

seventy-first days after bleeding. The results are summarized in Table II.

SUMMARY OF TABLE II.
Hemorrhagic Anemia.

| | Before Bleeding | | 24 Days After Bleeding | | | No. of Rats |
|--|-----------------|-----------|------------------------|-----------|-------|-------------|
| Control Group (Practically iron-free) | 0.899 | | 0.787 | | 0.886 | 49 |
| | 0.821 | Var 0.731 | 0.708 | Var 0.627 | 0.817 | |
| Copper and iron group | 0.898 | | 0.809 | | 0.914 | 51 |
| | 0.803 | Var 0.725 | 0.716 | Var 0.633 | 0.791 | |

The results are very different from those obtained in the nutritional group. The rats in the sub-group receiving copper and iron in addition to the diet had a concentration of 0.803 before and 0.791 on the seventy-first day after bleeding. An intermediate reading was taken to determine the rate of change. In other words, in this group the hemoglobin was almost exactly the same on the seventy-first day as in the beginning, whereas in the nutritional group, a marked improvement occurred as early as the twenty-fourth day, which remained at substantially the same level on the fifty-fourth day. In the sub-group used as control, we find a concentration of 0.821 before and 0.817 on the seventy-first day after bleeding. Expressed in another way, the group treated with the addition of iron and copper to a standard diet did not improve either more rapidly or to a greater degree than those on a standard control diet alone.

Conclusions: In rats with nutritional anemia the addition of iron and copper to the diet produces definite and prompt improvement in the hemoglobin concentration. In rats with anemia produced by repeated abstraction of blood the addition of iron and copper to the diet produces no perceptible effect.

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Fermentation of Glucosamine Hydrochloride by Bacteria.

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With the exception of a few observations on glucosamine the fermentation reactions of micro-organisms on the hexosamines have not been studied. Inasmuch as these nitrogenous sugars contain a carbohydrate and an amino group, both of which are necessary for the growth of bacteria, an investigation of the metabolic activities

of different organisms on these substances might be of value. Abderhalden and Fodor¹ reported that *B. tenuis* fermented glucosamine hydrochloride. Meyer² found that glucosamine hydrochloride was fermented by such organisms as he used in the same manner as glucose is fermented with the exception of *B. paratyphosus* A, which formed acid only in glucosamine. Noble and Knacke³ found variations in the ability of different strains of *B. diphtheriae* and diphtheroid organisms to ferment glucosamine hydrochloride but no relationship between these differences and virulence. The organisms capable of fermenting glucose all fermented glucosamine hydrochloride except *B. mesentericus*, yeast and possibly *B. proteus*. The amount of acid formed by *B. proteus* was slight, the reaction delayed and transient, and therefore doubtful whether or not due to the fermentation of the glucosamine. *B. paratyphosus* A formed gas in glucosamine hydrochloride. This is contrary to the findings reported by Meyer.

B. coli and *B. mucosus capsulatus* grew well in a medium containing only inorganic salts and glucosamine hydrochloride. There was an accumulation of free ammonia in the medium indicating that deaminization is one part of the mechanism by which bacteria split a molecule of glucosamine. It is probable that it is the initial step leaving the hexose free to be acted on as such because in all cases observed, when an organism ferments glucosamine, the fermentation proceeds in the same manner as when that organism is acting on any monosaccharide.

There is still a doubt whether the sugar in the glucosamine molecule is glucose or mannose. With this in mind parallel tests were made with the same organisms using glucose, mannose and glucosamine hydrochloride.

The results are somewhat confusing. *B. mesentericus* ferments glucose but not mannose or glucosamine hydrochloride, *B. anthracis* ferments glucose and glucosamine hydrochloride but not mannose, and yeast ferments glucose and mannose but not glucosamine hydrochloride. Thus we have organisms that can ferment glucose unable to ferment glucosamine hydrochloride and also organisms able to ferment mannose that can not use glucosamine hydrochloride. It would therefore appear that the kind of sugar in the glucosamine molecule is not the only factor that determines whether an organism can or can not utilize this substance. The presence of the amino group and especially the carbon atom to which it is attached may be

¹Abderhalden and Fodor, *Zeit. f. Physiol.*, 1913, **87**, 214.

²Meyer, *Biochem. Zeit.*, 1913, **57**, 297; 1914, **58**, 415.

³Noble and Knocke, *J. Bact.*, 1928, **15**, 53.

the inhibiting factor. In glucosamine the amino group is attached to the second carbon atom. There are other hexosamines in which the amino group is attached to the first, the third and the sixth carbon atom. These hexosamines are being prepared and the action of bacteria on them will be investigated.

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Effect of Diathermy on Concentration of Complement and Normal Opsonins.

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The increasing use of diathermy has augmented the need for greater knowledge of its effect upon the body. Such knowledge may aid in the elucidation of some controversial points in immunology since a temperature is produced without the injection of protein or other foreign substance. Nine cases of allergic asthma were treated with diathermy according to the method described by Neymann.¹ The investigation was limited to a study of the concentration of complement and natural opsonins, and the frequency and choice of time of procuring samples were restricted. The number of treatments was 1 to 4, the time of second treatment, 2 hours to 17 days, and the change in opsonic index in 10 cases was 0.1 to 0.4. Serum from a normal adult, the same throughout the experiment, was used as a control.

Complement concentration of the patient's serum was determined in the usual way employing sheep's red blood cells and rabbit anti-sheep cell serum with appropriate controls. The results of such tests demonstrated that no significant change in the complement content of the serum was effected by diathermy and such slight alterations as did occur are probably explainable by normal variation in complement or slight differences in the suspension of blood cells.

Opsonins. A modification of the opsonic test used by Tunncliffe² was selected as most applicable for the conditions and material at hand. A heat killed laboratory strain was used, leukocytes from a normal control and unheated serum from patients and control. The opsonic index was obtained by dividing the percentage phagocytosis

¹ Neymann, *J. Am. Med. Assn.*, 1931, **96**, 7.

² Tunncliffe, *J. Am. Med. Assn.*, 1926, **87**, 625.