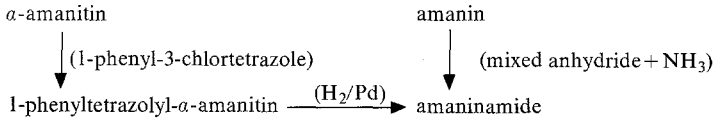


Scheme 1.

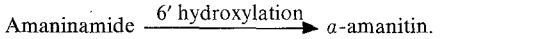
ammonia gas was passed in and allowed to react over 2 h. After evaporation, the residue was applied to a small DEAE Sephadex column (OH^- form) and developed with water; 5 mg (56% yield) of amaninamide was eluted as a single component, while the starting material and byproducts remained bound.

Results and discussion. The new toxin was found in extracts of *A. virosa* both from Europe and Northern America in amounts of 0.5–0.9 mg/g dry weight. During the adsorption chromatography on Sephadex LH 20 the toxin eluted midway between the newly detected group of virotoxins[2] and the group of neutral phallotoxins. In many of its properties, the toxin is indistinguishable from amanin; this accounts for the typical UV-spectrum of α-indolylsulfoxides as well as its reaction with cinnamic aldehyde/hydrochloric acid, which yields a characteristic blue-gray color. Also, the amino acid analysis displayed the pattern typical for amanin including aspartic acid, glycine, hydroxyproline and isoleucine in a ratio, 1:2:1:1. In addition, the lactone formed from the hydroxylated amino acid was that of γ,δ-dihydroxyisoleucine as is found in amanin. However, on high voltage paper electrophoresis, the toxin proved to be dissimilar to amanin, in that it was a neutral cyclic peptide rather than an acidic one.

Most of the amatoxins thus far isolated from *A. phalloides*, and whose structures have been elucidated by Th. Wieland and coworkers[7], exist in both acidic and neutral forms. In all cases, this is the result of the substitution of asparagine for aspartic acid. The pairs of neutral and acidic amatoxins include β-amanitin and α-amanitin, ε-amanitin and γ-amanitin, amanullin and amanullinic acid. Correspondingly, we were compelled to expect the new toxin from *A. virosa* to be amaninamide, the partner of the acidic toxin amanin. Amaninamide has not yet been detected in *A. phalloides*.

The simplest way to confirm the structure was by semisynthesis of amaninamide. This has recently been achieved by Buku et al.[6] by hydrogenation of 1-phenyltetrazolylether (formula) of α-amanitin. We have now also prepared amaninamide via amanin by reaction of amanin-mixed carbonic anhydride with ammonia.

Both preparations of amaninamide yielded identical products, which in turn proved to be identical with the newly isolated unknown toxin. This was confirmed by silica TLC, using different solvent mixtures: (R_f = 0.24 in I; R_f = 0.24 in II; R_f = 0.32 in III), as well as by the identity of the UV-spectra, electrophoretic behaviour and amino acid analysis. The *A. virosa* sample from Europe contained a considerable amount of α-amanitin in addition to amaninamide. This was in contrast to the sample from North America, which contained amaninamide exclusively. β-Amanitin was absent in both samples. Therefore, it seems reasonable to suggest that in *A. virosa* amaninamide is the precursor of α-amanitin, the hydroxylation reaction of the indole ring being incomplete in the case of the European sample and totally absent in the case of the North American sample.



Scheme 2.

To some extent this is supported by the results of Yocum and Simons[8], who recently reported the analysis of various *Amanita* species of North America, including 4 samples of *A. virosa* from different areas in the U.S. These authors found α-amanitin to be present in 2 samples while absent in the other 2. However, in the latter 2 samples amanin was detected. Their identification of amanin was by UV-spectrum only and, consequently, had to be a tentative one, because an authentic sample of amanin was not available. Since amanin has been detected in none of our samples of *A. virosa*[2,9] we believe that the toxin found by Yocum and Simons was, in fact, the new toxin amaninamide.

