cerebrospinal fluid) were carried through the entire procedure in every experiment. This is important, not only for the calculation of the recovery, but also for the qualitative determination, because the presence of organic material usually causes small changes in the Rf-values. By this method spots have been observed corresponding in position and colour to HVA and VMA added to cerebrospinal fluid. The amounts of the acids were estimated semiquantitatively on the chromatograms. The recoveries of HVA and VMA were about 40%. After correction for this, the normal concentration of HVA in cerebrospinal fluid was estimated to about 75 ng/ml and of VMA to about 25 ng/ml. DOPAC and DOMA were not found on the chromatograms. Nor was it possible to detect them spectrophotofluorimetrically after condensation with ethylenediamine[3]. The recoveries of added DOPAC and DOMA varied between 50 and 60%.

The catecholamines in the central nervous system have two inactivating pathways. Dopamine and noradrenaline are transformed by monoamine oxidase (MAO) to dihydroxylated phenolic acids, DOPAC and DOMA, respectively. This reaction seems to take place mainly in the synthesizing cell[4], but the enzyme can probably degrade also extracellular amines. The catecholamines can also be inactivated by catechol-O-methyl transferase (COMT), which seems to be localized far away from the synthesizing sites[4]. At their passage to the ventricles and subarachnoidal space, the 3-O-methylated catecholamines and dihydroxylated phenolic acids are converted to the corresponding 3-O-methylated acids by MAO and COMT, respectively. The catecholamines and their metabolites are normally so slightly lipid soluble that they can only with difficulty diffuse through the lipid-like blood-brain barrier. This may be the reason why HVA and VMA occur in the cerebrospinal fluid in such high concentrations. They probably leave the central nervous system by filtration through the arachnoidal villi[5]. An active transport from cerebrospinal fluid to blood in the choroid plexus may also be possible[6,7].

Zusammenfassung. Es wird papierchromatographisch gezeigt, dass Cerebrospinalflüssigkeit gesunder Menschen sowohl Homovanillinsäure als auch 3-Methoxy-4-hydroxymandelsäure enthält. Die Konzentration der ersteren ist etwa 75 ng/ml und die der letzteren etwa 25 ng/ml.

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[3] E. ROSENGREN, Acta physiol. scand. *49*, 370 (1960).
[4] A. CARLSSON and N.-Å. HILLARP, Acta physiol. scand. *55*, 95 (1962).
[5] L. D. PROCKOP and L. S. SCHANKER, Life Sciences *4*, 141 (1962).
[6] J. R. PAPPENHEIMER, S. R. HEISEY, and E. F. JORDAN, Amer. J. Physiol. *200*, 1 (1961).
[7] L. D. PROCKOP, L. S. SCHANKER, and B. B. BRODIE, J. Pharmacol. *135*, 266 (1962).
[8] *Acknowledgment.* This work has been supported by grants from Leo Ltd., Hälsingborg, and from the Medical Faculty, University of Göteborg (Sweden). For technical assistance we are indebted to Mrs. I. OLOFSON.

The 'brown spots' (bsp) Character of *Drosophila melanogaster* and its Relation to Copulation

The *'brown spots'* (*bsp*) character arose spontaneously in the wild stock *Aspra 52* of *Drosophila melanogaster* in 1959.

The phenotypical manifestation is strictly limited to the female sex; it consists in the formation of brown-coloured areas, variable in shape and size, localized in the pleurae; the pigmentation (probably due to melanin) (DI PASQUALE[1]) affects the hypodermic cells only (DI PASQUALE[2]).

The character is transmitted by the male as well as by the female; it is due to the presence of one or more recessive factors localized in the 2nd chromosome, and segregates normally in F_2 (DI PASQUALE[3]). The penetrance varies in time from 60% to 90%.

The phenotypical manifestation shows a clear relation to mating. Females kept apart from males, and females isolated from them when all the phases of courtship are complete but copulation has not yet taken place, never show spots.

A high incidence of manifestation (63.3% sp. and 21.6% d.)[4], on the other hand, is found when females are allowed to pair with males and are removed from them soon after the first copulation. Moreover, repeated copulations barely increase the degree of spotting.

bsp females mated with males of nine different stocks (*cn*, Chieti-*v*, *Cyl/Pm*, Varese, *tu* B3, *tu* A2, Oregon, Urbana, *y w*) show in all cases typical spots generally with a high frequency; significant differences are, however, noticeable to a greater or lesser extent in the incidence in the *bsp* stock; the mating with *y w* males, in which the incidence of the spots is particularly low (9.5% sp. and 2.2% d.), is a peculiar case.

