chromatograms also contained a few unidentified spots, one of which may represent histamine, observed on the free amino acid chromatograms of the pollen mixture.

An analysis of pollen collected directly from dandelion plants gave the same results as those obtained from the dandelion collected in the beehive.

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The Formation of Monoiodotyrosine From Radioiodine in the Thyroid of Rat and Man[1]

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A further investigation of the multiplicity of radioactive substances found in filter paper chromatograms of thyroid hydrolysates (*1*) has indicated that monoiodotyrosine acts in the metabolism of iodine by the thyroid.

Ten rats were given an intravenous or intraperitoneal injection of 0.05–1 mc of carrier-free I^{131} and sacrificed from 1 min to 10 days later. The thyroids received from about 0.5 to 40,000 rep (roentgen equivalents, physical) of beta radiation, delivered at rates varying from about 30 to 10,000 rep/hr. Portions of the thyroids were hydrolyzed in sealed tubes at 100° C with 8% $Ba(OH)_2 \cdot 8H_2O$ for 6 hrs, 2N NaOH for 10 hrs, or 6N HCl for 24 hrs. The hydrochloric acid hydrolysate was evaporated as a small spot directly on a sheet of filter paper. The sodium hydroxide hydrolysate was adjusted to approximately pH 4 with 6N H_2SO_4, and a butyl alcohol extract of this solution was applied to the paper. The $Ba(OH)_2$ hydrolysate was adjusted to approximately pH 8 with 6N H_2SO_4, centrifuged, and the supernatant used for preparing the chromatogram.

Two-dimensional chromatograms were developed at 26° C with phenol as the first solvent and the upper phase from a secondary butyl alcohol, tertiary butyl alcohol, and water mixture (4, 1, and 4½ vols, respectively) as the second solvent. After the phenol run, the paper was washed with methyl alcohol except for a narrow strip below the original spot.

Radioautographs were prepared to show the exact positions of radioactive substances, the corresponding portions of the filter paper cut out for Geiger counter measurements, and the chromatograms then sprayed with ninhydrin. In each of the 40 chromatograms prepared as outlined a highly radioactive spot appeared with R_F values between those of tyrosine and diiodotyrosine. When monoiodotyrosine[2] was added either before hydrolysis or directly to the filter paper, it gave a ninhydrin spot which corresponded exactly both in *position* and *shape* with that of the above-mentioned radioactive spot.[3] The total radioactivity in the monoiodotyrosine spot ranged from about one-third to two-thirds of that shown by the adjacent diiodotyrosine spot.

A chromatogram was prepared from a $Ba(OH)_2$ hydrolysate of a thyroid biopsy specimen taken from a patient with an adenoma of the thyroid 8 days after the oral administration of 12 mc of I^{131} (*5*). It likewise showed a radioactive spot corresponding to monoiodotyrosine and containing about half the amount of radioactivity present in the diiodotyrosine spot.

As a check on the possibility that radioactive monoiodotyrosine might have been formed by exchange with, or decomposition of, other iodine-containing substances of the thyroid during the processing of the tissue, normal rat thyroids were hydrolyzed with 2N NaOH in the presence of carrier-free I^{131} or in the presence of various radioactive substances isolated from chromatograms. Radioiodide under these conditions gave a chromatogram similar to those obtained when iodide is chromatographed alone, mono- and diiodotyrosine yielded small amounts of iodide, and about 10% of the thyroxine broke down to diiodotyrosine and iodide. A mixture of two or more radioactive compounds with R_F values between those of diiodotyrosine and thyroxine yielded small amounts of iodide, monoiodotyrosine, and diiodotyrosine. The relatively slight amount of radioactivity associated with monoiodotyrosine in these control studies, in contrast to the *in vivo* findings described above, strongly indicates that the compound was present before the processing of the tissue. The possibility remains, however, that iodine-containing amino acids bound in their normal peptide linkage may be more subject to decomposition than were the free amino acids used as markers in these control experiments. This question is under investigation.

Considering the large amount of radioactivity associated with the monoiodotyrosine spot after administration of radioiodine and the reportedly low concentration of this compound in thyroglobulin (*4*), it seems probable that monoiodotyrosine attains a high specific activity. Preliminary tests indicate that by use of radioactive reagents it may be possible to determine the concentration of a number of the iodine-containing compounds on an

[1] Published with permission of the chief medical director, Department of Medicine and Surgery, Veterans Administration, who assumes no responsibility for the opinions expressed or conclusions drawn by the authors.

[2] The authors are indebted to S. Woislawski for preparing a supply of 3-iodotyrosine according to the method of Harington (*2*) and to C. E. Dent for sharing a sample of the compound given to him by C. R. Harington.

