TABLE II.

Group No.	Supplement to basal diet 113GN	No. chicks	No. died	Avg wt 4 weeks, g	No. black-tongue
1	5% gelatin	6	0	175	2
2	10% "	6	2	133	5
3	As 1 + 10 mg % nicotinic acid	6	0	235	0
4	As 2 + 10 " % " "	6	0	244	0
5	As 1 + 15% zein	6	1	155	5
6	As 1 + 15% amino-acid mixture*	6	5	61	6
7	As 1 + 11.5% modified amino-acid mixture†	6	1	110	5
8	As 5 + 10 mg % nicotinic acid	6	0	221	0
9	As 6 + 10 " % " "	6	0	226	0

* *l*-Glutamic acid 3.90, *l*-leucine 3.60, *dl*-alanine 1.55, *l*-proline 1.45, *dl*-phenylalanine 1.00, *l*-tyrosine 0.85, *d*-isoleucine 0.65, *dl*-aspartic acid 0.50, *dl*-methionine 0.35, *dl*-threonin 0.35, *dl*-valine 0.35, *dl*-serine 0.15, *l*-histidine 0.15, *l*-arginine 0.15.

† This mixture contained glutamic acid, leucine, alanine, proline, and phenylalanine at the same levels as used in the above amino-acid mixture.

reason that zein was less depressing on growth than the amino-acid mixture was probably due to the presence of small amounts of naturally occurring tryptophane[14] and/or nicotinic acid in zein. The most significant point to observe, however, is the counteraction of the effects of zein and of the amino-acid mixture with low levels of nicotinic acid (compare Groups 5, 6, 8, and 9). Furthermore, these data demonstrate that nicotinic acid is involved in some manner in the metabolism of amino acids.

Since more than maximal growth depression was obtained with the amino-acid mixture simulating the protein zein, it is logical to assume that the commercially prepared zein was not contaminated with a "pellagragenic" agent. Furthermore, it is safe to state that a large part of the inhibitory action of corn in chick pellagra may be attributed to its predominant protein, zein, and that the presence of a special "pellagragenic" agent reported to exist in corn plays a relatively minor part, if any.

Summary. By use of an amino-acid mixture simulating zein, it was found that chick-pellagra symptoms in chicks caused by the feeding of zein were due to the cumulative action of the amino-acid constituents of this protein. Glutamic acid, leucine, alanine, proline, and phenylalanine, the amino acids occurring in greatest amounts, seem to be principally involved in this connection.

[14] Block, R. J., and Mitchell, H. H., *Nutr. Abst. and Rev.*, 1946-47, **16**, 249.

16554

Inhibition of Testicular Hyaluronidase Activity by Rutin.*

BENJAMIN A. LEVITAN.† (Introduced by B. P. Babkin.)

From the Department of Physiology, McGill University, Montreal.

The term vitamin P was introduced by Armentano, Szent-Gyorgyi, and co-workers[1] to designate an essential substance other than ascorbic acid, regulating capillary permeability and fragility. In the guinea pig[2]

* Aided by a grant from the Cooper Fund of the Faculty of Medicine, McGill University.

† Fellow in Medicine, American National Research Council.

[1] Armentano, L., Bentsah, A., Beres, T., Rusnyak, S., and Szent-Gyorgyi, A., *Deut. Med. Wochsch.*, 1936, **62**, 1325.

and in the rat,[3] vitamin P deficiency produces a decreased capillary resistance. Scarborough[4] has shown that in the human, lack of vitamin P leads to spontaneous petechial hemorrhages, lassitude and rheumatic pains. The condition rapidly responds to vitamin P, while ascorbic acid is without effect.

Parrot and Lavollay[5] have suggested that the vitamin acts indirectly by inhibiting the oxidation of some product of adrenalin metabolism. This as yet unidentified compound is said to maintain normal capillary resistance. This action is supposedly antagonized by histamine which decreases capillary resistance. The normal capillary state is believed to be a balance between these two factors. Chambers and Zweifach[6] have cast doubt on the theory that histamine normally influences capillary permeability. Injections of histamine locally or intravenously, in doses sufficient to produce arteriolar dilatation, fail to increase capillary permeability. Only when the concentration of histamine is sufficient to produce visible endothelial damage, does increased capillary permeability occur.

There is evidence to suggest that hyaluronidase might replace histamine in the schema of Parrot and Lavollay. Duran-Reynals[7] has recently reviewed the experimental findings which indicate that hyaluronidase increases capillary permeability. He believes that small amounts of hyaluronidase are normally present in most, if not all tissues. Wislocki, Bunting and Dempsey[8] have shown that arteries, arterioles and veins contain a metachromatic ground substance, probably chondroitin sulfate. Meyer and Ragan[9] have suggested that this substance which is subject to attack by hyaluronidase, may be a constituent of capillary walls. In view of the foregoing, the possibility suggested itself that widely distributed hyaluronidase normally maintains tissue and capillary permeability and its overfunction is prevented by vitamin P. Thus a deficiency in vitamin P would lead to an excessive hyaluronidase activity which would weaken all ground substances including that present in capillary walls, and consequently would increase tissue as well as capillary permeability. With this hypothesis in mind, it was decided to test the possible antihyaluronidase activity of rutin, a vitamin P-like flavonol derivative.

