

of life in the adrenalectomized animal. The gonadotropic principles contained in the material are probably the active agents.

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Comparison of the Corner-Allen and Clauberg Tests for Assay of Progesterin.

LAWRENCE E. YOUNG. (Introduced by George W. Corner.)

From the Department of Anatomy, The University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

At the present time two methods for assaying progesterin are in use, one involving the use of mature female rabbits, and the other, immature female rabbits. In connection with their work which showed that progestational proliferation is due to a specific corpus luteum hormone, Corner and Allen¹ developed a method for the standardization of corpus luteum extracts. The test devised consists of injecting the extract over a period of 5 days into a sexually mature doe castrated 18 hours after mating. A rabbit unit (Corner-Allen unit) is then defined as the minimum quantity of progesterin which, divided into 5 equal daily doses, produces on the sixth day a state of the uterus equal to that of the eighth day of a normal pregnancy. A uterus in this state shows a +++ or ++++ proliferation.

Allen² discovered that, in the immature rabbit weighing from 575 to 1647 gm., progestational proliferation can be induced in the uterus by injections of progesterin, provided the uterus is first influenced by a series of oestrin injections. Only a small percentage of the immature animals responded to the progesterin treatment when it was not preceded by the oestrin injections. Clauberg^{3, 4} confirmed the findings of Allen² on immature rabbits and established the Clauberg unit which is measured in the same way as the Corner-Allen unit except that 600 gm. rabbits are used and are primed with a total of 80 international units of oestrin, administered in 8 daily injections of 10 international units each, preceding the 5 daily injections of progesterin.

¹ Corner, G. W., and Allen, W. M., *Am. J. Physiol.*, 1929, **88**, 326.

² Allen, W. M., *Am. J. Physiol.*, 1930, **92**, 612.

³ Clauberg, C., *Zbl. f. Gynäk.*, 1930, **54**, 2757.

⁴ Clauberg, C., *Klin. Wschr.*, 1931, **10**, 1949.

McPhail⁵ modified the Clauberg test slightly when he defined a unit, for use in his own laboratory, as that amount of progestin which injected over a period of 5 days, following the injection of 150 international units of oestrin over 6 days, produces an average proliferation of ++ in a group of immature rabbits of 750 to 950 gm. weight. A slight modification of the Corner-Allen test was used by Fevold, Hisaw and Leonard,⁶ who defined a unit of progestin as that amount which, injected over a period of 4 days into an oestrous rabbit castrated previous to the first injection, produces complete proliferation on the fifth day. The Corner-Allen and Clauberg units, however, have been most widely adopted, the former being in general use among American workers, while the latter is more commonly used in the German laboratories.

Allen² found the immature rabbit uterus fully as sensitive as that of the mature rabbit to progestin treatment, and unpublished experiments of Corner of this laboratory and Elden of the Department of Obstetrics of this school, suggested that the Clauberg unit is equal to more than one-half but less than a whole Corner-Allen unit. Fels⁷ concludes from his experiments on mixtures of Lutetosteron C and D that the Clauberg unit is smaller than the Corner-Allen unit, but he does not set up an exact numerical relationship between the 2 units because of the great spread which he observes in the Clauberg test. He found that a small amount of a given solution often causes a stronger reaction than a larger amount of the solution, when injected into several immature rabbits at the same time. Although these experiments give some idea of the relationship between the two units, no direct and adequate comparison of the Clauberg and Corner-Allen units had been made prior to that reported in this paper.

A given extract, 0.10 cc. of which contained 13.2 mg. of solids, was assayed on both mature and immature rabbits according to the Corner-Allen and Clauberg methods. The extract was dissolved in benzene and prior to injection was diluted with Mazola (commercial maize oil) so that the total dose was contained in 1.0 cc. Eight mature animals were mated, castrated, injected, and autopsied according to the method described by Corner and Allen.¹ The results of this assay are recorded in Table I. Eleven immature rabbits were primed with 80 international units of oestrin in the

⁵ McPhail, M. K., *J. Physiol.*, 1934, **88**, 145.

