mental conditions: (*a*) In matings of ♂ and ♀ on various nutrient media; (*b*) in water culture matings; (*c*) in matings on agar in which the two sexually opposed strains were separated by a dialyzing Cellophane membrane; and finally (*d*) in single water cultures in water in which the opposite strain had previously grown, the water having been passed through a Seitz bacteriological filter. In this particular case a ♀ will only react when placed in water in which a ♂ has previously given the initial sexual reaction.

(3) The effects of variations in the composition of the medium offers a third body of evidence. By changing the composition of the medium the reaction can be stopped at times coinciding with the specific actions of the several hormones. These effects may depend either on the production of the substances involved or the ability of the opposed strains to react to them.

(4) Further evidence is furnished by interspecific matings between this species and *Achlya bisexualis* Coker. In each of the two reciprocal matings, *A. bisexualis* ♂ × *A. Sp.* ♀ and *A. bisexualis* ♀ × *A. Sp.* ♂, partial incompatibility is encountered. In the former the reaction stops at the time of differentiation of antheridia, while in the latter the female fails to produce oogonial initials in response to the substance produced by the exceedingly plentiful antheridial branches. These differences indicate a rather high degree of specificity of the hormones. Complete incompatibility would result if the ♂ were unable to react to hormone A of the ♀.

All the plants used in this work remain perfectly sterile in single culture, oogonia and antheridial branches forming only when ♀ and ♂ mycelia are brought together. Adequate controls have been employed throughout. A full account of the work which is summarized here will be published in the near future with complete data and methods.

The role of hormones as activators and coordinators in the sexual reaction is outlined in the accompanying diagram. On different media and under different experimental conditions the time and space intervals involved are considerably varied.

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VITAMIN C TREATMENT IN LEAD POISONING

IN a certain industrial plant where the lead hazard was exceptionally great, due largely to dust and spray, 400 workmen were examined clinically and with a differential blood count. About 40 per cent. showed distinct lead absorption or poisoning.

Of these 160, a group of 34 was selected for treatment with vitamin C (ascorbic or cevitamic acid), a treatment suggested by the bad condition of gums common to scurvy and severe lead poisoning. Half of this group was given 100 mg of vitamin C daily (but no extra calcium) for several weeks. With practically all of them there was a marked gain in vigor, color of skin, cheerfulness, blood picture, appetite and ability to sleep well. The basophilic aggregation test of Dr. C. P. McCord also indicated improvement. A typical case is reported below.

Workman Number One: Clinically diagnosed by one of us (E. J. A., a physician) as chronic lead poisoning. The man showed marked tremors, sleeplessness, a bad blood picture (differential count of white cells), nervousness. He was underweight and easily fatigued and had a sallow complexion. After five weeks of vitamin C treatment the tremors had disappeared, his complexion had greatly improved, his blood picture was encouraging, he enjoyed normal sleep, was cheerful and not easily tired.

The other half of the group of 34 continued their previous calcium gluconate treatment to drive toxic lead into the bones and supplemented this treatment with 100 mg vitamin C daily. They gained in health but not so well as the 17 men given vitamin C alone.

At this stage the research was transferred to Oberlin College, where 14 local house painters were examined. Judged by the combined evidence of a clinical examination and a differential blood count, half of these men showed enough lead absorption to call for treatment or greater precautions. Three of them were studied intensively for several weeks with careful analysis of urine for lead and vitamin C. Each was given 200 mg of vitamin C daily, in one case totaling 6,800 mg.

The chart for Painter Number One, as rather typi-

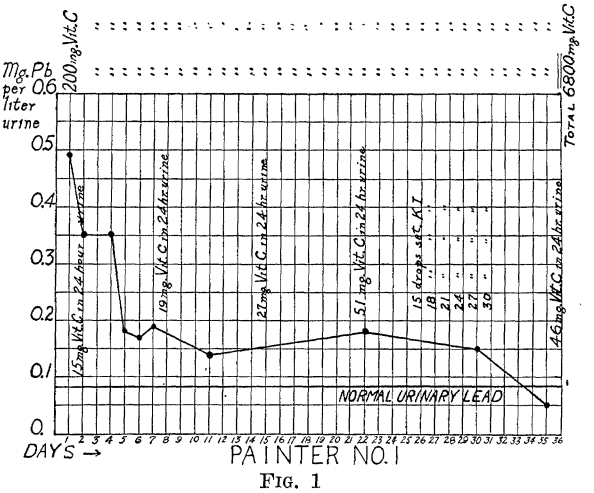

FIG. 1

cal of all three, is reproduced here. This man, a painter and sprayer, was diagnosed by a physician experienced in observation of lead poisoning as a chronic case. After only four days of vitamin C treatment he felt pleasantly lazy and sleepy but not

tired. In a few days more he "felt fine," ready for work and fresh at the end of a hard day. The differential white cell count showed decided improvement, and lead excretion fell from nearly 0.5 mg per liter of urine to normal.

