This conclusion does not hold for the subjective sensations of irritation produced by cigarette smoke. In blind tests, the average smoker had no difficulty in arranging these cigarettes in the order of increasing nicotine content on the basis of increasing irritation of the smoke.

The results obtained with nicotine solutions were quite similar. As seen in Table II, edema failed to result at any of the nicotine concentrations used. Tests at higher concentrations were not completed owing to the appearance of rather severe symptoms of systemic nicotine poisoning.

Again the lack of edema production bore no relation to the evidence of subjective irritation. Even though the rabbits were well depressed by morphine, the struggling that followed application of the higher concentrations of nicotine indicated a severe subjective irritation far exceeding that which in our experience results from application of smoke in amounts sufficient to produce massive edemas.

This lack of edema formation following nicotine application brings to mind the observation by Bardy[13] that local application of nicotine to the eye of the rabbit will block further production of conjunctivitis on administration of mustard oil. From this and other evidence, Bardy arrived at the conclusion that conjunctivitis produced by mustard oil was reflex in character and that nicotine interrupts the reflex path through acting on peripheral synapses. Carrying this argument further could lead to the postulate that nicotine tends in this manner to inhibit its own production of conjunctivitis. However this may be, it seems certain that while nicotine may markedly influence the subjective sensations of irritation from cigarette smoke it does not significantly contribute *per se* to its edema-producing properties.

Conclusions. Neither nicotine nor its products of combustion contribute significantly *per se* to the edema-producing properties of cigarette smoke, although it may definitely increase the subjective sensations of irritation.

We are indebted to Mrs. Sarah Guerry for technical assistance in performing the reported experiments.

[13] Bardy, H., *Skand. Arch. Physiol.*, 1915, **32**, 198.

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Studies on "Vitamin P." I. Topically Applied "Vitamin P"-like Substances on the Mammalian Capillary Bed.*

THOMAS J. HALEY, WILLIAM G. CLARK,† AND T. A. GEISSMAN.

From the Department of Pharmacology, School of Medicine, University of Southern California, and the Department of Chemistry, University of California at Los Angeles.

Since the introduction of the concept of "vitamin P" by Szent-Györgyi and his collaborators,[1] an extensive and controversial literature has appeared on the subject.[2] Recently, interest has been reawakened in the subject by the observations of Griffith, Couch and Lindauer[3] that rutin, a flavonol glycoside, decreases capillary fragility in hypertensive patients; and by the work of Lavollay, Par-

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† Present address: The Scripps Metabolic Clinic, La Jolla, Calif.

[1] Armentano, L., Bensáth, A., Bères, T., Rusznyak, I., and Szent-Györgyi, A., *Dtsch. med. Wchnschr.*, 1936, **62**, 1326.

[2] Anon., "Bibliography of Vitamin P" (256 refs.), *Nutrition Research*, 1945, **5**, No. 1 and 2.

[3] Griffith, J. Q., Jr., Couch, J. F., and Lindauer, M. A., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 228.

rot and their co-workers[4-9] who postulate that "vitamin P" exerts its various antihemorrhagic effects by the inhibition of oxidation of circulating epinephrine or adrenochrome, which then is capable of decreasing capillary fragility, "capillary permeability,"‡ and bleeding time, through a vasoconstrictive or tonic action on precapillary blood vessels.

In studying the direct or indirect effects of "vitamin P" on the filtration of fluid through the capillary wall, it is of importance to first describe possible effects on the vasomotion of the small blood vessels, since capillary blood pressure and capillary diameter may influence fluid filtration through the capillary walls. A few studies indirectly bearing on this subject have been reported,[11-13] in which vasoconstriction was observed in Laewen-Trendelenburg preparations of amphibian and mammalian extremities perfused with quercetin, quercitrin, rutin, myricetin and a quercetin diglucoside. One group of workers, however, reported dilatation by quercetin.[14]

In the light of recent concepts of the epinephrine-sparing action of "vitamin P,"[9,15,16] it became of interest to determine if several compounds claimed to have "vitamin P" activity, would show any microscopically observable vasomotor effects on the *intact* mammalian capillary bed when applied topically in the absence of extrinsic epinephrine.

