volume, 5.16 ± 0.55 cc; plasma volume, 3.13 ± 0.57; and red cell volume, 2.03 ± 0.41 cc per 100 sq cm of surface area. A comparison of these values with those of the intact rats shows no significant difference for the total blood volumes. There is, however, an appreciable increase in the plasma volume and a corresponding decrease in the red cell volume, due to the anemia developed in these animals.[7]

Comment. The results obtained in this work for intact rats are similar to those reported by Went and Drinker.[10] These workers, using a micro-dye method obtained an average total blood volume of 7.4 cc per 100 g of body weight and 5.1 cc per 100 sq cm of surface area.

Griffith and Ingle[6] recently reported that hypertensive partially nephrectomized rats showed an increase in total blood volume, which would appear to be contrary to the findings reported in this paper. On inspection of their procedure for estimating blood volume,[11] it appears that they neglected to take the red cell volume into consideration and the values reported by them probably represent plasma volumes. In the present experiments, the increased plasma volumes in partially nephrectomized rats agree with the reinterpreted data of Griffith and Ingle.

Summary. Plasma and total blood volumes were determined in 33 normal male and 20 normal female rats. In the hypertensive partially nephrectomized rat, the plasma volume was increased, the red cell volume decreased and the total blood volume remained unchanged. It is concluded that hypertension is not associated with increased blood volumes in these experiments.

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Relationsip of Moscow 2 Virus of Equine Encephalomyelitis to Rabies.

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The virus designated as the Moscow 2 strain by Vichelessky and coworkers[1] was placed by them in the group of neurotropic agents

10 Went, Stephan, and Drinker, C. K., *Am. J. Physiol.*, 1929, **88**, 468.

11 Griffith, J. Q., and Campbell, R., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 38.

1 Vichelessky, R. S., Naskov, A., Soukhof, M., and Moutovine, V., *Rec. med. vet. l'École d'Alfort*, 1935, **3**, 357.

causing encephalomyelitis in horses. The Russian workers had recognized a serological relationship to the virus of rabies but were unable to demonstrate clearly defined Negri bodies in the tissue cells of the animals examined.

In order to determine the extent of this relationship, further comparisons have been made with a known fixed rabies virus.* The earlier observations were confirmed by these studies and several animal experiments have been made in addition.

Anti-rabic immune serum was mixed with an equal amount of the Moscow 2 virus, diluted 1:5,000 and 1:10,000 respectively, placed in the refrigerator over night and inoculated intracerebrally into white mice the next day. Neutralization occurred on each of several occasions, while the control mice given mixtures of virus diluted in normal serum and broth, succumbed to the disease. In like manner, complete protection occurred when the Moscow 2 hyperimmune rabbit serum was tested against the known fixed rabies virus in dilutions of 1:5,000 and 1:10,000 respectively. These results could also be repeated.

A guinea pig hyperimmunized against the Russian strain was found to be immune to an intracerebral inoculation of undiluted rabies virus which killed the two control animals in 4 days. Four white mice immune to the rabies virus failed to succumb to intracerebral injection of the undiluted Moscow 2 virus which killed 3 control animals in 5 to 6 days.

Serum from rabbits immunized with suspensions of live rabies virus isolated from a dog in San Francisco gave strong reciprocal reactions with the Moscow 2 strain. The former virus had been recovered from an animal showing an atypical clinical appearance, a slow paralytic form of disease rather than the acute, furious type. Reciprocal reactions occurred, however, with both the immune serums and viruses, thus establishing the identity of the two viruses. In the same manner, the Moscow 2 antiserum protected against another strain of rabies isolated from a duck and against yet another somewhat atypical one. The latter was recovered under rather unusual circumstances and did not produce intracytoplasmic inclusions in the brain cells of any animal tested except the dog.

Previously no reciprocal reactions of any kind had been demonstrated for this Russian strain. In a former report[2] it had been shown that the Moscow 2 virus was serologically different from both

* Both the rabies virus and the anti-rabic immune serum first used were kindly sent by Dr. Anson Hoyt of the University of Southern California.

[2] Howitt, B. F., *J. Inf. Dis.*, 1938, **63**, 269.

American strains of equine encephalomyelitic virus, about the only point in common being the recovery from the horse. There was no cross tissue immunity with either the eastern or the western equine strains, nor any cross neutralization or complement fixation with these or with the viruses of lymphocytic choriomeningitis, Japanese B and St. Louis encephalitis, respectively. It differed from the 2 viruses of equine encephalomyelitis in its larger size, its ability to induce a lower fever reaction in the guinea pig, its inability to be readily cultivated on the membranes of the developing chick, its absence in the blood stream and visceral organs and the non-transmission of immunity to the young guinea pig through the placenta. It did, however, have the same 4-day incubation period in the guinea pig characteristic for the western equine strain and often induced similar clnical symptoms, although occasionally animals showed a difference by manifesting extreme spasticity and a convulsive state. In mice the incubation was longer than for the western virus. White rats, the cotton rat *(Sigmodon hispidus eremicus)*, cats, mice, rabbits and monkeys were found to be susceptible in the same manner as they were for the 2 equine strains. A 6-pound duck and two 3-pound chickens failed to respond to intracerebral inoculation after 7 weeks' observation. Three chicks of one week and 2 of 2 weeks' age, hatched in the laboratory, however, became prostrated or died in 2 to 3 weeks, respectively, after intracerebral injection of undiluted virus. The virus was recovered from the brain of the chicks.

