response to filling and distention has been studied by subjecting innervated and denervated intestinal segments of unanesthetized dogs to identical distending forces. Distention acts as a stimulus to the smooth muscle of the denervated intestine so that an active pressure increase may be induced by the stretch and passive pressure resulting from a standard amount of filling. The stimulatory effect of filling is not equally evident in the innervated intestine. This result may be interpreted as indicating that the degree of motility observed at the site of a distention in the innervated intestine depends, in part, upon a balance between direct stimulatory effects and reflex inhibitory effects.

## 11704

## Cobalt Color Reaction of Barbiturates.*

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Of the numerous analytical methods employed for the determination of barbiturates, those making use of specific color reactions have been most extensively studied. Handorf[1] employed the murexide reaction for the detection of veronal, and Zwikker,[2] Bodendorf,[3] Herwick[4] and Koppanyi, *et al.*,[5, 6] have developed modifications of a color reaction with cobalt salts in conjunction with various bases.

Kozelka and Tatum[7] attributed this color formation to a specific reaction with substances containing one or 2 imide groups. In an investigation of amytal (isoamylethyl barbituric acid) excretion by dogs, using a modification[9] of the reaction designed by Koppanyi

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1 Handorf, H., *Z. f. d. ges. exp. Med.*, 1922, **28**, 56.

2 Zwikker, J. J. L., *Pharm. Weekblad.*, 1931, **68**, 975.

3 Bodendorf, K., *Arch. Pharm.*, 1932, **270**, 290.

4 Herwick, R. P., *Arch. internat. de Pharm. et de Therap.*, 1933, **45**, 160.

5 Koppanyi, T., *et al., ibid.*, 1933, **46**, 76.

6 Koppanyi, T., *et al., Am. Pharm. Assn.*, 1934, **23**, 1074.

7 Kozelka, F. A., and Tatum, *J. Pharm. and Exp. Therap.*, 1937, **59**, 54.

9 Krause, R. F., and Riley, Richard F., unpublished work.

*et al.*,[6] employing cobalt acetate and isopropylamine made up in absolute methanol solutions, it was observed in this laboratory, that of the more probable breakdown products of amytal[8] only isoamyl-ethyl acetic acid gave the characteristic color reaction. Since this compound contains no imide grouping, it was apparent in this case that color formation was not due to such a group.

This finding led to an examination of various classes of compounds a number of which have not been reported upon by other investigators. A partial listing is given in Table 1.

Since the acids tested gave a color apparently identical with that obtained with amytal, a number of these test solutions were submitted to spectrophotometric analysis.

The spectrophotometric curves given herewith were made with a Bausch and Lomb medium quartz spectrograph and sector photometer. The length of the absorption cells was 5 cm. To cobalt acetate and isopropylamine mixtures were added respectively, solutions of amytal, isoamyl ethyl acetic acid ($RRC_2H_2O_2$) and stearic acid in amounts sufficient to give an intensity of color suitable for measurement.

It is noted that the addition of isopropylamine to the cobalt acetate produces a shift of the absorption band from 5150 to 5450 AU, and then for the other 3 compounds there is a further shift for each to 5650 or 5700 AU. It is also interesting to note that the absorption

TABLE I.
Compounds Tested for Color Reaction.

| Compound tested | Results | |
|---|---|---|
| Acetic acid | + | Compounds soluble in chloroform and possibly present in urine specimens |
| Propionic acid | + | |
| Butyric acid | + | |
| Isoamyl-ethyl acetyl urea | — | |
| Isoamyl-ethyl acetamide | — | |
| Isoamyl-ethyl acetic acid | + | |
| Hippuric acid | + | Compounds insoluble in chloroform and possibly present in urine specimens |
| Creatinine | + | |
| Uric acid | — | |
| Allantoin | — | |
| Histidine | — | |
| Theobromine | — | |
| Caffeine | — | |
| Diphenyl amine | — | Miscellaneous compounds |
| Benzoic acid | + | |
| Benzaldehyde | + | |
| Heptaldehyde | + | |
| Stearic acid | + | |
| Methyl propionate | — | |

[8] Shonle, H. A., *et al.*, *J. Pharm. and Exp. Therap.*, 1933, **49**, 393.

maxima exhibited by the 2 acids and by amytal are nearly the same. This may indicate that the colored substances formed are of similar structure in the 3 cases. This together with the fact that all 3 are acidic compounds with ionizable hydrogen atoms may be considered presumptive evidence for salt formation as the mechanism of this reaction.

