Sprague Dawley from 111 to 225 g. On April 6, 1938, after 209 days of feeding the oils, there were no palpable tumors; the dose of the oils was then increased to 3 cc per rat per day for 22 days. During this time 2 Sprague Dawley rats which had received the crude oil were found dead, but autopsy revealed no macroscopic tumors. Then for 27 days each of the remaining 10 rats received 3 cc of the crude oil daily by stomach tube. The refined oil was mixed with the diet as before. This forced feeding was followed by a drop in food consumption resulting in emaciation. At the end of this period both groups of rats were killed, but there was no evidence of tumors on autopsy.

The vitamin E content of the refined and crude wheat germ oils was approximately the same, 1.5 g of each sufficing for a successful gestation.

Conclusions. Tumors have not been observed in 20 albino rats of 2 strains, Wistar and Sprague Dawley, which were fed a crude and a refined wheat germ oil for 258 days. Perhaps all wheat germs do not contain the sarcogenic agent. Certain nutritional conditions may influence tumor production, and there may be subtle factors, as yet unappreciated, incidental to the preparation of the crude wheat germ oil.

10323

Hemoglobin-Methemoglobin and KCN.

MATILDA MOLDENHAUER BROOKS.*

From the University of California, Berkeley.

In a previous paper[1] it was found that the addition of comparatively small amounts of KCN to oxyhemoglobin in solution had no effect on the absorption maxima of the spectrum, as determined by spectrophotometric studies. The maxima for oxyhemoglobin solutions and for oxyhemoglobin containing KCN were identical. It was also found that when KCN was added to reduced hemoglobin, reduced by adding Stoke's reagent, the absorption spectrum again reverted to that of oxyhemoglobin. These facts were then inter-

* I wish to express my thanks to Dr. Kubowitz for his assistance.

1 Brooks, M. M., *Am. J. Physiol.*, 1935, **114**, 160; PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1228.

preted to mean that KCN must form a compound with the bivalent form of hemoglobin. But since there was no change in color and no evolution of O_2, it was assumed that the change could not have occurred by the addition of the CN radical to the prosthetic group and it was suggested that perhaps some rearrangement took place in the uncolored portion of the hemoglobin compound.

Further experiments which were done this summer at the Kaiser Wilhelm Institute with the kind coöperation of Dr. Otto Warburg have helped to clear up some of these questions.

The KCN-oxyhemoglobin absorption spectrum. For these experiments pure crystals of oxyhemoglobin freshly prepared from rat blood were used. The crystals were dissolved in either glycocoll buffer at pH 9.0 or phosphate buffer at pH 7.4 or 5.5. Dilutions were made from 10 to 1%.

The spectroscope used was a Schmidt and Haensch hand spectroscope with scale. No ratios between designated wave lengths could be measured and therefore the results are not quantitative as would be obtained by means of a spectrophotometer. Reagents were used in the same concentrations as described previously.[1]

The absorption spectrum of oxyhemoglobin solution alone was the usual one, with 2 maxima, the greater at 576 mμ and the lesser at 543 mμ.

The addition of an equivalent number of moles of KCN to oxyhemoglobin had no effect upon the absorption maxima. When the solution was reduced by bubbling in argon the absorption spectrum changed to that of reduced hemoglobin with the maximum at 555 mμ. This method was eminently satisfactory, while in the former experiments an attempt was made to reduce the defibrinated blood and cyanide mixture by vacuum. Because of the inadequacy of this procedure the experiment was unsuccessful.

When oxygen was again admitted to replace the argon, the spectrum changed again to that of oxyhemoglobin. These processes could be repeated. This experiment shows that KCN does not combine with the prosthetic group of the bivalent form of hemoglobin. This is in keeping with such old observations as those of Preyer,[2] v. Zeynek,[3] Kobert.[4]

The KCN-methemoglobin absorption spectrum. In one experiment (Table I) oxyhemoglobin was treated with K ferri CN or $NaNO_2$ to produce methemoglobin. In the former case the spectrum

[2] Preyer, W., *Die Blusäure*, 1868.

[3] v. Zeynek, R., *Z. physiol. Chem.*, 1901, **33**, 426.

[4] Kobert, R., *Arch. f. Physiol.*, 1900, **82**, 603.

TABLE I.
Experiments with Oxyhemoglobin.

	Wave length of maximum absorption, in mμ
A. Oxyhemoglobin alone	576, 543
Added K Ferri CN	543
" KCN to above	543
B. Oxyhemoglobin alone	576, 543
Added $NaNO_2$	576, 543, 620
C. Oxyhemoglobin alone	576, 543
Added KCN	576, 543

gave only the wave lengths at 543 mμ and the band at 576 mμ had disappeared, showing that hemoglobin was completely oxidized. In the latter case, when $NaNO_2$ was used in neutral or acid solution, both bands at 543 mμ and 576 mμ appeared. This showed that the $NaNO_2$ had not completely oxidized all of the hemoglobin and that some oxyhemoglobin was still present. The band at 620 mμ appears in acid or neutral solutions of methemoglobin but not in alkaline solutions.

TABLE II.
Experiments with Reduced Hemoglobin.

