Comparative Methods of Diagnosis of Rabies in Animals*

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NEGRI announced his microscopic test for rabies in 1903 which has since been generally followed in routine diagnostic work. A considerable amount of investigation concerning the pathological changes in the central nervous system had already been published. As early as 1892 Babes[1] described his "rabic tubercles." Ganglionic changes occurring in the disease were described by van Gehuchten and Nélis,[2] and McCarthy and Ravenel.[3]

Following the announcement of Negri's discovery earlier microscopic methods of diagnosis were gradually succeeded by the Negri body test. It is known that Negri bodies are not always demonstrable in the brains of rabid animals. Joseph Koch[4] states that 10 to 12 per cent of the brains which are positive on animal inoculation will fail to show Negri bodies when the customary method of examination is employed. This percentage of error, he claims, would be reduced if impressions or smears were taken from ganglion cells and various parts of the brain instead of from the Ammon's horn alone. The percentage of error will probably vary also with the prevalence of the disease in the geographical areas from which the material for diagnosis is obtained. In regions of high endemicity one might expect a high percentage of Negri negative specimens proving positive on animal inoculation.

Negri found peculiar intracellular bodies occurring with a high degree of constancy in different parts of the central nervous system, especially in the Ammon's horn of the hippocampus major or in the Purkinje cells of the cerebellum. These bodies exhibit marked variations in size; the diameter varying from 1 μ to 27 μ. In form they may be round, oval, or, in the larger bodies, pear shaped. The internal structure is reticulate and usually contains one or more vacuoles. The bodies are surrounded by a clearly defined membrane.

The injection of brain emulsion from rabid animals into rabbits and guinea pigs is uncertain in its results after incubation periods of 2 to 8 weeks. In consequence of the long incubation period and uncertainty of infectivity in these animals, their use has been very limited in recent years.

Mice have been tried as test animals by European workers, but have not proved satisfactory. Babes[5] in 1887

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reported that they were susceptible to rabies. He stated that they develop the furious type of the disease and that great care should be exercised in handling the animals during the late stages of the disease because of the danger of being bitten. Gerlach [6] reports a series of 26 white mice injected with street virus from Negri positive brains, only 17 of which died of rabies. He does not state how the injections were administered and does not recommend the white mouse as a test animal.

A questionnaire was sent to all state laboratories in the United States asking if confirmatory animal inoculations were employed with Negri negative material. Replies were received from 39 states, and 5 reported that all Negri negative material was being injected into rabbits or guinea pigs. Five laboratories stated that they occasionally checked their Negri negative material by animal inoculation. Among the above 10 states employing animal inoculation for the diagnosis of rabies, 5 listed the guinea pig as the animal of choice and 4 the rabbit, while one laboratory uses the guinea pig and rabbit on each specimen examined. The reasons given for not employing the confirmatory animal test included the following: (1) delay in obtaining a result, (2) inadequate facilities for housing animals, (3) cost of laboratory animals.

Webster and Dawson [7] have described an improved method of diagnosis by mouse inoculation. They make use of a special strain of white mice supposedly bred for special susceptibility to neurotropic viruses. Four to 6 weeks old white mice are highly susceptible to the rabies virus when inoculated intracerebrally with brain emulsion from a rabid animal. The mouse technic as practised in this laboratory is as follows:

Material, when not examined fresh, is preserved in full strength glycerin. A small portion of the Ammon's horn is ground up in a sterile mortar and 9 parts of hormone broth added to 1 part of ground brain material. This is thoroughly emulsified and then centrifuged at 2,000 to 2,500 revolutions per minute for about 5 minutes. With a 0.25 c.c. tuberculin syringe, 0.03 c.c. of the supernatant fluid is injected into the brain through the skull of a 4 to 6 weeks old mouse. A 27 gauge needle, ¼" long is employed, and the injection made slightly to one side of the mid line of the skull and half way between the eye and ear. The site of inoculation is moistened with alcohol before injecting. The mice are lightly etherized before the operation. It has been our practice to inoculate 4 mice with each brain specimen. When the injections are performed by experienced technicians, one can expect a mortality of 0.5 to 1.0 per cent due to mechanical injury. In our series of 1,032 brain specimens we have employed 5,339 mice with a mortality of 1.7 per cent due to mechanical injury. This is higher than usual and may be explained by the fact that many of the injections were performed by persons learning the technic.

Table I gives an analysis of the findings on 1,032 specimens from various animal brains received from Alabama, Georgia, West Virginia, Connecticut, and Havana, Cuba. Of the total received, 338 specimens were reported positive by the diagnostic laboratories, 690 negative, and 4 questionable. Among the 338 specimens reported positive for Negri bodies, 3 proved negative on mouse inoculation; 83 of the 690 reported negative (12.0 per cent) produced typical symptoms of paralytic rabies in the mouse, and Negri bodies were demonstrated in the mouse brain. Three of the 4 specimens reported as questionable by the diagnostic laboratories proved positive on intracerebral mouse inoculation.

TABLE I

Comparative Diagnostic Results by the Usual Laboratory Methods and the Mouse Inoculation Technic

Animal	Number Examined	Reported +	Reported + Found —	Reported —	Reported — Found +	Per cent Reported — Found +	Reported Question-able	Reported Quest. Found +
Dogs	815	307	3	506	67	13.2	2	1
Cats	135	14	0	120	5	4.2	1	1
Cows	44	12	0	31	4	12.9	1	1
Hogs	12	1	0	11	3	27.3	0	0
Mules	11	1	0	10	1	10.0	0	0
Human	4	1	0	3	2	66.7	0	0
Foxes	3	1	0	2	0	0.0	0	0
Horses	3	1	0	2	1	50.0	0	0
Chickens	2	0	0	2	0	0.0	0	0
Rat	1	0	0	1	0	0.0	0	0
Squirrel	1	0	0	1	0	0.0	0	0
Goat	1	0	0	1	0	0.0	0	0
Totals	1,032	338	3	690	83	12.0	4	3

In an effort to determine the relative susceptibility of the true field mouse (Peromyscus polionotus polionotus—Wagner) and the Webster-Dawson strain of white mouse, a group of white mice and field mice were injected with brain emulsion from 11 different animals. The same supernatant fluid from each specimen was injected at the same time into mice of the two groups.

