phagia. A differentiation of these individual mechanisms of the action of barbiturates – the nervous and humoral component of the regulation of food intake, based hitherto assembled clinical results, is not yet possible.

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Zusammenfassung

Im Gegensatz zum Chlorpromazin- und bedingt reflektorischen Heilschlaf löst die Medikation von kleinen Dosen kurzwirkender Barbiturate während der 14tägigen Schlafperiode bei Geschwürkranken und Neurotikern eine zeitweilige Hyperphagie verschiedenen Grades aus. Der relative Anteil von einzelnen mitbeteiligten Mechanismen wird diskutiert.

PRO EXPERIMENTIS

Ein Nomogramm zur Kombination mehrerer Ergebniswahrscheinlichkeiten

Für die zusammenfassende Beurteilung der gemeinsamen Wahrscheinlichkeit mehrerer voneinander unabhängiger Versuchsergebnisse gibt es verschiedene methodische Möglichkeiten[1,2], von denen die Kombination mittels der χ^2-Werte nach FISHER[3] wohl die unter den gewöhnlichen Umständen vorteilhafteste[1] und deshalb auch die verbreitetste[4] sein dürfte. Sie beruht darauf, dass die Grösse $-2\sum_1^k \log_e P_i$ wie χ^2 mit $2k$ Freiheitsgraden verteilt und daher exakt testbar ist.

Das angeführte Nomogramm gestattet nun die schnelle und hinreichend genaue graphische Bestimmung der kombinierten Totalwahrscheinlichkeit P_t bei der Zusammenfassung von 2, 3 oder 4 Versuchen. Der Ablesungsvorgang ergibt sich aus der Rechentafel selbst, bzw. ist aus den beigefügten Schemen zu erkennen.

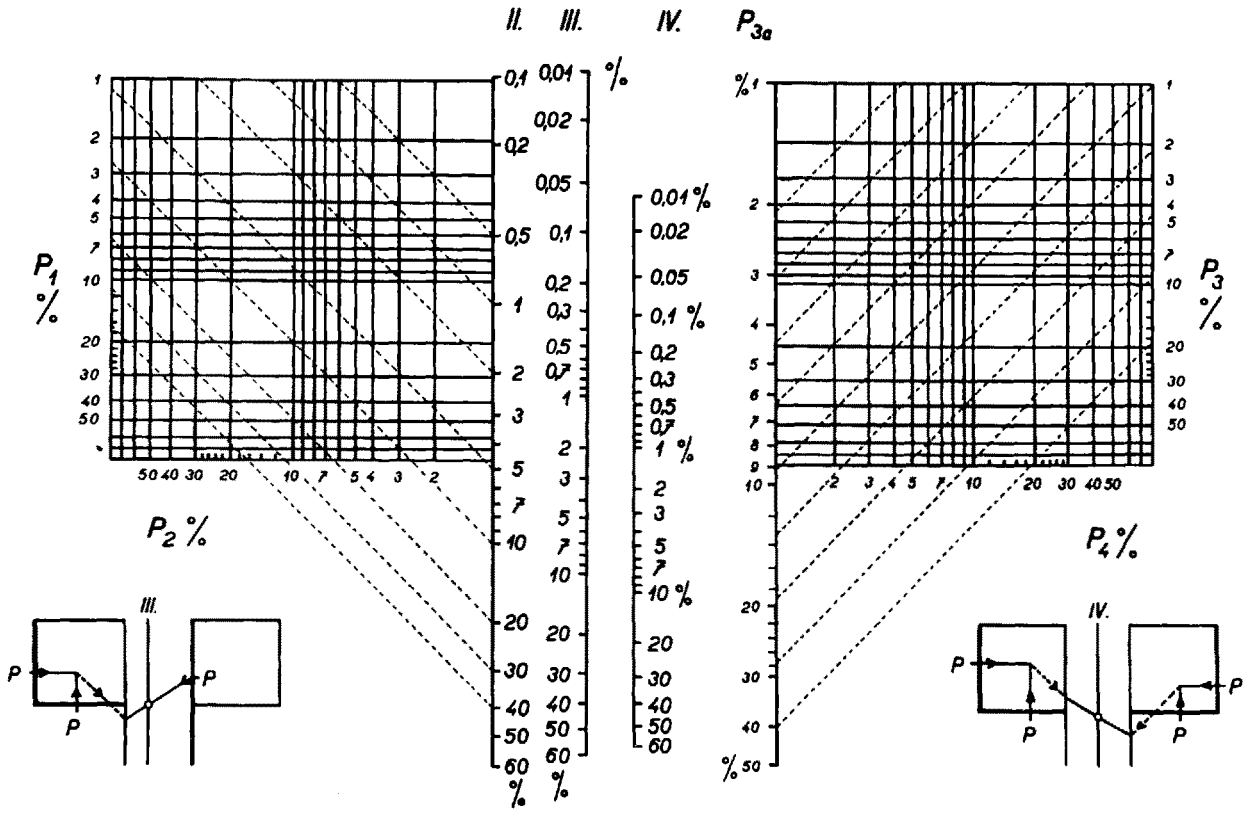
Im Falle zweier Versuche genügt die linke Bildhälfte: man geht mit P_1 und P_2 in die linke Netztafel ein und erhält das gesuchte P_t mittels deren Schnittpunktes, bzw. genauer durch dessen Verschiebung parallel zu den gestrichelten Leitlinien bis auf die *Resultatleiter II*.

