ferent pathological changes are arranged concentrically. In the center is the thread or the space through which it passed, surrounded or filled by necrotic masses. Next are reactive and reparative processes of inflammatory type, then hyaline muscle fibers, and outside these an occasional layer of calcified (previously hyalinized?) muscle fibers. Still further out are "muscle giant cells" or fiber clubs with proliferated nuclei, then thin muscle fibers with less marked nucleosis, and finally, normal muscle with some interstitial reaction. As may be expected, there is often some overlapping of these zones. If the injury and reactions are slighter, the hyalinization, calcification, and nuclear proliferation in the muscle fibers may be absent.

Following these injuries the effects of colchicine and other substances may be determined. If, after local injury to muscle, colchicine is given in repeated doses over a 5- to 10-day period, nucleosis will be slight or entirely absent. But if a single dose is administered 5 to 10 days after the injury and the animal killed 4 to 9 hours later, nucleosis will be present. In neither case have any mitotic figures been found. Sodium cacodylate is reputed to have an action similar to colchicine. Its use in these tests failed to bring out mitotic figures in the damaged muscle or to influence nucleosis, though enlargement of many nuclei occurred. When quinine sulphate or quinine chloride was given to animals with local injuries of skeletal muscle, an increased nucleosis was observed. X-ray irradiation of locally injured muscle had so far no influence on nucleosis.

In carrying out the experiments with colchicine, sodium cacodylate, and quinine, it was found desirable to bring these substances into more intimate contact with the tissue, showing nuclear proliferation. Prior to insertion, the threads were impregnated with the test substance which was used in aqueous or aqueous-gelatinous solution.

The above-described procedure by which muscle tissue is injured locally and acted upon more or less contemporaneously (a) through the general circulation, by introducing drugs either orally or hypodermically, (b) locally, by impregnating the introduced threads with the drug, and finally (c) by the combination of (a) and (b), should prove helpful as a biological test to ascertain the influence of certain substances on pathological processes in skeletal muscle.

Addendum: Since this paper was submitted for publication, W. E. Le Gros Clark has published an article (*J. Anat.*, 1946, **80**, 24) in which he describes the use of colchicine for ascertaining the lack of mitotic division in injured skeletal muscle. No mention is made of the influence of the drug on amitotic proliferation.

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On the Fluorometric Determination of Nicotinamide[1]

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After Najjar's detection (*8*) of F_2, a metabolite of nicotinamide, fluorometric methods for its determination were developed (*2, 6*). Although F_2 is measured fluorometrically, investigators are still obliged to measure concentrations of nicotinamide colorimetrically. A simple means of measuring both substances fluorometrically is, therefore, desirable.

When it was shown that F_2 is an N-methyl-β-formamidopyridinium salt (*3, 4, 7*), it became evident that the fluorometric methods for the determination of F_2 can be used for nicotinamide if the latter substance can be converted to F_2. N-methylation of nicotinamide was carried out (*5*) under reflux conditions for six hours with excess methyl iodide. It has now been found that this reaction can be effected more simply by allowing dilute methanolic solutions to stand overnight at room temperatures in the presence of excess methyl iodide. The excess methyl iodide is readily removed by evaporation under a current of air. The product, treated with alkali and isobutanol, may then be measured fluorometrically according to the method described by Najjar (*6*). Thus, given a solution of nicotinamide and N-methyl-nicotinamide chloride, the metabolite is measured as usual, and after treatment with methyl iodide, a second analysis gives the sum of the metabolite and the nicotinamide.

In the course of this work, which has since been discontinued, a simpler and more rapid method for the fluorometric measurement of nicotinamide was found. The method involves treatment of aqueous solutions of nicotinamide with cyanogen bromide according to Bandier and Hald's colorimetric method (*1*). Instead of adding the metol solution to complete the color reaction, alkali is added; the product is extracted with isobutanol, and readings are taken fluorometrically as described by Najjar (*6*). Unlike pyridoxal, the pyrimidine moiety of thiamine and certain alkaloids, the following do not interfere: pyridine, nicotinic acid, methyl nicotinate, pyridoxine, and pyridoxamine. When the test is performed as described,

[1] Thanks are due Mr. J. C. Hornberger for his assistance.

