exposure to approximately 15,000 r of X-rays. Both sources of ionizing radiations produced similar phenotypic effects in the plants grown from the irradiated seed, but the bomb produced relatively more chlorophyll deficiencies and dead tissue occurring as sectors than did the treatments with X-rays.

Crystalline Human Myoglobin: Some Physicochemical Properties and Chemical Composition

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Most of the researches on myoglobin refer to the horse (*2, 3, 4, 8*), the ox (*2, 6*), and the pig (*5*). In 1947 the writer (*7*) succeeded in obtaining from human skeletal muscles pure crystallized myoglobin (1–2 gr), utilizing a procedure previously described for animal myoglobin (*6*).

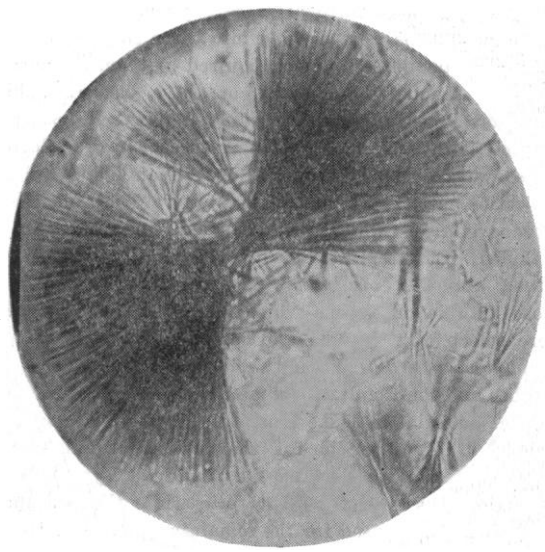

FIG. 1. Crystals of myoglobin prepared from human skeletal muscle (×370).

Human myoglobin crystals consist of long, very thin needles, tied together in subparallel bundles or in radiated, fibrous spheroidal masses (Fig. 1). The lengthening of the crystals is negative, parallel to α. Extinction is at right angles.

Crystals of metmyoglobin are clearly double-refracting, and they show an evident pleochroism with α′ of a reddish-brown color parallel to the lengthening and γ′ of a pale yellow color perpendicular to the lengthening; the refractive index is greater than 1.514. The iron content is 0.34%, the prosthetic group is probably the same as for hemoglobin, and the N content is 16.5%.

Spectrophotometric determinations gave maxima of 5,815 A and 5,426 A for the α and β absorption bands of oxymyoglobin, respectively.

The ratio between the absorption coefficients at the two maxima for myoglobin is different from that for hemoglobin. Myoglobin is comparatively stable in an alkaline medium. The formation of hemochromogen in alkaline reducing solutions may be followed spectrophotometrically. While in 0.4 N NaOH human oxyhemoglobin is rapidly transformed to hemochromogen; the oxymyoglobin still presents the two typical α and β bands of oxymyoglobin. To obtain, in this case, hemochromogen from myoglobin, the strength of the NaOH solution has to be increased to 3 N. Myoglobins from different animals (horse, ox) behave differently toward alkali—another distinguishing feature between myoglobin and hemoglobin which is, very probably, due to the chemically different composition of the two globins.

TABLE 1

	Nitrogen			
	Hemoglobin (mg)	Myoglobin (mg)	Hemoglobin (%)	Myoglobin (%)
Amide N	1.79	1.95	5.86	6.57
Humin N	1.20	1.38	3.93	4.66
Cystine N	0.153	0.138	0.50	0.47
Arginine N	2.40	1.18	7.86	3.98
Histidine N	4.08	4.10	13.37	13.85
Lysine N	2.85	3.88	9.34	13.10
Filtrate NH_2 N (monoamino acids)	16.63	15.32	54.52	51.75
Filtrate non-NH_2 N (imino acids + ½ N tryptophane)	1.26	1.50	4.13	5.06
Total N recovered	30.36	29.44	99.55	99.44

Chemical determinations carried out with myo- and hemoglobin emphasize this difference. The nitrogen distribution and the amino acid composition have been determined on 184 mg of pure crystallized human myoglobin and 186 mg of human hemoglobin prepared in the microcrystallized form (Drabkin) by a micromodification (*3*) of the Van Slyke procedure (*10*). The nitrogen distribution in human hemoglobin and myoglobin is shown in Table 1.

TABLE 2

Amino acids	Hemoglobin	Myoglobin
Cystine	0.71	0.65
Arginine	4.00	1.98
Histidine	8.09	8.22
Lysine	7.98	11.00

In Table 2, percentages of some amino acids expressed as g% of the total amount of protein are given for both proteins.

