the intervals is ensured by the 60-cycle alternating current.

Both impactors follow a path of motion which is oblique to the deflection of the styli levers. The object achieved by this disposition of the impactors is the dissipation of sufficient energy by frictional contact to prevent rebound, which would cause the tracing of fuzzy division lines between intervals. The impactors are light in weight and do not strike the styli until they have reached their maximum velocity. Their high velocity is then transmitted to the styli, ensuring sharp graduations.

Records made with this instrument are shown in Fig. 2. It is apparent that, at either moderately slow or moderately fast speeds of the kymograph drum, the time scale traced on the paper can be read clearly. Due to the nature of this time scale no adjustment of the intervals traced by this instrument is necessary when changing drum speeds within very wide limits.

A new nonadjustable interval timer is described which traces a characteristic time scale on kymograph records (Fig. 2). The distinctive nature of the time scale traced allows its use on most records of moderate speed. It records intervals from 1/5 sec to 1 min. The timer described is a self-contained unit which requires no dry cells or signal magnet but merely an electricity supply of 110-volt, 60-cycle alternating current.

## Induction of Cytogenetic Changes and Atypical Growth by Hexachlorocyclohexane

**Dontcho Kostoff**

***Institute of Applied Biology and Organic Development, Academy of Sciences, Sofia, Bulgaria***

We have treated with insecticides containing hexachlorocyclohexane the seedlings of the following plants: *Zea mays, Triticum vulgare, T. monococcum, T. compactum, Secale cereale, Setaria italica, Panicum miliaceum, Helianthus annuus, Crepis capillaris, Vicia faba, V. sativa, Brassica nigra,* etc. The insecticides chiefly used were: Agrocide 7, Agrocide 3, hexachlorane, 666. The main active substance of the first two is the gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane. The others contain gamma isomer and some of the other isomers. Crystals of almost pure gamma isomer display the same activity as these.

The cytological studies of the affected root, stem, and coleoptile tissues show that the agents act first upon the cytoplasm and interfere with the cytoplasmic processes involved in the formation of achromatic figures. The chromosomes do not arrange in an equatorial (metaphase) plate after prophase, but remain scattered approximately as they are during the prophase. They appear less bent than usual. The thickening of the chromosomes and their reproduction and splitting proceed, regardless of the fact that the processes involved in the formation of achromatic figures are highly disturbed or even entirely inhibited. Poles are not formed. After reproduction, the chromatolytic (nucleoproteolytic) processes proceed, and a nucleus is formed containing twice as many chromosomes as at the start of the abnormal mitotic processes. The main trend of the processes of chromosome doubling recalls those induced by colchicine, acenaphthene, and other polyploidizing agents.

Since the insecticide continues to act further, the next abnormal (Ab-) mitosis ends with a second chromosome doubling, and so on. Thus tetraploid and octoploid cells and cells of a much higher polyploid degree are formed. Along with these, certain diploid cells that have not yet undergone Ab-mitosis can still be found.

Chromosome multiplication leads to increase in the size and occasionally in the number of the nuclei and further to increase in the size of the cells. Thus the cells grow instead of multiplying and differentiating, bringing about the swelling of the roots, stems, and coleoptiles.

The chromosome reproduction and separation in *Zea mays* should be considered as a somewhat special case. We have observed in a series of cells that the chromatids of the somatic chromosomes bend at the centromeres reciprocally toward each other, each being shaped like a V, and together forming a X, the chromatids being attached at the centromere. These figures can be interpreted by postulating certain repulsion forces existing between the chromatids, the reproduction (or division) of the centromere being somewhat delayed. This phenomenon occurs when the chromatic figure is highly or completely disturbed. In other words, it does not seem to be regulated to a very great extent by the forces exerted by the achromatic figure.

The solubility of hexachlorocyclohexane in water is very low; therefore, it is applied in the form of small solid particles. The particles act when they are in contact with the plant tissue. It has a specific odor, but the experiments failed when we tried to induce specific atypical growth from a distance. In this respect its effect differed from that of acenaphthene. For the sublimating particles of the acenaphthene act even when the crystals are not in contact with the plant tissue.

The effect of hexachlorocyclohexane is so striking that it can be used as a polyploidizing agent, especially when one considers that it is much cheaper than other such agents.

In certain cases one or more chromosome groups may move slightly in various directions into the cytoplasm. Such a slight separation may occasionally end in the formation of two or more aneuploid nuclei; thus polynucleate cells or cells with monstrously deformed nuclei arise. In certain cases a cell wall is formed between such nuclei. This leads to the formation of cells with aneuploid chromosome numbers. Dead cells were occasionally found in the roots, stems, and coleoptiles; they may have been aneuploid.

All these phenomena are due to the activity of the agent upon the cytoplasm.

The active agent may also induce certain changes in the nuclear elements, i.e., in the chromosomes. Chroma-

tid and chromosome fragments were also observed, although rarely.

Such insecticides or fungicides, when applied, may increase hereditary changes in cultivated varieties (''pure lines''), leading thus to more rapid degeneration of the highly bred, uniform varieties. This means that when such insecticides or fungicides are applied the seeds of the propagated varieties should be changed more often so as to secure new nondegenerated stocks.

