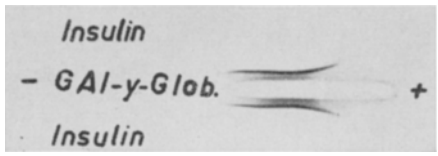
benen Methode isoliert: in GAIS wird bei 4 °C unter kräftigem Rühren tropfenweise ein Aliquot von 3,2 *M* Ammoniumsulfat (in NaCl 0,9%) eingebracht, der Niederschlag in der Kälte abzentrifugiert, zweimal gewaschen, in NaCl 0,9% aufgenommen und über eine Sephadex-G-50-Säule entsalzt; abschliessend erfolgen Reinigung über DEAE-Sephadex A 50 und Konzentrierung mittels Druckfiltration (Ultrafilter Lsg 60 Sartorius Göttingen). Das gewonnene GAI-γ-Globulin ist eine elektrophoretisch einheitliche Fraktion; die Ausbildung einer starken Präzipitationslinie mit Rinderinsulin in der Immunelektrophorese (Figur) weist einen hohen Anteil von PIAK im GAI-γ-Globulin aus.

Mit unseren Befunden ist erneut der Beweis erbracht, dass es gelingt, präzipitierende IAK zu erzeugen und dazu eine Spezies heranzuziehen, die grosse Mengen eines einheitlichen hochtitrigen Antiserums zu gewinnen erlaubt. An der Isolierung der PIAK aus GAI-γ-Globulin und deren physikochemischer Charakterisierung wird gegenwärtig noch gearbeitet[11].



Immunelektrophoretischer Nachweis der PIAK im GAI-γ-Globulin.

Summary. Antiserum was produced against beef insulin in male goats (goat anti-insulin-serum). It contains precipitating insulin antibodies; they were identified by immunelectrophoresis as a fraction of γ-globulin.

M. Ziegler und H. G. Lippmann

Institut für Diabetes «Gerhard Katsch», Bereich experimentelle Diabetesforschung, 2201 Karlsburg/Greifswald (DDR), 23. September 1968.

[1] K. Brunfeldt und T. Deckert, Acta endocr., Copenh. *47*, 353 (1964).
[2] E. R. Arquilla und J. Finn, J. exp. Med. *122*, 771 (1965).
[3] P. J. Moloney und M. A. Aprile, Can. J. Biochem. Physiol. *37*, 793 (1959).
[4] O. J. Birkenshaw, S. S. Randall und P. C. Risdall, Nature *193*, 1089 (1962).
[5] Y. Hirata und H. T. Blumenthal, J. Lab. clin. Med. *62*, 683 (1963).
[6] H. Finger, W. Schaeg und H. Niemann, Z. Immun. allg. klin. Immun. *134*, 293 (1967).
[7] J. H. Humphrey und R. G. White, in *Immunology for Students of Medicine* (Blackwell Scientific Publications, Oxford 1965), p. 446.
[8] J. Freund und M. V. Bonanto, J. Immun. *48*, 325 (1944).
[9] O. Ouchterlony, Acta path. microbiol. scand. *26*, 507 (1949).
[10] H. Voss, G. Henneberg, R. Herrmann, H. Pichl, S. Schulte-Oberberg und H. Werner, Zbl. Bact. Orig. *1*, 203 (1967).
[11] Die Untersuchungen wurden mit Mitteln eines Forschungsauftrages des Ministeriums für Gesundheitswesen der DDR durchgeführt.

Modified in vitro Assay for Melanocyte-Stimulating Hormone

A method for increasing the sensitivity of the in vitro bioassay[1] for melanocyte-stimulating hormone (MSH) has been recently reported[2]. The present communication describes some modifications of this assay method which decrease its variability.

Methods. The equipment used for this assay has been described by Long and Guillemin[3]. The gray enamel standard reflects approximately 28% of the light which is supplied by the Photovolt Photoelectric Reflection Meter model 610 and is used to set the reflectance scale at 100. The procedure of Geschwind and Huseby[2] is followed, except that reflectance readings with MSH standard (Δ_1) are obtained before addition of the unknown sample (Δ_2) and the treated skins are rinsed twice with Ringer's solution during the 1 h equilibration periods.

A 3 factor factorial experiment was designed to evaluate the effects of the following on the variation of the assay procedure: (A) constant illumination of the frogs, (B) pretreatment of the frog skins with MSH, and (C) application of 2 rules for selection of suitable skins.

Exposure of the frogs to constant illumination (900 foot candles) was accomplished by means of fluorescent lights placed approximately 15 cm above the frogs for at least 48 h. Control frogs were kept in darkness for the same period of time.

Pretreatment of each frog skin with 1 unit of MSH for 1 h was followed by a rinse with Ringer's solution for 1 h. This preceded the usual addition of 1 unit of MSH standard which resulted in the Δ_1 reading.

The first selection rule requires that a frog skin must give an initial response of at least 8 reflectance units to qualify for use in the assay. The second skin selection stipulates that 1 h after treatment with 1 unit of MSH standard, the light reflectance of the frog skin must be similar to its original value. Since the failure of a skin to return exactly to its baseline state of pigmentary dispersion will be a function of its initial sensitivity to the standard MSH, the following arbitrary values were used for the second rule: if the initial response (Δ_1) to the 1 unit of MSH standard is 8–10 reflectance units, the frog skin must return exactly to the original value; if 11–20, it must return to within 1 reflectance unit; if 21–30 to within 2 reflectance units; if over 30, to within 3 reflectance units.

In order to examine the potential effects and interaction of the 3 sources of variation just indicated, an analysis of variance of the within cell variances was performed. Since sample variances are distributed as χ square variables, a square root transformation was made, thus satisfying the requirement of normality by the analysis of variance. The sampling provided the required independence. The response to 0.5 units of MSH was utilized as the 'unknown' and designated Δ_2.

