enzyme destroys hypertensin after a short incubation period, but does not affect the integrity of tyramine and epinephrine, since both amines retain their vasoconstrictor action despite incubation for several hours at 37° at pH 6-8 in the presence of hypertensinase. In contrast, tyrosinase inactivates epinephrine and tyramine rapidly (Fig. 5 and 6). Besides, if hypertensin or epinephrine are placed in contact with tyrosinase under anaerobic conditions for a long period, both substrates are not attacked, while hypertensinase is equally active in the presence or absence of O_2 (Fasciolo and coworkers;[8] Bing and coworkers[5]). A similar difference between hypertensinase and tyrosinase may be demonstrated when these two enzymes act on hypertensin in the presence of cyanide (Fig. 7). Tyrosinase is totally inhibited while hypertensinase is unaffected. The amine oxidase obtained from cuttlefish liver is inhibited by octyl alcohol. Renal hypertensinase is unaffected by the presence of this alcohol (Fig. 7). The sensitivity of hypertensin in respect to tyrosinase and amine oxidase under anaerobic conditions has been interpreted as the possession of a common chemical grouping by tyramine and hypertensin. Hypertensin, like pepsitensin, is a polypeptide within all probability an end amino phenol group. Hypertensor extracts of the kidneys, even when highly purified, have been shown in previous investigations to behave like polypeptidases (Croxatto and Croxatto[6]).

Summary. The vasoconstrictor action of hypertensin and of the vasoconstrictor principle formed anaerobically by the action of renal tissue on dopa have been the subjects of study. It was shown that hypertensin has no vasoconstrictor effect in the Loewen-Trendelenburg test at a level producing a marked hypertension effect in the cat. Renal hypertensinase behaves similarly both aerobically and anaerobically, and its activity is not affected by cyanide or octyl alcohol, from which it is concluded that there is a clean-cut difference between this enzyme, amino oxidase and tyrosinase. These findings support the finding that hypertensinase has the characteristics of a polypeptidase (Croxatto and Croxatto[6]).

[8] Fasciolo, J. C., Leloir, L. F., Muñoz, J. M., and Braun Menendez, E., *Rev. Soc. Argent. Biol.*, 1940, **16**, 643.

14027

Active Immunization of Rats Against *Nippostrongylus muris.*

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The fact that acquired immunity against the nematode, *Nippostrongylus muris,* could be induced in white rats by the injection of the homologous worm material was first demonstrated by Chandler.[1,2] This investigator employed vaccines prepared from larvae and adult worms killed by heat; 3% H_2O_2; saturated salt solution, and by drying, then suspending in saline. Therefore the vaccines were in the nature of a suspension of worm material.

The purpose of the present study was to test the effect of parenteral vaccination with two types of antigens, prepared by filtering aqueous extracts of the dried adult worms and of larvae of *N. muris,* upon the resistance of pied stock rats, in an effort to determine whether immunity could be developed in these animals with the particle-free filtrate.

Plan of Study. The strain of parasites was isolated from a single wild Norway rat captured in 1939, and maintained in this labo-

[1] Chandler, A. C., *Am. J. Hyg.*, 1932A, **16**, 750.
[2] Chandler, A. C., *Am. J. Hyg.*, 1936B, **24**, 129.

ratory by passing it through rats at appropriate intervals.

The rats used were from the pied stock inbred in this department for the past 3 years. They were raised and maintained on a diet consisting of "Purina" dog-chow biscuits and water. Twice weekly the rats were given fresh carrots. The animals used were of the same age and from closely related litters.

Infective larvae (isolated by means of Baermann's apparatus from 9- to 10-day moist-chamber cultures of infected rat feces) were washed repeatedly with tap water in 50 cc centrifuge tubes, and the number estimated.[3] A rat, to be infected, was anesthetized lightly with ether; the hair was clipped from the abdomen, which was then soaped and rinsed. Five-tenths cc of the thoroughly agitated suspension of larvae was then spread over this area and left undisturbed for 30 min.

The infection was followed by dilution counts of the eggs of *N. muris*.[4] The egg counts were supplemented by counting the number of worms in the small intestines and lungs. At autopsy the rat was killed with chloroform, and the small intestine was detached at the pylorus and ileocaecal junction. The intestine was placed in distilled water, slit lengthwise with small scissors. It was then agitated so as to free the living worms into the suspending fluid. The whole of the fluid was examined for the presence of adult worms, small portions being transferred to a counting chamber[3] and searched with a binocular microscope. The intestine was agitated in fresh water until no more worms were recovered.

