TABLE I. Effect of Molybdenum on Fluoride Retention in the Rat.

Group	Mean wt gain (g)	Carcass fluoride		Femur fluoride	
		Total (μg)	Conc. (μg/g)	Total (μg)	Conc. (μg/g)
Control	87	145 ± 21	26.4± 3.1	6.37± 1.03	35.5± 5.9
50 ppm Mo	87	212 ± 12	39.9± 3.7	15.37± 1.48	88.3± 7.0
50 " F	70	8200 ± 550	1640 ±65	392 ±23	2340 ±165
50 " Mo + 50 ppm F	98	9820 ± 390	1830 ±66	464 ±24	2560 ±115

Values include stand. dev.

When the carcass was used to evaluate fluoride retention, 145 μg of F was present in control animals, while animals receiving 50 ppm Mo had 212 μg of F. When animals received 50 ppm F, 8200 μg of F was found in the carcass, and when both Mo and F was fed 9820 μg was found. Similar ratios are evident when the femur is used as index of skeletal fluoride retention.

Discussion. The presence of Mo seems definitely associated with increasing retention of fluoride in the rat. This is demonstrated by 2 types of evidence. (1) It increases retention by apparently increasing availability of both fluoride present, as a constituent of the diet and also that small fraction present in drinking water; and (2), it increases retention of that available fluoride added directly to drinking water as sodium fluoride. When total fluoride in the carcass is used to evaluate fluoride retention, a 32% increase is found when 50 ppm Mo is added to low-fluoride drinking water as compared to group receiving no Mo. When both Mo and F are added to drinking water there is approximately 17% more fluoride in the carcass than when only F is present. Both of these differences have probabilities in excess of 0.01. These data suggest that one of the functions of Mo is to increase the availability of the fluoride ion.

Conclusions. The presence of 50 ppm Mo as ammonium molybdenate is associated with significantly more fluoride retention in skeletal and carcass of the rat, than when Mo is absent from drinking water. These data suggest that Mo may act metabolically to increase availability of fluoride ion.

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Spatial Summation of Pain. (24949)

FRED B. BENJAMIN
*Dept. of Physiology, Medical School, University of Pennsylvania, Philadelphia**

An old textbook concept, probably largely based on Hardy's work(1) maintains that pain does not show spatial summation. Recently however, Green(2) has shown that pain summation occurs for small areas at pain threshold. The present investigation is an attempt to resolve this problem. The approach used is crude. However it allows a comparative evaluation over a wide range of experimental conditions, practically impossible to obtain with more refined technics. A constant heat stimulus was applied and the elapsed time determined until the subject reported the first pricking pain (threshold). Then the size of the stimulation area was changed and threshold time was again determined. This was done for 3 different sizes of stimulation area. The entire procedure was repeated with the subject reporting the maximum bearable pain (supra-threshold pain). All the above procedures were then repeated for 2 other levels of heat stimulation. The heat stimulus

* Present address: Biological Research Laboratories, Colgate Palmolive Co., New Brunswick, N. J.

TABLE I. Effect of Intensity and Area of Stimulation (Contact Heat) on Threshold and Supra-Threshold Pain.

Stimulus, °C	Area 70 cm²			Area 14 cm²			Area 3 cm²			Mean		
	t(s)	T(°C)	T.D.*	t(s)	T(°C)	T.D.*	t(s)	T(°C)	T.D.*	t(s)	T(°C)	T.D.*
A. Threshold pain†												
51	2.6	45.1	.32	4.7	46.9	2.21	9.7	47.9	9.25	5.7	46.6	2.15
55	1.3	44.1	.08	2.3	46.4	.75	4.1	48.3	5.36	2.6	46.3	.79
60	.7	44.0	.04	1.3	46.2	.36	2.4	48.6	3.90	1.5	46.3	.45
Mean	1.5	44.4	.11	2.8	46.5	1.00	5.4	48.3	7.05	3.3	46.4	1.01
B. Supra-threshold pain†												
51	4.9	46.9	2.31	11.8	48.2	14.4	48.2	48.3	62.9	21.6	47.8	18.7
55	2.5	46.7	1.16	4.4	48.5	6.65	7.9	49.6	26.5	4.9	48.3	5.79
60	1.5	46.7	.61	2.9	49.2	3.26	4.0	50.4	23.9	2.8	48.8	5.25
Mean	3.0	46.8	1.31	6.4	48.6	10.4	20.0	49.4	57.8	9.8	48.3	12.8

* T.D. = Tissue damage in mΩ (see text).
† Each value represents mean of 24 experiments in 12 subjects.

