activity of protamines was also observed by McClean.[7] Annau, *et al.*, believe that the pharmacological effects of histones are associated with the arginine radical present in the molecule in a peptide linkage. Free arginine having a free carboxyl group is entirely ineffective pharmacologically. It seems probable that the presence of arginine radicals in peptide combination conveys properties to the histone and protamine molecules which are to some extent similar to those of cationic detergents. The mechanism by which these substances exert their toxic effects on cells is not clearly understood. Whether or not the reactivity of these substances with insulin can be taken as suggestive of the mechanism of their toxic action remains to be investigated.

Summary. The order of the toxicity of the proteins investigated is as follows: protamine > histone > globin. Globin was found to be devoid of appreciable toxicity when injected into animals or tested on tissues and on monocellular organisms *in vitro*. Protamine and histone were found to be highly toxic to trypanosomes. Factors which may contribute to the toxicity of these proteins were discussed.

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Origin of Ionized Iron after Action of Acids on Blood and Influence of Carbon Monoxide.

GEORG BARKAN* AND OTTO SCHALES. (Introduced by F. H. Pratt.)

From the Evans Memorial, Massachusetts Memorial Hospitals, and the Department of Biochemistry, Boston University School of Medicine, and from the Medical Clinic, Peter Bent Brigham Hospital, and the Department of Medicine, Harvard Medical School.

When dilute acids are added to hemolyzed blood, a small amount of iron is split off in ionized form,[1-4] varying from 14 to 18 mg per liter of human blood.[5, 6, 7] It was assumed that this easily split-off

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* Partially aided by the Rockefeller Foundation.

We gratefully acknowledge the assistance of the Ella Sachs Plotz Foundation in furtherance of our work.

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[5] Schwarz, L., and Deckert, W., *Klin. Woch.*, 1935, **14**, 601, 900.

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iron was due to the presence in the erythrocytes of iron-containing bile pigment proteids (pseudohemoglobins[8, 9]). The presence of non-hemoglobin iron in the erythrocytes has been confirmed by a number of recent investigators, using different methods.[10–19] In the above experiments,[1, 3–9] the determination of the liberated iron was carried out in protein-free filtrates obtained from hemolyzed blood solutions, which had been incubated at 37°C for 24 hours with 0.4% HCl. The low concentration of acid was considered necessary in order to avoid any attack on the iron in the hemoglobin molecule itself. Some recent data[18, 20] have shown, however, that incubation with HCl in concentrations lower than 0.4% results in even higher values for the easily split-off iron.

These observations and other reports[21, 22] make it doubtful that the values for the easily split-off iron obtained with the customary method[23–25] give a true picture of the amount of pseudohemoglobins originally present in the blood.

The purpose of the present investigation was to determine whether labile iron can originate from the action of dilute acid on hemoglobin[2, 22] and if so, whether it is possible to differentiate between the liberation of iron from hemoglobin and from preformed pseudohemoglobin.

Action of acids on native and synthetic hemoglobin. The effect of various concentrations of acid on solutions of human blood was studied. Venndt[20] had found that 1.3% HCl solution liberated less and 0.1% HCl solution liberated more iron than did 0.4% HCl solu-

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25 Barkan, G., and Walker, B. S., *J. Biol. Chem.*, 1940, **135**, 37.

TABLE I.

HCl%	12	6	4	3	2	1	0.4	0.12	0.10	0.05
Fe mg/liter blood sol. 1:10	1.03	0.92	1.13	1.32	1.25	1.56	1.84	4.50	4.89	2.11

tion. These results have been confirmed and extended. A series of iron determinations on diluted blood (1:10) incubated for 48 hours at 37°C, in HCl solutions varying from 12 to 0.05%, was done. Table I shows an example with human blood containing 15.63 g Hb per 100 ml; "Liquoid" (Roche) was used as an anticoagulant.

An incubation time of 48 hours was chosen in order to secure a more complete reaction.[20] The iron determinations were carried out electrophotometrically with o-phenanthroline.[25] A correction had to be made for the brownish color of the protein-free filtrates obtained from mixtures with HCl above 2%.