[3] From the data available there seems little doubt that the substance in question actually is monoiodotyrosine, but it is hoped that additional evidence may be obtained by means of the electron diffraction pattern (*3*) when the installation of an electron microscope at this laboratory is completed.

ultramicro scale and thus obtain data on specific activities in studies of the type described here.

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Some Factors Involved in Oxygen Evolution From Triturated Spinach Leaves

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Now that a technique has become available for studying quantitatively the evolution of oxygen by isolated chloroplasts and by fragments of chloroplasts, it is possible to determine some of the factors involved in the reactions. Hill (*5*) made the first advance toward quantitative measurements of oxygen produced by isolated chloroplasts, and Warburg and Lüttgens (*7*) simplified the procedure by using a single substance, *p*-benzoquinone, as the hydrogen acceptor in place of the complex mixture employed by Hill (*6*). Warburg and Lüttgens also demonstrated that pieces of chloroplasts 0.5 μ in diameter were just as efficient as intact chloroplasts in the photochemical yield of oxygen. That other types of quinones could be used as the oxidant was shown by Aronoff (*1*), the rate of oxygen production being roughly proportional to the redox potential of the quinone.

Young spinach leaves were ground in the Waring blendor for 2 min in a solution of M/20 phosphate buffer of pH 6.9, using 5 ml of buffer/gm fresh weight of tissue. This triturate was then filtered twice through glass wool and centrifuged for 20 min at 3,000 times gravity. The clear, yellowish-green supernatant was the "suspension" used in all experiments carried out in the Warburg manometric apparatus containing an atmosphere of purified nitrogen. Illumination of 2,000 foot-candles was provided by 9 fluorescent lamps mounted below a glass-bottomed water bath designed and built by G. F. Somers, of the Federal Nutrition Laboratory at Cornell University. All experiments were performed at 25° C.

Repeated microscopic examination under the oil immersion lens (magnification, 1,000 ×) failed to disclose any particles in the suspensions within the visible range, thus indicating that the active material from the leaves was in colloidal solution. Further evidence for this conclusion was provided by centrifuging the supernatant solution in a Beams air-driven ultracentrifuge at an estimated relative centrifugal force of 400,000 times gravity. No sedimentation of the material occurred when centrifuged for periods sufficiently long to bring down any of the larger particles. That the chlorophyll was firmly adsorbed or chemically combined with protein was revealed by dialysis, ultracentrifugation, and "salting out" procedures much the same as those employed by numerous other workers. Evidence that one or more of the constituents of the material here described is enzymatic in nature was furnished by the behavior toward heat and heavy metals. As little as 0.1 ppm of copper completely inactivated the suspensions.

The cell-free and chloroplast-free suspensions evolved more oxygen under identical experimental conditions than did small pieces of intact leaves, ground leaf tissue, or whole chloroplasts—an observation which has already been made by Aronoff (*2*). Calculated on the basis of oxygen produced by intact leaf tissue, the whole chloroplasts produced approximately 100% more oxygen and the suspension of triturated material 150% more oxygen over a period of 30 min of illumination. A plausible explanation of this difference in activity between various tissue fractions might be based on the lower permeability of the intact cells or chloroplasts to the reactants.

Use of higher concentrations of *p*-benzoquinone than the 2 mg/2 ml of solution employed by Warburg and Lüttgens enhanced the rate of oxygen evolution. Doubling the amount of quinone to 4 mg/2 ml of suspension increased the rate by 35%, and quadrupling the concentration caused a 60% increase in rate. Some of the quinone is probably lost in side reactions.

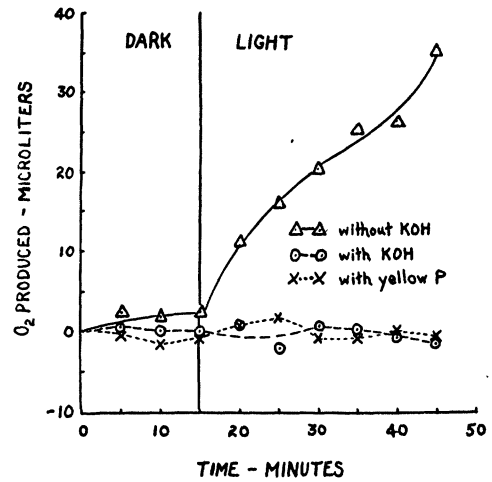

FIG. 1. Necessity of CO_2 for oxygen evolution.

One of the most significant observations to come out of this work was the apparent necessity of minute quantities of CO_2 to bring about oxygen evolution. When commercial nitrogen was purified for use in the manometers and vessels and when 0.1 ml of 10% KOH was placed in the center well of the vessels to take up CO_2, the pressure change remained near zero, but when the KOH was left out, considerable gas pressure developed. The pressure developed in the manometers without KOH could not have been due to evolution of CO_2 because, when yellow phosphorus was placed in the side arm of a

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