the oral route, it is hundreds of times as potent a progestin(8). Thus for an effective progestational dose of MAP one gives an infinitesimal amount of androgenic potential compared with that of an effective progestational dose of ET.

Summary. When tested for ability to stimulate seminal vesicles and prostates of castrate, immature rats, a series of progestational steroids produced results as follows: 1) None of the compounds has androgenic activity approaching that of standard androgens, methyltestosterone or testosterone propionate. 2) All compounds tested *orally* produced some stimulation of the prostate, although it was extremely slight in the case of 17α-acetoxy progesterone and 6α-methyl-17α-acetoxy progesterone. Significant stimulation of seminal vesicles was seen only with 17α-ethinyltestosterone, 17α-ethinyl-19-nortestosterone, and EN. 3) Of compounds tested *subcutaneously,* only 17α-(2-methallyl)-19-nortestosterone failed to stimulate the seminal vesicle. All compounds produced some stimulation of the prostate, although EN and 17α-(2-methallyl)-19-nortestosterone produced minimal responses.

Samples of Enovid and 17α-(2-methallyl)-19-nortestosterone were generously supplied by Dr. Lee Hershberger and Dr. V. A. Drill respectively, G. D. Searle and Co. All other steroids used were provided by Dept. of Chemistry, The Upjohn Co.

1. Lamar, J. K., *Anatomical Record,* 1937, v70, suppl. 45.

2. Wilkins, L., Jones, H. W., Holman, G. H., Stempfel, R. S., *J. Clin. Endo. Metab.,* 1958, v18, 559.

3. McGinty, D. A., Djerassi, C., *Ann. N. Y. Acad. Sci.,* 1958, v71, 500.

4. Saunders, F. J., Drill, V. A., *ibid.,* 1958, v71, 516.

5. Babcock, J. C., Gutsell, E. S., Herr, M. E., Hogg, J. A., Stucki, J. C., Barnes, L. E., Dulin, W. E., *J. Am. Chem. Soc.,* 1958, v80, 2904.

6. Deanesly, R., Parkes, A. S., *Biochem. J.,* 1936, v30, 291.

7. Korenchevsky, V., Dennison, M., *ibid.,* 1934, v28, 1486.

8. Barnes, L. E., Schmidt, F. L., Dulin, W. E., submitted to PROC. SOC. EXP. BIOL. AND MED.,

9. Buckner, F., Herrmann, W., *Yale J. Biol. and Med.,* 1958, v30, 446.

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Particle Counts of Meningopneumonitis Virus by Phase Microscopy.* (24691)

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A rapid and simple technic for counting psittacosis virus particles would be of obvious value. Gogolak(1) described a modification of the method of Backus and Williams(2) for counting murine pneumonitis virus particles in which virus was mixed with a suspension of known concentration of *E. coli,* sprayed onto a glass slide and stained with Gram stain. The ratio of virus particles to bacteria was determined by counting each by light microscopy. This method was criticized by Crocker(3) on the basis that proof was lacking that the proportions of virus particles and bacteria are unchanged by the staining procedure, and that it does not allow differentiation between virus and non-infectious, non-elementary body forms. The need for a simple and rapid method for counting unpurified and partially purified virus preparations prompted the present study. The technic of a method and confirmation of its validity by egg infectivity tests, electron microscopy counts, and nitrogen determination is reported here.

Materials and methods. (1) *Preparation of virus suspensions.* Meningopneumonitis virus from the American Type Culture Collection was passed serially approximately 20 times in

