thyroid gland has been removed *in toto* might suggest some utilization of this finding in the future treatment of patients with peripheral vascular disease. At least it appears that the increased dilatation response is as great as that achieved by operations upon the sympathetic apparatus, whether central in type, that is, ganglionectomy or ramisection, or peripheral in type, that is, decortication of the arteries.

Moreover, it would appear that, even with observations carried on for only 4 months, we have already indicated as permanent a vasodilatation response as the various sympathetic procedures have given us up to this time.

## 7300 C

## Cobalt Glutamate in Nutritional Anemia.*

ERWIN BRAND AND CHARLES J. STUCKY.

*From the Department of Chemistry, New York State Psychiatric Institute and Hospital, New York City.*

While our studies on milk anemia[1, 2] were in progress, evidence appeared in the literature[3, 4] which indicated that cobalt stimulated blood regeneration in nutritional anemia. Therefore, experiments were conducted to determine the effect of cobaltous glutamate as compared to inorganic cobalt in hematopoiesis, when supplementing a whole milk (klim)-iron-copper diet. The technique was as previously described.[1]

In preliminary experiments it was found that rats, which had recovered from anemia by supplementing the whole milk (klim) ration with Fe and Cu showed renewed hematopoietic activity when the diet was further supplemented with 0.5 mg. cobalt as cobalt nitrate. Thus at the end of a feeding period of 6 weeks, a group of 3 "cobalt" rats gave average values of 18.6 gm. and 11.0 millions for the hemoglobin and erythrocytes respectively as compared to

* In coöperation with the Research Department of the Calco Chemical Co., Bound Brook, N. J.

1 Brand, E., and Stucky, C. J., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 627.

2 Brand, E., and Stucky, C. J., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 689.

3 Orten, J. M., Underhill, F. A., Mugrage, E. R., and Lewis, R. C., *J. Biol. Chem.*, 1932, **96**, 11.

4 Orten, J. M., Underhill, F. A., Mugrage, E. R., and Lewis, R. C., *J. Biol. Chem.*, 1933, **99**, 457.

14.6 gm. and 8.4 millions for a group of "non-cobalt" controls. Both groups gave approximately equal values for these constituents at the beginning of the experiment.

Accordingly, further tests were performed with young anemic animals. Four groups of 3 rats each were employed. The animals were distributed in each group according to sex and litter in order to obtain more representative results. Groups 1 and 3 received 0.5 mg. of ferrous glutamate plus 0.1 mg. of copper glutamate and served as controls for groups 2 and 4 respectively. The cobalt (0.5 mg.) was administered as a bivalent compound of glutamic acid in group 4 and as cobalt nitrate in group 2.

The average results for the groups are shown graphically in Figures 1 and 2 and it can be seen that all the animals showed rapid blood regeneration. However, in the "cobalt" rats, the hematopoietic response was much more extensive, the individual hemoglobin levels reaching values of 18 to 24 gm. and the erythrocytes increasing to 13 to 16 millions. There is no significant difference between the two cobalt groups (*i. e.*, groups 2 and 4) as regards the degree of polycythemia. However, the rats which received cobalt as the inorganic salt (group 2) did not grow as well as the control rats (group 1) and the growth was below that exemplified by the

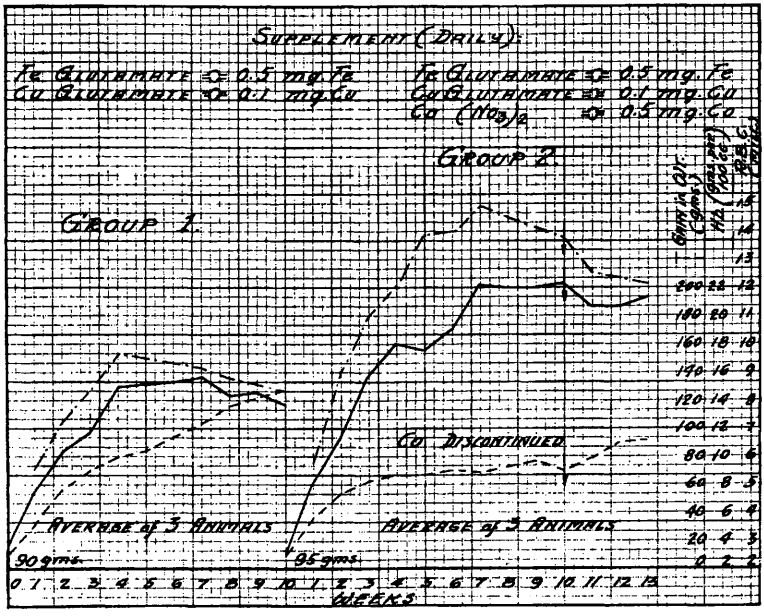


FIG. 1.
Effect of Cobalt Nitrate on Hb, R.B.C. and Growth.