To determine the number of larvae in the lungs, these organs were removed at autopsy, placed in a petri dish, and stored in the refrigerator for 24 hr. They were then cut into small pieces, pressed between 2 plate-glass slides (2″ x 3″), and searched for the presence of larvae under the microscope.

[3] Scott, J. A., *Am. J. Hyg.*, 1928, **8**, 158.

[4] Winfield, G. F., *Am. J. Hyg.*, 1933, **17**, 168.

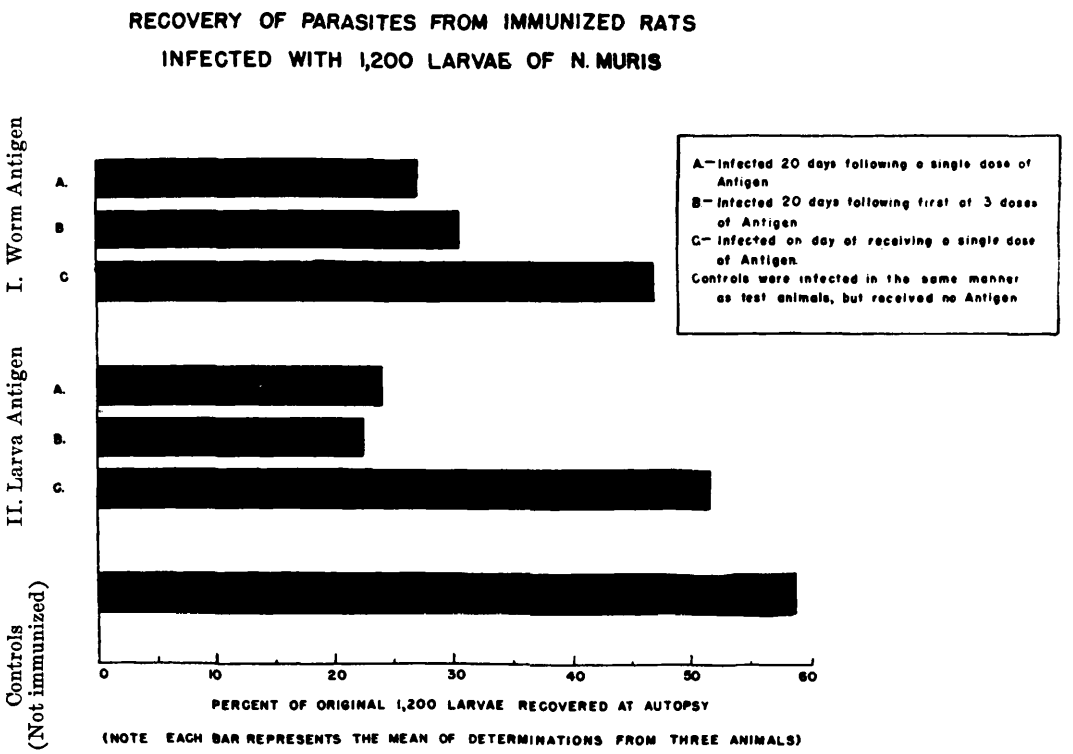

CHART 1.

Preparation of Antigens. The adult worms from the intestines of the rats were washed repeatedly with distilled water. After each washing, the parasites were allowed to settle and the supernatant fluid was discarded. By means of a 1-cc pipette the parasites were finally drawn off and placed in watch glasses, then dried in a desiccator containing phosphorus pentoxide. Drying was continued for 3 to 4 days; when thoroughly dried, the mass was pulverized in a mortar and weighed.

The extraction strength was calculated on the basis of the dried powder in a neutral 0.85% solution of sodium chloride; 1:50 was usually the basic dilution used. Before extraction was carried out, the mixture was ground in an electric ball-mill for 4 to 5 hr until a fine suspension was obtained. The extraction was first carried out at room temperature for 24 hr, then continued in the refrigerator for 5 to 6 days. Following this, the entire mixture was thoroughly agitated in a shaking machine for an hour, then passed through a Seitz filter. The pH of the extract after filtration was between 7.2 and 7.3, as determined by means of a glass-electrode pH meter. The stock extracts were kept in the refrigerator for ready use upon proper dilution.

