West Nile (Egypt 101 strain), dengue (Hawaiian strain), and Japanese B encephalitis viruses. The sera from monkeys infected or hyperimmunized with the respective viruses inhibited HA, while the control samples of serum taken prior to infection or immunization failed to do so. Furthermore, it can be seen that 2 different pools of normal mouse serum did not affect HA with dengue (Hawaiian strain) or Japanese B encephalitis viruses; nor did an Eastern equine encephalitis hyperimmune mouse serum inhibit HA with Japanese B encephalitis virus. The hyperimmune homotypic mouse sera, however, inhibited agglutination in dilutions from 1:160 (for Japanese B encephalitis) to 1:640 (for dengue, Hawaiian strain).

*Summary.* A simple, easily reproducible method for preparation of high-titer hemagglutinins with several arthropod-borne viruses is described. The method is based on the use of brains from newborn mice infected with virus as the source material, and on treatment with acetone and ether for the elimination of nonspecific factors. With this method, stable specific HA has been found associated with the following viruses: dengue (Hawaiian type), dengue (New Guinea B type), Ilhéus, Japanese B encephalitis, Ntaya, West Nile (Egypt 101 strain), and yellow fever (Asibi strain). As yet, no agglutination has been uncovered associated with Anopheles A, Eastern equine encephalitis, poliomyelitis (Type 2, MEF 1 strain), Russia Far Eastern encephalitis, and West Nile (original strain) viruses.

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## Failure of Rutin and Catechin* to Affect Vascular Lesions of Experimental Hypertension or Hypersensitivity.† (20300)

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In a previous communication, one of the authors, (J.L.O.), in collaboration with several others, reported the effectiveness of rutin in decreasing the gross hemorrhage in experimental malignant hypertension in dogs(1). Shortly after that work was completed, Moss, Beiler and Martin(2) reported the effectiveness of another flavonol, catechin, in preventing anaphylactic shock in guinea pigs. Because we were interested in both hypersensitivity arteritis and malignant hypertension, it was decided to test the effectiveness of each of these two compounds in hypersensitivity arteritis and also in the arteritis of malignant hypertension.

*Methods.* Seven rabbits and 6 dogs were given a solution of rutin in 0.1 N NaOH with

* Catechin was generously supplied by the National Drug Co., Philadelphia, Pa.

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0.5 cc of 1% procaine subcutaneously, at the rate of 20 mg/kg/day. These injections were given to the rabbits for 12 days before the first injections of horse serum, and to the dogs for from 15-20 days before the stenosis of the renal arteries. In both groups the treatment was continued until the animals died or were sacrificed. This schedule of injections was used in all of the following groups. Seven rabbits and 5 dogs were given a solution of catechin in 0.1 N NaOH with 0.5 cc of 1% procaine subcutaneously. The dogs were given only 8 mg/kg/day, the dosage used by Moss, Beiler and Martin in guinea pigs, but it was decided to give the rabbits the catechin in the same dosage as the rutin, that is, 20 mg/kg/day. The third group of 7 rabbits and 5 dogs were given only 0.5 cc of 1% procaine subcutaneously per day and served as the control group. The rabbits were sensitized by a single intravenous injection of 10 cc/kg of sterile horse serum, and were sacrificed 14 days later. In preparing dogs for the experiment, control blood pressures were measured 3 times per week by a direct femoral puncture until mean blood pressures were consistently below 135 mm Hg for at least 2 weeks. Malignant hypertension was then produced by stenosis of both renal arteries. The stenosis was produced by including a stilet 0.5 mm in diameter within a silk ligature around the artery, the stilet then being withdrawn. After stenosis of the renal arteries, blood pressures were taken daily. All animals were autopsied, and the heart, aorta, lungs, liver, gall-bladder, spleen, pancreas, adrenals, kidneys, urinary bladder, stomach, intestine, colon and mesentery examined both macroscopically and microscopically. The blocks for microscopic examination were taken in the same manner as described previously(3). An arbitrary system was established for the rough quantitative grading of vascular disease in these animals. Since the rabbits had no grossly visible lesions, only grading of microscopic lesions could be made. The lesions in each organ were graded from 0 to 3+ and the total number of plus signs was determined for each animal. A scale for comparison was then made by dividing the total number of plus signs for the animal having the most lesions into 4 equal parts. The scale was then ½ to 3 = +; 3½ to 6 = 2+; 6½ to 9 = 3+; 9½ to 12 = 4+. The use of a final 4+ scale for the rabbits was decided upon since one rabbit had much more extensive lesions than any of the others. This fact could be appreciated in the chart only when a 4+ scale was used. The results for the dogs were similarly computed, except that a final 3+ scale was used.

