

THE INDUCTION OF SELF-CURE AND PROTECTION,
WITH SPECIAL REFERENCE TO EXPERIMENTAL
VACCINATION AGAINST *HÆMONCHUS*

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FOREWORD

BEING the concluding speaker on this symposium, I shall be allowed to pause a moment, not alone to express again my (and our) appreciation for the opportunity to share in the program, but to add my (and our) congratulations to Rice Institute for two reasons:

First, because of the new facilities for biology teaching and research. We have a way of thinking of physics, chemistry and biology together. But physics and chemistry are in the real heyday of productive activity at this interglacial moment in the so-called ascending spiral of civilization. Biology is only on the threshold, and yet eventually its voice must be heard above the others. Anthropocentric man is first of all a living organism, and from every biological angle needs more understanding of himself and his associated hundreds of thousands of species. So, as has been stated before on this weekend, these new facilities will help in significant and timely fashion to equip those who help to find the biological answers.

The second congratulation to Rice is for Dr. Chandler himself. This transplanted New Jerseyite, with a gift for finding biologically interesting and workable projects (imagine it—from bacteria in cutting compounds, through acquired immunities, to the anatomy of *Mesocestoides*, et cetera) has headed and catalyzed one of the—shall I say three?—most stimulating laboratories of parasitological research and training in this country. After extricating himself early from the momentarily unresolvable quagmire of hookworm in man, he has tenaciously, resourcefully and in good humor probed problems cognate to central puzzles in parasitology. The work of himself and his students has pushed back the limits that fenced in the field of host-parasite relations. And he has with lucidity presented his ideas to an international audience of young and old in the subject, to their profit in understanding some of the intricate interdigitations of the phenomenon of parasitism.

Not only Rice, Houston, and Texas each has a right to be proud of him, but the parasitological fraternity of the whole world—if I may make bold to speak for it—shares that pride and affection.

INTRODUCTION

It was my original plan in this paper to undertake an up-to-date review of the status of our knowledge of acquiring resistance, or acquiring immunity, or "self-cure and protection," against the gastro-intestinal nematodes, especially of large mammals. The review was not to have been oriented toward detailed immunologic phenomena such as discussed by my colleagues. It was rather to have been concerned with overall net effects of interplay between worm and host, in terms of long-run survival and well-being of both species. And it was to have had the aim of evaluating any encouraging hints or leads toward developing a more productive approach than the present orthodoxy, against such entities as hookworm, ascaris and whipworm parasitism in *Homo sapiens*.

If it is a reasonable and defensible calculation that these three together encompass two-thirds of the helminthic infections in man (Stoll, 1947), so it may be conservatively concluded they account for more than half the verminous miseries of mankind. For none of these three is there now a program to be mentioned in the same breath with that against malaria, of which you heard Thursday evening from Doctor Russell. Yet it would seem to be among the simplest of prophecies that the future will find means, besides the ones we practice today, of breaking the grip that these worms and man have on each other. Such means, one predicts, will involve bolstering the capacity of the host to self-cure and protect himself, through aiding him antigenically, nutritionally, and anthelmintically—each or all three, perhaps even as simply as by supplying him iodized salt or fluoridated water. What more reasonable then, than to inquire whether there is in present knowledge of worm-host relationships, or, if you will, just beneath the surface of such knowledge, something to contribute to a shortening of that future?

Instead, however, I shall present an experimental study that has no immediate interest in the human problem, but is concerned with vaccination against *Haemonchus contortus*. It was undertaken shortly before the war, interrupted by it, and has hitherto been reported only in abstract (Stoll, 1942). The post-war sequel of the closing of the Princeton laboratory of the Rockefeller Institute has so far made it inappropriate to explore the problem further.

A somewhat better picture of the purely *Haemonchus*-sheep interrelationship is available for orientation now than the one in 1941 when this vaccination study began. Let me refer you particularly to J. S. Andrews (1942) and Stewart (1950 a, b, 1953, 1955). As a preliminary to it may I review briefly the conception one had achieved by about the time it was initiated.

In 1927 a first experiment had been set up, which grouped what were

then four novel factors for furthering the understanding of how a nematode-large host system interacted, in this case *Haemonchus* and sheep. These factors were the availability of lambs reared helminth-free by Smith and Ring (1927), their infection established solely with *Haemonchus contortus*, their exposure to reinfection on a limited pasture area, and the visualization of their infections as a continuing process by means of egg counts—a sort of X-ray time-lag movie of their changing parasitic status. What came about can appropriately be re-stated at this time in the words used in my initial report before the Helminthological Society of Washington on 17 March, 1928. "This type of study resulted in the rather striking discovery of a self-cure phenomenon on the part of the hosts, evidently through elimination of the parasites. Moreover, animals which had gone to a self-cure were protected almost completely against reinfection. The possibility was advanced that perhaps certain special conditions of the experiment produced this effect, although there are numerous anomalous observations in the literature bearing not only *Haemonchus* infection, but on other gastro-intestinal nematodes as well (see for instance Herrick, March 1928, Am. J. Hyg.) which are most easily harmonized with this conception of the intervention of self-cure and protection on the part of the host. The more likely possibility, therefore, is that there occurs, in nematode infections of the type of *H. contortus*, the development of a resistance which is independent of an age resistance per se, and which acts to protect the host to the same end (and which may or may not have the same modus operandi) as obtains for typical bacterial infections." (Stoll, 1929a).

The term, "self-cure and protection" summarized both a logical and biological definition of what had happened. Some new expression had seemed called for, because immunity versus worms was not then in the language of parasitologists. Even my chief, Theobald Smith, expressed skepticism that the result of the experiment was due to acquired immunity. He cautioned that it was more apt to be due to an abnormal worm strain, or to lambs abnormal because bottle-fed.

It is helpful to date this period of change in our thinking, which in a way is well-marked by the fact that Taliaferro (1929) in his "The Immunology of Parasitic Infections," was able to cover the subject of "Acquired Immunity in Infections with Metazoan Parasites" in just 2 pages of a 400-odd page book.

Nowadays we take for granted a host will develop protective response to the presence of a worm species, and we would ask for evidence to prove exceptions. Then, however, there was little or no traffic in the idea. The reversal in regard to it is sufficiently distinct that it is as if professional parasitologists themselves underwent some sort of self-cure and protection against the *absence* of the idea.

