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.

1. Hardy, J. D., Wolff, H. G., Goodell, H., *Pain Sensation and Reactions,* Williams and Wilkins, Baltimore, Md., 1952.

2. Green, L. C., Hardy, J. D., *J. Appl. Physiol.,* 1958, v13, 457.

3. Stoll, A. M., Hardy, J. D., *Rev. Scient. Instr.,* 1954, v25, 184.

4. Henriques, F. C., Moritz, A. R., *Arch. Path.,* 1947, v43, 489.

5. Benjamin, F. B., *J. Appl. Physiol.,* 1959, in press.

Received April 21, 1959. P.S.E.B.M., 1959, v101.

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

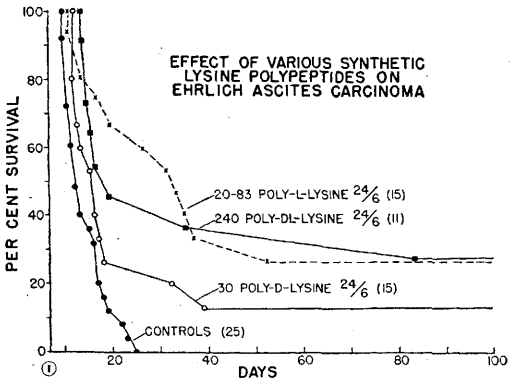
Synthetic polypeptides of lysine reduce infectivity of tobacco mosaic virus(1); inhibit multiplication of bacteriophage(2,3); and protect chick embryos for a period after inoculation with animal viruses such as mumps virus(4), infectious bronchitis, Newcastle disease viruses(5), and influenza B virus(6). 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

* Presented in part before Am. Soc. Biol. Chem., Atlantic City, Apr. 15, 1959. This investigation supported in part by research grant from Nat. Microbiol. Inst., N.I.H., U.S.P.H.S., and Research Committee of Graduate School, Univ. of Wisconsin from funds of Wisconsin Alumni Research Fn.

† Predoctoral Research Fellow of Nat. Cancer Inst.

weighing 20 to 25 g were each transplanted intraperitoneally with 5 to 10 million tumor cells in isotonic saline as a 1 to 10 dilution of the packed cell volume. Treatment of mice was begun 24 hours after tumor transplant. Polymers were administered in isotonic saline so that each injection volume was 0.2 to 0.4 ml. Solutions were adjusted to pH 7.2 with sodium bicarbonate and then sterilized by passage through bacterial filter. Unless stated otherwise treatment schedule consisted of 2 intraperitoneal injections of the polypeptide solution daily for 6 days. An increased survival time of treated mice over control mice was used as the criterion of activity. The poly-DL-lysine (DP = 240) was also screened against the solid forms of Sarcoma 180 and Carcinoma 755 and against the ascites form of Leukemia L-1210. Screening procedures were essentially those outlined in Cancer Chemotherapy Report No. 1(15). Polypeptide solutions were prepared as before, and treatment schedule consisted of 2 injections daily for 7, 11, and 15 days for Sarcoma 180, Carcinoma 755, and Leukemia L-1210, respectively. Dosage in each case averaged 12 mg/kg for each injection. Criteria of effectiveness of treatment were the weights of excised solid tumors and survival time of mice with Leukemia L-1210. The polylysine used in cytological screening was poly-DL-lysine (DP = 240). The tumor used was the Ehrlich ascites carcinoma hyperdiploid Buffalo, chromosome mode (45-46) (16) maintained in this laboratory for 18 fluid transfer generations. Mice inoculated with 20 x 10^6 tumor cells were divided into 2 equal groups, a control group receiving injections of saline and a study group receiving polylysine 12 mg/kg/day i.p. This regimen was carried out over 6 days, daily weights of mice being used as estimate of tumor development. Mice were sacrificed on seventh day, and ascitic fluid withdrawn. Smears were made using Carnoy's fixative and stained by Feulgen's technic(17), with Toluidine Blue (18), or with Naphthol Yellow S(19) to investigate any changes in DNA, RNA and total protein of tumor cells. An aliquot of the ascites fluid was also treated after the method of Yoshida(20) to demonstrate chromosome



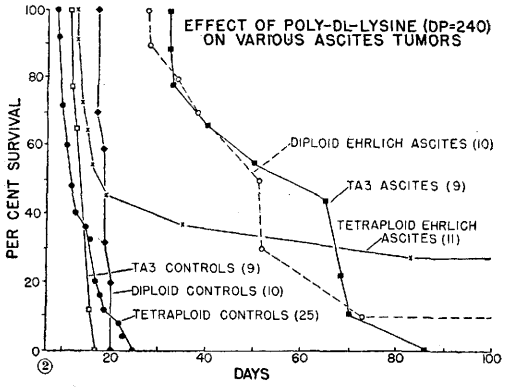


FIG. 1. Effect of various synthetic lysine polypeptides on survival of mice bearing Ehrlich ascites carcinoma.

