

cal action. The basic experiment to support this idea is the beating of an isolated frog's heart when perfused with a certain solution of a mixture of salts, one of which has to be potassium chloride. The amount of potassium in this solution is so small that a simple calculation indicates that only about one potassium atom per hour radiates in the frog's heart. Since the whole experiment can be performed in a much shorter time, it is absurd to connect in this experiment the necessary presence of potassium with its radioactivity.<sup>2</sup>

**Conclusion.** The above remarks are meant to show that tracer experiments with radioactive elements, if carefully executed, give definitely reliable information about the behavior of the normal element. If unexpected results are found they should certainly not be ascribed to a difference in the properties of the radioactive isotope.

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### PSEUDOMETHEMOGLOBIN AND ITS REACTION WITH CARBON MONOXIDE AFTER REDUCTION

In a discussion in this journal<sup>1</sup> recently, E. Amundsen published her observations on the presence in the blood of "a kind of hemoglobin" combining with carbon monoxide only after reduction. With reference to that substance the author emphasizes that "for the present its composition and the cause of its appearance will have to be looked upon as unknown." In this connection the following facts may be of interest.

The normal presence of substances in human and animal blood not identical with hemoglobin but combining with carbon monoxide in a similar way was described by Barkan and Berger<sup>2</sup> in 1928. These authors pointed out that of the "easily split-off" blood iron ("leicht abspaltbares" Bluteisen) discovered by Barkan<sup>3</sup> (1925) one portion E combines with O<sub>2</sub> and CO following the distribution equation, while another portion E' does not react with those gases. In 1937 we<sup>4</sup> demonstrated that both fractions of the "easily split off" blood iron are pseudohemoglobins which are intermediates in the formation of bilirubin from hemo-

globin and accompany the hemoglobin within the red cells of the circulating blood. Pseudohemoglobins, according to our definition, are chromoproteins in which the protein is probably an unaltered globin, while the prosthetic group is an iron-containing heme or hemin derivative with an opened porphyrin ring, similar to that in Lemberg's<sup>5</sup> "verdohemochromogen." The two fractions E and E' differ by the valency of the iron. The latter is bivalent in E, which combines with oxygen and carbon monoxide and hence was designated as a pseudohemoglobin *in sensu strictiori*. The iron is trivalent in E', which does not combine with O<sub>2</sub> and CO and was designated as a "pseudomethemoglobin." By reduction with Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> we were able to transform the pseudomethemoglobin into pseudohemoglobin, which does combine with carbon monoxide.

Our work was published in different original articles<sup>4, 6</sup> and, in addition, some reviews<sup>7</sup> have been given. Taylor and Coryell<sup>8</sup> in an article (1938) dealing with the magnetic susceptibility of the iron in ferrohemoglobin confirmed our work. Using the same method as was later used by E. Amundsen,<sup>1</sup> they found too that in normal blood and in oxyhemoglobin solutions there are present hemoglobin-like compounds which combine with carbon monoxide only after previous reduction.

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### AN EXPERIMENT ON RETINAL AFTER-IMAGE AND JUDGMENT OF SIZE

By an almost unconscious combination of elements, we judge the size of an object from the image it produces on the retina and from our judgment of its distance from the eye. If there is uncertainty in this latter judgment, there is corresponding uncertainty in judgment of size because we do not then know how far away to consider the object to be and do not know how to interpret the retinal image. It is common experience that we are incapable of estimating the

<sup>2</sup> A. J. Glazko and D. M. Greenberg (*Am. Jour. of Physiol.*, 125: 405, 1939) have shown that radioactive sodium can not replace the potassium in this experiment. Compare also a critical article by S. G. Zondek, *Biochem. Zeits.*, 121: 76, 1921.

<sup>1</sup> SCIENCE, 90: 2338, 372-373, October 20, 1939.

<sup>2</sup> G. Barkan and E. Berger, *Arch. exp. Pathol. u. Pharmacol.*, 136: 278-299, 1928; *Klin. Wochenschr.*, 7: 1868, 1929.

<sup>3</sup> G. Barkan, *Zeits. physiol. Chem.*, 148: 124-154, 1925; *ibid.*, 171: 179-193 and 194-221, 1927; *Biochem. Zeits.*, 224: 53-62, 1930; *Zeits. physiol. Chem.*, 216: 1-16 and 17-25, 1933; *ibid.*, 221: 241-251, 1933; *ibid.*, 236: 198-200, 1935. See also G. Barkan and O. Schales, *ibid.*, 244: 81-88, 1936; *ibid.*, 244: 257-265, 1936.

<sup>4</sup> G. Barkan and O. Schales, *Zeits. physiol. Chem.*, 248: 96-116, 1937.

<sup>5</sup> Cf. R. Lemberg, *Ann. Rev. Biochem.*, 7: 421-448, 1938; and original papers cited therein.

<sup>6</sup> G. Barkan and O. Schales, *Zeits. physiol. Chem.*, 253: 83-103, 1938.

