Japanese group following the maximum swelling state of chloroplasts in the light. Preliminary investigations to explore the cause of this discrepancy provided evidence that growth conditions and the age of spinach leaves might be the clue.

The mechanism by which swelling is accelerated by light has not yet been conclusively explained. However, the fact that light-induced swelling is associated, at least during the first 30 min of incubation, with energy-dependent ions[7,14] and water[1] uptake points to a close relationship of these phenomena to photochemical reactions in chloroplasts. The Table illustrates such a correlation. In 45 min chloroplasts doubled their volume in the light (experiment 1) while oxygen evolution by the same light-incubated chloroplasts diminished to 40% of its initial rate (experiment 4). Also, photophosphorylation was dramatically affected, showing a 73% drop in its original rate (experiment 5). Although less affected, light-triggered ATPase was reduced 48% (experiment 6). After 135 min, all of the above photochemical activities had almost completely disappeared. Since the bleaching of chlorophyll during the experimental period might have been the cause of the inhibition of the photochemical reactions, it was determined by estimating the optical density changes at 652 nm of the diluted chloroplast suspension (experiment 2). The degree of chlorophyll bleaching was of a very much smaller magnitude than the observed inhibition of photochemical activities. Thus, it appeared that a 135-min light incubation of chloroplasts at 20 °C obliterated the main energy transfer reactions which are necessary for subsequent CO_2 reduction.

However, the photoreduction activity was an exception to this general pattern in that it was not affected by a 45-min light treatment and was only 14% inhibited after 135 min (experiment 3). This might suggest that chloroplasts are uncoupled when incubated for a long period of time in saturated light.

In darkness, a condition under which chloroplast swelling occurs at a much slower rate than in the light and where chlorophyll bleaching is negligible, the ability of chloroplasts to carry out photochemical reactions also diminished but to a lesser degree than in the light (Table). After a 135-min incubation period, chloroplasts still retain 50% of their capacity to evolve O_2 and to phosphorylate. Only after 5 h, when chloroplast volume was the same as in the light, had the chloroplasts lost these activities. In darkness, as in the light, chloroplasts did retain their photoreduction capacity.

Thus, it appears that maximum swelling of chloroplasts corresponds to the abolition of all the measured photochemical reactions carried out by these organelles with the exception of photoreduction. This strengthens the view that this phenomenon is a deteriorative process. Such a process occurs slowly in the dark (natural aging) and is accelerated in the light. The causes and the conditions under which such a deteriorative process occurs and its relation to swelling are under investigation[16].

Résumé. Une incubation prolongée de chloroplastes isolés d'épinard à l'obscurité provoque un gonflement des plastides et une inhibition concomitante de leurs activités photochimiques (à l'exception de la photoréduction du ferricyanure). Un traitement lumineux accélère ces phénomènes qui, probablement, sont associés à des processus de détérioration de l'appareil photosynthétique.

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The Intermediate Role of 18-Hydroxycorticosteroids in Aldosterone Biosynthesis

The role of 18-desoxycorticosteroids as intermediates of the aldosterone biosynthesis is well established[1-3]. It still remains unclear, however, which of the possible 18-hydroxycorticosteroids is (are) the essential precursor(s) of aldosterone[4].

Quarters of left and right adrenals of male Sprague-Dawley rats were incubated separately in Krebs-Ringer bicarbonate glucose (200 mg%) solution for 4 h with either 0.2 μC 4-^{14}C-progesterone (57 mC/m*M*) or 0.5 μC 1,2-^{3}H-11-desoxycorticosterone (10 C/m*M*) added per 100 mg tissue. 4 μg/mg tissue of either 11-desoxycorticosterone, corticosterone, 11-dehydrocorticosterone, 18-hydroxyprogesterone, 18-hydroxy-11-desoxycorticosterone or 18-hydroxycorticosterone were added to the right adrenals prior to incubation. The sample with the left

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adrenals served as control with no further additions. 18-Hydroxy-11-desoxycorticosterone, corticosterone, 18-hydroxycorticosterone and aldosterone were isolated from the incubation media and the radioactivity incorporation into each of the 4 steroids was estimated[5]. Inhibition of radioactivity incorporation (radioactivity dilution, competitive or product inhibition) into 1 or several of the isolated corticosteroids by a particular unlabelled steroid added was expected to provide information about the intermediate role of this steroid in aldosterone biosynthesis.

