The results of the hemoglobin determinations which are presented in Table 1 show that the administration of either β-pyracin or LCF alone is of some value in hastening the regeneration of hemoglobin after severe hemorrhage, but that the administration of both these factors together is markedly more effective, causing a return of the hemoglobin level to the "pre-bleeding" value in five to six days, whereas in the hens receiving no treatment only a slight increase in hemoglobin level was observed in that length of time. Furthermore, the hemoglobin level in the hens receiving both β-pyracin and LCF continued to rise in all experiments and reached the normal level in eight to nine days, while the controls showed an increase in hemoglobin of approximately 1.5 grams per 100 cc. of blood in the same period of time.

In Experiment 3, the hens were held after the vitamin injections were discontinued and periodic hemoglobin determinations were made in order to ascertain the ultimate effect of the removal of approximately one-third of the blood supply. The results are presented in Fig. 1. The results show that in all cases

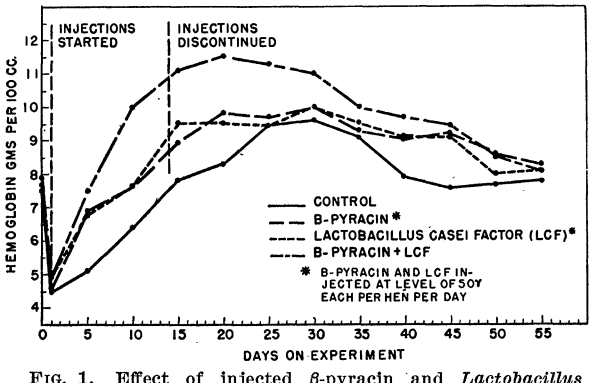

FIG. 1. Effect of injected β-pyracin and *Lactobacillus casei* factor upon the rate of recovery from hemorrhagic anemia.

the hemoglobin levels rose above the "pre-bleeding" values and then declined to approximately the original level. The rate of increase was much more rapid and the level reached was higher in the lot receiving both β-pyracin and LCF than in the other lots. In this lot and the lots receiving β-pyracin and LCF singly, the higher hemoglobin levels were maintained for some time before declining. The rise in hemoglobin levels above the "pre-bleeding" values, which was observed in all lots, may possibly be explained by an overcompensatory activity of the hematopoietic organs under conditions of extreme demand.

SUMMARY

In severe experimental hemorrhagic anemia in hens, the injection of β-pyracin and *Lactobacillus casei* factor, alone and in combination, was found to hasten the regeneration of hemoglobin. When these factors were administered together, however, the rise in hemoglobin was much more rapid and the level reached was higher than in the lots administered the factors alone.

The hemoglobin levels in the control lots rose above the "pre-bleeding" values and then began an almost immediate decline to approximately the original level. In the lots receiving β-pyracin and *Lactobacillus casei* factor, alone or in combination, the hemoglobin levels also rose above the "pre-bleeding" values but were maintained for some time after injections were discontinued before declining to the original values.

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The Site of Action of DDT in the Cockroach[1]

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Yeager and Munson (*4*) have obtained indirect evidence that DDT (*1*-trichloro-2,2-bis(*p*-chlorophenyl)-ethane) may act on motor nerve fibers of the cockroach, raising their irritability until spontaneous discharges result in muscle twitches. Others have confirmed this, observing the twitching which occurs in amputated DDT-treated legs.

If an emulsion[2] containing .5 μg. DDT is injected into the body cavity of an adult cockroach (*Periplaneta americana*) weighing 1.0 to 1.5 grams, the insect shows typical symptoms of poisoning within 5 or 10 minutes. Briefly, the symptoms consist of coarse clonic spasms of the trunk and appendicular muscles and increased but initially well-coordinated activity. Within a few hours the spasms become continuous and the insect is unable to stand, although the legs may continue to twitch feebly for 48 hours.

The dose producing tremors within 5 to 10 minutes is in the neighborhood of 5 to 10 mg./kg. If it is assumed that DDT is distributed evenly among the body tissues, the toxic concentration at the site of action must be approximately 5 parts per million. Even if DDT is selectively accumulated in certain

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Tufts College.

