catalyzes the exchange of α-galactose-1-phosphate with uridinediphospho-glucose, forming α-glucose-1-phosphate and uridinediphospho-galactose, is absent in blood from galactosemic subjects. It is known that this enzymatic exchange is an important step reaction by which administered galactose is used in general carbohydrate metabolism. Several of the metabolic manifestations of the disease might readily be explained on the basis of this enzymatic defect.

Our thanks are due to Drs. G. T. Cori, V. O'Donnell, J. Kety, H. H. Mason, R. Harris, and A. Hartmann for their kind help in obtaining the galactosemic cases, and to Miss Bodil Waage-Jensen, who rendered valuable assistance as a trainee under the Scandinavian-American Foundation through a grant-in-aid generously made available to one of us (H. M. K.) by the Eli Lilly Laboratories.

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THE COMBINING POWER OF MYOGLOBIN FOR ALKYL ISOCYANIDES AND THE STRUCTURE OF THE MYOGLOBIN MOLECULE

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Communicated November 26, 1955

It was found by St. George and Pauling[1] that, although the combining powers of ferroheme with ethyl isocyanide, isopropyl isocyanide, and tertiary butyl isocyanide are essentially the same (the extreme values of the equilibrium constants differing by only a factor of 3), hemoglobin combines far more strongly with ethyl isocyanide than with *t*-butyl isocyanide, corresponding to a factor of 200 in the equilibrium constants, with isopropyl isocyanide having an intermediate value. These facts led St. George and Pauling to the conclusion that there is steric hindrance between the alkyl groups of the isocyanides and a part of the globin in the hemoglobin molecule. Inasmuch as it is known that the heme group is attached to globin by

way of an imidazole ring of a histidine side chain of the globin on the side opposite that to which the alkyl isocyanide molecule is attached, it was concluded that the heme groups of hemoglobin are buried within the protein molecule.

The rather close similarity in properties of myoglobin and hemoglobin suggests a general similarity in structure. The myoglobin molecule, with molecular weight 17,000, is, however, only one-quarter as large as the hemoglobin molecule, and it is conceivable that the myoglobin molecule might differ from the hemoglobin molecule in having the heme attached on the surface of the globin of myoglobin; it would then be predicted that myoglobin would have nearly the same combining powers for alkyl isocyanides differing in size and shape of the alkyl group, whereas if the heme were buried in the globin the combining powers would be different, as in the case of hemoglobin.

We have determined equilibrium constants for combination of horse myoglobin with ethyl isocyanide, *n*-propyl isocyanide, isopropyl isocyanide, and *t*-butyl isocyanide and have obtained the values 2.0×10^5, 1.1×10^5, 1.4×10^4, and 1.0×10^3, respectively. The value for ethyl isocyanide is accordingly 200 times greater than that for *t*-butyl isocyanide. This great dependence of the value of the combining constant on the nature of the alkyl group has a reasonable explanation in steric hindrance, and we conclude that in myoglobin, as in hemoglobin, the heme group is not attached to a surface of the globin molecule, but is buried within it.

Experimental Procedure and Results.—Myoglobin was prepared from horse heart according to a method adapted from Theorell[2] and Morgan.[3] A 4.5-kg. heart, obtained from the abattoir about 12 hours prior to use and kept refrigerated during this time, was washed, dissected free of fat and connective tissue, and cut into small pieces. Weighed portions together with half their weight in water were triturated in a Waring Blendor for between 30 and 45 seconds. This mixture was refrigerated for about 15 hours, and the fluid was removed by filtration, yielding 2.1 l. of extract with pH 5.8. The extract was neutralized with 1 *N* NaOH, and 540 ml. of 1 *F* basic lead acetate solution[4] was added slowly with stirring. The precipitate that formed was removed by centrifugation, and the supernatant fluid was brought to pH 6.6 and made 3 *F* in phosphate by adding a suitable mixture of KH_2PO_4 and K_2HPO_4;[5] this amount of phosphate saturated the solution at 4° C. The resulting precipitate, containing excess lead (as phosphate) as well as hemoglobin,[3] was removed by centrifugation, and the supernatant solution was dialyzed against several changes of saturated ammonium sulfate. The precipitated protein was removed by filtration, washed with saturated ammonium sulfate, dissolved in a minimum volume of water, and then again dialyzed against saturated ammonium sulfate. Microscopic examination of the resulting precipitate disclosed that, although some amorphous material was present, fanlike clusters of needles predominated. The precipitate was dissolved in a minimum volume of water, and the solution was dialyzed against 0.133 *F* KH_2PO_4-Na_2HPO_4 at pH 6.8. Spectrophotometric examination of this solution disclosed maxima and minima in close agreement with those given by Theorell[6] for ferrimyoglobin; following reduction with sodium dithionite, the absorption spectrum for ferromyoglobin was found; and after treatment with carbon monoxide the distinctive absorption spectrum of carbonmonoxymyoglobin was revealed. Electrophoretic examination in barbital buffer, ionic strength 0.1 and pH 8.6, disclosed essentially a single component

(mobility, 1.95×10^{-5} cm^2/volt sec), with the exception of a small contaminant having a negative refractive index contribution, possibly a lipoprotein, which moved off rapidly (mobility, 1.81×10^{-4} cm^2/volt sec) toward the anode. Concentrations of myoglobin solutions determined spectrophotometrically, using extinction coefficients given by Theorell,[6] agreed very closely with those determined by differential refractometry.