1 Communication No. 57 of the series: Components of the green deathcap toadstool Amanita phalloides. No. 56: E. Munekata, H. Faulstich and Th. Wieland, Liebigs Ann. Chem., in press.
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Diurnal fluctuation in the rate of synthesis of a specific protein fraction in the rat brain

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Summary. A protein fraction has been identified in microsomes prepared from the rat hypothalamus whose rate of synthesis fluctuates diurnally in ovariectomized animals.

The mammalian brain controls many functions that follow tightly controlled circadian rhythms. The suprachiasmatic nucleus, a small area of the hypothalamus positioned just above the optic chiasma, has recently been shown to play an important role in generating such rhythms. Rats, in which this nucleus has been surgically destroyed, show randomized drinking and locomotor activity[1], loss of adrenal and pineal rhythmicity[2,3] and disrupted reproductive cyclicity[4]. It has also been demonstrated that the rate of uptake of [^{14}C] deoxyglucose (a measure of functional activity) into the suprachiasmatic nucleus fluctuates diurnally, although it was not reported whether this fluctuation occurred only in this part of the hypothalamus[5].

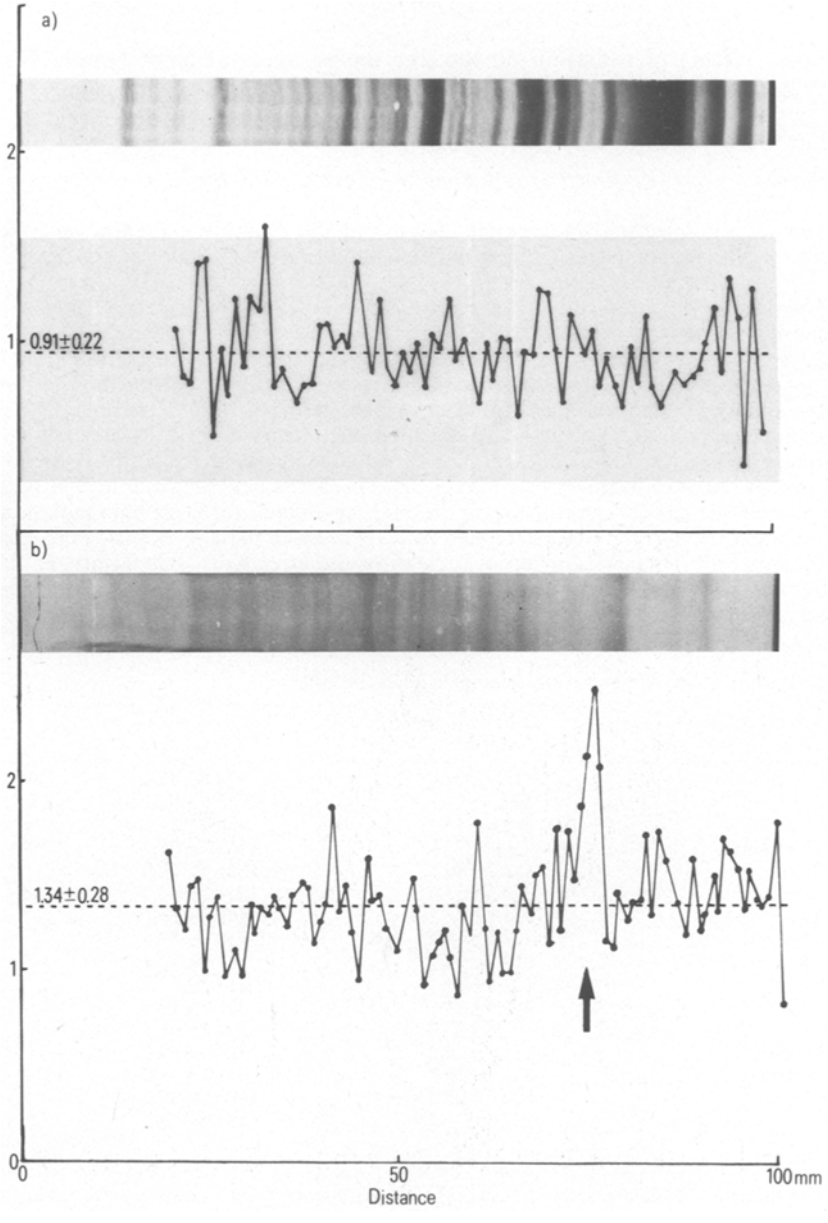
The findings presented here were made during the course