Let me illustrate the earlier stage thrice. First illustration. With, among others, Ackert, Augustine, Cadbury, Grant, Hausheer, Hill, Oldt, G. C. and F. K. Payne, Riley, Schapiro, Sweet, and Tseng, I had been associated with Cort during a six-year period of hookworm studies in the Caribbean, China, Panama and Baltimore, in which none of us was led to propose the idea of an acquired immunity being involved in the host-parasite relations we observed. Nor in the great anti-hookworm programs of the quarter-century then ending had other workers interpreted puzzling phenomena they encountered, where *Necator* and *Ancylostoma* met man, as involving anything much more than such forces as *Drosophila* meets in a milk bottle. Thus Sawyer (1925) did not suggest that the problem of acquired immunity had anything to do with the "factors that influence the rate of increase of hookworm infection." Later, from his dog hookworm work, Herrick (1928) found increased resistance after reinfection as one of the factors involved in his results; and Fülleborn, Dios and Zuccarini (1928) and Fülleborn (1929), following hookworm studies in the Argentine, more frankly raised "the question of immunity." However, my friend Chandler seems not to have brought forward any such possibility in either his summary paper on hookworm in India (1928) or on "The rates of loss and acquisition of hookworms" (1929). Three years afterward "The temptation to speculate along these same lines on human hookworm disease is too tempting to resist" was noted by Cort (1932) and in 1940 Cort and Otto observed "although we know of no clear-cut demonstration of the presence of a specific immunity in man acquired through previous infection with hookworms, there is a considerable body of evidence that strongly suggests that host reactions must play an important role in the regulation of human hookworm infection." J. M. Andrews (1942) re-emphasized these possibilities, but except for epidemiological implications, the first (and shall I say only?) evidence from man appears to be that furnished by Sheldon and Groover (1942), of Sarles' type precipitates in serum, which seemed so irregular as to be discouraging. There has not been much added since. The situation in relation to "immunity against canine hookworm disease" has been well summarized by Otto (1948).

Second illustration. Faust and Meleney (1924) in their classical monograph on schistosomiasis japonica mentioned in passing, but omitted from the chapter reference list, the article by Fujinami (1916) on "erworbene Immunität." This summary in German of Fujinami's striking experiment and post mortem findings on the development of acquired immunity in the horse against *Schistosoma japonicum*, constitutes the first competent evidence encountered on the subject in relation to typical worm parasites.

Third illustration. Sandground (1928) in "some studies on susceptibility, resistance and acquired immunity to infection with *Strongyloides stercoralis* in dogs and cats" ended his conclusions by affirming that the devel-

opment of the immunity he observed "is an index of the biological *mal-adjustment* of the parasite to the host." (Italics mine). This point of view was further set forth in a review article (Sandground, 1929).

Perhaps one should add a fourth illustration—the neglected experiments of Ducas (1921) on *Trichinella spiralis*.

In such a climate it seemed prudent to me (Stoll, 1928, 1929b) to follow Stiles wise rule for taxonomy, that if you've found something and cannot be sure of its identity, give it a new name. His thesis was that it is preferable to have an extra synonym than to cover too much territory with one nomen.

To what degree are "acquired immunity" and "self-cure and protection" synonymous? Stewart in Australia has particularly applied himself to resolving the question for the *Haemonchus*-sheep system with the aid of complement-fixation studies, and if you are interested in it, his two papers on "The Nature of the 'Self-Cure' Phenomenon" (1953, 1955) become required reading. In any event self-cure and acquired immunity now exhibit so many features in common that we are not apt to be led astray if we use the expressions somewhat interchangeably.

To return to the Princeton sheep and *Haemonchus*. It was possible to confirm the type of results of the 1927 field study in a new reinfection experiment the following season. Four sheep, aged one and two years, were placed in a fenced one-quarter-acre pasture enclosure. One, lightly infected, seeded the area with *Haemonchus* eggs, and all four became subject to re-infection as they grazed.

You recall that *Haemonchus contortus* has a life cycle substantially like hookworm, with the exception that infection of the host results not from skin penetration by the infective third-stage larvae, but by their ingestion on forage, and that there is no wandering in the adult host, but mature parasitic development on the mucosa of the abomasum, the fourth or true stomach of ruminants. Damage from this parasite is referable to the loss of blood resulting from the feeding habits of the worms (Fourie, 1931; Martin and Ross, 1934; J. S. Andrews, 1942).

In these observations net damage to the host is attested by the well-being or death of the sheep. Increase or decrease of the worm population is inferred from the rise and fall of the egg count, with final autopsy checks.

In the 1928 experiment (Stoll, 1940a), one animal (previously tested in an indoor reinfection experiment) showed little rise in egg count. The other three, with a previous history of just-detectable *Haemonchus* infection, were now subjected to serious reinfection for the first time. One of these exhibited no substantial egg count, another developed an acute infection but became immunized and discharged it, and the last, after a fulminating infection with almost the theoretical additive curve of in-

creased egg output, died with a worm load of over 40,000 *Haemonchus*. No other worm species was involved.

These results did two things. They attested the pathogenicity of the worm strain; and, pertinent to today's consideration, they epitomized what, in retrospect, might be labelled characteristic reactions of pure *Haemonchus* infections as observed in sheep.

The later history of the three animals surviving the 1928 season's exposure to reinfection is equally instructive. They were introduced to a regular pasture flock of sheep which contained numerous species common in New Jersey. Besides a predominant *Haemonchus* infestation there were present gastro-intestinal nematodes of the genera *Bunostomum*, *Capillaria*, *Chabertia*, *Cooperia*, *Nematodirus*, *Oesophagostomum*, *Ostertagia*, *Strongyloides*, *Trichostrongylus* and *Trichuris*, as well as lungworms and cestodes. Each of the three animals was followed until its death, none, of course, ever receiving anthelmintic treatment.