Antigen was prepared in the same manner from larvae obtained from cultures, as described above.

Results. Experiment I. This experiment was undertaken to compare the number of worms recovered from the intestines of vaccinated and control rats 12 days after infection. The results are summarized in Chart 1. Eighteen rats were divided into groups (I-A, I-B, I-C, II-A, II-B, and II-C) according to the type of antigen, the number of injections of the antigen, and the time when the antigen was given. Each of these rats was vaccinated by the following procedure: 2 cc of antigen of 1:200 dilution (per 100 g

TABLE I.

Number of Larvæ from Lungs, and Adult Worms from Intestines, Recovered from Vaccinated and Control Rats 27 Days after Infection with 1,200 Larvæ of *Nippostrongylus Muris.*

Group	Rat No.	No. injections of antigen given	Days after vaccination	Larvæ found in one lung	Worms found in intestines	
					Male	Female
I. Worm Antigen						
A—	1	single	20	14	14	1
	2	"	20	20	18	0
	Mean	—	—	17	16	0.5
B—	3	3	20	18	10	0
	4	"	20	15	16	1
	Mean	—	—	16.5	13	0.5
C—	5	single	same	10	19	25
	6	"	"	7	11	32
	Mean	—	—	8.5	15	28.5
II. Larva Antigen						
A—	7	single	20	12	13	0
	8	"	20	14	15	0
	Mean	—	—	13	14	0
B—	9	3	20	22	13	1
	10	"	20	12	10	1
	Mean	—	—	17	11.5	1
C—	11	single	same	8	17	20
	12	"	"	11	9	17
	Mean	—	—	9.5	13	18.5
Controls	13			1	10	4
	14			0	6	7
	Mean			0.5	8	5.5

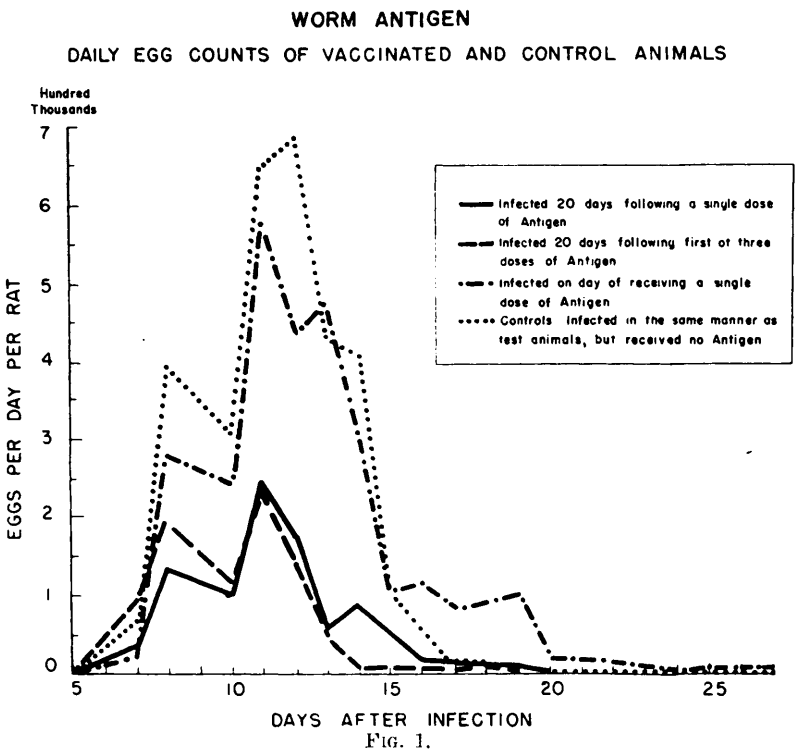
WORM ANTIGEN
DAILY EGG COUNTS OF VACCINATED AND CONTROL ANIMALS
Hundred Thousands
EGGS PER DAY PER RAT
DAYS AFTER INFECTION
Infected 20 days following a single dose of Antigen
Infected 20 days following first of three doses of Antigen
Infected on day of receiving a single dose of Antigen
Controls Infected in the same manner as test animals, but received no Antigen

FIG. 1.