[2] Twenty ml. of an oil solution (petrolatum mineral oil, 90.5 per cent; oleic acid, 8.5 per cent; and DDT, 1.0 per cent) is passed through a hand emulsifier with 80 ml. of an aqueous solution (insect saline, 99.0 per cent; triethanolamine, 1.0 per cent). The emulsion minus DDT had no effects on cockroaches.

tissues, it seems improbable that the toxic concentration at the site of action is more than 10 times greater or less than this figure. The following experiments were carried out in order to discover a site of action sensitive to a direct application of DDT in an approximate concentration of 0.5 to 50 ppm.

Motor nerves and muscles. Yeager and Munson found that the injection of a 10-per cent solution of DDT in corn oil locally into the leg of a roach, or into the region of the ganglion of the same segment, produced twitching in the leg muscles within 5 to 10 minutes even after the motor nerves had been severed medial to the injection site. If DDT emulsion (1,600 ppm DDT) is applied directly to the exposed, intact internal ventral muscles of the cockroach after removal of the abdominal ganglia, a series of isolated and often partial contractions of the individual fibers can be observed. These contractions were noted only one to three hours after exposure to emulsion containing 1,600 ppm DDT. In view of the times of onset and high concentration of DDT necessary, it seems doubtful that the direct action of DDT on muscles or on the peripheral stumps of motor nerves can account for the symptoms observed in the intact insect.

Central nervous system. Since decapitation or separation of the body segments causes no decrease in DDT tremors, no specific nerve center can be affected. To determine whether DDT has a general excitatory effect on the central nervous system, the thoracic ganglia were dissected in saline and mounted on silver electrodes. The spontaneous electrical activity was amplified and counted electrically over continuous periods of many hours. This method has been used previously to study the central action of excitant and depressant drugs and ions (*3*). Application of solid DDT crystals, 2 per cent DDT in mineral oil, and DDT in emulsion (1,600 ppm) had no detectable effect on the level of spontaneous activity. In view of the concentrations used, the central action of DDT, if any, is of no significance in the genesis of typical DDT tremors.

Sense organs. Silver electrodes were placed in contact with an exposed section of the ventral nerve cord of an otherwise intact cockroach. Potentials were fed to a Grass amplifier and recorded with a cathode-ray oscilloscope and camera. Impulses were also counted electrically and monitored with a loudspeaker. The number of impulses per second became relatively constant except during struggles of the insect. If an effective dose of DDT was injected, the number of impulses per second increased 700 per cent as the typical symptoms of poisoning developed. Sectioning the cord above and below the site of the electrodes revealed that the DDT-induced impulses were in ascending fibers. Since the ganglia themselves are not apparently affected by DDT, the ascending impulses must be sensory in origin.

To obtain direct evidence of a sensory effect, the large crural nerve in the metathoracic segment was exposed from its root at the ganglion to the point where it enters the coxa. This nerve is designated as nerve 5 by Pringle (*1*) who showed by physiological methods that it contains afferent fibers from the campaniform sensilla and the hair sensilla in the leg, and a few efferent fibers to the muscles of the coxa. Using a manipulator, two silver electrodes were placed below the nerve between the ganglion and coxa. The electrode nearest the ganglion was pinched tightly around the nerve to crush it at this point. This prevented the nerve from slipping off the electrodes and rendered the afferent impulses monophasic. To eliminate any possibility of motor activity the ganglion was removed, and all other nervous connections with the leg were severed.

Normal electrical activity in the afferent fibers consists of a steady asynchronous series of axon spikes, which is replaced by a complex high-frequency burst of impulses if the leg is moved passively or the coxa and femur are subjected to torsion (*1*). The electrical picture is similar but somewhat simplified if the distal third of the femur is amputated. If DDT emulsion is injected into the cut end of the femur so that the solution passes completely through the leg, there is an immediate and striking change in the electrical activity. In place of the random spikes, a series of impulse trains appears and persists for hours. Each train seems to involve a single fiber which fires repetitively at a frequency of 300 to 400 spikes per second. The train declines slightly in frequency and dies out in 0.1 to 0.5 seconds. The spike height in each train is constant, indicating the involvement of a single axon, although a number of different trains may occur simultaneously. Comparison with normal oscillograms suggests that while the untreated sense cell discharges a single spike at various intervals, under the influence of DDT each single spike is replaced by a short train, likewise repeated at intervals. Even at the height of the DDT-induced discharge no spontaneous movements of the leg were detected in well over 100 preparations and the increased electrical activity seems to be confined to the afferent fibers.

