Die ersten vergleichenden Untersuchungen zwischen synthetischem und dem verwendeten natürlichem ACTH-Präparat (Cortrophin Organon) sprechen dafür, dass sie bezüglich Wirkungseintritt und Wirkungsmaximum sich wahrscheinlich voneinander unterscheiden. Die Zunahme der Ausscheidung der 17-Hydroxycorticoide im Urin setzt mit dem synthetischen Corticotropin schneller ein, hält aber nach Ende der Infusion weniger lange an.

Ausscheidung der 17-Hydroxycorticoide im Urin nach 8stündiger Infusion von 12,5 Einheiten ACTH während der ersten Urinsammelperiode von 8–16 h bei einer 53jährigen Patientin

	8–16	16–24	0–8	Total 24 h
β^{1-24}Corticotropin	12,5	3,3	5,2	21,0 mg
Cortrophin	8,1	7,1	23,6	38,8 mg

Es ist denkbar, dass der Abbau des kürzeren synthetischen Polypeptides schneller einsetzt als derjenige der längeren Polypeptidkette des natürlichen Hormons. Das β^{1-24}Corticotropin hätte nach intravenöser Verabreichung demnach eine kürzere biologische Halbwertszeit.

Summary. Synthetic β^{1-24}Corticotropin (CIBA 30920-Ba) administered intravenously over 8 h to humans produced a significant rise in free plasma 17-hydroxycorticoids, urinary 17-hydroxycorticoids and 17-ketosteroids. The logarithmic dose response curve of the urinary 17-hydroxycorticoids was linear from 3 to 25 units. The comparison of the steroidogenic effect of the synthetic and natural ACTH (Cortrophin) revealed a more rapid but less sustained activity of the synthetic product.

A. WALSER und F. KOLLER

Medizinische Universitätsklinik Basel (Schweiz), 30. April 1963.

DISPUTANDUM

The Free Amino Acid Pattern in Tadpole Tails

While studying the marked increase of cathepsin in regressing tadpole's tail (*Xenopus laevis*), WEBER[1] pointed out that nothing definite was known about the factor controlling catheptic activity. (This increase in catheptic action means the release of lysosomal enzymes. BRACHET[2] attempted to generalize this phenomenon in order to explain the regression of Muellerian ducts in chick embryo and of the tails of tadpoles, suffering metamorphosis.) However, WEBER noticed that paper chromatography of methanol extracts of tails altered remarkably during the regressing stage. More recently DEUCHAR[3] detected a considerable increase in catheptic activity in chick embryo following treatment with an amino acid (leucine) analogue. Further, DINGLE[4] and FELL et al.[5] have shown that vitamin A releases protease from lysosmes and WEISSMAN[6] has detected regression of tadpole's tail due to hypervitaminosis. Similarly, BHATTACHARYA[7] and BHATTACHARYA and MEDDA[8] detected a role of vitamin B_{12} in the metamorphosis of tadpoles. Now, amino acids may be involved in vitamin production, e.g. biosynthesis of vitamin A might utilize a pool of leucine through the intermediary of some isoprene like precursor[9]. Again, GOODWIN and MCEVOY[10] found certain amino acids to inhibit riboflavin biosynthesis.

Thus, amino acids may play a role in the rupture of lysosomes, either directly, as the findings of WEBER or DEUCHAR might suggest, or indirectly, by being involved in vitamin production. With this in view, I repeated WEBER's work with a local anuran species, *Bufo melanostictus*.

Instead of using methanol extracts of tails, I used the classic method of HADORN and MITCHELL[11], namely to crush the tissue directly on whatman paper No. 1. The usefulness of this method has been emphasized by BUZZATI-TRAVERSO[12] who pointed out, on the basis of a remarkable investigation by CHEN and BALTZER[13], that direct crushing gives a more correct picture. I have also found[14] that in case of snails, amino acids are lost in the process of extraction.

In most cases I have tried (insects, snails, seeds, plants), the resolution following direct crushing was poor or absolutely nil (i.e. one long streak). Fortunately, there was quite a good resolution of ninhydrin positive spots on whatman paper No. 1 after direct crushing of tadpole tails and developing with the solvent, n-Butanol: acetic acid: water, 4:1:1. Therefore, in the case of tadpole tails, one dimensional chromatograms could conveniently be used.

On the basis of the results obtained, it is very clear that, unlike WEBER's case, there is practically no change in the pattern of free amino acids in regressing tails of *Bufo melanostictus*. It is interesting that in the first set of experiments there were four spots in the chromatograms developed from non-regressing tails, but two of them grew very faint in case of regressing tadpoles. However, as all these tadpoles were either given no food at all or fed very sparingly, the experiment was repeated with tadpoles *freshly caught* from a big pond. A large number of spots were now detected in the chromatogram, though the resolution was no longer so good. A number of chromatograms was developed after crushing on the same paper a