Procedure. Forty-four albino rats were used, weighing 250-400 g. Ten ainmals were given rutin‡ intraperitoneally 3.5 hours before the hyaluronidase. The dose was 200 mg dissolved in 1 cc propylene glycol. Ten animals were similarly given 1 cc propylene glycol, but no rutin. Fourteen animals were not pretreated. The hyaluronidase was prepared in a 2% solution in 0.9% saline. The dry powder assayed 75 T.R.U. per mg and was purchased from the Tremond Company, New York.

The freshly prepared enzyme solution was gently mixed with an equal volume of doubly filtered Higgins india ink and 0.1 cc of the final solution was injected intradermally into the right flank of all animals. Into the left flank 0.1 cc of a solution of equal volumes of ink and saline was injected intradermally. Twenty-two hours later, the rats were killed with ether and skinned. The margins of the black areas on the inner surface of the skins were traced out and transposed onto cellophane paper. The enclosed areas were then measured with a planimeter. The results are presented in tabular form and analyzed statistically.

Summary. From the data it can be seen that rutin inhibits the spread of intradermally injected india ink to which hyaluronidase or saline is added. The effect is statistically highly significant. From the analysis of

2 Zacho, C. E., *Acta. Path. Microbiol. Scand.*, 1939, **16**, 144.

3 Rusznyak, S., and Benko, A., *Science*, 1941, **25**, 94.

4 Scarborough, H., *Lancet*, 1940, **239**, 644.

5 Parrot, J., and Lavollay, J., *Compt. Rend. Acad. Sc., Paris*, 1944, **218**, 211.

6 Chambers, R., and Zweifach, B. W., *Physiol. Rev.*, 1947, **27**, 436.

7 Duran-Reynals, F., *Bact. Rev.*, 1942, **6**, 197.

8 Wislocki, G. B., Bunting, H., and Dempsey, E. W., *Am. J. Anat.*, 1947, **81**, 1.

9 Meyer, K., and Ragan, C., *Modern Concepts of Cardiovasc. Dis.*, 1948, **17**, No. 2.

‡ Furnished by the Charles E. Frosst & Co., courtesy Dr. E. Lozinski.

TABLE I.
Area of Spread of Intradermal India Ink (sq. inches).

Controls		Propylene glycol		Rutin in propylene glycol	
Ink	Ink-Hy'ase	Ink	Ink-Hy-ase	Ink	Ink-Hy'ase
.14	1.20	.35	1.14	.11	.40
.28	1.10	.21	.87	.12	.22
.44	1.41	.25	.84	.12	.35
.22	1.20	.24	.45	.07	.38
.27	2.00	.49	.90	.10	.39
.25	2.50	.25	1.28	.13	.40
.22	2.52	.30	.96	.14	.30
.30	1.92	.28	.98	.14	.43
.28	2.20	.33	.85	.13	.33
.28	1.00	.32	.80		
.35	1.20				
.10	1.30				
.17	.59				
.20	1.05				
.25	1.51	.302	.907	.117	.355
.030	.196	.041	.118	.C16	.085

Differences Between Means in Ink-Hyaluronidase Data.

Mean (controls) — Mean (propylene glycol) = 0.603* ± 0.228

Mean (propylene glycol) — Mean (rutin in propylene glycol) = 0.552† ± 0.145

Differences Between Means in Ink Without Hyaluronidase Data.

Mean (controls) — Mean (propylene glycol) = 0.052 ± 0.050

Mean (propylene glycol) — Mean (rutin in propylene glycol) = 0.185† ± 0.043

Analysis of Variance.

	F (actual)	F (0.01 signif.)
Ink-Hyaluronidase	19.2†	5.39
Ink	16.3†	5.39

* Significant.
† Highly significant.

variance it is evident that the results are due to rutin treatment and not variations within the individual animals. Propylene glycol, which was used as the rutin solvent, inhibits the spreading power of hyaluronidase and this inhibition is increased by a highly significant factor when rutin is also present. Propylene glycol produces slight shock and the resultant liberation of adrenalin probably is responsible for inhibition of hyaluronidase activity. Favilli[10] and Homburger[11] have shown that adrenalin has this property.

Conclusions. Rutin markedly inhibits the spreading activity of intradermally injected inked hyaluronidase. The spread of control injection of ink is also inhibited. Propylene glycol exerts a similar but less intense effect on hyaluronidase and does not reduce the spread of control injections of ink.

10 Favilli, H., quoted in 11.

11 Homburger, F., *Yale J. Biol. Med.*, 1944-45, **17**, 479.