apparent photosynthesis by DCMU, the rates of photorespiration were about 60% lower than in the control. The magnitude of this inhibitory effect of DCMU on photorespiration was similar in 1 as in 100% oxygen.

However, the stimulating effect of oxygen on this part of photorespiration which was not inhibited by DCMU was clearly pronounced. The relative values of the stimulation of photorespiration in 100% O_2 were similar during photosynthetic absorption of CO_2 and after suppression of this process by DCMU.

The results presented above have confirmed and extended our previous studies[1,4], showing that also this part of CO_2 evolution in light, which was not inhibited by DCMU in concentrations applied, represents a different process from dark respiration. This conclusion is supported by the fact that, in the absence of photosynthesis, photorespiration was stimulated by oxygen relatively to the same degree as during photosynthesis. Because of the discrepancy in the response of photorespiration and dark respiration to simultaneous action of DCMU and oxygen concentration, these experiments suggest that CO_2 output in light must come from a different source from that in dark respiration. Moreover, it supports the conclusion made earlier[1,2,5] that dark respiration was inhibited in light and replaced by a photorespiration[6,7].

Effect of 3-(3,4-dichlorophenyl)-1,1.dimethylurea (DCMU) and of oxygen concentration on the rates of apparent photosynthesis (APS), dark respiration (DR) and photorespiration (PR) in detached wheat leaves. Light intensity 90×10^3 ergs/cm^{2}sec^{-1}.

		μg CO_2/min g fresh weight		
	O_2%	APS	DR	PR
Control	1	121.8	13.0	1.0
(without DCMU)	100	22.0	14.6	32.4
$10^{-6}M$ DCMU	1	12.6	12.0	0.3
	100	0.0	15.9	9.9
Control	1	114.6	14.6	0.6
(without DCMU)	100	21.4	15.6	28.3
$10^{-5}M$ DCMU	1	9.4	13.4	0.2
	100	0.0	15.8	7.8

Zusammenfassung. Es werden der Einfluss des Photosynthesehemmstoffs DCMU und des O_2-Partialdrucks auf die Photosynthese und die Licht- und Dunkelatmung untersucht. Die Ergebnisse zeigen erneut, dass Licht- und Dunkelatmung 2 verschiedene Prozesse sind.

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Histochemistry of Ovarian 20α-Hydroxysteroid Dehydrogenase in Mature Hypophysectomized Rats

The activity of ovarian 20α-hydroxysteroid dehydrogenase (20α-HSD) can be histochemically demonstrated only in the corpora lutea (C.L.) 4–5 days after their onset in normal cycling rats and 24 h before parturition in pregnant animals[1–4].

A possibility of hypophysial control on the appearance of this enzymatic activity has been suggested in previous papers but it has not yet been clarified.

A blocking effect in the newly formed C.L. was obtained by treatments which inhibit gonadotropic incretion, such as the administration of natural or synthetic estrogens and of progestin-estrogen association as well[5,6]. On the other hand Balogh et al.[7,8] were able to induce this enzymatic activity in ovarian interstitial and thecal cells of hypophysectomized immature female rats by administration of HGC and in the C.L. of superovulated rat ovaries by administration of luteinizing hormones.

The task of the present work has been to evaluate the appearance of the 20α-HSD activity in the C.L. of ovaries from mature female rats deprived of their pituitaries. For this purpose the hypophysectomies were performed in rats in the different phases of the estrous cycle, i.e. with newly formed C.L. in different stages of maturation, before the onset of the 20α-HSD activity. In these experiments albino Sprague-Dawley female rats were used, weighing 150 g, with regular 4–5 days estrous cycles, controlled by daily vaginal smears for at least 3 cycles before making the operation. The animals were hypophysectomized by traspharyngeal approach and killed by decapitation 2, 4, 8 and 10 days after the hypophysectomy. The ovaries from single animals were quickly dissected out and frozen with CO_2. The 3β-hydroxysteroid dehydrogenase (3β-HSD) and the glucose-6-phosphate-dehydrogenase (G-6-PD) were determined at the same time with the 20α-HSD in order to obtain a more com-

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plete pattern of the ovarian enzymatic situation. The histochemical determinations were performed following the techniques referred to in previous papers[2,5]. At sacrifice, a control of the hypophysectomy was performed by microscopical inspection of the sella turcica, in order to detect whether it was complete.

Results. Two days after hypophysectomy the follicular granulosa cells are consistently devoid of 3β-HSD and G-6-PD activities. On the contrary a normal pattern is shown by these enzymes in the interstitial and thecal cells. The newly formed C.L., 20α-HSD negative, are present only in those animals that were hypophysectomized in proestrus (Figures 1 and 2) or in estrus and in only one of these hypophysectomized in metestrus (see Table). Four days after hypophysectomy the follicles are small, and none show 3β-HSD and G-6-PD activity in their granulosa cells. These activities are strongly reduced both in interstitial and thecal cells. All the C.L. still possess marked G-6-PD, 3β-HSD and 20α-HSD activities (Figures 3 and 4).