Males of two different stocks, X/Y^{Lc} and X/O, sterile because of the non-motility of spermatozoa, the factors contained in the short arm of the Y chromosome being absent, also determine the appearance of spotting in *bsp* females. The same result is also obtained by pairing *bsp* females with X/X; *tra/tra* individuals. These, genotypically females, are transformed into males owing to the presence of *'transformer'*, a recessive gene localized in the 3rd chromosome (STURTEVANT[5]); they possess external genitalia and secondary sexual characters of male type, copulate regularly with females, have normal paragonia and rudimentary testes, and therefore do not produce spermatozoa.

Interspecific mating between *bsp* females and *Drosophila simulans* males also produces spotting, but with a low incidence (10.9% sp. and 1.1% d.).

These investigations, therefore, prove that the observed response to mating is a general phenomenon; it is also independent of the sterility of the male, whether due to the non-motility of the spermatozoa or to their absence.

Recent experiments using paper chromatography (CHEN and DIEM[6]) emphasize the presence of a ninhydrin-

[1] A. DI PASQUALE, Atti A.G.I. *7*, 138 (1962).
[2] A. DI PASQUALE, Atti A.G.I. *5*, 117 (1960).
[3] A. DI PASQUALE, Atti A.G.I. *6*, 233 (1961).
[4] In these experiments it was preferred to classify separately the females with spots (sp.) and with very small pigmented areas (dottings, d.).
[5] A. H. STURTEVANT, Genetics *30*, 297 (1945).
[6] P. S. CHEN and C. DIEM, J. Ins. Physiol. *7*, 289 (1961).

positive substance, previously described by Fox et al.[7] as 'sex peptide', peculiar to the male; this is found only in the paragonia and its amount increases in concentration from the 1st to the 9th day of the adult stage.

According to this finding the following experiment was set up to investigate whether differences in incidence of spots could be related to the amount of paragonial substance in the glands of the copulating male.

Individuals of the same age, aged from 2 to 8 days, bsp males and *bsp* females, isolated on emerging, were allowed to mate when the desired age was reached; when one copulation had taken place, the male was removed.

The results of counting are reported in Figure 1. It can clearly be seen that the frequency of mating increases with age; both frequency and size of spots increase too, reaching their maximum on the 6th day, while dotting frequency, higher on the 2nd day when the spots are few, decreases during the successive days, showing a symmetrical pattern.

Control males and females were mated in single pairs soon after emergence and left together until counting. The incidence of the spots was not very high and the size rather small; the mortality among females was particularly high.

To investigate whether the detected increase in spots really depends on the age of the male, the following experiment was made: males aged from 2 to 9 days were allowed to mate with females of a fixed age, 5 days old; reciprocally, females aged from 2 to 9 days were paired with 5-day-old males. The mode of collecting, isolating and pairing flies was the same as in the previous experiment; the age of 5 days was chosen, owing to the high incidence of spots previously found.

The following results were obtained. (a) *males of 2 to 9 days* × *females of 5 days* (Figure 2): the frequency of mating, high on all days, progresses regularly, showing two low points on the 3rd and the 6th day. The frequency of the spots is constantly very high, and decreases significantly on the 3rd and the 6th day; on the other hand, the frequency of dotting is low, and it follows a trend opposed to that of the spots. The size of the spots, fairly large, is more or less constant in time.

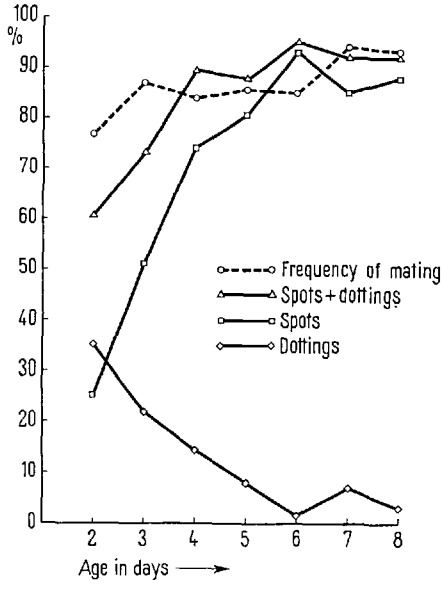

Fig. 1. Diagram showing the frequency of spots and dottings and the frequency of mating between individuals of the same age, in different days.

