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.

A. DI PASQUALE and L. ZAMBRUNI

Istituto di Genetica, Università di Milano (Italy), February 11, 1963.

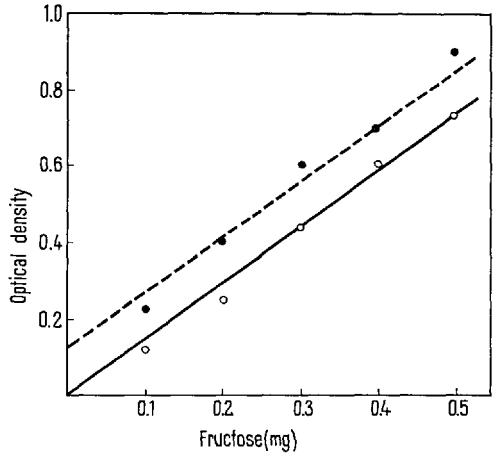
[8] This investigation was supported financially by the Consiglio Nazionale delle Ricerche, Roma.

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).

Experiments were carried out to find the effect of adding different amounts of tryptophane to fructose and then determining the sugar by the resorcinol method. As seen from the Table, with the increase in the amount of tryptophane there was a corresponding increase in the fructose values as estimated by the resorcinol method. So these results made it evident that the error observed in the estimation of seminal fructose by the resorcinol method would depend on the concentration of tryptophane present in semen.

It has already been shown that, in the cnromatographic method, fructose became separated from other reducing substances, particularly tryptophane, and thus the fructose values obtained by using the chromatographic method would be more accurate. These observations will hold true for any biological material containing tryptophane in which fructose is to be estimated by the resorcinol method. Results strongly indicate that the chromatographic method should prove of greater value in estimating fructose in the presence of tryptophane[9].

Effect of varying concentrations of tryptophane on the estimation of fructose by the resorcinol method

Tube No.	Fructose mg	Tryptophane mg	Optical density	mg fructose obtained in the presence of tryptophane	Difference in fructose (mg) in the presence of tryptophane
A	0.5	—	0.700	0.50	—
B	0.5	0.1	0.750	0.53	0.03
C	0.5	0.2	0.900	0.64	0.14
D	0.5	0.3	1.100	0.78	0.28
E	0.5	0.4	1.200	0.85	0.35

Résumé. Nous avons essayé de chercher la nature des substances réduisantes dans la semence humaine. Ces substances interviennent dans l'estimation quantitative du fructose d'après la méthode de Roe, utilisant le resorcinol. L'existence du tryptophane dans la semence humaine est déjà démontrée. En présence du tryptophane, la quantité du fructose obtenue est plus élevée que la quantité du fructose obtenue en absence du tryptophane.

Il semble, selon nos résultats, que l'estimation quantitative du fructose selon la méthode de Roe dépend de la quantité de tryptophane présente dans la semence humaine.

A. R. Sheth and Shanta S. Rao

Reproductive Physiology Unit, Indian Cancer Research Centre, Parel, Bombay (India), August 30, 1962.

[9] We are deeply indebted to the Director, Dr. V. R. Khanolkar, for his help and criticism during the course of this work. We are also grateful to Shri N. A. Dhonde for his technical assistance.

Occurrence of Hibernation in the Golden Hamster, *Mesocricetus auratus* Waterhouse

Introduction. In the course of an investigation of hibernation in the golden hamster, with particular attention paid to the endocrine glands (Smit-Vis, in press), the dates of entrance into the cold environment (refrigerator, $+5°C \pm 0.5$) and into hibernation respectively were registered as a matter of routine. Although, therefore, not collected for the purpose of studying the occurrence of hibernation, these data will be presented here as they are thought to give an explanation of the varying length of the prelethargic period (i.e. time in cold environment before hibernation). A hypothesis may be put forward, which allows the approximate length of the prelethargic period during the course of the year to be predicted.

Observations and Discussion. The dates of the entrance into the refrigerator and of the first hibernation period, together with the length of the prelethargic period for 26 hamsters, are summarized in the Table. The animals were kept in separate cages. Food and water were supplied *ad libitum* and sufficient hay was given for nestbuilding. Twice a day the animals were inspected and at the same time the shallow refrigerator was ventilated. At the beginning of the experiments, the age of the hamsters varied between three and six months. The animals are grouped according to the month in which they came into the cold environment. For each group an average of the prelethargic periods with its standard deviation is given in the right column of the Table. The shortening of the prelethargic time from November up to February (leaving out the divergent value for hamster 162; the reason for this will be discussed later) is noteworthy. Using Student's test, the differences between the group averages were proved to be statistically significant at the 5% level (Nov./Dec., $t_0(13) = 2.27 > t_{0.975}(13) = 2.16$; Dec./Jan., $t_0(11) = 2.22 > t_{0.975}(11) = 2.20$; Jan./Feb., $t_0(5) = 3.28 > t_{0.975}(5) = 2.57$.

In consequence of the shortening of the prelethargic periods, the observed hamsters entered hibernation in the course of February, with only a few exceptions, regardless of the date of entrance into the refrigerator (Figure 1).

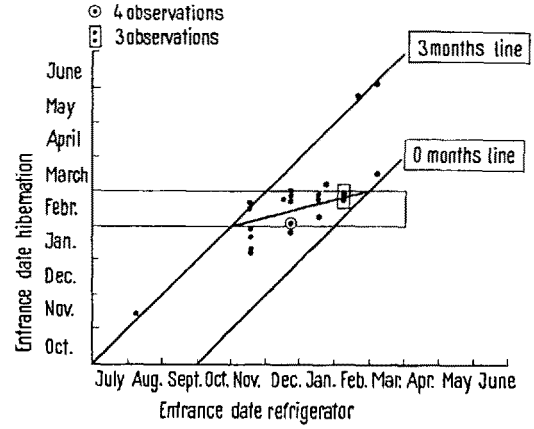


Fig. 1

The divergent value found for the prelethargic time of hamster 162 can be considered as an extreme value in the group of 'February' hamsters, because Dixon's test showed that for hamster 162 the following expression holds:

$$r_{10} = 0.94 > r_{R(n=4,\alpha=0.01)} = 0.889.$$

This test could not be applied to the 'March' hamsters, the number of observations being too small. Nevertheless,