the bulk of the sperm and hyaluronidase remain in the uterus following copulation and no mass movement of the enzyme from the uterus into the tubes seems to play a part in the process of denudation and fertilization. We are of the opinion, however, that hyaluronidase is produced by those sperm which gain access to the tubes, and with the aid of this enzyme, the sperm traverse the follicle cell mass to fertilize the ovum. Later the concentration of enzyme is sufficient to denude the ova as was observed 24 hours post-coitus.

Conclusion. Fertilization of the rat ovum occurs before mass displacement of the surrounding follicle cells; denudation of the ova occurs subsequently. Since hyaluronidase, introduced into the uterus, does not pass into the tubes to denude the ova, it seems that only the enzyme associated with the sperm which reach the oviduct disperses the follicle cells.

16142

Influence of *M. varians* on Oral Infectivity of Mouse-Hamster (M-H) Virus.*

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During the course of experiments on the susceptibility of white Swiss mice (Webster strain) to orally-administered M-H (Mouse-Hamster) virus, an unexplained reduction in the usual mortality of the mice occurred. During the period of January 5 to June 15, 1946 eight experiments involving 214 mice were conducted. The mortality to an orally-administered, highly concentrated mouse-brain suspension of the M-H virus in these 8 experiments varied between 48 and 80%, or an average mortality of 60%. Quite unexpectedly on July 26, the administration of the same dose of the M-H virus that had been used in the previous experiments now produced a mortality of only 20%. No explanation for this reduced mortality was available at that time as the virus end-point by intracerebral or foot-pad inoculation had not altered as compared with the previous 8 experiments. This reduced mortality to the M-H virus occurred again on December 21, 1946 (Experiments 12, 13, Table I). Thus, in the 13 experiments conducted during this year, there were only 2 in which the mortality to the usual dose of the virus was less than 44%-80%, and these mortalities were only one-half to one-fourth of the lowest mortality previously achieved in the other 11 experiments. A review of the protocols of the 13 experiments and the method of preparation of the virus used in the oral feeding experiments shed some light on the problem.

Up to May 15th, consecutive passages of the M-H virus by intracerebral and foot-pad inoculation into the mouse had been performed until a seventh-passage virus was obtained on March 20, 1946. Contemplating a large number of experiments, subsequent to April, 1946, 200 mice were obtained and were inoculated subcutaneously into the foot-pad with a fatal dose of the seventh passage M-H virus. The brains were harvested when paralysis or death occurred, and a 25% emulsion in Ringer's solution was prepared. This total volume of the brain emulsion was then divided into 10 equal portions and stored in sterile lusterloid tubes in the CO_2 icebox at -40° C until needed in the future. Thus, the first experiment conducted with one of the samples of this new batch of virus was done in May, 1946, approximately 4 days after

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TABLE I.

Mortalities of 13 Groups of Swiss Mice Fed M-H Virus Brain Suspension. The concentration of the virus suspension fed was 10% except in Exp. 4 and 5 when 2.5% was used. The virus end-point, by subcutaneous injection, in Exp. 1 to 11 was 100% of the animals when 0.03 cc of 10^{-6} dilution was given. In Exp. 12 and 13 the end-point was 10^{-7}.

		Virus			Mice			
Exp. No.	Date	Amt fed	Passage	No. of feedings	Age, days	Sex	No.	Mortality, %
1	1– 5–46	.5–.9	3	1	28	Mixed	26	65
2	25	.5–.9	4	1	180+	Males	50	48
3	2–13	.5–.9	5	1	50	Mixed	40	47
4	18	.5–.9	6	3	28	"	25	80
5	18	.5–.9	6	3	28	"	25	48
6	3–24	.5–.9	7	2	52	"	10	60
7	5–19	.5	8	1	28	"	18	77
8	6–15	.5	8	2	40	"	20	55
9	7–26	.5	8	3	48	"	20	*20*
10	9–21	.5	8	1	48	Males	20	45
11	21	.5	8	1	48	Female	27	44
12	12–21	.5	8	2	48	Males	28	*17.8*
13	21	.5	8	2	48	Female	20	*10*