Results and discussion. The results of the analysis of variance of the experimental variances after light, pre-

[1] K. Shizume, A. B. Lerner and T. B. Fitzpatrick, Endocrinology *54*, 553 (1954).
[2] I. I. Geschwind and R. A. Huseby, Endocrinology *79*, 97 (1966).
[3] J. M. Long and R. Guillemin, Experientia *17*, 132 (1961).

treatment with MSH, and application of the skin selection rules are presented in Table I. It can be seen that previous exposure of the frogs to constant illumination and pretreatment of the frog skin with 1 unit of MSH did not significantly decrease the variation despite indications to the contrary in preliminary experiments. The interaction of the effect of rules with the effect of light probably reflects a slight tendency for light to reduce the experimental variation.

Application of the first of the 2 rules for selection of frog skins resulted in the greatest reduction in the amount of variation among the Δ_2/Δ_1 observation ($p < 0.01$). Use of the second rule caused a further statistically significant reduction in variation ($p < 0.01$). The marginal means of the transformed variances for the rules are as follows: no rule = 0.3236; rule 1 = 0.1704; rule 2 = 0.1312. The index of precision (λ) = 0.204 when the rules are applied.

It is obviously desirable that the application of the rules should not change the mean value of the observations within any given treatment. To determine whether the rules did actually comply with this requirement, an analysis of variance was performed upon the observations. The results of this analysis are presented in Table II. It is evident that none of the treatments changed the observed means.

Table I. Analysis of variance of transformed variances

Source	*df*	Mean squares	*P*
Light	1	0.00015194	NS
Pretreatment	1	0.00000850	NS
Light × pretreatment	1	0.00058427	NS
Rules	2	0.04103082	< 0.01
Light × rules	2	0.00529057	< 0.025
Pretreatment × rules	2	0.00090760	NS
Residual	2	0.00006908	–

Table II. Analysis of means
Analysis of variance

Source	Mean squares	*P*
Light	0.00160777	NS
Pretreatment	0.00086530	NS
Light × pretreatment	0.00204624	NS
Rules	0.00226067	NS
Light × rules	0.00056293	NS
Pretreatment × rules	0.00022678	NS
Light × pretreatment × rules	0.00174125	–
Error	0.00084365	–

Table of means and sample sizes

	No rule		Rule 1		Rule 2	
	No MSH	MSH	No MSH	MSH	No MSH	MSH
No light	0.6106 (123)[a]	0.5675 (126)	0.5176 (87)	0.5738 (82)	0.5832 (39)	0.5427 (38)
Light	0.5929 (125)	0.6480 (121)	0.5813 (72)	0.5934 (80)	0.5283 (46)	0.5904 (55)

[a] Sample size.

Efficient application of the rules requires that MSH standard be applied to the frog skins as the first treatment so that only suitable skins be used for assay of the unknown solution. Those investigators[1,2,4] who test for the MSH activity of the unknown solution (Δ_1) before addition of the standard (Δ_2) could still apply the rules after completion of their assay.

This in vitro method can detect MSH activity in the plasma of normal rats and has been used to measure changes in this hormonal activity after various treatments[5]. Although 50 and 100 µl of rat plasma are usually employed in the assay, a linear log-dose response curve from 50–400 µl of plasma has been obtained consistently. The addition of up to 1 ml of rat plasma failed to diminish the response of the frog skins to standard MSH. No difference was detected in the MSH activity of hemolyzed rat plasma when compared with non-hemolyzed plasma.

Although the assay described here did not find the inhibitory effects of rat plasma upon frog skin response to MSH noted by GESCHWIND and HUSEBY[2], unextracted human plasma did seem to possess these properties. Whereas 50 µl of human plasma did not affect the response of the skins to standard MSH, as little as 100 µl (10% of the added volume) was found at times to exert an inhibitory effect. MSH activity could not be measured in normal human beings with this bioassay despite the detection of MSH activity in untreated albino rats.

For convenience, accuracy, and precision, the in vivo bioassay method for MSH[6] is still preferred. However, neither it[6] nor the usual in vitro method[1] are able to detect MSH activity in normal rat plasma. The modified in vitro bioassay reported here, therefore, provides a suitable and improved assay system for measurement of MSH activity in the plasma of rats[7,8].

Résumé. L'activité de l'MSH dans du sérum de rat peut être mesurée maintenant grâce à une méthode nouvelle, très sensitive, de test biologique in vitro. Cette méthode a été modifiée de façon à réduire à un minimum acceptable des variations expérimentales.

A. J. KASTIN, M. C. MILLER III and A. V. SCHALLY

Endocrinology Section of the Medical Service and Endocrine and Polypeptide Laboratories, Veterans Administration Hospital and Departments of Medicine and Biostatistics, Tulane University School of Medicine, New Orleans (Louisiana 70140, USA), 16 July 1968.

[4] D. P. ISLAND, N. SHIMIZU, W. E. NICHOLSON, K. ABE, E. OGATA and G. W. LIDDLE, J. clin. Endocr. *25*, 467 (1965).
[5] A. J. KASTIN, A. V. SCHALLY, S. VIOSCA and M. C. MILLER Endocrinology, January 1969.
[6] A. J. KASTIN and G. T. Ross, Experientia *29*, 467 (1964).
[7] Supported by NIH Grant Nos. NB 07664 (AJK) and AM 07467 (AVS).
[8] Acknowledgments: The editorial assistance of Dr. E. B. FERGUSON Jr. and Dr. M. PALMER, and technical aid of Mrs. S. VIOSCA, Miss J. BRAUN and Miss B. JERRY are appreciated.