3-phosphoglycerate were also present as impurities, but their amounts, determined through optical methods[13], were so low that they did not interfere in our experiments.

The hydrolytic activity of acyl phosphatase on 3-phosphoglyceryl phosphate was measured by the above mentioned optical test, which estimates the residual substrate after suitable periods of time. The enzymatic reaction was stopped by adding ammonium sulphate in final concentration 0.4 *M*: this concentration completely inhibits muscle acyl phosphatase[6,10]. In our conditions, the estimation of enzymatic hydrolysis, by using the method of LOWRY and LOPEZ[14] for the inorganic phosphate, is not suitable due to the catalytic effect of molybdate on the hydrolysis of the 1-radical of 3-phosphoglyceryl phosphate[15].

Results. The Table reports the acyl phosphatase activity on both acetyl phosphate and 3-phosphoglyceryl phosphate, expressed as relative rate of enzymatic hydrolysis.

Figure 1 shows a LINEWEAVER–BURK[16] plot, from which the Michaelis constant for 3-phosphoglyceryl phosphate at pH 5.3 was evaluated: this value is also reported in the Table.

It can be seen (Table) that 3-phosphoglyceryl phosphate is easily hydrolyzed by the enzyme, furthermore the hydrolysis rate is higher and the Michaelis constant is lower than with acetyl phosphate.

The effect of pH on the enzymatic hydrolysis of 3-phosphoglyceryl phosphate is reported in Figure 2. The optimum pH for acyl phosphatase activity on this substrate results in about 5.3, as previously obtained with other acyl phosphates[3,4,17].

These results add some quantitative information about the acyl phosphatase action on 3-phosphoglyceryl phosphate; this hydrolytic activity can explain the increase both of alcoholic fermentation[5] and that of glycolysis[18,19], under particular conditions[20].

Riassunto. Viene studiata l'attività idrolitica dell'acilfosfatasi purificata da muscolo sull'acido 1,3-difosfoglicerico. L'ottimo di pH è uguale a quello ottenuto per altri acilfosfati. Il valore della costante di Michaelis è dello stesso ordine di grandezza di quello trovato per l'acetilfosfato.

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[13] *Methods of Enzymatic Analysis* (Ed. H. U. BERGMEYER; Verlag Chemie Academic Press, New York and London 1963), p. 528 and p. 224.
[14] O. H. LOWRY and J. A. LOPEZ, J. biol. Chem. *162*, 421 (1946).
[15] H. WEIL-MALHERBE and R. H. GREEN, Biochem. J. *49*, 286 (1951).
[16] H. LINEWEAVER and D. BURK, J. Am. chem. Soc. *56*, 658 (1934).
[17] I. HARARY, Fedn Proc. Fedn Am. Socs exp. Biol. *16*, 192 (1957).
[18] V. BACCARI, A. GUERRITORE, G. RAMPONI and M. P. SABATELLI, Boll. Soc. ital Biol. sper. *36*, 360 (1960).
[19] I. HARARY, Fedn Proc. Fedn Am. Socs exp. Biol. *21*, 87 (1962).
[20] We are indebted to Mr. G. CAMICI for his skilful technical assistance.

Abnormalities of the Eye Pigments (Pteridins and Ommochromes) Induced in *Drosophila melanogaster* by the Inhibitor of Xanthine Dehydrogenase 4-Hydroxypyrazolo (3,4 d) Pyrimidine

When wild-type strains of *Drosophila melanogaster* are grown on media containing the inhibitor 4-hydroxypyrazolo (3,4 d) pyrimidine (HPP), phenocopies are obtained which mimic the *ma-l* and *ry* mutants. In fact, loss of isoxanthopterin and uric acid with accumulation of the corresponding precursors 2-amino-4-hydroxypteridine and hypoxanthine, as well as diminution of the red pteridins of the eye, have been observed[1,2]. These phenomena do not exclude the possibility that the inhibitor might affect other mutants, with different patterns of eye pigments metabolism.

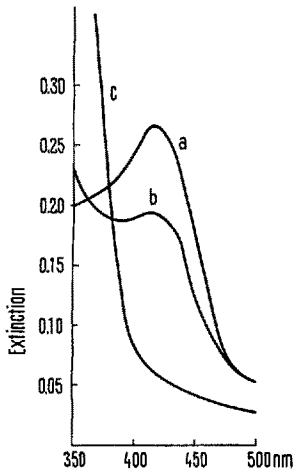
Strains of the *cl* and *se* mutants were raised, according to KELLER and GLASSMAN[1], on different concentrations of HPP[3]: 0.0; 0.01; 0.02; 0.03; 0.04; 0.06; 0.08 and 0.1 g%. Eye colours were recorded daily when the adults emerged. Flies, 3–5 days old, were placed in boiling water for 1 min, the heads dissected and homogenized in AEA (30% ethanol and HCl q.s. to pH 2[4]), 0.01 ml for each head. Aliquots of 0.01 ml of the homogenates were laid down on Whatman No. 1 filter paper; the chromatograms, developed by ascending chromatography in *n*-propanol and 2% ammonium acetate in water (1:1) and also in 3% NH_4Cl in water[5], were dried and observed in the visible light and under an UV-lamp emitting mainly at 365 nm. The remaining portions of the homogenates were centrifuged (20,000 *g*/30 min); the limpid supernatants, quantitatively collected, were diluted 1:10 and the absorption was measured with a Beckman DU spectrophotometer. The bodies also were treated in the same manner.

In the *cl* mutant, grown on HPP, the eye colour remains dark maroon. Loss of isoxanthopterin and accumulation of 2-amino-4-hydroxypteridin, mainly in the bodies of the male flies, occur at about the same concentrations of HPP as in wild-type strains. Partial loss of the drosopterins is more prominent for neodrosopterin than for iso- and drosopterin; correspondingly accumulation of biopterin, but not of sepiapterins, could be observed.