the preparation of this batch of virus. The end-point of this fresh virus by foot-pad inoculation was still 10^{-6} and the mortality to the same large oral dose was 77% (Experiment 7, Table I). One month later on June 15, 1946, a second vial of the virus suspension was removed from the CO_2 icebox for the conduction of Experiment 8 (Table I). At this time the usual high mortality of 55% was obtained with the same dose and concentration of the M-H virus used previously. However, on July 26, 1946, a third tube of the virus suspension prepared in April was removed from the icebox for the conduction of Experiment 9 (Table 1). In spite of the fact that in this experiment the mice received 3 consecutive feedings of the same dose of virus as in previous experiments the mortality was only 20%. On Sept. 21, 1946, another vial was removed from the icebox for Experiments 10 and 11, and a good mortality was obtained. Again on Dec. 21, 1946, when Experiments 12 and 13 were conducted, mortalities of only 17.8% and 10% were obtained.

Fortunately, there was remaining from Experiments 12 and 13 (Table I) a small amount of the original 25% mouse-brain virus suspension that had been used in these 2 experiments. It was noted that this suspension had a rather orange or amber color and that particulate matter had settled out. For this reason the material was cultured on various media, and an organism with the following properties was found. This organism tolerated a temperature of 60° C for 30 minutes. It reduced nitrates to nitrites and fermented all of the usual 6-carbon sugars, dextrins and glycerol with the production of acid only. Starch was not hydrolyzed. The inoculation of pure cultures of the organism intraperitoneally and intracerebrally into white mice produced no signs of peritonitis or meningitis. The mice remained well for the 14-day observation period subsequent to inoculation. The organism grew well on nutrient agar, the colonies having a faint yellow color when grown at room temperature, but not when grown at 37° C. When grown in a mouse-brain suspension in Ringer's solution at 4° C or 11° C the colonies at the meniscus have a faint pinkish tinge. The brain suspension also developed a slight orange-pinkish color. This organism has been identified as *Micrococcus varians*.

A relationship of this organism to the reduced oral infectivity of the M-H virus was postulated, and the following experiments were done.

An amount of eighth-passage virus, (that used in Experiments 12 and 13 and containing the *Micrococcus varians* sufficient to feed 18 mice was left. This was prepared as a 10% suspension and fed in 0.5 cc amounts for 2

TABLE II.

Comparison of the Oral Infectivity and the Subcutaneous Foot-pad Titer of Eighth and Ninth Passage M-H Virus. A 10% brain virus suspension was fed. Eighth passage virus contained *Micrococcus varians*, ninth passage virus was sterile by culture.

Mice			Virus				
Sex	Age (days)	No.	Passage No.	Amt fed	No. feedings	Subcutaneous end point	Mortality, %
Mixed	32	18	8	0.5	2	10^{-6}	16.6
"	32	18	9	0.5	2	10^{-4}	33.0

consecutive days. For controls, a small amount of this virus was passed subcutaneously into young Swiss mice whose brains were harvested in order to obtain a ninth-passage virus, free of the organism. Thus, a comparison of the original micrococcus containing eighth-passage virus, and micrococcus free ninth-passage virus could be made. The eighth-passage virus material produced a heavy growth of the micrococcus by aerobic culture while the ninth-passage virus material was sterile. A titration, by foot-pad, of each of the viruses was done, and a suitable group of animals, (18 in number), was fed an 0.5 cc dose of this ninth-passage virus as a 10% suspension.

Table II presents the results obtained in this experiment. The 18 male mice, fed on 2 consecutive days, a dose of 0.5 cc of the 10% eighth-passage virus, had a mortality end-point by foot-pad inoculation of 10^{-6} and a mortality of only 16.6% to oral administration of the virus. On the other hand, the mice fed the same dose of ninth-passage virus, which had the higher oral infectivity, had a lower end point (10^{-4}) by foot-pad inoculation.