The ovaries of 8 animals sacrificed 8 and 10 days after hypophysectomy show a reduction in number and size of follicles and C.L. These latter are 32.3 and 24.4 per ovary after 8 and 10 days respectively.

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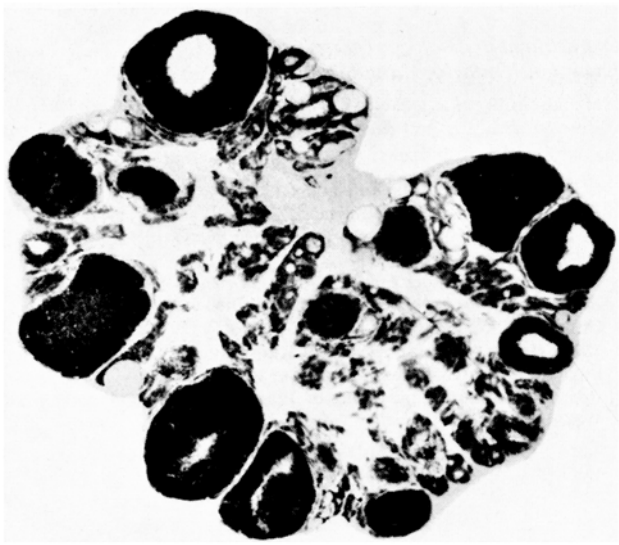

Fig. 1. Ovary of rat hypophysectomized in proestrus and sacrificed 2 days afterwards. Marked 3β-HSD activity in all the C.L. and in interstitial cells. × 25.

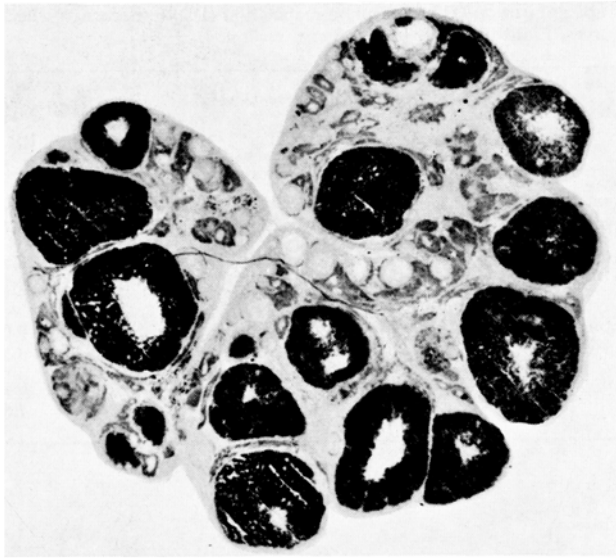

Fig. 3. Ovary of rat hypophysectomized in proestrus and sacrificed 4 days afterwards. The 3β-HSD activity is marked in all the C.L. and reduced in interstitial cells. × 25.

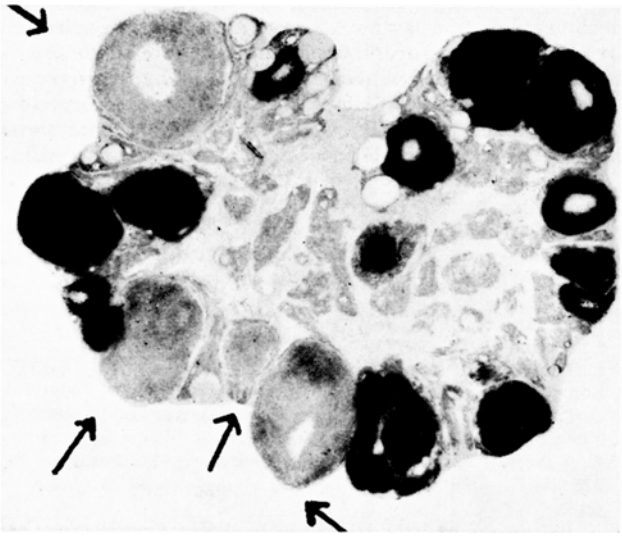

Fig. 2. Section contiguous to that of Figure 1. The arrows show the newly formed C.L. 20α-HSD negative formed after hypophysectomy. × 25.

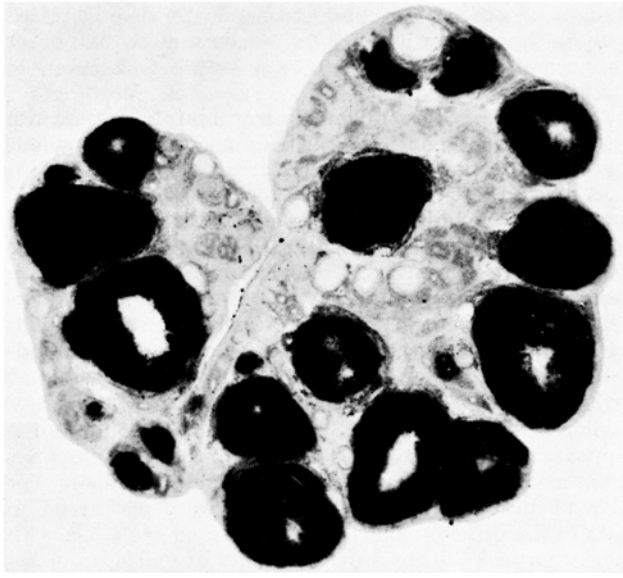

Fig. 4. Section contiguous to that of Figure 3. All the C.L. are 20α-HSD positive. × 25.

