vated by orthodromic as well as by antidromic impulses (*7, 11*), it may be well to abandon the term *antidromic* inhibition; *recurrent* inhibition is offered as a functionally more meaningful name. Because the system generally inhibits neighboring motoneurons, it has been assigned a nonspecific anticonvulsant function (*9*). In the normal animal, however, recurrent inhibition may play an important coordinating role. Monosynaptic reflexes, that represent stretch reflexes, ordinarily are confined to the path of afferent origin (homonymous reflexes). However, if the motoneurons of synergists are sufficiently excited, they may also be discharged (heteronymous reflexes). If the inhibitory system normally is a factor in restricting the field of stretch reflex action, then a reduction in the inhibition, as by the action of DHE, should result in an increase in heteronymous reflex transmission. To test this supposition, monosynaptic reflex discharges were elicited, before and after injection of DHE, by repetitive stimulation at frequencies of 50 to 200 per second. These frequencies were chosen because high-frequency stimulation is a convenient means for securing heteronymous reflex discharge (*12*) and because each response of the series will normally fall into the period of inhibition caused by its predecessor. Figure 3, taken from an experiment that is representative of many carried out with various flexor or extensor nuclei in spinal or decerebrate cats, shows a plot of the amplitudes of homonymous and heteronymous reflexes elicited in, and recorded from, the peripherally cut lateral and medial branches of the gastrocnemius nerves in a decerebrate cat. Administration of 1 mg of DHE per kilogram increased heteronymous, but not homonymous, reflexes. This discrimination requires no special distribution of Renshaw cells but, instead, follows from the fact that heteronymous reflexes usually are smaller than homonymous ones; an equal amount of inhibition exerted on both types would depress the smaller ones more. This discrimination varies from one experimental situation to another. In some experiments injection of DHE increased not only heteronymous reflexes but also homonymous ones. In several tests it was possible to evoke heteronymous reflexes after injection of DHE when they had been absent before.

It is concluded from the evidence described above that the recurrent inhibitory system tends to confine stretch reflexes to their paths of afferent origin.

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Occurrence of Unequal Amounts of Free Methionine in Male and Female Drosophila melanogaster

An investigation by means of two-dimensional paper chromatography (*1*) of the free amino acids occurring in *Drosophila melanogaster* has revealed that wild-type females contain twice the amount of free methionine found in males. In previous chromatographic studies of the free amino acids of *Drosophila* (*2–5*), only one qualitative difference in the sexes has been reported—the occurrence exclusively in males of an unidentified substance presumed to be a peptide (*3*). Quantitative differences among amino acids have been cited but not documented (*4*). In the study discussed in this report, the difference in methionine content was confirmed by microbiological assay. In addition, the identification of this substance was made, and proof of its homogeneity was established, by isolation and chromatographic study of a dinitrophenyl derivative.

In the original chromatographic studies, whole flies were extracted with ethanol and the extracts were evaporated to dryness and redissolved in saturated picric acid to yield a protein-free solution. Chromatograms were developed, phenol-ammonia-water being used in the first direction, and lutidine-water in the second direction. Prior to development, the spotted samples were treated with ammonium molybdate and hydrogen peroxide.

This procedure converts essentially all the methionine in the extract to methionine sulfone, in which form it migrates as a distinct spot well separated from valine and alanine. The presence of Ninhydrin-reactive material at this position, as a result of the oxidation procedure, associated with a corresponding decline in amount of material at the position normally occupied by methionine, greatly facilitated the identification of the latter substance. In five different wild-type stocks, visual examination of chromatograms indicated the occurrence of twice as much methionine sulfone (and therefore of methionine) in females as in males.

To make sure that the larger amount of material at the methionine sulfone position in chromatograms of extracts of females was attributable solely to the presence of a higher level of this substance, the material was isolated and identified. Two-dimensional chromatograms of extracts of females were prepared in the usual way but were heated without spraying with Ninhydrin. The amino acids could then be seen as fluorescent spots when viewed under ultraviolet light.

The material in the methionine sulfone position was cut out and eluted with water, and a dinitrophenyl derivative was prepared. Chromatography of the reaction product in four solvents revealed the existence of a single substance whose R_f corresponded in each case with that of authentic dinitrophenyl methionine sulfone. This showed that the isolated material was methionine sulfone, and that the larger amount of material observed at the methionine sulfone position in chromatograms of extracts of females must be due entirely to the presence of a larger amount of this substance. It should be clearly recognized that the material present in the original extracts of flies is free methionine.

A bacterial assay was made in an effort to secure independent evidence that levels of free methionine differ with sex. For this purpose, tungstic acid filtrates of frozen flies were prepared by adaptation of methods previously described (*6*). The extracts were assayed in accordance with standard microbiological procedures (*7*) in which *Leuconostoc mesenteroides* P-60 and a completely synthetic methionine-free medium (*8*) were used. The amounts of free methionine found in extracts of wild-type male and female flies are shown in Table 1. Two experiments were per-

Table 1. Free methionine content of protein-free extracts of *Drosophila melanogaster*.

Expt. No.	Batch	Male (μg/g*)	Female (μg/g*)
1	1	200	475
1	2	190	420
1	3	360	430
Av.		250	442
2	1	300	585
2	2	305	610
Av.		303	598

* Wet weight.

formed, in each of which 2- to 3-day-old flies were collected as separate batches representing successive hatchings from a set of culture bottles. In both experiments extracts of females were found to contain twice the amount of methionine detected in extracts of males. With one exception the amounts found in the separate batches of a single experiment were relatively constant for each sex. However, the amounts of methionine found in the second experiment were higher in both sexes. The amounts of free methionine found are among the highest so far reported to have been found in an animal tissue (*9*). Separate studies have eliminated the possibility that the difference in males and females is attributable to the contents of the gut. The possibility that males and females have a differential ability to metabolize methionine is now under investigation.