Im Falle dreier Versuche wird die Leiter II zur Dreh- oder Zapfenlinie[5]: von ihr aus wird eine Ablesegerade durch den Wert für P_3 auf Leiter *P3a* (nicht P_3!) gelegt, deren Schnittpunkt mit der *Resultatleiter III* das gesuchte P_t ergibt.

Im Falle von vier Versuchen geht man wie bei II, aber beiderseits symmetrisch von P_1 und P_2 links, bzw. P_3 und P_4 rechts aus vor, wobei zu beachten ist, dass nunmehr auch Leiter *P3a* zur Zapfenlinie wird. (Ihre Skala entspricht nur dem P eines einzigen Versuches, nämlich dem des dritten von dreien, und ist daher als solche zur Ablesung des kombinierten Wertes von P_3 und P_4 allein nicht verwendbar.) Die gesuchte Totalwahrscheinlichkeit P_t wird in diesem Falle mittels einer durch die entsprechenden Punkte auf II und 3*a* gelegten Ablesegeraden auf der *Resultatleiter IV* ermittelt.

Abschliessend wird noch darauf hingewiesen, dass zwecks Vermeidung überflüssiger Dezimalstellen alle P-Werte *in Prozenten* angegeben sind und daher zum Beispiel der Ausdruck «0,1» einem Zehntelprozent, das heisst einer Wahrscheinlichkeit von 0,001 entspricht.

[1] A. BIRNBAUM, J. Amer. stat. Ass. *49*, 559 (1954).
[2] K. PÄTAU, Biol. Zbl. *63*, 152 (1943).
[3] R. A. FISHER, *Statistical Methods for Research Workers* (Edinburgh 1934).
[4] G. W. SNEDECOR, *Statistical Methods*, 5th Ed. (Ames 1956).
[5] P. LUCKEY, *Nomographie* (Leipzig 1953).



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Summary

A nomograph is presented, which gives (according to FISHER's formula) the pooled probabilities for the combination of two, three, and four independent experiments respectively.

PRO EXPERIMENTIS

A Method for the Separation and Estimation of Catechol Amines in Urine

Adrenaline, noradrenaline, and a third catecholamine, namely, hydroxytyramine are constituents of normal urine[1–3]. While biological methods are available for determination of catecholamines in blood and urine, these methods have obvious limitations for routine work and can only be employed for the determination of the pharmacologically active hormones, adrenaline and noradrenaline. A differential fluorometric method[4] has been used in the past for determination of all three catecholamines. In this method the catecholamines are estimated by a combination of the fluorometric methods of WEIL-MALHERBE and BONE[5] and of VON EULER and FLODING[6]. The former method estimates the sum of the three catecholamines. Adrenaline and noradrenaline are estimated separately by the latter, and hydroxytyramine is obtained by the difference in results between the two methods.

It seemed desirable to develop a method for simultaneous estimation of all three catecholamines. In previous experiments[7,8], it was shown that it is possible to acetylate catecholamines quantitatively in biological tissue and urine. These acetylated derivatives of adrenaline, noradrenaline, and hydroxytyramine have been shown to be stable, whereas the free compounds are known for their instability. In the procedure described below we acetylated the catecholamines in urine and separated the acetylated derivatives by paper chromatography.

