TABLE I. Statistical Variability in the Standardization of I^{131}.

ml of I^{131} sol. taken*	Activity in c/m minus background	Activity in rutherfords	Total activity of I^{131} soln. in rd.†	Deviation from mean
.1	41 ± 2	.0187	9.35 ± .47	—.25
.1	40 ± 2	.0184	9.20 ± .46	—.40
.5	197 ± 5	.0906	9.06 ± .23	—.54
.5	207 ± 5	.0954	9.54 ± .23	—.06
1.0	402 ± 5	.185	9.25 ± .12	—.35
1.0	402 ± 5	.193	9.65 ± .11	+.05
2.0	863 ± 12	.398	9.95 ± .14	+.35
2.0	869 ± 12	.400	10.00 ± .14	+.40
3.0	1306 ± 15	.601	10.00 ± .12	+.40
3.0	1300 ± 15	.598	9.96 ± .11	+.36
Standard (2.0)	113 ± 3	.0522‡		
		Mean	9.60 ± .11§	
Standardized against RaD beta standard†			7.93 ± .16	
			7.80 ± .20	
		Mean	7.87	

* Make up to a total volume of 3.0 ml with distilled water.
† Stand. dev. based on counting error only.
‡ Calculated from data supplied by the National Bureau of Standards.
§ Stand. dev. of the mean for 10 samples.

the Geiger-Mueller tube. Even at this close distance geometry errors were less than 2% as shown by repeated counts on the same sample following repositioning of the ampoule.

It is seen from Table I that for low intensity samples the counting precision appeared to be the limiting source of error while for higher intensity samples other factors such as measurement of volume become more predominant. The agreement between the mean values obtained by the 2 methods of standardization was considered to be satisfactory, in view of the fact that small differences in the geometries and absorption coefficients of the I^{131} beta sample and RaD standard were in the direction to give a low result.

Summary. A rapid and convenient method for the standardization of I^{131} solutions is described. A statistical evaluation of the method showed that the standard deviation of the mean for 10 samples was approximately 1%.

The authors acknowledge the technical advice and help of R. R. Edwards, Ph.D., Institute of Science and Technology, University of Arkansas, Fayetteville, Ark., and R. G. Holmes, Pediatric Research Assistant, School of Medicine, Little Rock, Ark.

Received May 9, 1950. P.S.E.B.M., 1950, v75.

Maintenance of Pregnancy in Hypophysectomized Rats with Placental Implants.* (18080)

STUART C. AVERILL, ESTEN W. RAY, AND WILLIAM R. LYONS

From the Division of Anatomy and the Institute of Experimental Biology, University of California, Berkeley, Calif.

Using the placentoma reaction Astwood and Greep(1) demonstrated that the rat placenta contains a luteotrophic substance capable of stimulating the corpora lutea to secrete pro-

* Aided by grants from the Research Board of the University of California.

1. Astwood, E. B. and Greep, R. O., PROC. SOC. EXP. BIOL. AND MED., 1938, v38, 713.

gestin. No mention was made of the age of the placentae used, but it might be inferred from the work of Pencharz and Long(2) that the placenta of mid-gestation is able to take over the function of secreting gonadotrophins comparable to those of the pituitary. In the following experiment in which pregnancy was maintained in hypophysectomized rats during the critical 6-12 day phase further proof of a chorionic luteotrophin is presented; and support is also lent to Selye's indirect evidence for a substance in the rat placenta which stimulates the ovary to secrete estrin(3).

Experimental. Nulliparous, Long - Evans rats, 2-7 months old were used. Rats in proestrus were caged with a male in the afternoon and when sperm were identified in the vaginal smear on the following morning this was considered day 1 of pregnancy. The test rats or recipients were hypophysectomized† on day 6 and injected or implanted from day 6 to 11. Necropsy was performed on day 12. Oöphorectomy in a control experiment was performed immediately after hypophysectomy. Tests were made on placentae taken from donors on days 10, 12 and 15 of gestation. Average weights of such placentae were 38, 55 and 250 mg respectively. Sixty tests were made on different amounts of placental tissue from the 3 stages of pregnancy. In some instances the placental implants were supplemented by subcutaneous injections of estrone‡ in sesame oil. In a few experiments, tests were made on separated fetal and decidual parts of the implantation mass. Except as noted in the Table the placentae were implanted within an hour of the removal from the donors. Two types of controls were used, namely, rats receiving no implants, and rats oöphorectomized as well as hypophysectomized receiving implants. For confirmatory and comparative purposes, pituitary lactogenic hormone alone and with estrone was injected into another series of similar test animals. As shown earlier(4) lactogenic hormone did not maintain pregnancy unless combined with estrone.

TABLE I. Effect of Various Hormonal Combinations on Maintenance of Pregnancy in Rats Hypophysectomized on Day 6, Treated for 6 Days and Sacrificed on Day 12.

No. of rats	Placentae implanted daily	Day of gestation of donor	Dose of estrone	Rats with living emb.
6	0	—	—	0
6	2 whole	10	1 μg day 7	0
12	1-4 whole	12	—	3
10	5-8 "	12	—	8
3*	5-7 "	12	—	0
7	1 "	12	1 μg day 7, 9	5
3†	2 "	12	.5 μg daily	1
5†	2 fetal	12	.5 " "	2
5†	1-2 maternal	12	.5 " "	2
4	1-2 whole	15	—	0
4	1 "	15	1 μg day 7, 9	0
1	2 "	15	1 " " 9	1

* Rats also oöphorectomized.
† Placentae used in these cases had been stored in a frozen condition 1-14 days.

