Chloromycetin, an Antibiotic With Chemotherapeutic Activity in Experimental Rickettsial and Viral Infections

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A new antibiotic, Chloromycetin (*1*), has been found to have a remarkable chemotherapeutic effect on a number of rickettsial agents and on one of the viruses. This substance was prepared in the Research Laboratories of Parke, Davis & Company and submitted to this Department as highly purified crystalline material; preliminary tests (*1*) had indicated that Chloromycetin possessed activity against *Rickettsia prowazeki*.

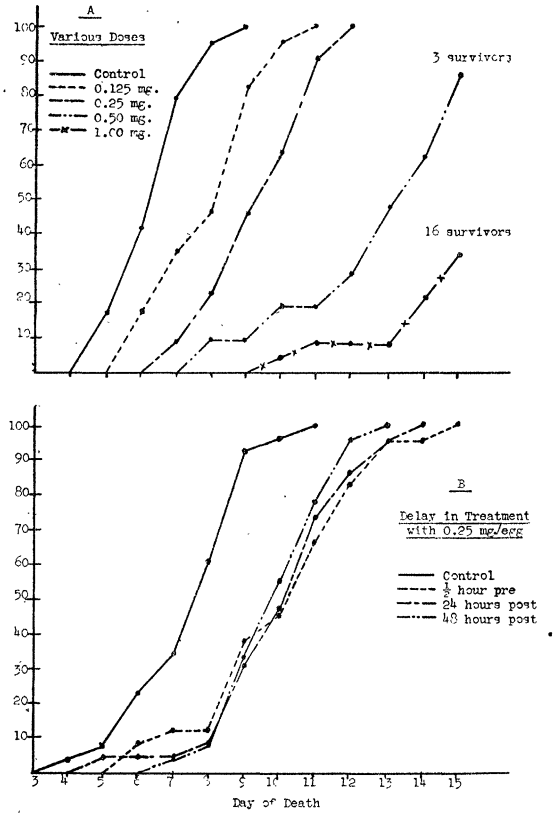

FIG. 1. Rickettsiostatic effect of drug in embryonated eggs infected with *R. orientalis*.

Initial tests were performed using groups of 24 seven-day embryonated eggs which were infected by the yolk sac route with the Gilliam strain of *R. orientalis* and treated by the same route with varying amounts of drug given at different times. Eggs were examined daily and the time of death of embryos noted. Data are summarized in Fig. 1 as the percentage mortality in the various groups on different days after infection. The experiment summarized in A, Fig. 1, shows the effect of varying amounts of drug given in a single injection ½ hour prior to infection. It is evident that the smallest dose used, namely, 0.125 mg./egg, resulted in a definite prolongation of life of the embryos. The beneficial effect increased step by step as the dose was increased. All of the embryos in the group receiving 1.0 mg. were alive when the last of the control eggs died. Furthermore, 16 of the 24 embryos were still alive in this group on the 15th day after infection, when the experiment was terminated since hatching was to be expected. Eight chicks did hatch from this group of 16 survivors. Section B of Fig. 1 summarizes an experiment in which 0.25 mg. doses of drug were given to each of 24 eggs in three different groups; one group was treated ½ hour prior to infection, another 24 hours, and a third 48 hours, after infection. It is apparent that all embryos were equally benefited by the treatment; the average prolongation of life in each of the groups was in the neighborhood of 2½ days.

The chemotherapeutic effect observed in treated mice infected with scrub typhus was as satisfactory as that obtained in experiments with embryonated eggs. Table 1 summarizes the results of studies on mice infected by the intraperitoneal route with 25–100 m.l.d. of the Karp strain of *R. orientalis*.

TABLE 1

CHEMOTHERAPEUTIC EFFECT OBTAINED IN MICE INFECTED WITH *R. orientalis*

Experiment	Treatment			Dilution of infectious inoculum		
	Mg./day/mouse	Route	Day begun in relation to infection	10^{-5}	10^{-6}	10^{-7}
1	Controls	Injected intraperitoneally		8/8	8/8	2/7
	2.5		1 post	0/8		
	1.5		1 "	0/8		
	0.75		1 "	2/8		
	0.5		1 "	5/8		
	0.25		1 "	5/8		
	0.10		1 "	3/8		
	5.0	Fed in water	1 pre	0/8		
	5.0		2 post	1/8		
	3.0		1 pre	0/7		
	1.5		1 "	1/8		
2	Controls	Injected intraperitoneally		7/8	7/8	3/8
	2.5		1 post	0/8		
	2.5		5 "	0/8		
	2.5		8 "	1/8		
	2.5		10 "	3/8		
	0.75		1 "	2/8		
	0.75		5 "	2/8		
	0.75		8 "	6/8		
	0.75		10 "	7/8		

Animals were treated either by a single daily intraperitoneal injection or by oral administration of the designated amount of drug; in the latter instance it was dissolved in the drinking water. Animals were given their first injection 18 hours after infection or at a later date designated in the table. Mice which were fed the drug generally were started on it the day before inoculation of infectious material. In all instances mice were treated from the designated time through the 20th day after infection. The data indicate that a single daily intraperitoneal dose of 1.5 mg. of the drug protected all of the mice. Half this amount protected the majority, while doses as small as 0.1 mg./mouse had some chemotherapeutic effect. All of the mice which received 3.0 mg. of the drug by mouth per day sur-

vived, as did practically all of those receiving half this amount. It is to be noted that oral administration of 5.0 mg./day begun two days after infection protected 7 of the 8 mice from death. The fact that an excellent chemotherapeutic effect can be obtained even when administration of the drug is delayed for 10 days after infection is indicated by the results of the second experiment (Table 1). Seven of the 8 untreated control mice which received the 10^{-5} dilution of infectious material died between the 14th and 18th day. In contrast, only 3 of 8 mice succumbed in the group which received 2.5 mg. of the drug beginning on the 10th day after inoculation; these died between the 15th and 20th days. The majority of the mice which received 0.75 mg. daily were protected when the drug treatment was begun as late as 5 days after infection but not when it was begun 8 days afterward.