(b) *Females of 2 to 9 days* × *males of 5 days* (Figure 3): the frequency of mating is high and constant, and shows a significant decrease on the 3rd and the 6th day. The frequency of the spots, low on the 2nd and the 3rd day, increases on the following days, except on the 6th day, where a decrease is noticeable. The size of the spots, very small on the 2nd and the 3rd day, becomes larger during the following days. The frequency of dotting shows a symmetrical pattern opposed to that of spots.

Controls gave the same result as in the previous experiment.

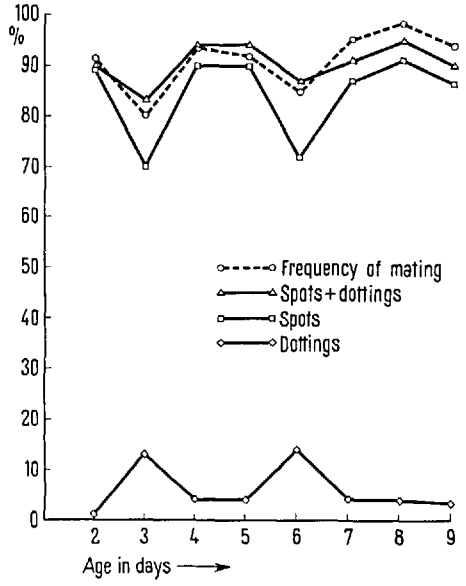

Fig. 2. Diagram showing the same phenomena as Figure 1; mating between 2–9 days old males and 5 days old females.

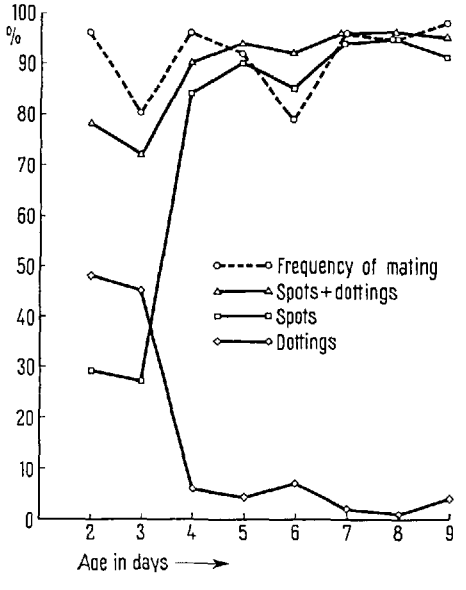

Fig. 3. Diagram showing the same phenomena as F gure 1 and 2; mat ng between 5 days old males and 2–9 days old females.

[7] A. S. Fox, C. D. Mead, and I. L. Munyon, Science *129*, 1489 (1959).

Discussion. From the results presented so far, it is clear that the phenotypical manifestation of the 'brown spots' character is strictly dependent on copulation.

The data are not yet sufficient to explain the nature of the phenomenon; they allow us, however, to hypothesise the existence of one of two different mechanisms, either of which for the moment appears equally likely.

(a) It might be suggested that the sperm fluid is responsible for inducing the spotting process. The fluid could produce a reaction in the *bsp* females owing to the presence in it of a peculiar substance; otherwise, an infecting agent present in the fluid could give rise to spots formation when introduced in the *bsp* female. In this last case it would be necessary to postulate a widespread agent; in fact it would be present in all males of the tested stocks.

One might also suppose that the sperm fluid merely activates a substance or an infecting agent already present in the *bsp* females.

(b) According to another mechanism, it could be assumed that spotting may be due to a reaction brought about in the female by her active participation in copulation, irrespective of the fluid introduced. In this case we should be confronted with a hormonal reaction.

Because of the clear connection between the age of the female at the moment of copulation and the frequency and size of spots, we must postulate that the contribution supplied by the male is constant throughout life.

At the same time we must conclude that the response supplied by the female is more or less intense according to her age, the mechanism at work being due to a specific cause contained in the sperm fluid or to a hormonal reaction.

