CATEDRA DE PARASITOLOGIA Y PATOLOGIA INFECCIOSA
Catedrático Prof. Dr. M. CORDERO DEL CAMPILLO

STUDIES ON THE IMMUNOLOGICAL TOLERANCE TO Trichinella spiralis INFECTION IN RATS

By A. Martínez Fernández
M. Cordero del Campillo
B. Aller Gancedo

The lack of immunological response of a specific nature to an antigen means in parasitic infections that hosts afford the pathogenic activity of parasites.

Examples of this type of phenomenon have been described in several Trichostrongylidae by GIBSON (1962) and LÒPEZ and URQUHART (1967); in *Trichomonas foetus* by KERR and ROBERTSON (1954); in *Cysticercus bovis* by SOULSBY (1963) etc. This process is being exhaustively studied in *Nippostrongylus brasiliensis* infections in rats by JARRET *et al.* (1966, 1968b, 1968c) and by KASSAI and AITKEN (1967) and KASSAI (1968a, 1968b).

In mice infected with *T. spiralis*, immunological tolerance could not be achieved with antigenic stimulation of the animals at birth by either metabolic antigenes, sonicated larvae, adult worm antigenes or actual infection. EWERT and OLSON (1960), and EWERT (1961), BASS and OLSON (1965), OLSON and HILL (1966).

However, considering that rats react to *T. spiralis* infection with strong immunity (McKOY, 1931) and that each immunitary system can vary according to the type of host, even with the same parasite, we have

Paper presented to II International Trichinellosis Conference in Wroclaw, Poland. June 26 to 28, 1969.

carried out a series of experiments on the kinetics of experimental *T. spiralis* infection in rats, in order to clarify the peculiarities of *T. spiralis* infection during the intestinal period, with reference to the reactive state of the animals, both in primary and challenge infections.

MATERIALS AND METHODS

Wister albino rats bred in our laboratory were the experimental animals. The strain of *T. spiralis* was GM-1, registered in the WHO International Register, mantained in our laboratory by regular passages in rats. Larvae for infections were obtained by digesting infected rat muscle in acidified pepsin. Digested material was passed through sieves (320 on 40 microns) to remove debris, and larvae cleaned and selected by baermannization. Larvae were counted in McMaster slides and dosage adjusted to the desired concentration with 10 % gelatine. Throughout the period of counting and administration of the infective doses, the suspension of larvae was magnetically stirred.

Primary infection was produced in young animals of ages varying from birth up to 25 days old, by way of 300-700 gelatine/larvae in 0.5 ml. using either/or stomach-tube, intragastric or intraperitoneal injection. Reinfection was produced by 1,000 gelatine/larvae in 1.0 ml 27 days after primary infection. The number of adult *T. spiralis* in the intestine was determined by DENHAM's method (1968) in this case using 320 on 125 micron sieves.

The kinetics of the intestinal process in primary infection, as well as in reinfection, was determined by counting the number of adult worms in the intestine 4, 7, 14 and, in some cases, 21 days after the induced infection under consideration.

The number of encysted larvae was determined, where necessary, by peptic digestion of 10 g. specimens of minced carcase from each animal, or from a pool of the group.

The number of adult worms found in experimental and control groups were statistically analized in accordance with STUDENT's t-test, or variance analized, no significance being given to differences with a probability greater than 0.05.

Each experimental group was subdivided into smaller groups with a minimum of six animals whenever possible, for purposes of considering the results.

The development of the intestinal process of an experimental trichinellosis was considered to have the following characteristics in common:

The *intestinal settlement phase*, determined by the number of adult worms on the 4th day after infection, and evaluated by the difference between this figure and the number of larvae given by way of infection.

The *intestinal infection plateau*, determined by the number of adult worms found on the 7th day after infection.

The *elimination phase*, determined by the difference between the number of adult worms on the 7th and 14th days after infection.

Finally, the *remaining infection*, being the number of adult worms on the 14th or 21st days after infection.

RESULTS

Experiment 1.

49 animals born with a difference of 24 hours between litters, made up the experimental group. When 23 days old, 31 animals were infected with 500 larvae *per os*, the infection being suppressed on the 6th day after infection with 500 mg/kg of Methyridine. The next day, the elimination of intestinal adult worms was checked in 5 animals taken at random.