Methods and Materials. The following substances were selected as representative compounds of the "vitamin P" group: rutin, sodium rutin acid succinate, sodium rutin acid phthalate, hesperidin, sodium hesperidin acid succinate, sodium hesperidin acid phthalate, methylated hesperidin chalcone, esculin ("vitamin P_2"),[17] *d*-catechin, epimerized *d*-catechin ("vitamin P_1"),[17] and *l*-epicatechin. In addition it was thought of interest to test "catechin red" (catechin tannin, catechin anhydride, "antivitamin P")[18] and adrenochrome.

The rutin was obtained from Dr. J. F. Couch, U. S. Department of Agriculture, Eastern Regional Laboratory, Philadelphia, and Dr. George Hazel, Abbott Laboratories, North Chicago. The hesperidin and rutin acid phthalates and succinates were synthesized by methods developed from suggestions contained in a Department of Commerce report on the activities of the I. G. Farbenindustrie,[19] for example:

Rutin Acid Succinate. A solution of 4.0 g rutin and 6.0 g succinic anhydride in 25 cc dry pyridine was heated 4 hours on a steam bath. After removal of the pyridine *in vacuo*, the residue was dissolved in 5% $NaHCO_3$, poured into dilute acetic acid, and the resulting clear solution saturated with ammonium sulfate and extracted with isopropanol. Re-

4 Parrot, J., *Gaz. méd.*, 1946, **53**, 157.

5 Galmiche, P., *La rèsistance et la perméabilite des vaisseaux capillaires et vitamine P*, 1 vol., 144 pp., Le Franeois, Paris, 1945.

6 Lavollay, J., *L'autoxydation des diphénols, en particulier de l'adrénaline. Etat actuel du problème de la structure et du rôle fonctionnel de la vitamine P*, 1 vol., 138 pp., Hermann & Cie, Paris, 1943.

7 Parrot, J., and Galmiche, P., *Le bull. méd.*, 1945, **59**, 413.

8 Paraf, A., *La presse méd.*, 1944, **52**, 134.

9 Javillier, M., and Lavollay, J., *Helv. Chim. Acta*, 1946, **29**, 1283.

‡ The term "capillary permeability" has been loosely used in medical literature, and is frequently confused with "capillary fragility," "capillary filtration," "extravasation," etc. The subject has been discussed recently by Landis.[10]

10 Landis, E. M., *Annals N. Y. Acad. Sci.*, 1946, **46**, Art. 8, 713.

11 Fukuda, T., *Arch. exp. Path. u. Pharmakol.*, 1932, **164**, 685.

12 Czimmer, A. G., *ibid.*, 1936, **183**, 587.

13 Jeney, A. V., Méhes, J., Sokoray, L., and Czimmer, A. G., *ibid.*, 1937, **187**, 553.

14 Sokoray, L., and Czimmer, A. G., *ibid.*, 1938, **190**, 622.

15 Muset, P. P., and Garcia-Valdecasas, F., *Estudiòs sobre la vitamina P. I. Acción antioxigeno de la vitamina "P"*, Consejo superior de investigaciones cientifícas, Madrid, 1944.

16 Muset, P. P., *Introducción al estudio de la vitamina P*, 1 vol., 125 pp., Monografias Miguel Servet, Barcelona, 1945.

17 Lavollay, J., *C. R. soc. biol.*, 1945, **139**, 270.

18 Parrot, J., and Cotereau, H., *ibid.*, 1945, **139**, 1051.

19 U. S. Department Commerce Rept. No. PB-981, p. 79, July, 1945.

moval of the isopropanol *in vacuo,* solution of the residual syrup in 15 cc of methanol and addition of this solution to 200 cc ether, gave a filterable solid. This was dried in the vacuum desiccator and ground in a mortar with several portions of fresh ether. The product was a yellow powder, soluble in $NaHCO_3$ solution.

The phthalates were synthesized by similar procedures, for example:

Hesperidin Acid Phthalate. Thirty-six grams of pure hesperidin were treated with 72 g phthalic anhydride in 150 cc dry pyridine, substantially as described above for rutin. The product was purified in much the same manner as in the above case, using butanol extraction and, after triturating the solid product with ether, was finally reprecipitated by acidification of its solution in $NaHCO_3$. The nearly white product was analyzed for phthalic acid residues and was found by 2 different methods to contain very close to 2 acid phthalate groups per molecule of hesperidin.