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the allantoic cavity before use in this study and was known to reach a maximum titer in allantoic fluid 5-6 days after injection. Allantoic fluid suspensions of virus were stored in 3-5 ml volumes at −20°C for use as seed. For use, seed virus was thawed, diluted 1-10 in nutrient broth, and 0.2 ml volumes were injected into the allantoic cavity of 7-day chick embryos. All eggs were incubated for 5-6 days, candled and the dead embryos discarded. The remaining eggs were chilled for 30 min. at −20°C and the allantoic fluids were collected separately. The allantoic fluids were centrifuged at 1,000 rpm for 15 min at 5°C and supernatant fluids were removed by suction and kept at 4°C until titrated. Particle counts and egg titrations were completed within 5 hours after harvest. (2) *Preparation of latex particles.* A polystyrene (latex) particle suspension† composed of particles 0.81 micron in diameter was counted in a Petroff-Hausser bacteria counting chamber and diluted in distilled water to contain $10^{9.11}$ particles per ml. The standard suspension was found to be stable when kept at 4°C for up to two months. There was little aggregation of particles during storage, and a particle count at the end of this time was almost identical to that originally obtained. Bacterial growth was not apparent. (3) *Particle counts.* Five-tenths ml amounts of virus suspensions were added to 0.5 ml of the latex particle suspension. After thorough mixing, thin smears approximately 15 mm in diameter were made with a transfer loop on clean slides, air dried and fixed by heat. These smears were stained 5 min with freshly prepared and filtered 0.25% aqueous basic fuchsin, washed gently in tap water for about 3 seconds, dipped briefly into 0.5% citric acid and washed again very briefly in tap water. Dried smears were examined under oil immersion using phase contrast microscopy. A grid was placed inside one ocular to facilitate counting. The number of latex particles and the number of virus particles in several grid areas were determined. No difficulty was encountered in distinguishing between the latex and virus particles. Sufficient grid areas were counted so that no less than 90 of one or the other type particles was counted. The ratio of the virus count: latex count was determined and the virus particle count per ml of allantoic fluid was calculated. (4) *Egg infectivity titration.* Two fold dilutions of infected allantoic fluids were prepared in phosphate buffered saline (PBS) after the virus particle count was determined as above. Four 6-7 day embryos were injected with each dilution *via* yolk sac route. All eggs were candled after 48 hours and dead embryos were discarded. Deaths were recorded for 10 days. Egg LD_{50} per ml was calculated by method of Reed and Muench(4). (5) *Total nitrogen determination.* The method of Lanni, Dillon and Beard(5) was used.

† Obtained from Dow Chemical Co.

Results. 1. *Precision of method.* Reproducibility of the counts obtained by this method was studied by making 10 separate counts on a single slide prepared as above. The ratios of virus: latex particles ranged from 1.47:1 to 2.0:1 with an average of 1.76:1. The maximum deviation from the average was 16%. Three separate experiments were performed on different days to compare the number of egg infectious units and virus particles per ml of allantoic fluids in individual eggs. The virus counts were made first and the dilutions for egg infectivity tests were based on these counts. These results are shown in Table I. The number of particles per egg LD_{50} varied from 83 to 240 with an average of 145. This is comparable to the findings of Crocker(3).

2. *Effect of washing on the virus:latex particle ratio.* The possibility that particles of either type were washed off the slides during

TABLE I. Relationship of Particle Counts by Phase Microscopy to Egg Infectivity of Meningopneumonitis Virus.

Exp.	Egg No.	Log of virus particles/ml	Log of egg LD_{50}/ml	No. of virus particles per egg LD_{50}
A	1	9.14	7.01	135
	2	9.21	7.29	83
	3	9.01	6.76	178
B	1	9.68	7.30	240
	2	9.57	7.50	118
	3	9.42	7.28	138
C	1	8.81	6.41	174
	2	9.21	7.25	91
	3	8.70	6.43	186

TABLE II. Effect of Prolonged Washing of Smears on the Virus : Latex Ratio.

Time of washing	Ratio of virus particle: latex particles	Log of virus particles/ml allantoic fluid
5 sec.	1.76	9.39
25	1.80	9.39
2 min.	1.68	9.37
10	1.58	9.34

the staining process was tested by preparing 4 smears from the same virus:latex mixture. All smears were treated the same except for the terminal washing procedure, when the 4 slides were washed for 5 sec, 25 sec, 2 min and 10 min respectively. The virus particle:latex particle ratio was determined on each preparation as above and is shown in Table II. In a second experiment to determine whether washing affected the virus:latex ratio, two smears were prepared from the same preparation, allowed to air dry and were heat fixed. One slide was examined directly and the other was stained before examination. Counts were made on both slides using phase contrast microscopy. Virus particles were plainly visible in the unstained preparations but were counted with more difficulty than in the stained preparation. Triplicate counts were done on each slide. The ratios of virus:latex particles in the unwashed preparation were 0.39, 0.43, and 0.37 (average = 0.40). In the stained preparations the ratios were 0.34, 0.43, and 0.45 (average 0.41). Results from both experiments indicate that no significant loss of particles occurred during the washing process or that both virus and polystyrene particles were washed away at exactly the same rate.

