The humped curve of nitrate reductase activity during ontogenetic development of the frog might suggest phylogenetically the existence of a nitrate respiration period, which would be supposed to come between the fermentation and the aerobic period of respiration.

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Observations on the Connexion between Intermedin and Adrenocorticotropic Hormone

Cutaneous pigmentations similar to those found in Addison's disease have been described after prolonged treatment with adrenocorticotropic hormone. It has been suggested that such pigmentation might be caused by an admixture of the pituitary melanophore-expanding hormone intermedin. We have therefore tried to determine the amount of intermedin in the blood of patients suffering from Addison's disease and in different adrenocorticotropic hormone preparations.

The injection of adrenocorticotropic hormone into the dorsal lymph sac of a frog (*Rana temporaria*) that has been adapted to light produces the same reaction as intermedin. As small an amount as 1/20,000 I.U. of the hormone still gives a clearly visible expansion of the melanophores, and the degree of expansion is independent of varying content of vasopressin or oxytocin in the preparations. Control experiments on hypophysectomized animals or with isolated skin samples have given the same results.

No difference was found in the properties of adrenocorticotropic hormone and intermedin when both were isolated from the pituitary glands of cattle and pigs. Both hormones are similarly affected by a number of substances; thus both are inactivated by nitrite, formaldehyde, trypsin and homogenates of liver, kidney and adrenal.

A peptide that gives the adrenocorticotropic activity was prepared by Li's method by hydrolysis of adrenocorticotropic hormone with pepsin and final separation from inactive components by paper chromatography. It shows the same activity when tested on the melanophores as it does when tested by Sayers's method. The biologically inactive components were also inactive in the melanophore test.

Intermedin was tested by paper chromatography using phenol – water as solvent. It gave only one spot, which had the same biological activity and R_F value as the adrenocorticotropic hormone peptides.

The effect on a normal human of a hormone fraction from the pituitary prepared by the technique used for the isolation of intermedin was observed. This intermedin was administered in daily doses corresponding to 20 mgm. of adrenocorticotropic hormone, in a single dose on the first day and in four doses of 5 mgm. during the two following days; this intermedin preparation was tested by the frog's melanophore test. After administration of intermedin, corresponding to 20 mgm. adrenocorticotropic hormone, the eosinophil leucocytes decreased from 376 per c.mm. to 127 per c.mm. (66 per cent). The corticoids in the urine rose from an initial value of 0·4 mgm./24 hr. to 1·1 mgm./24 hr. and the potassium excretion in the urine increased more than 100 per cent (from 74 mgm. to 218 mgm.). There was, however, no significant increase in the excretion of 17-ketosteroids. The test was repeated with corresponding doses of adrenocorticotropic hormone. It was found that even then there was no increase in the excretion of 17-ketosteroids, and the corticoids rose from 0·6 mgm. only to 0·9 mgm. during administration of this hormone.

Serum from fifteen normal persons and from five patients with cutaneous pigmentation but with a normally functioning adrenal cortex (according to the adrenocorticotropic hormone test) had no melanophore-expanding effect. Serum from five of six patients with Addison's disease caused a marked melanophore expansion. The serum from one patient with Cushing's disease, in whom no adrenal tumour could be found at operation, showed a positive reaction before operation. Four patients, who had been cholecystectomized, showed a moderate increase in the melanophore hormone content of their serum on the day after the operation. The serum from two cases with myocardial infarction showed the same result on the second day after the onset of the disease.

The hormone was concentrated from the serum in the following way: 10 ml. serum from each patient was treated with trichloroacetic acid, the filtrate precipitated with phosphotungstic acid and the resulting precipitate treated with barium hydrate. Three frogs were injected for each test.

These experiments seem to show that intermedin and an active adrenocorticotropic hormone peptide are closely related or identical. The frog test seems to be a rapid and simple method of testing for adrenocorticotropic hormone. The method is well suited for the demonstration of an increased content of the hormone in serum.

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Effects of Substituents on Ultra-violet Absorption Spectra

Substituents in a conjugated system do not always shift the absorption bands of the system in the one direction. If we consider only the absorption band of lowest frequency, then the most commonly observed effect is a shift to still lower frequencies[1]. However, Plattner and his co-workers[2] found that