nachgewiesen werden konnten. Möglicherweise treten diese Metabolite nur intrazellulär auf und werden dann rasch weiter metabolisiert.

Diskussion. Auffallend ist die sehr kurze Halbwertszeit der Elimination aus dem Blutplasma, die auf den raschen Oxydationsprozess zurückzuführen ist. Hierbei spielen zweifellos vorläufig nicht näher definierbare Organfermente eine Rolle, denn bei Inkubation von Natulan in defibriniertem Blut ist lediglich eine langsame, über Stunden protrahierte Autoxydation zu registrieren. Die rasche Oxydation bzw. Umwandlung der Hydrazingruppe in eine Azogruppe könnte der Grund sein, warum die N-Acetylierung, die in der Regel bei der Entgiftung von aromatischen Aminen (Sulfonamide) und aromatischen Hydraziden (Isoniazid) im Vordergrund steht, beim Natulan von sekundärer Bedeutung ist.

Als wahrscheinlich darf angenommen werden, dass bei der Oxydation der Hydrazingruppe in vivo ähnlich wie bei der Autoxydation in vitro HO-Radikale gebildet werden. Die Ergebnisse der vorliegenden Untersuchungen liefern demnach neue Hinweise für die Richtigkeit der aus der spezifischen Reaktionsfähigkeit der Verbindung in vitro abgeleiteten Auffassung über ihre Wirkungsweise als Cytostaticum[8].

Summary. The metabolism of the tumour-inhibiting methylhydrazine-derivative Natulan proceeds according to a similar pattern in man, dog and rat. Initially, a very rapid oxidation of the hydrazine-group with the formation of an azo-compound takes place, then splitting of the molecule and further oxidative degradation occurs. The major portion of the drug is excreted in urine as N-isopropyl-terephthalamic acid. Some implications of the results for elucidating the mechanism of the antitumour activity of the drug are pointed out.

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Abteilung für experimentelle Medizin der F. Hoffmann-La Roche & Co. AG, Basel (Schweiz), 26. Oktober 1964.

Succinic-Dehydrogenase Activity in the Neuromuscular Spindles of the Chick

During our studies on the location of the succinic-dehydrogenase in the muscle of the chick[1], we observed elements with intense reaction for this enzyme which are interpreted according to their morphological characteristics as neuromuscular spindles. Such elements are mostly found in the muscle erector spinae and tibialis anterior. Sections of the fresh material were cut on the freezing microtome. The medium for incubation was the following: 10 mg of nitro BT dissolved in 10 ml of distilled water, 5 ml of 0.2*M* sodium succinate and 5 ml of 0.2*M* pH 7.7 phosphate buffer. Incubation time was 10–15 min at 37°C, followed by fixation in 10% formalin. The sections were mounted in Apathy's syrup or in glycerine jelly.

In the longitudinal sections of the intrafusal fibres, we observed three zones with different kinds of reactions: (a) with thin grains disposed in longitudinal rows all along the breadth of the fibre (see Figure 1A); (b) with clusters leaving spaces among them which are probably occupied by nucleus (see Figure 1B); (c) with a very positive reaction all along the breadth of the fibre without staining the nuclei disposed in several rows (see Figure 3). Three kinds of reactions are observed in the transversal section: (a) little grains disposed all along the breadth

[1] N. I. Germino, H. D'Albora, and J. P. Wahrmann, in press.

The Figures correspond to sections of skeletal muscle of chick, with the technique to show the activity of the succinic-dehydrogenase.

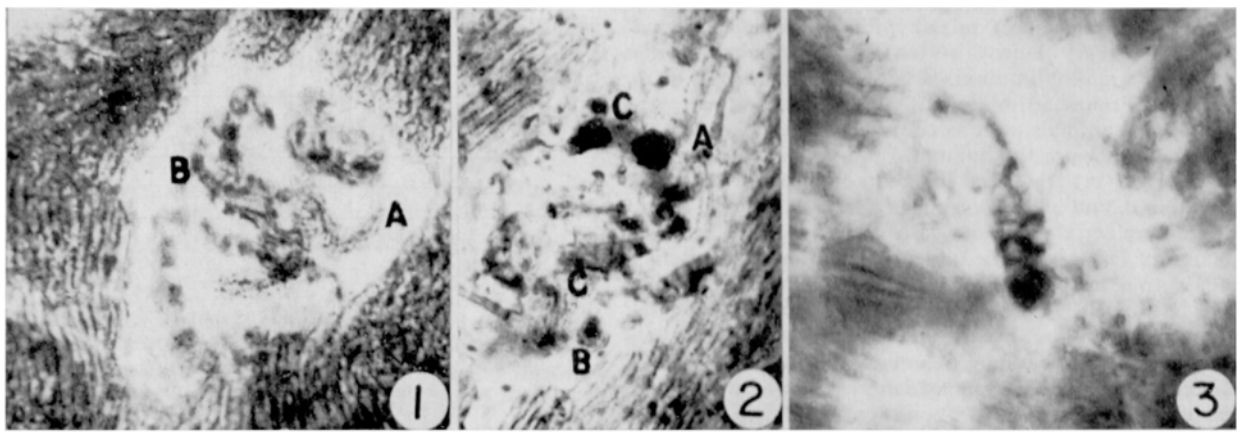

Fig. 1. Longitudinal sections of several intrafusal fibres: A – with granular reactions in rows; B – with thick clusters leaving limpid spaces among them, which are probably occupied by nucleus.

Fig. 2. Complex muscle spindle: A – a fibre with grains disposed in rows; B – transversal section fibre with only one central cluster; C – transversal and longitudinal section of zones with 'nuclear bags'.