The reinfection to be studied was carried out 27 days after the primary infection with 1,000 larvae *per os*. At the same time, a control group of 18 animals of the same age (50 days) was infected. Groups of six animals, both experimental and control, were killed on days 4th, 7th and 14th *p. i.*

Results are summarized in table I.

A statiscally significant difference was found between the number of adult worms in the experimental animals as compared with the control group ($p < 0.05$).

The following facts appear significant; 1. Settlement loss in the experimental group reached 78 %, whereas in the control one it was only 28 %. 2. Intestinal infection plateau is lower in 83 % for experimental

animals *vis á vis* the control group. 3. Experimental animals were found free of infection sooner.

This difference in response showed that these experimental animals had reacted to reinfection with a certain immunity.

Experiment 2.

As in the 1 st experiment, we attempted to study the response of animals which had been subjected to a primary infection at an early age, when challenge took place.

7 groups with 18 animals in each, were selected, their ages ranging from 10 to 17 days. Six groups were infected at different ages and with different doses, as shown in Table II, one group being kept as control for reinfection, which took place 27 days later. All these animals were more than 37 days old at the time of challenge.

In Table II the details of primary infection and reinfection, the level of muscular parasitism produced by primary infection, and results of intestinal reinfection are summarized. As may be seen, some groups were treated with Maretin.

On the basis of the number of adult worms on the 7th day after infection, comparing the experimental and control groups, we confirmed that a succesful infection at an early age (12, 15 and 17 days) produces a notable protection against reinfection. On the 7th day *p. i.*, the difference in the number of adult worm was statistically significant ($p < 0.01$).

In this case also, over 90 % of administered larvae failed to settle in the intestine of animals suffering a normal infection (without any treatment), whilst in groups whose infection was curtailed, between 85 and 90 % failed to settle, the number of adult worms in both groups being compared to those which settled in the control group. Maretincurtailed infection produced also a statistically significant reduction ($p < 0.01$).

While the number of adult worms on the 7th day is greater in groups with curtailed infection than in the normally infected ones, no statistical difference was observed between them. For this reason we are unable to state that the protection given by total infection is greater than that obtained with curtailed infection.

Finally, intraperitoneal infection did not have any apparent effect on reinfection. There was no statiscal difference with control animals. In both groups, a typical self-cure phenomenon was observed.

Under the condition of this experiments, no tolerance to reinfection was observed.

Experiment 3.

In the light of previous experiments, it has been concluded that in young animals a moderate primary infection (1,700 larvae per g.) produces a notable resistance to reinfection, and that even a single intestinal infection, or this together with a slight muscular infection, produces resistance to reinfection. As it was quite possible that the initial dose and ages of animals had some effect on their reaction to reinfection, Experiment 2 was repeated with higher initial doses.

Two groups, one with 18 and other with 20 animals, were infected with 700 larvae *per os* at 19 and 16 days of age, respectively. 27 days after primary infection, they were reinfected with 1,000 larvae by the same route. Results appearing in Table III were obtained. Batches of mice were used to control the infection potential of larvae administered in reinfection, which was as expected.

The response to reinfection was one of marked immunity, as in previous experiments. However, digestion of 10 g. specimens from the carcase pool of each group gave a higher level of infection (an average of about 2,300 larvae per g.) than expected from the initial dose. This fact led us to believe that, for some reason, the initial infection had been more severe than normally, as if the animals had tolerated the primary infection, which facilitated the pathogenic action to the highest degree.

Immunological unresponsiveness was therefore suspected to a greater o lesser extent. New experiments were carried out in order to verify whether this phenomenon occurs in primary infection induced with light and heavy doses and, finally, to see whether it would continue in reinfection.

Experiment 4.

In order to find out whether a delay in self-cure occurs in young animals after primary infection, and whether this has any relation to larvae doses and animal age, some further experiments were carried out.

A group of 36 animals eighteen days old, were infected with 200 larvae *per os*, this group being subdivided into batches with five animals

each, which were killed on the 4th, 7th, 10th, 13th, 16th and 19th days after infection. A statistically equal number of adult worms were found up to the 13th day (96 adult worms). During the remaining days of the experiment, an equal number of adult worms (32.2) was also found. It would appear that partial selfcure took place between the 13th and 16th days, leaving a constant threshold.

