body and iris of the beef eye, and from colored human skin. These preparations were characterized with the electron microscope.

2. The melanin-containing pseudoglobulin from the S-91 melanoma was shown to consist of melanin granules unchanged in shape or size with respect to granules obtained by centrifugation.

## 16114

## Evaluation of a New Test for the Effect of Vitamin P on Capillaries.*

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In our work with rutin[1] we felt the need for a quick test for vitamin P like effects on the capillaries. The method described by Majovski *et al.*[2] appeared to have the advantage of speed and simplicity necessary for the assay of a series of compounds.

The test proposed by Majovski *et al.*[2] consists of the following procedures: Control and test mice are placed in a small jar which is connected by means of a stop-cock and pressure tubing to a five-gallon bottle. The larger bottle is evacuated by means of a vacuum pump to 45 mm of mercury pressure. Upon opening the stop-cock, the pressure in the smaller jar containing the mice quickly drops to 70 mm of mercury pressure. After a 60-second exposure to this decreased pressure the mice are removed and observations are made on the extent of pulmonary hemorrhage. Control mice exhibited a greater degree of pulmonary hemorrhage than did those "protected" by prior administration of certain citrus peel fractions. Thus, it was assumed that this method affords a means of measuring changes in vascular fragility or permeability as influenced by various agents.

In the report of this method, the authors made mention of control animals which failed to conform to the usual reaction and indicated that there seemed to be some correlation between the "presence or absence of food in the stomach and these peculiarities." This observation was repeated by Kibrick and Goldforb[3] who reported "protection" afforded by starving the animals prior to their use in the test as compared with fed mice. Besides, Kibrick and Goldforb[3] found that a number of preparations of the hesperidin group did not afford protection to the mice against pulmonary hemorrhage. They admit, however, that the drugs used may have had varying and unknown contents of vitamin P activity. We felt that the observations of Kibrick and Goldforb did not obviate the usefulness of this method as a test for vitamin P activity, as feeding and fasting of the mice were factors easily controlled.

A few preliminary trials with two groups of fed mice, one of which had received rutin, showed that the animals which had been fed only exhibited gross pulmonary hemorrhages, while those which had been fed and which also had received prior treatment with rutin, showed no pulmonary hemorrhages. Thus it appeared that control of the feeding-fasting factor retained the method's capacity for testing for vitamin P activity.

We have found, however, in the course of our work, that the method is not specific for

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1 Raiman, R. J., Later, E. R., and Necheles, H., *Science*, in press.

2 Majovski, G. J., Lesser, A. J., Hanson, H. C., Carne, H. O., and Thienes, C. H., *J. Pharm. and Exp. Therap.*, 1944, **80**, 1.

3 Kibrick, A. C., and Goldforb, A. F., *J. Pharm. and Exp. Therap.*, 1944, **82**, 211.

TABLE I.

| Agent | Amt of drug intraperitoneally | Protection | No. of mice |
|---|---|---|---|
| Simultaneous controls of all tests | Untreated | No | 48 |
| Acetate buffer, pH = 7.75 | 0.15 cc | " | 3 |
| Rutin in acetate buffer, pH = 7.75 | 1 mg in 0.15 cc | Yes | 4 |
| Propylene glycol | 0.15 cc | " | 6 |
| Rutin in propylene glycol | 1 mg in 0.15 cc | " | 12 |
| Ethylene glycol | 0.15 cc | " | 4 |
| Glycerol | " " | No | 3 |
| Ethyl alcohol | " " | Yes | 4 |
| Oxalic acid | 2 mg in 0.15 cc | No | 3 |
| Hypertonic saline | 0.15 cc | * | 5 |
| Glucose | 2 mg in 0.15 cc | Yes | 4 |

* Questionable.

vitamin P and are reporting our results here in order to save other workers time and labor.

*Observations.* On running control mice which received as prior treatment only the fluids used in dissolving the agents which we desired to test for their effects on vascular permeability and fragility, we found that some solvents by themselves afforded equal protection against the hemorrhages induced by exposure of the animals to decreased pressure as the specific agents believed to decrease vascular fragility and permeability. Therefore, we tried the effect of several other agents. Table I lists the agents tested and their ability to protect adult white mice of 20-25 g body weight and of both sexes against the pulmonary hemorrhages. No effect of sex was noted in the tests.

Our method deviated from that of Majovski *et al.* only in that the pressure to which the animals were subjected was slightly higher. We used a pressure of 85 mm instead of 70 mm of mercury.

No apparent pulmonary hemorrhages in treated mice as compared with grossly hemorrhagic lungs of untreated controls simultaneously exposed to the decreased pressure was called "protection," and similar degrees of hemorrhage in control and test animals was declared "no protection."

All agents were administered by intraperitoneal injection 35 minutes before exposure to the decreased pressure.

It might be mentioned that most of the "protected" animals survived significantly longer in the decreased pressure and afterwards than did the controls, but the correlation between survival time and protection is not absolute.

Hypertonic saline afforded "questionable" protection because complete protection was never achieved, but in several trials, the mice given hypertonic saline exhibited significantly less hemorrhage than did the controls.

Except rutin, the agents which were found to protect the animals by this method are generally accepted to have no effect on vascular fragility or permeability. Most of the solvents used for rutin afforded as much protection as did rutin itself. Also glucose, which is an integral part of the molecule of both hesperidin and rutin, afforded protection against the pulmonary hemorrhages.

We conclude therefore, that this test is non-specific for the vascular effects of vitamin P.

*Summary.* The test proposed by Majovski *et al.* for evaluating the effects of vitamin P on capillaries was subjected to a critical study.

The experience of others that previous feeding affects the results of the test was confirmed, but this objection can be overcome by using starved mice only.

The present tests demonstrate that Majovski's method is non-specific, however, because a number of solvents used for the rutin, as well as other substances, gave mice the same protection against low atmospheric pressure as rutin did. The drugs which protected mice are not known to have any effect on capillaries; they were: propylene glycol, ethylene glycol, ethyl alcohol and glucose.