FIG. 2. Effect of poly-DL-lysine (DP = 240) on survival of mice bearing various ascites tumors.

morphology. Cell volumes were measured by phase microscopy. Cells were maintained at 37°C unfixed, bathed in the ascites serum in hanging drop preparation. Diameters were measured by eyepiece micrometer and volumes calculated as spheres from this measurement. Total cellular nitrogen was estimated by Kjeldahl analysis with Nessler's reagent as described by Klein(21), cellular RNA and DNA by method of Schneider(22).

Results. In preliminary screening all of the polypeptides except the lysine polypeptides were ineffective against tetraploid Ehrlich ascites carcinoma. In initial experiments poly-L-lysine preparations of DP 20 and DP 83 inhibited growth of tetraploid Ehrlich ascites carcinoma. There was no significant difference between the 2 treated groups and an average of 27% of treated mice survived for 100 days, apparently free of tumor (Fig. 1). The untreated control mice all died between

TABLE I. Effect of Poly-DL-Lysine* on *In Vivo* Mitotic Cycle of Ehrlich Ascites Tumor Cells.

	% of cells in different phases of mitosis				
	Prophase	Metaphase	Anaphase	Telophase	Mitotic index
Control†	1.64	1.06	.19	.47	3.4 ± .6
Treated†	1.21	.23	.006	.10	1.4 ± .7

* DP = 240. † 17,500 cells sampled.

10 and 25 days following transplantation. The effect of molecular size of the polypeptide and optical configuration of its lysine residues was studied. The results obtained with poly-DL-lysine (DP = 240) and with poly D-lysine (DP = 30) against the tetraploid Ehrlich ascites carcinoma are presented in Fig. 1. The data indicate that the DL isomer had about the same activity as the L isomer with average 27% survival at 100 days after transplantation without evidence of tumor formation. The poly-D-lysine (DP = 30) was the least effective with only 13% of mice surviving at 100 days with no apparent tumor.

Poly-DL-lysine (DP = 240) also showed activity against the diploid strain of Ehrlich ascites carcinoma: 10% of treated mice survived 100 days without any sign of tumor. Survival time of some mice bearing TA3 ascites tumor was extended to 86 days after transplantation (Fig. 2). It would appear that the poly-DL-lysine is most effective against tetraploid Ehrlich ascites carcinoma. This difference might be a function of tumor cell volume, for in the 1 to 10 dilution of the packed cell volume of tumors, a greater number of diploid and TA3 cells are transplanted. Generally, mice in the preceding experiments that died after first 3 to 4 weeks succumbed from solid tumors outside the peritoneal cavity rather than to recurrent ascites tumors. Subsequent development of solid tumors near site of implant were frequently observed.

The poly-DL-lysine (DP = 240) had no significant effect on growth of Sarcoma 180, of Carcinoma 755, nor on survival time of mice bearing Leukemia L-1210.

On removal of the ascitic fluid from treated and control mice, transplanted with Ehrlich ascites carcinoma, a noticeable difference in volume of ascites cells was seen, estimated as a packed cell volume from pooled samples. The treated mice showed half the volume of that of control mice. This finding is in agreement with daily weighings of the mice, controls showing usual increase in weight, whereas the treated group showed little or no weight increase. It should be noted that the dose of polylysine used in the cytological experiments was half of that used in survival time studies. The higher dose when used in preliminary studies did not allow multiplication of a sufficient number of cells for this study.

Analysis of mitotic activity of these tumor cells showed a marked decrease in later phases of the mitotic cycle, which would indicate that polylysine acts on tumor cells prior to these phases. From results tabulated in Table I it seems that this effect is most noticeable after prophase. There was little difference between control and treated groups at prophase. When the cell commences the mitotic cycle, the polylysine may inhibit at or previous to this stage and result in arrest at prophase. Overall, the mitotic index is reduced from 3.4 ± 0.6 for control value to 1.4 ± 0.7 for treated value.

In this examination, the frequency of multinucleated cells and mitotic abnormalities were also quantitated. The number of multinucleated cells showed a reduction from 1.27% in the control to 0.67% in treated animals.

