electric field the steady-state transport equation becomes

$$e\mathcal{E}\frac{df}{dk} = \alpha\frac{\partial f}{\partial E} - \frac{1}{S}f. \tag{8}$$

For $\mathcal{E} = 0$ the solution is

$$\begin{aligned} f_0 &\propto e^{E/S\alpha} \text{ for } E < E(A) \\ &= 0, \qquad E > E(A). \end{aligned} \tag{9}$$

Thus on this special model the unperturbed distribution of injected carriers simulates a negative temperature distribution and it becomes possible in principle to have a negative resistance element, if the relevant masses are principally negative.

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[3] Similar considerations have been carried out independently by Dr. Peter Kaus of the University of Southern California. Dr. S. Rodriguez of the University of Washington has successfully extended our argument to motion in three dimensions in a magnetic field. I am grateful to both workers for the private communication of their results. Cyclotron resonance experiments bearing on the negative mass regions have been carried out by Dousmanis and coworkers.

SEX INDUCED WITH ECDYSONE

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Introduction.—This paper deals with the induction of sex in the protozoan flagellates of the wood-feeding roach *Cryptocercus punctulatus*. Except for a few days following hatching, the hind-gut of all individuals of this insect is always filled to its utmost capacity by these symbiotic protozoa without which the roach cannot live.[1] These parasitic flagellates, many of which are unusually large cells, represent a great diversity of structure, comprising 9 families, 14 genera, and over 30 species.

However, their diversity in structure, great as it is, is surpassed by the number of very different types of sexual cycles these organisms undergo.[2]

Sex in them occurs only during the molting period of their insect host. Except in the first three instars, there is usually only one molting period a year; sex never occurs in the protozoa of adults and intermolt nymphs. Any procedure that prolongs the molting period affects sex in the same way; and any procedure that prevents molting, prevents sex.

Some genera of these flagellates have sexual processes that are as well developed as those of higher organisms; that is, they have highly differentiated types of gametogenesis, fertilization, and meiosis. In other genera, sex is less well developed,

and in some it is simple. However, all of these types are induced, as the experiments described here prove, by the molting hormone ecdysone.

Under natural conditions some of the genera of flagellates begin their sexual cycles much earlier in the molting period than others.[3] As shown in Table 1, *Barbulanympha*, *Saccinobaculus*, and *Oxymonas* begin their sexual cycles 40–50 days before their host undergoes ecdysis, while *Trichonympha* does not begin its sexual cycle until 5–6 days before ecdysis; yet, except for three species, they all complete their cycles at the same time. In all of these cycles, gametogenesis is the first sexual process. In the haploid genera, gametogenesis and meiosis are separate processes, but in diploids they occur at the same time. Thus, in haploids the sexual cycle ends with meiosis, while in diploids it ends with fertilization.

The data shown in Table 1 were obtained by isolating over 500 individual roaches whose protozoa were beginning a certain stage in their sexual cycles. Each roach was observed many times daily and the time of its ecdysis noted. The data after ecdysis were obtained by killing roaches at frequent intervals and noting the stages of development of their protozoa. By such a procedure an accurate timetable of normal events in sexuality of the different genera of protozoa becomes available for comparison with what occurs under experimental conditions. This table enables one to compare the sexual behavior, in time, under natural conditions with that which is produced by the injection of different amounts of the insect hormone ecdysone. This hormone has been variously termed, growth and differentiation hormone, molting hormone, and prothoracic gland hormone.

There is general agreement among insect endocrinologists that ecdysone, extracted by Butenandt and Karlson,[4] is produced by the prothoracic glands of insects. The corpus allatum produces the juvenile hormone. The hormone produced by the neurosecretory cells of the insect brain serves to activate the prothoracic glands. When the neurosecretory cells are removed, the prothoracic glands do not continue the production of ecdysone, and gametogenesis in *Trichonympha*, the genus studied most thoroughly, stops in less than 2 hours, followed by degeneration within 6–10 hours.[5] This means the prothoracic glands must be continuously activated by the brain hormone if the development of the sexual cycles is to continue. Sex in these flagellates, then, is indirectly dependent on the neurosecretory cells of their host.

