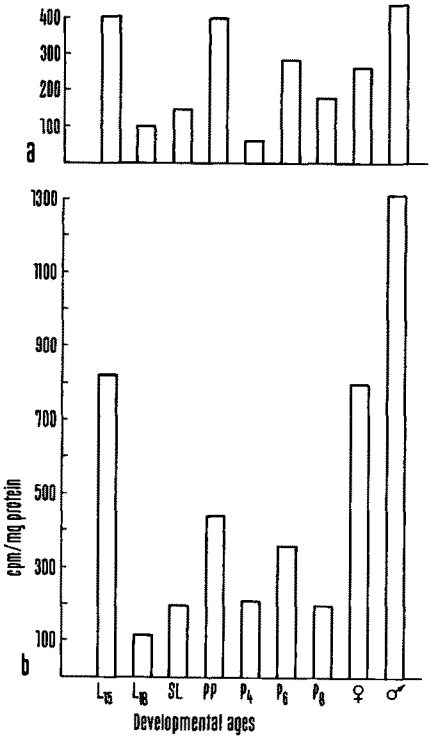
counter. The radioactivity was measured with an automatic Nuclear Chicago gas flow counter. Proteins were determined by the method of LOWRY[10].

Results and discussion. From the Figure it is seen that there are significant differences in the incorporation of radioactive leucine at several of the developmental stages of the insect. Thus, in the early larval stage L_{15}, the uptake by total animal is greater than in the late larval stage L_{18}. This finding parallels results of PRICE[6,7,11], who reported that in blowfly larvae cultured at 25 °C the high rate of protein synthesis in the fat body decreases rapidly in the late larvae (aged from 4 to 6 days). Further, CHIPPENDALE and KILBY[9] found that the relative in vivo protein synthesis in mid-fifth instar *Pieris brassicae* larvae is high in the fat body and midgut, which may be considered active sites of protein synthesis. In the pupal stage we found 2 other smaller peaks of increasing rate of protein synthesis. These appeared in the stages of pre-pupa and 6-day-old pupa. This observation can be correlated with the changes occurring in the fat body. BUTTERWORTH et al.[12] observed changes in the fat body in the late pre-pupal stage when proteinaceous granules, which were stained with fast green, began to appear. On the other hand, WALKER[13] reported that during the pharate pupal stage, each fat body cell becomes packed with many granules and fat vacuoles. CHIPPENDALE and KILBY[8] suggested that fat body stores haemolymph proteins during the pharate pupal stage.



Incorporation of C^{14}-leucine into protein of different developmental stages of *Plodia interpunctella*. C^{14}-leucine was injected (a) 2 h and (b) 4 h before the animals were sacrificed.

A significant increase of protein synthesis was observed in the adults. This finding supports the suggestion of CHIPPENDALE and KILBY[9] that some other tissues may also participate in haemolymph protein synthesis. Thus, except for the fat body, other cells or differentiated tissues like midgut become possible active sites for haemolymph protein synthesis. It is of interest that the males showed a rate of protein synthesis twice as high than the females. No ready explanation is available for this finding.

Zusammenfassung. Die Einbaurate für Leucin-^{14}C in die Körperproteine ist in 15tägigen Larven und Adultinsekten von *Plodia interpunctella* bedeutend höher als in den Zwischenstadien. Bei letzteren zeichnen sich Vorpuppen und 6tägige Puppen durch eine höhere Einbaurate aus. Männliche Falter inkorporieren mehr Leucin als weibliche.

H. A. PATARYAS[14] and H. GELTI-DOUKA

Department of General Biology, University of Athens, Athens (Greece), 2 October 1970.

[10] O. H. LOWRY, N. J. ROSEBROUGH, A. LEWIS FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[11] G. M. PRICE, J. Insect Physiol. *15*, 931 (1969).
[12] F. M. BUTTERWORTH, D. BODENSTEIN and R. C. KING, J. exp. Zool. *158*, 141 (1965).
[13] P. A. WALKER, J. Insect Physiol. *12*, 1009 (1966).
[14] Present address: Department of Medicine, University of Washington, Division of Medical Genetics, Seattle (Washington 98105, USA).

