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Study on the Photo-Oxidation of Blood Plasma and Tissues in Buckwheat Disease.

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The effect of irradiation of animals fed on buckwheat is well known and has been described in detail by several authors.[1-6] The majority of the workers in this field assume that buckwheat disease is due to a substance contained in this plant which after ingestion renders the animals susceptible to light within the visible range of the spectrum.[1-5]

Since photo-dynamic action is characterized by the oxidation of an acceptor in presence of a sensitizer during irradiation, studies were made on the oxygen consumption of irradiated blood plasma and tissues of animals fed on buckwheat as well as of controls. It was thought that if a photodynamic substance were present, there should be a greater oxygen consumption during irradiation of plasma and tissues of sensitized animals than during irradiation of similar substrates of controls.

Materials and Technic. Thirty-six guinea pigs, weighing from 600 to 800 g were fed exclusively on fresh buckwheat plants and seeds from 2 weeks to 3 months. The animals were kept in cages covered with black cloth. Thirty-three control guinea pigs were fed on stock diet and also kept in the dark. All animals seemed entirely well as long as they were kept in the dark; they gained weight and some females had litters during this period; the young appeared normal. In order to prove that animals were sensitive to light, albino animals fed on buckwheat and controls were exposed to sunlight in glass jars covered with glass tops. It was found that guinea pigs exhibited typical symptoms of photo-sensitivity after 4 to 6 days of feeding on buckwheat. Heart punctures of sensitive animals and controls were made from time to time and the blood plasma or serum was examined through the spectroscope or the spectropho-

1 Oehmke, W., *Zentralbl. f. Physiol.*, 1909, **22**, 685.

2 Meridan, L., *Arch. f. Anat. u. Physiol.*, 1915, **39**, 161.

3 Sheard, C. H., Caylor, H. D., and Schlotthauer, C., *J. Exp. Med.*, 1928, **47**, 1013.

4 Hausmann, W., and Zaribnicky, *Klin. Wchnschr.*, 1929, **8**, 74.

5 Hausmann and Haxthausen, *Strahlentherapie*, 1929, Sonderband XI.

6 Lutz, H. E. W., and Schmid, G., *Biochem. Z.*, 1930, **226**, 67.

tometer. Samples of plasma or serum were placed in respirometers and the oxygen consumption before, during and after exposure to light was studied in the water bath at 37.5°C. The details of the apparatus, temperature control and light source are given in a previous communication.[7] Experimental animals and controls were sacrificed at intervals and certain organs were finely sliced with a sharp razor blade. After weighing, tissue slices were placed in respirometers containing 1 cc of Ringer's solution of pH 7 and the oxygen consumption was studied before, during and after exposure to light in the water bath. Routine autopsies were performed on all animals.

Results. Spectroscopic and spectrophotometric examinations of plasma or serum of guinea pigs fed on buckwheat from 2 weeks to over 3 months never showed any abnormal absorption bands; traces of hemoglobin were frequently encountered.

The results of the studies on the oxygen consumption during irradiation of tissue slices and plasma of animals fed on buckwheat as well as of controls are presented in Table I. The figures give the average of cmm of oxygen consumed during 10 minutes of a 30-minute period. Each figure represents the mean of from 9 to 30 observations or an average of 17 experiments. The column showing the difference between the readings divided by the difference of the probable errors always pertains to the figures before and during irradiation.

It can be seen that there is no significant difference between the oxygen consumption of organs of animals fed on buckwheat during irradiation and controls. There is the usual increase in oxygen consumption of plasma during irradiation, which occurs in both experimental animals and controls.

Autopsies of guinea pigs fed on buckwheat never showed any characteristic lesions. The gall bladder of the experimental animals was usually larger and contained more bile than that of controls which was probably due to the feeding of green plants. Spectrophotometric studies of the bile of the gall bladder of animals fed on buckwheat never showed any absorption bands different from those seen in the bile of controls.

In the absence of any demonstrable difference in the oxygen consumption of plasma and tissues between animals fed on buckwheat and controls, the question arises whether buckwheat disease is due to the presence of a photodynamic substance. The behavior during irradiation of guinea pigs fed on buckwheat is, however, identical to that of animals effectively sensitized by the parenteral injection of a photosensitizing substance, for instance, hematoporphyrin.

[7] Smetana, H., *J. Biol. Chem.*, 1938, **124**, 667.

TABLE I.
Oxygen Consumption of Blood Plasma and Tissues of Guinea Pigs Fed on Buckwheat and Controls.
Average of cmm of O_2 consumed per mg of tissue during 10 min of 30 min period.