Ecdysone is measured in Calliphora units, one unit being 0.0075 μg crystalline α-ecdysone. In the experiments reported here purified amorphous hormone concentrate was used. It was dissolved in insect Ringer and injected either into the thorax or the abdominal cavity of the roach by means of a special syringe which measured accurately amounts of $^1/_{10,000}$ ml. Both sites of injection gave the same results.

A total of 67 experiments was carried out. Adults and all nymphal instars except the first two were inoculated with ecdysone. In most of the experiments, 4th and 5th instar nymphs were used. The detailed results of only certain experiments are reported in this paper. A further report will be given later elsewhere.

Experiments.—In the first series of experiments 4th and 5th instar nymphs, in which *Barbulanympha*, *Saccinobaculus*, and *Oxymonas* had just begun gametogenesis, were each injected with 100 units of ecdysone. Ecdysis occurred 7–8 days later, instead of 42–47 days later as would have been the case under natural con-

ditions (Table 1). When 500 units were administered under these same conditions, ecdysis occurred after 6–7 days. In both of these experiments (100 and 500 units) the sexual cycles of the protozoa in every roach used were in exactly the same stage of development at ecdysis as under natural conditions. In other words, when a roach which would normally undergo ecdysis in 45 days is made to do so in 7–8 days the sexual cycles of its protozoa are affected in no discernible way other than time. The activities of the protozoa are closely tuned to those of their host.[6]

When only 50 units of ecdysone are given under the same conditions as were 100 and 500, ecdysis takes place on the natural schedule, i.e., 42–47 days later and all the sexual cycles of all the genera of protozoa save one, *Barbulanympha*, are also on their normal, natural schedule. This experiment, like others not reported here, shows that *Barbulanympha* is more sensitive to ecdysone than the other genera. It completes the sexual cycle 7–8 days after ecdysone is injected into its host. But a further reduction in ecdysone to 25 units will not induce sex in this genus.