These results represent the first contribution to the knowledge of the chemical constitution of human myoglobin. As may be seen from the analytical data (Tables 1 and 2), the chemical composition of human myoglobin is different from that of hemoglobin. The most conspicuous differences are observed in the arginine and lysine content and also in the monoamino acids.

In Table 3 the more probable distribution of the amino acids in myoglobin and hemoglobin of man and horse is

TABLE 3

	Number of amino acid molecules for 4 atoms of iron				Amount of basic amino acid for every atom of iron
	Cystine	Arginine	Histidine	Lysine	
Horse hemoglobin (4)	3	14	39	37	22
" myoglobin (4)	4	8	40	60	27
Human hemoglobin	4	16	35	37	22
" myoglobin	4	8	36	52	24

given, taking as molecular weights 68,000 and 17,000, respectively.

These data show that myoglobin of different animal species contains about half as much arginine and twice as much lysine as hemoglobin of the same animal and that the total amount of basic amino acid radicals is greater in myoglobin than in hemoglobin.

Addendum: Drabkin (*1*) and Theorell (*9*) recently mention having crystallized human myoglobin from cardiac muscle, but the writer has not yet seen these articles.

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Differential Effects of 2,4-D on Aerobic, Anaerobic, and Facultative Anaerobic Microorganisms

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2,4-Dichlorophenoxyacetic acid (2,4-D) is recognized biologically as an auxin (plant hormone) or growth-regulating substance. It is well known that 2,4-D has a selective activity on the growth rate of certain plants. The mode of action of the auxin is still a matter of conjecture, but several workers indicate that 2,4-D is involved in some way in the process of plant respiration (*1, 6*). Hsueh and Lou (*3*) developed this idea more fully in their experiments with germinating rice and barley seeds, which they treated with various concentrations of 2,4-D before allowing them to germinate under aerobic and anaerobic conditions. The germination of barley, a typical aerobic seed, was entirely inhibited, whereas that of rice, a seed known to be able to germinate anaerobically, was only partially inhibited. The results of their experiments seemed to indicate that the rate of germination of the treated barley seeds closely paralleled that of the untreated barley seeds which were grown in an anaerobic atmosphere. It appeared as if oxygen were no longer available to the treated seeds.

That 2,4-D can display bacteriostatic and bacteriocidal properties has been shown by Stevenson and Mitchel (*5*) and Lewis and Hamner (*4*). Dubos (*2*) observed that a number of synthetic, unsaturated, ring-containing acids, including 2,4-D, endowed with auxin activity exerted a bacteriostatic effect on the growth of certain microorganisms. However, no correlation has been made between the effects of 2,4-D on bacteria with respect to the oxygen requirements of bacteria.

The purpose of this report is to demonstrate the effects of different concentrations of 2,4-D on those organisms which vary in their utilization of oxygen.

The organisms were divided into three groups, depending on the amount of oxygen required by them for normal growth. Among the aerobes, or those bacteria which must have free oxygen, *Rhizobium trifolii, R. phaseoli, R. japonicum,* and *Azotobacter chroöcoccus* were selected. These were obtained from the American Type Culture Collection at Georgetown University. The anaerobes, those organisms which will grow only when oxygen is excluded, were represented by *Clostridium welchii, Cl. tetani,* and *Cl. botulinum.* The facultative anaerobes, those aerobic organisms which can grow anaerobically, were selected at random and included *Escherichia coli, Staphylococcus albus,* and *Candida albicans.* The anaerobes and the facultative anaerobes were obtained from clinical material in Duke Hospital. It was necessary to employ different media for the various organisms. A special formula supplied by the American Type Culture Collection was used for *R. trifolii,* while the Waksman medium #79 was used for the remaining aerobes. Brain-heart infusion agar was used for the anaerobic and facultative anaerobic organisms. All of the media employed were adjusted to a pH of 7.4.

Sodium 2,4-dichlorophenoxyacetate, obtained from the J. T. Baker Chemical Company, was used throughout the present study. Solutions of the 2,4-D were prepared and diluted with distilled water to concentrations of 5, 1, 0.1, 0.01, and 0.001% and autoclaved for 15 min. at 18 lbs pressure. Varying amounts of these solutions were measured into a series of tubes of the agars used to give a final volume of 5 cc of agar-2,4-D solution/tube. The final concentrations of 2,4-D, after taking into account the dilution factor of the agar, amounted to 2, 1, 0.2, 0.02, 0.002, and 0.0002%/5 cc of diluent. The number of grams of 2,4-D/5 cc of agar were 0.1, 0.5, 0.25, 0.001, 0.0001, and 0.00001. The agar containing the auxin was poured into level Petri dishes which contained a base of approximately 10–15 cc of the corresponding type of agar.