Fig. 3. A zone of 'nuclear bags' with an intense internuclear reaction.

of the fibres (see Figure 2A); (b) only one cluster of diformazan which occupies the central zone of the fibre (see Figure 2B); (c) a big and compact cluster, where no grains are individualized, occupying the fibre entirely (see Figure 2C).

These three kinds of reactions are explained as different zones, disposed all along the fibre and coinciding with the ones described morphologically[2]: (a) A zone with well-differentiated motor innervation and myofibrils, whose reaction for the succinic-dehydrogenase is similar to the completely developed extrafusal fibres. (b) A zone with nuclei disposed in the central zone of the fibres, whose reaction for the succinic-dehydrogenase is perinuclear, and having predominance at the poles similar to the embryonary myotubes[1,3]. (c) A zone in which the nuclei are disposed in several rows with intense internuclear reaction for the succinic-dehydrogenase, and that resembling zone b is a zone where there are sensitive neural ends. The distribution of the enzyme is similar to the description that we have given of it for the muscle bud ends in vitro[3].

Briefly, we think it of interest to narrate these discoveries because they relate, as we believe for the first time, the distribution of the succinic-dehydrogenase with the three morphological zones in the intrafusal fibres which have been described for a long time. Yet we do not dismiss the possibility that some of our observations correspond to the presence of red and white fibres in the neuromuscular spindles as suggested by neurophysiological work[2]. However, at this point of our studies, we think it too soon to advance this hypothesis for certain.

Zusammenfassung. Mit der Succinodehydrogenasetechnik werden 3 verschiedene Zonen in den Muskelfasern der Neuromuskelspindeln des Hühnchens beschrieben.

N. I. GERMINO and H. D'ALBORA

Facultad de Medicina, Departamento de Histologia y Embriologia, Montevideo (Uruguay), June 8, 1964.

2 S. COOPER, in *Structure and Function of Muscle* (Ed. G. H. BOURNE, 1960), vol. 1, p. 381.
3 N. I. GERMINO, J. P. WAHRMANN, and H. D'ALBORA, in press.

Ageing and Antibody Production in the Rat

Little precise experimental data deals with the immunological activity of aged organisms[1]. This study, however, concerns many problems both in the biological and in the clinical fields: antibody synthesis, homeostasis of the reticulo-endothelial system, resistance to infection, ageing mechanisms.

This communication deals with the comparative study of antiprotein antibody production in two groups of WAG (Wistar) rats, one aged three months (called 'young'), the other twenty-two months (called 'aged').

All animals were submitted to the same experimental conditions: identical balanced diet, same immunization techniques and identical dates of blood samplings.

Each group consisted of ten animals (seven females and three males) immunized against bovine serum albumin (BSA) (Behringwerke) mixed with incomplete Freund adjuvant (IFA) (Difco), of ten animals (seven females and three males) immunized against BSA mixed with complete Freund adjuvant (CFA) (Difco), and of two non-immunized animals used as controls.

For each group, the immunization procedure consisted successively in: (1) A first injection of 10 mg of BSA dissolved in 0.3 ml of physiological saline solution and emulsified in an equal volume of IFA or CFA. (2) A second injection of BSA emulsified as above, followed by a series of six injections of BSA without adjuvant. (3) A last injection of BSA emulsified in CFA. Each hind paw received half the dose by intramuscular injection.

The first blood sample (T_1) was taken four weeks after the first injection. The second sample (T_2) one week after the sixth injection of BSA without adjuvant, and the third and fourth samples (T_3 and T_4) respectively four and seven weeks after the last injection of BSA-CFA. The entire immunological study covered a period of over five months. The titer of anti-BSA serum antibodies was determined by the passive haemagglutination test as described by BOYDEN[2] and modified by STAVITSKY[3].

The results are presented in Figures 1 and 2, which clearly show a difference in antibody production in young and aged rats. The following facts can be pointed out:

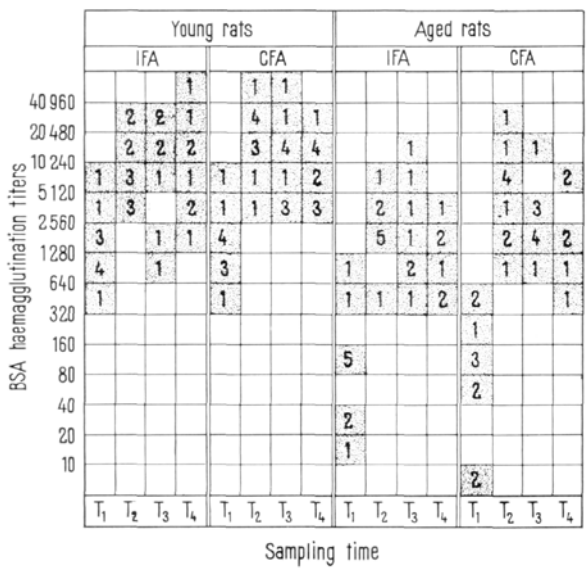

Fig. 1. In the black squares: number of animals showing identical BSA haemagglutination titers. IFA: rats immunized against bovine serum albumen mixed with incomplete Freund adjuvant. CFA: rats immunized against bovine serum albumen mixed with complete Freund adjuvant.

1 K. STERN, Gérontologia *7*, 118 (1963).
2 S. V. BOYDEN, J. exp. Med. *93*, 107 (1951).
3 A. B. STAVITSKY, J. Immunol. *72*, 360 (1954).