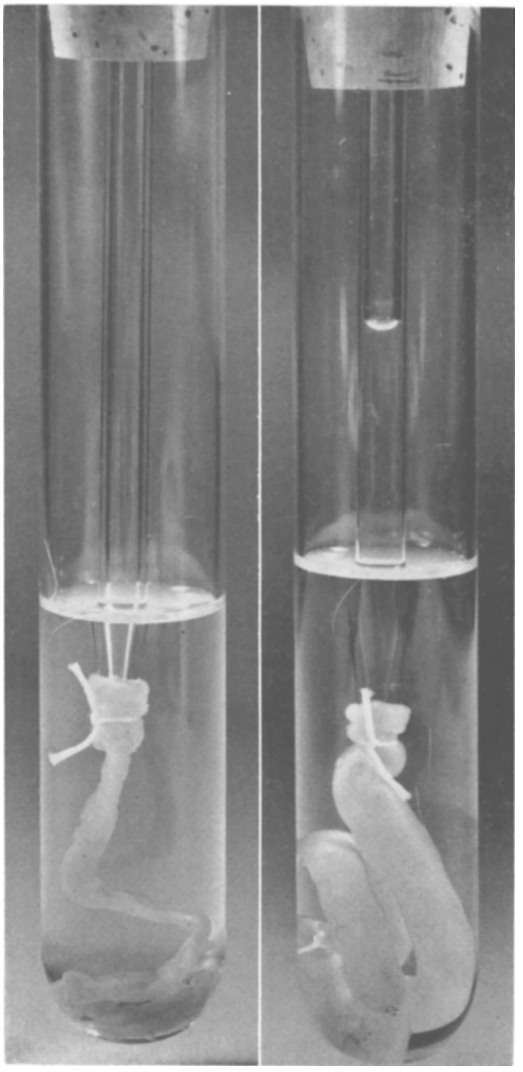

FIG. 1.

TABLE II. Effect of Drug Concentration on Inhibition of K Release.

Drug conc., mg/ml	No. exp.	1 hr after distention	S.E.	2 hr after distention	S.E.
.005	4	98.7	7.1	101.9	8.2
.01	4	79.9	6.4	87.3	5.9
.05	9	56.6	4.9	77.0	5.1
.5	4	87.0	8.0	92.1	7.7

Values for 3 drugs (phenacetin, acetanilid, aminopyrine) are combined and expressed as % of control.

Results. Table II gives the effect of various drug concentrations. In higher concentrations of drugs there was a slight change of pH which was corrected by adding $NaHCO_3$.

If we assume that the therapeutic dose in man is equally distributed throughout the extracellular fluid space, concentration would be about 0.015 mg/ml. This corresponds fairly well to the dose of maximum effectiveness in our experiments.

Tables III and IV give the results of 9 experiments analyzed according to wet weight

TABLE III. Effect of Analgesic Agents on K Release (Wet Wt).

	Control	Phen.	Control	Acet.	Control	Amin.
1 hr after distention	407	224 †	388	231 †	329	182 †
S.E.	74	44	60	29	44	67
% of control		55.		59.4		55.3
2 hr after distention	808	711 *	787	554 †	783	568 *
S.E.	102	49	61	70	85	73
% of control		88.		70.4		72.5

Values expressed as microequivalents/g wet wt, and are means of 9 experiments. S.E. refers to stand. error within the group, while significance indicated by asterisk and dagger is based on paired analysis.

* 95% probability. † 99% probability.

TABLE IV. Effect of Analgesic Agent on K Release (Dry Wt).

	Control	Phen.	Control	Acet.	Control	Amin.
1 hr after distention	3.63	2.23†	4.41	2.96†	3.80	2.68*
S.E.	.36	.33	.49	.31	.47	.63
% of control		61.4		67.1		70.5
2 hr after distention	7.60	6.40*	9.94	6.54†	8.58	7.06*
S.E.	.59	.56	.39	.56	.62	.76
% of control		84.2		65.8		82.3

Values expressed as milliequivalents/g dry wt and represent means of 9 experiments. S.E. refers to stand. error within the group, while significance indicated by asterisk and dagger is based on paired analysis.

* 95% probability. † 99% probability.

and dry weight respectively. Mean dry weight is 13.3% of mean wet weight.

Analysis of the intestinal wall showed that mean concentration for 4 controls was 2.60 meq/l and mean for 6 preparations with analgesic was 5.01 meq/l.

To determine effect of analgesics on K release from blood cells, washed human erythrocytes were suspended in physiological saline. The concentration was approximately 125 million cells/ml. Analgesic agents were used in a concentration of 0.05 mg/ml. Two tubes were used for each analgesic agent and a total of 4 controls. All tubes were exposed to a temperature of 7°C for 60 minutes. Then they were centrifuged at the same temperature and supernatant fluid was analyzed. Results in microequivalents/l showed:

Acetanilid 172 Phenacetin 231
Aminopyrine 273 Control 494

A problem arising from these data is the degree of specificity of inhibition of K release for analgesic agents. Table V shows that salicylic acid and cholesterol have no significant effect. Obviously only a greatly extended series of experiments can answer this point adequately.

TABLE V. Effect of Salicylic Acid and Cholesterol on K Release of Intestine.

Agent	Conc., mg/ml	No. exp.	1 hr after distention	2 hr after distention
Sal. acid	.25	9	99.2	98.0
Cholesterol	.25	6	96.1	97.8

All values expressed as % of control and none are significantly different from control.

Summary. *In vitro* distention of intestinal tract of guinea pigs causes release of intracellular K. This effect is decreased when bathing fluid contains non-narcotic analgesic agents (acetanilid, phenacetin, aminopyrine) in pharmacological concentrations. The release of K on exposing washed human erythrocytes to cold is similarly inhibited by these agents.

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