⁶ Fevold, H. L., Hisaw, F. L., and Leonard, S. L., *J. Am. Chem. Soc.*, 1932, **54**, 254.

⁷ Fels, E., *Zbl. f. Gynäk.*, 1935, **59**, 2420.

TABLE I

Group	Animal No.	Extract Injected	Degree of Proliferation	Aver. Proliferation for group
A	x-943	cc. .20	++++	4.0
	1003	.20	++++	
B	1016	.15	++	2.5
	1017	.15	+++	
C	x-930	.10	++	2.0
	1002	.10	++	
D	1001	.05	++	1.5
	1015	.05	+	

TABLE II.

Group	Animal No.	Body Weight at Beginning of Treatment	Extract Injected	Degree of Proliferation	Aver. Proliferation for Group
A		gm.	cc.		
	1004	585	.075	+++	2.33
	1005	515	.075	+++	
	1010	530	.075	+	
B	1006	515	.050	+	2.0
	1007	550	.050	++	
	1012	580	.050	++	
	1014	580	.050	+++	
C	1008	520	.025	0	0.75
	1009	645	.025	0	
	1011	530	.025	+++	
	1013	580	.025	0	

form of oestra diol mono benzoate (Progynon-B, manufactured by the Schering Corporation) previously assayed on 5 rats, injected with the corpus luteum extract, and autopsied after the method of Clauberg.⁸ The daily dose of oestrin was varied slightly in rabbits 1004, 1005, 1006, 1007, 1008, and 1009 with no noticeable effect on the subsequent growth and proliferation. The results with the immature rabbits are shown in Table II.

A Corner-Allen unit of this particular extract, based on the assay recorded in Table I, may be set at 0.15 cc. to 0.20 cc. since the proliferation averages for these 2 doses were 2.5 and 4.0 respectively. A +++ to ++++ reaction is required for the unit.

Comparison of groups B and C of Table I with groups A and B of Table II shows that doses of 0.15 cc. and 0.10 cc. of the extract produced proliferation averages of 2.5 and 2.0 respectively in the mature rabbits, whereas doses of 0.075 cc. and 0.050 cc., or exactly

one-half the above, produced the very similar proliferation averages of 2.33 and 2.0 in the immature rabbits. This comparison indicates that the Clauberg unit is equal to approximately one-half of a Corner-Allen unit.

It is evident from Table II that while the average proliferation of each group of immature animals corresponds rather well to the amount of progestin injected, the variation within each of the 3 groups is considerable (from + to +++ in groups A and B and from 0 to +++ in group C). This is in great contrast to the relatively uniform group reactions shown in Table I. It must also be noticed in Table II that in rabbit 1010 a dose of 0.075 cc. produced a reaction of only + whereas 0.025 caused a proliferation of +++ in rabbit 1011. Fels⁷ reports this same sort of variation or spread and concludes from this that the Clauberg test is unreliable for measurement of potency of the corpus luteum hormone. McPhail⁸ reports a similar variation within each group of 5 or 6 immature animals, but also shows that when the average proliferation of the group is taken, the proliferation indices can be plotted against the doses in a rough standardization curve. Clauberg does not give the details of his experiments, but one may conclude from the work of Fels and McPhail and the experiments reported in this paper, that assays based on proliferation in the uterus of the immature rabbit do not afford accuracy comparable to that of the Corner-Allen test unless large numbers of animals are used and group averages taken. In selecting a method of assay, this disadvantage of the immature rabbit test must be weighed against its obvious advantage in not requiring oophorectomy.

Summary. 1. The Corner-Allen and Clauberg methods for assay of progestin were compared by assaying a given extract on 8 mature and 11 immature rabbits. 2. The uterus of the immature rabbit showed much greater variation than that of the mature rabbit in its reaction to progestin. 3. The accuracy of the Corner-Allen test can be approached by the Clauberg test only if large numbers of animals are used and group averages taken. 4. A Clauberg unit, based on group averages, was found to equal approximately one-half of a Corner-Allen unit.