in Group 2, and highest in Group 3. These differences between the groups were highly significant ($p < 0.001$). The nitrogen content on the other hand, was significantly higher ($p < 0.001$) in Group 1 than in Group 2 or Group 3. The difference between the latter 2 groups was not significant.

Discussion. The youngest, most rapidly growing rats released the most FFA per unit weight of the tissue *in vitro*. The nitrogen content per unit weight was also the highest in this group. The difference between the groups was much less striking if release of FFA was expressed per nitrogen content of the tissue. Tissue triglyceride content was highest in the oldest group and lowest in the youngest group. These differences became much more marked when expressed per nitrogen content of the tissue. Since FFA release by adipose tissue is under the influence of tissue lipases, it could be assumed that either concentration and/or activity of these lipases was greater in the younger animals than in the older ones.

It is not clear at the present time, what bearing these findings have on *in vivo* conditions. It might be speculated that there is a relationship between more extensive accumulation of adipose tissue with advancing age and the decreased release of FFA from the adipose tissue.

It might also be suggested that the young rapidly growing rats have an abundant supply of growth hormone which could be responsible for the increased release and decreased accumulation of FFA, but this cannot be determined with presently available information. Finally, it has been observed[17] that tissues from young animals are more responsive to the metabolic effects of insulin than tissues removed from older rats. The connection, if any between this finding and the presently reported observations has not been clarified.

Zusammenfassung. Die Absonderung freier Fettsäuren aus dem Fettgewebe wurde bei verschieden alten Ratten (1. Gruppe unter 100 g, 2. Gruppe 180–300 g und 3. Gruppe 380–500 g) *in vitro* ermittelt. Für das Fettgewebe der Nebenhoden ergab sich in der 1. Gruppe die stärkste Absonderung freier Fettsäuren und deren wirksamste Förderung durch Epinephrin. Hingegen war der Gehalt an 3-Glyceriden in der 1. Gruppe am niedrigsten, in der 3. Gruppe am höchsten.

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On the *in vitro* Corticotropin Releasing Factor (CRF) Activity of the Heptapeptide: Methionyl-Glutaminyl-Histidyl-Phenylalanyl-Arginyl-Tryptophyl-Glycine

In a preceeding publication[1], we have mentioned the *in vitro* CRF activity measured by the method of SAFFRAN et al.[2] of the heptapeptide H-Met-Glu(NH_2)-His-Phe-Arg-Try-Gly-OH synthesized by KAPPELER and SCHWYZER[3,4]. This heptapeptide was already known for its melanocyte stimulating activity (MSH activity); it represents a sequence of seven amino acids common to the hormones of the corticotropins (ACTH) and MSH group.

Later on, the heptapeptide: H-Met-Glu-His-Phe-Arg-Try-Gly-OH which differs from the preceeding one only by the absence of an amide group, was synthesized by LI et al. and found to have both melanocyte stimulating and corticotropin releasing activities (MSH and CRF activities)[5].

In the present note, we describe a series of *in vitro* assays of the heptapeptide H-Met-Glu(NH_2)-His-Phe-Arg-Try-Gly-OH performed in our laboratory between December 1959 and October 1961. A large number of determinations made during this period gave consistant results, some of the most significant of which have been collected in the Table.

The experimental details are those described by SAFFRAN et al.[2]. The CRF activity may be expressed either: (1) as the minimal dose of active material which is able to provoke a statistically significant increase of the secretion of ACTH by the anterior pituitary of rats, versus non-stimulated anterior pituitaries; (2) as the dose of active material able to produce a response equal to the response given by a constant standard preparation.

At the present time, both methods of expression are used; in the Table we have used uniformly the second mode of expression so that recent results may be compared to the former ones calculated according to the second mode. The rats utilized for the assays were male animals of the Wistar U.S.A. strain, weighing 150 g.

Different doses of the heptapeptide were tested versus a constant uniform dose of 4 μg of CRF 91[6]; this dose of 4 μg of CRF 91 was considered as a provisionnal unit of CRF activity. The activity of any unknown material was expressed by the ratio between the amount of ACTH released by this material versus the amount of ACTH released by 4 μg of CRF 91. Generally speaking this ratio varies between a rather narrow range and several hundred assays performed so far have shown that: a ratio 0.5 means no stimulation; a ratio between 0.5 and 1.0 means weak stimulation; a ratio between 1.0 and 1.5 means medium stimulation; a ratio between 1.5 and 2.0 means high stimulation.

