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].

Flies were kept at -20 °C until they were used for analyses. The method of Pant and Agrawal[12] was used for the preparation of amino acid extracts from adult fruit flies. Free amino acids in the tissue extracts were separated and determined quantitatively by 2-dimensional paper chromatography according to the method detailed in the preceding paper by Boctor and Salem[13]. For each sample, 6 chromatographic separations were carried out, and the average and experimental errors were calculated (table).

Results and discussion. Few studies have been made regarding the effect of radiation on the free amino acids of insects. Radiation affects the rates and patterns of protein synthesis and would thus be expected also to alter free amino acid pools. In the present study, 16 amino acids have been identified on the 2-dimensional paper chromatogram of both normal and irradiated adult fruit flies. In 6-day-old female fruit flies, irradiation was seen to have caused a decrease in the quantities of glycine, serine, proline, lysine, histidine and methionine and an increase in the amounts of threonine, valine, aspartic acid, glutamic acid, glutamine, tyrosine, ornithine and cystine. The results also show that levels of alanine and leucine were not changed by irradiation. In 2-day-old female fruit flies, the radiation effect on the free amino acids was an increased concentration of both individual amino acids and the total pool. Methionine was the only amino acid which was lower (18.3%) in the irradiated group.

Several investigators demonstrated that X- and γ-irradiation have been found to cause variations in the amounts of amino acids in organisms, but there seems to be little consistency. For instance, in yellow mealworm embryos *Tenebrio molitor* arginine, serine, histidine, phenylalanine, threonine, tryptophan and valine increased due to X-irradiation, and alanine, aspartic acid, cystine, glutamic acid, methionine and proline decreased[15]. Richardson and Myser[16] showed that large doses of irradiation (20 krad) increased the total concentration of amino acids in the haemolymph pools of prepupae of the honeybee and last-instar larvae of the greater waxmoth, *Galleria mellonella* (L.); lesser amounts raised the levels of particular amino acids such as lysine, the most radiosensitive in their study. Recently Meyer et al.[17] reported abnormalities in the free amino acids of adult hornflies irradiated as pharate adults. They found no effect of irradiation on the total concentration of amino acids in either their physiological or total amino acid analyses.

In the present investigation, most of the free amino acids of 6-day-old female fruit flies changed in titre as a result of irradiation, but the total concentration of amino acids was almost the same in both normal and irradiated flies. In 2-day-old female fruit flies, irradiation caused a higher increase in the total concentration of amino acids. The increased free amino acid concentration after γ-irradiation may be attributed to a decreased capacity of the tissues to utilize the free amino acid pool particularly with respect to protein synthesis.

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A logical approach to the isolation of lactate dehydrogenase isozyme X from human testes: A general rationale for the isolation of homotetrameric LDH isozymes[1]

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Summary. Immunoadsorbent and oxamate-Sepharose chromatography were used to isolate electrophoretically homogeneous LDH-X from human testes with a final specific activity of 125 IU/mg and good yields: other applications of this approach are discussed.

Lactate dehydrogenase isozyme X^{2-4} (LDH-X) is found only in the mature testes and spermatozoa of mammalian and avian species, and differs from the 5 major isozymes (LDH-1-LDH-5) in its kinetic[5], chemical[5] and immunological properties[6]. These properties make LDH-X a possible target for fertility regulation and in this connection, active[7] or passive[8] immunization of female mice with mouse LDH-X has been shown to decrease fertility. Although human LDH-X has been separated from other LDHs by us using DEAE-cellulose chromatography[9] and by others using AMP-Sepharose chromatography[10] the differences in charge properties and affinity for nucleotides are not sufficient to predict such separations a priori. Here we present a generally applicable procedure for the isolation of LDH-X from human testes, using immunoadsorbent chromatography to separate LDH-X from other LDHs and oxamate-Sepharose affinity chromatography to free LDH from other proteins.

Materials and methods. Human tissues (heart, liver and testes) were obtained by autopsy from accident victims with the cooperation of the Police Hospital, Bangkok. Homogenous LDH-1 (sp. act. 168 IU/mg) and LDH-5 (sp. act. 283 IU/mg) were isolated from human heart and from human liver as described[11]. Antisera to these proteins were obtained by immunizing rabbits (LDH-1 was acetylated prior to immunization[12]). Gamma globulins were isolated from antisera by the method of Fleischman et al.[13] and were coupled to Sepharose (1:30 by wet weight) by the cyanogen bromide procedure[14]. The immunoadsorbent used in the present experiments was an equal volume mixture of anti-