Effect of Hepatectomy on the Growth of the Foetal Rat Liver

The regeneration that follows partial surgical removal of the rat liver is initiated either by the dilution of an inhibitor[1], or by the concentration of a stimulator in the blood[2,3]. Experiments with parabiotic rats were used as a system of studying these specific growth factors in the blood. It was reported that there was a mitotic response in the intact partner of a parabiotic rat when one member was subjected to partial hepatectomy[4]. The findings, when the serum or plasma from a partially hepatectomized rat was injected into normal recipient rats, or the rats that had undergone partial hepatectomy, are conflicting. It was reported that these injections either enhanced[5] or inhibited mitosis[6] in the regenerating rat liver, while McDONALD and ROGERS[7] failed to confirm the existence in the blood of stimulating or inhibiting factors.

To analyse the action of these growth factors during the embryonic life, a series of experiments were carried out during which the pregnant female rat was partially hepatectomized and the weight and the DNA content of the foetal liver were subsequently measured. It was DOLJANSKI[8] who proposed the analysis of changes in the total hepatic cell population by DNA determination instead of the mitotic index, which is in his opinion a criterion of limited value.

One series of pregnant females underwent only laparatomy[9] and represented our control series; the other one partial hepatectomy[4]. The operations were carried out between 2 and 4 p.m. during the 16th day of pregnancy. The rats were sacrificed 24 h after operation, and on day 19 or 21 post-conception. The embryos were weighed and the livers isolated. The homogenate was delipidated and DNA extracted after SCHNEIDER[10]. DNA was determined in the appropriate fraction of the tissue after BURTON[11]. The method standardized with DNA isolated from the calf thymus after ZAMENHOFF[12]. The sample was analysed for phosphorus after CHEN et al.[13] (Table I).

Four of all hepatectomized females had more absorption than the intact ones and were excluded from the present analysis. The difference in weight of the 21-day-old embryos (without the liver) as well as the difference in their livers, is statistically significant. Our purpose was to analyse whether the weight of the liver decreased in the same manner as the weight of the embryos. We therefore applied the analysis of covariance[14] which showed that (1) when testing differences in 'corrected' means ($F = 0.656$) F was not significant, which means that if the liver weights are compared, after the weights of the embryos are adjusted, they do not significantly differ; (2) when testing whether one regression line can be used for all the observations ($F = 0.568$) F was not significant, which means that one regression line can be used for both series of observations. The relative weight of the liver is the same in both series.

As far as DNA measurements are concerned (Table II), the series of hepatectomized rats had a lower total organ content than the series after laparatomy; but the differences, although constant, are not significant. On the other hand, in both series, even in the intact one[9], the difference of the total organ DNA content between the

Table I. Weight of embryos in mg

Day of gestation	Laparatomy	Hepatectomy	No. of embryos
16.5	439 ± 9.3[a]	399 ± 13.5	19 and 16
19	1780 ± 55	1692 ± 49	10
21	4084 ± 64	3724 ± 115	24

[a] Standard error of the mean.

Weight of liver in mg

Day of gestation	Laparatomy	Hepatectomy
16.5	33 ± 0.34	28.75 ± 0.36
19	173 ± 6.72	175.00 ± 7.87
21	350 ± 10.50	298.00 ± 17.00

t (embryos of 21st day) = 2.74 $P < 0.01$.
t (livers of 21st day) = 2.53 $P < 0.02$.

Table II. Total DNA organ content in γ

Day of gestation	Laparatomy	Hepatectomy	No. of samples
16.5	325 ± 14	294 ± 14	14
19	1155 ± 57	1153 ± 58	9
21	970 ± 44	879 ± 61	15

Difference between the 19th and 21st day
t = 2.57 $P < 0.02$ t = 3.26 $P < 0.01$

Liver DNA γ/100 mg

Day of gestation	Laparatomy	Hepatectomy
16.5	988 ± 19	1014 ± 31
19	680 ± 14	679 ± 21
21	292 ± 10	280 ± 7

