substance for demonstration of the enzymatic formation of the sulfate derivative.

Materials and methods. 4-Methylumbelliferone sulfate (MUS) was synthesized by the method of SHERMAN and STANFIELD[2], or bought (Koch-Light). No difference between the two products was observed.

From ATP and sulfate-ions in the presence of Mg^{2+}, the enzymatic formation of active sulfate, 3'-phosphoadenosine 5'-phosphosulfate (PAPS) is possible. Such a PAPS-generating system was prepared in the lyophilized form from sheep brain[3]. For the activation and for the transfer of sulfate from PAPS to the accepting phenol, we used a lyophilized preparation from rat liver[4]. This preparation contains, however, both the sulfate activating and transferring enzymes. The former can be inhibited by the addition of EDTA to the incubation mixture. In this way it is possible to use this preparation for both reactions.

Our incusation circumstances do not differ essentially from those used by others for the sulfation of other compounds e.g.[3-6] and the conditions are optimal for the system described here. The sulfate activating enzyme system in our experiments contained 21 mg enzyme preparation, either from sheep brain or from rat liver, in 4.5 ml *tris*-HCl buffer 0.4 *M*, pH 7.4, containing 2.2 m*M* $MgCl_2$, 11 m*M* K_2SO_4 and 3.3 m*M* ATP. After incubation for 1 h at 37 °C the raction was stopped by heating for 60 sec in boiling water. After cooling and centrifugation 1.5 ml of the clear supernatant was added to 1 ml of a solution of MU and EDTA in *tris*-HCl buffer 0.4 *M*, pH 7.4, containing 14 mg rat liver preparation.

The final concentration of MU being 0.4 m*M* and of EDTA 50 m*M*. This mixture was incubated at 37 °C for 45 min. The reaction was stopped by heating in a boiling water-bath for 1 min. The supernatant was collected after centrifugation. This fluid was investigated for the presence of MUS.

Results. Thin-layer chromatography on silicagel (Merck, Darmstadt, 1.5 mm thickness) revealed under UV-light one spot, MU. To detect MUS on the chromatogram the thin-layer plates were suspended in fumes of HCl in a closed vessel for approximately 10 min. The chromatograms were then exposed to a current of air for a short period, thereafter they were examined under UV-light again. In this way MUS was hydrolyzed and could be detected as MU.

In the Table the Rf values of authentic and enzymatically formed MUS are presented, after thin-layer chromatography in some developing fluids. As can be seen from this Table, the Rf values of authentic and enzymatically formed MUS are in excellent agreement.

After electrophoresis on cellulose acetate (Gelman, Sepraphore III) at pH 8.6 (veronal buffer 0.05 *M*) at 350 V for 10 min, MU was left at the application line, MUS being displaced some centimeters to the anode. Hydrolysis by HCl fumes revealed MUS as MU, being visible under UV-light. The product formed by incubation appeared at the same place as authentic MUS.

Hydrolysis of the formed product with a highly specific arylsulfatase (Sigma, type III) revealed again MU, as demonstrated by chromatography and electrophoresis. Next to all this, the formed MUS and the hydrolysis products exhibit the same excitation and emission spectra on an Aminco-Bowman spectrofluorometer as do the authentic compounds.

Discussion. From all these experiments we consider the enzymatic formation of MUS proved. In other experiments we investigated the essentiality of the cofactors mentioned above. ATP, SO_4^{2-} and Mg^{2+} were found to be necessary for the activity of the system, as were, of course, the enzyme preparations. We reached the conclusion, based on the above-mentioned results, that the development of a very sensitive assay of sulfotransferase (E.C. 2.8.2) should be possible. The only question to be solved remains the quantitation of the amount of MUS formed. Preliminary experiments in our laboratory promise excellent results from the use of a Dowex H^+ cation exchanger retaining MU, all of the MUS passing the column. After acid hydrolysis the formed MUS can be very sensitively measured as MU[8].

Zusammenfassung. Die Aktivität von Sulfo-Transferasen wird mit Hilfe der 4-Methylumbelliferon-Methode nachgewiesen.

G. M. J. VAN KEMPEN and G. S. I. M. JANSEN

Biochemical Laboratory, Mental Hospital 'Endegeest', Oegstgeest (The Netherlands), 7 September 1970.

TLC of authentic and enzymatically formed MUS and of MU

Developing fluid	Rf value MUS Formed	MUS Added	MU
Butanol-ethanol-ethylmethylketon-water (3:3:3:1)[7]	0.6	0.6	0.8
Butanol-ethanol-ethylmethylketon water (4:2:3:1)	0.3	0.3	0.8
Butanol-ethanol-ethylmethylketon (4:2:3) saturated with water	0.4	0.4	0.8
Butanol-ethanol-water (3:3:1)	0.8	0.8	1.0
Butanol-acetic acid-water (10:2:5)	0.6	0.7	1.0

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8 The technical assistance of Mr. R. VAN ELK and Mr. F. VAN DER MARK is greatfully acknowledged.