In addition. 4 groups of 30 animals, whose ages were 50, 35, 27 and 15 days, respectively, were infected with 100 larvae *per os*, and killed in batches of 5 animals on the 8th, 10th, 12th, 14th and 18th days after infection. The relevant results appear in Table IV. From this experiment it was concluded that the animals behaved in different ways according to their age. The 50 and 35 days old rat were able to cure themselves of this slight infection to such an extent that the moment of self-cure was missed in the case of the 50 days old rats owed to the timing of the experiment. The 27 days old rats cured themselves later on, between the 14th and 18th day, whilst in the case of the 15 days old rats, this had not yet taken place on the 18th day.

We therefore concluded that tolerance to infection is not only a result of age, but it also depends on the degree of infection; a mild infection is easily overcome if animals are almost mature.

Experiment 5.

Several groups of animals were infected with varying doses. Infection intensity was ckecked in order to know whether primary infection is really tolerated, and whether initial doses have any influence on it.

At the same time, parallel groups were intraperitoneally infected.

First part. A group of 42 animals, aged 22-23 days, were infected *per os* in order to follow the progress of primary infection and also that of reinfection. A second group (12 days old animals) was infected *per os*, and a third one (24 days old rats) injected by the intreperitoneal route. All these animals were infected with 300-325 larve at the same time. After 27 days. they were reinfected prior to treatment with Methyridine, with a similar dose of 300-325 larvae *per os*. A group of control animal, whose ages ranged from 47 to 60 days, were also infected whith identical dose.

The progress of primary infection was checked in the 22-23 days old group by killing batches of 6 animals on the 4th, 8th, 12th and 16th days

after infection, an average of 172, 174, 192 and 148 adult worms being recovered, with a sex ratio (females: males) of 0.8, 1.2, 1.5 and 0.7 respectively. This means that even a moderate infection is tolerated without self-cure taking place at the normal time. By using variance analysis, no statistical difference between the number of adult worms found on the above mentioned days was observed ($F = 0.61$).

Results of the infection given in Table V show that those groups which underwent complete primary infection reacted with immunity to reinfection. whilst the control and the intraperitoneally infected ones responded with the self-cure phenomenon.

Second part. Two experimental groups were used, one of 18 days old animals, infected *per os*, and the other of 24 hours old animals infected by intraperitoneal injection. The level of infection was tested in the former group 5 days after infection. It was found an average of 440 adult worms. 27 days after primary infection, both groups were reinfected, as well as a control one, made up of 45 days old animals, with 600-650 larvae.

Experiment 6.

Three groups of experimental animals were simultaneously infected at 14, 8 and 6 days of age, the first two *per os*, and the third one by intragastric injection of anaesthetized animals with 700 larvae in 0.5 ml of gelatine.

Four days before reinfection, they were treated with Methyridine. 27 days after primary infection, reinfection was carried out with 1,000 larvae *per os*. At the same time, two control groups were infected, their ages being 35 and 21 days.

Results which appear in Table VI, show that the fall in the number of adult worms on the 14th day is not excessive, except for the 35 days old control group which reacted with the self-cure phenomenon. The 14 days old group tolerated reinfection only to a relative extent. There was no statistical difference in the number of adult worms on the 7th and 14th days ($p < 0.1$), although this was nearly the case.

The 8 days old experimental group reacted with absolute tolerance to reinfection. That cannot be atributed to age, as this was similar to that of the 35 days old control group when reinfection took place. No statistical differences were observed by variance analysis in the number of

adult worms on days 4, 7, 14 and 21 after reinfection ($F = 0.076$), that is to say, the populations were equal. This clearly demonstrates the existance of an immunological tolerance.

Group infected by intragastric injection when 6 days old, did not tolerate reinfection. In the first place, the initial population that settled was greater in 500 worms than that which appeared as an average in the 8 days old experimental group. Secondly, there was a significant statistical difference in the worm population on the 7th and 14th days after infection ($p < 0.05$). Thirdly, the encysted larvae level produced by primary infection, was quite a lot less: 2,000 fewer larvae per g. of carcase, than those found in the 8 days old experimental group, which tolerated infection.

The control groups reacted in a different way, although their only difference was in age. The 21 days old control group tolerated infection, in view of the fact that there was no statistically significant difference in the worm populations found on days 4, 7 and 14 after infection. Besides, the number of adult worm that settled (average = 516) was lower by 237 than the figure obtained for the 35 days old control group, although greater than the 8 days old experimental group tolerated.

Finally, the 35 days old control group —adult rats by the time of infection— reacted with self-cure, the statistical difference being $p < 0.01$ on the 7th and 14th days.