Alkyl isocyanides were prepared from alkyl iodides by the silver cyanide method,[7] using two moles of silver cyanide per mole of alkyl iodide, instead of equimolar quantities as stipulated in the published method. (The authors are indebted to Dr. Jake Bello for synthesizing the isocyanides used in this work.) Binding curves were obtained at 25° C. by adding serial increments of the solutions of isocyanides to the myoglobin, which had first been reduced from the ferrimyoglobin to the ferromyoglobin form with sodium dithionite. Three milliliters of 0.16 per cent myoglobin solution was placed in a suitable cuvette, and solutions of the isocyanides were made of such concentration that a maximum of 32 μl. of isocyanide solution (delivered from an ultramicroburette) was required to saturate the myoglobin.

With the addition of the isocyanides to the myoglobin, absorption maxima appeared at 532 and about 563 mμ. Since the change in optical density with increments of isocyanides was greater at 532 than at 563 mμ, measurements at 532 mμ were used to determine the amount of binding of isocyanide by the myoglobin. Percentage saturation was calculated from the ratio of the change observed to the total change in optical density at saturation. Assuming a molecular weight of 17,000 for myoglobin and the binding of one mole of isocyanide per mole of myoglobin at saturation, the amount of isocyanide bound and the concentration of free isocyanide were calculated for each addition of this material.

As a preliminary to the isocyanide-binding study, the spectral characteristics of myoglobin and some of its derivatives were studied in the visible range from 475 to 675 mμ. Table 1 summarizes the absorption maxima found in this range. As

TABLE 1

Compound	Absorption Maxima (Mμ)	Compound	Absorption Maximum (Mμ)
Ferrimyoglobin	502, 631	*n*-Propyl isocyanide–myoglobin	532, 563
Ferromyoglobin	556	Isopropyl isocyanide–myoglobin	532, 563
Carbonmonoxymyoglobin	542, 579	*t*-Butyl isocyanide–myoglobin	532, 562
Ethyl isocyanide–myoglobin	532, 566		

indicated in the table, the β band at 532 mμ was not influenced by the size of the alkyl isocyanide; however, the α band, at 566 mμ for the ethyl isocyanide–myoglobin complex, shifted slightly toward the blue with increasing size of the isocyanide alkyl group.

Using the symbols "M" for myoglobin, "I" for isocyanide, and "MI" for myoglobin-isocyanide, the equilibrium constant K for the combination of myoglobin and isocyanide is equal to [MI]/[M][I]. For each of the four isocyanides, values of the equilibrium constant were calculated for each of a half-dozen experimental determinations in the range 20 to 84 per cent saturation of the myoglobin. In order to eliminate a trend in the values of K obtained for ethyl isocyanide, a 2 per cent correction in the concentration of the stock solution was made; a similar correction

was also made for *n*-propyl isocyanide, which was suspected, because of its color, to be contaminated with a small amount of *n*-propyl iodide. The values of the equilibrium constants were averaged, with the concentration of free isocyanide as a weight factor. The equilibrium constants obtained in this way are 2.0×10^5 for ethyl isocyanide, 1.1×10^5 for *n*-propyl isocyanide, 1.4×10^4 for isopropyl isocyanide, and 1.0×10^3 for *t*-butyl isocyanide, the units in each case being liters per mole.

* Contribution No. 2059.

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THE CONTROL OF A CANCER GROWTH IN EMBRYONATED EGGS

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Communicated November 29, 1955

Most of the investigations in our laboratories having to do directly with cancer and cancer tissues in the last ten years have centered around the development by one of us[1, 2] of a convenient method for inoculating and propagating cancer tissue in the yolk sac of embryonated eggs. This method has been developed to a point such that it is now possible to inoculate a series of eggs quantitatively and to harvest several days later a relatively uniform series of egg-grown cancers, along with chick embryos which have grown alongside, utilizing the same blood supply. Each fertile egg will bear a tumor, and without dealing with large numbers it is generally possible to get satisfactory replicate results from different sets of eggs.

Although there is the expected variation in the sizes of tumors harvested at any given time, this variation is not prohibitively large and does not include zero values. The following results may be regarded as typical. When eggs were inoculated with a suitable tumor suspension on the fourth day of incubation, the tumor weights, expressed in grams, at the end of the tenth day were 0.19, 0.12, 0.17, 0.08, 0.05, 0.09, and 0.04. At the end of thirteen days, in a similar set, the respective tumor weights in grams were as follows: 0.6, 0.9, 0.6, 0.4, 0.3, 0.5, 0.6, and 1.0.

This relative uniformity and consistency of growth make it possible to subject various sets of eggs to different treatments and, by comparison with controls, to determine the effects of different treatments both on the cancer growth and on the growth of the chick embryos. Many thousands of experiments involving hundreds of thousands of eggs have been carried out in our laboratories using this technique.