One ewe, on pasture demonstrated to be heavily infested through the fact that haemonchosis developed in susceptible animals, withstood two seasons. Each spring she showed some rise in egg count, the first season during the period a lamb was suckling, the next in the couple of months before a lamb was born. During the heaviest reinfection periods, summer and fall, she showed no rise the first season, but developed an overwhelming infection at the end of the second season and at death was found to harbor about 7,500 worms, of which approximately 7,000 were *Haemonchus*. On its face this looks like poor protection, and that may be true. However, this ewe had been twice injured by dogs, in one case receiving a broken leg, in the other severe head injuries including the loss of an ear. At autopsy it was found that the head injury had disturbed the set of her jaw so that the teeth did not meet properly. Thus a ridge of high, sharp points developed which eventually prevented rumination. Diagnosis: Emaciation due to food deprivation, with secondary haemonchosis. This would be a perfect case for Whitlock's (1949) thesis.

In a ram there were two subsequent egg count rises, one evidently due to the drain of breeding the flock, a characteristic result with rams. The net result of reinfection in seven successive pasture years, following his first *Haemonchus* exposure, was good, with no sign of essential damage. Then, challenged with a single dose of a quarter million *Haemonchus* larvae (about 4,500/kg), there was no resulting change in egg count; when killed a few months later this ram showed about 500 worms, counting all species. He was to be regarded as adequately immune to *Haemonchus*.

The last animal, a ewe, season after season underwent pasture reinfection conditions with so little infection acquired as to fail sometimes to show a single egg on floatation examination. Year after year she produced vigorous lambs, once a single, twins five times, triplets once. Near the

end of her 8th helminthic reinfection season she developed an acute case of hemorrhagic septicemia (bacteriologically confirmed), and died in a few days after being discovered ill. At autopsy less than 50 worms, involving several species, were found.

It was, of course, possible to repeat the observation on self-cure and protection, or oppositely, a fatal result, with known doses of larvae in indoor experiments.

We had, then, under conditions of exposure to infection on pasture or indoors, sheep hosts undergoing self-cure and protection, against a pathogenic strain of *Haemonchus contortus* capable of producing fatalities; furthermore, these immunized animals could successfully stand exposure to the mixed nematode species on New Jersey pastures year in and year out without treatment (Stoll, 1936a, 1940b).

Studies around the world on ovine and bovine gastro-intestinal nematodes have been made mostly on hosts exposed to just such mixed infections, usually with *Haemonchus* and one or two other species dominating the picture. In trying to untangle the complex problem thus presented by the sheep host reacting to numerous worm species more or less simultaneously, no one has shown more realism than Tetley (1949) in his masterly "Rhythms in nematode parasitism of sheep." Fruitful observations on all or part of the complex, primarily from the epidemiologic standpoint, have been made by many workers, including Ross (1932), Kauzal (1933), Gordon (1948, 1949, 1950), and Stewart (1950 d) in Australia; Taylor (1934 a, b), and Crofton (1955) in England; Morgan, Parnell and Rayski (1951) in Scotland; Swales (1940) in Canada; and Marsh (1936), Hawkins et al. (1944), Whitlock (1949, 1955), and Levine et al. (1956) in this country. Also by Roberts, O'Sullivan and Riek (1952) on cattle in Australia.

Eventually, of course, the mosaic of protective mechanisms in the domestic ruminants will need to be patiently unravelled through study of individual worm-host systems. There are already a number of positive experiments indicating specific immune responses to other species than *H. contortus*. In addition such responses may be allowably inferred from certain investigations designed primarily to improve animal husbandry, to demonstrate life histories, or to test pathogenicity of given species with huge challenge infections. The impact of some of the latter on the unprepared host suggest a resemblance to the impact of an automobile driven at 60 miles an hour when it is ordinarily in operation at a reasonable fraction of such speed, unless and until both driver and car are conditioned to potentially cataclysmic events. We would be in error to believe there are any host resistances that cannot be broken, including potential immunities before they get into gear. Learning to elicit im-

munities may require more subtlety and difficulty than to break them. If, however, they exist, biological interest in the host will continue to encourage their discovery.

It should be added ruminants are not always available, or easy, to study under adequately controlled experimental conditions. Nevertheless, as indicated, a view is emerging of specific acquired immunities in ruminants resulting from the parasitism of members of many of the genera earlier mentioned. They are illustrated, for instance, in the observations of Mayhew (1940), Sprent (1946), and Lucker and Neumayer (1946) for *Bunostomum*; Ross and Kauzal (1933), and Threlkeld (1948) for *Chabertia*; Andrews (1939 b) for *Cooperia*; Tetley (1935), Kauzal (1937), Kates and Turner (1953), and Herlich (1954) for *Nematodirus*; Mayhew (1940), Andrews and Maldonado (1943), and Goldberg (1951) for *Oesophagostomum*; Threlkeld and Downing (1936), Stewart, Miller and Douglas (1937), and Porter and Cauthen (1946) for *Ostertagia*; Vegors (1954), and Turner (1956) for *Strongyloides*; Mönnig (1927), Andrews (1939 a), Stewart, (1950 c), Gibson (1952), and Stewart and Gordon (1953) for *Trichostrongylus*; and, perhaps also to be included, Seddon (1931) and others for *Moniezia expansa*. *Capillaria* and *Trichuris* spp. appear to be undefined in this relation.

In my own work all attempts continued to fail to induce a state of resistance to *Haemonchus* or to diagnose its presence, by means other than reinfection itself. This experience with sheep thus did not parallel that of Chandler (1932) in measurably protecting rats against *Nippostrongylus muris*; or of Sheldon (1937) in similarly protecting rats against *Strongyloides ratti* by subcutaneous injections of infective larvae heat-killed at 55-58°C.; or of Watt (1943) inducing some immunity in rats against *N. muris* with Seitz-filtered extracts from dried larval or adult worms; or of McCoy (1935) in partially protecting rats against *Trichinella spiralis* by intraperitoneal injections of heat-killed larvae; or of Spindler (1937) inducing some resistance to intestinal trichinosis in small laboratory animals "by feeding metabolic products of encysted trichinae."

Nor could I convincingly demonstrate Sarles' (1938) precipitates forming at the oral or other apertures of exsheathed infective *Haemonchus* larvae in serum from immune or hyperimmune sheep, although such precipitates did form in the serum of guinea pigs 25 days after receiving *Haemonchus* larvae intraperitoneally. Such precipitates also formed in the serum of calves, especially if they were *Haemonchus*-positive. Hawkins and Cole (1945) have since reported such precipitates forming in the serum of sheep with mixed nematode infections. Stewart's (1950 a) complement fixation test was still a long way off.