Mitotic anomalies, although quantitatively small, appear significant rising to 0.33% in treated animals from 0.15% in the controls. The most frequent of these anomalies appeared at telophase; these may be described as (a) unsynchronized divisions to daughter cells, (b) daughter cells which, although in very late telophase, are still joined, (c) an occasional unipolar type of mitosis. In the control group the mitotic cycle showed a small percentage of mitotic anomalies characteristic of this tumor(23). Chemical analyses for nucleic acids and total proteins in treated and control cells showed no significant difference (Table II). Morphologically, resting cells of both groups were comparable, and there is

TABLE II. Cytological Analyses of Ehrlich Tumor Cells Treated *In Vivo* with Poly-DL-Lysine.*

	Cell vol, μ^3	DNA	RNA	Nitrogen
		$\mu\mu$g/cell		
Control	1150 ± 60	9.6	18.5	36.4
Treated	1240 ± 58	9.7	19.6	38.1

* Treated with 12 mg/kg/day of poly-DL-lysine (DP = 240) for 6 days.

no significant change in volume. Control cell volumes were 1150 μ^3 ± 66 μ^3 as compared to treated cell volumes of 1204 μ^3 ± 58 μ^3. Cytochemical preparations and morphological studies also indicated that there is no change in cellular volume, nucleic acids or proteins.

Discussion. Only the polypeptides of lysine had an inhibitory action on growth of ascites tumors. When the positive charge of the polylysine was dispersed as in leucine-lysine or valine-lysine copolymers, and in protamine sulfate, the antitumor action was lost. The ineffectiveness of polyglutamic acid against the tetraploid Ehrlich ascites carcinoma suggests that the high positive charge of polylysine may be necessary for activity. It has been reported that tumor cells develop an abnormally high negative charge when compared to homologous cells from which they are derived(24). The effectiveness of polymers with high positive charge, as polylysine, in inhibiting tumor development might involve an electrostatic interaction with the tumor cells. Such a specific interaction of polyethylene imine with Walker tumor cells has been demonstrated(25).

The antitumor action of lysine polypeptides apparently requires an intimate contact of polylysine with the ascites tumor cells within the peritoneal cavity. The refractoriness of solid tumors to polylysine might mean that the polypeptide does not reach the solid tumor in a concentration high enough to exert its inhibitory action. The high affinity of polylysine for red blood cells and tissue proteins could limit the systemic availability of polylysine. In the case of highly invasive leukemia L 1210, the tumor might be too highly metastatic to be significantly affected by the polylysine.

The observation that many treated mice either survived without development of ascites tumors or succumbed to solid tumors rather than to recurrent ascites tumors indicates a marked cytotoxic effect of polylysine against ascites tumor cells within the peritoneum.

Cytological studies of cells from the peritoneum showed marked tumor growth inhibition by polylysine as evidenced by decreased number of tumor cells in mitosis and a marked mitotic inhibition at the prophase level. The mitotic index in treated animals was about half that of controls and there is a marked mitotic inhibition at the prophase stage (Table I). Polylysine treatment does not change cell volume or nucleic acid or protein content (Table II), as is seen when Ehrlich ascites carcinoma is treated with 5-fluorouracil(26).

Summary. Synthetic polypeptides of lysine, of glutamic acid, of valine and lysine, of leucine and lysine, and commercial protamine sulfate were tested against tetraploid Ehrlich ascites carcinoma in Swiss mice. Only the synthetic polypeptides of lysine inhibited ascites tumor development. Up to 27% of mice treated with polylysine survived for 100 days apparently free from tumor. Mice that died after 3 to 4 weeks usually did so from metastatic solid tumors or solid tumors at site of implant. Polylysine also had a marked inhibitory activity against the diploid strain of Ehrlich ascites carcinoma and TA3 ascites carcinoma in Swiss and BAF1 mice, respectively. Survival time of some BAF1 mice was increased to 86 days, while 10% of Swiss mice survived 100 days with no sign of tumor. On cytological analysis, mitotic arrest at prophase was found. The nucleic acid and protein content/cell remained unchanged.

We wish to thank Dr. G. A. LePage for advice and assistance and Dr. B. E. Kline for assays on Sarcoma 180, Carcinoma 755 and Leukemia L-1210.

