variabilité. La plus petite des deux doses a déjà entraîné une augmentation maximale des poids *utérins* si bien que les poids observés après la dose plus grande étaient du même ordre de grandeur. Les chiffres indiquent cependant que l'extrait administré a stimulé la croissance de l'ovaire et la sécrétion d'œstrogènes par cet organe. Nous avons tâché d'étudier la nature de la réaction ovarique par un *examen histologique*.

Les ovaires prélevés ont été fixés dans la formaline à 4% et coupés en séries. Les coupes de 6 $\mu$ d'épaisseur ont été colorées à l'hématoxyline-éosine. Chez le témoin impubère hypophysectomisé non traité (fig. *a*), les follicules sont de grandeur variable mais tous relativement petits. Chez les trois rates hypophysectomisées ayant reçu 460 $\mu$g (17 U) du HMG-$J_2$ (fig. *b*), nous avons vu des follicules plus larges que chez le témoin non traité, mais nous n'avons décelé ni une lutéinisation des follicules ni la formation de corps jaunes. Enfin, les ovaires des deux animaux traités par la double dose (920 $\mu$g = 34 U) de l'extrait (fig. *c*) contiennent non seulement des follicules en maturation à grande cavité, mais également des corps jaunes typiques, démontrant la présence du facteur lutéinisant. Cette image se rapproche donc de celle qu'on voit chez l'animal intact âgé de 20 mois (fig. *d*).

Ce travail a bénéficié de l'appui du Fonds national suisse de la Recherche scientifique. Nous tenons à remercier la CIBA S.A. à Bâle pour les rates hypophysectomisées et Melles K. Schnider et E. Simon pour leur précieuse assistance technique.

R. Borth, B. Lunenfeld,
G. Riotton et H. de Watteville

*Laboratoire d'Hormonologie de la Maternité, Genève, le 30 novembre 1956.*

*Summary*

In agreement with earlier observations on hypophysectomized male rats, a purified extract from pooled menopausal urine has been shown to contain follicle-stimulating (FSH) as well as luteinizing (LH) activity. Its administration to immature hypophysectomized female rats increased ovarian and uterine weights and induced follicle growth and the formation of corpora lutea. If the activity of the extract is measured in standardized units by bioassay in intact female mice using the uterine weight, the dose needed for corpus luteum formation in the hypophysectomized female rat seems to be approximately ten times higher than the dose producing interstitial cell repair and complete spermatogenesis in the hypophysectomized male.

## Adenosine Triphosphate Concentration in Guinea Pig Muscle after Denervation

It has been reported in a previous paper[1] that the ATP content is strongly decreased in the muscles of guinea pigs treated with tetanus toxin. ATPase activity of isolated myofibrils is increased. It is known that tetanus toxin acts mainly on the cells of the nervous system and that tetanus does not occur in denervated muscles. It therefore seemed interesting to study the effects of denervation on ATP content and on ATPase activity of the muscles of normal guinea pigs and of guinea pigs treated with tetanus toxin.

Male guinea pigs weighing 250–300 g fed on a standard diet were used. They were divided in three groups. The first one consisted of normal animals, in which the sciatic nerve of the right leg was cut and removed for the length of 1 cm. These animals were killed by decapitation at different times after denervation. The second group received a similar treatment, but 10 lethal dosis of tetanus toxin[2] were injected intramuscularly in the left leg of the animals immediately after denervation of the other leg. These animals were killed after the development of a generalized tetanus. The third group consisted of guinea pigs which were submitted to the same surgical operation as other animals, but without cutting the nerve. These animals were retained as normal controls.