The enzymohistochemical pattern is, on the contrary, similar to that observed in the animals killed 4 days after hypophysectomy.

As far as 3β-HSD is concerned the above results are in agreement with the conclusions by LEVY et al.[9], TAYLOR[10], and FUHRMANN[11], i.e. that the presence of these enzymes in the ovaries depends on the hypophysial incretion. It is to be pointed out that the loss of this enzymatic activity runs at different rates in the different ovarian structures. The same is to be said for the G-6-PD.

First of all the ovarian granulosa, blocked in its maturation, do not acquire the 3β-HSD and the G-6-PD activities. Then the thecal and interstitial cells show a reduction of their enzymatic activities. The C.L. enzymes are, according to LEVY[9], the most resistant. Only later is 3β-HSD activity strongly reduced in C.L. as observed by TAYLOR[10] in rats 3 months after hypophysectomy.

As for 20α-HSD activity it must be said that hypophysectomy does not prevent the onset of new C.L. 20α-HSD negative when the animals are hypophysectomized in estrus or in proestrus, whereas it blocks the ovulation and the consequent formation of C.L. in those animals submitted to hypophysectomy in metestrus and diestrus. In no animal does the hypophysectomy inhibit the appearance of the 20α-HSD activity both in the C.L. already present at the time of the operation and in those that appear after it (rats in estrus and proestrus).

Looking at these results, consistent with the fact that the C.L. of pregnancy are 20α-HSD negative[1,4], we may suggest the possibility that the gonadotropins will play their role mostly in inhibiting rather than inducing the onset of 20α-HSD activity in the C.L.[12].

Corpora lutea (C.L.) scored in ovaries of hypophysectomized rats

Cycle phase at the moment of hypophysectomy	2 days after hypophysectomy			4 days after hypophysectomy		
	No. of animals	C.L./ovary		No. of animals	C.L./ovary	
		Total[a]	20α-HSD negative		Total[a]	20α-HSD negative
Proestrus	5	45.1	3.4	5	41.4	0
Estrus	5	38.4	5.7	5	36.2	0
Metestrus	5	42.3	1.8	5	35.7	0
Diestrus	5	39.4	0	5	36.7	0

[a] Identified by the visualization of the 3β-HSD activity.

Riassunto. È stato studiato il quadro enzimatico dei corpi lutei (C.L.) di recente formazione nelle ovaie di ratte ipofisectomizzate. In particolare si è constatato come la comparsa dell'attività 20α-idrossisteroide deidrogenasica non sia impedita dall'ipofisectomia. Si suggerisce la possibilità che l'ipofisi eserciti una azione inibitrice sulla comparsa di questo enzima.

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[12] The authors are greatly indebted to Dr. C. SECHI who performed the hypophysectomies, to Dr. E. SCRASCIA who supplied the animals controlled by vaginal smears and to Miss G. E. CACCIA for her most valuable technical assistance.

Hydrolysis of Amino Acid β-Naphthylamides by Aminopeptidases in Human Parotid Saliva and Human Serum

The enzymatic hydrolysis of leucyl β-naphthylamide in serum[1], urine[1], and cerebrospinal fluid[2] had been reported. MÄKINEN[3] recently found in whole human saliva, and in human dental plaque, the aminopeptidases which hydrolyze various amino acid β-naphthylamides including leucyl β-naphthylamide. He suggested that the aminopeptidases are mostly produced by the plaque organisms. However, specific data on the presence of an enzyme hydrolyzing amino acid β-naphthylamides in fluid from the salivary glands is lacking. We tried to measure the activities of aminopeptidases in human parotid salivary secretions by means of a photometric procedure[1], but the activities were too low to be measured by this method. We subsequently found that the activity could be exactly measured by a sensitive fluorometric assay[4,5]. This communication reports the presence of aminopeptidases in human parotid saliva and the comparison of the substrate specificities with the enzymes in human serum.

Parotid saliva was collected from 4 subjects aseptically by means of a cannula devised by UMEMOTO et al.[6]. Salivary flow was elicited by the stimulation of dilute acetic acid. The substrate amino acid β-naphthylamides, which were synthesized as described by GLENNER et al.[7], were kindly supplied from Dr. G. G. GLENNER. The incubation mixture contained 90 μmoles *Tris*-maleate buffer, pH 7.0, 0.15 ml of parotid saliva, 0.45 μmole amino acid β-naphthylamide and water to 0.90 ml. The activity for the hydrolysis of α-L-aspartyl β-naphthylamide and α-L-glutamyl β-naphthylamide was measured in the presence of 10 m*M* of Ca^{2+}. Incubation was carried out at 37 °C for 60 min. Increase of fluorescence intensity

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