only a small number of eggs are deposited by females, and the parasite is discarded with the sloughed skin each time the host molts. Clearly, further comparison between wild and domestic populations is desirable to determine ecological factors that influence host-parasite relationships under each of these widely divergent situations.

In view of these facts, it may be postulated that the sustaining hosts of *Ophionyssus natricis* in nature are reptiles that repeatedly visit areas that are compatible with the ecological requirements of the nonfeeding stages of the mites. Only those reptiles with small home ranges that are either abundant or gregarious will satisfy these conditions.

With the discovery of *Ophionyssus natricis* in nature, the opportunity is also presented to investigate the natural ecology of hemorrhagic septicemia in snakes, a disease entity that, like *O. natricis*, has heretofore been studied only in the laboratory (*5*).

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5. This investigation was supported by the Microbiology Branch of the Office of the Naval Research under contract No. NONR-1809(00). The opinions and assertions contained herein are mine and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large. I am grateful to Harry Hoogstraal, head of the department of medical zoology, NAMRU-3, for advice and facilities; to G. W. Wharton, head of the department of zoology, University of Maryland, for help and advice; to J. H. Camin, curator of invertebrates, Chicago Academy of Sciences, for active interest and suggestions during the course of this work; and to Hymen Marx, Chicago Natural History Museum, for identification of hosts.

23 August 1956

Action of Guinea Pig Serum and Human Gamma Globulin on the Growth of a Rat Tumor

The growth of the fibrosarcoma ACMCA2 that was transplanted into the AxC9935 strain of the Irish gray rat was found to be inhibited by repeated intraperitoneal injections of normal guinea pig serum following tumor transplantation. This inhibition is enhanced by a single injection of heterologous gamma globulin given at the time of implantation.

Preliminary experiments have shown

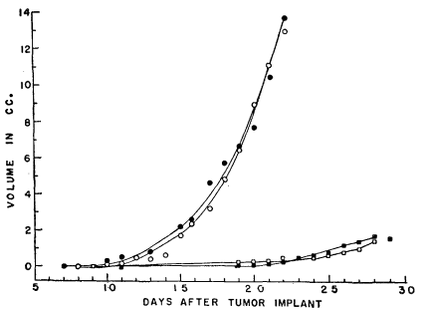

Fig. 1. Growth curves of the tumor in treated and untreated animals. Injections: ○ 5 ml of Ringer's solution daily; ◉ single injection of 28 mg of human gamma globulin plus daily injections of 5 ml of Ringer's solution; ■ 5 ml of guinea pig serum daily; □ single injection of 28 mg human gamma globulin plus daily injections of 5 ml of guinea pig serum.

that for this tumor, unlike other tumors that have been studied (*1*), multiple injections of guinea pig serum, rather than single or short-term injections, are required to produce the maximum inhibition. Treatment was therefore continued for 20 to 25 days (*2*).

Three series of experiments, with a total of 82 animals, were carried out (*3*). Thirty-two control animals were each given daily injections of 5 ml of Ringer's solution. Twenty-six animals were treated with daily injections of 5 ml of pooled guinea pig serum. Nineteen animals were given a single injection of 28 mg of human gamma globulin intramuscularly in the thigh in addition to the daily injections of guinea pig serum. Five animals were given a single intramuscular injection of 28 mg of human gamma globulin and daily injections of 5 ml of Ringer's solution. The animals were grouped so that litter mates were distributed in the different groups. The guinea pig serum was obtained commercially from heterogeneous colonies and stored in a Deep-freeze for 5 to 30 days. Human gamma globulin was obtained from Hyland Laboratories and dissolved in Ringer's solution with a concentration of 140 mg/ml.

In the first two series of experiments, the tumor was cut into pieces of weight 1.0 to 2.0 mg and implanted by trochar. In the third series, the Bernfeld and Homburger (*4*) modification of Snell's (*5*) cytosieve procedure was employed, and 0.1 ml of a 15-percent suspension was injected. In all cases the tumor was implanted subcutaneously in the back. Daily measurements were made in three dimensions with a vernier caliper during the course of treatment and for a short time afterward. The volumes were calculated from these data.