Table II shows a slightly greater resistance to the rabies virus on the part of the field mice. This may be due in part to the fact that some of the field mice were well over 6 weeks of age, as could be determined by the ossification of the skull. The needle was forced through some of the skulls of field mice with greater difficulty than was the case with the younger white mice of known age. The field mice were much more excitable and difficult to handle, and in some cases the furious type of rabies was produced, whereas among the 5,339 white mice inoculated, none has shown the furious type.

When the disease appears among a group of inoculated mice, in the great majority of instances all mice in the group succumb. One survivor has been observed from each of 4 groups, and in 3 groups there were 2 survivors in each of 4 inoculated. The survivors may show early symptoms and later recover, but no recoveries are recorded after the disease reaches the paralytic stage.

After the development of paralysis, death follows on an average of approximately 48 hours. Sluggishness, roughening of the fur and loss of hair luster appear as the earliest symptoms, followed by photophobia in many cases and a conjunctivitis with sero-purulent discharge. Paralysis, most frequently of the hind legs, appears and the legs are extended either to the sides or straight back. At this stage convulsions are frequently seen, especially when the animal is disturbed by jarring or noise. Death is preceded by complete prostration and labored and irregular respiration. Of the 2,095 mice injected with rabies positive brain material, the majority died in from 10 to 12 days.

An occasional mouse inoculated intracerebrally with brain emulsion from a

TABLE II

Comparative Susceptibility of the Field Mouse and the Webster-Dawson Strain of White Mouse

SPEC. Nº	MOUSE	MORTALITY AFTER INJECTION (IN DAYS)																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
510	White						1+				II	III											
	Field						1−	1−					1+			III							
511	White	I					1−		1+		II			I									
	Field				I		1−							III+		I	I						
513	White						1+			I	I	II	I										
	Field	II								I			II+	I	I								
514	White						1+				I	II	I	I									
	Field	I					1+						II			I	I	I					
897	White										1+			I			III		I				
	Field	I									1+								I	I	I		I
915	White								III+	III+													
	Field	I							1+			II				II							
932	White							I	II+		I	II											
	Field	II		II					1+					I									
933	White							IIII+	I	I													
	Field							II+		III				I									
937	White							IIII+		I													
	Field							1+		II		I		II									
948	White							1+	II	II				I									
	Field							1+		I	I	I			II								
971	White								I	II+	I	I	I										
	Field	I								1+			I	I	I								

| + Indicates mouse sacrificed and found Negri +
| — Indicates mouse sacrificed and found Negri —

positive case showed Negri bodies as early as the 5th day after injection. On the 6th day following intracerebral inoculation with Negri positive brain, 226 of 289 (78.2 per cent) mice examined were Negri positive and 3 doubtful.

It has been the experience of this laboratory that in brain material from animals vaccinated against or treated for rabies, Negri bodies are more difficult to demonstrate in the original material. The disease, however, can be produced in mice, with the production of Negri bodies, by inoculating with material from the brain.

A human brain from an adult dying of rabies following a face bite inflicted by a proven rabid dog was submitted for examination. This patient started treatment the day following the bite and received 3 injections a day for 15 days. The patient developed rabies 21 days after exposure, and the brain was found negative for Negri bodies on repeated examinations. Mouse inoculation with this material proved positive, and Negri bodies were present in the mouse brain on the tenth day after intracerebral injection. When a vaccinated dog develops the disease, it is usually of the paralytic or "dumb"

type, and the experienced veterinarian's diagnosis is of the utmost importance, as the Negri diagnosis is unreliable. In these cases the intracerebral injection is of particular value.

The mouse test offers a cheaper and more rapid method of diagnosis than is the case with the guinea pig or rabbit. Mice purchased from dealers cost 15 cents each but can be bred with little difficulty, thus reducing the cost. Reactions to rabies virus when injected intracerebrally into the mouse are very consistent and offer a reliable means of verifying the routine microscopic Negri technic.

SUMMARY

A technic for the earlier diagnosis of rabies by mouse inoculation is described. In addition to shortening the period required for animal diagnosis the method has the advantage of considerably reducing the cost. Comparative results from brain material of 1,032 animals diagnosed by the usual methods in several laboratories and then injected into mice confirmed the positive results obtained by the usual methods, but disclosed an error of 12.0 per cent for the brains reported negative for Negri bodies.

The vaccination or treatment of animals with rabies vaccine apparently increases the difficulty of diagnosis by the discovery of Negri bodies.

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Animal and Human Diseases

. . . Animal disease has a close connection with human disease, and students of public health will find much to think over in the Ministry of Agriculture's *Report of Proceedings under the Diseases of Animals Acts for the Year 1936*. Bovine tuberculosis is but one of the many subjects dealt with. It was reported to exist on 26,744 premises, and after examination by veterinary inspectors, 23,802 animals were ordered to be slaughtered, an increase of nearly 1,500 on 1935. The number of Attested Herds was 55 at the beginning of the year, and rose to 140 on 31st October, after which a rapid increase brought the total to 192 at the end of the year. (During the first quarter of the present year 58 further herds were added to the register.)—*J. Roy. Inst. Pub. Health & Hyg.*, 1, 2 (Nov.), 1937.