Trains appear in the afferent fibers when solutions containing as little as 0.01 ppm DDT are perfused through the leg. Effects have been obtained with diluted DDT emulsion, suspensions of DDT made by a series of saline dilutions from a 1-per cent solution of DDT in ethyl alcohol, and a saturated aqueous solution of DDT in saline made by allowing saline to stand in contact with pure DDT crystals overnight

at 22° C. The time interval between perfusion and the appearance of trains is related to the DDT concentration perfused (Table 1), and comparison of the figures suggests that the DDT concentration in the aqueous solution was between 0.1 and 0.01 ppm. Although the trains take longer to appear at very low concentrations, they may be marked and persistent.

TABLE 1

	Diluted emulsion			DDT suspension in saline				Saline saturated with DDT
DDT in ppm	16.0	1.6	0.16	10.0	1.0	0.1	0.01	?
Individual times (in minutes) from perfusion to appearance of trains	3.0	4.0	16.0	3.0	10.0	13.0	43.0	20.0
	7.0	8.0	9.0	2.0	3.0	21.0	52.0	42.0
			13.0	3.0	2.0	32.0	36.0	35.0
			25.0	4.0	12.0	19.0	32.0	12.0
				3.0	10.0	23.0	27.0	30.0
					16.0	31.0	31.0	
					18.0		29.0	
					17.0		32.0	
					9.0			
Av. times			16.0	3.0	9.6	23.1	35.2	27.8

The sensitivity of the sensory response is such that the DDT concentrations reached within the body of a cockroach receiving the minimum lethal dose would be more than sufficient to generate similar trains of impulses in the afferent nerves. It is concluded that in the cockroach the tremors characteristic of DDT poisoning are due to an intense and patternless bombardment of the motor neurones by trains of impulses originating in sensory endings.

It has not been possible to identify the sensory structures upon which the DDT acts. Progressive amputation of the leg reveals that both normal electrical activity and DDT trains disappear if the trochanter is removed, although a considerable length of nerve 5, including several motor branches, remains intact in the coxa. Removal of the trochanter would eliminate all the groups of campaniform sensilla described by Pringle, and the general impression gained is that the proprioceptive sense organs are the structures in which the DDT trains originate. A similar series of trains can be obtained if DDT is injected into the cut cercus, and the electrical activity in the cercal nerve is recorded. The DDT trains are much less marked in this nerve and are somewhat masked by a large, spontaneous background of electrical activity which seems to be unaffected by DDT. Although campaniform sensilla are located on the cercus, they are greatly outnumbered by hair sensilla sensitive to air currents and sound vibrations (*2*). This suggests that DDT does not have its characteristic effect on all sensory structures, but acts to some extent specifically on a particular group of sense organs, as yet unidentified.

Summary

(1) Although DDT undoubtedly affects motor nerves and muscle fibers in concentrations greater than 1,000 ppm, this action cannot be directly responsible for the clonic tremors in the DDT-poisoned cockroach which can be produced by internal concentrations of the order of 5 ppm.

(2) DDT has no significant action on the cockroach central nervous system.

(3) DDT emulsion perfused through the leg of the cockroach in concentrations as low as 0.01 ppm causes the appearance of a series of high frequency trains of axon spikes in the afferent fibers.

(4) It is concluded that the tremors characteristic of DDT poisoning are due to an intense afferent bombardment of the motor neurons.

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Molecular Weights and Other Properties of Viruses as Determined by Light Absorption

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Virus solutions exhibit visible opalescence due to light scattered by the virus particles, and this results in a decrease in light transmission. It can be shown by an application of Einstein's theory of light-scattering of mixtures (*1*) that the molecular weight, M, of uniform spherical particles, small compared with the wave length of light, λ, is given for dilute solutions by the expression $M = 1.69 \times 10^{22} \frac{D\lambda^4}{c\left(\frac{\partial n^2}{\partial c}\right)^2}$, where c is the concentration of the particles expressed in grams per milliliter of solution, D is the optical density (the absorption coefficient divided by 2.303 for a cell of 1 cm. thickness) of the solution, and n is the index of refraction of the solution. Since $\frac{\partial n^2}{\partial c}$ is nearly equal to $2n_0\left(\frac{n-n_0}{c}\right)$, where n_0 is the index of refraction of the medium, the latter expression can be used. Therefore, it is possible to secure molecular weight values if, for dilute solutions, the change in optical density with wave length and the indices of refraction of the solvent