Dissociation of Avidin-Biotin Complex in vivo

Avidin, a toxic glycoprotein, which renders biotin unavailable for animal and microbial growth combines firmly with biotin to yield an avidin-biotin complex with a dissociation constant of $10^{-15}M$[1]. The avidin-biotin complex was found to be extremely stable. It is stable over a wide pH range[2,3] and towards heat, even steaming at 100 °C for a short period[4,5]. Furthermore, biotin, when combined with avidin, cannot be liberated from the avidin-biotin complex by ordinary proteolytic enzymes[6].

In contrast to the effectiveness of avidin to cause egg white injury upon oral administration, GYÖRGY and ROSE[7] found that its injection was shown to have a curative effect on the same deficiency disease. This was explained by FRAENKEL-CONRAT and FRAENKEL-CONRAT[8] that biotin in the avidin-biotin complex presumably is released under physiological conditions and then fellows a path similar to that of free biotin. In the present investigation tracer techniques have been used to further confirm this problem.

Pure avidin used in this study was prepared by the method of MELAMED and GREEN[9] with an activity of 13.4 U/mg.

Avidin-radiobiotin complex (A–B*) was prepared from appropriate amount of avidin saturated with excess amount of D-biotin-carbonyl-^{14}C. The excess free radiobiotin was removed from the solution by dialysis against $0.2M$ ammonium carbonate. The undialyzed material, A–B*, was lyophilized and dissolved with appropriate amount of normal saline. 4 male rats (Sprague-Dawley

Table I. Daily urinary excretion of radioactivity in rats after i.p. injection of avidin-radiobiotin complex (1.28 μc)

Day	No. of animals	% of administrated dose (mean ± S.E.)
1	4	20.24 ± 2.07
2	4	19.49 ± 3.49
3	4	5.10 ± 0.59
4	3	2.16 ± 0.33
5	4	1.52 ± 0.39
6	4	1.41 ± 0.14
10	2	1.19 ± 0.03
20	4	0.42 ± 0.03

Table II. Dialysis experiments

Composition	dpm per ml Inside	Outside
24 ml diluted urine	2773	2671
24 ml diluted urine + 2 mg avidin (excess)	2800	60

The urine collected from the first day period after the i.p. injection of avidin-radiobiotin complex (1.28 μc) was diluted with water to 25 ml. An aliquot of 24 ml was then dialyzed, with or without addition of avidin, against 76 ml of 0.2 *M* ammonium carbonate for 48 h.

strain) of approximately 300 g were injected i.p. with 4 ml of the A–B* solution (1.28 μc) and immediately placed separately in metabolic cages. The urine was collected each day and the radioactivity was measured by the method of BLOOM and NELP[10] in a liquid scintillation counter. The result is shown in Table I. The excretion of radioactivity during the first and the second days corresponded to 20.24% and 19.49% of the total dose respectively. The excretion was graduately decreased probably because the radiobiotin is getting into the metabolic pool of biotin and then follows the same path of free biotin. In order to know whether or not the

[1] N. M. GREEN, Biochem. J. *89*, 585 (1963).
[2] R. E. EAKIN, E. E. SNELL and R. J. WILLIAMS, J. biol. Chem. *136*, 801 (1940).
[3] P. GYÖRGY, C. S. ROSE and R. TOMARELLI, J. biol. Chem. *144*, 169 (1942).
[4] R. D. WEI and L. D. WRIGHT, Proc. Soc. exp. Biol. Med. *117*, 341 (1964).
[5] C. H. PAI and H. C. LICHSTEIN, Proc. Soc. exp. Biol. Med. *116*, 197 (1964).
[6] P. GYÖRGY and C. S. ROSE, Proc. Soc. exp. Biol. Med. *53*, 55 (1943).
[7] P. GYÖRGY and C. S. ROSE, Science *94*, 261 (1941).
[8] J. FRAENKEL-CONRAT and H. FRAENKEL-CONRAT, Biochim. Biophys. Acta *8*, 66 (1952).
[9] M. D. MELAMED and N. M. GREEN, Biochem. J. *89*, 591 (1963).
[10] P. M. BLOOM and W. B. NELP, J. Lab. clin. Med. *65*, 1030 (1965).