Table III presents the results of a more critical experiment in which the mortalities of Swiss mice fed the following preparations were determined: (1) 10% normal mouse brain suspension. (2) 10% normal mouse brain plus 1 cc of *Micrococcus varians* culture. (3) 10% M-H virus mouse brain suspension. (4) 10% M-H virus mouse brain suspension plus 1 cc of *Micrococcus varians* culture.

The method of preparation of the mouse brain suspensions for this experiment was as follows: 10% normal mouse brain in Ringer's solution was prepared. One hundred cubic centimeters of this solution was divided into 2 aliquots, each aliquot containing 50 cc. To one aliquot, 1 cc of a 25 day culture of the micrococcus was added. (This preparation of the *Micrococcus varians* was prepared from 20% guinea pig brain suspension in which the micrococcus had been inoculated and incubated for 25 days at 11° C). The second aliquot of the normal mouse-brain suspension received no *Micrococcus varians* inoculum. These 2 brain suspensions served as the controls. The brain suspensions containing the M-H virus were prepared by making 100 cc of a 10% M-H infected mouse-brain suspension. This volume was divided into 2 aliquots of 50 cc each. The first aliquot was inoculated with 1 cc of the *Micrococcus varians* culture while the second aliquot of the M-H brain suspension was not inoculated. All 4 aliquots were then cultivated, both aerobically and anaerobically, on blood agar plates at the time of preparation and all were found to be sterile except the 2 which had been inoculated with the micrococcus. These 4 specimens of mouse brain; namely, (1) normal mouse brain, (2) normal mouse brain plus *Micrococcus varians*, (3) M-H brain, and (4) M-H mouse brain plus *Micrococcus varians* were incubated at 11° C for 29 days. Ten days after the inoculation of the brain suspension with the *Micrococcus varians*, all of the tubes were cultured on blood agar. Growth existed only in the *Micrococcus varians* inoculated tubes. The other 2 control tubes were sterile. Twenty-nine days after inoculation with the micrococcus, the 4 tubes of mouse brain were fed to 4 groups of normal male mice. Each mouse was given 0.5 cc of the 10% mouse-brain suspension for three

TABLE III.
Influence of *Micrococcus varians* on the Oral Infectivity of the M-H Virus.

	No. of mice	Mortality
Normal brain suspension	25	0
" " " + *Micrococcus varians*	25	0
M-H brain suspension	26	65.0
" " " + *Micrococcus varians*	24	33.3

TABLE IV.
Comparison of the Inhibiting Effect of *M. varians* Organisms and *M. varians* Culture Filtrate on the Oral Infectivity of the M-H Virus.

	No. of mice	Mortality, %
M-H virus + *M. varians* (incubated 97 days)	21	23.8
M-H virus + washed *M. varians* (incubated 48 hr)	20	35.0
M-M virus + *M. varians* filtrate (incubated 48 hr)	20	50.0
M-H virus only	17	59.4

successive days. These experiments presented in Table III demonstrated that when the M-H virus was incubated with a growing culture of the *Micrococcus varians* its oral infectivity was reduced by one-half.

A virus titration of the specimen without bacteria was carried out by foot-pad inoculation at the time the brain samples were prepared and when fed. Likewise, the normal mouse brain and the normal mouse brain containing the *Micrococcus varians* were inoculated into 5 mice at the time of feeding. All mice survived.

At the time of the feeding of the virus, samples of brain suspension were removed from the bottom of each of the 4 tubes and cultured on individual blood agar plates. *Micrococcus varians* was found only in the 2 samples that had been inoculated 29 days previously.

After incubating for 29 days the sample of brain suspension containing the virus only and the sample of virus brain suspension which had been inoculated with the *Micrococcus varians* were titrated by foot-pad method in replicates of 5 each of 4-week-old mice. The volume of the dilution inoculated into each mouse was 0.03 cc. All samples of the M-H virus brain suspension had the same end point; a subcutaneous dose of 0.03 cc diluted to 10^{-7} killed 100% of the animals.