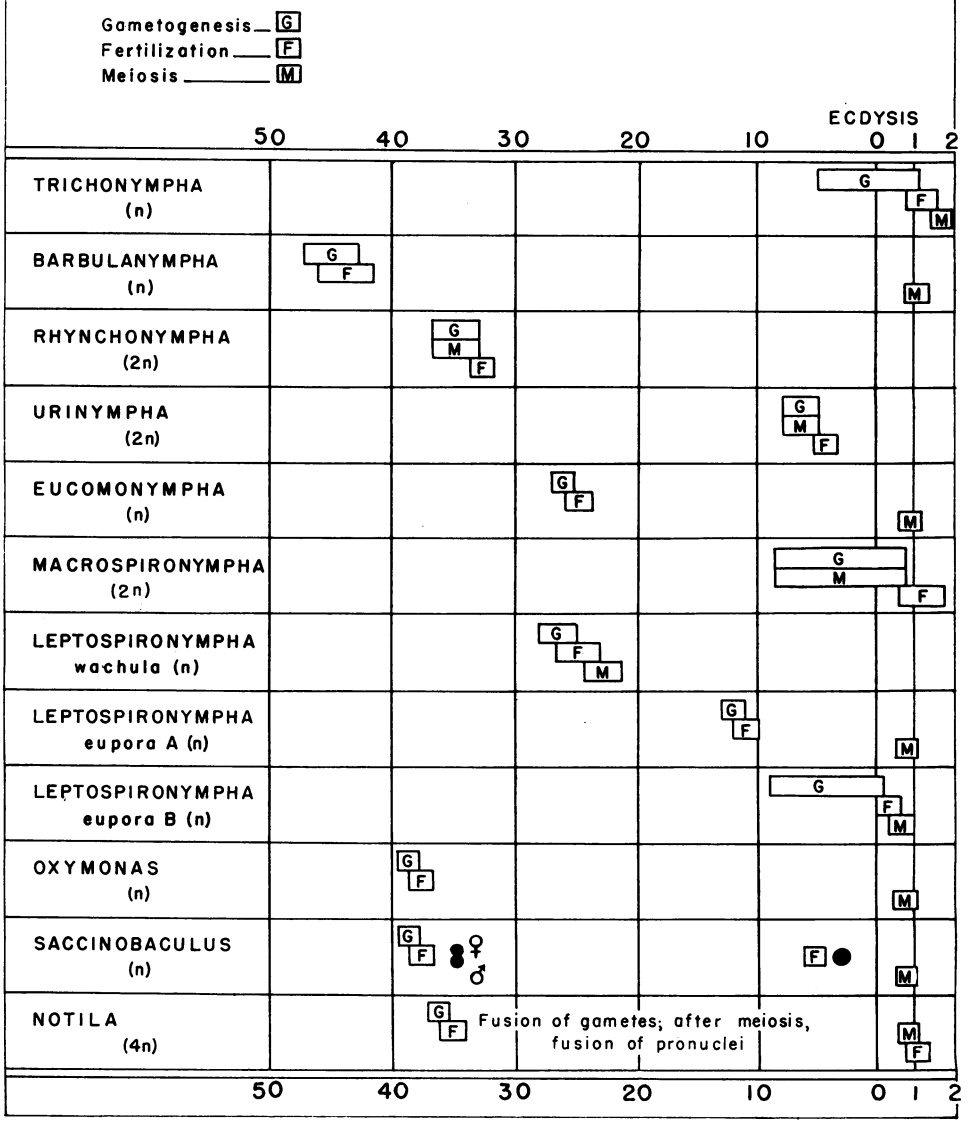
The second series of experiments dealt with intermolt 4th and 5th instar nymphs in which none of the protozoa had begun a sexual cycle. When nymphs of this type were given 100 units of ecdysone, no ecdysis occurred, even in 100 days (most of the ecdysone, as indicated by repeated small doses, probably disappears within 10 days). However, sexual cycles occurred in the protozoa, and all the cycles were completed within 7–8 days after ecdysone was given. This means the protozoa are more sensitive to ecdysone than their host. One hundred units is not enough to carry such nymphs through the molting period to completion (ecdysis), but it is enough to induce sex in their protozoa. The difference in the results of the first and second series of experiments lies in the fact that the nymphs of the first series of experiments were producing ecdysone of their own when this hormone was given them. The amount given, plus what they were producing themselves, was enough to produce ecdysis, and in a much shorter time than would have occurred otherwise. The administered ecdysone served as a booster shot.

The second series of experiments shows that, under experimental conditions, it is not necessary for a roach to undergo ecdysis to induce sex in its protozoa. Beginning about 4 days before ecdysis, the fluid of the hind-gut gradually becomes more viscous. This is true of both natural and experimental ecdyses. But this increase in viscosity did not occur in the intermolt nymphs of the second series of experiments; yet sex occurred just the same. Hence, increase in viscosity plays no role in the induction of sex in the protozoa. It was also shown that the number of organisms present in the hind gut is totally unrelated to the induction of sex. This was done by removing the protozoa from a nymph with oxygen[7] and replacing them with a small number of protozoa from an adult roach (for methods of procedure *vide infra*).

The third series of experiments are the most crucial ones of all. When adult roaches were each given 2,000 units of ecdysone, sex was induced in their protozoa, and with amazing rapidity. Most of the individuals of three genera, *Barbulanympha*, *Saccinobaculus*, and *Oxymonas*, underwent haploid gametogenesis and many examples of fertilization were present within 3 hours after ecdysone was injected. The other genera, as under natural conditions (Table 1) were slower in beginning and completing their sexual cycles. Since adults have no prothoracic glands and hence, unlike nymphs, are incapable of producing any ecdysone of their own, this series

TABLE I

Relation, in days, of the molting period of *Cryptocercus* to sexuality in its protozoa



of experiments shows clearly that ecdysone alone has the ability to induce sex in the protozoa of *Cryptocercus*.