The following scheme was employed in the development of the cobalt color for quantitative measurement. To 0.2 ml of 1% cobalt acetate in absolute methanol, in a colorimeter cup, are added in the following order: 0.6 ml of 5% isopropylamine in absolute methanol, 2.0 ml of chloroform, and 1.0 ml of the chloroform extract. The solutions were well mixed by rotation, and the intensity of the color produced read in terms of an arbitrary scale on a photoelectric colorimeter.

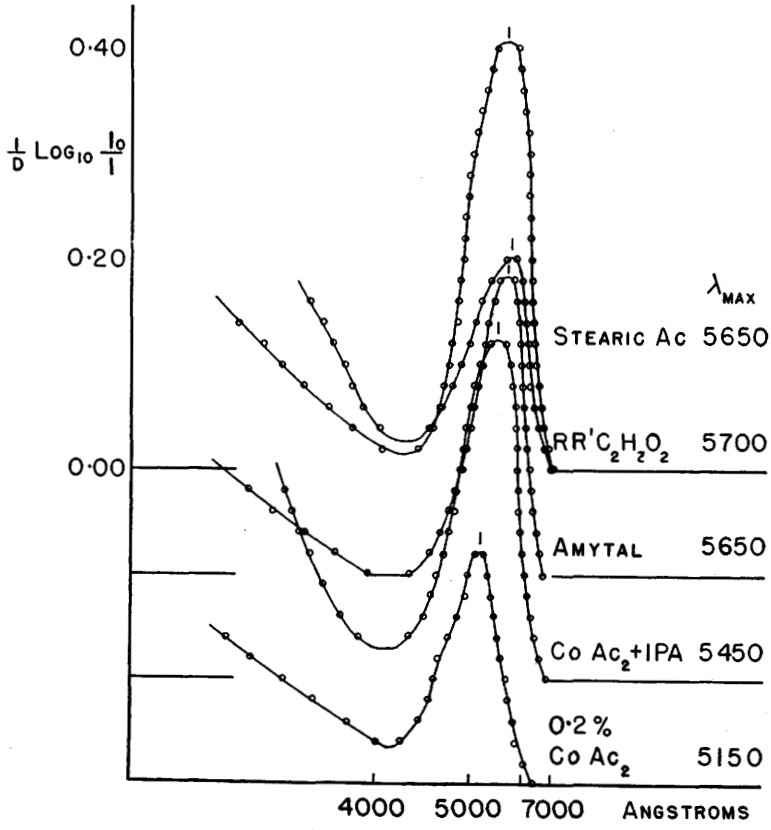


FIG. 1.

Spectrophotometric curves of cobalt acetate and cobalt acetate plus isopropylamine and these reagents plus amytal, isoamyl ethyl acetic acid ($RR^1C_2H_2O_2$), and stearic acid.

Using this technique, chloroform extracts of urine that contain no barbiturates have been examined. It was found that the developed color showed a maxima of 5600 and 5750 AU, for copper sulfate treated[6] and untreated urine, respectively. We have found that such extracts contain chromogenic substances[9] which may possibly be of acidic character. Inasmuch as these factors produce similar absorption spectra, and can give artifactual excretion values, results obtained using the cobalt acetate and isopropylamine test in the study of barbiturate excretion in urine must be considered cautiously and accepted with reservation.

## 11705

## Variations in Concentration of Certain Electrolytes of Blood Serum During Induced Hyperpyrexia.

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The remarkable constancy of the potassium content of the blood under pathologic conditions has been long recognized, but recent studies have brought forth evidence that this constancy may be disturbed greatly in cases of shock of various kinds. However, no uniformity of opinion has emerged from studies of the potassium fluctuations in shock. Different workers have reported different changes in the potassium content of the blood in cases of induced shock, and a variety of conclusions as to the role which such variations play has been drawn. The question whether variations in the blood potassium may be the primary cause of shock, and whether terminal shock may actually result from potassium poisoning caused by release of potassium from intracellular spaces has remained unanswered.[1-3] Furthermore, the theory that changes in the blood potassium level are not the cause of shock, but that such changes are incidental to alterations in blood volume has not been substantiated.

The present work was undertaken to ascertain what variations in the potassium level take place during shock caused by induced hyperpyrexia. This report includes experiments on patients who were

1 Scudder, J., Zwemer, R. L., and Truszkowski, R., *Surgery*, 1937, **1**, 74.

2 Scudder, J., Zwemer, R. L., and Whipple, A. O., *Ann. Surg.*, 1938, **107**, 161.

3 Bisgard, J. D., McIntyre, A. R., and Osheroff, W., *Surgery*, 1938, **4**, 528.