Results are summarized in Figure 1 as differences in radioactivity incorporation by the control (left) adrenals and by those adrenals (right) which had been exposed to unlabelled steroid substrate. The differences are expressed in % of the control incorporation (%-changes). Each bar represents the averaged results of 5 or 6 independent experiments. Proof for the validity of the left-right comparison, also referred to previously[5], has been achieved by independent incubation of left and right adrenals under identical conditions (unlabelled substrate added: 'none'). The incorporation of 4-^{14}C-progesterone into the 4 steroids isolated from the incubation media of either left or right adrenals did not differ significantly.

11-Desoxycorticosterone showed the most remarkable inhibitory effect on the incorporation of both radioactive precursors into each of the 4 corticosteroids analyzed. This confirms the importance of 11-desoxycorticosterone as key intermediate of the biosynthesis of 18-hydroxy-11-desoxycorticosterone, corticosterone, 18-hydroxycorticosterone and aldosterone, and it also confirms the validity of our experimental design. Since the incorporation of 1,2-^{3}H-11-desoxycorticosterone was inhibited by 11-desoxycorticosterone as well as that of 4-^{14}C-progesterone this inhibition must be due to radioactivity dilution rather than to product inhibition of the progesterone-21-hydroxylation. Corticosterone did not significantly change the incorporation of either radioactive precursor into 18-hydroxy-11-desoxycorticosterone nor into corticosterone. Since corticosterone is the major product of the rat adrenal cortex, larger amounts of corticosterone may be required to cause product inhibition. Radioactivity incorporation into 18-hydroxycorticosterone and aldosterone was reduced significantly by corticosterone, most probably due to radioactivity dilution. This result points to the fact that corticosterone is a precursor of 18-hydroxycorticosterone and aldosterone[4,6], and it confirms again the validity of our working hypothesis.

11-Dehydrocorticosterone almost resembled corticosterone in its effects. There were no significant changes in the incorporation of ^{14}C or ^{3}H into 18-hydroxy-11-desoxycorticosterone and corticosterone, while the incorporation into 18-hydroxycorticosterone and aldosterone was significantly diminished. These data might suggest participation of 11-dehydrocorticosterone in the formation of 18-hydroxycorticosterone and aldosterone. Incorporation of 4-^{14}C-11-dehydrocorticosterone into 18-hydroxy-11-dehydrocorticosterone and aldosterone by rabbit adrenal slices has been reported by FAZEKAS and KOKAI[7]. In similar studies with bull frog adrenals, DE NICOLA, KRAULIS and BIRMINGHAM[8] failed to find any incorporation of 1,2-^{3}H-11-dehydrocorticosterone into 18-hydroxy-11-dehydrocorticosterone or aldosterone. In our experiments with 11-dehydrocorticosterone large amounts of UV-absorbing material were observed at the chromatographic site of corticosterone, thus suggesting that a great portion of the 11-dehydrocorticosterone added was reduced to corticosterone during incubation. Therefore, we tend to the view that the inhibitory effect of 11-dehydrocorticosterone is in fact due to radioactivity