[1] R. Weber, Exper. *13*, 153 (1957).
[2] J. BRACHET, *The Biochemistry of Development* (Pergamon, 1960).
[3] E. M. DEUCHAR, Develop. Biol. *2*, 129 (1962).
[4] J. T. DINGLE, Biochem. J. *79*, 509 (1961).
[5] M. B. FELL et al., Biochem. J. *83*, 64 (1962).
[6] G. WEISSMAN, J. expt. Med. *114*, 581 (1961).
[7] G. C. BHATTACHARYA, Science and Culture *19*, 571 (1954).
[8] G. C. BHATTACHARYA and A. K. MEDDA, Science and Culture *23*, 380 (1958).
[9] S. KIT, in *Fundamental Aspects of Normal and Malignant Growth* (Ed. Nowinski, Elsevier 1960).
[10] T. W. GOODWIN and D. MCEVOY, Biochem. J. *71*, 742 (1959).
[11] E. HADORN and M. K. MITCHELL, Proc. Nat. Acad. Sci. *37*, 650 (1951).
[12] A. A. BUZZATI-TRAVERSO, in *New Approaches in Cell Biology* (Ed. Walker, Academic Press, 1960), p. 95.
[13] P. S. CHEN and F. BALTZER, Nature *181*, 98 (1958).
[14] R. L. BRAHMACHARY and A. BHATTACHARYA, Exper. *19*, 143 (1963).

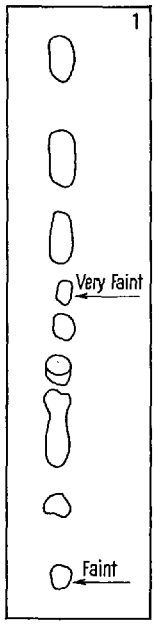

Fig. 1. The free amino acid pattern of the tail of a freshly caught tadpole.

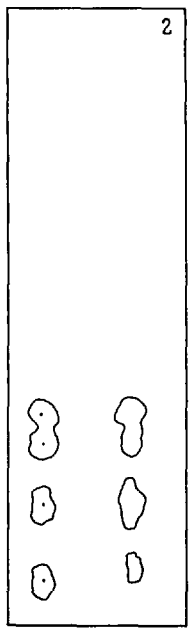

Fig. 2. After 5 days of complete starvation.

pair (or two pairs) of tails, one of which was regressing and the other non-regressing. Thus the chromatograms of tails at different stages of regression could be compared and a marked similarity was noticeable. However, after a few days of starvation the number of amino acids decreased very much (see Figures). Thus, unlike the snail[14] *Limnaea*, where there is a 'stubborn' free amino acid pattern, the tadpole tail has a very labile pattern which depends on the feeding conditions.

Certain attempts were made to compare the amino acid patterns of tails and other non-regressing tissues (such as head) and a very slight difference was noted.

Résumé. Les acides aminés libres dans la queue du têtard *Bufo melanostictus* subissent une perte remarquable après l'inanition, comme ce n'est pas le cas chez les escargots (gastéropode) *Limnaea*. Mais, au contraire, ils ne subissent aucune altération pendant la métamorphose, et ce résultat est bien différent de celui signalé par WEBER avec *Xenopus laevis*.

R. L. BRAHMACHARY

Research and Training School, Indian Statistical Institute, Calcutta (India), November 13, 1963.

PRO EXPERIMENTIS

Thin Layer Chromatography of 2,4-Dinitrophenylhydrazones of Aliphatic Carbonyl Compounds

The separation and identification of 2,4-dinitrophenylhydrazones of formaldehyde and other C_2 and C_3 carbonyl compounds has been tried by paper chromatography, adsorption chromatography, counter-current distribution, or liquid–liquid partition. These methods are time-consuming and tedious, and good results have generally been obtained only in cases with two components.

During researches on the oxydation products of some 10-(dialkylamino-alkyl)-phenothiazines, we had the problem of separating and identifying some carbonyl compounds with 1–3 carbon atoms, isolated as 2:4-dinitrophenylhydrazones.

We have used thin layer chromatography for the separation of 2:4-dinitrophenylhydrazones of formaldehyde, all C_2 and a few C_3 carbonyl compounds. These separations are fast and quite good for all the compounds tried. The spots are well separated and sharp when nitrobenzene is present in the eluent mixtures.

We have tried several adsorbents, such as magnesol, silicagel, polyamide and alumina at varying pH, and found neutral alumina ('Woelm' for thin layer chromatography) best.

In the Table the Rr values are given, the derivative of acetone is assigned the value 1. We are pursuing the research with various C_3 and other low molecular weight carbonyl compounds of biological interest.

2,4-Dinitrophenylhydrazone of	Eluents I	II
Formaldehyde	0.80	0.71
Acetaldehyde	0.94	0.88
Glycolaldehyde	0.06	0.04
Glyoxal	0.67	0.10
Glyoxylic acid	0.01	0.01
Propionaldehyde	0.98	0.94
Acetone	1.00	1.00

I: Cyclohexane/nitrobenzene (2:1)
II: Hexane/chloroform/nitrobenzene (8:2:1)
Elution time: 1 h

Zusammenfassung. Für die Trennung und Identifizierung niedermolekularer Carbonylverbindungen eignet sich die Dünnschichtchromatographie ihrer 2,4-Dinitrophenylhydrazone auf Aluminiumoxid mit Nitrobenzol als Bestandteil der Entwickler.

G. M. NANO and P. SANCIN

Istituto di Chimica Farmaceutica e Tossicologica dell'Università di Torino (Italy), January 4, 1963.