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Elution of 3,4-Benzpyrene and Related Hydrocarbons from Soots by Plasma Proteins

The carcinogenic polycyclic aromatic hydrocarbon, 3,4-benzpyrene, has been identified as a constituent of soot. Its action in inducing skin cancers has been explained on the basis of the lipid solvent action of sebaceous secretions in eluting 3,4-benzpyrene from soot particles. Renewed interest in the carcinogenic properties of soot has followed the epidemiologic observation that the risk of development of lung cancer is greater among urban residents than among rural residents. Any description of the role soot may possess in the pathogenesis of human lung cancer must include an explanation for its biological activity in the lung.

In an earlier study we demonstrated that the carcinogenic hydrocarbon, 3,4-benzpyrene, which is routinely demonstrably adsorbed on soot, is absent in soot-laden human lungs (*1*). The morphologic demonstration of the intracellular entry of soot particles of appropriate size made it advisable to determine the effect of cellular and plasma proteins on the elution of benzpyrene from soot.

Soots of two representative sizes were studied in vitro for quantitative hydrocarbon determinations. One soot, of an average particle size of 80 mμ, was freed of essentially all polycyclic aromatic hydrocarbons by appropriate extraction methods and enriched by the adsorption of 3,4-benzpyrene onto the particles in a ratio of 1 μg of 3,4-benzpyrene to 1 mg of soot. The second soot was a commercial carbon black with an average particle size of 500 mμ. In their natural state these large particles contain a number of adsorbed polycyclic hydrocarbons.

The soots of the two sizes were handled in essentially the same manner. Ten-milligram samples of the enriched soot and 50- and 100-mg samples of the large-sized soot were placed in contact with an aqueous test medium consisting of 25 or 50 ml of sterile human plasma and were incubated at 37°C. The solutions were shaken constantly for 90 minutes in the 1.5- and 16-hour experiments and for 5-minute intervals to a total of 60 minutes of shaking in the remaining experiments. Incubation periods varied from 3 hours to 8 days. After exposure of the soot to the test medium, centrifugation or filtration was used to separate the soot from plasma. Three or four extractions with hot acetone were necessary for the removal of the adsorbed hydrocarbons from the soot.

Table 1. Percentage recovery of polycyclic aromatic hydrocarbons from 500 mμ soot and plasma after incubation of various durations; *t* trace; *p*, present.

Compound (μg/100 mg of soot)	1.5 hours			16 hours			96 hours			192 hours			Saline soot
	Soot	Plasma	Total	Soot	Plasma	Total	Soot	Plasma	Total	Soot	Plasma	Total	
Pyrene (8.4)	9	81	90	34	61	95	9	50	59	6	61	67	100
Fluoranthene (1.0)	*t*	≈ 100		*p*	*p*		0	*p*		*p*	*p*		100
Compound X (2.8)	0	100	100	*t*	93		0	*p*		0	39	39	100
1,2-Benzpyrene (2.9)	*t*	52		17	41	58	0	21	21	*t*	21		100
3,4-Benzpyrene (1.7)	18	82	100	41	59	100	6	23	29	18	18	36	100
1,12-Benzperylene (9.2)	31	66	97	67	33	100	5	15	20	11	13	24	100
Anthanthrene (2.4)	25	54	79	54	33	87	*t*	17		13	13	26	100
Coronene (10.0)	40	36	76	66	26	92	12	8	20	15	10	25	100

Although separation of the enriched small-sized soot from plasma was laborious, analysis for but a single carcinogenic hydrocarbon made the procedure relatively simple. In marked contrast, separation of large-sized soot from plasma was readily accomplished; however, analysis was more complicated because of the presence of several polycyclic aromatic hydrocarbons. In all instances, chromatography on a short column of activated alumina was necessary before accurate qantitation was possible.

Analysis for 3,4-benzpyrene was carried out with a Beckman DK-2 spectrophotometer. Fluorescence spectroscopy was used for the differentiation of 3,4-benzpyrene and 1,12-benzperylene. The plasma phase was similarly extracted, with ether as the solvent, and 3,4-benzpyrene quantitation was undertaken by the chromatographic and spectrophotometric methods described above. Control experiments were identical to the test ones except for the substitution of saline as the medium in the former.

After exposure to plasma proteins, the 3,4-benzpyrene remaining on the small-particle soot decreased slowly. After 3 days of incubation, 60 percent of the benzpyrene remained on the soot; and after 8 days, the quantity of 3,4-benzpyrene present dropped to 35 percent.

The elution pattern of the various aromatic polycyclic hydrocarbons from the large-sized carbon black (500 mμ) by plasma could readily be determined. Both the amounts eluted by plasma and the amounts retained on soot for the polycyclic aromatic hydrocarbons studied are shown in Table 1. Of the eight hydrocarbon compounds detected by the spectrophotometric technique, the following were found to be readily extracted by plasma: pyrene, fluoranthene, compound X, and 1,2-benzpyrene. The remaining identified polycyclic aromatic hydrocarbons in soot—3,4-benzpyrene, 1,12-benzperylene, anthanthrene, and coronene—were eluted at a lesser rate. Complete retention of 3,4-benzpyrene on the