The results of the three series were combined, and the treated and untreated animals were compared using three criteria: (i) the positive or negative "take" of the tumor at the end of 65 days; (ii) the latent period, as determined by the time after implantation when a palpable tumor was first discernible; (iii) the time necessary for the tumor to reach a volume of 0.2 cm^3, at which time it was well into the logarithmic phase of growth.

The growth curves of the four groups are compared in Fig. 1. It will be seen that the treatment not only approximately doubled the time before the tumor entered the logarithmic phase of growth but also induced a slower rate of growth of the tumor.

The most significant finding is that the tumors in seven of the 19 animals that received guinea pig serum plus gamma globulin showed no growth at the end of the 65-day period. Two of these seven animals, however, had shown a small tumor which then regressed; one of these reappeared subsequently and again regressed. One of the 24 animals that was given guinea pig serum alone was also negative. One of the control animals was negative after implantation, and it remained refractory on subsequent implants.

The negative animals were reimplanted after 65 days (that is, 40 days after cessation of treatment), and all the tumors but one grew at a normal rate. The one grew to a volume of about 5 cm^3 and then regressed.

The effect on the latent period is graphically represented in Fig. 2. The appearance of the tumors is plotted on a probability scale. The lowest curve shows that the appearance of the normal tumor is a linear function of time. However, the curves of the treated animals deviate not only in the time of appearance, but in the rate of appearance as well.

When the time necessary for the tumor

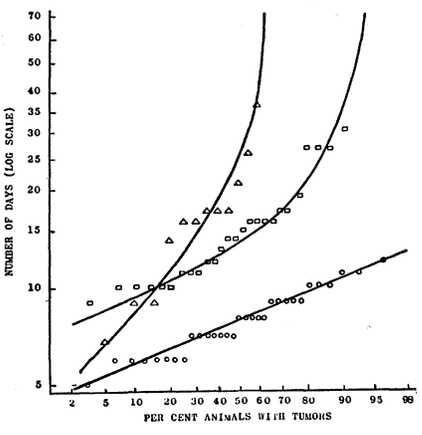

Fig. 2. Effect of treatment on the latent period of the tumor. The percentages of tumors are plotted on a probability scale. Injections: ○ 5 ml of Ringer's solution daily; □ 5 ml of guinea pig serum daily; △ 28 mg of human gamma globulin plus 5 ml of guinea pig serum daily.

to reach a volume of 0.2 cm^3 was plotted on a probability scale and compared with the curves of the treated groups, it was found that the curves were almost parallel. The curves of the two treated groups overlapped. The mean time for the tumors in the treated animals to reach this volume was again about double that of the controls.

The gamma globulin appears to act in one of several ways. By itself, in the dose used, it has no effect on tumor growth, although larger doses have a stimulating effect on the growth. When it is given in conjunction with guinea pig serum, it may suppress the growth of the tumor so that it does not appear at all, or it may have no added effect on the inhibitory action of guinea pig serum. Here also there is an optimum dose. The course taken is probably dependent on the tumor-host relationship.

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24 September 1956.

Cholinergic Effects of β-Substituted Derivatives of Pyruvic Acid

As part of a continuing search for inhibitors of lactic dehydrogenase, fluoropyruvic acid (*1*) was studied *in vitro* in systems containing lactic dehydrogenase and then *in vivo* in rats (*2, 3*). Within 5 minutes after intraperitoneal administration of 150 mg/kg of fluoropyruvic acid, rats exhibited sialorrhea, lacrimation, and rhinorrhea, accompanied by wheezing and audible rhonchi. These effects were followed by lethargy, head drop, a decreased sensitivity to pain, occasional clonic movements, hyperpnea, labored breathing, and ultimately death of the animals in periods ranging from 30 minutes to 12 hours. In doses of 1 mg/kg, atropine suppressed the muscarinic effects of these compounds.

The specificity of the compounds causing these effects was investigated.

Table 1. Specificity of β-substituted pyruvic acids in producing cholinergic effects.