In conclusion, this experiment indicates that it is possible to provoke tolerance to reinfection as long as primary infection occurs at an early age and is strong enough. Besides, it made us to suspect that animals tolerated a primary infection, this being responsible for the strong muscular infection. Those intragastrically infected rats, did not reach such a high level of infection, as a result of which did not respond in the same way as the tolerant ones. The response of the 21 days old control group again indicated that primary infection at an age of less than 30 days can be tolerated, without prejudicing the acquisition of resistance to reinfection.

DISCUSSION

Rat immunity to *T. spiralis* is demonstrated in adult animals by a decrease in the number of parasites capable of settling and completing their endogenous cycle in reinfection.

The progress of a primary infection in adult rats can be considered

as a self-cure process of the same type as that shown in *Nippostrongylus brasiliensis* infection, taking into account the differences between these two nematodes. This statement would seem to be in agreement with DENHAM's definition (1968) of self-cure: «the expulsion from the intestine of nematodes if there is reasonable evidence that this expulsion is of an immune nature». We do not consider it neccesary to use a new term such as *«spontaneous cure* resulting from antigenic stimulation of the host, followed by resistance» (D. F. STEWART quoted by GORDON, 1968), a term preferred by CAMPBELL (1968) seeing that this is self-cure according to STOLL's original definition (1929). Finally, what takes place in sheep and rabbit trichostrongylosis and what occurs in *N. brasiliensis* infection in rats is not essentially different, as it was pointed out by SOULSBY (1966). It is for this reason that authors who study the kinetics of intestinal infection by *N. brasiliensis* call this self-cure (JARRET, W. F. H. *et al.*, 1968; JARRET, E. E. E. *et al.*, 1968a).

From the work of MCCOY (1931) and later authors, it is known that resistance to reinfection is much more marked in rats than in mice, and the fundamental fact that makes this evident is not the earlier beginning of self-cure, but the intensity of initial settlement loss. about 90 % or more of the administered dose.

The progress of *T. spiralis* infection in adult rats consists in: initial settlement loss, which may be up to 47 % (GOURSCH, 1949), a plateau of settled intestinal population, which remains constant up to the 9th day and then is dramatically lost between the 9th and the 12th days after infection, to be totally eliminated at about the 18th day *p. i.* (LARSH, 1963).

The statistical difference between the number of adult worms found on the 7th and the 14th days after infection can therefore be considered as a clear evidence of self-cure. This occurred in our experiments when adult rats were infected. On the other hand, a leck of statistical difference between the adult worms populations on the 7th and the 14th days can be considered an indication of tolerance.

Immunological unresponsiveness can be induced by submitting immunologically immature animals to an antigen, the continuance of this state depending upon the persistence of the antigen. At first sight it appears that this can be casily provoked with *T. spiralis*, because it is possible to infect new-born animals and because some form of antigenic stimulus remains in the animal. The experiments of OLSON *et al.* (*loc. cit.*) with mi-

ce failed to produce immunological unresponsiveness to reinfection, but viable infection of new-born mice produces resistance to reinfection.

The above mentioned authors only sensitized new born mice, but not through the complete suckling period in which the animals probably continue being immunologically unresponsive but, however, capable of overcoming primary infection. On the other hand, perhaps the explanation of these results lies in the inadequeacy of the intestinal antigenic stimuli, as they were unable to produce strong infection in any experiment. They attributed the failure of strong infection settlement to physiological differences in the gut of the newborn and adult mice (e. g. nutrition, intestinal elimination rate, surface area) and perhaps, we suggest, owing to the possible limiting effect of intestinal flora in newly born animals (STEFANSKI and PREZJALKOWSKI, 1964).

It can be deduced from our experiments that adult rats which are over 30 days old react to primary infection wit self-cure. Young animals, however, tolerated primary infection for longer that adult ones, and self-cure does not generally takes place on the 14th day *p. i.*

The reinfection response depends not only on the age of the animals subjected to the antigenic stimulus of primary infection, but also on the intensity of the same. If the stimulus is not very strong, there may be a response of marked resistance. whether the life cycle of the worm has been completed or terminated by means of anthelmintics. As can be seen in graph I (from experiment 5, part I) even in the case of tolerance to primary infection, there is a response of resistance to reinfection.

If initial antigenic stimulus of young animals is strong enough, giving rise to marked muscular infection, there may be a response of notable tolerance to reinfection (graph II, from experiment 6).

