HCG and 20, 100 or 200 µg of C.c., respectively, in 2 ml normal saline.

The mode of injection followed the scheme given above. The animals were sacrificed on the fourth day following the first injection, and the wet weight of the uteri was recorded to the nearest 0.1 mg.

Result and discussion. The results are summarized in Tables I and II. There was a significant difference ($p < 0.001$) in plasma FSH-activity between ovariectomized, oestrogen-progesterone blocked animals and those who had been ovariectomized but not been blocked. The injection of C.c. at the dose levels of 300 and 600 µg per animal caused a significant rise of plasma FSH-activity as compared to ovariectomized, oestrogen-progesterone blocked controls ($p < 0.001$). The difference between the 2 dose levels, however, was statistically not significant ($p > 0.05$). The plasma FSH-activity of C.c. treated animals was in the same order of magnitude as the one observed in ovariectomized, non-blocked animals. This seems to indicate that a maximal release of FSH was achieved at these dose levels.

Table I. Effect of clomiphene citrate upon FSH-release in the ovariectomized, oestrogen-progesterone blocked rat

Treatment (ovariectomized rats)	Dose of clomiphene citrate (µg)	Mouse uterine weight (mg)	*P v.* control
None	0	46.4 ± 1.3	< 0.001
50 µg oestradiol + 25 mg progesterone	0	29.3 ± 1.3	control
50 µg oestradiol + 25 mg progesterone	300	49.3 ± 2.9	< 0.001
50 µg oestradiol + 25 mg progesterone	600	47.0 ± 2.7	< 0.001

Determination of plasma-FSH according to IGARASHI and MCCANN[8].

Table II. Effect of clomiphene citrate and HCG upon uterine weight of infantile mice

Treatment	No. of mice	Uterine weight (mg)	*P v.* control
None	21	5.8 ± 0.2	–
2 ml normal saline	10	6.1 ± 0.5	–
0.25 IU HCG	11	19.1 ± 1.2	control
0.25 IU HCG + 20 µg clomiphene	7	20.9 ± 0.6	> 0.05
0.25 IU HCG + 100 µg clomiphene	7	20.2 ± 0.3	> 0.05
0.25 IU HCG + 200 µg clomiphene	7	19.8 ± 0.5	> 0.05

A direct uterotropic effect of C.c. still present in the plasma at the time of sacrifice could be excluded. As shown in Table II, there was a significant rise of uterine weight in immature mice after injection of a total dose of 0.25 IU HCG as compared to controls. No further increase of uterine weight was seen when 20, 100 or 200 µg of C.c./animal were injected together with HCG.

It is concluded, therefore, that the increased plasma FSH-activity observed in ovariectomized, oestrogen-progesterone blocked rats after i.v. injection of C.c. was due to an effect of this compound on the releasing mechanism for FSH. The results presented here are in agreement with the findings of IGARASHI et al. It appears likely that C.c. stimulates the release of FSH-RF in the rat, which leads to increased production and secretion of pituitary FSH.

Zusammenfassung. Der Effekt von Clomifendihydrogenzitrat auf den FSH-Release der Ratte wurde untersucht. Die i.v. Injektion von 300 bzw. 600 µg Clomifendihydrogenzitrat/Tier führte bei oophorektomierten, weiblichen Ratten, die mit Östradiol und Progesteron blokkiert worden waren, zu einer signifikanten Erhöhung der FSH-Aktivität im Plasma. Eine Beeinträchtigung des FSH-Nachweises durch noch im Plasma befindliches Clomifendihydrogencitrat konnte ausgeschlossen werden.

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Liver Regeneration After Partial Hepatectomy in Rats Exposed Before the Operation to the Stress Stimulus

Some metabolic changes, for example the changes of proteosynthesis[1] and the changes of ATP metabolism[2] developing in the liver in the first 12 h after 65–70% hepatectomy, can be found even in the intact liver after the administration of different stress stimuli[1–4]. The conditions under which the development of these changes in the liver tissue is stimulated probably differ after partial hepatectomy (PH) from those after simple stress stimulus. Only the development of these changes after the application of stress stimulus can be prevented by adrenalectomy[1]. After PH the onset of the changes of proteosynthesis and of the changes of ATP metabolism precedes the development of the increased DNA synthesis[2,5]. To understand better the relationship between these changes, we decided to find out whether the changes of DNA synthesis are influenced if PH is carried out in time when the stress reaction is fully developed.