The results of these determinations were evaluated by standard statistical tests[8], the limits of confidence were calculated for the ratios as well as the 'departure from parallelism'. The assays reported in the Table do not show any departure from parallelism exceeding the critical values. The data of the Table show that the heptapeptide is able to release an amount of ACTH greater than the amount released by 4 μg of CRF 91 at the doses of 4, 2, 1,

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Amounts of ACTH released by various levels of heptapeptide ($\varkappa$ µg) versus the amounts of ACTH released by 4 µg of CRF 91

Date	amounts of heptapeptide $\varkappa$ µg	ACTH released by $\varkappa$ µg / ACTH released 4 µg CRF 91	Limits of confidence	F	λ
29. 2. 1960	4.0	2.02	1.65–2.58	274.0	0.039
7. 3. 1960	2.0	1.32	0.85–2.23	49.3	0.096
4. 10. 1960	1.0	1.43	0.73–3.17	24.0	0.140
8. 3. 1960	0.5	1.43	1.10–1.93	137.7	0.057
9. 3. 1960	0.1	1.04	0.66–1.78	47.6	0.098
6. 10. 1961	0.1	1.16	0.74–1.87	54.0	0.092
15. 12. 1959	0	0.66	0.51–0.82	205.0	0.047
12. 10. 1961	0	0.51	0.33–0.73	105.9	0.066

and 0.5 µg; the dose of 4 µg of heptapeptide doubles the release of ACTH obtained with 4 µg of CRF 91. By lowering progressively the doses of heptapeptide one reaches the point where 0.1 µg of this material is still able to release an amount of ACTH equal to the amount released by 4 µg of CRF 91. When the hypophyses receive no stimulation they release only one half of the amount of ACTH released by 4 µg of CRF 91 and one fourth of that released by 4 µg of the heptapeptide.

In this series of experiments we were thus able to demonstrate that the heptapeptide H-Met-Glu(NH_2)-His-Phe-Arg-Try-Gly-OH was capable of releasing corticotropin *in vitro*. No definite conclusion can be drawn at present concerning the *in vivo* activity of this peptide and its possible physiological role.

Résumé. Au cours d'une série d'expériences décrites en détail nous avons observé le phénomène suivant: l'heptapeptide H-Met-Glu(NH_2)-His-Phe-Arg-Try-Gly-OH est susceptible d'augmenter *in vitro*, de façon statistiquement significative, la libération de corticotropine par des antéhypophyses de rat isolées.

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Laboratoire de Chimie Biologique de la Faculté des Sciences, Paris (France), le 3 novembre 1961.

Cumulative Record of Motor Activity. (A New Procedure and its Use for the Study of Central Stimulant Drugs)

The motor activity of small animals is measured in a variety of ways. The rotations of a squirrel-wheel or a revolving cage[1], or the interruptions of light beams[2] are counted with impulse counters over standard periods of time[3]. The movements of a jiggle-box are measured on a sooted drum[4] or recorded electrically[5]. The movements of a platform of an animal container are converted into electrical currents with a piezo electric crystal[6] and registered on a kymographion or electronic recorder. The various methods have recently been critically reviewed[7].

Counts over standard periods of time are easy to use for measuring the potency of drugs on motor activity, but have the disadvantage that information on how motor activity proceeds in time is lost. Continuous measurements of animal induced movements do indeed give a time course relationship but it is often difficult to obtain statistical data from such recordings.

In operant behaviour research[8], extensive use has been made of cumulative recorders (Gerbrands recorders), to which square pulses of 24 V and 35–40 msec duration are fed, resulting in excursions of a pen in steps of 0.25 mm. If rotations of wheels, interruption of lightbeams or movements of animal-cages can be converted into pulses of standard duration, a cumulative record of motor activity can be obtained on a cumulative recorder. A cumulative record has the advantage that a complete time-response curve is obtained, while motor activity can easily be determined from such a curve. A procedure for integration pulses over standard periods of time can as well be used. With such a procedure practically the same information can be obtained as with the cumulative procedure provided that integration is carried out over short periods of time (30 sec or less).

The lightbeam method is in use in our laboratory and we therefore developed pulse-shapers for converting an interruption of a beam into a pulse of 40 msec duration. A diagram of the transistorized pulse-shaper is given in Figure 1.

The animal cage consists of a box (dimensions 36 cm l; 24 cm d, and 20 cm h) situated in a ventilated soundproof box and illuminated with a house light of constant intensity. The dimensions of the cage are not critical but are chosen for studying groups of two mice, or one or two rats. For larger animals or larger groups the cage should be extended. Three lightbeams were placed 6 cm apart over the depth of the cage and opposite to them 3 CdS photoelectric cells. Each photoelectric cell is connected with the input of a pulse-shaper. The output terminals of the three pulse-shapers are connected with the input terminal of the stepping device of a Gerbrands recorder. If less than 100 pulses/min are recorded, there is no

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