The following experiment was done in an attempt to determine whether or not this reduction in mortality was due to a direct action of the micrococcus on the virus and thus to a reduction in the number of virus particles available for absorption, or perhaps due to the action on the virus of some agent in the culture filtrate produced by the micrococcus. Four groups of 8-week-old male mice were fed for 2 consecutive days 0.5 cc of the following 10% mouse brain virus suspension: (1) M-H brain-virus suspension plus micrococcus organisms (incubated at 4° C for 97 days). (2) M-H brain-virus suspension plus washed *Micrococcus varians* organisms (incubated at 4°C for 48 hours. (3) M-H brain-virus suspension plus *Micrococcus varians* filtrate (incubated at 4°C for 48 hours). (4) M-H brain-virus suspension only. These results are tabulated in Table IV.

Both the M-H virus suspensions containing the *Micrococcus varians* organisms had a lower mortality than that obtained with the M-H virus brain suspension alone. The filtrate from the *Micrococcus varians* culture did not significantly reduce the infectivity of the M-H virus. It appears that the action of the *Micrococcus varians* organism on the M-H virus is a direct one, possibly related to its metabolic activity or to some absorption phenomenon.

Summary. Suspensions of mouse brain tissue infected with the M-H (Mouse-Hamster) virus when incubated with a pure culture of the *Micrococcus varians* organism were found to have a markedly reduced oral infectivity for the white Swiss mouse. In spite of this reduction in oral infectivity there is no change in the infectivity of the virus material con-

taining the micrococcus when it is injected subcutaneously. Filtrates of the micrococcus culture do not appear to inhibit the oral infectivity of the virus. It is suggested that the M-H virus may have been absorbed on the *Micrococcus varians* organism, and is thus not as readily available for invasion through the intestinal tract or that the virus itself may have been modified so that its oral infectivity has been reduced while its infectivity by subcutaneous inoculation is unchanged.

16143 P

Allantoin Clearance as a Measure of Glomerular Filtration Rate in Man.*

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In a recent study,[1] the renal clearance of allantoin in the rat and dog was found to be equal to that of creatinine. This equivalence held true despite variations in both urine flow and plasma concentration of allantoin. It seemed probable therefore that the clearance of allantoin could serve as a measure of glomerular filtration rate in these two species. Furthermore, it seemed possible that the clearance of this same substance might serve as a measure of glomerular filtration rate in man. Therefore it was thought advisable to determine the renal clearance of allantoin in normal human subjects.

Methods. Five normal men and one woman received 10 g of allantoin dissolved in 500 cc of fresh orange juice by mouth at 8:00 A. M. on the day of the experiment. Each subject was kept at bed rest until 9:30 A. M. at which time they were catheterized and given 1000 cc of H_2O by mouth. At 10:00 A. M., the bladder was emptied, washed out with normal saline solution, a blood sample taken and the first urine collection begun. At 10:30 A. M., the bladder was emptied again, washed out with saline solution, and a second blood sample was obtained. The second urine collection began immediately after the bladder had been emptied and washed. At 11:00 A. M., the second and final collection period was terminated, and a third blood sample was obtained.

Urine and plasma samples were analyzed for allantoin by the method of Christman, Foster and Esterer,[2] modified as described in a previous study.[3]

The allantoin clearance of each subject then was calculated as an average of the clearances obtained for the 2 collection periods and corrected to 1.73 square meters of surface area.

Results. As Table I indicates, the allantoin content of plasma, 120, 150 and 180 minutes after oral ingestion of 10 g of this substance remained approximately constant. The average stability of the plasma allantoin during the entire clearance study of course eliminated any need for supplementary oral or parenteral administration of allantoin.

The average plasma allantoin clearance of the 6 subjects was found to be 123 cc per minute (See Table I). Individual clearances varied from 107 to 137 cc per minute. It should be mentioned that no ill effects were observed in any patient during or after the clearance study.

Discussion. Similar to the results obtained in the rat and dog,[1] the present study indi-

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