Nor could I observe any immobilization or debilitating effects on in-

fective *Haemonchus* larvae immersed in serum from immune sheep, even though no precipitates were formed. Nor, of course, did parenterally administered extracts made from larval or adult *Haemonchus* result in any protective immunizing effect against infection in sheep. Later Stewart (1950 b) also found these fruitless. Mayhew (1944, 1949 b) has reported similar negative results in tests with *Haemonchus* in the bovine host, although he had earlier been able to develop immunity in calves by oral infection with live larvae (1941), and later to confirm it (Mayhew, 1953).

May I digress a moment to note that in 1928 it had been possible also to show that self-cure and protection was not a function alone of the blood-sucking *Haemonchus*, but could be demonstrated (Stoll, 1932), under conditions of natural reinfection in *Oryctolagus cuniculus*, the domestic rabbit, against *Trichostrongylus calcaratus*. A later investigation by Sarles (1932) indicated that this worm-host system was amenable to controlled experimental results that had many of the aspects of the overall picture in sheep. This was particularly gratifying at the time because Sarles came to Princeton in skeptical mood about the reaction, his work on dog hookworm in Baltimore having led him rather to the conclusion that age immunity was the significant factor. Indeed, this skepticism held up to the next to the last measured worm dose he gave his first rabbit prior to its reacting against the worms, the rabbit having shown unmodified susceptibility up to then. It is such a result and the somewhat parallel experience of Otto, Kerr and Landsberg (1937) with the dog hookworm, that makes one wonder whether Beaver's observation on the apparent failure to immunize a human volunteer against hookworm may not have stopped one or two immunizing doses too soon (Beaver, 1945).

For more information on the experimental work of this period and later, which engaged the attention of a number of investigators, and with special reference to the nematode parasitisms of small mammals, I refer you to such reviews as those in Chandler (1937, 1948), Clapham (1933), Culbertson (1938), Taliaferro (1940, 1943), and Wetzel (1952).

The bulk of this work was indicating that while acquired immunities could be secured against nematodes, it had been successful primarily as a result of the process of infection or reinfection. Against nematodes vaccination procedures had not developed much promise. At this point, then, it seemed probable that the protective immunizing effect was being produced in the host against the secretions and excretions of living worms, as the current studies of Thorson (1953, 1954) with *Nippostrongylus muris*, and Campbell (1955), Chute (1956), and Chipman (1957) with *Trichinella spiralis* have now demonstrated for those two species.

But if secretions and excretions of the living worms were to be em-

ployed, how make them available in useful quantities as a vaccine? The possibility that a vaccine (in my definition, an inoculable immunizing agent) might be had in the accumulated metabolic products of the worms was one of the factors that lent encouragement to attempting the in vitro culture of the parasitic stages of *Haemonchus*. This Glaser and Stoll (1938) were able to carry only part way. The axenic work did, however, among other things, give practice in the sterilizing or axenizing of normally-grown infective *Haemonchus* larvae (Glaser and Stoll, 1940). Indeed, producing such larvae that had been obtained from fecal cultures and subsequently rendered bacteria-free to the degree that several hundreds or thousands in a broth tube sterility test would fail to show any contaminative growth, was turned into a routine laboratory procedure. The availability of this new biological reagent, namely bacteria-free infective larvae, permitted the final demonstration that *Haemonchus contortus* does not wander in the body of the sheep host (Stoll, 1943). And this led naturally to the next idea, that such bacteria-free, non-wandering *Haemonchus*, might have the capacity to elicit a host reaction favorable to protection against similar parasites later reaching the host by the normal oral route.

The remainder of my remarks are concerned essentially with a partial test of that idea. They can appropriately be introduced in the recent words of Chandler (1953), "If functional immunity is due, even if only in part, to reaction against metabolic products of parasites, it would seem that investigation of the possibilities of immunization by the use of metabolic products might be a fruitful field of investigation." In these experiments bacteria-free third-stage larvae of *Haemonchus contortus*, that do not wander in the usual nematode meaning, are emplaced and allowed to deliver metabolites as living organisms parenterally, for an ultimate effect brought to bear at an enteral target site.

MATERIAL AND METHODS

The sheep hosts here reported were taken from their dams and bottle-raised (Smith and Ring, 1927). Reared indoors they were demonstrated to be helminth-free by repeated floatation examinations, and by Baermann isolations of fecal cultures, up to the time of entering the experiments. The two bull calves had nursed from their dams for two days, and then were weaned and fed milk from a pail. No helminth eggs were detected in either of them until one became positive for *Haemonchus* in the course of the experiment.

Live larvae for parenteral injection and oral test were cultured from fecal pellets of seed animals (sheep) with pure *H. contortus* infections. Oral test doses of larvae were sheathed infective 3rd stages, washed, and

administered by pipette in a few ml. of tap water to the back of the mouth held open by an attendant.

Larvae for injection were similarly grown, then exsheathed and sterilized by the method of Glaser and Stoll (1940). They were demonstrably bacteria-free before use. Injections were in a few ml. of tap water. Subcutaneous injections were usually given under the skin in the wool-free areas on the inner aspects of the hind legs near the groin, half the dose on each side.

On a given day and in a given experiment, recipients of challenge doses received fair samples from a single pool of sheathed larvae, and those injected received fair samples from a single pool of sterile exsheathed larvae.

For occult blood tests of the feces the method of Thevenon and Rolland was employed, as described by Arnaud (1932) in his study of hookworm-infected natives near Leopoldville, Belgian Congo. This makes use of an alcoholic solution of pyramidon (amidopyrine) with a few drops of glacial acetic acid and hydrogen peroxide. The violet color developed varies in intensity with the amount of blood present. The "unknown" tested was a standard amount of the aqueous fecal suspension prepared for the floatation examination for *Haemonchus* eggs.

Jugular blood drawn into oxalated tubes, or, in anemic animals, blood from the ear obtained by needle prick, was used for hemoglobin determinations (Talquist) and stained smears (Wright's).

