The American Physiological Society has elected the following officers for 1946: Wallace O. Fenn, president; Maurice B. Visscher, secretary; D. B. Dill, treasurer; and Henry C. Bazett, councilor.

Recent Deaths

Jean-Marie-Eugène Derscheid, ornithologist and conservationist, was imprisoned by the Germans in 1941 and executed on 13 March 1944. He had made several trips to the Congo, one of them with the American expedition for studying gorillas, which were headed by Carl Akeley. Dr. Derscheid was the principal promoter of Réserves Naturelles in the Belgian Congo and effected establishment of Le Parc National Albert, of which he was made director. He was an enthusiastic aviculturist, studying in particular the Anatidae, and he was the artist who designed the fine Gyrfalcon on the cover of *Le Gerfaut.* In this journal (1945, **35**, 109–111) Charles Dupond gives an account of Dr. Derscheid's contributions to science and conservation.—*Margaret M. Nice* (Chicago, Illinois).

In the Laboratory

Nucleosis of Skeletal Muscle: Its Value as a Biological Test[1]

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Damage to skeletal muscle which is not severe enough to cause necrosis provokes not only alterations in the sarcoplasma but intense proliferation of subsarcolemmal nuclei (nucleosis). Such damage may be caused by mechanical or toxic agents, by ischemia or avitaminosis-E; its nature may be unknown, as in either progressive muscular dystrophy or in denervation. The nuclear proliferation is frequently so active that the nuclei fill the sarcolemma producing the "nuclear tubes" of Waldeyer. If nucleosis occurs only in restricted zones of the fiber, it results in "muscle giant cells" or in "fiber clubs."

This proliferation may be interpreted as regenerative or as reactive in nature. It may, however, constitute the initial stage of a metaplasia of skeletal muscle into connective tissue. There is no agreement as to the manner in which the numerical increase of muscle nuclei occurs. While some authors believe that the proliferation is due to mitosis (A. M. Pappenheimer, *3*; Chor, Dolkart, and Davenport, *2*), others regard the process as "amitotic" (Tower, *4*; Altschul, *1*). Whether amitosis occurs at all, whether it is the division of damaged cells, or whether it is limited to syncytia are questions that are still under discussion. A solution of this problem regarding skeletal muscle would not only have a theoretical interest but might explain certain features in muscle degeneration and lead to an improvement of measures for the prevention of muscle degeneration.

If mitosis of subsarcolemmal nuclei does occur in denervated or otherwise damaged muscle, it should be easy to find mitotic figures amongst the great number of recently proliferated nuclei. The writer has examined several hundred sections showing nuclear proliferation in muscle fibers but has been unable to find clear evidence of mitosis; yet there have been many indications of amitosis, in the form of constrictions and, even more frequently, of fissures. These latter were especially clear if the sections were stained with cresyl violet instead of the standard hematoxylin-eosin.

Since the apparent lack of mitotic figures and the presence of morphological changes, suggesting amitosis, are insufficient to justify a final decision on the nature of this nuclear proliferation, an attempt has been made to obtain more conclusive evidence by the use of colchicine. The procedure and preliminary findings were as follows: The skeletal muscles of rabbits, guinea pigs, and white rats were denervated or locally injured by the insertion of catgut or cotton threads. Three months after denervation nucleosis and other histological changes are very pronounced. Subcutaneous injections of 0.2–0.3 mg. of colchicine per 100 grams of body weight, 4 or 9 hours before the animal was killed, failed to arrest a hypothetical mitosis, and the "colchicine effect," with its apparent or real numerical increase of mitotic figures, was lacking. The decomposition of proliferated nuclei was more pronounced than in control cases.

Focal injury by threads permits one to observe and analyze the degree of muscle damage and of tissue reactions. It is found in cross-sections that the dif-

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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.

[2] Affiliated with Merck and Company, Rahway, New Jersey, when this article was prepared.