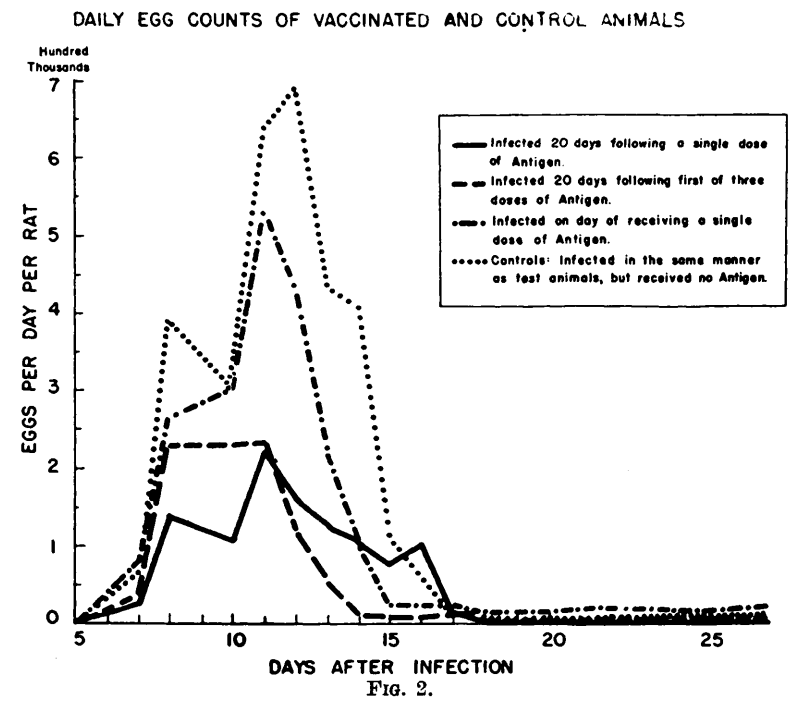
LARVAE ANTIGEN
DAILY EGG COUNTS OF VACCINATED AND CONTROL ANIMALS
Hundred Thousands
EGGS PER DAY PER RAT
DAYS AFTER INFECTION
Infected 20 days following a single dose of Antigen.
Infected 20 days following first of three doses of Antigen.
Infected on day of receiving a single dose of Antigen.
Controls: Infected in the same manner as test animals, but received no Antigen.

FIG. 2.

rat body weight) was administered subcutaneously. Infection was carried out with 1,200 *N. muris* larvae. Post mortem determinations revealed that vaccinated rats, particularly those given antigens 20 days before infection, yielded considerably fewer adult worms than did the controls. Expressed as percentages of the number of larvae given in the original infection, the average yields by the vaccinated and control groups were, respectively: Groups I-A, 27.11%; I-B, 30.35%; and I-C, 46.67%. Groups II-A, 24.03%; II-B, 22.35%; and II-C, 51.33%. Controls, 58.84%. (See Chart 1).

These data seem to imply that in the vaccinated rats, a greater proportion of the parasites failed to complete their development and migration to the intestines at the usual time, than in the normal rats.

Experiment II. This study was devised to compare the egg production and the number of larvae and adult worms recovered from the lungs and intestines of the vaccinated and control rats 27 days after infection. The results are summarized in Table I, shown graphically in Fig. 1 and 2. Fourteen rats were vaccinated and divided according to the methods used in Experiment I. The vaccinated and control animals, simultaneously given test infections of 1,200 larvae of *N. muris,* had total 24-hr egg counts made on them from the seventh to the twenty-seventh days, inclusive, after infection. These counts seem to indicate that vaccinated rats in all groups produced considerably fewer eggs during the period between the 10th and 13th days than did the control animals. Average daily outputs for this period were: I-A, 245,000; I-B, 244,000; and I-C, 576,500. II-A, 224,000; II-B, 253,000; and II-C, 538,000. In the controls, on the other hand, the egg counts averaged 688,500 for the period in question. The graphs of the average daily egg output over the entire period for all the animals are shown in Fig. 1 and 2.

On the 27th day all the rats, including the controls, were autopsied; the intestinal worms and larvae in the lungs were counted. At this time the egg counts had fallen to a relatively low level. Post mortem determinations revealed:

(a) Higher average numbers of larvae in the lungs of the vaccinated than in the control animals, as given below: Groups I-A, 17; I-B, 16.5; and I-C, 8.5. Groups II-A, 13; II-B, 17; and II-C, 9.5. In the control animals there was only 0.5. (Table I).

