an increase of the voltage and decrease of the duration of arousal in the 1st h after injection. However, in the next $^1/_2$-h the duration of arousal was normalized but the value of amplitude was still significantly higher comparing with the control value. Clearly this suggests the existence of the time differences in the recovery of the structures which are involved in the synchronisation and arousal after the administration of a CNS depressant drug.

Discussion. In spite of the fact that averaged signals of linear characteristics give better visual presentation of the amplitude variation in arousal than normal EEG records, we considered them to be insufficient criteria for the determination of the end of arousal. This is due to the fact that oscillating averaged line can, for short periods of time, be equal or greater than the pre-arousal averaged amplitude value, thus giving a false impression that arousal has been terminated. Evidence that arousal has indeed not terminated came from the integrated amplitude value, which in a period of 10 sec did not reach the control pre-arousal value. For this reason we suggest the simultaneous use of both parameters – integrated and averaged signals – as a more accurate way to determine the end of arousal.

The use of the integrated EEG-signals together with an averaged amplitude line gives not only objective criteria for determination of the end of arousal, but also the possibility to compare the changes in the duration of the arousal and amplitude values during the pre-arousal and arousal period. Using this method in experiments with the rats treated with the GABA-like substance HA-966, we have shown that the increase of the amplitude in the pre-arousal period was not followed by changes in the duration of arousal reaction in the recovery time. This indicates that certain drugs may have a 'dissociative' effect, showing that synchronisation in the pre-arousal period and duration of the arousal might be two independent phenomena not necessarily correlated. It is of interest to note that the substance HA-966 has earlier been described to induce dissociation between EEG-pattern and behaviour[8]. In this respect it would be of importance to conduct similar studies with other 'dissociation' inducing drugs.

In addition we should like to point out that the method described for the determination of the duration of arousal with the parallel registration of amplitude can be applied to any other animal with any type of stimulus for the induction of activation pattern and is particularly suitable for laboratories without computer facilities.

In our experiments we intended to exclude the small spontaneous variation of the amplitude, thus we chose the relatively small number of resets (20) to correspond for 150 μV in 1 min period time. However, the possibility still exists of using a higher number of resets (60–80 etc). If this variation is applied than the arbitrary end of arousal should be considered when the post-arousal amplitude of EEG signals reaches the 90% of control pre-arousal voltage value. The exact point of the end of arousal within the 10 sec time interval already selected is determined by the averaged line as described before.

Résumé. On décrit une méthode établie pour déterminer la durée de la désynchronisation corticale en utilisant les changements d'amplitude de signaux électro-corticographiques. Un intégrateur Grass a été utilisé pour enregistrer d'amplitude moyenne et pour intégrer les signaux EEG. La phase de désynchronisation est considérée comme terminée quand l'amplitude des signaux, avant l'excitation, réapparaît et reste à ce niveau pendant 10 sec. Le point culminant de la ligne de moyenne pendant la période de 10 sec (qui n'exède pas le point culminant de la ligne de moyenne en la phase précédant l'excitation) est choisie comme indiquant la fin de la phase de désynchronisation. L'usage de la méthode démontra que les effets d'un nouveau congénère du GABA, l'HA-966 sur la synchronisation de l'EEG et sur le raccourcissement de la réaction d'éveil peuvent exister indépendamment l'un de l'autre.

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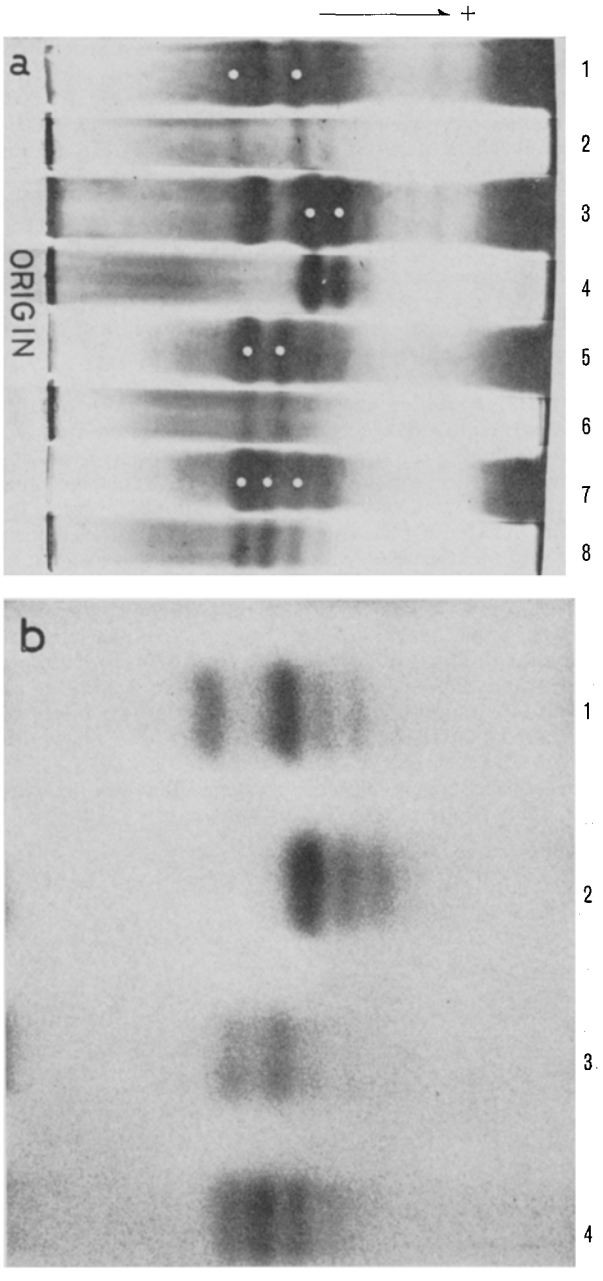
Improved Method for Separation and Identification of Serum Transferrins: Thin Layer Acrylamide-Gel Electrophoresis with Acrinol Pretreatment

For the separation and identification of serum transferrins, starchgel electrophoresis[1] has been widely employed in combination with the labelling of the protein with radioactive iron (Fe^{59}) followed by autoradiography[2]. It is still desirable, however, to develop more rapid, accurate and safe method for these purposes. A combination of acrinol pretreatment and thin layer acrylamide-gel electrophoresis seems to fulfill the above requirements, as demonstrated in the present study. Using this method one can identify transferrin bands on the gel clearly and rapidly without any use of the hazardous radioactive isotope and subsequent autoradiography.

