sorption or utilization of the two forms of iron. All three groups received 0.025 mg copper per rat daily as copper sulfate. Supplementation was carried on for a total of 9 wk. Blood hemoglobin determinations were made on tail blood at six different intervals during the supplementation period.

The average blood hemoglobin levels in Table 1 clearly indicate that the iron from the iron chelate was absorbed from the gastrointestinal tract and utilized for hemoglobin regeneration in the anemic rat at the same rate as iron from ferrous sulfate. Using the average figure of 6.7 ml of blood per 100 g body weight for the white rat, supplied by Cartland and Koch (*6*), and the generally accepted figure of 0.335 percent iron for the iron content of hemoglobin (*7*), an approximate calculation indicated that the rats were approaching 100-percent utilization of administered iron from both forms of iron during the early weeks of the experiment. The percentage utilized tapered off as the blood hemoglobins began to approach normal levels.

Fecal examinations were made several times during the course of the experiment. Feces from the ferrous sulfate group were usually slightly darker in color than those of the control group. Feces from the iron chelate group were usually softer and lighter in color than those of the controls. At autopsy, the lining of the stomach and gastrointestinal tract of the animals in both groups showed blackened areas. In the case of the iron chelate animals, this indicates that at least some of the complex was being broken down in the gastrointestinal tract, since the unbroken complex will not release iron to form sulfides of iron. Animals maintained on a milk diet with only the copper supplementation do not develop blackened areas in the gastrointestinal tract.

The effect of iron chelate administered parenterally was also explored in a preliminary way. When the control group had continued 6 wk beyond their depletion period on the iron-free diet, they had very low blood hemoglobin values, two had died, and the remaining eight were in poor condition. These eight were divided into two groups of four each, and intravenous therapy was instituted at a dosage level of 4 mg iron per kilogram twice weekly. One group received a solution of saccharated iron oxide (*8*). The other group received a sterile solution of 1.0 percent ferric sodium ethylenediamine tetraacetate (Table 2). Both groups continued to receive copper supplementation in the milk diet.

The poor hemoglobin response of the rats that received chelated iron intravenously is in accord with the postulate of Brendel *et al.* Once introduced into the body parenterally as the heavy metal complex, the metal ions remain attached to the complexing nucleus and are not readily available to the body. The parenteral results in turn lend further support to our suspicion that, following oral administration, iron is released from the chelate by some mechanism in the gastrointestinal tract before being absorbed. This mechanism is presently under further study.

References and Notes

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Received February 15, 1954.

Studies of Copper Chlorophyllin-Odorant Systems[1]

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The use of chlorophyllins (water-soluble derivatives of chlorophyll) has been reported (*1*) for the effective reduction of breath odor, but no explanation of a mechanism by which such odor reduction is possible has been forthcoming in terms of the principles of physical chemistry.

By independent measurements, we have found that buffered (*2*) "solutions" of potassium copper chlorophyllin (*3*) will take up appreciable quantities of odorant, which can be estimated by titrating the solution with odorant to an olfactory end-point. In addition, we have measured the physical adsorption of gases onto powdered chlorophyllin (Table 1). In view of the marked difference between the two experimental techniques, the results are in surprisingly good agreement.

This observed adsorption is both physical and chemical, and where both occur, they may be of approximately equal magnitude. The physical adsorption is of the Langmuir type. Surface area by the BET method for several preparations of chlorophyllin is of the order of 15 m^2/g.

The fact that chlorophyllin does not form true water solutions but rather polyelectrolyte dispersions can be demonstrated by determinations of molecular-weight distribution and of intrinsic viscosity, and by precipitation with alcohol and with electrolytes. Chlorophyllin is about 75 percent nondialyzable through Cenco cellophane tubing; the particle weight is, therefore, primarily greater than 12,000. The particle weight of 3.6 percent of a sample is larger than 1-million as determined by sedimentation in a preparative centrifuge. The precipitation of chlorophyllin by ethanol increases linearly with the concentration of alcohol, and this suggests a continuous range of particle weights. Viscosity data (*4*) give straight-line functions for c/η_{sp} vs. $\sqrt{c}$ and fit a Fuoss Formulation giving $[\eta] = 0.33$; this indicates that particles are

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[1] This paper was delivered at the meeting of Section C of the AAAS in Boston, Dec. 27, 1953.