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nicotinamide gives rise to a greater fluorescence intensity than an equivalent weight of N-methyl-nicotinamide chloride. This unexpected increment is quite pronounced, and, as a result, one may measure as little as one microgram of nicotinamide. The fluorescence is stable, and measurements are reproducible. The method appears to be simpler, more sensitive, and more reproducible than existing colorimetric methods.

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Chloride-free Filter Paper

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In recent years considerable emphasis has been placed on the purity of reagent chemicals—so much, in fact, that manufacturers have found it necessary to include the analysis on the labels. Nevertheless, no restrictions upon the purity of the filtering media used in analytical work have been mentioned in laboratory procedures. Small quantities of impurities have little significance in macroanalytical work, but become proportionately serious when microanalytical procedures are used.

Benedetti-Pichler (*1*) has recommended Schleicher and Schuell filter papers No. 589 (black or blue ribbon) and No. 601 (spot test paper) in the experiments which he has outlined. In no instance has he indicated the degree of chemical purity of the above papers, although it is stated that "as a rule, the efficiency of the tests is greatly affected by the type of paper used." In an earlier work the above author and Spikes (*2*) have referred the reader to the silver test of the caution group in a discussion of the identification of the chloride ion. No caution concerning possible contamination by the filtering medium was mentioned. Chamot and Mason (*3*) in their descriptions of filtering methods for chemical microscopy, have not indicated that the filtering medium must be of any particular quality. It may be that the false concept that filter paper is pure cellulose still persists.

The recent Federal specifications (*6*) for filtering paper have not mentioned the chemical purity of the product; however, by private communication it has been found that they refer to the usual analytical operations and that no consideration was given to special tests such as might be required in biological or microanalytical work.

There are some analyses in the biological and metallurgical fields where the presence of certain ions, particularly chloride ion, in the filter paper could lead to false conclusions. An example of ion interference in metallurgical work is that of the chloride ion in corrosion studies. Here microanalytical filtering technique is employed together with microscopical identification methods. In studying the corrosion of metals, particularly that of stainless steel, the test for chloride ion is very important. Filter paper is often necessary when analyzing the minute quantities of films and deposits by the various techniques of chemical microscopy. Inasmuch as such corrosion studies call for definite negative results as well as uncontaminated positive results, it can be seen readily that traces of chloride ion would introduce serious interference.

If a circle of a commercial filter paper is shaken with redistilled water until it is well pulped, the fact that chloride is present can be easily confirmed. When the water is removed by decantation or centrifuging and tested for chloride ion, a positive result will be obtained.

Proper and Green (*4*) have stated that a hypochlorite bleaching is usually used in the preparation of pulp for filter paper, and that all of the bleach must be removed with pure water. W. Schmid (*5*) has indicated that chlorine gas as well as hypochlorite is used in the manufacture of high-grade Swedish filter papers, and that thorough washing must be done to remove the chlorides. Nevertheless, chloride ion has been found in all of the papers tested.

Since chloride-free filter papers are not available commercially, a method for obtaining these has been worked out. The demand for a filter paper of this type would normally be rather small, and, since it would not be practicable to store much material for long periods of time, the method has been so arranged that any chemist can prepare his own filter paper as required.

The Federal specifications have called for 100 per cent rags as raw material. But, the history of the rags being unknown, it would be quite possible for traces of chloride to be present from previous bleaching operations. Therefore, unbleached sulfite wood pulp was chosen as the raw material.

The pulp was bleached with sodium peroxide, although any bleaching agent which contained no chlorine could have been used. First, 5 per cent sodium peroxide, based on the pulp weight, was dis-