tion this globulin is made or freed in excess in order that there may be plenty of it present to fix all the antibody activity that may develop. This could be answered by investigating the parallel development of antibodies and s(16) globulins in horses undergoing immunization; against it is the fact that the antibody concentrates here studied, coming from very different sources, have the same protein antibody ratio, *i.e.*, k of Table I is practically the same. If the alternative, that the globulin is the antibody, is true, then it is of interest to find that in a bivalent concentrate containing equal quantities of Types I and II antibodies (A, I+II and B, I+II) the protein content is not increased by the presence of the additional Type II antibodies: this indicates that a single globulin molecule can exhibit the characteristics of more than one antibody.

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EARTH WORMS AS TEST OBJECTS FOR DETERMINING THE VALUE OF DRUGS TO BE USED IN HUMAN INTESTINAL HELMINTH INFESTATIONS[1]

THE earthworm has for many years enjoyed the reputation of being the test object *par excellence* for determining the efficacy of "anthelmintics." In attempting to foretell the effect of any drug in man, it is necessary to make tests on lower animals with the hope of avoiding unpleasant complications. Every experimenter is, however, aware of the fact that toxicity or lethality experiments carried out in such animals can not be transferred to man with the assurance that identical effects will be produced in this species. It would seem, however, that our present state of knowledge is such as to require that we use some judgment in the choice of biological test objects. An animal without a heart would not appear to be the best object on which to study cardiac drugs, or *Macrocanthorhynchus hirudinaceus,* which has no alimentary canal, for the investigation of cathartics.

It is doubtful, however, if a more diversified group of animals was ever brought together under one heading than those designated as "worms." This is well illustrated in J. Arthur Thomson's[2] description of these animals: "It is hopeless at present to arrange with any definiteness those heterogeneous forms to which the title 'worm' is given. For this title is little more than a name for a *shape,* assumed by animals of varied nature who began to move head foremost and to acquire sides. There is no class of 'worms,' but an assemblage—a mob—not yet reduced to order."[3]

It is bad enough when we assume that an anthelmintic should act equally well on unattached roundworms, tapeworms and blood-sucking hookworms, but it would seem as if the time had come when we should give up the indiscriminate use of a biological test object of any nature as long as it looks like a worm. However, leeches, vinegar eels and above all earthworms are used by many investigators in testing "anthelmintics," but those who have chosen for this purpose test objects with other shapes, as fishes and frogs, have been ridiculed. But would not a frog, whose feet admittedly differ in shape from those of an earthworm or even a butterfly serve as well as an earthworm for determining the ascaricidal value of a group of chemical substances?

The human Ascaris is a parasitic animal living in the gut of man. It has no respiratory or circulatory system in any way related to that of an earthworm. It can live under anaerobic conditions. It is covered with a chitinous coat which is very sensitive to certain substances that have been shown to effectively kill the animal by acting on or through it. The earthworm is a free-living species inhabiting not man, but the ground. It feeds on substances in the soil rather than those of the human gut. It has no chitinous coat. It is dependent upon a circulatory system with not merely a single heart but five pairs of these organs through which circulates blood containing both corpuscles and hemoglobin. Except for its shape, there is nothing which under any consideration could be used as an excuse for taking such an animal as a test object for ascaricides, especially when one can obtain with great ease pig Ascaris, which are morphologically indistinguishable from the human Ascaris.

In spite of this, a publication has just appeared on "Rational Use of the Earthworm for the Evaluation of Vermicides."[4] No explanation is given as to why this is considered a rational use of these animals, unless it is the four pages of mathematical calculations used in comparing the "rate of change of 'apparent' speed of fatality with change in molar concentration" of three substances.

During the past year we published an article on "Methods of Testing the Anthelmintic Properties of Ascaricides."[5] Our conclusions were based on experi-

[1] The funds for carrying out this work were given by the International Health Division of the Rockefeller Foundation.

[2] J. Arthur Thomson, "Outlines of Zoology," page 10.

[3] This opinion regarding the group of "worms," the "Vermes" of Linnaeus, is not confined to recent writers alone, and some of the most modern authorities consider the grouping made by Aristotle some two thousand years ago to be superior. They speak of the so-called "phylum Vermes" as a "wastebasket," a "Rumpelkämmer" or "attic," corresponding to that place where everything that had been respected for a hundred years was piled together to save it for future use which never came.