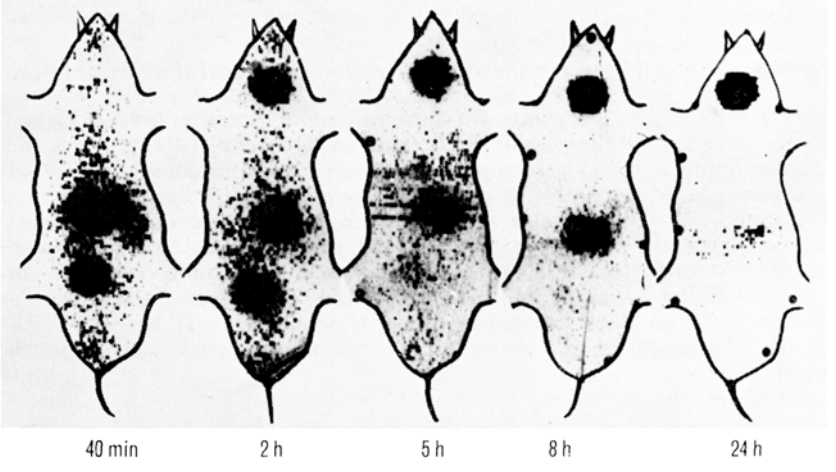


Fig. 1. Serial scans of a rat after i.p. injection of 42 μc of radioavidin-biotin complex, showing the ^{131}I was concentrated in the liver and urinary bladder at 40 min. Disappearance of the radioactivity from the liver into the thyroid proceeded graduately. Most of the radioactivity was found in the thyroid at 24 h.

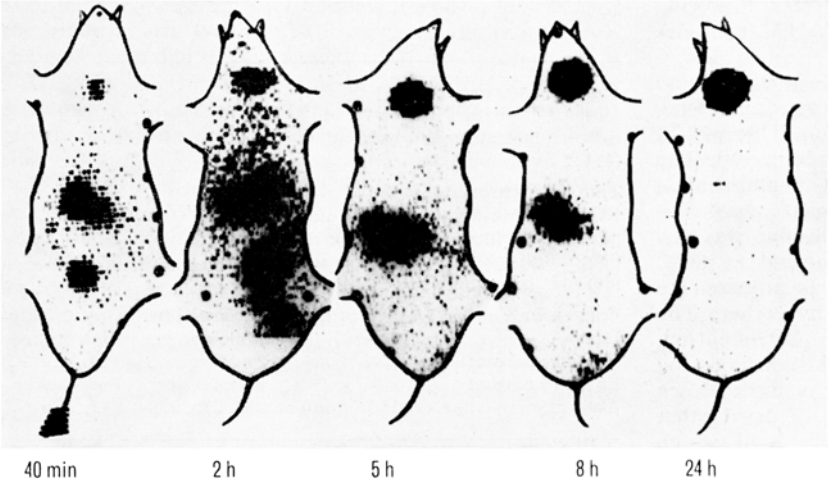


Fig. 2. Serial scans of a rat after i.v. injection into tail of 42 μc of radioavidin-biotin complex, showing the rapid concentration of ^{131}I in the liver, urinary bladder and even in the thyroid, at 40 min. Disappearance of the radioactivity from the liver into the thyroid proceeded graduately. Most of the radioactivity was found in the thyroid at 24 h.

excreated radioactivity was derived from the degradation of A–B*, the urine collected from the first day period was dialyzed against 0.2*M* ammonium carbonate. Data are given in Table II. The specific radioactivity of the solution in the membrane was equal to the dialysate. This reveals that a decomposition was occurred in the A–B*. To ascertain how much of the excreated radioactive material was unchanged biotin or processed biotin activity, excess avidin was added to an aliquot of the urine which was then dialyzed. This experiment indicated that 92% of the total radioactivity contained in the urine was in the form of avidin-combinable.

In another way of approach, radioavidin-biotin complex (A*–B) was prepared by the labelling of avidin part of avidin-biotin complex with ^{131}ICl followed the technique of BALE et al.[11]. The free radioiodide was removed by DEAE Sephadex and the pure A*–B solution (42 µc) was injected i.p. or i.v. into male rats. The rats were then scanned with Magnascanner III (Picker X-Ray Corp., Cleveland, Ohio) at 40 min, 2 h, 8 h, and 24 h after the injections. Figure 1 shows that A*–B was decomposed and the radioiodine was started to be deposited in thyroid gland at 40 min after the i.p. injection. At the end of 24 h almost all of the radioactivity was uptaken by the gland. The experiment of i.v. injection gave similar result, as shown in Figure 2, except that the radioactivity uptaken by the thyroid was faster than the experiment of i.p. injection.

Further studies on the in vitro dissociation of avidin-biotin complex in minced or sliced tissues are now under investigation. The preliminary results showed that marked degradation of the complex occurred in the presence of liver tissue and less with kidney, when they were incubated with medium 199 (NIH) at 37 °C under an atmosphere of 95% O_2 – 5% CO_2[12].