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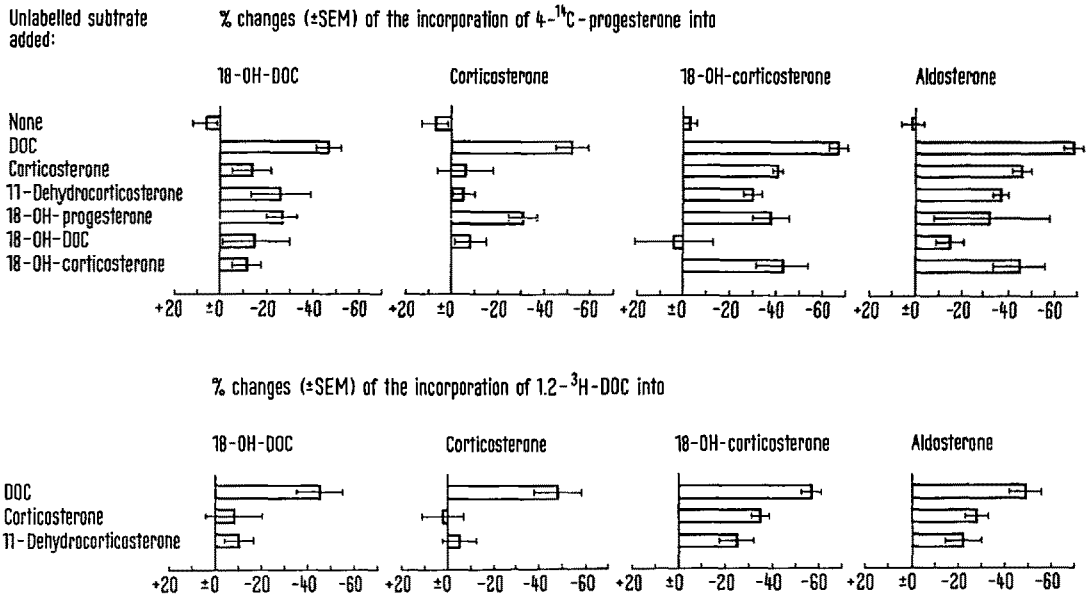

Fig. 1. Effects of unlabelled substrates added to the incubation media on the radioactivity incorporation from 4-^{14}C-progesterone and 1,2-^{3}H-11-desoxycorticosterone.