(b) More male than female worms were found in the intestines of rats in groups I-A, I-B, II-A, and II-B; more immature female than immature male worms in rats of groups I-C and II-C than in the control animals. The average numbers of worms found from different groups of animals were as follows: I-A, male 16 and female 0.5; I-B, male 13 and female 0.5; and I-C, male 15 and female 28.5. I-A, male 14 and female 0; II-B, male 11.5 and female 1; II-C, male 13 and female 18.5. In the controls there were male 8 and female 5.5 (Table I).

This sequence of events seems to suggest that the worms had not been adversely affected permanently during their sojourn in the lungs and intestines of the vaccinated rats (I-C and II-C) due to the slowness in response to the antigenic stimulation on the part of the host, but had merely been inhibited in their development. The larger number of larvae in the lungs, and the smaller number of adult female worms in the intestines and low egg production in groups I-A, I-B, II-A, and II-B, may be explained by the early loss of worms in the skin, lungs, and intestines.[5]

Conclusions and Summary. Results obtained from this series of experiments seem to warrant the following conclusions: 1. Immunity can be engendered to a marked degree in pied stock rats against *Nippostrongylus muris* after vaccination with antigens prepared by extracting the homologous worms. 2. Immunization of pied stock rats with these two types of "improved" antigens precludes any possibility of the condition described as "premunition", as in the case of the results

[5] Taliaferro, W. H., and Sarles, M. P., *J. Inf. Dis.*, 1939, **64**, 157.

[6] Sarles, M. P., and Taliaferro, W. H., *J. Inf. Dis.*, 1936, **59**, 207.

[7] Taliaferro, W. H., and Sarles, M. P., *J. Inf. Dis.*, 1942, **71**, 69.

obtained by a previous investigator.[1,2] 3. The immunity produced by this method is effective against the lung and intestinal phases, and possibly the skin phase, of the parasite, in the same manner as those reported by previous investigators after active (by prior infection) and passive immunization against this parasite.[5,6,7]

14028 P

Use of Chicken Serum in the Species and Type Identification of Neisseria.*

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The Commission on Meningococcal Meningitis, of the Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, has been concerned with the problem of identifying and subclassifying meningococci in connection with the occurrence of meningococcic meningitis in the Army. Certain difficulties were encountered in the preparation and use of monovalent sera. Rabbits are extremely susceptible to the toxic effects of the meningococcus and small, carefully graduated doses must be employed. A long period of time is usually required and animals frequently react poorly. The sera obtained are not sharply specific and will commonly show group agglutinins with the other types. Furthermore, with the technics usually recommended[1] the agglutination reactions are difficult to interpret because of low titer differences and fine clumping which must be read with a magnifying glass.

It was decided to investigate the agglutinin response of other animals and of fowl. Five young dogs were given intravenous injections of large numbers of living meningococci of the various types once a week for a period of 6 weeks. Although they tolerated the toxic effect, sera taken during and after this period contained no agglutinins. On the other hand, 5 chickens treated in a like manner not only exhibited similar tolerance but furnished sera with highly specific agglutinins. This lead was followed with gratifying results, and a preliminary report of the procedure is herewith presented.

Ten chickens of the Rhode Island Red variety were injected with *N. intracellularis* (Groups I, II, Types II alpha and IV), and with *N. gonorrhoeae* at weekly intervals, using the wing vein. The schedule was so arranged that the chickens received at the beginning of the first and second weeks 2 billion cocci, the third and fourth 4 billion, and the fifth and sixth 6 billion. They were bled from the heart at the beginning of the fourth and seventh weeks, and the titer of the agglutinins in each serum was ascertained for each of the strains employed.

The agglutination technic adopted was a modification of that suggested by Noble.[2] The antigens were prepared by suspending the organisms from a 5- to 24-hr culture in a physiological saline solution containing 0.05% potassium cyanide and standardizing to match the No. 8 tube of the McFarland nephelometer. The potassium cyanide was added because it was thought that the resulting suspensions were more homogeneous and did not settle so rapidly. The chicken

* These investigations were aided through the Commission on Meningococcal Meningitis, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Division, Office of the Surgeon General, United States Army.

1 Branham, Sara E., *The Meningococcus. Diagnostic Procedures and Reagents*, 1st ed., Am. Pub. Health Assn., 1941, 60-84.

2 Noble, A., *J. Bact.*, 1927, **14**, 287.