The table shows no significant change in the amount of iron split off with concentrations of HCl between 12 and 2%, but there is a definite increase below this concentration with a sharp peak at about 0.1% HCl. The amount of iron given off in this range of low acid concentrations represents about 10% of the total blood iron. Such an amount of non-hemoglobin iron is not in accordance with known facts, and at least in this range a considerable part of the iron found originates from the hemoglobin molecule itself.

For a dependence of the breakdown of hemoglobin on the acidity of the solution, an explanation can be given. On the addition of acid to oxyhemoglobin solutions active oxygen is liberated instantly,[26, 27] which probably then forms hydrogen peroxide.[28] At low acid concentrations a considerable amount of pigment with intact protein-hemin linkage will exist for a time and this acts as a peroxidase, catalyzing the oxidative self-destruction of a part of the pigment with the formation of open-ring compounds with labile iron. At higher acid concentrations, however, the protein moiety of hemoglobin will be attacked immediately and hemin is set free. This is a much less active peroxidase than hemoglobin, but has about the same catalatic activity[29] as the intact blood pigment. Consequently at higher acid concentrations any hydrogen peroxide formed will be destroyed rather than utilized for the destruction of blood pigment.

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TABLE II.

Material	Total iron mg/liter	Iron split off by HCl 0.4% (48 hrs at 37°C) mg/liter	Iron split off by HCl 0.1% (48 hrs at 37°C) mg/liter
"Synthetic" methemoglobin (2.4 g/100 ml)	80.3	0.23	0.25
Blood solution (3.13 g Hb/100 ml)	105.0	3.68	9.78

(The results for "synthetic" methemoglobin are corrected for the Fe content of the globin solutions from which it was prepared.)

For an iron-containing bile pigment-globin compound (pseudohemoglobin) such a dependence of the liberation of its iron on the acid concentration is improbable. The results obtained with 2-12% HCl then would represent the amount of iron derived from preformed pseudohemoglobins, equal to only about 2% of the total iron. This would agree in the order of magnitude with the amount of bile pigments isolated by Lemberg and associates[30] from red blood cells.

In contrast to the experiments of Legge and Lemberg,[22] methemoglobin prepared by combining native bovine globin[31] with crystalline chlorhemin,[32] did not split off more than 0.3% of its total iron under conditions in which the yield of liberated iron from blood solutions is highest (Table II).

As methemoglobin in contrast to oxyhemoglobin does not form active oxygen or hydrogen peroxide on addition of acid, a destruction similar to that suggested for oxyhemoglobin in 0.1% HCl will not take place.

Influence of carbon monoxide. When blood solutions are treated with CO before adding HCl, a much smaller amount of iron is split off.[33] A blood solution saturated with CO and incubated in 0.4% HCl yields only about ⅓ of the amount of iron liberated without carbon monoxide treatment. With amounts of CO not sufficient

TABLE III.

Human blood, Hb 16.7 g/100 ml. Dilution 1:10 digested 24 hrs with 0.4% HCl at 37°C.

Fraction of total Hb present as HbCO (determined spectrophotometrically) %	Iron split off mg/liter	Amount inhibited original blood	Inhibition in % of that in sol. saturated with CO
0	17.1	0	0
36	9.6	7.5	65
71	6.3	10.8	94
100	5.6	11.5	100

30 Lemberg, R., Lockwood, W. H., and Legge, J. W., *Biochem. J.*, 1941, **35**, 363.

31 Anson, M. L., and Mirsky, A. E., *J. Gen. Physiol.*, 1929-30, **13**, 469.

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33 Barkan, G., and Berger, E., *Arch. exp. Path. u. Pharmakol.*, 1928, **136**, 278.

TABLE IV.

