throughout the thyroid parenchyma of all rats may also be indicative of exhaustion from excessive thyrotrophic stimulation.

The possibility that the trapped I^{131} might initiate neoplastic changes in the thyroid gland has been recognized by many clinical investigators(10-13) who have expressed the need for caution in the use of radioiodine for diagnosis and treatment of Grave's disease. The discovery of 2 highly suspicious tumors among the 10 rats that were treated with radioactive iodine further emphasizes the need for caution.

Summary. Examination of 10 rats sacrificed 18 months after a single injection of 400 μc of radioactive iodine revealed the following changes in the thyroid gland: 1. Hürthle-like elements (previously reported by us in thyroids of rats sacrificed 8 months after a single I^{131} injection) were evident in the parenchyma of all glands. 2. Multiple adenomata, apparently benign in nature, were found in the thyroids of 2 rats. 3. Two types of anaplastic, nonencapsulated neoplasms, one of which was invading adjacent thyroid tissue, were also found in the thyroids of these 2 rats.

We are indebted to Dr. Stuart Lindsay of the Division of Pathology for suggestions and advice in the preparation of this paper. The technical assistance of Mr. F. Pasantino is also gratefully acknowledged.

Received January 11, 1951. P.S.E.B.M., 1951, v76.

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Japanese B Encephalitis in the Rat. (18559)

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It has been reported that the rat can be infected with the virus of Japanese B encephalitis if the virus is injected intratesticularly (1). However, this finding has not been confirmed and it is generally believed that the rat is resistant to infection with Japanese B encephalitis virus(2). The observation that 7- and 8-day-old albino rats are uniformly susceptible to St. Louis encephalitis virus whereas 21-day-old rats fail to develop a clinically apparent infection even though the virus is injected directly into the brain(3,4) suggested that experiments be conducted to determine whether rats of different ages behave toward the related Japanese B encephalitis virus in a similar manner.

The purpose of the present report is to record the data obtained from a study designed to determine the behavior of rats of different ages following injection of Japanese B encephalitis virus.

Materials and methods. Virus and animals. The Nakayama strain of Japanese B encephalitis virus which had been maintained by mouse-brain passage was used.† Mouse brains infected with the virus were ground in a mortar with sand and sufficient of a mixture composed of equal parts of rabbit serum and

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† This strain of virus was obtained through the courtesy of Doctor A. B. Sabin.

TABLE I. Susceptibility of Rats of Different Ages to Japanese B Encephalitis Virus Injected Intracerebrally.

Age, days	No. tested*	Wt		Results		
				Mortality		
		Avg, g	Range, g	Total	%	Day of death†
7	9	7.6	6.5 to 8.4	9	100	5, 5, 5, 6, 7, 7, 7, 8, 8
7	7	11.5	10.0 to 12.5	7	100	5, 5, 5, 5, 5, 5, 6
12	7	13.4	11.0 to 14.6	7	100	5, 5, 5, 5, 6, 8, 8
12	8	12.9	11.7 to 13.7	8	100	4, 4, 5, 5, 5, 5, 7, 7
12	10	14.0	12.5 to 15.0	1	10	5, S, S, S, S, S, S, S, S, S
21	6	25.9	20.0 to 31.5	0	0	S, S, S, S, S, S
21	6	22.1	17.8 to 24.4	0	0	S, S, S, S, S, S
27	15	36.9	30.0 to 39.1	0	0	S, S, S, S, S, S, S, S, S, S S, S, S, S, S

* At least 2 litters in each age group.
† Numeral indicates day of death. S indicates survival and no evidence of infection.

saline to make a 10% suspension of the infected mouse brains. The suspension was centrifuged at about 2000 R.P.M. for 5 minutes to sediment gross particles. The supernatant liquid was then removed and titrated intracerebrally in 3- to 4-week-old Swiss mice using a mixture of 10% rabbit serum in saline as a diluent. Immediately after the supernatant liquid was removed from the sediment it was distributed in 2 cc amounts in 5 cc glass vials. The vials were sealed and the virus therein was then frozen in a mixture of dry-ice and alcohol. The frozen virus was stored in a dry-ice cabinet. As virus was needed one or more vials were thawed in a water bath at 37°C just prior to the time it was to be used. A single preparation of virus was used in the studies. The mouse intracerebral LD_{50} titer(5) of the freshly prepared virus suspension was 1 x $10^{-8.8}$. The Fischer strain of albino rats was used.‡ The rats used in the experiments were raised in our animal quarters and an accurate record of their ages was available. All of the rats which were younger than 21 days of age when tested were allowed to remain with the mother. The rats were weighed just prior to inoculation and the average weight and the weight range determined. The inoculated animals were observed daily for 21 days.