In a later work it will necessary to determine the limits of both age and intensity of initial antigenic stimulus within which tolerance to reinfection takes place. It will also be of interest to know the duration of tolerance to reinfection through the animal's life.

SUMMARY

The progress of intestinal infection of rats with *T. spiralis* was studied. Statistically significant differences between the numbers of adult *T. spiralis* found on the 7th and 14th days after infection are considered a sign of self-cure. This phenomenon occurs in immunologically adult animals which are more than 30 days old.

There is a certain tolerance to primary infection in young animals younger that 30 days, this being revealed by a considerable delay in the self-cure. Reaction to reinfection is, however, one of resistance (loss of about 90 % of administered larvae and earlier cure). If the primary infection stimulus in animals of less than 15 days is strong, with heavy muscular parasitism, reinfection 27 days later can be tolerated, without statistically significant fall in the number of intestinal adult worms on the 21st day *p. i.* Both age and infection intensity seem to condition the possibility of immunological tolerance to *T. spiralis* infection in rats.

RESUMEN

Se ha estudiado la marcha de la infestación intestinal de ratas con *T. spiralis*. Las diferencias estadísticamente significativas entre las cifras de *T. spiralis* adultos halladas en los días 7.º y 14.º después de la infestación, se consideran como signo de autocuración. Este fenómeno se presenta en animales de más de 30 días de edad, adultos desde el punto de vista inmunológico.

En animales jóvenes, de menos de treinta días de edad, se observa una cierta tolerancia ante la primo-infestación, revelada por la considerable dilación con que aparece el fenómeno de la autocuración. No obstante, la reacción ante la reinfestación es típica de resistencia (eliminación del 90 % de las larvas administradas, aproximadamente, y curación temprana). Si el estímulo de la infestación primaria, en animales de menos de quince días, es fuerte, con intenso parasitismo muscular, puede tolerarse la reinfestación realizada veintisiete días más tarde, sin significación estadística en el descenso del número de adultos intestinales hallados a los veintiún días *p. i.* Tanto la edad como la intensidad de la infestación, parecen condicionar la posibilidad de establecer la tolerancia inmunológica de las ratas a *T. spiralis*.

RESUME

On a étudié le cours de l'infestation intestinale causée par la *T. spiralis* dans des rates. Les différences statistiquement significatives entre les chiffres de *T. spiralis* adultes trouvées le 7ème et le 14ème jours après l'infestation, sont considérées comme un signe d'autoguérison. Ce phénomène se présente dans des animaux âgés de plus de 30 jours, qui sont des adultes sous le point de vue immunologique.

Dans des animaux âgés de moins de 30 jours on observe une certaine tolérance à la première infestation, révelée par le grand délai ou retardement qui accompagne le phénomène de l'autoguérison. Cependant, la réaction à la réinfestation est typique quant à la résistance (élimination d'environ un 90 % des larves administrées et une guérison rapide).

Si le stimulus de la première infestation, dans des animaux âgés de moins de 15 jours, est fort et avec un parasitime musculaire intense, la réinfestation effectuée 27 jours plus tard peut être tolérée sans aucune signification statistique dans la diminution du nombre d'adultes intestinaux trouvés après 21 jours *p. i.* L'âge, aussi bien que l'intensité de l'infestation, semblent conditionner la possibilité d'établir la tolérance immunologique des rates à la *T. spiralis*.

REFERENCES

Bass. G. K. and Olson, L. J. (1965).—*Trichinella spiralis* in newborn mice: Course of infection and effect on resistance to challenge. *J. Parasit* **51** : 640-644.

Campbell, W. C. (1968).—Effect of anti-inflamatory agents on spontaneous cure of *Trichinella* and *Trichuris* in mice. *J. Parasit.* **54** : 452-456.

Denham, D. A. (19668).—Immunity to *Trichinella spiralis*. III. The longevity of the intestinal phase of the infection in mice. *J. Helminth.*, **42** : 257-268.

Ewert. A. and Olson, L. J. (1960).—Immunological tolerance studies with mice and *Trichinella*. *J. Parasit.* **46** : 849-854.

Gibson, T. E. (1952).—The development of acquired resistance by sheep to infection with the nematode *Trichostrongylus axei*. *J. Helminth.*, **26** : 43-53.

Gordon, H. McL. (1968).—Self-cure reaction. *Proc. 3rd Int. Conf. World Ass. Adv. Vet. Parasit.* Lyon, 1967, 452-456.