was a metal container filled with hot water which was maintained at a constant temperature (60°, 55°, and 51°C). Under these conditions the outside of the container had a temperature of 57.6, 53.0, and 49.6°C, respectively. These temperatures were determined with a Stoll-Hardy radiometer(3) and were practically identical over the entire field of stimulation. Exposure area was varied by covering part of the container with heat-insulating material, leaving free an area of 70 cm², 14 cm², and 3 cm². The site of stimulation was the volar side of the forearm, which was held by the experimenter in firm contact with the heat source. Twelve students and faculty members served as subjects. Final temperature at the site of the pain receptor was calculated. For this purpose pain receptors were assumed to lie at an average depth of 200 μ below the skin surface. Using this calculated skin temperature, intensity of heat stimulation and time of exposure, temperature at the pain receptor was calculated using Henriques' formula(4). As pain may be more directly related to tissue injury than to time of exposure or final temperature, tissue damage was similarly calculated(4). However, the unit used for damage was one thousandth of Henriques' omega, or one millomega: $1\ m\Omega = 3.1 \times 10^{101} \times t \times e^{-75{,}000/T}\ t + 273$. One "millomega" corresponds to damage caused by application of 45°C for 8.64 seconds.

Experimental *results* are summarized in Table I. For statistical analysis each subject was used as his own control. All the differences of stimulation areas are significant ($p < 0.05$) except the temperature values for supra-threshold pain resulting from stimulus temperatures of 51° and 55°. The differences of mean values are highly significant ($p < 0.01$) except those for temperature values at supra-threshold pain, which are significant only for $p < 0.05$.

When the skin is exposed to constant heat energy, an immediate and rapid temperature increase is observed. Gradually this diminishes until a balanced state is reached where heat gain equals heat loss. Threshold pain occurs at an early part of this curve. Therefore differences produced by spatial summation will be more clearly defined if expressed in terms of tissue temperature. At supra-threshold pain the heating curve is flatter, and differences of stimulation area come out clearer when time is being measured. Intensity of stimulation has a similar effect, and modifies the effect obtained by change of pain intensity. The interaction of these factors explains largely the time and temperature variability of the data shown in Table I. The evidence for spatial summation of pain is considered clear cut, since it occurs at all intensities of stimulation used for 2 widely different pain intensities, and over the entire range of areas examined. From common life experience we know that a burn the size of a needle prick is hardly felt, while a burn of the same intensity covering a large area may cause intense pain. Therefore, spatial sum-

mation of pain is a matter of every day experience. The denial of spatial summation for pain is probably due to the false assumption that injury is the stimulus for pain. Undoubtedly severe injury can occur with very little or no pain. This difficulty was explained recently when it was shown that pain is not caused by injury as such, but by the tissue reaction to injury(5). Therefore, scientific evidence as well as common experience indicate that spatial summation is an inherent characteristic of pain sensation.

Summary. Spatial summation of pain was determined by applying contact heat to the volar side of the human forearm. Evidence indicates that summation of pain occurs for large fields as well as for small fields, for threshold pain as well as for supra-threshold pain, and for high intensity stimuli as well as for low intensity stimuli. Therefore pain summation is considered an inherent characteristic of pain sensation.

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Action of Polylysine on Some Ascites Tumors in Mice.* (24950)

T. RICHARDSON,† J. HODGETT, A. LINDNER AND M. A. STAHMANN
Depts. of Biochemistry and Clinical Pathology, University of Wisconsin, Madison

Synthetic polypeptides of lysine reduce infectivity of tobacco mosaic virus(1); inhibit multiplication of bacteriophage(2,3); and protect chick embryos for a period after inoculation with animal viruses such as mumps virus(4), infectious bronchitis, Newcastle disease viruses(5), and influenza B virus(6). The antibacterial activities of synthetic polylysines and copolymers containing lysine have been demonstrated by a number of investigators(7-11). Because of activity of polylysine against viruses and bacteria it seemed worthwhile to screen polylysine, along with other synthetic polypeptides, against various tumors in mice. This communication reports increased survival time of mice bearing Ehrlich ascites carcinoma and TA3 ascites tumors after treatment with synthetic lysine polypeptides, and describes some cytological effects of polylysine on tumor cells.

Materials and methods. The polypeptides were synthesized from amino acid N-carboxyanhydrides. Lysine polypeptides isolated and tested as their hydrochlorides were prepared as described by Becker and Stahmann(12) and Tsuyuki, Tsuyuki, and Stahmann(13). Average degree of polymerization(DP) was determined from α-amino nitrogen analyses. Random copolymers containing lysine in combination with valine or leucine were prepared by copolymerization of corresponding N-carboxyanhydrides. Polyglutamic acid was synthesized as described by Green and Stahmann(14); Protamine sulfate was obtained from the Eli Lilly Co. The above polymers were tested against tetraploid Ehrlich ascites carcinoma in female Swiss mice. However, only the poly-DL-lysine (DP = 240) was used in additional assays against diploid Ehrlich ascites carcinoma and mammary adenocarcinoma TA3 ascites tumor in female Swiss and male BAF1 mice, respectively. In screening procedures, young adult mice

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† Predoctoral Research Fellow of Nat. Cancer Inst.