TABLE I. Degree of Vascular Damage Induced by Horse Serum in Treated Rabbits. 7 animals in each series.

| Type of pretreatment | No. of animals with degree of arteritis | | | | |
|---|---|---|---|---|---|
| | 0 | + | ++ | +++ | ++++ |
| Control (procaine only) | 4 | 1 | 2 | 0 | 0 |
| Catechin and procaine | 4 | 1 | 2 | 0 | 0 |
| Rutin and procaine | 3 | 0 | 1 | 2 | 1 |

*Results.* As shown in Table I, rabbits made hypersensitive to a large intravenous dose of horse serum varied greatly in the amount of vascular damage induced. In spite of this variation, the catechin-treated group and the control group showed essentially the same results. The rutin-treated group, however, developed even greater vascular damage than the control group.

The changes in the dogs could be seen grossly as hemorrhages and microscopically as acute necrosis of arterioles and myocardial necrosis. Accordingly, a rough quantitation of both the macroscopic and microscopic changes was made. As in the rabbits with hypersensitivity, neither rutin nor catechin had any effect on the lesions of dogs with malignant hypertension. These results are presented in Tables II and III.

The results reveal no difference in incidence of lesions between the control and flavonol-treated animals. The only apparent variation is in the rutin-treated group of dogs. Two dogs in this group were completely free of lesions. However, these two animals, in contrast to all of the others, did not develop hypertension after renal artery stenosis and are, therefore, considered to be failures in experimental technic. When this factor is con-

TABLE II. Degree of Hemorrhage Seen Grossly in Treated Dogs with Malignant Hypertension.

| Type of pretreatment | No. of animals | No. of animals with each degree of hemorrhage 0 | + | ++ | +++ |
|---|---|---|---|---|---|
| Control (procaine only) | 5 | 1 | 3 | 1 | 0 |
| Catechin and procaine | 5 | 1 | 3 | 1 | 0 |
| Rutin and procaine | 6 | 2 | 1 | 2 | 1 |

TABLE III. Arteriolar Necrosis and Myocardial Necrosis in Treated Dogs with Malignant Hypertension.

| Type of pretreatment | No. of animals | No. of animals with each degree of arteriolar necrosis 0 | + | ++ | +++ |
|---|---|---|---|---|---|
| Control (procaine only) | 5 | 0 | 3 | 2 | 0 |
| Catechin and procaine | 5 | 0 | 2 | 3 | 0 |
| Rutin and procaine | 6 | 2 | 0 | 4 | 0 |

sidered, the apparent difference in the rutin-treated group loses its significance.

*Discussion.* Two reviews of the literature pertaining to the flavonol compounds indicate wide variations in the effectiveness of these substances in hemorrhagic states and other vascular disease(4,5). Contrary to the previous observations of one of us (J.L.O.), the results obtained in this experiment fail to indicate any effectiveness of rutin in malignant hypertension in dogs. The only differences between this experiment and the previous one using rutin are the use of different samples of rutin and a different injection schedule. The injection schedule used previously was three times daily, whereas in this experiment, the injections were given once daily. It is possible that either or both of these factors may have caused the difference in results.

Rutin was also found to be ineffective in the prevention of arterial lesions in hypersensitivity. Catechin was ineffective in both types of vascular lesions. It would appear, therefore, that if either flavonol has any effect, it must be too slight to be demonstrated by the procedures used.

*Summary.* Neither rutin nor catechin altered the vascular lesions of hypersensitivity arteritis in rabbits or of experimental malignant hypertension in dogs.

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## Myotrophic Activity of 19-Nortestosterone and Other Steroids Determined by Modified Levator Ani Muscle Method.* (20301)

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Many steroids produce both androgenic and protein anabolic effects(1). Protein anabolic effects have been measured by body weight gain(2), by nitrogen retention(3-6), and by renotropic activity(7,8). Eisenberg and Gordon(9) have proposed a myotrophic test for the assay of protein anabolic activities of androgens. Steroids which had the greatest protein anabolic activity as measured by the classical nitrogen retention methods, also had the greatest myotrophic activity as measured by a levator ani muscle assay(9,10).

In this study a modification of the Eisenberg and Gordon myotrophic assay is proposed which eliminates the 23-day post-castration

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