[4] G. L. Jenkins and L. L. Manchey, *Jour. Am. Phil. Asn.,* 25: 194–201, March, 1936.

[5] P. D. Lamson and H. W. Brown, *Am. Jour. Hyg.,* 23: 85–103, January, 1936.

ments in which we tested several hundred substances on several hundred thousand pig Ascaris *in vitro,* collected the parasites passed, as well as those found at autopsy, in many hundreds of dogs infested with Ascaris, and egg-counted over 3,000 human cases of ascariasis before and after treatment.

During the course of these experiments we checked as a matter of interest, but with no intent of publication, the effect of 121 substances on both earthworms and pig Ascaris, using the *in vitro* method described in our "Methods" paper. The earthworms were tested in distilled water at room temperature, while the Ascaris were kept at 35° in saline. Of these 121 chemicals chosen from *inorganic substances, hydrocarbons, halogenated hydrocarbons, alcohols, phenols, ethers, organic acids* and their *salts, organic sulfur compounds, terpenes, sesquiterpenes, alkaloids, enzymes, glucosides, plant products, dyes, substances liberating oxygen* or *iodine,* as well as a miscellaneous group, only 6 per cent. showed a fairly close correspondence of anthelmintic action on earthworms and pig Ascaris. In 58 per cent. the Ascaris were alive after twenty hours, while the earthworms had died in from two minutes to six hours. Sixty-seven per cent. killed earthworms in thirty minutes or less, while only 8 per cent. of these substances killed Ascaris in that time.

Conclusion

(1) The use of earthworms as a test object for evaluating the activity of anthelmintics to be used in human intestinal helminth infestation is irrational.

(2) A comparative study of the lethality of 121 widely diversified chemical substances on both earthworms and pig Ascaris shows no correlation of action.

(3) *In vitro* tests of human ascaricides on pig Ascaris, which is morphologically indistinguishable from human Ascaris, are of value.

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CATALYSIS OF FORMALDEHYDE TO REDUCING SUGARS BY ASCORBIC ACID

Kusin[1] has recently shown that compounds capable of forming en-diol groups in alkaline solution (glucose, fructose, benzoin, etc.) markedly catalyze the production of reducing sugars from formaldehyde in the presence of calcium hydroxide. According to Haworth's formula ascorbic acid contains the en-diol group and, if Kusin's theory is correct, might be expected to catalyze the reaction. The writers have investigated this possibility and have found that ascorbic acid is a very active catalyst. Table 1 summarizes the results of experiments in which the rate of formation of reducing substances was followed in mixtures of formaldehyde and calcium hydroxide both with and without the addition of ascorbic acid. Ascorbic acid and excess formaldehyde were removed from the solutions before sugar estimations.

TABLE 1

Reactants	Time Minutes	Red. Subs. as glucose mg. per 100 cc
Formaldehyde + ascorbic acid + $Ca(OH)_2$	0	24.
"	30	220.
"	60	391.
"	90	312.
"	120	269.
"	180	199.
Formaldehyde + $Ca(OH)_2$	0	0.
"	60	0.
"	120	11.
"	180	154.
"	240	94.

The disappearance of formaldehyde from the solutions was about complete at the time of maximum reduction.

It will be noted that in the reaction catalyzed by ascorbic acid maximum reduction was attained before the appearance of reducing substances in the control. In both cases the curves show a maximum after which the reducing values decrease. This is possibly due to higher reducing equivalents of the smaller molecules formed early in the reaction followed by polymerization of these to larger molecules of lower reducing values.

Kusin has isolated an intermediate compound of benzoin and formaldehyde from reaction in an alkaline solution. We have obtained evidence that ascorbic acid combines with formaldehyde even in an acid solution. The suggestion has been made that simple sugars play a catalytic rôle in the photosynthetic production of carbohydrates from formaldehyde in green leaves. The evidence presented here suggests that ascorbic acid may be an active photosynthetic catalyst.

Further work is in progress and this, with experimental details, will be published elsewhere.

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THE CONDENSATION OF β-CYCLOCITRAL WITH DIMETHYLACROLEIN

According to the principle of vinylogy[1] the condensation of β-cyclocitral (I) with two moles of

[1] A. Kusin, *Ber.* 68: 619, 1494 and 2169 (1935).

[1] Fuson, *Chem. Rev.,* 16: 1, 1935.