pletely dispense with that gland.

*Summary.* Pregnancy has been maintained in rats hypophysectomized on day 6 of their first pregnancy and injected daily subcutaneously for 6 days with placentae from rats, 12 days pregnant. Five placentae or approximately 275 mg daily accomplished this in the majority of the test rats. One 12-day placenta plus 0.5 μg of estrone proved equally efficacious. Pregnancy was not maintained by similar treatment if the rats were also oöphorectomized.

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## Capillary Fragility and Vitamin 'P' Protective Action Against Radiation. (18081)

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(Introduced by Walter H. Eddy)

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When Armentano and associates(1) announced their discovery of a new vitamin 'P', or "permeability vitamin," they did not define what they understood under the term of increased capillary permeability. The absence of any specification concerning vitamin 'P' activity became a source of considerable confusion, which was further increased by the theory of Javillier and Lavollay(2) and Haley *et al.*(3) that increased capillary permeability and fragility are phenomena of a purely neurogenic nature. Yet neither of these investigators made a distinction between the terms of increased capillary permeability and capillary fragility. Haley *et al.*, using a mammalian capillary bed (a rat meso-appendix preparation) for the estimation of the activity of vitamin 'P', measured this activity by visible vasomotion of the arterioles and metaarterioles and concluded that only catechins among the flavonoids possess "permeability activity" interpreted in terms of vasomotor response.

The purpose of our present investigation is to show that the flavonoids described by Szent-Gyorgyi and others under the name of vitamin 'P' exert their effectiveness on the capillary wall mostly if not exclusively in cases when increased capillary fragility is present. We define the term "increased capillary fragility" as a condition when chemical lesions in the capillary wall are apparent, while increased capillary permeability of a temporary nature might occur due to neurogenic factors. In most cases of hemorrhagic diathesis either due to toxic condition or in radiation injury chemical changes in the capillary wall may be detected microchemically(4).

*Experimental.* A vitamin 'P' compound, isolated from citrus fruit and containing 4 identified flavonoid substances† was tested for capillary permeability activity. In the first series of experiments we used Menkin's method(5). He found that an inflammatory exudate induces an increase in capillary permeability, manifested by the accumulation of trypan blue from the circulation in a cutaneous area previously injected with an exudate. This effect was duplicated and even accentuated by leukotaxine, a nitrogenous substance extracted from inflammatory exudation. Ac-

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1. Armentano, L., Bentsath, A., Beres, T., Rusznyak, B., and Szent-Gyorgyi, A., *Deut. med. Wochschr.*, 1936, v62, 1326.

2. Javillier, M., and Lavollay, J., *Helvetica Chim. Acta.*, 1946, v29, 1283.

3. Haley, T., Clark, W., and Geissman, T., PROC. SOC. EXP. BIOL. AND MED., 1947, v65, 202.

4. Sokoloff, B., and Redd, J. B., "Capillary Permeability and Fragility" Monograph, Florida Southern College, 1949.

† Four factors present in this compound were identified as chalcone hesperidin, glucose-hesperidin, eriodictin and quercitrin-like substance. This compound is similar but not identical to the original "citrin" of Szent-Gyorgyi.

5. Menkin, V., *Am. J. Physiol.*, 1940, v129, 691.

TABLE I. Dye Penetration Rates.

| |
|---|
| Control rabbits, time of dye penetration in sec: 180, 120, 195, 103, 110, 90, 60, 80, 90, 135 (avg 1 min. 57 sec.) |
| Vit. 'P' rabbits, time of dye penetration in min. 23, 60, 48, 22, 51, 46, 47, 41, 24, 52, 39, 38, 38, 44, 59, 43, 44, 45, 46, 48 (avg 42 min. 54 sec.) |

cording to Menkin its action can be inhibited by an extract of adrenal cortex.

Rabbits averaging 2 kilo in weight were given 10 mg per kilo of weight of a flavonoid substance 20 minutes before injection of 3 mg of leukotaxine. Immediately afterward 12 cc of 1% trypan blue in saline solution was introduced into the marginal ear vein. The capillary permeability was gauged by the degree of dye accumulation in the various skin areas. We found that the citrus vitamin 'P' compound when given in this amount wholly inhibited the effect of leukotaxine of increasing capillary permeability. The citrus vitamin 'P' compound was effective in inhibiting the injurious effect of leukotaxine whether it was injected separately or mixed together. Thus it exerted an effect on capillary permeability similar to the cortical extract of Menkin's experiments.