The differences in spot frequency related to the aging of the female could merely consist in a capacity to produce brown pigment which changes with age.

In any case, the differences in incidence obtained in mating *bsp* females to males of various stocks demonstrate that the male genotype is responsible up to a point for the extent of the manifestation[8].

Riassunto. Le autori dimostrano che la manifestazione del fenotipo nelle femmine omozigoti per il carattere *bsp* è provocata dalla copulazione. L'accoppiamento con maschi di ceppi non *bsp* e con maschi sterili (X/Y^{Le}, X/O, X/X; *tra/tra*) determina pure la comparsa delle macchie nelle femmine *bsp*, dimostrando che il fenomeno è indipendente dalla presenza degli spermatozoi nello sperma. Esiste una relazione fra frequenza della manifestazione ed età della femmina al momento dell'accoppiamento.

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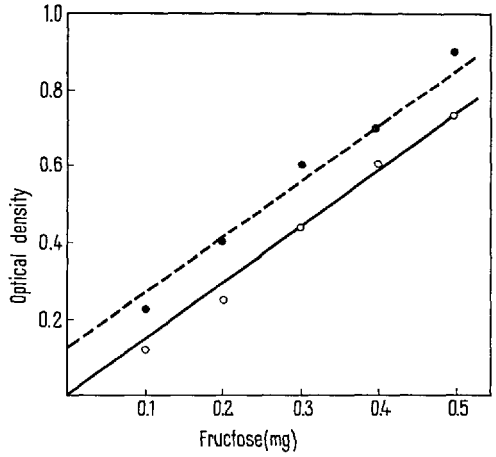
[8] This investigation was supported financially by the Consiglio Nazionale delle Ricerche, Roma.

The Interference of Tryptophane in the Estimation of Fructose by the Resorcinol Method

In our earlier studies with human semen, it was observed that fructose values of semen as estimated by the resorcinol method of ROE[1] were always higher than those obtained by the chromatographic method (SHETH and RAO[2,3]). The yeast fermentation tests carried out with human semen have also indicated that the resorcinol method is not very specific for the estimation of fructose in human semen (SHETH and RAO[3]). In the present investigation, attempts were made to find out the nature of the substances present in human semen which interfere with the estimation of fructose by the resorcinol method.

JORDAN and PRYDE[4] and POGELL[5] have shown that fructose reacts with tryptophane in the presence of concentrated hydrochloric acid to form a purple coloured product. As shown earlier, tryptophane was found to be present in human semen (SHETH and RAO[6]). Analyses of 9 semen samples have shown that the tryptophane content of the semen, estimated according to the method of FISCHL[7], was anywhere from 0.6 to 2.4 mg per ml.

Experiments were therefore carried out to see whether tryptophane would interfere with the estimation of fructose by the resorcinol method. Preliminary experiments showed that 0.2 mg of tryptophane by itself did not form any colour with resorcinol. In the subsequent experiment, fructose was estimated in the presence of tryptophane. As seen from the Figure in the presence of 0.2 mg of tryptophane, fructose values were always higher (0.07 to 0.16 mg/ml) as compared with the fructose values obtained in the absence of tryptophane. For the estimation of seminal fructose by the resorcinol method 0.1 ml of semen is employed (MANN[8]). As already shown, this quantity of semen would contain 60 µg to 240 µg of tryptophane. The results reported indicate that this concentration of tryptophane would seriously interfere with the estimation of fructose by the resorcinol method.



Graph showing the interference of tryptophane in the estimation of fructose by the resorcinol method. ● – ● Fructose values in the presence of 0.2 mg of tryptophane. o – o Fructose values in the absence of tryptophane.

[1] J. H. ROE, J. biol. Chem. *211*, 143 (1934).
[2] A. R. SHETH and S. S. RAO, Exper. *15*, 314 (1959).
[3] A. R. SHETH and S. S. RAO, Indian J. med. Sci. *16*, 709 (1962).
[4] R. C. JORDAN and J. PRYDE, Biochem. J. *32*, 279 (1938).
[5] B. M. POGELL, J. biol. Chem. *211*, 143 (1954).
[6] A. R. SHETH and S. S. RAO, Indian J. med. Sci. *15*, 24 (1961).
[7] J. FISCHL, J. biol. Chem. *235*, 999 (1959).
[8] T. MANN, Lancet *254*, 446 (1948).