The sodium salts of the succinates and phthalates were prepared by neutralizing the acid salts with sodium bicarbonate, and were quite soluble and suitable for parenteral administration, in contrast with their parent compounds.

The esculin was purchased from the Mercantile Export and Import Co. of New York, and was a purified preparation. The hesperidin and its methylated chalcone were obtained from Mr. W. E. Baier of the California Fruit Growers' Exchange, Ontario, California. The hesperidin was purified by the formamide method of Pritchett and Merchant,[20] and the methylated chalcone was prepared by the method of Higby.[21] The *d*-catechin was a purified product prepared from gum gambir§ by the method of Freudenberg,[22] and had a melting point 93.5°, which corresponds with the value described by Freudenberg[22] for the hydrated molecule. The epimerized *d*-catechin was supplied by Hoffmann-LaRoche, Inc., and was a brownish colored powder with an optical rotation of $[\alpha]_D^{25}$ + 23.2 (C = 5, in 96% ethanol), equivalent to ca. 33% *d*-epicatechin. In addition, a brownish colored sample of epimerized *d*-catechin, "Catèchin actif" was obtained through the courteous cooperation of Prof. Jean Lavollay. Unfortunately, the sample was too small for an optical rotation determination. The *l*-epicatechin was a small sample obtained from Dr. Edwin F. Bryant of the California Fruit Growers' Exchange, Corona, California, who prepared it in Freudenberg's laboratory from *Acacia catechu* heartwood. "Catechin red" was prepared by the method of Etti,[23] and was rendered catechin-free by ether extraction. The adrenochrome was supplied by Hoffmann-LaRoche, Inc., and was prepared by the method of Buchnea,[24] by oxidation of epinephrine in methanol by means of silver oxide: $C_9H_9O_3N$, calc. C = 60.33, H = 5.06; found C = 59.03, H = 5.23, 5.22.

The capillary bed used was the rat mesoappendix preparation of Chambers and Zweifach.[25] The animals were anesthetized with 45 mg per kg of sodium pentobarbital intraperitoneally, and the bed exposed according to Zweifach's directions[26] and with the helpful suggestions of Dr. Chester Hyman of the Department of Aviation Medicine, University of Southern California. Inasmuch as it had been shown by Zweifach, Hershey, Rovenstine and Chambers[27] that the anesthetic had a deleterious effect on the

§ Supplied by the E. S. Miller Laboratories, Inc., Los Angeles.

20 Pritchett, D. E., and Merchant, H. F., *J. Am. Chem. Soc.,* 1946, **68**, 2108.

21 Higby, R. H., *J. Am. Pharm. Assn.,* Sci. Ed., 1943, **32**, 74.

22 Freudenberg, K., in G. Klein's *Handbuch der Pflanzenanalyse,* Bd.III, Spezielle Analyse, Teil, II, Organische Stoffe II, Julius Springer, Wien, 1932; see also Mason, F. A., *J. Soc. Chem. Ind.,* 1928, **47**, 269T.

23 Etti, E., *Liebig's Annalen der Chemie,* 1877, **186**, 327.

24 U. S. Dept. Commerce Rept. No. PB-47, 1946.

25 Chambers, R., and Zweifach, B. W., *Am. J. Anat.,* 1944, **75**, 173.

26 Zweifach, B. W., personal communication (directions for instructing new assistants).

27 Zweifach, B. W., Hershey, S. G., Rovenstine, E. A., and Chambers, R., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 73.