	Exposure to Light (400μ Amps.) Experimental Animals				Controls			
Organs	Before	During	After	Diff. PE Diff.	Before	During	After	Diff. PE Diff.
Skin	1.08±.04	1.12±.05	.84±.06	.62	1.3±.08	1.6±.11	1.3±.11	2 1
Subcut. tissue	.73±.04	.79±.1	.67±.03	.06	.7±.04	.7±.03	.6±.06	0
Liver	13.2±.18	13.6±.14	13.1±.16	1.7	12.2±.16	12.3±.16	11.7±.12	.043
Spleen	11.0±.12	11.1±.14	12.8±.21	.05	12.6±.34	12.4±.49	10.7±.92	0
Kidney	27.6±.27	27.7±.33	26.5±.21	.03	28.2±.28	26.5±.34	26.9±.32	0
Brain	7.9±.21	7.0±.29	6.5±.54	0	7.8±.45	7.0±.52	5.6±.28	0
Blood Plasma	.4±.05	1.86±.13	.4±.09	9.73	.4±.062	1.85±.15	.4±.1	8.95

Since symptoms in buckwheat disease are very pronounced and start almost immediately upon exposure of animals to sunlight one would expect a measurable concentration of a photosensitizing substance in the blood as well as in some of the tissues, if this substance were the cause of this disease. If such a substance does exist, its concentration in the blood plasma and in the tissues is too low as to effect the oxygen consumption during irradiation *in vitro*. Compared with the effect of concentrations of hematoporphyrin on plasma and tissues this would be the case in the neighborhood of concentration 10^{-6}; such a low concentration of sensitizer on the other hand would hardly produce the marked actual effect *in vivo*.[7, 8] Since all experiments *in vitro* were carried out with the exclusion of ultraviolet and infrared rays the question arises whether the effective rays, causing symptoms of buckwheat disease, lie in the invisible part of the spectrum. This was quickly disproved by a few experiments where irradiation of plasma and tissues of sensitive animals was carried out in quartz containers, using a light source rich in infrared and ultraviolet rays, which was projected on the respirometers through a quartz window. The results were in no way different from those obtained when identical material was irradiated in glass respirometers. Besides, effective irradiation of sensitive animals was carried out in glass jars covered with glass tops. Moreover, most workers in this field[2-6] have found that the invisible parts of the spectrum were ineffective in producing symptoms of buckwheat disease.

Although many of the authors[1-5] suggest the presence of a photodynamic substance in buckwheat disease, no such substance has as yet been conclusively demonstrated. The suggestion that phylloporphyrin or cholehematin may be the photodynamic substances causing buckwheat disease[3] was based on absorption bands found in a single sample of swine serum. It has been ruled out by several authors[3, 6] that ordinary chlorophyl or a specific chlorophyl of buckwheat was the photodynamic substance causing the disease. Lutz and Schmid[6] left the question open whether buckwheat disease is due to a photosensitizing substance or to intoxication.

Because of the failure to demonstrate any photodynamic substance responsible for the sensitivity to light of animals fed on buckwheat, it might be suggested that feeding of buckwheat may possibly cause a change in the tegument of the animal, rendering the skin more susceptible to irradiation, similar to the effect of sunlight in relation to skin lesions in pellagra. Payne and Perlzweig[9] have shown that there

[8] Smetana, H., *J. Biol. Chem.*, 1938, **125**, 741.

[9] Payne, S. A., and Perlzweig, *J. Clin. Invest.*, 1935, **12**, 899.

exists an abnormal sulphur metabolism in pellagra which is reflected in the susceptibility of the skin to irradiation and is specifically related to the dermatitis of this disease. According to these authors the relatively high cystine content in wool and hair is of physiological importance in the protection of the organism against harmful effects of prolonged exposure to light. In this respect the changes and the falling out of hair of animals fed on buckwheat, as reported by Oehmke,[1] are of interest and perhaps of significance.

Conclusions. 1. There is no abnormal increase of the oxygen consumption during irradiation of plasma and tissues of various organs of guinea pigs made susceptible to light after prolonged feeding with buckwheat. 2. There is no conclusive evidence that the susceptibility to light of animals in buckwheat disease is due to a photodynamic substance.

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Cause of Increased Fragility of Erythrocytes in Congestive Heart Failure.

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Increased fragility of the red blood cells has been noted in certain patients with severe congestive heart failure.[1, 2] With recovery from heart failure the minimum osmotic resistance of the red cells to hypotonic salt solutions returns to normal.[2, 3] It was of interest to study the effect of anoxemia and stasis on blood in the capillaries and in the veins in producing these changes.

The studies were made on 10 convalescent ward subjects with no demonstrable cardiac, respiratory or hematologic disease. Using the hypotonic saline method of Daland and Worthley,[4] the minimum osmotic resistance of the red blood cells obtained from the finger tips was observed. Complete circulatory arrest was produced by means of a standard blood pressure cuff applied to the right upper arm and rapidly inflated to a level 50 mm of mercury above the systolic pres-

1 Franceson, M., *Minerva Med.*, 1936, **37**, 255.

2 Waller, J. V., and Blumgart, H. L., to be published.

3 Butler, G. G., *Quart. J. Med.*, 1912-13, **6**, 145.

4 Daland, G. A., and Worthley, K., *J. Lab. and Clin. Med.*, 1935, **20**, 1122.