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‡ The rats used in the study were kindly supplied by Dr. W. F. Dunning.

Experimental. Behavior of rats of different ages following intracerebral injection of the virus. Rats varying in age from 7 to 27 days were injected intracerebrally with 0.03 cc of the 10% suspension of virus. The results appear in Table I. Of the 16 animals tested when they were 7 days of age all developed a fatal encephalitis. The first signs of infection in these young animals appeared 4 days after the virus was injected. At this time there were no symptoms which would indicate that there was involvement of the central nervous system. However, the animals appeared weak and slightly emaciated. Nine of the 16 animals were found dead on the morning of the 5th day. In those animals which survived longer than 5 days, tremors of the head, ataxia and prostration were observed before death occurred. It is interesting to note that a rat weighing 12.5 g was found to be as susceptible as an animal weighing only 6.5 g. Twenty-five rats were injected intracerebrally with the virus when they were 12 days old. These 25 rats represented 3 different litters. In 16 of the 25 animals a clinically apparent infection occurred and in all 16 the infection had a fatal termination. It is interesting to note that in 2 of the litters tested the mortality was 100%, whereas, in the 3rd litter only 1 of 10 rats injected died of the infection. The course of the disease in rats of this age in which a clinically apparent infection manifests itself, followed by death from the infection, is essentially the same as that described for 7-day-old rats. It

TABLE II. Susceptibility of Young Rats to Japanese B Encephalitis Virus Instilled Into the Nose.

Age, days	No. tested	Wt		Results		
				Mortality		
		Avg, g	Range, g	Total	%	Day of death
7	7	10.7	10.2 to 10.9	7	100	6, 6, 7, 7, 7, 7, 7
12	4	13.3	9.6 to 14.2	4	100	4, 7, 12, 13
12	6	15.1	14.4 to 15.6	4	67	11, 11, 12, 13, S,* S*

* On the 11th day after the virus was instilled into the nose, these 2 animals were weak, emaciated and walked with a staggering gait, but on the 18th day were completely recovered.

should be noted, however, that 2 animals in the 12-day-old group died 4 days after inoculation, whereas, none of the 7-day-old rats died before the 5th day. It will be noted (Table I) that the weights of the 12-day-old rats tested varied from 11.0 to 15.0 g. It would not appear, however, that weight alone had any influence on the susceptibility since there was not a great difference in the average weights of the 3 litters. However, in 2 litters the mortality was 100% and only 10% in the 3rd. The 12 rats which were injected when they were 21 days of age never showed any clinical evidence of infection. The same was true of the 15 rats which were inoculated when they were 27 days of age.

From the data presented in Table I, it is evident that Japanese B encephalitis virus is able to initiate a lethal infection in the rat providing young enough animals are tested. At 7 days of age rats are uniformly susceptible to intracerebral injection of the virus. However, at 12 days of age, while some rats can be infected, others fail to develop clinical signs of infection. Rats which have attained the age of 21 days do not develop an apparent infection even though the virus is injected by the intracerebral route.

Behavior of 7- and 12-day-old rats following nasal instillation of the virus. In this experiment 0.03 cc of the 10% suspension of virus was instilled into the nose of 7- and 12-day-old rats. The results appear in Table II. It will be seen that the virus is highly infectious for these animals when inoculated by this route. All of the 7-day-old rats developed symptoms of encephalitis and the infection terminated fatally in every case. The symptoms observed were the same as those described for the 7-day-old rats which had received the virus by intracerebral injection. Of the ten 12-day-old rats tested 8 developed a fatal encephalitis. In the two 12-day-old rats which survived, the virus apparently invaded the brain and caused encephalitis from which the animals recovered. When these 2 animals were observed on the 11th day after the virus had been instilled into the nose, they appeared weak and emaciated and walked with a staggering gait. Their condition improved gradually on the ensuing days and by the 18th day after inoculation they had recovered. Generally speaking, death occurred earlier in the 7-day-old rats following nasal instillation of the virus than in the 12-day-old group. All of the 7-day-old animals were dead by the 7th day, whereas, in the 12-day-old rats death occurred as late as 13 days after the inoculation of the virus although 1 animal in this age group succumbed on the 4th day. Reference to Table I reveals that only 16 of 25 (64%) rats which were 12 days old when injected intracerebrally with the virus died of the infection. On the other hand, 8 of 10 (80%) of the 12-day-old animals which had the virus instilled into the nose succumbed. This might be interpreted to mean that the 12-day-old animals are more susceptible to the virus when it is instilled into the nose than after intracerebral injection. The probable explanation, however, is that at or near 12 days of age rats begin to become resistant to the virus and the number which are susceptible may vary from litter to litter.