capillaries, it was always given in a volume of not more than 0.1 cc. After 30 minutes, a booster dose of half the amount of the original dose was given to maintain the same depth of anesthesia. The preparation was bathed with Locke's adjusted to pH 7.6 with $NaHCO_3$, and containing 1% gelatin. The temperature of the solution, which dropped on the preparation at the rate of approximately one drop per second, was $37.5\pm0.2°C$ at the preparation. The animals used weighed from 120 to 244 g, with the majority weighing 150 ± 20 g. The meso-appendix mesenteric membrane of heavier animals contained chains of fat which obscured the smaller vessels. All drugs were dissolved in Locke's solution. The *d*-catechin, epimerized *d*-catechins, as well as the adrenochrome were oxygen- and photo-sensitive. Decomposition of the catechin compounds was observed as a red-brown discoloration and the formation of a colored precipitate (catechin tannins). However this did not decrease the vasoconstrictor properties of these compounds within the periods of storage of the solutions investigated. The oxygen- and photo-sensitive solutions were freshly prepared for each experiment. All solutions were kept in the refrigerator in the dark when not in use. The solutions were placed on the membrane in doses of 0.1 cc, alternating between 0.05 γ of epinephrine (0.1 cc of 1:2 $\times10^6$) and the different dilutions of the compounds being tested, starting with the highest dilutions. The solutions were left on for 5 minutes, during which time the stream of the bathing fluid was interrupted. The animal preparation was considered "normal" only if a consistent and reversible vasoconstriction of the arterioles and meta-arterioles occurred with 0.05 γ of epinephrine, in accordance with one of the criteria established by Zweifach.[25-27] A total of 76 animals were employed.

Results and Conclusions. A saturated solution of rutin or hesperidin had no effect on the capillary bed. As these substances were very poorly soluble, all further work with them was done with the soluble succinates and phthalates. The other compounds investigated were relatively soluble. The results are summarized in Table I. The numbers signify the number of active vasoconstrictions observed over the total number of animals used. The heavy lines in Table I are drawn in the form of lateral histograms, to simplify comparison of relative activity of the various compounds.

The results indicate that some of the "vitamin P"-like substances were highly active vasoconstrictors when topically applied to the mammalian capillary bed, under the experimental conditions employed. This is in accord with the perfusion experiments of Fukuda,[11] Czimmer[12] and Jeney, Mehes, Sokoray and Czimmer,[13] but not with those of Sokoray and Czimmer[14] who reported vasodilatation by 1:5000 quercitrin in the isolated, perfused mesentery. If these workers had used the intact animal the results may have been different, but on the other hand intravascular contact by the compounds may differ from the topical effects described above. In agreement with Lavollay, Parrot *et al.*,[4-9] the catechins were the most active substances tested. According to these workers, *d*-epicatechin is the most active "vitamin P"-like compound, in decreasing capillary "permeability," capillary fragility, hemorrhagic diatheses and even shock symptoms. The present study indicates that epimerized *d*-catechins are no more active than pure *d*-catechin, and that a sample of *l*-epicatechin was as active or more so, at least in its direct effect upon the mesenteric capillary preparation investigated. Rutin, hesperidin, sodium rutin acid succinate, sodium hesperidin acid succinate, methylated hesperidin chalcone, esculin, and adrenochrome were inactive in the concentrations tested. Rutin and hesperidin sodium acid phthalates were slightly active, although negligibly so in comparison with the catechins.

The catechins were active even after partial decomposition. After decomposition had progressed, however, the catechins were damaging to the capillary bed at doses of 0.1 to 1.0 γ, since after they had been applied, the bed was unresponsive to epinephrine. Doses of 0.01 γ of the decomposing catechins were

TABLE I.
Effects of Topically Applied "Vitamin P"-like Substances on the Mammalian Capillary Bed.

Compound tested	No. of rats	Gamma added (in 0.1 cc) 100	10	1	1x10-1	1x10-2	1x10-3	1x10-4	1x10-5	1x10-6	1x10-7	0	Remarks
		No. vasoconstrictions observed, over No. of rats used											
Na-rutin acid succinate	5	0/5	0/5	0/5	0/5	—	—	—	—	—	—	—	No effect in concns. studied
" phthalate	3	1/3	0/3	0/3	0/3	—	—	—	—	—	—	—	Constricts less than epinephrine
Na-hesperidin acid succinate	4	0/4	0/4	0/4	0/4	—	—	—	—	—	—	—	No effect in concns. studied
" " phthalate	4	3/4	1/3	0/4	0/4	—	—	—	—	—	—	—	Constricts less than epinephrine
Methylated hesperidin chalcone	4	0/4	0/4	0/4	0/4	—	—	—	—	—	—	—	No effect in concns. studied
Esculin	4	0/4	0/4	0/4	0/4	—	—	—	—	—	—	—	Same
Adrenochrome	3	0/3	0/3	0/3	0/3	—	—	—	—	—	—	—	Same
d-catechin	6	4/4	4/4	6/6	6/6	2/2	2/2	2/2	0/2	—	—	—	Sensitizes to subsequent doses
Epimerized *d*-catechin	6	2/4	2/4	2/4	6/6	6/6	0/2	—	—	—	—	0/2*	Same
"Catèchin actif"	5	3/3	3/3	5/5	5/5	5/5	2/2	2/2	0/2	—	—	0/2†	Same
l-epicatechin	3	—	3/3	3/3	3/3	3/3	2/2	3/3	1/1	1/1	1/1	—	Lowest dose not determined
Catechin red	2	0/2	0/2	0/2	0/2	—	—	—	—	—	—	—	Vasodilates, with stasis