A Method for Determining the Maximal Stimulus Strength for Massive Stimulation of Skeletal Muscle

Massive stimulation with square pulses is now widely used in experiments where the simultaneous excitation of all parts of a muscle, or a muscle fiber preparation, is desired[1-4]. The preparation is usually placed longitudinally between 2 parallel platinum or silver-silver chloride plate electrodes surrounded by a physiological salt solution. Excitation is effected by passing brief electric pulses through the solution.

The suitability of a stimulator for maximal massive stimulation is usually tested by determining if the

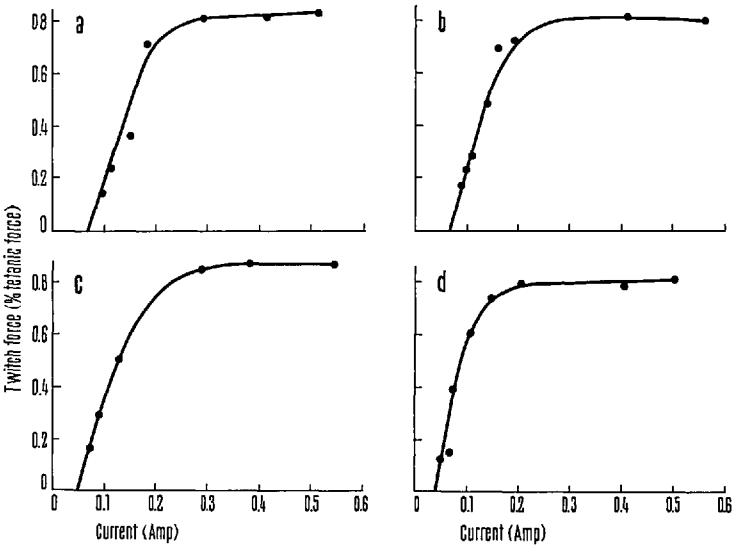

Fig. 1. Strength-response curves of frog sartorious muscle at different stimulus durations: a) 0.18 msec; b) 0.22 msec; c) 0.27 msec; d) 0.42 msec.

instrument is capable of delivering a minimal stimulus which results in a maximal response from the preparation under investigation. This test requires that the stimulator be capable of delivering a supramaximal stimulus. There is, however, no technique for determining the power and current capability which a stimulator must have in order to stimulate maximally under the existing experimental conditions if the available instrument is inadequate. The experiments described in this report were performed with a view to solving this problem.

Sartorious muscles of *Rana pipiens* were used in all the experiments. The preparations were suspended between 2 platinum plate electrodes immersed in frog Ringer solution kept at 6 °C. The muscle bath used was essentially the same as that described by McCrorey, Gale and Alpert[5]. The electronic switch described previously[6] was used for stimulation.

Each experiment was performed at the length of maximum contractility of the preparation, determined experimentally. The preparation was stimulated once every 5 min throughout the duration of the experiment, whether or not any records were being taken. The force developed, if any, was recorded on a Tektronix storage cathode ray oscilloscope, model 564, from which the twitch forces were read and photographic records made.

The experimental data were collected as follows: A stimulus duration was selected by setting the duration dial of the Grass stimulator. (However, due to the inaccuracy of the controls of the Grass S8 stimulator, the actual stimulus duration was read on the cathode ray oscilloscope.) The stimulus current was then increased in steps until a definite plateau was reached. A twitch and a tetanus were then recorded using a supramaximal current of 1.4 amp applied for 0.2 msec. The tetanic force was obtained with a stimulus frequency of 40 Hz applied for 400 msec.

Figure 1 shows typical stimulus response curves obtained for 1 preparation at different stimulus durations. Analysis of the data obtained from 4 different preparations showed that, at any stimulus duration, the twitch force rises hyperbolically as a function of the current flowing through the muscle bath. This relationship was best described by the equation $P = P_o - K/I$, where P_o is a constant which is equal to the tetanic force obtained with a supramaximal stimulus, P is the twitch force obtained with a submaximal stimulus, I is the current in amperes flowing through the muscle bath and K is a constant. The slope of the regression of P on $1/I$ was found to be highly significant at all stimulus durations. Plots of such regression lines obtained for 1 preparation are shown in Figure 2. The values of P_o obtained by extrapolation, as shown in Figure 2, were found to be identical with the corresponding recorded values of tetanic forces. The mean ratio of prediction to observation was $1 + 0.01$ SE(14).

