TABLE 2

DISAPPEARANCE OF CARBON DIOXIDE IN THE COURSE OF THE OXYHYDROGEN REACTION IN ALGAE*

Material	Time hrs.		cmm O_2 introduced	cmm H_2 consumed	cmm CO_2 present before exp.	cmm CO_2 present after exp.
Rhaphidium polymorphium ...	20	exp.	196	567	109	50
		contr.	0	43	115	112
Scenedesmus D 3	18	exp.	218	620	118	65
		contr.	0	6	116	125
Scenedesmus D 3	20	exp.	417†	871	173	50
		contr.	0	14	163	179

* About 0.03 cc of cells in 3 cc of slightly acid phosphate solution. Gasphase: 99 per cent. H_2, 1 per cent. CO_2. Temperature: 25°.

† Exp. was stopped before all the oxygen had been consumed.

in the dark, photochemical reduction with hydrogen donors in the light and photosynthesis as specified by the liberation of oxygen, may occur in the same plant cell.

The decomposition of carbon dioxide in plants is generally believed to be intimately linked with, and absolutely dependent upon, the effect of light on chlorophyll. The finding here reported suggests, however, that the photochemical process and the reduction of carbon dioxide are more widely separated reactions than has been hitherto assumed.

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INACTIVATION OF PITUITARY LACTOGENIC HORMONE BY IODINE[1]

OUR preparation of pituitary lactogenic hormone has shown itself to be a homogeneous substance with respect to electrical charge.[2] Solubility studies[3] have also indicated that both beef and sheep preparations are almost if not actually pure. Although we have not yet been able to secure this protein in *uniform* crystalline state, the above mentioned physico-chemical behavior seemed to justify further chemical studies.

There is no indication that the hormone has a prosthetic group; yet the study of effects on biological activity of specific modifications of the molecule by chemical reagents might enable us to detect "functional" groups in the molecular makeup, for example, specific spatial arrangements of amino acids.

We have already reported the results obtained by the action of ketene[4] and nitrous acid[5] on the lactogenic hormone. These studies indicated the essentiality of the free amino groups for biological action of the hormone. This conclusion has recently been confirmed by Bottomley and Folley[6] in their experiments using phenyl isocyanate. Question as to the essentiality of the tyrosine molecule is next in line.

It has already been demonstrated that tyrosine is an essential component of certain protein hormones, enzymes and antigens.[7] Harrington and Neuberger[8] found that iodinated insulin retains only 5–10 per cent. of the physiological activity of the parent substance. Herriott[9] obtained an iodinated pepsin which has less than 1 per cent. of the original proteolytic activity. The iodination of these two substances showed that only the tyrosine molecule changed into the diiodo-compound which gives no Millon reaction. The present study also demonstrates that iodine acts only on the tyrosine component of lactogenic hormone.

Lactogenic hormone (L 283) as prepared from beef[10] pituitary contains 5.84 per cent. tyrosine and 1.34 per cent. tryptophane as determined by Lugg's modification of the method[11] of Folin and Ciocalten. Iodinated lactogenic hormones were prepared by the treatment of 100 mgm L 283 with 0.024 N iodine solution in 10 cc 0.5 M phosphate buffer of pH 7.0. The reaction was completed within an hour. The excess iodine was removed by a few drops of 0.2 N thiosulphate reagent. The colorless suspension was then dialyzed and its iodine[12] and nitrogen contents were analyzed. As shown in Table I, decrease in biological potency accompanied the absorption of iodine.

TABLE I

Material	N per cent.	I_2 per cent.	Tyrosine per cent.	Tryptophane per cent.	Med* mgm
L 283	15.26	0	5.84	1.34	0.2
Iodinated for ½ hr. (L 41)	F: 14.50 T: 14.52	F: 4.96 T: 5.38	2.00	1.38	1.0
Iodinated for 1 hr. (L 36A)	F: 14.00 T: 14.12	F: 7.90 T: 8.18	0.00	1.30	<1.5

* The minimum effective dose is defined as the smallest amount of the preparation which on intramuscular injection in 30-day-old squabs (4 daily injections of 0.5 cc) causes the minimum crop sac reaction in at least two out of three squabs.

The "F" and "T" in Table I denote the found and theoretical values. The latter is calculated and based

[1] Aided by grants from the board of research of the University of California, from The Rockefeller Foundation and from Parke, Davis Company. Assistance was rendered by the Works Progress Administration, Project No. OP 665-08-3-30, Unit A-5.

[2] C. H. Li, W. R. Lyons and H. M. Evans, SCIENCE, 90: 622, 1939; *Jour. Gen. Physiol.*, 23: 433, 1940.

[3] Results to be published.