<sup>7</sup> G. Barkan, Lecture in the Wiener Biologische Gesellschaft, June 14, 1937; *Klin. Wochenschr.*, 16: 1265-1268, 1937; *Dtsch. Mediz. Wochenschr.*, 64: 638-640, 1938; Kongressbericht II des XVI Internat. Physiol. Kongresses, Zürich (Schweiz), 1938, p. 250; G. Barkan and O. Schales, *Arch. Ital. di Sc. Farmacologiche*, Vol. Jubil. ad Benedicenti, 1937; O. Schales, Research Meeting of the Rockefeller Institute, Copenhagen, December 3, 1938.

<sup>8</sup> D. S. Taylor and Ch. D. Coryell, *Jour. Am. Chem. Soc.*, 60: 1177-1181, 1938.

size of the moon, for example, because, although we receive a clear image which subtends a certain angle, we receive no accompanying sense experience which might enable us to judge the distance of the object. (Astronomically determined distances are not, of course, perceived directly.) Elementary observations in optics show that whenever a real image is formed by a lens, as in the case of images formed on the retina by the lens of the eye, the size of the image decreases as the distance between lens and object increases. We learn in infancy to interpret this relationship between image size and distance of object. The following paragraphs describe a simple experiment which is, in a sense, the converse of our common experience with retinal images.

It is well known that exposure of the eye to bright light produces retinal fatigue and that after one has gazed intently for several seconds at a bright source, there may appear an after-image of the source soon after the eyes are shifted to some less brightly illuminated area. This after-image is commonly seen in a color complementary to that of the source, a fact which has heretofore played a part in hypotheses of color-vision, but which is, for the present, of secondary importance. It is the *size* of the after-image rather than its color to which the writer wishes to direct attention; for this persistent image may be used to produce the illusion of an external object whose apparent distance from the eye may be varied at will, with the result that the illusory "object" changes size, and its apparent size depends entirely upon the observer's judgment of its distance from the eye.

Let the observer, with one eye closed, look with the other eye steadily for ten seconds upon the bright filament of an unfrosted 60-watt lamp situated, say, two feet from the eye. If the gaze is then shifted to some less bright region, there soon appears a pronounced after-image of the lamp filament which may persist clearly for a minute or two because of the fatigue of that portion of the retina which has been so strongly exposed to light. If the observer has difficulty in discovering the after-image, it may be "developed" or made more apparent by blinking the exposed eye occasionally or by exposing it indirectly to intermittent flashing of a low-power lamp (15 or 25 watt). Once the image is seen, it may be followed from spot to spot about the room as the observer shifts his gaze; it may appear as a large object on a distant wall or as a small object on a piece of paper held close at hand. The change in apparent size of the illusory object is immediately evident; it is amusing, for example, to "fix" the object on a finger tip held not far from the eye and to observe the increase in its apparent size as the finger is moved farther away. By observing the after-image on a scale and by recording its appar-

ent size as a function of the observer's distance from the scale, it is possible to check with reasonable accuracy that this apparent size of object is directly proportional to the distance between the eye and the scale on which the after-image appears to be situated.

Because of the well-known phenomenon of irradiation and because of involuntary movements of the eye during exposure to the light, the after-image on the retina is somewhat extended and looks larger than the lamp filament. However, the horseshoe-shaped filament of a 60-watt lamp gives an image which is readily recognized and is sufficiently definite in outline to serve for measurement. A frosted lamp may be used if it is obscured from view except for a small aperture of sharp outline and characteristic shape. The color of the after-image is not always the same but appears to depend upon length of exposure and upon external illumination. It may, at first, be dark blue (roughly complementary to the bright yellow of the source); but in many cases, especially under periodically changing illumination, it may change from dark blue to bright yellow, and by this reversal of color the after-image may be easily detected. In many cases it appears as a bright yellow object like the lamp filament, but with a blue border. When this experiment was tried with a group of twenty students at once, it was found that nearly all of them observed the effects described herein. The experiment may serve to demonstrate to students of physics and psychology certain fundamental facts of vision by the simple expedient of using a strong retinal after-image for purposes of measurement and observation. It is recognized that there may be danger to the eye in prolonged exposure to an intense source of light, but the writer has personally repeated the experiment a great many times without any observed harmful effects.

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#### THE OCCURRENCE OF THE TWO RARE GENERA, *PROTOHYDRA* AND *PRO-* *TODRILUS*, ON THE EAST COAST OF NORTH AMERICA

THESE two genera, so far as can be ascertained, have never before been reported from the North American continent. The first form, *Protohydra leuckarti*, probably represents the most primitive coelenterate of the class Hydrozoa. It was found in a small brackish water creek formed at low tide on the mud flats in New Haven harbor together with numerous Protozoa, rotifers and Turbellaria. Only one specimen was found which after several hours' isolation divided by transverse fission. The two individuals thus formed lived for several weeks in the laboratory, when for some unaccountable reason they disintegrated over night. The animals, which lack tentacles, feed on small