Gursch, O. F. (1949).—Effects of digestion and refrigeration on the ability of *T. spiralis* to infect rats. *J. Parasit.*, **35** : 19-26.

Jarret, E. E. E., Jarret, W. F. H. and Urquhart, G. M. (1966).—Immunological unresponsiveness in adult rats to the nematode *Nippostrongylus brasiliensis* induced by infection in early life. *Nature*, **211** : 1310-1311.

———, ——— ———. *(1968a).—Quantitative studies on the* kinetics of stablishment and expulsion of intestinal nematode populations in susceptible and immune hosts. *Nippostrongylus brasiliensis* in the rat. *Parasitology*, **58** : 625-639.

———, ——— ———.(1968b).— Immunological unresponsiveness in *Nippostrongylus brasiliensis* infection. *Proc. 3rd Int. Conf. World Ass. Adv. Vet. Parasit.*, Lyon, 1967: 242-249.

———, ——— ———. (1968c).—Immunological unresponsiveness to helminth parasites. I. The pattern of *Nippostrongylus brasiliensis* infection in young rats. *Exp. Parasit.*, **23** : 151-160.

Jarret, W. F. H., Jarret, E. E. E., Miller, H. R. P. and Urquhart, G. M. (1968).—Quantitative studies on the mechanisms of self-cure in *Nippostrongylus brasiliensis* infections. *Proc. 3rd. Int. Conf. Worls Ass. Adv. Vet. Parasit.*, Lyon 1967, 191-198.

Kassai, T., and Aitken, I. D. (1967).—Induction of immunological tolerance in rats to *Nippostrongylus brasiliensis* infection. *Parasitology*, **57** : 304-418.

———. (1968a).—Immunological tolerance to *Nippostrongylus brasiliensis infection in rats. Proc. 3rd. Int. Conf. World Ass. Adv. Vet. Parasit.*, Lyon 1967, 250-358.

———. (1968b).—Examination of some phenomena concerning parasitic immunity in rat. *Nippostrongylus brasiliensis* model. *Parasit. Hung.* **1** : 37-56.

KERR, W. R. and ROBERTSON, M. (1954).—Passively and actively acquired antibodies for *Trichomonas foetus* in very young calves. *J. Hyg.*, **52** : 253-263.

LARSH, J. E. (1963).—Experimental trichinosis. *Adv. in Parasitology*, **1** : 213-286.

LOPEZ, V. and URQUHART, G. M. (1968).—The immune response of Merino sheep to *Heamonchus contortus* infection. *Proc. 3rd Int. Conf. World Ass. Adv. Vet. Parasit*, Lyon, 1967, 151-159.

MCCOY, O. R. (1931).—Immunity of rats to reinfection with *T. spiralis*. *Am. J. Hyg.*, **14** : 484-494.

OLSON, L. J. and EWERT, A. (1961).—Further studies on immunological tolerance with mice and *Trichinella*. *Tex. Repts. Biol. Med.*, **19** : 866-868.

——— and HILL, M. (1966).—Resistance of mice injected at birth with extract of adult *Trichinella spiralis* to subsequent infection. *J. Parasit.*, **52** : 821.

SOULSBY, E. J. L. (1963).—Immunological unresponsiveness to helminth infections in animals. *Proc. 17th World Vet. Congr.*, Hannover, 1963, vol. **1** : 761-767.

——— (1966).—The mechanism of immunity to gastrointestinal nematodes *Biology of Parasites*, pp. 225-276, Academic Press, London.

STEFANSKI, W. and PRZYJAIKOWSKI, Z. (1964).—L'influence de certaines bacteries sur l'établishement des trichines dans le tube digestif de la souris. *Bull. Acad. Vétér. France*, **3** : 131-134.

STOLL, N. R. (1929).—Studies with the strongyloid nematode *Haemonchus contortus*. I. Acquired resistance of hosts under natural reinfection conditions out-of-doors. *Am. J. Hyg.*, **10** : 384-418.