Results essentially identical with those just described have been obtained in embryonated eggs and mice infected with the agent of rickettsialpox (*R. akari*). Furthermore, the results of experiments in which embryonated eggs were infected with *R. prowazeki*, *R. mooseri*, *Dermacentroxenus rickettsi*, or the 6-BC or P-4 strains of psittacosis duplicated almost exactly those summarized in Fig. 1. A good chemotherapeutic effect was demonstrable in mice infected with the 6-BC strain. The antibiotic has been ineffective in the treatment of mice infected with Japanese encephalitis virus and in eggs infected with variola virus and influenza A virus.

Inasmuch as control mice and embryonated eggs which received amounts of the drug comparable to the maxima used in the above tests remained unaffected, Chloromycetin appears to be of low toxicity. Its low toxicity, its absorption from the alimentary tract, and its beneficial effect when given relatively late in disease suggest that it may be valuable in the treatment of patients.

Reference

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Initiation of the Cone Gall of Witch Hazel

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The control of growth and differentiation leading to the orderly development of the plant and animal body continues to be the central problem of biology. From Darwin and Herbert Spencer to the present time, theories of heredity have demanded some sort of controlling substance, whether envisaged as a physiological unit, a gemmule, a gene, or even as a hierarchy of biophores, determinants, and ids. In theories of the last 50 years the location of these units has been placed in the chromatin of the nucleus, and only recently have these specific, controlling, and self-propagating entities been ascribed to a location in the cytoplasm, the "plasmagenes" of Wright (*1*). A substance initiating, stimulating, and directing development and differentiation has been found in the cells of the cone gall of witch hazel (*Hamamelis virginica* L.). It is injected into the young leaf by "stings" of an aphid (*Hormaphis hamamelidis* Fitch) which feeds on the neoplasm induced by its presence.

The process of stinging is quite different from that of feeding. When stinging, the stem mother inserts her stylets into and between the cells of the young leaf and injects minute drops of a substance secreted by glands opening into the stylar canal. The sting substance cannot be confused with the salivary secretion because it shows characteristic staining reactions and properties of refraction.

Cells receiving the injected sting material dedifferentiate, undergo rapid mitotic divisions, and redifferentiate, not into cells of the normal witch-hazel leaf tissues, but into cells of other and different tissues which organize the gall. The cells show the effects of the stings immediately, and when about 150 stings have been made over a small circular area of the leaf, the cells of this area first become etiolated and then, by growth in size and number and by tissue differentiation produce the highly specialized cone gall. This is not a haphazard excrescence on the leaf, but in symmetry and harmonious unity of cells and tissues shows the same phenomena of functional adaptation that are familiar in the individual. It differs from an individual in its inability to reproduce.

The injected material, when stained with acid fuchsin and jod green consists of a blue ground substance in which minute crystalloids are embedded. The material of the crystalloids seems to pass readily from cell to cell, especially from phloem parenchyma to adjoining cells. The crystalloid is a tiny, refractory, deeply staining body characterized by its faceted appearance and showing a reddish or purplish cast in such stains as gentian violet, hematoxylin, safranine, congo red, acid fuchsin, basic fuchsin, Feulgen, and pyronine.

The behavior of the injected material is somewhat different when injected directly into the cytoplasm and when injected into intercellular spaces. When injected directly, it is seen first in the cytoplasm as a single globule containing, usually, one refractory crystalloid. The globule enters the nucleus and then the nucleolus, where the crystalloid breaks up into smaller bodies.

When the injection is intercellular, the globule is usually much larger and at first does not show crystalloids, though a small body showing the same staining reactions may be present. Crystalloids form within the globule which enters the leaf cells, occasionally the inner epidermal and spongy parenchyma cells, but much more often the phloem parenchyma. From the phloem parenchyma the sting material passes into palisade or even into cells of the upper epidermis. During its existence in the cytoplasm of the phloem parenchyma it may break up into smaller globules, each with its crystalloid or may lose its crystalloids singly into the cytoplasm. Whether injection is intra- or intercellular, the end result is the entry of the crystalloids into the nucleolus. There they may fuse to form a larger, single crystalloid which breaks up into smaller ones when the nucleus enters prophase.

As mitosis approaches, the bodies become more numerous, and are distributed to the daughter nuclei, where they are found in the nucleolus as they were in the mother nucleus. They appear not to be capable of self-propagation. For gall formation to continue normally, additional sting material must be injected repeatedly into the leaf. During the entire growth of the gall the stem mother continues to sting in intervals of feeding. Only the stem mother can initiate gall