In *se* strains, grown on HPP (more evidently at the higher concentrations) the eyes are more pale, having a brown-beige colour, and usually smaller as compared with control flies. Loss of isoxanthopterin is in *se*, as in wild-strains, almost complete at the concentrations of HPP higher than 0.04%; accumulation of 2-amino-4-hydroxypteridine seems to be present in somewhat

[1] E. C. KELLER and E. GLASSMAN, Nature *208*, 202 (1965).
[2] P. BONI, B. DE LERMA and G. PARISI, Experientia *23*, 186 (1967).
[3] We are indebted to 'Wellcome Italia S.p.A.', Rome, for a generous gift of HPP (Allopurinol®).
[4] B. EPHRUSSI and J. L. HEROLD, Genetics, Princeton *29*, 148 (1944).
[5] M. VISCONTINI, E. HADORN and P. KARRER, Helv. chim. Acta *40*, 579 (1957).

smaller amounts. There is a partial loss of the yellow sepiapterins in the eyes of both sexes, as shown in the Figure; correspondingly the probable precursor biopterin accumulates chiefly in the bodies. Visual examination of the pellets from homogenates of *se* heads has shown that in either sex the colour of the pellets relative to inhibited flies is lighter than in control flies. Resuspending the pellets in 3% HCl in methanol, freshly saturated with SO_2 gas, red hydroxanthommatin is extracted, the colour intensity of which appears to be markedly less in the inhibited flies than in the control ones[6,8].



Light-absorption curves of AEA extracts of the heads of the mutant *sepia* (10 heads/1 ml): (a) for control flies; (b) for flies grown on HPP 0.06%; (c) for control flies, the extract of which has been irradiated 24 h under UV-light. The maximum at 420 nm present in the curves (a) and (b) corresponds to that of the sepiapterins.

Riassunto. Quando ceppi dei mutanti *se* e *cl* di *D. melanogaster* sono fatti crescere su terreni contenenti l'inibitore della xantino-deidrogenasi 4-idrossipirazolo (3,4 d) pirimidina, si osserva una perdita parziale dei pigmenti dell'occhio: tale perdita riguarda non soltanto i pigmenti pteridinici rossi (drosopterine), fenomeno questo già descritto, bensì anche i pigmenti pteridinici gialli (sepiapterine) nonchè i pigmenti ommocromici dell'occhio.

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[6] Notwithstanding that the diminution of the ommochromes visually observed is a very clearcut one, we were not able to perform a quantitative determination of the pigment in the form of hydroxanthommatin according to Butenandt et al.[7], because the colour intensities obtained after extraction of the pellets with 2*N* HCl and butanol were unstable and the absorption maximum frequently shifted to the yellow.

[7] A. Butenandt, E. Biekert, H. Kübler and B. Linzen, Hoppe-Seyler's Z. physiol. Chem. *319*, 238 (1960).

[8] This research was supported by the Consiglio Nazionale delle Ricerche, Rome (Italy).

Two Different Hemodynamic Patterns Underlying Hypotension during Desynchronized Sleep[1]

We have previously reported detailed studies of arterial pressure changes during natural sleep in the cat, showing that blood pressure falls more markedly during sleep with a desynchronized electroencephalogram and rapid eye movements than during sleep with synchronized electroencephalographic patterns[2]; that the hypotensive effect of desynchronized sleep is strikingly exaggerated by sino-aortic deafferentation[2]; and that this exaggeration is caused by abolition of the buffering action of chemoceptive impulses from the carotid and aortic bodies[3].

The experiments summarized below have been devised with the aim of further clarifying the hemodynamics of sleep, with particular reference to desynchronized sleep (DS). Most of the operating, recording and statistical techniques were as previously reported[2,3]. All cats were studied in a sound-attenuating cage. Systolic and diastolic pressure was recorded throughout the wakefulness-sleep cycle from a cannulated femoral artery; electroencephalogram, cervical electromyogram, and eye movements were also monitored on a Grass P7 polygraph. Details of other procedures are mentioned below.

In a first group of cats, most of them with previous sino-aortic deafferentation, arterial pressure changes during sleep have been compared before and after various kinds of heart denervation (bilateral stellatectomy, bilateral cervical vagotomy, and combined bilateral stellatectomy and vagotomy). In a few cats blockade of cardio-inhibitory vagal fibres was obtained by large doses (1 mg/kg i.v.) of methylatropine. Statistical analysis showed that during sleep arterial pressure undergoes changes of the same type and of corresponding size both when the heart innervation is intact and when it is destroyed. This means that the neural control of the heart is not basically involved in the blood pressure fall occurring in sleep.

These experiments, however, though implying that hypotension during sleep is mainly dependent on peripheral vascular phenomena, do not indicate whether the fall in blood pressure is basically due to a decreased vascular resistance or to a reduced venous or pulmonary return resulting in a lower cardiac output. This problem has been the object of a second series of experiments, in which, besides arterial pressure, aortic flow has been continuously monitored in sleeping cats by means of an electromagnetic flowmeter probe (Statham 4000A flowmeter, chronic K-probes) chronically implanted around the ascending aorta. The probe was calibrated with saline or blood before and after the experiment. Electronic integration of the aortic flow curve (Grass 7P10 integrator

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[2] M. Guazzi and A. Zanchetti, Science *148*, 397 (1965); Archs. ital. Biol. *103*, 789 (1965).

[3] M. Guazzi, G. Baccelli and A. Zanchetti, Science *153*, 206 (1966).