For our experiments, 75 female rats, aged 3–4 months, were used. At PH liver weight was reduced by 65–70%[6]. As the stress stimulus 8 h before PH the i.p. injection of the water suspension of hyflo-super-cell (5 mg/0.5 ml of saline/100 g of body weight), of compound similar to

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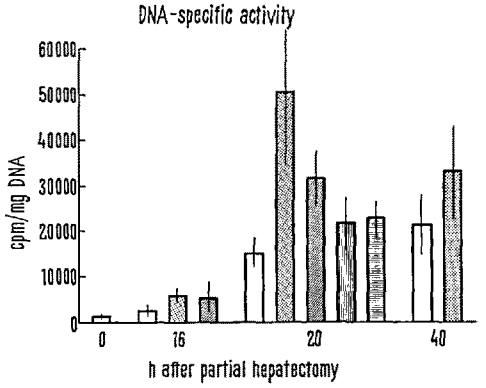
celite used to the same purposes by other authors[1,2], was administered. The other rats received at the same interval before PH the injection of ACTH (corticotrophin, SPOFA, Czechoslovakia) (8 U/100 g of body weight i.m.) or hydrocortisone (natrium 21-succinicum, SPOFA, Czechoslovakia) (5 mg/100 g of body weight i.m.). In 1 group laparotomy was carried out. The content of liver triglycerides was estimated according to VAN HANDEL and ZILVERSMITH[7], the content of DNA by the method of DISCHE[8]. For the estimation of DNA synthesis tritiated thymidine (5 μc/0.5 ml of saline per 100 g of body weight, (sp. act., 25 c/m*M*) was injected into the femoral vein of each rat. 1 h later the rats were killed by decapitation. The nuclei of the liver cells were isolated[9], then washed on filters with trichloracetic acid (5%), alcohol, ether and finally dissolved by hyamin before the scintillation fluid was added. The radioactivity of samples was measured in liquid scintillation counter Mark I (Nuclear, Chicago). The sp. act., cpm/1 mg of DNA was then calculated. The results were evaluated statistically using Student's *t*-test.



Sp. act. of hepatic DNA before and after 65–70% hepatectomy in control rats (□) and in rats in which 8 h before the operation the stress stimulus (5 mg of hyflo-super-cell/100 g of body weight) (▨), ACTH (8 U/100 g of body weight) (▨), hydrocortisone (5 mg/100 g body weight) (▨), or laparotomy (▨) were applicated. Means and the confidence limits for $p = 0.05$ are given.

Liver weight and content of liver triglycerides after PH in rats exposed 8 h before the operation to the stress stimulus (5 mg of hyflo-super-cell/1 ml of saline per 100 g body weight i.p.) or to the administration of ACTH (8 U/100 g body weight i.m.)

After PH (h)	Groups	Liver weight g/100 g body weight	Glycerol of triglycerides mg/g of liver
	Normal (a)	3.01 ± 0.28	1.06 ± 0.33
0	Control rats (b)	0.97 ± 0.20	–
20	Control rats (c)	1.83 ± 0.08	8.01 ± 1.15
	Stress stimulus (d)	2.16 ± 0.19	11.93 ± 1.49
	ACTH (e)	2.00 ± 0.21	10.21 ± 1.49
40	Control rats (f)	2.08 ± 0.23	7.59 ± 3.39
	Stress stimulus (g)	2.73 ± 0.37	7.21 ± 1.41

Means and the confidence limits for $p = 0.05$ and the statistical significance between the groups are given. L.w. c:d, $p < 0.01$, gl. c:d, $p < 0.001$, l.w. f:g, $p < 0.01$, gl. c:e, $p < 0.05$.

In rats in which 8 h before PH the stress stimulus was applied, the DNA synthesis in the liver tissue 16 ($p < 0.01$) and 20 h after the operation ($p < 0.001$) was significantly higher than in the control rats (Figure). In rats which received 8 h before PH the injection of ACTH, the DNA synthesis 16 ($t = 2.12$; $p > 0.05$) and 20 h after the operation ($p < 0.001$) was more marked than in the control rats. Injections of hydrocortisone and laparotomy itself were not effective in this sense. In rats in which the stress reaction was provoked, the weight of the liver and the accumulation of liver triglycerides after PH were more marked than in the control rats (Table). The injection of ACTH before PH had the same effect.

These experiments have shown that, during the stress reaction, the conditions accelerating and intensifying the regeneration of liver tissue after PH had been created. A similar effect was observed if the endogenous secretion of glucocorticoids was stimulated by ACTH administration. The possible role of glucocorticoids in the preoperational development of conditions favouring the stimulation of liver regeneration after PH could be connected with their stimulatory effect on the synthesis of RNA[10], proteosynthesis[11] and on the induction of enzymes synthesis in the liver tissue[12]. Even the higher accumulation of triglycerides in the liver found in these experiments after PH can be linked to the permissive effect of glucocorticoids on lipomobilization[13], or to their direct effect on the metabolism of fatty acids in the liver tissue[14]. It is interesting to note that the increased lipid accumulation in the tissue did not interfere with the more intensive liver regeneration after partial hepatectomy. We suppose that the results of our experiments should be taken into account on consideration of the role of stress reaction developing regularly after PH as the consequence of the operational intervention.

Zusammenfassung. Es wird nachgewiesen, dass bei Ratten, die 8 h vor partieller Hepatektomie einer Stress-Stimulation ausgesetzt wurden, die Intensität der DNS-Synthese postoperativ im Vergleich mit den Kontrolltieren grösser war. Ebenso waren Veränderungen des Lebergewichtes und des Triglyzeridinhaltes in der Leber nach der partiellen Hepatektomie dieser Ratten stärker ausgeprägt. Vergleichbare Resultate wurden auch bei Ratten gefunden, die in der gleichen Zeit vor der partiellen Hepatektomie eine ACTH-Injektion erhielten.

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