Since the regression line of P on $1/I$ at any stimulus duration can be obtained without knowing the magnitude

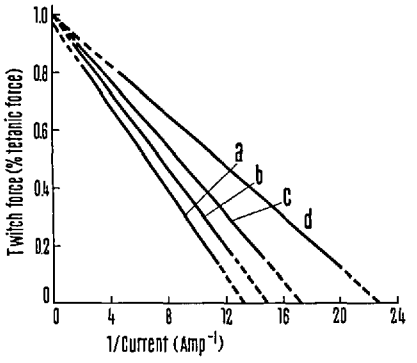

Fig. 2. Regression lines of the submaximal twitch force on the reciprocal of the current flowing through the muscle bath for the same preparation discussed in Figure 1. The coefficients of determination (r^2) were 0.91, 0.92, 0.97 and 0.93 for a, b, c, and d respectively. Extrapolations of the regression lines are shown as dashed lines.

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of the maximal twitch force, and the maximal tetanic force can be obtained from the regression analysis, the value of the maximal stimulus strength can be determined even if the available stimulator is inadequate for massive stimulation, provided that the maximal twitch to tetanus ratio can be estimated by direct stimulation or from the literature. The magnitude of the minimal current giving the maximal response – usually called the maximal stimulus – is calculated from the value of $1/I$ which corresponds to the maximal twitch force. The maximal stimulii obtained by this method were found to be in good agreement with those obtained experimentally[7].

Zusammenfassung. Eine Methode zur Bestimmung des zur maximalen, anhaltenden Reizung von Froschmuskeln benötigten Reizstroms wird beschrieben.

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Department of Medical Physiology, Nairobi University, Nairobi (Kenya), 10 September 1970.

[7] This study was supported in part by USPHS Training Grant No. GM 00439-08. The experimental part of this study was done at the University of Vermont, Burlington, Vermont, USA.

CONGRESSUS

Hungary
Second International Congress of Psycho-Neuroendocrinology

in Budapest, 1–3 July 1971

The program will consider the following topics: Developmental neuroendocrinology; Biosynthesis and release of pituitary trop-hormones; Drug actions on neuroendocrine and brain mechanisms; Hormonal effects and brain mechanisms; Developments in clinical neuroendocrinology.

Each session will be followed by a free discussion of 6 min duration and presentation of new unpublished data by registered participants.

A registration fee of U.S.$ 20.– or of 20 rubels (for participants from socialist countries) will be requested from participants till March 31, 1971, at the following address: Hungarian National Bank, in favour of the IBUSZ account No. RCS 505019.

Further information from: Secretariat of the Congress of the International Society of Psycho-Neuroendocrinology, Motesz, Aprod-ut. 1–3, Budapest (Hungary).

Switzerland
Third International Congress for Stereology

in Berne 26–31 August 1971

Under the auspices of the International Society for Stereology the meeting shall comprise interdisciplinary sessions on basic stereological methods, their mathematical foundations and their application to various disciplines. Analysis of shape, topological properties, size distribution and number of particles on microscopic sections shall receive special attention. Further topics include sampling problems and instrumentation, particularly automatic image analysis and data processing. Information and provisional program by: Third International Congress for Stereology, Anatomisches Institut der Universität, Bühlstrasse 26, CH-3000 Bern (Switzerland).

CONSTRUCTIONES

European Training Awards in Brain and Behaviour Research

In cooperation with the Organization for Economic Cooperation and Development, a group of European Scientists have initiated an experimental schema under which younger scientists working on Brain and Behaviour can apply for awards to enable them to acquire training in a specialized area. The money to finance this training program has been provided by the Max-Planck-Gesellschaft. Successful applicants will receive travel and living expenses to enable them to study in selected laboratories. The normal duration of an award will be three months, but some longer term awards can be made.

Eligibility. To be eligible for an award, a candidate must already by undertaking research in the field of Brain or Behaviour in a laboratory situated in a member country of O.E.C.D. Applicants must produce evidence that their own research will benefit by the training for which they apply. In making the awards, preference will be given to candidates applying for a type of training that will assist them to follow an interdisciplinary approach in their own research. Candidates are expected to return to their original laboratory at the expiry of their training.

Nature of training courses. Some of the training programs incorporate formal course work, others involve the learning of techniques whilst undertaking closely supervised research on a particular problem. Training programs exist in the following subjects: Animal behaviour, brain biochemistry, brain modelling, ethology, experimental psychology, histochemistry, morphology, neuroanatony, neuropharmacology, neurophysiology etc.

Method of application. Further details of the scheme (including a list of laboratories participating in the training programs) and application forms can be obtained from:

The Executive Office, Foundation FUNGO, Laan van Meerdervoort 53D, Den Haag (The Netherlands).