The Russian workers recognized the resemblance of the Moscow 2 strain to rabies virus, yet they placed it in the equine group of neurotropic viruses largely because of failure to infect dogs or to find Negri bodies in the tissues examined. In order to make further observations in this regard, two 6-weeks-old puppies were inoculated with the undiluted Moscow 2 virus, one with 0.4 cc intracerebrally and the other with 2 cc subcutaneously. The former animal was sacrificed after complete prostration in 5 days, the virus being recovered from the brain and cord. The other dog became very weak in the hindlegs, although with some spastic reactions, then developed a spastic paralysis of the forelegs, combined with a generalized convulsive twitching, and finally became prostrate on the 23rd day after the injection. The virus was demonstrated in the brain and cord after autopsy.

Vichelesky and coworkers[1] did not report the presence of Negri bodies within the nerve cells for the Moscow 2 strain, yet, because of the short incubation period for most animals, it is likely that the

inclusion bodies were not sufficiently developed to be readily seen. It is known that these inclusions are not always demonstrable in tissues from rabid animals, especially in the acute type of the disease. That rabies may appear in several forms, both unusually acute as well as with a prolonged course, has been recognized for some time. The virus reported by Koritschoner[3] and that by Jonnesco[4] both manifested extremely short incubation periods and failed to produce Negri bodies in small laboratory animals. It was not until repeated passage in the dog that they were found with the latter strain. Later Pawan[5] recovered a rabies virus having a 2-day incubation period from rabbits bitten by a vampire bat. No Negri bodies were demonstrated until after the incubation was prolonged by intrasciatic inoculation of rabbits with the virus.

As previously reported,[2] small intracytoplasmic bodies were found by Covell in the brain cells of a cat and a monkey given the Moscow 2 strain, but not in those of the guinea pig and the rabbit. They were again demonstrated in cells from the brain of the dog given the subcutaneous injection.

To determine if more typical inclusion bodies could be produced, the Russian virus was inoculated into the sciatic nerve of a monkey. After 9 days it developed violent convulsions, frothing at the mouth and the general appearance of a rabid animal. It was sacrificed and examination revealed the presence of the virus in the brain and cord at different levels after passage to white mice. Medium sized, round acidophilic bodies were found by Covell in the cytoplasm of the brain cells when stained with rosaniline methylene blue by the Schleifstein method.[6] The inclusions were different from the usual Negri bodies, however, in that no basophilic granules were noticed, but appeared more like the bodies described for rabies by Manouelian.[7] The inclusions demonstrated in the tissue cells of the other animals examined, dog, cat, and monkey, were all of this general type, rather small and compact and not of the appearance usually seen in the cells of dogs infected with the common street virus. Since it is known that typical Negri bodies are not usually observed with fixed virus material and since the Moscow 2 strain has been passed for a number of years, showing a definite incubation period it is not unlikely that it has become "fixed" for laboratory animals and thus the original form of inclusion has been modified.

[3] Koritschoner, *Wien. klin. Wschr.*, 1923, **36**, 385.

[4] Jonnesco, D., *Ann. l'Institut. Past.*, 1932, **49**, 435.

[5] Pawan, J. L., *Ann. Trop. Med. and Parasitol.*, 1938, **32**, 35.

[6] Schleifstein, J., *Am. J. Pub. Health*, 1937, **27**, 1283.

[7] Manouelian, Y., *Ann. l'Institut. Past.*, 1914, **28**, 233.

References may be found in the literature to various atypical forms of the rabies virus, yet Remlinger and Bailly[8] have not recognized any so-called "plurality of viruses". They rather prefer to account for the differences among strains as due to the differences in activity and invasiveness, especially if a serological and immunological relationship has been demonstrated. That the infectiousness may vary considerably has been illustrated by the reports previously mentioned of Koritschoner,[3] Jonnesco,[4] and Pawan.[5] In these instances the short incubation periods and the failure to demonstrate Negri bodies readily were disturbing factors, remedied only after the serological and immunological experiments had shown a definite relationship to rabies virus. For this reason Remlinger and Bailly consider it misleading to rely solely on the finding of Negri bodies, because of the occasional acceleration of the incubation time with coincident failure to produce intracytoplasmic inclusions in many animals. They would depend largely upon the serological and immunological tests to establish the relationship of an unknown strain to that of rabies as was done for the viruses mentioned above.

While the relationship of the Moscow 2 strain to that of rabies has been demonstrated by the serological, immunological and cytological experiments, the particle size, 80 to 130 millimicrons,[9] would also place this virus closer to rabies than to the American strains of equine encephalomyelitis. The range of the latter lies between 20 to 30 millimicrons,[2] while that of rabies is placed by Galloway and Elford[10] at 100 to 150 millicrons for fixed rabies virus; Levaditi and coworkers[11] have found higher figures, 140 to 210 millicrons for the fixed virus and 160 to 240 millicrons for the street virus.

Summary. From the serological and immunological reactions, from the difficulty in filtration, from the particle size, from the absence of virus in the blood and organs, from the ability to infect dogs both intracerebrally and subcutaneously, and from the presence of intracytoplasmic inclusions, although slightly atypical and not inclusive for all animals, the Moscow 2 virus may now be classified with the rabies strains rather than with the American viruses of equine encephalomyelitis as has formerly been suggested.

8 Remlinger, P., and Bailly, J., *Bull. Inst. Past.*, 1931, **29**, 1.

9 Lazarus, A., and Howitt, B., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 595.

10 Galloway, I., and Elford, W. J., *J. Hyg.*, 1936, **36**, 532.

11 Levaditi, C., Paic, M., and Krassnoff, D., *C. R. Soc. Biol.*, 1936, **123**, 866.