Dilution and floatation egg counting methods have been earlier described (Stoll, 1930).

VACCINATION EXPERIMENTS WITH SHEEP

In three experiments designed to determine whether *Haemonchus* larvae administered by the IP (intraperitoneal) or SC (subcutaneous) routes would assist development of self-cure and protection against single challenge oral infections, 13 sheep were utilized. These had been born in early spring and, when given their oral test doses of normal, sheathed, infective *Haemonchus* larvae in Sept. to Feb., 4 were 7 months, 4 were 8-9 months, and 5 were 11-12 months old. The 3 rams, 2 wethers (castrates) and 8 ewes weighed 57 to 101 pounds (25.8 to 45.8 kg) each, and included 3 pair of twins (ram 881 and ewe 882; ram 890 and ewe 891; ewes 899 and 900).

Five animals received IP injections of live, exsheathed, bacteria-free 3rd stage *Haemonchus* larvae 7 to 11 weeks before oral test. All showed a successful resistant response to challenge infections, a response that contrasted strikingly with the fatal haemonchosis developing in 2 uninjected controls otherwise similarly handled. No greater resistance appeared in 2 of the animals given 4 injections spaced one week apart, than in the

3 that were given single IP injection of larvae. (In one of the former, ewe 897, the timing of the initial appearance of eggs in the feces gave evidence of entry into the rumen of at least part of the first and fourth injections). Each animal received as challenge a single oral dose of 10,000 to 15,000 larvae per kg. body weight. The IP injections totalled 3600 to 22,000 larvae per kg. body weight.

Five animals received single SC injections of larvae. Two injected 5 weeks before, one injected 9 weeks before, and one injected less than an hour before challenge infection, showed a successful resistant response, again in striking contrast to the severe but, in this instance, non-fatal haemonchosis that developed in the uninjected control No. 892, otherwise similarly handled. The fifth animal, a year-old pregnant ewe No. 891, received the SC injection 3 weeks before oral test, lambed prematurely 3 weeks after the test, and succumbed 3 weeks afterward. The SC injections were 3100 to 4100 larvae per kg. body weight, the single oral challenge doses 15,000 larvae per kg., and the animals a year old at test (except one at 7 months).

The other three animals were given no injections, but were challenged orally in a similar manner.

Following the challenge doses, of the 9 animals successfully protected after IP or SC larvae (that is, all but one of the 10 injected), 6 showed little or no blood loss, as measured either by hemoglobin readings of the circulating blood, or by OBT (occult blood tests) of the feces. In only two did the egg count exceed about one MEPD (million eggs per day), these two reaching, respectively, 2 MEPD, and 5 MEPD, both transiently.

These 6 animals that were best protected by the parenterally emplaced larvae comprised 2 rams and 4 ewes. Ewe 899 had not at any time been positive by OBT, her egg count had barely reached one MEPD, and when autopsied 12 weeks after challenge harbored only 279 *Haemonchus* adults, i.e., about 0.05 per cent of her test dose. This was the only one of the 4 coming to autopsy after parenteral injection of larvae which exhibited sign of the injection sites. Ewe 899 had received SC larvae once, 5 weeks before oral test. Now, 4 months after SC, there was at the site of injection in each groin a single subcutaneous "cyst" measuring about $1\frac{1}{2} \times 1 \times 1$ cm.; each had a thick capsule and caseous contents, indicating probable bacterial contamination at the time of injection.

Ram 890 (twin to ewe 891, the only injected animal that died) after becoming negative to a lightly positive OBT, and to an egg count that reached 5 MEPD on one examination, was killed 14 weeks after challenge. Only 3 *Haemonchus* could be found. This ram had received SC larvae once, one hour before oral test. At autopsy there was no sign of even the exact site of injection.

The second ram, 895, having scarcely exhibited sign of infection by

OBT, and with a transient egg count maximum of 2 MEPD for only one week, was released 2 months after challenge for sale in the war effort.

The other 3 ewes, Nos. 882 (twin of ram 881 mentioned below), 897 and 900 (twin of ewe 899 mentioned above), were returned to the pasture flock 2 to 4 months after challenge, this being January for 882 and 897, and June for 900. These 3 had not at any time since the oral test dose shown a distinctly positive OBT, and their egg counts had scarcely reached one MEPD.

The other 3 injected animals, IP wether 899, IP ram 881, and SC ewe 888, had shown considerable blood loss temporarily after the challenge doses, as indicated by lowered hemoglobins and OBT. However, the *Haemonchus* egg counts, considered by themselves, were neither very high nor prolonged. Their records were as follows:

Wether 889 had 4 IP injections at weekly intervals, and was challenged 4 weeks after the last. He became negative within 10 weeks after oral test, and remained so for 3 weeks both by OBT and egg count, one later examination showing about 4 MEPD. Then he exhibited a "relapse" by again showing a positive OBT and reaching about the same egg count as previously. This manifestation of renewed pathogenicity of residual (and temporarily retarded?) worms, or of a lapse of resistance, lasted all told about 5 weeks. It then ended by OBT and egg counts again becoming negative without intervention by the investigator. Wether 889 was killed a week later to determine whether *Haemonchus* still persisted in his abomasum. Not one worm was found nor was there any trace of the IP injection.

The possibility of contaminative reinfection here as the cause of the "relapse" is remote. Rigid sanitary precautions had been early established by Theobald Smith for routine cleaning of animal quarters at the Princeton laboratory of the Rockefeller Institute. That they were effective in these experiments with *Haemonchus* is attested, for instance, by a helminth-free wether of similar age having been maintained in the unit during this experiment for 16 weeks. He retained a helminth-free status as determined by failure to demonstrate nematode infection even by Baermann isolation of fecal cultures.