In our next series of experiments, using the technic of Ambrose and DeEds(6) which consists in the application of chloroform to the skin by means of a cotton-tipped applicator, we tested the citrus vitamin 'P' compound. A dose of 10 mg per kilo of weight was injected subcutaneously 20 minutes before the injection of trypan blue (10 cc) in the marginal ear vein and the application of chloroform wheal to the skin. The average time of the appearance of the dye for 20 tests was 43 minutes as against about 2 minutes in the control batch of 10 rabbits (Table I).

These 2 tests, although reliable for a qualitative testing of vitamin 'P' activity, are not well adapted for quantitive bio-assaying of the potency of these compounds. In search for such a test we began experimentation with a bacterial polysaccharide. The polysaccharide isolated by Shear(7) from *Serratia marcescens* produces an extensive hemorrhage in the tumors of animals and, when the dose is sufficiently large, can even cause death. Normal rats and mice are much less susceptible to the toxic effect of this compound and can survive a much larger dose than can the cancerous animals. Four or 5 hours after the bacterial polysaccharide is injected into the cancerous animals, profuse hemorrhages are observed in the adrenal gland. For our experiments we used August Rat Carcinoma, a fast growing tumor. When a rat bearer of a large tumor is given 0.3 mg per 100 g of weight of P-25, a bacterial polysaccharide which we received from Dr. Shear, death occurs in about 7 hours preceded by profuse hemorrhages in the adrenal gland. If a small dose, 3 mg per 100 g of weight, of the citrus vitamin P compound is given subcutaneously, one or two hours before the injection of polysaccharide, the life of the animal is prolonged up to 18-24 hours. A larger dose of the same vitamin 'P' compound given twice, before and shortly after the injection of P-25, will prevent the hemorrhages not only in the adrenal gland but also in the tumor itself. We were able to estimate with a considerable degree of accuracy the exact dose of vitamin 'P' compound which inhibits the effect of polysaccharide on the capillary system. Five mg per 100 g of weight of vitamin 'P' compound, given twice, is the protective dose against the near-lethal dose of polysaccharide. The hemorrhages in the tumor and in the adrenal gland do not occur immediately after the injection of polysaccharide but develop gradually as the result of increasing capillary fragility in the affected area. Apparently this compound causes an injury to the capillary wall in a similar if not identical manner as leukotaxine.

In our next series of experiments, radiation injury to the capillary wall was investigated. One hundred rats were submitted to a total

6. Ambrose, A. M. and DeEds, F. L., *J. Pharm. Exp. Therap.*, 1947, v90, 359.

7. Shear, M. J., *J. Nat. Cancer Inst.*, 1943, v4, 81.

TABLE II. Control Groups (40 rats).

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of days of survival | 11, | 12, | 13, | 14, | 15, | 16, | 17, | 18, | 19, | 20, | 21, | 22, | 23 |
| No. of rats succumbed | 1, | 3, | 3, | 2, | 3, | 3, | 3, | 3, | 2, | 4, | 2, | 1, | 2 = 32 |
| Mortality rate: 80% | | | | | | | | | | | | | |

TABLE III. Rats Given 4 mg of Vit. 'P' for 10 Days (20 rats).

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| No. of days of survival | 17, | 18, | 19, | 20, | 21, | 22, | 23, | 24, | 25 |
| No. of rats succumbed | 1, | 1, | 2, | 0, | 0, | 1, | 1, | 1, | 1 = 8 |
| Mortality rate: 40% | | | | | | | | | |

TABLE IV. Rats Given 5 mg of Vit. 'P' for 30 Days (40 rats).

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| No. of days of survival | 18, | 19, | 20, | 21, | 22, | 23, | 24, | 25, | 26 |
| No. of rats succumbed | 1, | 0, | 0, | 0, | 1, | 0, | 0, | 1, | 1 = 4 |
| Mortality rate: 10% | | | | | | | | | |

body, near-lethal dose of radiation. The radiation factors were: 250 kv, with 0.5 mm Cu and 3.0 mm Bakelite Filters. Target distance was 27.5 cm and 210 r/min dose rate. All rats received 800 r total body X radiation in a single exposure. Forty rats served as control. The mortality rate in the control group was 80% (Table II). All survivors manifested gross hemorrhages of various degrees of gravity. Of the treated animals 20 were given 4 mg of citrus vitamin 'P' for 10 days, 3 days prior to radiation and 7 days post radiation. This dose gave a partial protection. The mortality rate was reduced to 40% (Table III). Forty rats were given 5 mg of vitamin 'P' compound for 30 days, with a total amount of 150 mg. In this group the mortality rate was reduced to 10% (Table IV).