Results. The results of the various experiments may be seen in Table I. Pregnancy was considered to have been maintained only when fetal viability was demonstrable at necropsy. When injections or implants were inadequate, blood and mucus always appeared in the vagina in more copious amounts than seen in the usual placental sign. In untreated controls, in implanted, oöphorectomized rats, and in rats implanted with placentae from the tenth day of gestation, this usually occurred on day 8 of pregnancy, within 2 days of hypophysectomy. In other rats inadequately treated, bleeding in excess of the placental sign was detected between days 9 and 12. At necropsy such rats showed evidence of partial success in maintaining the conceptus by the presence of placentae of various sizes and in some instances dead fetuses.

None of the uninjected rats, nor the injected rats that had been oöphorectomized

2. Pencharz, R. and Long, J. A., *Am. J. Anat.*, 1933, v53, 117.

3. Selye, H., McKeown, T., *Proc. Roy. Soc.* (B), 1935, v119, 1.

† We are greatly indebted to Mrs. Luree Hughes for hypophysectomizing these animals.

‡ The estrone and progesterone used in this study were generously supplied through the courtesy of Dr. Daniel A. McGinty of Parke, Davis and Company.

4. Lyons, W. R., Simpson, M. E. and Evans, H. M., PROC. SOC. EXP. BIOL. AND MED., 1943, v52, 134.

as well as hypophysectomized maintained their embryos or placentae. During the progress of the experiment it became evident that the 12-day implantation sites, preponderantly decidual, were adequate in maintaining gestation if a sufficient number were implanted. Generally 5 or more of these placentae or approximately 275 mg daily were required. Double this weight of 15-day placentae proved ineffective. It was then decided to limit the experiment to the relatively potent 12-day placenta and to attempt to enhance its pregnancy-maintaining value by the addition of estrone. Histologic study of the ovaries, uteri and vaginae of the rats receiving only a single 12-day implant showed evidence of luteotrophic action even though pregnancy was not maintained. This was also true of the animals receiving purified lactogenic hormone alone in this and in the earlier series. When the rats were injected with 0.5-1 μg of estrone daily as well as lactogenic hormone, pregnancy was maintained. As may be observed in Table I this dose was equally efficacious when given with a single 12-day placenta. In an attempt to localize the luteotrophic activity in the parts of the placenta composed mainly of either decidual or trophoblastic tissue, it was found possible to make a separation of the 12-day sites by discarding the embryo in its amnion and lifting the small trophoblastic part or träger away from the cupped, decidua basalis. The latter in turn was readily isolated by forceps as a compact button. Histologic sections of such parts of the placentae showed very few small nests of trophoblast in the decidual buttons and predominantly trophoblastic cells in the fetal part. However the latter also contained yolk sac epithelium and cells of the decidua capsularis. It will be noted in the Table that both fetal and maternal parts showed luteotrophic activity; but the maternal part of a 12-day rat placenta weighs approximately 40-45 mg in contrast to 10-15 mg for the fetal part. Further experiments with 10- and 15-day placentae supplemented by estrone proved unsuccessful except in a single instance in which 2 fifteen day placentae (500 mg) were administered with estrone.

Discussion. The above data support the earlier finding(1) of a luteotrophic substance in the rat placenta§ and place the initiation of its secretion at mid-pregnancy. In the Astwood and Greep investigation the question of whether or not estrin formation was induced in the ovary was not raised, probably since allegedly pure progesterone alone suffices to cause deciduoma formation in the traumatized uterus. In our previous experiments(4,5) the importance of the synergism between estrin and progestin in the maintenance of pregnancy has been emphasized; and the present investigation brings further proof of that synergism. Finding that a single 12-day placenta weighing 55 mg plus 0.5 μg of estrone accomplished the same result as five 12-day placentae or 275 mg suggested that the equivalent of 0.5 μg of estrone is present in the extra 4 placentae (220 mg) or else a substance capable of inducing the rats' ovaries to form this amount of estrogen may be formed there. Another possibility would be that the 12-day rat placenta contributes some of the necessary estrin and secretes a gonadotrophin capable of causing the ovaries to contribute the rest. The same argument might be used in support of a combined secretion of progestin by ovaries and placenta.

To have proven that the pituitary's gonadotrophic triad, FSH, ICSH and lactogenic hormone may be completely replaced by the placenta during the critical 6-12-day period of gestation in no way leads to the implication that these gonadotrophins are not responsible for the maintenance of the conceptus until mid-term. But it does allow one to surmise that in its earliest existence the rat placenta probably begins to supplement the functions of at least these three pituitary hormones so that by mid-term it may com-

§ Astwood and Greep were unable to detect other than luteotrophic activity in the rat placentae. We have been able to elicit positive local tests in squabs' crops with acid-acetone extracts of 12-day rat placentae in doses as low as 2 mg equivalents of wet tissue. However, this is an extremely high dose as compared to 0.001 μg of purified lactogenic hormone required for a positive reaction.

5. Lyons, W. R., PROC. SOC. EXP. BIOL. AND MED., 1943, v54, 65.

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.

Received June 8, 1950. P.S.E.B.M., 1950, v75.

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

BORIS SOKOLOFF, JAMES B. REDD, AND RAYMOND DUTCHER*
(Introduced by Walter H. Eddy)

From the Southern Bio-Research Laboratory, Florida Southern College, Lakeland, Fla.

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-

* Carll Tucker Fellow.

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.