croscopy are mounted dry on albumin-coated slides to avoid destruction of some of the enzyme which occurs when water is used to flatten paraffin sections). The enzymatically liberated phosphate is converted to the lead salt, and the usual treatment with ammonium sulfide and careful rinsing with water follows. It is necessary to substitute lead for the cobalt of the alkaline phosphatase procedure, since cobalt sulfide is insufficiently soluble in the HCl required subsequently.

TABLE 1

Duration of incubation (hr)	Optical density of methylene blue*	μg Sulfide (approx.)	μg Sulfide†/hr since previous determination
0.5	.60	.195	
1.0	.75	.244	.098
2.0	.94	.305	.061
4.0	1.21	.393	.044
6.0	1.48	.480	.044
8.0	1.64	.530	.025

* Final volume 277 μl.

† Includes sulfide derived from preformed (nonenzymatic) phosphate.

To the tube containing a dry section stained with lead sulfide, there is added 135 μl of water and 25 μl of a solution of 0.1 g *p*-amino dimethyl aniline sulfate (Eastman Kodak Company) in 100 ml of 5.0 *N* HCl. After standing 20 min with occasional stirring to achieve solution of the sulfide, there is added 5 μl of 0.023 M ferric chloride in 1.2 *N* HCl. This is stirred and allowed to stand for 20 min and the methylene blue is determined in a spectrophotometer at 670 mμ using microcuvettes. For larger or heavily stained sections, final volumes of 3.0 ml may be more suitable. Appropriate lead sulfide standards are also prepared.

Experiments. Serial sections of rabbit appendix 10 μ thick and 14 sq mm in area were incubated in Gomori glycerophosphate substrate for alkaline phosphatase pH 9.5 for from ½ to 8 hr at 26° C. Microscopically, this gave a range from faintly stained sections at ½ hr to generalized overstaining after 8 hr. The amount of sulfide found after the various periods of incubation is shown in Table 1. From the table it is evident that in this material the rate of deposition of sulfide decreased appreciably after 6 hr of incubation under these conditions. Both the amount of sulfide formed and the time scale of decrease in enzymatic activity are in fair agreement with expectation based on independent quantitative microestimations under standard quantitative conditions.

The method should be applicable to any of the histochemical procedures involving deposition of a sulfide soluble in hydrochloric acid.

Reference

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Comparison of Electroencephalographs of Young Rats from Dams on Synthetic and on Normal Diets[1]

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Richardson and Hogan (*1*) observed hydrocephalus in young rats born of dams on a synthetic diet, and concluded that the abnormality was due to a deficiency in the maternal diet. Young rats with the same nutritional history, though free from any sign of hydrocephalus, learn their way through a maze more slowly than do those from the stock colony (unpublished data). In a search for other differences between these two groups of rats, their electroencephalographs were compared. There were no significant differences; however, since to our knowledge observations of this kind on the rat have not been published, it seemed desirable to have

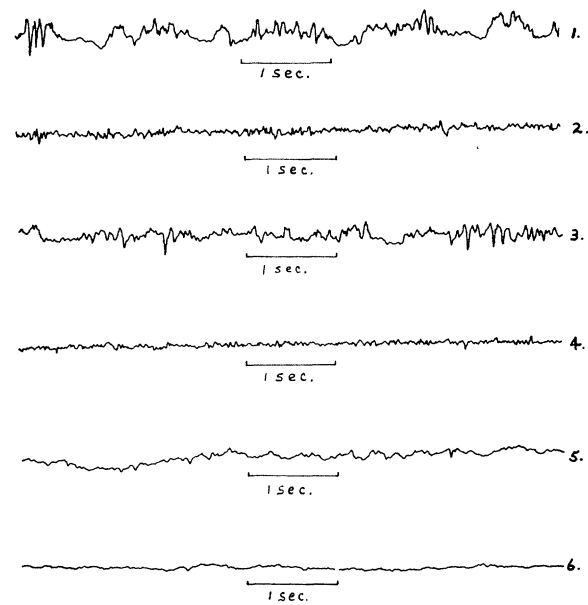

FIG. 1. Electroencephalographs of a normal rat from dam on the stock diet, a rat from dam on the synthetic diet and a hydrocephalic rat from dam on the synthetic diet. 1—Lead I, and 2—Lead II; normal rat from dam on the stock diet. 3—Lead I, and 4—Lead II; rat from dam on the synthetic diet. 5—Lead I, and 6—Lead II; hydrocephalic rat from dam on the synthetic diet.

a typical electroencephalograph of this animal described in the literature.

