earlier by Folin and Looney;[9] recent improvements in the procedures of estimating these amino acids have frequently given results which are at variance with those obtained by the original technique of Folin and Looney.† The glutamic acid, aspartic acid and proline values are, so far as it has been possible to determine from the literature, the first recorded quantitative determinations of the amounts of these amino acids yielded by thyroglobulin.

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Experimental Rabies in White Mice. Studies on Passive Immunization I.*

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The subject of passive immunization against rabies has received relatively little attention, even though Fermi,[1] Pfeiler[2] and others have reported striking experimental demonstrations of the efficacy of anti-rabic serums. In the current report, which summarizes 12 protection tests performed in the past 2 years, the protective properties of such serums have been studied using white mice as experimental animals.

A fixed virus strain, obtained through the courtesy of the Cutter Laboratories, was passed through rabbits and preserved at ice-box temperature, either fresh or in 50% glycerine, for periods which did not exceed 2 weeks. Details of preparation varied, but in effect the virus was ground to an initial dilution of 1/20 in normal saline and centrifuged 10 minutes at 2200 R.P.M. The supernatant fluid was then filtered through "Whatman No. 1" paper and final dilutions of from 1/100 to 1/800 were made. The virus was injected intracerebrally, at first in amounts proportional to the body weights of the mice (0.005 cc./gm. or 0.001 cc./gm.), later in a constant dose of 0.02 cc.

The serums were prepared by hyperimmunizing rabbits and goats

[9] Folin, O., and Looney, J. M., *J. Biol. Chem.*, 1922, **51**, 421.

† For examples and amplification of this statement, see references 4 and 7.

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[1] Fermi, C., *Cent. f. Bakt., Parasit. u. Infektskr.*, Orig., 1909, **52**, 576.

[2] Pfeiler, W., *Berliner Tierarzt. Wochenschr.*, 1913, **29**, 269.

with either 1/10 fresh fixed virus or 1/20 fixed virus preserved with ½% phenol. Serums were developed which would neutralize equal volumes of 1/200 fixed virus prepared as above to a final titer of 1/256, when the mixtures were incubated at 37° C. for 2 hours and tested by intracerebral mouse injection. Corresponding normal serums showed no *in vitro* neutralizing properties. The serums were filtered and inactivated before use.

In the experiments reported the serums were injected intraperitoneally, the first dose being given at intervals ranging from 48 hours to 1 hour before the administration of virus. Doses for each serum injection varied from .05 cc./gm. body weight to .15 cc./gm. Serum was usually given only once, but at times as many as 2 subsequent injections were made, the maximum total of serum given to any mouse being .45 cc./gm. in a period of 5 days. Control mice, either untreated or receiving corresponding amounts of normal serum, were included in all experiments. Titrations of the intracerebral infectivity of the virus and of the neutralizing titer of the serum against the virus employed were run simultaneously with most experiments. The virus often infected in dilutions as high as 1/6400. All mice were observed for symptoms at 12-hour intervals.

Any mouse that remained symptom-free for more than 2 weeks was classified as a survival. The results† of the separate tests evidence a reliability sufficient so that they may be assumed to indicate a normal statistical expectancy. Their characteristics have been examined and it has been determined that the separate tests can be combined by totaling and, considered, in the aggregate, as one experiment, which includes 155 treated and 198 control mice.

The mice contracting rabies in the different tests were also considered. It was found that the mean incubation period of each treated group was longer than that of its control group. An inspection of the corresponding standard deviations showed that these variations are significant.

From a study of the results of the experiments it is obvious that some evidence of passive protection, apparently due to specific serum therapy, was found in each test. Such protection was demonstrable in one or both of the following ways: (a) A higher percentage of treated mice (39%) survived than of the corresponding controls (12%). (b) A longer mean incubation period was shown by the treated mice that contracted rabies than by their corresponding controls. We are continuing this study in an effort to evaluate the maximum efficacy of anti-rabic serum.

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