Zusammenfassung. Es wurde mittels des mit ^{14}C markierten Biotins und mit ^{131}J markierten Avidins gezeigt, dass der Avidin-Biotin-Komplex, i.p. oder i.v. in Ratten injiziert, dissoziert war.

R. D. WEI, D. H. KOU and S. L. HOO

Department of Biochemistry and the Kohlberg Laboratory, National Defense Medical Center and Medical Research Laboratory, Veterans General Hospital, Taipei, Taiwan (China), 29 July 1970.

[11] W. F. BALE, R. W. HELMKAMP, T. P. DAVIS, M. J. IZZO, R. L. GOODLAND, M. A. CONTRERAS and I. L. SPAR, Proc. Soc. exp. Biol. Med. *122*, 407 (1966).

[12] This work was supported by a grant from the China Medical Board of New York, Inc. (Grant No. 70-127).

The in vivo and in vitro Formation of 2-Amino-3-Hydroxyacetophenone from 2-Aminoacetophenone

It was reported by DALGLIESH[1] that 2-amino-3-hydroxyacetophenone-*O*-sulfate was excreted in small amounts in some normal human urines, and in appreciably larger amounts in certain pathological urines. ALLEN et al.[2] demonstrated the possibility of 2-amino-3-hydroxyacetophenone (AHA) being the cause of cancer of the bladder in man. Recently, we have isolated and identified AHA from the urine of rats, and 2-aminoacetophenone (AA) and AHA from the urine of hens, following ingestion of tryptophan[3]. However, the biosynthetic pathway of these compounds remains obscure. It is assumed that AHA is produced either by the degradation of 3-hydroxykynurenine or by the direct hydroxylation of AA, which may be formed from kynurenine.

This communication describes the isolation and identification of AHA from the urine of rats following ingestion of AA, and the formation of AHA from AA by microsomes isolated from rat liver.

Isolation and identification of AHA. Male Wister albino rats, weighing 100–150 g, received daily for 4 days 15 mg of AA per kg of body wt. by i.p. injection. Three 24-h-urine collections (200 ml) were pooled and filtered. The filtrate was adjusted to pH 3 with acetic acid and shaken with ether-ethanol (3:1). The organic phase was evaporated to dryness under nitrogen. The residue was dissolved in 20 ml of 1*N* HCl and hydrolyzed at 80 °C for 1 h under nitrogen. The solution was adjusted to pH 3 with NaOH and treated with ethyl ether. The ether extract was washed with 0.1% Na_2CO_3 solution, dried over sodium sulfate, anhydrous and concentrated under a stream of nitrogen gas. The sticky dark brown residue was dissolved in a small volume of dried ether and applied to a column (1 × 9 cm) of silicic acid, which was then eluted by 30 ml of dried peroxide-free ether. The eluate was evaporated to dryness under nitrogen. The residue was dissolved in a small volume of ethanol and partially purified by paper chromatography. Whatman No. 3 MM papers were used with the solvent system of MASON and BERG[4] containing 1 ml of glacial acetic acid per 100 ml of the solvent. *n*-Butanol-acetic acid-water (4:1:1) was also used. The fluorescent area corresponding to AHA on the chromatograms was cut out and treated with methanol-ether (1:2) to extract the fluorescent material. The extract from pa[illegible]r strips was

Chromatographic properties of a reaction [illegible]oduct with authentic 2-amino-3-hydroxyacetophenone

	Product	Synthesized
Paper chromatography[a]		
Mason-Berg with 1% acetic acid, Rf	0.91	0.91
n-Butanol-acetic acid-water (4:1:1 by volume), Rf	0.88	0.88
Thin layer chromatography		
Ethylacetate-isopropanol-28% ammonia water (9:6:4 by volume), Rf	0.88	0.88
Chloroform-ethylacetate-formic acid (60:40:1 by volume), Rf	0.72	0.72
Fluorescence at 3650 Å	Greenish blue	Greenish blue
DSA[b]	Pink orange	Pink orange
Ekman's reagent	Purple	Purple
Ehrlich's reagent	Pink orange	Pink orange
Absorption maxima at pH 7.0 (nm)	233, 270, 378	233, 270, 378

[a] Whatman No. 3 MM filter paper. [b] Diazotized sulfanilic acid.