IP ram 881 developed the highest egg count, about 6 MEPD on one day, and more blood loss than any of the other injected animals (except ewe 891 that died). Nevertheless, by 8 weeks after challenge he had become negative by OBT, and so remained throughout the succeeding 14 months during which he was examined. Sixteen weeks after challenge, when a barely detectable egg count remained as the result of the challenge dose, this ram was tested with a succession of large oral doses of

infective *Haemonchus* larvae for a period of 16 weeks. On 26 occasions, at intervals of 2 to 7 days, he was given larvae at the rate of 14,000 to 30,000 per kg. body weight, until he had received 15 million larvae. Five days after this series 350 ml. of blood from the jugular (about 13 per cent of blood volume) was withdrawn; the next day he received 500,000 more larvae by mouth, and a week after that 10,500,000 additional in a single dose. Throughout the whole period of his getting these 26 million larvae, and for the 8 months thereafter that he was under examination, he remained untroubled by this rigorous *Haemonchus* assault. He showed a continuously negative OBT, was negative or nearly negative to floatation fecal examination, regained normal hemoglobin values after the setback due to his original challenge oral infection, and gained weight. He was thus demonstrated to be magnificently protected.

SC ewe 888's history of blood loss and egg count following challenge roughly paralleled that of ram 881, but was less pronounced. She was returned to pasture in January, 16 weeks after oral test, as having undergone self-cure.

There had been three fatalities as an immediate result of the experiments. Ewe 891, the 10th injected animal, has already been noted as a pregnant ewe lambing prematurely after an oral challenge infection 3 weeks after SC larval injection. She died 6 weeks after the oral test with a profound anemia when passing about 13 MEPD, and 15,270 *Haemonchus* were recovered from the abomasum at autopsy, i.e., 2.7 per cent of the oral test dose. There was no sign discovered of the SC injections she had received.

The other two fatalities were among the uninjected controls, following oral tests. Ewe 886 succumbed 25 days after receiving 15,000 larvae per kg. orally, and before becoming egg-count positive. Her worm count was 29,130 *Haemonchus*, i.e. 6.6 per cent of the oral test dose. Ewe 901 died 21 weeks after receiving 10,000 larvae per kg. orally, and after a continuously high egg count which exceeded 20 MEPD during most of the period. Her worm count was 9690 *Haemonchus*, i.e. 3.0 per cent of the oral test dose.

The third uninjected control, wether 892, did not succumb after receiving the test infection of 15,000 larvae per kg. orally, but showed typical severe haemonchosis. The egg count reached 14 MEPD the 7th week, and continued high, falling off less than half in the next 10 weeks. As with fatal cases there was a markedly lowered hemoglobin, and strongly positive OBT. By 20 weeks after the oral test the OBT had disappeared and did not reappear, although the egg count remained about 4 MEPD. Then placed in pasture (late June) the egg count fell sharply in about 6 weeks to 0.2 MEPD. When killed 3 months later this wether then had

only 22 *Haemonchus*, but had 15,200 *Trichostrongylus axei* and 120 *Oesophagostomum*. This was 41 weeks after the oral test dose indoors, of which the last 18 weeks had been under out-door conditions exposed to mixed species in pasture.

To summarize these three experiments, of the 10 sheep 7 to 12 months of age that were parenterally injected with live, exsheathed, bacteria-free 3rd stage *Haemonchus* larvae, 9 showed evidence of enhanced protection, 6 of them to a high degree, against the challenge of a single huge oral dose of larvae. (The 10th, yearling ewe 891, demonstrated no increased resistance, probably because of the complication of parturition 3 weeks after oral challenge.) Of these 9, the protection afforded to 6 was, as stated, striking, whether judged by blood hemoglobin values, OBT of the feces, egg counts, or the worm counts of the 2 killed for examination. In 3 other animals the protection afforded by the parenteral larval injections was less in degree, but nonetheless effective, one of the 3 autopsied 19 weeks after test being *Haemonchus*-free. In contrast, 3 uninjected controls of similar ages, and handled similarly throughout except for the omission of parenteral injections, developed severe cases of haemonchosis from which 2 died with post-mortem worm counts of 9690 and 29,130 *Haemonchus*, respectively, and the third survived only after a lengthy convalescence.

Of the 9 injected animals, besides the 3 killed for examination, one (ram 881) was not fazed later by a protracted series of oral doses that totaled 26 million larvae in 16 weeks, and 5 were returned to the pasture flock as self-cured. One of these latter, ram 895, was shortly sold and his later history is unknown. The 4 ewes were kept under observation during their initial pasture season, but were given only occasional fecal examinations. These indicated that their reaction to the mixed species on pasture, including *Haemonchus*, was not troublesome, and typical of animals that had undergone self-cure. Two of the ewes lambed the following season, i.e. after their first opportunity to be bred.

VACCINATION EXPERIMENT WITH CALVES

One experiment was performed to determine whether *Haemonchus* larvae of sheep origin administered parenterally to a calf would assist the development of self-cure and protection against a single, oral challenge infection.

Two Guernsey bull calves, S2120 born 25 April, and S2119 born 21 April (hereafter referred to as A and B), were brought to the laboratory 29 April, placed together in a large stall, and started on 2 pounds of dry milk and one pound of grain mixture per day, together with mixed hay. On 21 May they weighed, respectively, 87 and 113 pounds (39.4 and 51.2 kg.).

The milk was discontinued for both animals on 13 June, 23 days after SC injection of calf A. As this was 19 days before the challenge infections of both calves it is unlikely that the milk feedings played any role in later decreasing their parasitization by *Haemonchus*, such as was observed by Porter (1941).

On 21 May, the smaller calf A, then 26 days old, received subcutaneously 145,000 live, exsheathed, bacteria-free 3rd stage *Haemonchus* larvae of sheep origin, representing 3700 per kg. body weight. The larvae, in sterile tap water, were divided into 3 portions of 2 ml. each, for injection sites on the inner aspects of each hind leg, in the region of the groin, and at the right side of the neck. The injection sites showed nothing of interest and were difficult to identify when examined 12 and 22 days later. Calf B was left uninjected.

On 2 July, 6 weeks after the SC injection of A, each calf was given a dose of normal, sheathed, infective *Haemonchus* larvae of sheep origin, administered by pipette into the back of the mouth in about 8 ml. of water. The calves were now 10 weeks old and weighed, respectively, 127 and 165 lbs. (57.5 and 74.7 kg.), each having gained 46% over his weight on 21 May. Calf A received 875,000 larvae and calf B, the uninjected control 1,125,000, each infection representing 15,000 larvae per kg. body weight.