TABLE I

Groups	Average number of adult worms Days after reinfection		
	4th	7th	14th
Experimental of 23 days	210	116	0
Control	820	702	6.5

TABLE II

Groups Agés.	Number of larvae Primary infection.	Nr. of larvae. Reinfec.	Mean Nr. of worms. (Days p. i.)			Muscular infection. Larvae/gm.
			4th	7th	14th	
Experim., 17 days	500	1.000	25.0	18.0	0.0	1,738
Experim., 17 days	250	1.000	4.5	0.0	0.0	748
Experim., 17 days	1.000 (M)	1.000	70.0	51.0	0.0	172
Experim., 15 days	500 (M)	1.000	110.0	68.5	0.0	73.3
Experim., 12 days	500 (M)	500	8.0	2.5	3.5	1,760.0
Experim., 10 days	500 (I)	1.000	630.2	443.3	3.5	0.0
CONTROL	—	1.000	768.2	507.2	4.1	0.0

M = Groups treated with 50 mg/kg Ibw of Maretin on day 6th *p. i.*
I = Intraperitoneally infected group.

TABLE III

Groups	Mean number of adult worms (days after reinfection)			Muscular infection larvae/gm.
	4th	7th	14th	
Experimental, 19 days	17.5	3.5	0.0	2,340.0
Experimental, 16 days	10.6	4.5	4.0	2.333.3

TABLE IV

Experimental groups		Mean number of adult worms (days after infection)				
Age	weight	8th	10th	12th	14th	18th
50 days	200 g	15.8	3.1	1.0	1.1	1.0
35 days	120 g	57.6	45.8	29.8	7.4	2.4
35 days	47 g	45.0	43.2	39.4	24.8	5.5
27 days	32 g	46.2	43.2	35.6	64.4	40.2

TABLE V

Groups, age	Mean number of adult worms Days after reinfection (sex rate)		
1 st part	4th	7th	14th
Experimental of 22-23 days	14.5	3.5 (3.5)	0.5
Experimental of 12 days	10.0	3.0 (2.7)	0.0
Experimental of 24 hour (I)	100.0	80.0 (2.3)	0.5
Control	200.0 (3.5)	140.0 (4.3)	2.0 (0.6)
2 nd part			
Experimental of 18 days	412.0	380.0 (0.9)	365.0 (0,9)
Experimental of 24 Hour (I)	450.0	430.0 (1.3)	53.0 (1.0)
Control	—	430.0 (1.3)	98.0 (0.4)

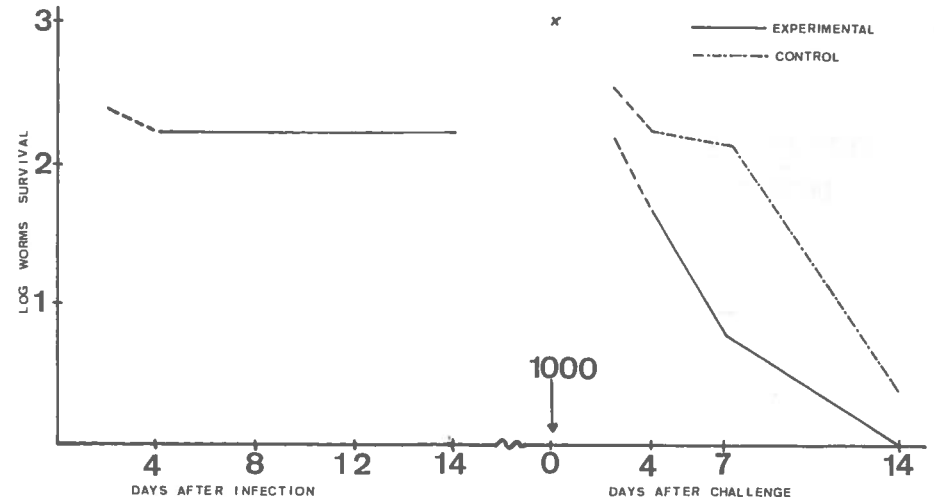
I = Group infected intraperitoneally

TABLE VI

Groups, age	Mean number of adult worms Days after reinfection (sex rate)				Muscular infection in larvae per grm.
	4th	7th	14th	21st	
Experimental of 14	450.0 (1.6)	388.0 (1.8)	146.0 (3.6)		3,652
Experimental of 8	393.5 (1.6)	393.3 (1.9)	350.0 (1.8)	205.5 (2.8)	3,077
Experimental of 6 (Ig)	860.0 (2.3)	844.0 (2.2)	253.5 (1.3)		792
Control of 21	620.0(1.6)	530.0 (1.4)	503.3 (2.8)		
Control of 35	820.0 (1.9)	754.0 (1.5)	50.0 (0.9)		

Ig = Group infected by intragastric injection

GRAPH 1



GRAPH 2