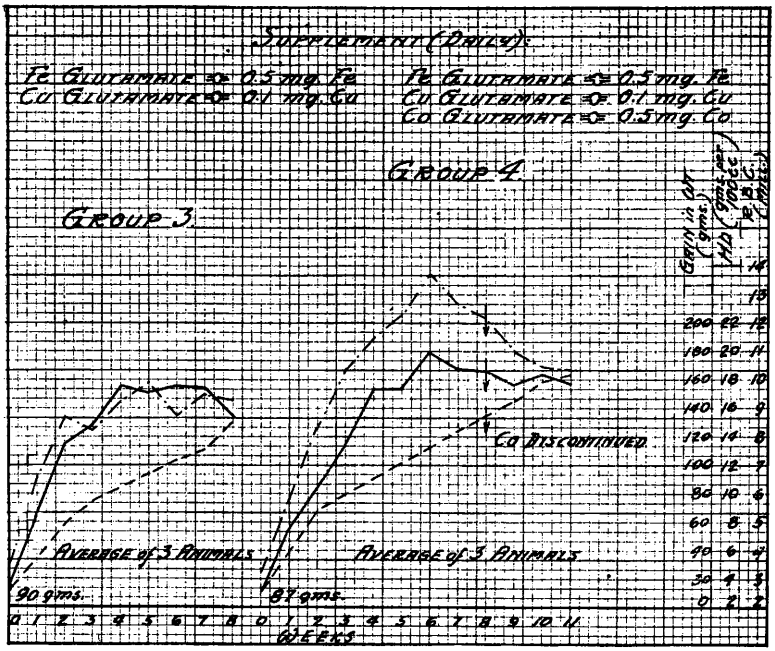


FIG. 2.
Effect of Cobaltous Glutamate on Hb, R.B.C. and Growth.

animals (group 4) which received the cobalt in the form of the glutamate compound. On the other hand, the cobaltous glutamate rats (group 4) grew at the same rate as the controls (group 3). Further experiments should be conducted to determine whether the retardation in growth of the rats which received cobalt in the form of the nitrate salt is significant.

The animals in group 4 gave values of 37% and 47% higher for Hb and erythrocytes respectively than those in group 3. Similarly, the rats in group 2 reached levels of 15% and 41% higher than those in group 1 for these same constituents.

In both cobalt groups there was a gradual decrease in the values for the blood constituents after the cobalt was discontinued. In the case of some of the rats there was a noticeable decrease even before the cobalt supplements were withdrawn. It is possible that this decline in Hb and erythrocytes during the period of cobalt feeding may indicate a toxic effect of this element due to its accumulation in the tissues. Further research may clarify this problem.

From these data it is clear that cobalt accelerates the hematopoietic activity of Fe and Cu in nutritional anemia. A marked poly-

cythemia is produced. No significant difference in this respect could be demonstrated between cobalt when administered as the glutamate or the nitrate.

*Summary.* Cobalt (0.5 mg.), as cobalt glutamate and as cobalt nitrate, produces a polycythemia in young rats, when fed as a supplement to a whole milk (klim)-iron-copper diet. The animals receiving cobalt glutamate showed the same growth as the control groups, while that of the cobalt nitrate group was retarded.

## 7301 C

## The Haptene-Protein Complex in Canine Anaphylaxis.

A. C. KURTZ AND HAROLD C. SOX. (Introduced by W. H. Manwaring.)

*From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.*

Recent studies of specific allergic and immune reactions against artificial protein-crystalloid conjugates have suggested that the superficial crystalloids of a protein molecule are the essential units of specific antigenicity. If so, the whole theory of specific immunity must be rewritten in terms of sub-colloidal antigenic "determinants".

Most of these studies, however, are inconclusive. This is due in part to the high haptene-protein ratios almost invariably used. In many cases the conjugates were "carriers" of no less than 150 to 200 haptene "determinants".[1] Moreover, from the point of view of clinical medicine, both rabbit antiserum and guinea pig anaphylaxis distort the quantitative perspective. We have, therefore, repeated some of the classical tests, using haptene-protein complexes of relatively low ratios, with dogs as the experimental animals.

The proteins used in this work were horse serum (HS), cow serum (CS), dog serum (DS), egg white (EW) and crystallized excelsin (Ex). Conjugation was made with benzoyl (Bz) and benzene-sulfonyl (Su) radicals, the quantitative relationships being such as to give final products with an average of from 25 to 40 haptene groups per protein molecule. This represents an approximate 20% haptene saturation.

Dogs were sensitized by a single intracardial injection of 40 mg.

[1] Kurtz, A. C., Sox, H. C., and Manwaring, W. H., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 138.