Four other painters, positively known to have selected for many years a diet unusually rich in vitamin C, were examined for lead and vitamin excretion in urine. Urinary lead was near that of the average man and vitamin C excretion high.

The conclusion, supported by test-tube experiment, is that vitamin C reacts with toxic lead ions to form a poorly ionized and much less toxic compound. Therefore lead destroys this vitamin, so necessary to buoyant health, while generous vitamin C supplements to the diet remove the lead from the field of action.

Any fear that the lead is stored dangerously by this reaction is met by Sollman's parallel observation that during deleading with potassium iodide, urinary lead generally decreases because the lead potassium iodide compound is absorbed by the liver and excreted in the feces with the aid of the bile. Analysis can justify a similar explanation for final disposal of the lead-vitamin C compound.

The obvious conclusion is that men exposed to lead hazard should be advised to include in their diet plenty of such rich sources of vitamin C as tomatoes (fresh or canned), raw cabbage, oranges or grapefruit, raw spinach (or even cooked, in very little water), raw turnips, green peppers, cantaloupe, etc. Or they may take 50 mg in a vitamin C tablet as an addition to the diet. The average healthy man excretes 25 to 35 mg vitamin C daily. Any excess intake is used to restore depleted reserves or is excreted.

A full report on this work is to be published in a medical journal later.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

IRON HEMATOXYLIN STAIN CONTAINING HIGH CONCENTRATION OF FERROUS IRON

IN the study of some recently prepared permanent mounts of tissue cultures, a slightly modified Janssens' iron hematoxylin was used as a stain. This stain was made up as follows: Water 100 cc, ferric ammonium sulfate, violet crystals, 40 g, hematoxylin (pinkish brown powder when finely ground) 1 gram dissolved in 25 cc absolute methyl alcohol, glycerin 25 cc.

In one of these mounts it was noted that the cell nuclei were beautifully stained a clear, deep blue, absolutely free from the opacity sometimes met with in some poor iron hematoxylin stains. The cytoplasm, however, was stained a distinct brown color.

From the appearance of this preparation it seemed obvious that in the Janssens' stain there were two quite distinct staining agents. This was also strongly suggested by the color changes in the staining solution itself. This freshly prepared staining solution was always a clear, deep violet blue and was most vigorous in its action. After 24 hours, however, this color changed to a more and more marked yellow shade of brown and the solution lost some of its staining vigor. From these observations it seemed that the two staining agents were probably iron salts of hematoxylin in different stages of oxidation. The deep and unusually translucent blue of the nuclear stain was so selective and so sharp that it seemed highly desirable to obtain this stain in a more reliable form and without the complicating and relatively opaque brownish stain.

It seemed likely that the blue stain was from a relatively reduced form of the iron hematoxylin salt, while the brown was from a relatively oxidized form. On trial it was found that the fresh blue Janssens' hematoxylin could be changed to a brown solution by addition of hydrogen peroxide, while addition of a reducing agent, sodium sulfite, resulted in some resumption of the blue color in a solution of Janssens' hematoxylin, which had aged to a brown color.

With this confirmation two lots of hematoxylin were made up according to the above formula except that while the first used 40 grams of *ferric* ammonium sulfate crystals, the second used 40 grams of *ferrous* ammonium sulfate crystals. Of these the ferric ammonium sulfate solution showed the usual deep violet blue which in 24 hours changed to brown. The ferrous ammonium sulfate solution showed an extremely pale washed-out blue, which, however, did not change to the brown color at the end of several days. A third lot was then made up in which half of the iron salts was in the ferrous and half in the ferric form. In this solution 20 grams each of ferric and ferrous ammonium sulfate were used. The resultant solution at once assumed a deep rich blue, even deeper in color than the freshly prepared Janssens' hematoxylin. This solution was still a deep violet blue with only a trace of brown color at the end of a number of weeks.

Staining tests with this solution, which we are now using routinely for sections and for whole mounts of tissue cultures, have shown it to be a highly selective, clear, transparent blue nuclear stain which requires little or no differentiation other than soaking for a few minutes in a number of changes of distilled water