In large single oral doses of larvae given to sheep there is demonstrable some immediate loss of worms that fail to stay in the abomasum. This was also demonstrable for these calves. Fecal samples from both calves, secured 23 hours after oral administration of larvae, showed about 20 larvae per gm. of feces by salt floatation. All these recovered larvae were now exsheathed, and half of them showed activity. As an extra prevention against fortuitous re-ingestion of larvae from fecally-soiled bedding, the animals were transferred to a clean stall 24 hours after the oral dose.

The results of the experiment may be followed by reference to Fig. 1.

Hemoglobin. Talquist readings (dash line, upon circles) on both animals remained at 90-95% for a fortnight after the oral dose. In calf A they were reduced to 70-75% at the 4th week, and then rose again to 90-95% at the 8th week. In control B the readings went lower, to 60-65% in both the 4th and 5th weeks, and then rose to 80-85%.

Occult blood. For calf A all OBT were negative (open circles on base line) except for some "possibly positive" on days 13 to 29 after oral challenge. In control B the OBT were positive in increasing intensity on days 11 to 18 (solid line), stayed high with some variation until day 32, then fell off to become negative on day 37, and remained so. It will be noted in Fig. 1 that the period of 30% fall in hemoglobin in control B coincided with the period of greatest blood loss determined by OBT. Also that the

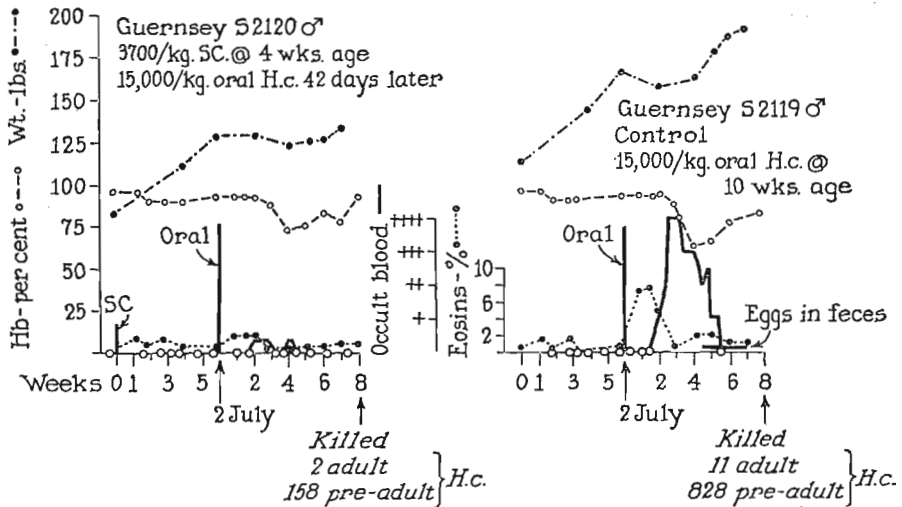


FIG. 1. Result of experimental vaccination of Guernsey bull calf S2120 by single subcutaneous injection of live, exsheathed, bacteria-free 3rd stage *Haemonchus contortus* larvae of sheep origin, and then challenged after 6 weeks with a single oral dose of sheathed infective *Haemonchus* larvae, as contrasted with uninjected control calf S2119 similarly tested. The comparative size of the SC injection (3700 larvae per kg. body weight) and of the oral challenge infections (15,000 larvae per kg. body weight) is indicated by the height of the respective vertical bars. The animals were of similar age, weighed 127 and 165 pounds (57.5 and 74.7 kg), respectively when challenged, and are referred to in the text as calf A and control B.

OBT became, and stayed, negative while eggs in the feces continued to appear for another 10 days.

Eosinophiles. In calf A throughout the experiment the eosinophiles (dotted line) did not rise above 2%. In control B this was true preceding the oral dose, but on days 6 and 11 after it they were at 7 and 7½%. Counts were on 200 leucocytes.

Fecal examinations for Haemonchus eggs. Both animals remained negative by floatation examination on weekly samples preceding the oral doses. The positive larval findings the day after the larval oral dose have been noted. Between days 7 and 54 of the post-oral-test period each animal received floatation examinations at 1 or 2-day intervals. For calf A these were uniformly negative in result. For control B they were positive on days 32 to 51 (negative 2 days before and after), shown with heavy line parallel to the abscissa in Fig. 1. Using the empirical formula of 20 times the bovine weight in pounds multiplied by the eggs per gram to represent the eggs per day (Stoll, 1936 b), the egg output per day of control B rose to 25,000 on the 36th day after oral infection, 50,000 on the 37th day, 100,000 on the 39th day, and was at 400,000 on the 41st to 46th

days. It then dropped 99% on the 48th, and was negative the 53rd day. While the absolute size of the counts was small, their numbers described the characteristic configuration of a self-cure curve.

Weights. The calves were weighed just before and at 2, 4, 5, 6 and 7 weeks after the oral test dose of larvae. As the dot-dash line in Fig. 1 shows, both temporarily failed to gain after the oral test, with apparent maximum losses of 3 lbs. for A and 7 for control B. By the end of the 7th week A was at 134 lbs., control B at 192 lbs., respectively, 105% and 116% of the pre-oral-test levels. The temporary damping of the weight curves is doubtless more significant in rapidly growing juvenile animals than the actual amount of change, when account is taken of the initial difference in weight of the two calves.

Autopsy findings. Calf A was killed 57 days after the oral test dose and was normal in appearance. Careful dissection of the subcutaneous areas of larval injection in the groin and neck sites failed to reveal even the exact places the inoculations were made. Thorough worm count of the abomasum showed 2 male adult *Haemonchus* and 158 immature worms about 2-3 mm long.

Control calf B was killed 54 days after the oral test dose and was likewise normal in appearance. Worm count of the abomasum revealed 2 male and 8 female adult *Haemonchus* and 828 immature 4th stages. One adult male was added by examination of the screened contents of the small intestine. Measured in physiological saline after refrigeration overnight the males were 10.3-13.0 mm., the females, all egg-bearing, 13.9-16.6 mm, and a fair sample of the 4th larval stages were 1.3-2.4 mm. long.