* Distilled water.
† Ringer-Locke, with gelatin.

not damaging and the toxic effects of Lavollay's "Catèchin actif" were less noticeable than the sample of epimerized *d*-catechin obtained from Hoffmann-LaRoche. Catechin red was especially damaging in doses greater than 0.01 γ. It is probable that the damaging effect of the partially decomposed catechin preparations was due to the presence of catechin tannins formed during the decomposition. This is also supported by the observation by Parrot and Cotereau[18] that these colored substances, which they term "antivitamin P," antagonize the action of active catechin ("vitamin P"), and increase capillary fragility.‖

It is probable that some of the biological and clinical effects ascribed to "vitamin P" can be explained on the basis of a vasoconstrictor effect on the capillaries rather than to an indirect effect on these vessels through an epinephrine-sparing action[4-9] or by a direct action on capillary walls.[16]

Summary. 1. A mammalian capillary bed preparation was modified from the rat mesoappendix mesenteric preparation of Zweifach and Chambers, and used to estimate the relative activities of various topically applied "vitamin P"-like compounds on microscopically visible vasomotor responses of the vessels.

2. Rutin, rutin sodium acid succinate, hesperidin, hesperidin sodium acid succinate, methylated hesperidin chalcone, esculin and adrenochrome were inert in the concentrations used.

3. The sodium acid phthalates of rutin and hesperidin were slightly active vasoconstrictors.

4. The catechins were highly active vasoconstrictors, including *d*-catechins, epimerized *d*-catechins, and especially *l*-epicatechin, in doses as low as 0.0001 γ or less.

5. Catechin red (catechin tannin or anhydride) was highly toxic, inducing irreversible vasodilatation and stasis.

‖ Preliminary investigations in these laboratories indicate that catechin tannins, like tannic acid, are powerful endothelium poisons, producing marked hemorrhagic symptoms when administered intravenously, which supports the observations of Parrot and Cotereau.[18]

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Relative Gastro-Intestinal Stability of Carotene and Vitamin A and Protective Effect of Xanthophyll.*

W. C. SHERMAN. (Introduced by W. D. Salmon.)

From the Laboratory of Animal Nutrition, Alabama Polytechnic Institute, Auburn.

Previous work[1,2] from this laboratory has demonstrated that carotene administered to vitamin A-deficient rats is destroyed in the gastro-intestinal tract by the simultaneous feeding of unsaturated fat acid esters. The gastro-intestinal destruction of carotene was prevented by feeding α-tocopherol. Similar results have been reported by Quackenbush, Cox and Steenbock,[3] and by Hickman, Harris and Woodside.[4] Hove[5] has shown that the mucosa of the rat stomach contains an enzyme, carotene oxidase, which greatly accelerates the destruction of carotene *in vitro* in the presence of methyl linolate.

Under conditions used in biological assay procedures for vitamin A, the potency of vita-

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1 Sherman, W. C., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 199.

2 Sherman, W. C., *Proc. Fed. Am. Soc. Exp. Biol.*, 1942, **1**, 134.

3 Quackenbush, F. W., Cox, R. P., and Steenbock, H., *J. Biol. Chem.*, 1942, **145**, 169.

4 Hickman, K. C. D., Harris, P. L., and Woodside, M. R., *Nature*, 1942, **150**, 91.

5 Hove, E. L., *Science*, 1943, **98**, 433.