	Wave length of maximum absorption, in mμ
A. Reduced hemoglobin in argon	555
Added KCN in argon	555
Replaced argon with O_2	576, 543
B. Reduced hemoglobin in argon	555
" " + $NaNO_2$ in argon	620, 543
Added KCN in argon to above	Around 550

In another experiment (Table II) oxyhemoglobin was reduced by means of argon gas. The addition of KCN did not change the spectrum. But when argon was displaced by O_2 the spectrum changed to that of oxyhemoglobin with the usual 2 maxima at 576 mμ and 543 mμ again present. When $NaNO_2$ was added to reduced hemoglobin in the presence of argon, the spectrum of neutral methemoglobin was produced, with the maxima at 543 mμ and 620 mμ. The addition of KCN to this (under an atmosphere of argon) gave a broad absorption band around 550 mμ. The band at 620 mμ failed to appear because the solution had become alkaline. These results show that KCN combines only with the trivalent form of hemoglobin.

In a third experiment (Table III) the presence of methylene blue, when added to hemoglobin reduced by argon, produced no change in the absorption maximum. When oxygen displaced argon

TABLE III.
Experiments with Methylene Blue.

	Wave length of maximum absorption, in $m\mu$
A. Reduced hemoglobin alone, in argon	555
" " + methylene blue	No change
Shook in oxygen	576, 543
Added KCN in presence of O_2	No change
Replaced O_2 by argon	555
Replaced argon by O_2	576, 543
B. Methemoglobin + KCN + methylene blue in argon	543
Replaced argon by O_2	543

the spectrum of oxyhemoglobin appeared. When KCN was added to this no change took place. When argon again replaced oxygen, the spectrum of reduced hemoglobin appeared. This shows that methylene blue produces its effect only in the presence of oxygen. When no oxygen was available methylene blue did not oxidize hemoglobin. In the short time of the experiment no methemoglobin produced by the action of methylene blue on oxyhemoglobin in the absence of reductants was observable.

Summary and Conclusions. These experiments show that KCN combines only with the trivalent form of hemoglobin. Since it does not combine with the bivalent form, the presence of CN^- in the blood system of animals poisoned by CN^- is not the factor which produces death; its lethal effect is rather due to its action on the oxidizing enzymes in the tissues.

Two substances have been used as antidotes for cyanide poisoning, methylene blue and $NaNO_2$ The action of these two substances is different and can now be clarified as follows:

In the case of $NaNO_2$, methemoglobin is formed in the blood stream, cyanide combines readily with this trivalent form and is taken out of the system as the blood circulates forming cyanmethemoglobin. This of course inactivates a certain percentage of hemoglobin, but the enzymes of the tissues are freed and are again able to function.

In the case of methylene blue, no appreciable concentrations of methemoglobin are produced in the blood stream as the oxidation-reduction potentials of the combined system of methylene blue plus reductants in the blood are too low.[1] Therefore the dye produces its action by catalysis, activating oxygen and actual substitution in place of the poisoned enzymes transferring oxygen to the tissues until the enzymes are again able to function.

K ferri CN and $NaNO_2$ are strong oxidants compared with methylene blue. The ferro-ferricyanide system, according to Clark's

scale[5] is far at the end of the scale near O_2 itself. $NaNO_2$ on addition to oxyhemoglobin still gives a weak band at 576 mμ, indicating that only a certain proportion of the oxyhemoglobin has been changed to methemoglobin. The methylene blue system is a weak oxidant located almost at neutrality in the pH-Eh oxidation-reduction scale.

10324 P

Growth of Small Numbers of Acid-fast Bacteria in Blood and in Serum.*

BROCKENBROUGH EVANS AND JOHN H. HANKS. (Introduced by Leland W. Parr.)

From the Department of Bacteriology, Hygiene and Preventive Medicine, School of Medicine, George Washington University, Washington, D. C.

The survival or growth of tubercle bacilli in blood is an important question from several points of view: the immunological action of serum and leucocytes, the investigation of tuberculous bacillemia, and the suitability of blood as a culture medium. Some investigators have emphasized the unfavorable action of blood on the growth of tubercle bacilli on solid media, while others have reported the growth of these organisms in blood. These points of view were reconciled when it was learned that the influence of blood in the two situations is quite different. This note indicates that, although blood interferes with the growth of tubercle bacilli on a solid substrate, it is actually an excellent medium.

Saline, defibrinated rabbit blood, or serum, .45 cc, were mixed with .05 cc volumes of 4 decimal dilutions of "clump-free" suspensions of human, bovine, avian, and timothy bacilli in 10 x 75 mm Pyrex tubes. Of these mixtures .05 cc was spread evenly on each of two slants of Corper's egg-yolk medium (in screw-capped tubes) which were free of surface moisture. The small tubes containing the mixtures were sealed with paraffined corks; both solid and liquid mediums were incubated with their surfaces horizontal.

From the results in Table I, several conclusions may be drawn: (a) Rabbit blood and serum were as favorable as the solid medium, with two exceptions: the irregular growth of bovine bacilli in blood,

[5] Clark, W. M., *Studies on Oxidation-Reduction.*

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