3. *Comparison of counts obtained by phase contrast and electron microscopy.* The morphology of particles counted by the phase contrast technic was studied by electron microscopy.‡ Two suspensions were prepared for counting by sedimenting the allantoic fluid virus at 10,000 rpm for ½ hr, resuspending in distilled water, repeating the process twice more and resuspending to original volume in distilled water. The usual mixture of virus suspension and latex was made and ratios determined by the phase contrast method. Samples from these same virus:latex mixtures were sprayed on collodion membranes and micro-droplets examined with the electron microscope. Latex particles, small-type virus particles, and large-type virus particles were counted separately. The results are shown in Table III. It is seen that the virus counts determined by the phase contrast method average 25% higher than those obtained by electron microscopy. This higher value is probably due to the counting of some virus fragments by the former method. Subsequent similar experiments have demonstrated that this difference is relatively constant. Stained smears of virus:latex mixtures were also found to be plainly visible and readily differentiated by darkfield microscopy. Particle count determinations on the same preparations by phase contrast and darkfield microscopy were found to be almost identical. The use of stained smears was much superior to unstained smears in both cases.

4. *Relationship between particle counts and nitrogen content of washed virus preparations.* In each of 2 experiments, pooled allantoic fluids from infected eggs were centrifuged at 1500 rpm for 30 min to remove cell debris. Supernatant fluids were centrifuged at 10,000 rpm for 30 min in the Spinco #30 rotor. The sediments were resuspended in PBS and washed twice by alternate centrifugation and

TABLE III. Comparison of Particle Counts Made by Phase Contrast and Electron Microscopy.

	Phase microscopy	Electron microscopy		
Exp.	Log virus particles/ml	Log elementary body forms/ml	Log large forms/ml	Log total virus/ml
1	9.32	9.08	8.72	9.24
2	9.27	9.04	8.61	9.18

‡ The electron micrographs from which these counts were done were obtained through the courtesy of Dr. D. Gordon Sharp.

resuspension prior to nitrogen determination. The results of these experiments are shown in Table IV. A very close correlation between

TABLE IV. Relationship between Nitrogen Content and Virus Particle Counts of Washed Suspensions.

Virus suspension No.	Particles/ml ($\times 10^9$)	μg N/ml	Particles/μg N ($\times 10^7$)
136-1	4.6	47	9.8
-3	6.0	68	8.8
137-1	10.0	97	10.3
-2	10.0	102	9.8
-3	9.1	96	9.5

virus counts and nitrogen content is evident, with the average being 9.7 x 10^7 particles per μg of nitrogen.

Discussion. These results have demonstrated that the virus particle counts of meningopneumonitis virus in allantoic fluid obtained by this technic are consistently parallel to egg infectivity titers and nitrogen content. In 9 separate determinations number of particles per egg LD_{50} ranged from 83 to 240 with an average of 145. In 5 separate determinations number of particles per μg of nitrogen ranged from 8.8 x 10^7 to 10.3 x 10^7.

It is certain that numerous non-elementary body forms have been included in the counts done by this technic but the proportion of these to elementary bodies is seldom more than 1:1 as seen by electron micrographs of virus in allantoic fluid and usually the large forms account for about ¼ of the countable particles in electron micrographs. For rapid estimation of virus content of allantoic fluids this technic has been of particular value, and allows the selection of high titer fluids as well as use of a narrow range of dilutions for egg LD_{50} determinations.

The use of latex particles for this procedure has several advantages. This material is stable on storage for long periods of time, is readily available in a variety of particle sizes and is easily standardized by counting in a bacteria counting chamber. The particles are easily visualized by phase contrast microscopy and are readily differentiated from the virus.

Summary. A rapid method for estimating the meningopneumonitis virus content of allantoic fluid suspension is described. Evidence is presented which indicates that counts obtained closely parallel egg infectivity titers and nitrogen content determinations. In 9 separate experiments one egg LD_{50} was found to contain 83-240 virus particles as counted by this method.

1. Gogolak, F. M., *J. Infect. Dis.*, 1953, v92, 248.
2. Backus, R. C., Williams, R. C., *J. Appl. Physics*, 1950, v21, 11.
3. Crocker, T. T., *J. Immunol.*, 1954, v73, 1.
4. Reed, L. J., Muench, H., *Am. J. Hyg.*, 1938, v27, 493.
5. Lanni, F., Dillon, M. L., Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 4.

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Hepatic and Splenic Uptake of Colloidal Radiogold in Rat after Partial Hepatectomy.* (24692)

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The subject of this study, *viz.*, phagocytic activity of hepatic and splenic macrophages in the rat after partial hepatectomy, has received only scant attention.

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Materials and methods. Healthy 2½ to 6 months old Wistar and Lewis rats were used. Twenty-four hours before hepatectomy or sham operation, they were injected intraperitoneally with 0.2 to 0.3 μc/g body weight of radiogold (Abbott's Aurcoloid). Removal of approximately ⅔ of liver was done according