Despite the effort made to gain complete worm counts on the calves post-mortem, the fact that so many were pre-adult stages naturally jeopardized the accuracy of examination in a large ruminant, although I think in these instances it was more theoretical than real. Thus while less than one-fifth the number of worms were recovered from previously injected calf A killed 3 days later than control B, the conservative position is that this may not have been a significant difference, even though it is in the right direction.

The worms recovered, as well as the passing of *Haemonchus* eggs by calf B, gave direct proof that *Haemonchus* of sheep origin are infective for calves, although possessing a lower grade of infectivity for the bovine than for the ovine host. This is in accord with the findings of Ross (1931), who also showed that *Haemonchus* of bovine origin infect sheep, as did Stoll (1936 b.). If, however, the determination by Roberts, Turner and McKevevtt (1954) that ovine and bovine "strains" of *Haemonchus* represent different species is true locally, the possibility needs consideration that some fraction of the *Haemonchus* larvae of sheep origin used in the ex-

periment actually represented the bovine species capable of maintaining residence in sheep.

To summarize, of two Guernsey bull calves of equal age, calf A parenterally injected with live, exsheathed, bacteria-free 3rd stage *Haemonchus* larvae of sheep origin, evidenced protection against the challenge of a single huge oral dose of sheathed infective *Haemonchus* larvae of sheep origin administered 6 weeks later, as contrasted with uninjected control calf B similarly challenged. Calf A exhibited minor depression of hemoglobin and negligible occult blood in the feces in contrast to calf B, which showed greater decrease of hemoglobin, with occult blood in the feces for 25 days and in large amount. No *Haemonchus* eggs were detected in the feces of calf A following challenge, whereas control B was egg-count positive for 3 weeks. There was temporary damping of the weight curves in both animals. At autopsy 8 weeks after challenge there were found 160 *Haemonchus* in calf A, 839 in calf B. These differences, showing protection in the injected animal, were more striking in that Calf A at challenge weighed 127 pounds, control B 165 pounds.

REGARDING PARENTERALLY EMPLACED HAEMONCHUS LARVAE

The fact that parenterally-injected, live, exsheathed bacteria-free 3rd stage *Haemonchus* larvae do not wander in the usual nematode fashion has been earlier reported (Stoll, 1943). By indirect evidence, their inability to reach the abomasum was attested in the present experiments by consistently negative floatation examinations of feces for the presence of larvae or ova in the period from the time of injection to two weeks after the oral challenge in 9 animals. The 5 SC-infected sheep received 40 such examinations (3 to 11 each), all negative. In IP injections the hazard of inadvertently injecting directly into the rumen has been mentioned in the instance of ewe 897. In the 4 other animals, one of which received 4 IP injections, the others one IP each, all of 93 floatation examinations (14 to 28 per animal), were negative. By direct evidence, the larvae appear to be trapped at the injection site where they are able to survive only temporarily.

A measure of this period of survival of parenterally-emplaced larvae was furnished by 3 hitherto helminth-free sheep examined at 3, 6 and 12 days after IP injection of larvae. In wether H38, 63 weeks of age and killed 3 days after IP injection, the worms, entangled in small hyaline nodules observed chiefly on the omentum, were for the most part found alive. In wether H85, 21 weeks of age and killed 6 days after IP, a few larvae were observed to be still alive in such nodules, but most were inactive and probably dead. In ram H52, 70 weeks of age and killed 12 days after IP, the nodules had lost their hyaline character and were firm,

were difficult to examine in squeeze preparations under the microscope, appeared well vascularized, and the larvae within them were vacuolated. Peritoneal fluid and washings of the peritoneal cavity in these animals revealed no larvae.

Photomicrographs of a squeeze preparation of a nodule from H38 shows (contrary to an item in Stoll, 1943) that many of the larvae were ensheathed for the first parasitic ecdysis terminating the 3rd larval stage, and at least one larva, already exsheathed, evidenced some development into the 4th larval stage.

Confirmatory observations were made in ram J17, 63 weeks of age. This animal was given SC injections on the inner aspect of the right leg near the groin 7 days before, and similarly of the left leg 3 days before being killed. There was an infiltrated mass of reaction tissue in both sites, firmer and more consolidated in the 7-day area. Photomicrographs of squeeze preparations of both the 3-day and 7-day sites show numerous larvae ensheathed preparatory to the first parasitic ecdysis; on the left side, injected 3 days earlier, some of these larvae have the appearance of having been alive when photographed; on the right side, at 7 days, all are vacuolated and of flaccid appearance.

These observations are direct evidence that the bacteria-free, parenterally-injected worms remain alive for a few days, during which many achieve their first parasitic ecdysis, before being overwhelmed and killed by the local reaction. Ample opportunity is thus afforded for metabolites in the form of secretions and excretions of the worms while alive to be absorbed by the host.

CONCLUSION

Maurice C. Hall once wrote, "parasitism is not a static and fixed thing." No more, it should be added, is our conception of it. Self-cure and protection against worms did not begin three decades ago. What then began was the recognition, as widespread, of a force that Nature had been generating for untold time to help balance hosts in relation to their worms.

Mobilization by a host of the self-cure and protection mechanism, or of acquired immunity, through the stimulation furnished by specific chemical products of metabolism of a worm parasite, is capable in its ultimate manifestation of preventing residence within the host of even a single helminth producing those specific metabolites. Usually, as happens characteristically with other types of etiological agents as well, something less than complete resistance or extirpation of every parasite is the result. In either event, the capacity of responding to the presence of the living parasite is quickened, or enhanced in degree, as compared to a host that has lacked such a specific biological experience.

It is submitted that experimental vaccination against *Haemonchus* by

live, parenterally-emplaced, non-wandering larvae appears capable of assisting to provide that experience for both the ovine and bovine host.

In the general scheme of things vaccination against worms could conceivably make a place for itself, as have immunities acquired through the normal process of infection and reinfection, and as have nutrition, and anthelmintics, and genetics, although none of these, in present view, can alone take over control of worm-host systems for the total protection of the host.

Moreover, *Haemonchus* is not only gastro-intestinal nematode unable to migrate from a parenteral injection site. Bacteria-free, infective larval stages of *Haemonchus*, and other non-wandering species, may therefore be taken to represent new biological reagents with which to explore further some of the phenomena of immunization against worms.

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