No.	Incubation time, hr	Iron split off by 0.4% HCl from sol. saturated with O_2 (mg/liter original blood)	Iron split off by 0.4% HCl from sol. saturated with CO (mg/liter original blood)	Iron split off by 0.4% HCl from sol. saturated with CO and reduced with $Na_2S_2O_4$ (mg/liter original blood)	$Na_2S_2O_4$ added per ml blood sol. 1:5, mg
1	24	16.8	2.8	1.4	.50
2	24	16.8	5.6	3.5	.33
3	48	22.0	6.5	2.7	.10

for complete transformation of HbO_2 into HbCO, the inhibition of the liberation of iron found is not proportional to the percentage of HbCO (see example in Table III).

The amount of iron split off becomes still smaller, if the solutions, after treatment with CO, are reduced and again treated with carbon monoxide. Table IV gives the results of some experiments of this type carried out with solutions (1:10) of defibrinated beef blood.

While the phenomena here involved are more complicated than previously assumed,[8, 22] the fact remains that the decrease by CO of the lability of iron from blood solutions gives a means of detecting smaller amounts of carbon monoxide in blood than are determinable by simple qualitative tests for HbCO. In 80 cases of carbon monoxide poisoning, Schwarz and Deckert[5] confirmed Barkan and Berger's[33] findings and noted that the decrease in labile iron would persist for several days after the acute exposure to carbon monoxide. Rooks[7] found the method valuable also for *post mortem* diagnosis of CO-poisoning. Schwarz and Deckert noticed that less labile iron was found in the blood of habitual smokers. With some reserve this was referred to as a carbon monoxide effect. Schwarz and Deckert's observation was confirmed by Olesk[6] and by Rooks.[7] These findings have also been confirmed here, as Table V shows. Twenty-four hours' digestion with 0.4% HCl and the photoelectric method for iron determination[25] were used.

Summary. 1. The amount of labile iron derived from preformed

TABLE V.

Group	No. of persons	Hemoglobin, avg, g/100 ml	Easily split-off iron, avg, mg/liter	Standard error of the mean, ±
Non-smokers	10	14.8	14.2	0.37
Smokers*	12	14.8	12.8†	0.34

*10-30 cigarettes a day.

†According to R. A. Fisher, *Statistical Methods for Research Workers*, 4th ed., the difference is significant. The probability for chance is about 0.0125 (see Table IV, table of t, *l.c.*).

pseudohemoglobins is not more than 2% of the total blood iron. 2. Under the influence of very dilute acids additional iron is split from the hemoglobin molecule itself. 3. Carbon monoxide inhibits the liberation of iron. 4. This inhibitory effect can be used for diagnostic purposes and confirms the presence of carbon monoxide in the blood of tobacco smokers.

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Permanent Devocalization of Dogs by Removal of both the True and the False Vocal Cords.

G. L. DONNELLY. (Introduced by W. deB. MacNider.)

From the Laboratory of Pharmacology, the Medical School, the University of North Carolina.

In many institutions where it has been necessary to devocalize the laboratory dogs the results have not been satisfactory. In 1932 the writer made a study of the usual devocalizing methods with the object in view of improving the results obtained from such methods.

By using a pair of Miles nasal cutting forceps it was found that the true vocal cords could be rapidly and completely removed.[1] The procedure was carried out on 20 dogs under morphine-ether anesthesia. There were no complications and the animals recovered within 24 hours. After about 3 months, however, each dog developed a hoarse, unpleasant bark. The immediate assumption was that the operative technic had been faulty and that the vocal cords had not been completely removed. Later, when some of the same animals came to autopsy, laryngeal examinations showed a complete absence of the true vocal cords. Further studies of the autopsy material revealed in each case a well developed pair of soft mucous folds or pockets at the base of the epiglottis and just above the true vocal cords. These folds were identified as false vocal cords and seemed to be the only remaining structures by the use of which sound might be made.

The surviving dogs whose vocal cords had been removed with poor results were again prepared for operation. The soft folds or pockets of the false cords were removed as completely as possible with a pair of straight, back-cutting Ostrom forceps. Upon recovery these animals were no longer able to produce any noise other than

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