1. Stahmann, M. A., Graf, L. H., Patterson, E. L., Walker, J. C., Watson, D. W., *J. Biol. Chem.*, 1951, v189, 45.

2. Watson, D. W., Bloom, W. L., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 29.

3. Shalitin, C., Katchalski, E., *Bul. Research Council Israel,* 1957, v6a, 314.

4. Green, M., Stahmann, M. A., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 852.

5. Green, M., Stahmann, M. A., Rasmussen, A. F., *ibid.*, 1953, v83, 641.

6. Rubini, J. R., Rasmussen, A. F., Stahmann, M. A., *ibid.*, 1951, v76, 662.

7. Burger, W. C., Stahmann, M. A., *Arch. Biochem. and Biophys.*, 1952, v39, 27.

8. Watson, D. W., Bloom, W. L., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 29.

9. Katchalski, E., Bichowsky-Slomnicki, L., Volcani, B. E., *Biochem. J.*, 1953, v55, 671.

10. Katchalski, E., Berger, A., Bichowski-Slomnicki, L., Kurtz, J., *Nature*, 1955, v176, 118.

11. Bichowski-Slomnicki, L., Berger, A., Kurtz, J., Katchalski, E., *Arch. Biochem. and Biophys.*, 1956, v65, 400.

12. Becker, R. R., Stahmann, M. A., *J. Am. Chem. Soc.*, 1952, v74, 38.

13. Tsuyuki, E., Tsuyuki, H., Stahmann, M. A., *J. Biol. Chem.*, 1956, v222, 479.

14. Green, M., Stahmann, M. A., *ibid.*, 1952, v197, 771.

15. Cancer Chemotherapy Rep. No. 1, publ. by U. S. Dept. of H.E.W., 1959.

16. Tjio, J. H., Levan, A., *Lunds Univ. Arsskr.*, 1954, N. F. Ard. 2, 50.

17. Feulgen, R., Rossenbeck, H., *Z. Physiol. Chem.*, 1924, v135, 203.

18. Herrman, H., Nicholas, J. S., Boricious, J. K., *J. Biol. Chem.*, 1950, v184, 321.

19. Deitch, A. D., *Lab. Invest.*, 1955, v4, 324.

20. Yoshida, T., *Ann. N. Y. Acad. Sci.*, 1956, v63, 211.

21. Klein, G., Forssberg, A., *Exp. Cell Research*, 1954, v6, 211.

22. Schneider, W. C., *J. Biol. Chem.*, 1945, v161, 293.

23. Love, R., Orsi, E. V., Koprowski, H., *J. Biophys. Biochem. Cytol.*, 1956, v2, 1.

24. Ambrose, E. J., James, A. M., Lowick, J., *Nature*, 1956, v177, 576.

25. Ambrose, E. J., Easty, D. M., Jones, P. C. T., *Brit. J. Cancer*, 1958, v12, 439.

26. Lindner, A., *Cancer Res.*, 1959, v19, 2, 189.

Received April 27, 1959. P.S.E.B.M., 1959, v101.

Intratubular Fluid Movement in Dog Kidney during Stop Flow. (24951)

AKIRA OMACHI AND ROBERT I. MACEY (Introduced by W. V. Whitehorn)
Dept. of Physiology, University of Illinois College of Medicine, Chicago

The stop flow technic introduced by Malvin, Wilde and Sullivan(1,2,3) provides a convenient method for localizing reabsorptive and secretory activities in the mammalian kidney tubule. Briefly, the technic consists of clamping the ureter for a short period (*e.g.*, 8 minutes) during the steady state that arises from constant infusion of an osmotic diuretic. The intratubular fluid at the end of this period of ureteral occlusion differs from intratubular fluid during free flow because the former has had longer contact with tubular epithelium and, accordingly, its composition reflects reabsorptive and secretory activities to a greater extent. Immediately following the period of stop flow, serial samples of urine are collected and analyzed. The first sample is considered to be representative of fluid which is located most distal to the glomerulus. Each succeeding sample approximates fluid lying in more and more proximal positions. The arrival of fluid that entered the tubules after release of the occlusion is signaled by the presence of an indicator (*e.g.*, inulin) which was injected intravenously during the occlusion period. In interpreting the data so obtained, it has been assumed that the column of tubular fluid is stationary during the stop flow period. The present investigation explored the possibility that the column of tubular fluid does, indeed, move during the period of stop flow as a necessary consequence of water reabsorption. Thus, tubular fluid would be replaced in part by new filtrate throughout the stop flow period. This possibility has been mentioned before(2,4) but the magnitude of the effect may necessitate more attention than has previously been given. To demonstrate movement of tubular fluid during stop flow, we have repeated the technic described by Malvin, Wilde and Sullivan(2) with the modification that 2 or 3 indicators of