The results of this series of experiments seem to indicate that the flavonoid compound, vitamin 'P', gives considerable if not complete protection against a total body near-lethal dose of radiation. The absence of hemorrhages and the state of the capillary wall of the treated animals suggests that this protective action of flavonoids concerns chiefly if not exclusively the capillary system. The striking fact is that injury to the capillary wall, whether it is caused by ionizing radiation or by leukotaxine, or by a bacterial polysaccharide, can be prevented to a considerable degree by the administration of the same flavonoid compound.

*Discussion.* All the available evidence indicates that the capillary wall in normal tissue is comparatively impermeable both to serum albumin and to serum globulin. Therefore, the average effective pore size of the capillary wall must be less than 6 millimicrons while the spaces between adjacent endothelial cells are greater than 15 millimicrons. It is established that pore size is defined by the intercellular cement lying between the endothelial cells. According to the work of Chambers and Zweifach(8) and others the cement dissolves under certain physical conditions such as the absence of calcium or at slightly acid pH. Danielli and Stock(9) pointed out: "It is quite certain that anything which affects the physical condition of the intercellular cement affects the pore size." This in turn will affect the effectiveness of the pores as "perfect sieves" and consequently induce what we know as increased capillary fragility. According to Danielli and Stock "under normal conditions the walls of the pores through this cement are coated with a layer of adsorbed serum protein which effectively reduces the area of pore through which filtration may occur." Displacement of this adsorbed serum protein by other molecules of small diameter may result in an increase in pore size and in a consequent injury to the capillary wall. In toxic conditions when increased capillary fragility is manifested such displacement might take place. Although Duran-Reynals(10) sug-

8. Chambers, R. and Zweifach, B. M., *J. Cell. Physiol.*, 1940, v15, 255.

9. Danielli, J. F. and Stock, A., *J. Physiol.*, 1945, v110, 81.

10. Duran-Reynals, F., *Yale J. Biol. Med.*, 1939, v11, 601.

gested that the "spreading factor," hyaluronidase causes an increase in capillary fragility by affecting the intercellular cement, other investigators were unable to confirm this observation. Danielli and Stock have stated that in all probability the injury to the capillary wall in toxic conditions is direct and not due to enzymic action.

It is even more unlikely that the "spreading factor" plays a role in the course of radiation injury. This impression is supported by the failure in the work of Rekers and Field(11) on dogs of such potent hyaluronidase inhibitors as dopa and sodium gentizate to influence the course of radiation. These results showed that the effect of vitamin 'P' factors seems also to be directly on the intercellular substance. They obtained no evidence that these factors inhibit heparin, for the heparin content was not altered by vitamin 'P' therapy to any great degree.

Rekers and Field also found that certain flavonoids gave considerable protection against a total body near-lethal dosage of radiation. They concluded that "previous misunderstanding of the nature of vitamin 'P' has arisen from both the failure to recognize that several flavonone analogues possess very similar anti-hemorrhagic activity and that ascorbic acid has the capacity to potentiate activity in other flavonones." They are inclined to conclude that vitamin 'P' factors affect the capillary system directly perhaps participating as a principal in the 'wear and tear' of a part or all of the capillary system, inhibiting degeneration and taking part in its regeneration. The results of our present investigation are in full accordance with their viewpoint and give further support to the original work of Armentano *et al.*(1) as to the role which the factors of vitamin 'P' play in increased capillary permeability. This term should be interpreted, however, as indicative of chemical lesions in the capillary wall, or more exactly as increased capillary fragility.

*Conclusion.* Administration of leukotaxine, a bacterial polysaccharide or an exposure to ionizing radiation induces increased capillary fragility. Injury to the capillary wall, whether it is caused by ionizing radiation, by leukotaxine, or by a bacterial polysaccharide, can be prevented to a considerable degree by the administration of a vitamin 'P' compound composed of flavonoids naturally present in citrus fruit.

11. Rekers, F. E. and Field, J. B., *J. Clin. Invest.*, 1949, v28, 746.

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## Occurrence of Endocarditis with Valvular Deformities in Dogs with Arteriovenous Fistulae.* (18082)

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The accidental finding of endocarditis in dogs has been reported to be extremely rare (1). Likewise the dog is generally believed to be a relatively resistant animal to the experimental production of endocarditis(2). It is therefore of interest that the application of an increased work load of a particular type to the canine heart was followed by a high incidence of endocardial vegetations without the necessity of intentional injection of any bacteria. The observations herein reported were made incidental to a study of heart failure in the dog induced by an increase in work load. This increase in work load of the heart has been accomplished by large

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1. Morehead, R. P. and Little, J. M., *Am. J. Path.*, 1945, v21, 339.

2. Clawson, B. J., Personal Communication, 1949.