Urine samples were acidified to pH 2 and hydrolyzed by refluxing at 100°C for 30 min. After cooling to room temperature, the urine sample was extracted four times with an equal volume of ethylacetate. The organic phase contained all the acidic catechols which could interfere with the separation of the catechol amines. The aqueous urine extract was adjusted to pH 4 by adding 0·5 *n* NaOH while stirring. The catechol amines in the extract were acetylated by adding 5 g $NaHCO_3$ and 2·5 cm^3 acetic anhydride in four equal portions with constant stirring until foaming ceased. Methylene chloride (50 ml) was added and stirring was continued for another 10 min. The mixture was transferred to a separatory funnel and the organic phase removed. The water was extracted three times with 50 ml of methylene chloride and the combined methylene chloride extracts were counter washed with 50 ml of water filtered through Na_2SO_4 and evaporated in a vacuum. The dry extract was dissolved in 0·5 ml of methyl alcohol and applied to Whatman No. 1 paper for chromatographic separation in the Bush system 'C'[9] using as solvents: toluene, ethyl acetate, methyl alcohol, and water in the ratio 10:1:5:5.

Table I
Comparison of R_f Values obtained after Paper Chromatography of Free Catechol Amines and their Triacetate Derivatives

Catecholamine	R_f Values	
	Free Compound*	Triacetate Derivative**
Noradrenaline	0·15	0·12
Adrenaline	0·28	0·50
Hydroxytyramine	0·33	0·82

* Solvent System: Phenol/HCl.
** Solvent System: Toluene: ethyl acetate: methyl alcohol.

Ascending chromatograms were developed after 24 h. The control area, which contained 100 γ of adrenaline acetate, noradrenaline acetate, and hydroxytyramine acetate, was developed by spraying first with 10% KOH in MeOH:H_2O (1:1) and then with a freshly prepared mixture of 1% $FeCl_3$ and 2% $K_3Fe(CN)_6$. Adrenaline acetate, noradrenaline acetate, and hydroxytyramine acetate were assumed to be opposite their control spots. These areas were cut out and a mixture of ethylenediamine and ethylenediamine hydrochloride was added to corresponding paper strips. A one step reaction was carried out by shaking and heating this mixture at 50°C, resulting in hydrolysis of the acetate derivative and condensation of the free compound with ethylenediamine. The fluorescent condensation product was extracted into isobutanol and a quantitative fluorometric determination using the WEIL-MALHERBE[5] method was carried out.

A comparison of the R_f values (see Table I), obtained from paper chromatography separation of the acetylated catecholamine derivatives and the free compounds, shows that an effective separation can be carried out by the use of this method. Another advantage of the method is the elimination of the adsorption of the catecholamines on alumina. Adsorption on alumina, which was an essential step in previous procedures, is known to be only a semi-quantitative operation.

A preliminary study of the acetylation method for the separation and determination of catecholamines has been completed on the urines of ten psychiatric patients with various diagnoses who were receiving different treatments. The results (see Table II) demonstrate a wide inter-subject variability among the psychiatric patients studied. Values obtained by this method are higher than those obtained by bioassay[10] or by specific fluorometric methods[6]. This may be the result of the elimination of alumina adsorption or may occur because of non-specificity of the condensation with ethylenediamine.

[1] P. HOLTZ *et al.*, Arch. exp. Path. Pharmakol. *200*, 356 (1942).
[2] U. S. VON EULER *et al.*, Biochem. J. *49*, 655 (1951).
[3] M. GOLDSTEIN and I. ABELIN, Helv. chim. Acta *39*, 158 (1956).
[4] H. WEIL-MALHERBE and A. D. BONE, J. clin. Path. *10*, 138 (1957).
[5] H. WEIL-MALHERBE and A. D. BONE, Biochem. J. *51*, 311 (1951).
[6] U. S. VON EULER and I. FLODING, Scand. J. clin. Lab. Invest. *8*, 288 (1956).
[7] M. HOGOPIAN, to be published.
[8] M. GOLDSTEIN, M. HOGOPIAN, and R. I. DORFMAN, IV. Int. Biochem. Congress, Vienna 1958.
[9] I. E. BUSH, Biochem. J. *50*, 370 (1951).
[10] U. S. VON EULER, Acta physiol. scand. *19*, 207 (1949).