[4] C. H. Li, M. E. Simpson and H. M. Evans, SCIENCE, 90: 140, 1939.

[5] C. H. Li, W. R. Lyons, M. E. Simpson and H. M. Evans, SCIENCE, 90: 376, 1939.

[6] A. C. Bottomley and S. J. Folley, *Nature*, 145: 304, 1940.

[7] C. R. Harrington, *Jour. Chem. Soc.* (London), 123, 1940.

[8] C. R. Harrington and A. Neuberger, *Biochem. Jour.*, 30: 810, 1936.

[9] R. M. Herriott, *Jour. Gen. Physiol.*, 20: 335, 1937.

[10] Lactogenic hormone from sheep pituitary invariably has a lower tyrosine content—about 4.5 per cent. (to be published).

[11] G. W. H. Lugg, *Biochem. Jour.*, 32: 775, 1938.

[12] We are indebted to Jane Conrat for iodine determinations.

on the amount of free tyrosine, while the theoretical value of nitrogen content is calculated from the iodine. Thus, since the theoretical values of both nitrogen and iodine checked very well with the analytical data, there is no doubt that the action of iodine on the protein is only on the tyrosine molecule.[13] In addition, it should be noted that the tryptophane content of the iodinated lactogenic hormone is not different from that of the parent substance. It is apparent, therefore, that any change found in the physiological activity of lactogenic hormone after treatment with iodine has been effected solely through modification of the tyrosine component of the hormone.

So far as we are aware, instances have not hitherto been detected of the dependence of physiological activity upon the presence of both free amino groups and tyrosine. Studies of pepsin and insulin show that tyrosine is an essential component of both these substances; yet free amino groups are not essential for their biological action. Lactogenic hormone would seem to be the first protein substance in which the essentiality of both tyrosine and free amino groups has been shown.

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[13] Kinetic data also support this conclusion. The kinetics of reactions between iodine, tyrosine and lactogenic hormone have been studied in some detail and will be reported later.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

MESOSTOMA EHRENBERGII WARDII FOR THE STUDY OF THE TURBELLARIAN TYPE

IN most laboratories of this country a species of Dugesia or some other common triclad is used in the laboratory as a Turbellarian type. The planarians have the advantages that they are large and are excellent material in which to demonstrate regeneration. However, they have many disadvantageous points which far outweigh the advantageous ones, it seems to me. They are thick, opaque; their reproductive organs are small and sometimes not present, and finally the other morphological features are difficult to distinguish.

Mesostoma ehrenbergii wardii, on the other hand, has the advantages of also being large; being thin, transparent; having proportionately large reproductive organs nearly continuously present; and lastly all morphological features are clearly visible in the living animal. The single disadvantage, *i.e.*, that *M. ehrenbergii wardii* has practically no powers of regeneration, is eliminated by simply collecting a few planarians for these experiments. This also enables one to emphasize the fact that two very closely related groups of animals have very different regenerative abilities.

In Fig. 1 I have attempted to show every feature that can be seen in the living animal with the use of very little technique and patience. A specimen of *M. ehrenbergii wardii* is placed in a small drop of water on a suitably sized coverglass. A second coverglass, which has been ringed with vaseline, is inverted over the drop and pressed down gently until it makes contact with the water. The pressure is then gradually increased until the animal is only able to move slowly. If it contains eggs, they will be caught between the coverglasses and the animal held comparatively quiet. The coverglasses may then be placed on a large slide under the dissecting or compound microscopes, and studied first under the lower powers and, after the animal has ceased struggling violently, under the 4 mm or oil immersion objectives. By inverting the coverglasses one may examine the dorsal or ventral surface of the body, a great advantage since many features are more easily seen from one side than the other. The magnificent flame cells, seen to best advantage in the region of 3 in the drawing, are probably the most striking thing present in these preparations, although in several very favorable specimens I have seen division figures in the testes.

A final exercise which is very simply done and gives good results is a demonstration of the chromosomes of this animal by means of an acetocarmine smear. The same specimen which has been studied during the period can be used for the smear. The animal is placed in a small drop of water on a slide, a drop of acetocarmine added and a coverglass placed over it. The animal is then mashed thoroughly by pressure on the coverglass with the blunt end of a needle holder. The excess liquid is taken off with absorbent paper, the slide warmed slightly and the coverglass ringed with paraffin or vaseline. The chromosomes are very large, up to 32 μ in length, and can be seen easily with high power. They are visible in the freshly prepared slide but are much more apparent after an interval of one or two hours.

M. ehrenbergii wardii is a member of the order Rhabdocoela, or forms with rod-shaped enteric cavities, and therefore belongs to an entirely different order of the Turbellaria from that of the planarians. It is, however, just as representative of the entire