of experiments designed to establish the metabolic consequence of the interaction of estradiol with its receptor in the hypothalamus. It was found that in the hypothalamus of control animals (ovariectomized but not treated with estradiol) a specific protein fraction was synthesized at a different rate depending on the time of day.

Methods. 2 colonies of rats were used. The animals in both colonies had been ovariectomized for at least 3 weeks and were kept under comparable conditions except that their lighting regimes were 180° out of phase. 3 rats from colony I (lights on 06.00–20.00 h) were paired with 3 rats from colony II (lights on 18.00–08.00 h) and the pairs killed at 17.50, 18.00 and 18.10 h 30 min after the administration into the right lateral ventricle of L-[2(n)-^{3}H]-methionine [0.2 μCi/g; 3.3 Ci/mmole] to animals from colony I and L-[U-^{14}C]-methionine [0.02 μCi/g; 285 μCi/mole] to animals from colony II. Blocks of hypothalamic tissue taken from these pairs were pooled, homogenized and microsomal and high speed supernatant fractions prepared from these homogenates[6]. Aliquots of identical subcellular fractions isolated from the 3 pairs of animals were then pooled to reduce interanimal variability, concentrated by freeze drying and fractionated on SDS - polyacrylamide slab gels, prepared by the method of Laemmli[7].

The strip of gel that contained the protein components of a particular sample was sliced into 1 mm sections using a Mickle gel slicer. Each section was placed in a separate scintillation vial containing a 3% solution of Protosol (New England Nuclear) in a toluene-based scintillant (5 ml). The vials were incubated at 37 °C overnight to promote leaching out of protein from the gel sections and then left to equilibrate in the dark at room temperature for 12 h before counting in a Beckman scintillation counter. The ^{3}H and ^{14}C disintegrations/min(dpm) were determined by the external standard ratio method and the ^{3}H:^{14}C ratios were calculated for each gel slice.

Since the labelled moieties of [2(n)-H^3] methionine and [U-^{14}C] methionine are not directly metabolized to a molecule that can be incorporated into protein, it is reasonable to assume that during the short time the animals were exposed to the radiolabel any incorporated label would be in the form of methionine. Any difference in the amount of radiolabelled methionine incorporated into protein during a 30-min exposure to it at the 2 different times of day



Relative labelling ratios (^{3}H:^{14}C) in hypothalamic cytosol (a) and microsomal (b) protein fractionated on SDS-polyacrylamide slab gels. Photographs of a typical gel stained with coomassie brilliant blue are included to demonstrate how many protein fractions are resolved by this technique. The mean labelling ratio (±SD) is shown for both subcellular fractions. The black arrow indicates the position of the microsomal protein whose rate of synthesis fluctuates diurnally.

chosen will change the $^{3}H:^{14}C$ ratio associated with that protein.

Results and discussion. The $^{3}H:^{14}C$ ratios associated with slices of a typical gel are given in the figure; the first 19 gel slices have been omitted because too few counts were detected in this section of the gel to allow calculation of a $^{3}H:^{14}C$ ratio. Slight fluctuation in this ratio is apparent across a broad spectrum of cytosolic and microsomal proteins. The significance of such variations is hard to assess since they are often small, caused by alteration in the isotopic ratio in only 1 gel slice, and not always apparent in every gel. A single fraction of microsomal protein does, however, show a marked increase in isotopic ratio which is detectable in 4 consecutive gel slices. In all of them the ratio falls outside the mean by more than 2 SD (fraction marked by arrow, mean and SD also indicated).

It is theoretically possible that extremely localized alterations in the specific activity of incorporable precursor or diurnal fluctuation in the rate of catabolism of this particular fraction could give rise to this observation, but it is much more likely that a microsomal protein fraction exists in the rat hypothalamus whose rate of synthesis fluctuates diurnally.