In the fourth series of experiments 100 units of ecdysone were given nymphs from 2–10 days following ecdysis. It should be noted that two days after ecdysis, the protozoa, as shown in Table 1, have completed their sexual cycles. All of the 10 nymphs used in these experiments failed to undergo ecdysis, but, just as in the intermolt nymphs of the second series of experiments, all genera of their protozoa

underwent sexual cycles. Thus, experimental sexual cycles may be induced immediately following the completion of natural ones. And if a large dose of ecdysone is given the induction of sex occurs quickly. For example, two 4th instar nymphs were given 500 units each 5 days after ecdysis. One, which was sacrificed 3 hours later, had many zygotes of *Saccinobaculus* and *Oxymonas;* the other, sacrificed 29 hours later, had *Barbulanympha* that had completed their sexual cycle, and *Trichonympha* that had nearly finished gametogenesis.

In a final experiment 2,000 units of ecdysone were given a last instar nymph 6 hours after ecdysis. Exclusive of three species, the protozoa of such a host still must carry out either meiosis or fertilization before their sexual cycles are completed. The haploids, except for *Leptospironympha wachula,* must undergo meiosis, and the diploids, except for *Urinympha* and *Rhynchonympha,* must undergo fertilization. *Trichonympha* must complete the cytoplasmic changes that differentiate its gametes into male and female, and undergo both fertilization and meiosis. We were not able, however, to determine precisely what happens to these uncompleted sexual processes, owing to the exhaustion of our supply of ecdysone at this point in the experiments. Only one definite observation was made: the nuclei of some genera become greatly enlarged.

Inability to continue experiments with ecdysone caused us to return to experiments which, save for a few details, were completed previously. Since these experiments dealt with the role of the molting hormone in the 3 phases of a sexual cycle, namely, gametogenesis, fertilization, and meiosis, they should be reported here. The two most suitable genera for such experiments are *Barbulanympha* and *Trichonympha.* Both are haploids and, as in all haploids, gametogenesis and meiosis are separate processes. A method had already been developed for transferring the protozoa from one host to another quickly and easily.[6] A thoroughly reliable method was worked out many years ago[7] for killing all the protozoa of the recipient host before the transfer from the donor was made. It thus became a simple matter to take protozoa after gametogenesis was completed in their nymphal host and implant them in the hind-gut of an adult host in which no molting hormone was present. The protozoa were removed from such adults at rather frequent intervals for microscopic examination (phase contrast and fixed and stained preparations) to determine whether the incomplete phases of the sexual cycles were completed or not. The result was that in *Trichonympha* both fertilization and meiosis can be completed in a host without molting hormone, and that in a similar host *Barbulanympha* is able to undergo meiosis. Hence, we were able to conclude that the molting hormone is responsible for only the gametogenesis phase of the sexual cycles of these two genera. The same is very probably true for the other genera in *Cryptocercus,* remembering, of course, that in the diploids gametogenesis and meiosis occur concomitantly. In diploids, then, all phases of the sexual cycle other than fertilization are induced by the molting hormone. And, since the molting hormone is ecdysone, we may conclude that ecdysone produces gametogenesis in haploids and both meiosis and gametogenesis in diploids.

Discussion.—There is general agreement that ecdysone is the growth and differentiation hormone that is responsible for carrying an insect through nymphal or larval development to an adult. The fact that this hormone is also capable of producing growth and sexual differentiation in the protozoan flagellates that live