TABLE 1. Adsorption of chlorophyllin.

Material	Temperature (°C)	Moles adsorbed per mole[†] chlorophyllin	Reversible
A. Amounts of odorants[*] taken up in "solutions" of potassium copper chlorophyllin as measured by an odor titration			
Amyl mercaptan		0.005	No
n-propyl mercaptan[‡]		.01	No
Diallyl sulfide		.01	No
Diisobutyl ketone[‡]		.03	
Vanillin		.08	
Trimethyl amine		.09	Yes
Limonene		.1	Yes
Ethyl acetate[‡]		.2	
n-valeric acid		.3	Yes
Thioglycolic acid		.3	No
Hexamethylene diamine		1	Yes
Hydrogen sulfide		5	No
B. Amounts of several chemicals adsorbed on solid chlorophyllin as measured by gas adsorption			
Ethyl acetate	35	0.2	Yes
Methyl ethyl ketone	35	0.1	Yes
Nitrogen	−196	$0.1_3 - 0.2$	Yes
Hydrogen sulfide	25	0.4_5	0.2_5 No, 0.2 Yes
Amyl mercaptan	35	0.3_8	0.3_4 No, 0.04 Yes

[*] The odorant taken up was corrected by the titration of a blank without chlorophyllin.

[†] A mole of chlorophyllin is taken as 690 g.

[‡] No buffer was used.

TABLE 2. Adsorption at the odor end-point of valeric acid and amyl mercaptan by an aqueous dispersion of materials of high specific surface.

Adsorbent	Surface area (m^2/g)	n-valeric acid (10^{-5} mole/m^2)	Amyl mercaptan (10^{-8} mole/m^2)
Carbon black	180	3	4
Activated charcoal	10	2.7	3
Attapulgus clay	3	4	5
Diatomaceous earth	0.8	< 8	5
Silica gel	300	0.04	0.09
Chlorophyllin	15	2.8	50

probably spherical with diameters possibly as large as 0.02 micron. Ultracentrifuge and light-scattering molecular-weight determinations have failed because of the interfering intense green color of chlorophyllin.

The adsorption of odorants by other surface active materials in aqueous dispersion has been measured for comparison by the odor titration technique (Table 2). All adsorption is reversible on heating, except on chlorophyllin. For n-valeric acid, all data are comparable, except for hydrophilic silica gel. For amyl mercaptan, the greater affinity of chlorophyllin can be explained by the nonreversible chemisorption.

These adsorption data can be explained by the principles of adsorption processes. Physical adsorption is reversible and minimized at low temperature; chemisorption, because of the high energies of adsorption, is nonreversible and minimized at low temperatures, because of the high activation energy required for chemical reaction. Interpretation of the foregoing data indicates that both types of adsorption by chlorophyllin are involved. For chemisorption, surface saturation is possible at all concentrations. For physical adsorption, monolayer formation may not take place at low concentrations, and the amount of adsorption may be very small. However, the data reported in Tables 1A and 2 depend on an olfactory end-point and indicate actual amounts of odor adsorbed at threshold concentrations of odorant.

Recent work (*5*) indicates that perceptible variations in odor perception can be expressed in chemical concentration as

$$C/C_{\text{th}} = 10^N,$$

where C is the detected concentration and C_{th}, the threshold concentration, and the perceived level N is limited to the following ranges: $N = 1$ (just detectable), $N = 2$ (weak), $N = 3$ (medium), $N = 4$ (strong), and $N = 5$ (very strong). Thus, a perceived odor reduction from medium to weak changes the concentration ratio from 1000 to 100 and 900 parts of the odor have been removed to reduce this perceptible difference.

This explains, for the first time, the reduction of odor by chlorophyllin in terms of the surface properties of adsorbent materials and the laws of odor perception. The help of Prof. Irwin W. Sizer, Biology Department, Massachusetts Institute of Technology, is gratefully acknowledged.

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3. 94.5 percent by optical assay, 2.5 percent yellow component, 4.36 saponification number, 0.60 percent free copper at *p*H = 3, 4.66 percent total copper. Analysis by F. D. Pickel, Evans Research and Development Corporation.
4. c/η_{sp} is the ratio of concentration to specific viscosity and $[\eta]$ is the intrinsic viscosity, $[\eta] = \lim_{c \to 0} \eta_{sp}/c$.
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Received March 2, 1954.

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