Immediately after the death of the animals, gastrocnemia muscles of both legs were removed, deprived of all tendons and aponevrosis, weighed and homogenized in the cold room at 2° with a Potter-Elvehjem homogenizer, 0·25 *M* sucrose being the suspension medium. The methods for the isolation of sarcosomes and of myofibrils, as well as those for the determination of nitrogen and of phosphorus and for the fractionation of organic phosphorylated compounds, were the same as have been reported in the previous paper[1]. Oxidative phosphorylation was determined according to Dianzani and Scuro[3]. The amount of sarcosomes used corresponded to 200 mg tissue. ATPase activity of sarcosomes and of myofibrils was studied in a medium containing 0·067 *M* borate buffer, pH 6·9, $1 \cdot 10^{-3}$ *M* $MgCl_2$; $1 \cdot 10^{-3}$ *M* $CaCl_2$; 0·03 *M* ATP potassium salt. This medium was made isotonic for red cells by preparing all solutions in 0·25 *M* sucrose. The final volume was 3 ml. The reaction mixture was incubated for 15 min at 37°. After this time the reaction was stopped by addition of 2 ml 40% cold trichloroacetic acid.

As shown in Table I, denervation produced a marked depletion of ATP from the muscles, as well as a corresponding increase of ADP. It is interesting to note that in guinea pigs treated with tetanus toxin, ATP decreased more in denervated non-contracted muscles than in those of the other leg which showed tetanus.

Table II shows that P:O ratios are markedly decreased in the denervated muscles and that this decrease is still consistent 12–16 h after cutting of the nerve. ATPase activity increases in the sarcosomes from the denervated muscle in the very first hours after denervation, while ATPase activity of myofibrils does not show any modification. 12–16 h after denervation, a decrease of ATPase activity occurs in both sarcosomes and myofibrils. No difference was found between normal guinea pigs and those treated with tetanus toxin, with respect to the behaviour of ATPase activity and of P:O ratios in the denervated muscles. It is interesting to remark that ATPase activity of the non-denervated leg, which showed tetanus, was increased in animals treated with toxin, as it has been pointed out in the previous paper[1]. Phase contrast examination of denervated muscles showed the presence of sarcosome swelling.

It seems evident from these experiments that the decrease of ATP occurring in denervated muscles is the consequence of at least two phenomena, *i.e.* decreased synthesis through oxidative phosphorylation, and increased destruction through ATPase action. It seems probable that these phenomena are concerned with

[1] L. Michelazzi, M. A. Mor, and M. U. Dianzani, Exper. *11*, 73 (1955).

[2] Istituto Sieroterapico Toscano, Siena, Italy.

[3] M. U. Dianzani and S. Scuro, Biochem. J. *62*, 205 (1956).

*Table I*

Influence of denervation on the phosphorus fractions in the homogenate of gastrocnemia muscles of normal guinea pigs and of guinea pigs treated with tetanus toxin. The data are given as mg/g wet tissue, ± standard deviation

| Phosphorus fractions | Normal guinea pigs | | Guinea pigs treated with tetanus toxin | |
|---|---|---|---|---|
| | Normal leg | Denervated leg | Normal leg | Denervated leg |
| Total P | 1·583 ± 0·022 | 1·625 ± 0·020 | 1·417 ± 0·089 | 1·305 ± 0·087 |
| Inorganic P | 0·969 ± 0·016 | 1·042 ± 0·026 | 0·677 ± 0·007 | 0·590 ± 0·048 |
| Phosphocreatine P | 0·086 ± 0·003 | 0·049 ± 0·002 | 0·059 ± 0·005 | 0·056 ± 0·008 |
| 7 min P* | 0·328 ± 0·004 | 0·249 ± 0·001 | 0·225 ± 0·007 | 0·192 ± 0·020 |
| ribose in this fraction | 0·830 ± 0·017 | 0·760 ± 0·025 | 0·658 ± 0·027 | 0·590 ± 0·064 |
| Molar ratio 7 min P/ribose | 1·92 ± 0·01 | 1·60 ± 0·01 | 1·67 ± 0·03 | 1·59 ± 0·06 |
| ATP** | 1·640 ± 0·021 | 0·814 ± 0·037 | 0·825 ± 0·036 | 0·617 ± 0·085 |
| ADP** | 0·180 ± 0·032 | 0·685 ± 0·027 | 0·511 ± 0·062 | 0·787 ± 0·157 |
| Hexose diphosphate P. | 0·032 ± 0·002 | 0·026 ± 0·0013 | 0·093 ± 0·012 | 0·072 ± 0·004 |
| AMP** | 1·430 ± 0·021 | 1·960 ± 0·025 | 2·300 ± 0·030 | 2·455 ± 0·138 |
| Number of experiments | 5 | 5 | 5 | 5 |

* P released after 7 min hydrolysis with 1 N HCl at 100°.

