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 exerction 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 rela-

tively 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

or in Van Giesen picrofuchsin. As an instance typical of the ease of use and reliability of the stain, a series of slides was stained for different lengths of time in a trial lot of this solution which contained only 10 grams each of ferrous and ferric alum, instead of the 20 as above described. The slides were then counterstained with Van Giesen picrofuchsin (5 minutes) in the usual manner. The slides stained 2 minutes were fairly satisfactory; those stained 4 minutes were nearly ideal; those stained 15 minutes could hardly be distinguished from those stained 4 minutes, and it was only when the staining was carried up to about an hour that the image showed signs of loss of detail from overstaining.

From these preliminary studies it is suggested that the combination of a high concentration of ferrous and ferric salts serves to maintain an oxidation reduction equilibrium in the solution which is far more suitable for the formation and preservation of the selective blue hematoxylin-iron salt than is either the ferrous or the ferric salt alone. It appears that the stain, above described, is highly selective. It is relatively insensitive to variation in staining time and may be used as practically a progressive stain with little or no differentiation. This differentiation, when required, may be done by time rather than by inspection. The solution is far more stable than either Weigert's or Janssens' hematoxylin, is easily prepared and does not require any aging. This stain has not given satisfactory results when used in contact with at least some metals, as, for instance, monel.

In conjunction with Dr. Ralph Lillie, of the division of pathology, a more detailed study of this stain is being carried on at this institute in order to more accurately define its properties. Even with the data above presented, however, the question is raised as to whether or not many, or possibly even all, of our current iron hematoxylin stains would not be substantially improved by substitution of a mixture of ferrous and ferric salts or some equivalent oxidation-reduction mixture for the ferric salts now in current use.

No studies have as yet been made on the action of this stain on such cell organs as mitochondria. These considerations will be left to a later date.

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## "SPOTTING" SPECIMENS FOR CATALOGUE NUMBERS

ENAMEL spots for catalogue numbers on minerals and fossils have been superseded in this department for some years by spots of clear Duco cement. Applied direct from the tube, the cement is more convenient to handle than paint, and it hardens more

rapidly. Its surface takes india ink well from a pen, and the inconspicuous character of the cement will be especially appreciated when numbers must be applied to transparent crystals.

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### THE CARE OF SKULLS AND SKELETONS OF SMALL ANIMALS

Before shipping small skulls and skeletons, or before allowing them to be cleaned by dermestid beetles, it is usually desirable to dry them as completely as possible.

The automobile, probably now used by the majority of collectors, makes an ideal desiccating machine. Small osteological specimens fastened under the hood by wire, in the hot air stream from the fan, become perfectly dry in a day or two, even in damp weather, if the automobile receives ordinary use. A minimum of preliminary cleaning is necessary, and brains need not be removed from skulls up to the size of a rat's. Enough muscle and tendon may be left on small skeletons to hold the bones firmly together. Fly eggs fail to hatch, and maggots quickly die under this treatment.

No claim to originality is made for this method, but it has proved so useful that it seems worthy of dissemination.

RICHARD M. BOND

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