in the roach *Cryptocercus* is of great interest. It is not just an occasional flagellate in the jam-packed insect gut that responds to this hormone; every individual flagellate present, except an occasional one of the genus *Trichonympha,* responds. But why do these protozoa respond to ecdysone by changing their method of reproduction from asexual to sexual? In the absence of ecdysone all reproduction is asexual, that is mitotic, and is asynchronized. Each parent cell produces daughters like itself. And not more than 1 to 2 per cent of the organisms present are ever in any stage of reproduction at the same time. On the other hand, the response to ecdysone is sexual reproduction which is closely synchronized in all species of each genus. A parent cell, under the influence of ecdysone, not only produces daughters that are greatly different from itself but also daughters much unlike each other, one being a male gamete and the other a female. These gametes, in both their morphology and their physiology, plainly differ from each other at three levels, cytoplasmic, nuclear, and chromosomal. In some genera, the gametes differ from the parent cell that produces them so much that if one did not observe this change one would place the parent cell in one genus and its gametic daughters in another.

At present, one cannot explain how ecdysone acts as such a potent cellular differentiating agent on insect cells and on protozoan flagellates. Protein binding may well be an essential feature of ecdysone activity. Further study should give information on its mode of action. The protozoa will probably provide better material for such a study than insect cells because their cells are immense in comparison to those of insects, and the tremendous differentiations which they undergo all occur in a single generation.

There will doubtless be some who will ask whether the action of ecdysone on these protozoa is direct or indirect. Our present knowledge is too meager, it seems to me, to warrant a discussion of this question. There are probably several profound chemical processes concerned in gametogenesis—in protozoa as well as higher organisms.

Is ecdysone responsible for the polyploidy so common in certain tissues of many insects? For example, the tissues of the gut and Malpighian tubules consist of a single layer of cells which do not divide from the first to the last instar. However, they do enlarge with each instar, at which time the polyploidy of their nuclei also increases. This increase, both in ploidy and cell size, occurs shortly before ecdysis, and thus coincides with a high titer of ecdysone. This behavior of cytoplasm and chromosomes may be produced in response to ecdysone. The cells of the fat body behave in a similar manner, and in addition there is differentiation in them.

The giant polytene chromosomes of the salivary glands may also be produced in response to ecdysone. Their size increases at the ecdysis of each instar, at which time they are subjected to a high titer of ecdysone.

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*MULTIPLE FORMS OF ENZYMES: TISSUE, ONTOGENETIC, AND SPECIES SPECIFIC PATTERNS**

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The fundamental metabolic activities of organisms are very similar and consequently enzymes catalyzing identical reactions may be found in many different organisms and in many different tissues within an organism. When subjected to a variety of physical,[1-4] chemical,[5, 6, 7] or serological[8-17] tests enzymes from different organisms are commonly found to be different from each other even though catalyzing the same chemical reaction. In view of the demonstrated genetic control of protein synthesis[18] it is not surprising that differences should exist in the structure of homologous enzymes or proteins synthesized by animals of different species[19-24] or even by animals of different genotype within the same species.[25-29] Rather surprising, however, is the evidence demonstrating that several enzymes exist in multiple molecular forms not only within a single organism but even within a single tissue. Among the enzymes that have been reported to exist in separate molecular types within a single tissue are esterase,[30, 31] ribonuclease,[32, 33, 34] pepsin,[35] chymotrypsin,[36] trypsin,[37] lysozyme,[38, 39] cytochrome C,[40] xanthine dehydrogenase,[41] malate dehydrogenase,[42, 43, 44] and lactate dehydrogenase.[2-4, 42-49] Likewise, in yeast distinct molecular types of phosphoglyceraldehyde dehydrogenase[50] and of enolase[51] have been identified. The existence of each of these enzymes as a family of closely related but distinguishable molecular types suggests the need for an extension of the classification of enzymes beyond that based on substrate specificity alone. We propose, therefore, to use the term *isozyme* to describe the different molecular forms in which proteins may exist with the same enzymatic specificity.

In the present investigation three dehydrogenase enzymes—lactate dehydrogenase (LDH),† malate dehydrogenase (MDH), and isocitrate dehydrogenase (IDH)—have been resolved into physically distinct forms, that is, into isozymes. In addition to the dehydrogenases, three enzymes with broad substrate specifici-