** ATP and ADP concentrations were calculated from the values of molar ratio 7 min P/ribose, that of AMP from total P of the Ba soluble-alcohol insoluble fraction.

*Table II.* – P/O ratios and ATPase activity in both normal and denervated muscles

| Hours after denervation | Normal leg | | | Denervated leg | | |
|---|---|---|---|---|---|---|
| | P/O | ATPase activity* | | P/O | ATPase activity | |
| | | Myofibrils | Sarcosomes | | Myofibrils | Sarcosomes |
| 1 | 2·48 | 102 | 125 | 2·36 | 103 | 140 |
| 6 | 2·39 | 109 | 93 | 2·21 | 120 | 144 |
| 12 | 2·57 | 123 | 117 | 1·72 | 82 | 305 |
| 18 | 2·50 | 110 | 110 | 1·54 | 75 | 145 |
| 24 | 2·27 | 124 | 122 | 1·25 | 76 | 106 |
| 36 | 2·64 | 107 | 125 | 1·13 | 70 | 62 |
| 48 | 2·70 | 98 | 120 | 0·83 | 50 | 58 |

* The values of ATPase activity are given as $\mu$g inorganic P released in 15 min by 1 mg nitrogen of enzymatic preparation.

sarcosome swelling. This could explain also the increase of oxygen uptake described by many authors in denervated muscles[4].

The fact that these biochemical changes occur very early after cutting the nerve, suggests that a nervous mechanism may be intimately concerned in the maintainment of the normal structure of the muscle. The fact that in guinea pigs treated with tetanus toxin the extent of decrease of ATP was higher in the denervated muscles than in those showing tetanus, shows that a low content of ATP is not entirely sufficient to explain the 'rigor' which occurs in tetanus.

L. MICHELAZZI, M. A. MOR, and M. U. DIANZANI

*Institute of General Pathology, University of Genoa, Italy, July 14, 1956.*

*Riassunto*

L'enervazione produce una notevole diminuzione dell'ATP nei muscoli gastrocnemi di cavia. In cavie trattate con tossina tetanica ed enervate immediatamente prima in uno dei due arti posteriori, l'ATP diminuisce più nell'arto enervato che in quello opposto, in preda a contrazione tetanica. I rapporti P/O del muscolo enervato diminuiscono rapidamente ed un notevole grado di disaccoppiamento delle ossidazioni dalle fosforilazioni è già in atto 12 h dopo l'enervazione.

L'ATPasi dei sarcosomi dell'arto enervato aumenta notevolmente già dopo le prime ore, poi diminuisce. L'ATPasi delle miofibrille tende invece a diminuire progressivamente. Si conclude che un basso contenuto di ATP nel muscolo non basta da solo a spiegare lo stato di contrattura dei muscoli tetanizzati.

[4] C. L. HOAGLAND, Adv. Enzymol. *6*, 193 (1946).

## Ein Beitrag zum Wirkungsmechanismus des Chlortetrazyklins

In einer vorhergehenden Arbeit gelang es einem von uns[1] zu zeigen, dass das Chlortetrazyklin eine zentral stimulierende Wirkung auf das Atmungs- und das Vasomotorenzentrum der Katzen ausübt. Es stand daher die Vermutung nahe, dass diese Substanz auch in das adrenohypophysäre System eingreift.

Wir haben deshalb den Askorbinsäuregehalt der Nebennieren von weissen Ratten 2 h nach einer intraperitonealen Gabe von Chlortetrazyklin (25 und 100 mg/kg) in Natriumglyzinatlösung mit der Methode von ROE und KUETHER[2] bestimmt und mit Kontrolltieren,

[1] V. SOBEK und Z. LOJDA, Čas lék. čes. *94*, 1396 (1955).

[2] J. H. ROE und C. A. KUETHER, J. biol. Chem. *147*, 399 (1943).