This study was not carried out using a regime which could establish that the rate of synthesis of this protein fraction follows a truly circadian pattern but it has provided the first evidence that diurnal fluctuations in protein synthesis occur in that part of the brain implicated in the maintenance of circadian functions. Such an indicator could prove invaluable in the biochemical dissection of the molecular mechanisms that underly 'clock' functions in the mammalian brain.

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Some effects of radiation on the free amino acids of adult female Mediterranean fruit fly, *Ceratitis capitata* Wied.

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Summary. Ceratitis capitata pupae, 2–3 days before adult emergence, were treated with gamma irradiation from a ^{60}Co source. The female fruit flies were extracted and analyzed for free amino acids.

The Mediterranean fruit fly *Ceratitis capitata* Wied. is considered now as one of the serious pests of fruit crops in Egypt. Recent advances in research concerning the fruit flies suggest their possible suppression. For example, Hafez and Shoukry[2] reported that fruit flies can be sterilized by gamma irradiation. They gathered considerable data on the longevity and fecundity of fruit flies exposed to varying doses of radiation from ^{60}Co source. Several investigators[3–8] reported the possible use of the sterile male technique for the eradication of the Mediterranean fruit fly in some parts of the world. However, in recent reviews of the effects of radiation on insects, it has been noted that little physiological or biochemical data have been amassed[9,10]. In the present paper, I therefore report an investigation of the changes in the free amino acids of adult female fruit flies after irradiation by a ^{60}Co source as 2–3-day-old pupae.

Materials and methods. The fruit flies used in the study were obtained from a permanent colony maintained in the laboratory on an artificial carrot medium[2]. Pupae 2–3 days before adult emergence were exposed to gamma irradiation from a ^{60}Co source at a dose rate of about 45 r/sec[11]. The gamma radiation dose used in the present study was 4000 r. Adults were allowed to emerge in small cages in the laboratory at 25 °C and 60–65% relative humidity. The female fruit flies were 2- and 6-day-old (post-irradiation) when they were collected, weighed and frozen (–20 °C).

Free amino acids of normal and irradiated adult fruit flies

Amino acids	2-day-old females Control*	^{60}Co	6-day-old females Control*	^{60}Co
Glycine	512.6±32.4	974.9±80.2	949.6±71.5	810.0±58.2
Alanine	493.5±26.7	825.6±60.1	796.2±30.3	797.2±46.1
Serine	282.0±13.2	356.9±16.5	538.3±25.7	305.5±12.6
Threonine	726.6±35.8	1069.7±35.7	760.8±35.4	821.6±40.7
Valine	182.2±10.6	1021.5±22.9	500.4±29.2	600.1±32.2
Leucine	364.3±15.5	1404.2±83.3	771.4±28.8	782.3±37.8
Aspartic acid	353.7±20.1	461.3±36.1	267.9±12.9	533.1±31.5
Glutamic acid	398.4±18.2	838.1±59.6	732.9±35.5	1033.6±60.8
Glutamine	219.2±10.7	609.6±32.8	423.9±25.7	529.3±22.1
Proline	123.7± 4.5	213.7± 9.5	165.8± 6.4	73.4± 3.2
Lysine	257.6±15.4	265.0±13.4	546.3±31.6	272.3±18.3
Histidine	665.6±28.7	799.1±31.7	1654.1±85.5	980.1±72.4
Tyrosine	115.7± 6.3	314.9± 9.5	357.8±25.1	725.9±40.0
Ornithine	432.9±29.2	657.1±44.0	568.8±22.4	627.2±27.7
Cystine	600.4±27.5	1670.7±99.2	1011.4±70.8	1329.5±91.1
Methionine	287.6±18.5	234.9±8.6	419.8±23.6	310.9±16.4
Totals	6016.0	11717.2	10465.4	10532.0

The values are given as µmoles amino acids/100 g of tissues. * Data reproduced from Boctor[14].