From the data so far presented it is evident that 7-day-old rats are highly susceptible to Japanese B encephalitis virus and that while some 12-day-old animals develop a fatal infection animals in this age group are not

TABLE III. Susceptibility of Young Rats to Japanese B Encephalitis Virus Injected Intraper.

Age, days	No. tested	Wt		Results		
				Mortality		
		Avg, g	Range, g	Total	%	Day of death
7	3	11.9	11.4 to 12.2	3	100	6, 7, 9
8	8	10.2	7.9 to 11.1	8	100	5, 5, 6, 8, 8, 8, 8, 9
8	8	8.6	—	8	100	5, 7, 9, 9, 9, 9, 9, 9
12	9	13.1	11.7 to 14.5	0	0	S, S, S, S, S, S, S, S, S
12	10	12.1	11.2 to 13.0	3	30	5, 12, 12, S, S, S, S, S, S, S

uniformly susceptible. However, rats which are 21 and 27 days of age do not develop a clinically apparent infection.

Behavior of 7-, 8- and 12-day-old rats following intraperitoneal injection of the virus. In this experiment 7- and 8-day-old rats were injected intraperitoneally with 0.1 cc of the 10% suspension of virus and 12-day-old rats were injected by the same route with 0.2 cc of the same virus suspension. The results appear in Table III. The 7- and 8-day-old animals were very susceptible to the virus inoculated by this route since all of the animals tested developed encephalitis and died of the disease. It is interesting to note that death occurred in the 7- and 8-day-old rats which had received the virus intraperitoneally as early as in the 7-day-old rats which had been injected intracerebrally (Table I). This would suggest that the virus, following intraperitoneal inoculation into these young animals, spreads rapidly to the brain to initiate a fatal infection. On the other hand, a high degree of resistance was observed in the 12-day-old rats which were injected intraperitoneally with 0.2 cc of the 10% suspension of virus since in only 3 of the 19 animals in this age group did the virus produce a fatal infection.

Discussion. From the data presented it is apparent that young rats are susceptible to infection with Japanese B encephalitis virus following either intracerebral, intranasal or intraperitoneal injection of the virus. However, as rats grow and develop they become increasingly resistant so that when they attain the age of 21 days they are no longer susceptible to the virus even though it is injected directly into the brain. Duffy and Sabin have reported a similar type of resistance to St. Louis encephalitis virus in the rat. Seven- and 8-day-old rats were found to be susceptible to the virus when it was inoculated by either the intracerebral or intranasal route. On the other hand, rats 21 days old did not develop a clinically apparent infection even though the virus was injected into the brain(3,4).

It would appear that the age-susceptibility pattern of the albino rat to both Japanese B encephalitis virus and St. Louis encephalitis virus is essentially the same since 7- and 8-day-old rats are uniformly susceptible and a high degree of susceptibility is seen in rats 12 days old. At 21 days of age, however, the rat appears to be resistant to both viruses in that rats of this age do not develop a clinically apparent infection, even though the virus is inoculated by the intracerebral route.

Summary. 1. A fatal encephalitis develops in 7-day-old albino rats following nasal instillation or intracerebral injection of Japanese B encephalitis virus. 2. The virus produces a uniformly fatal infection in 7- and 8-day-old rats when injected intraperitoneally. 3. While some 12-day-old rats are susceptible to the virus when inoculated either by the intracerebral, intraperitoneal or intranasal route they are more resistant than 7- and 8-day-old rats. 4. Rats 21 and 27 days of age appear to be resistant to the virus since when it is injected intracerebrally into animals of this age it does not induce a clinically apparent infection.

Received February 14, 1951. P.S.E.B.M., 1951, v76.