Fifty-seven young rats, 4–8 weeks of age, from dams on the synthetic diet, were paired according to age, weight, and sex with an equal number from dams on the stock diet. They were given an intraperitoneal injection

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of avertin (0.01 ml/g of body weight), their heads were shaved, and silver electrodes (3 mm in diam) moistened with electrode paste were attached to the skin with collodion. Two leads were taken from each animal. In Lead I, the first electrode was placed in the midline of the skull on a line between the external auditory meatuses; the second electrode was placed in the midline 12 mm in front of the first electrode. In Lead II, both electrodes were placed on the line between the external auditory meatuses, each one being 6 mm from the midpoint. The recordings were made with an Offner amplifier (type 140) and crystograph set at a speed of 2.5 cm/sec.

TABLE 1

FREQUENCY OF BRAIN WAVES IN THE RAT

No. of rats	Diet of dams	Average frequency per sec	
		Lead I	Lead II
57	Stock	30.8	32.4
57	Synthetic	30.2	31.9
3*	Synthetic	28.0	29.0

* Hydrocephalic rats.

A typical record from each group is shown in Fig. 1. The frequency per second was the measurement used in the analysis of the records. All countable waves were included and an average was taken of several counts on each recording. There was no significant difference in the frequency of the brain waves of the two groups of rats from dams on different diets, but the average frequency for three animals with well-developed hydrocephalus was somewhat lower than is normal. These results are shown in Table 1.

Reference

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Studies on the Life Cycle of *Syphacia obvelata*, a Common Nematode Parasite of Rats[1]

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Syphacia obvelata is an oxyurid nematode frequently occurring in the ceca of laboratory and wild rats and mice. It has been reported once from man (*4*). Considering its common occurrence and wide distribution, surprisingly few details are known regarding its life cycle. It has been assumed that this cycle is simple and direct, and that rats are infected from eggs in feces. The worms occur, usually in large numbers, in the cecum. Males and immature females are found most numerous in the tip of the cecum, although a few may be found in other parts of the cecum and in the large intestine. Gravid females are found throughout the cecum and large intestine, including the rectum. I have also collected them from washings of the body surface of the anal region. In scores of autopsies, there was no evidence that eggs are ever laid within the host. Repeated search, using a variety of the usual laboratory methods, revealed no eggs in the feces, although Philpot (*3*) found a few in the feces of mice. Lawler (*2*) seemed able to infect mice by feeding them with macerated gravid female worms from infected mice, although the number of mice used was small. He did not mention any period of incubation. Using *S. obvelata* from rats, I have not been able to infect rats by this method.

Attempts to infect rats by feeding whole gravid female *Syphacia*, freshly collected from an infected rat, were unsuccessful. The test rats used were previously treated with phenothiazine or carbon tetrachloride to rid them of any natural infection that might be present. Tests show that both of these drugs are effective anthelmintics. Attempts to infect rats by feeding eggs liberated from gravid female *Syphacia* were also unsuccessful. Finally, although it was impossible to demonstrate eggs in the feces of rats proved by autopsy to be heavily infected, feces from infected rats were fed to test rats. No infections resulted.

Several methods of incubating eggs to the fully embryonated, infective stage were tried. The eggs did not develop well in distilled water, tap water, or dilute formalin solutions, either at 20° or 37° C. Eggs were also cultured in moist air, as recommended by Deschiens (*1*). None of these methods produced eggs that were infective to test rats.

All these negative results led to the discovery of the natural location of embryonated eggs and the probable means of transmitting the infection. A rat was lightly anesthetized to prevent struggling, and its anal region was washed with 10% alcohol. The washing was caught in a glass container. This liquid was then centrifuged and the sediment examined. A fairly large number of embryonated eggs and some larvae measuring 0.14–0.22 mm in length were obtained in this way. In addition to unhatched embryonated eggs found on the anal region, about 25% of the eggs found were actually empty egg shells. Using water in place of the 10% alcohol, living larvae as small as 0.09 mm in length have been recovered. Whether the variation in size of what must be recently hatched larvae is due to swelling or to growth is not known at present. Thus eggs are liberated from the worms, become embryonated, and may hatch on the body surface of the anal region. Other rats may become infected by licking these embryonated eggs or the larvae from the body of an infected rat. Another possible method of infection is through the anus. Schüffner and Swellengrebel (*5*) showed that some eggs of *Enterobius vermicularis*, the human pinworm, hatch on the anal region of the host, and that some larvae may migrate back into the body through the anus and rectum, a method of infection which they termed "retrofection." Careful examination of the posterior inch of the large intestine

[1] Studies from the Department of Zoology, University of Nebraska, No. 242. The work was done under the direction of Dr. H. W. Manter.