## Rumen Bacteria in Cobalt-Deficient Sheep

**Lorraine S. Gall,[1] S. E. Smith, D. E. Becker, C. N. Stark, and J. K. Loosli**

*Cornell University, Ithaca, New York, and Ohio Experiment Station, Wooster*

In 1944, McCance and Widdowson (*4*) suggested that cobalt may function in connection with the rumen bacteria. This view was based on a communication from C. J. Martin who stated that sheep suffering from ''Coast Disease'' were cured by feeding cobalt but not by cobalt injection. Cobalt deficiency does not occur in horses, according to Marston (*3*). Attempts to produce cobalt deficiency in rats (*3, 8*) and in rabbits (*7*) have been unsuccessful. Thompson and Ellis believe that cobalt is needed only by ruminants and that it may be concerned with biological processes in the rumen. Recently, Ray *et al.* (*5*) observed a definite response in hemoglobin and gain in weight in cobalt-deficient sheep injected with cobalt, but the response was much less than in animals fed cobalt.

In an attempt to obtain direct evidence which might show with some certainty whether or not cobalt deficiency influenced the bacteria of the rumen, studies were undertaken to measure the bacterial activity of rumen samples. Twenty-one Western yearling sheep became cobalt-deficient after being fed a ration low in cobalt for 8 months. At this time the sheep were losing weight, and had poor appetites and low hemoglobin values (*6*). These sheep were then divided into three equal groups. The first group was fed 1 mg of cobalt per day, while the second received the same amount of cobalt by injection. The remaining seven sheep were kept on the deficient ration. After 1 week sheep fed cobalt were gaining weight, and had good appetites and increased hemoglobin values, while those on the basal ration alone or those injected with cobalt continued to decline. At this time the rumen contents of four sheep from each group were sampled by stomach tube for bacteriological study, and the remaining sheep were sampled the following week. Six additional sheep were maintained for 1 month on a restricted feed intake comparable to that of the cobalt-deficient sheep. These sheep were then sampled to determine the effect of lowered feed intake *per se* on rumen bacteria. The sheep received adequate cobalt in the ration and ate 60% or more of the ration eaten by the sheep fed cobalt. The kinds and number of bacteria present in the rumen contents of the four groups of sheep were compared by means of Gram stains, direct slide counts, and anaerobic cultures (*1*).

The results of the slide counts and cultural tests are summarized in Table 1.

TABLE 1

INFLUENCE OF DIET ON RUMEN BACTERIA

| Dietary group | No. of animals | Bacterial slide count | Cultural results—No. growing in dilutions $10^{-7}$ | $10^{-8}$ | $10^{-9}$ | $10^{-10}$ | $10^{-11}$ |
|---|---|---|---|---|---|---|---|
| | | *billions/gm* | | | | | |
| Cobalt-deficient | 7 | 30.2 | 7 | 7 | 2 | 0 | 0 |
| Cobalt injected | 7 | 30.7 | 7 | 5 | 3 | 0 | 0 |
| Cobalt fed | 7 | 54.6 | 7 | 7 | 7 | 5 | 2 |
| Restricted feed intake | 6 | 56.3 | 6 | 6 | 5 | 3 | 1 |

Culturally, there were major differences between the dietary groups. The samples from sheep fed cobalt gave the highest cultural counts, all cases showing growth in the $10^{-9}$ dilution, 5 in $10^{-10}$, and 2 in $10^{-11}$ dilution of rumen contents. In marked contrast, only 2 cobalt-deficient and 3 cobalt-injected animals showed bacterial growth in the $10^{-9}$ dilution, and neither group gave growth above this dilution. Bacteriologically, sheep on restricted feed intake but fed sufficient cobalt resembled those fed cobalt and unlimited feed. Both the kinds and numbers of bacteria in the rumen content of sheep fed cobalt resembled those of sheep fed normal rations (*1*).

It can be seen by slide count that cobalt-fed sheep had almost twice as many bacteria per g of rumen contents as cobalt-deficient and cobalt-injected animals. The bacterial slide count of sheep on restricted feed intake was about the same as that of sheep fed cobalt.

Gram stains of samples were identified only by number, which had no significance to the examiner, but after microscopic study it was possible, on the basis of the stains alone, to separate cobalt-deficient sheep from cobalt-fed animals. In 5 out of 7 cases, Gram stains from cobalt-injected sheep were grouped with cobalt-deficient sheep; while in the other two instances, the bacterial picture was not clearly typical of either group.

Gram stains from sheep fed cobalt were characterized by the usual wide variety of bacteria with large numbers of *Gram-positive, slender curved rods,* and *coccoid types* of bacteria covering the fibers, which were in an advanced stage of decomposition. The slides from cobalt-deficient and cobalt-injected sheep were recognized because of a complete lack of slender curved rods, and a great reduction in the numbers of coccoid forms on the fibers. The fibers were only slightly disintegrated. The differences in the bacterial picture between the groups lay more in the absence of these bacteria in cobalt-deficient and cobalt-injected animals than in a complete change of flora. Gram stains from sheep on

[1] Formerly at Ohio Experiment Station. (Present address: National Dairy Research Laboratories, Inc., Oakdale, Long Island, N. Y.)