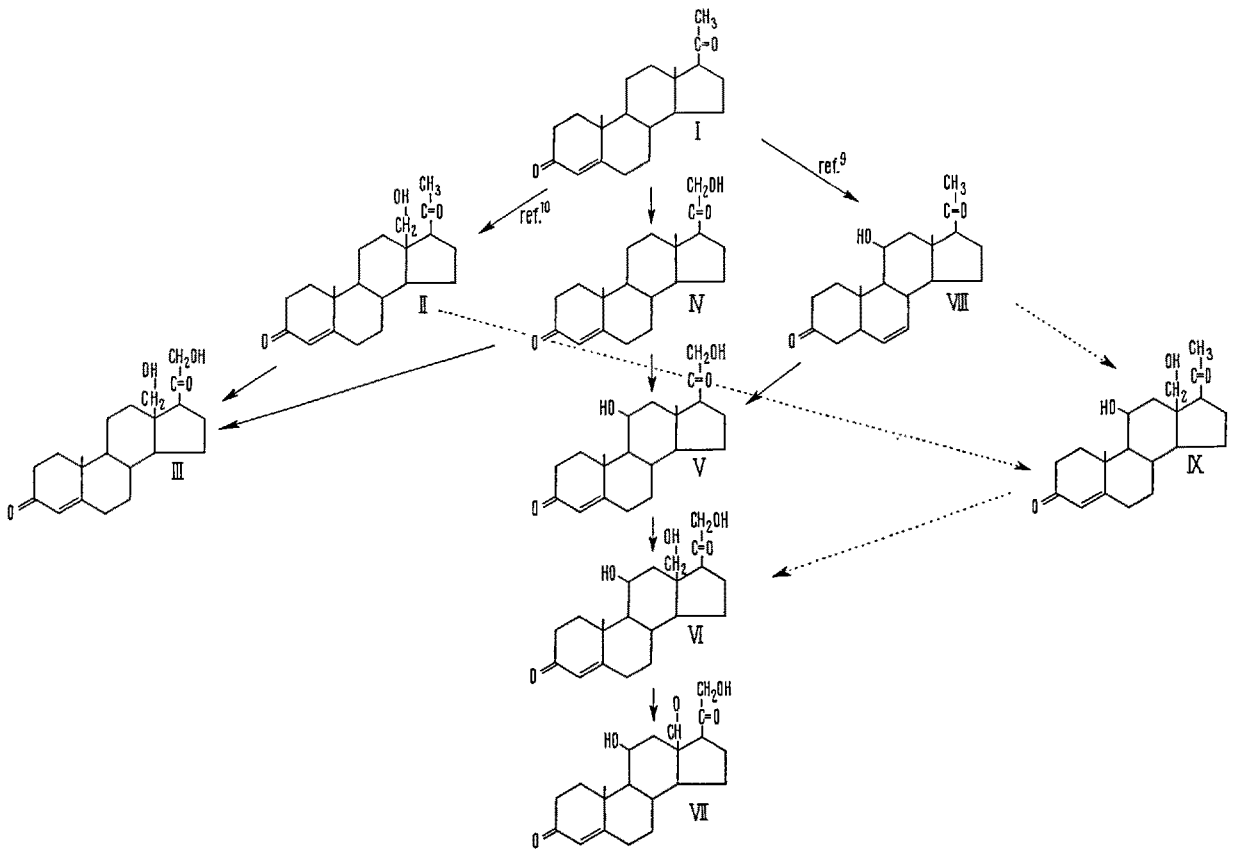

Fig. 2. Proposed synthesis scheme for the formation of aldosterone from progesterone in rat adrenals. I, progesterone; II, 18-hydroxyprogesterone; III, 18-hydroxy-11-desoxycorticosterone; IV, 11-desoxycorticosterone; V, corticosterone; VI, 18-hydroxycorticosterone; VII, aldosterone; VIII, 11β-hydroxyprogesterone; IX, 11β,18-dihydroxyprogesterone.

dilution by corticosterone formed from the added 11-dehydroderivative.

18-Hydroxyprogesterone inhibited the incorporation of 4-^{14}C-progesterone into 18-hydroxy-11-desoxycorticosterone, corticosterone and 18-hydroxycorticosterone; no statistical significance could be found for the inhibition of the 4-^{14}C-aldosterone formation. The fact that the 18-desoxy compound corticosterone was inhibited as well as 18-hydroxy-11-desoxycorticosterone and 18-hydroxycorticosterone suggests true inhibition of progesterone oxydation by 18-hydroxyprogesterone rather than radioactivity trapping.

18-Hydroxy-11-desoxycorticosterone did not influence the incorporation of 4-^{14}C-progesterone into either of the 4 corticosteroids analyzed. From this result it can be concluded that 18-hydroxy-11-desoxycorticosterone is not an essential intermediate of the 18-hydroxycorticosterone and aldosterone biosynthesis.

18-Hydroxycorticosterone distinctly inhibited the formation of radioactive 18-hydroxycorticosterone and aldosterone from 4-^{14}C-progesterone. Reduction of radioactivity incorporation into 18-hydroxycorticosterone by 18-hydroxycorticosterone may be considered as product inhibition. This assumption is supported by RAMAN et al.[6], who reported inhibition of 1,2-^{3}H-corticosterone incorporation into 18-hydroxycorticosterone by 18-hydroxycorticosterone during incubation of sheep adrenocortical mitochondria. The reduced formation of 4-^{14}C-aldosterone may be explained by additional radioactivity trapping, and it emphasizes the essential role of 18-hydroxycorticosterone as the direct precursor of aldosterone.

Summarizing results and conclusions of this study together with previous findings of other groups, the scheme outlined in Figure 2 may be proposed for the biosynthesis of aldosterone from progesterone in rat adrenals.

Zusammenfassung. Durch ihre Eigenschaft, bestimmte Stufen bei der Bildung radioaktiv markierten Aldosterons aus ^{14}C-Progesteron bzw. ^{3}H-11-Desoxycorticosteron zu hemmen, erwiesen sich 11-Desoxycorticosteron, Corticosteron und 18-Hydroxycorticosteron als wesentliche Zwischenstufen der Aldosteronbiosynthese. 18-Hydroxy-11-desoxycorticosteron hingegen scheint an der Bildung von Aldosteron nicht beteiligt zu sein.

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