[1] A. D. GLINOS, in *The Chemical Basis of Development* (Johns Hopkins Press, Baltimore 1958), p. 813.
[2] H. v. FRIEDRICH-FREKSA and F. G. ZAKI, Z. Naturforsch. *9b*, 394 (1954).
[3] H. WREA, M. RIFOLL-GOMEZ, and H. RANZ, Exp. Cell Res. *20*, 232 (1960).
[4] A. S. WENNEKER and N. SUSSMAN, Proc. Soc. exp. Biol. Med. *76*, 683 (1951).
[5] S. ADIBI, K. E. PASCHKIS, and A. CANTAROW, Exp. Cell Res. *18*, 396 (1959).
[6] F. J. MOYA, Exp. Cell Res. *31*, 457 (1963).
[7] R. A. MACDONALD and A. E. ROGERS, Gastroenterology *41*, 33 (1961).
[8] F. DOLJANSKI, Int. Rev. Cyt. *10*, 217 (1960).
[9] LJ. HOFMAN and N. ŠKREB, Bull. Sci., Conseil Acad. RSF Youg., in press (1965).
[10] W. C. SCHNEIDER, J. biol. Chem. *161*, 293 (1945).
[11] K. BURTON, Biochem. J. *62*, 315 (1956).
[12] S. ZAMENHOFF, in *Methods in Enzymology* (Academic Press, New York 1957), p. 696.
[13] P. S. CHEN, T. J. TORIBARA, and H. WARNER, Anal. Chem. *28*, 1756 (1956).
[14] W. DIXON and F. MASSEY, *Introduction to Statistical Analysis* (McGraw-Hill Book Company, New York 1957).

19th and 21st day of pregnancy is statistically significant. If we compare the concentration of DNA per 100 mg of the wet liver tissue, there is no difference between the two series.

Summarizing, we can say that the hepatectomy of the pregnant female rat slows down the growth of the embryo without any specific effect on the liver growth.

Résumé. Les auteurs ont utilisé deux lots des Rates portantes. L'un d'eux a été soumis à la laparotomie et l'autre à une hépatectomie partielle le 16ème jour de la gestation. Les animaux ont été sacrifiés à des dates échelonnées après l'intervention. Le poids des embryons et celui de leur foie, ainsi que la teneur en DNA du foie ont été mésurés.

L'hépatectomie de la mère ralentit la croissance des embryons sans aucun effet spécifique sur le foie embryonnaire.

N. Škreb, Lj. Hofman, and G. Luković

Institute of Biology, University of Zagreb (Yugoslavia), February 24, 1965.

The Influence of Cocaine and Reserpine on the Effect of Several Sympathomimetic Amines on the Mouse Iris

The various sympathomimetic amines can have direct, indirect (releasing of catecholamines as transmitter agent) or mixed (direct and indirect) actions on the various effector organs. With regard to the fact that the sympathomimetic drugs show certain differences of action on various effector organs (Trendelenburg[1], Holtz[2]), the behaviour of the mouse iris to sympathomimetic drugs after pre-treatment with cocaine or reserpine was investigated for determining the mode of action of fifteen sympathomimetic amines on this system.

Method. Determination of the mydriatic effect [E = maximal mydriatic diameter minus diameter before treatment (d_{bt} = 0.24 ± 0.016 mm)] according to Pulewka[3] on male mice of an average weight of 25 g, breed NMRI-Tübingen.

Results (see Figure). (a) Preceding injection (30 min s.c.) of cocaine significantly increased the mydriasis produced by the i.v. injection of norepinephrine, epinephrine or corbadrine, but did not influence the pupillary dilatation produced by i.v. injection of isoproterenol, alupent, norphenylephrine, *p*-hydroxyephedrine or effortil, and decreased significantly the mydriasis produced by ephedrine, *l*-phenylethanolamine, synephrine, buphenine, β-phenylethylamine, tyramine or pholedrine. (b) Preceding injection (24 h s.c.) of reserpine did not significantly influence

[1] U. Trendelenburg, Pharmacol. Rev. *15*, 225 (1963).
[2] P. Holtz, Acta neuroveg. *21*, 445 (1960).
[3] P. Pulewka, Arch. exp. Path. Pharmak. *168*, 307 (1932).

Direct, indirect, and mixed mode of action of various sympathomimetic amines

Agent	Substitution at						Mode of action	
	o	*p*	*m*	β	α	N	According to the chemical constitution (Fleckenstein et al.[4] etc.)	On the mouse iris
(a) Catecholamine derivatives								
Norepinephrine		OH	OH	OH			direct	direct
Epinephrine		OH	OH	OH		CH_3	direct	direct
Corbadrine		OH	OH	OH	CH_3		direct	direct
Isoproterenol		OH	OH	OH		$CH(CH_3)_2$		mixed
Alupent	OH		OH	OH		$CH(CH_3)_2$		mixed
(b) Intermediary agents								
Norphenylephrine			OH	OH			mixed	mixed
p-Hydroxyephedrine		OH		OH	CH_3	CH_3	mixed	mixed
Effortil			OH	OH		C_2H_5		mixed
Ephedrine				OH	CH_3	CH_3	mixed	indirect
l-Phenylethanolamine				OH			mixed	indirect
Synephrine		OH		OH		CH_3	mixed	indirect
Buphenine		OH		OH	CH_3	$CH(CH_3)CH_2CH_2$		indirect
(c) Neurosympatomimetic drugs								
β-Phenylethylamine							indirect	indirect
Tyramine		OH					indirect	indirect
Pholedrine		OH			CH_3	CH_3	indirect	indirect