For the acrinol pretreatment, a preparative method[3] of transferrin using acrinol and ethanol was modified to the smaller scale. As a routine procedure, 100 μl murine serum, mainly *Rattus rattus* in our case, was mixed with 1 μl of 0.6 m*M* $FeCl_3$ solution and diluted 4 times with 300 μl of 5 m*M* *Tris* buffer (pH 8.8). To that mixture was added 400 μl of 0.6% acrinol solution prepared just before use. After 30 min, coagulated proteins were removed by centrifugation at 3,000 rpm for 10 min at 0 °C. An equal volume of 95% cold (–10 °C) ethanol was added to the supernatant. After standing the mixture in an ice bath for 30 min, the protein fraction was spun down at 3,000 rpm for 10 min at 0 °C, after which it was dissolved in 100 μl of 5 m*M* *Tris* buffer and subjected to the thin layer acrylamide-gel electrophoresis.

The procedures for the thin layer acrylamide-gel electrophoresis essentially followed a previous report[4]. Acrylamide was dissolved at the final concentration of 3.8%, methylenbisacrylamide (BIS) 0.2%, tetramethylethylendiamine (TEMED) 0.3% and ammonium persulfate 0.036% in *Tris* citrate buffer (pH 7.4) containing

[1] O. Smithies, Biochem. J. *61*, 629 (1955).
[2] E. R. Giblett, C. G. Hickman and O. Smithies, Nature, Lond. *183*, 1589 (1959).
[3] E. W. Böttcher, P. Kister and Hs. Nitschmann, Nature, Lond. *181*, 490 (1958).



a) Acrylamide-gel electrophoretic patterns of black rat sera and the transferrin fractions partially purified with acrinol and ethanol. Sample Nos. 1, 3, 5 and 7: crude sera; 2, 4, 6 and 8: partially purified transferrins. 1 and 2: Tf-CF in *Rattus rattus* collected from Kanpur in India. 3 and 4: Tf-C_1 from Kandy in Sri Lanka. 5 and 6: Tf-CE from Islamabad in Pakistan. 7 and 8: Tf-DE from Kandy in Sri Lanka. b) Autoradiographic demonstration of transferrin bands on the acrylamide-gel. Sample Nos. 1, 3, 5 and 7 are correspond to those dotted in Figure a.

64 m*M* *Tris*-aminomethane and 11.2 m*M* citric acid. The mixed solution was poured into the lucite form (100 × 180 mm square inside and 1 mm thick) which was put on a glass plate (120 × 200 × 5 mm) overlaid by a wet cellophane. Then it was covered by a lucite lid with 10 slot formers (8 × 1 × 1 mm) at the starting positions. After standing at room temperature for 3 hours, gelation of the thin layer plate (100 × 180 × 1 mm) was completed. Usually the electrophoresis was conducted at the room temperature for 4.5 h with a constant current power supply at 1.0 mA/cm gel width. After staining for 30 min with amide black, the gel was washed repeatedly by 7% acetic acid.

We have applied the present method to analyse serum transferrin polymorphism in feral black rats, *Rattus rattus*, collected from India, Pakistan and Sri Lanka. Typical electrophoretic patterns are shown in Figure a, where Tf-C, -C_1, -D, -E and -F could be definitely identified following acrinol and ethanol treatment (See sample Nos. 2, 4, 6 and 8). In order to confirm that these bands are transferrins, the crude sera (Sample Nos. 1, 3, 5 and 7) were labelled with Fe^{59}. Position of transferrins on the gel was examined by autoradiography. Figure b indicates beautiful coincidence of the radioactive bands with the transferrin bands exhibited by the acrinol method. Detailed data will be published elsewhere[5]. The relative distances between these transferrin band were almost similar to those demonstrated by the starch-gel electrophoresis method for rats[6].

The improved method described here seems to be useful for the easier analysis of serum transferrin polymorphism, not only in the rodents but in many other vertebrates including man.

Zusammenfassung. Verbesserte Methode für die Separation und Identifihation von Serum-Transferin. Bei Behandlung von Rattenserum (*Rattus rattus*)mit Acrinollösung und Alkohol und darauffolgender Dünnschicht-Acrylamidgel Elektrophorese können 12 Transferrine gut separiert werden.

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[4] Z. OGITA, M. HASHINOTSUME and Y. KOSUGI, Sabco J. *2*, 58 (1966).
[5] K. MORIWAKI, K. TSUCHIYA, H. KATO, T. H. YOSIDA and T. SADAIE, Ann. Rep. Nat. Inst. Genet. *23*, 18 (1973).
[6] K. MORIWAKI, K. TSUCHIYA and T. H. YOSIDA, Genetics *63*, 193 (1969).
[7] Contribution No. 981 from the National Institute of Genctics, Japan. This study was supported in part by grant-in-aid Nos. 834043 and 748003 for fundamental scientific research from the Ministry of Education in Japan.

CONGRESSUS

Austria

1st International Congress on Human Ecology

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