Positive effects	No cholinergic effects
Fluoropyruvic acid	Pyruvic acid
Chloropyruvic acid	α-Ketobutyric acid
Bromopyruvic acid	Oxaloacetic acid
Hydroxypyruvic acid	Fluoroacetic acid
	Chloroacetic acid
	Glycolic acid
	α-Chloropropionic acid
	Sodium fluoride
	Potassium oxalate
	Potassium oxamate
	Acetic acid

Chloropyruvic acid was synthesized by the method of Garino and Muzio (*4*), and bromopyruvic and hydroxypyruvic acids were synthesized by the procedure of Sprinson and Chargaff (*5*). As is indicated in Table 1, each of these β-substituted derivatives of pyruvic acid induced cholinergic effects in rats following intraperitoneal injection of 75–150 mg/kg. The effects of 2 and 3 carbon analogs of these pyruvic acid congeners were also studied (Table 1). The convulsant effects of fluoroacetic acid are well known, but this compound, like chloroacetic acid, does not induce cholinergic effects in the rat. Pyruvic acid itself and its β-methyl analog, α-ketobutyric acid (Sigma Chemicals Company), did not induce cholinergic responses.

The effects of β-substituted pyruvic acids were studied in other animals. In the mouse, chloropyruvic acid subcutaneously caused death in 1.5 to 12 hours and was found to have an LD_{50} of 200 mg/kg, closely approximating the value of 250 mg/kg reported by Blank *et al.* (*1*) for fluoropyruvic acid. Chloropyruvic acid produced analgesia in the mouse in less than 30 minutes, and the AD_{50} by the method of Haffner (*6*) was also found to be 200 mg/kg. As in the rat, the spontaneous activity of the mouse decreased, but activity could be readily evoked by sensory stimulation.

When 50 mg/kg of chloropyruvic acid was injected intravenously into spinal vagotomized cats, there was an initial fall followed by an increase in blood pressure and pulse pressure. These immediate effects were followed by a prolonged depression of blood pressure and pulse pressure. The cat also exhibited marked salivation, lacrimation, and rhinorrhea. Although instillation of 0.2 ml or 5-percent bromopyruvic or chloropyruvic acid in the conjunctival sac did not induce miosis in the rabbit, miosis was generally observed following intravenous injection of these compounds. Administration of acetylcholine in doses of 2 μg/kg to the cat resulted in a depressor response without an accompanying bradycardia prior to the administration of chloropyruvic acid. Following the administration of chloropyruvic acid, a marked bradycardia accompanied the depressor response to acetylcholine. Approximately 0.5 hour later, the depressor response to acetylcholine was noted again, but the bradycardia failed to recur. Flexion and extension of rear extremities with superimposed tremor were also observed following administration of chloropyruvic acid to the spinal cat. Administration of 1 μg/kg of norepinephrine after the administration of chloropyruvic acid did not result in a pressor effect.

Immediately after injection of 150 mg/kg of chloropyruvic acid in the cat, the EEG, which had shown frequent spindle activity, became activated and remained so for 8 minutes. During this time, there was a gradual diminution of the amplitude of the high-frequency activity. After 10 minutes, the EEG was characterized by spikes, abortive spindles, and slow wave activity. The activating response to auditory stimuli was still present and was longer than in the control period. The amplitude continuously decreased, and within an hour the cortex was isoelectric; however, single auditory stimuli produced evoked cortical potentials in the frontal, parietal, and occipital leads. These potentials were not artifact and are presumptive evidence that the activating system was still capable of mediating impulses to the cortex.

To test the possibility that the effects of the halogenated pyruvic acids were due to the formation *in vivo* of haloacetylcholines, which might be the direct stimulatory compounds, fluoroacetylcholine was synthesized by the method of Gryszkiewicz-Trochimowski *et al.* (*7*). Fluoroacetylcholine, in doses of 50 μg/kg, in the unanesthetized cat produced the cholinergic effects observed with chloropyruvic acid as well as bradycardia and motor activity of the rear extremities. After intravenous administration to rats of 15 mg/kg of fluoroacetylcholine, hemodacryorrhea was observed in the first 15 seconds and was followed within a minute by the other cholinergic effects observed with β-substituted pyruvic acids. The same effects in the rat were noted with acetylcholine at the same dose levels (*8*).

Inasmuch as the possibility existed that these compounds functioned as anticholinesterases, their effects were determined on the rate of hydrolysis of acetylcholine by purified bovine erythrocyte cholinesterase (Winthrop-Stearns). Neither fluoropyruvic acid, chloropyruvic acid, nor fluoroacetylcholine significantly altered the rate of enzymatic hydrolysis of acetylcholine.