units) and rheumatic patients, active or inactive (250-200 units), of the same age group shows no significant difference. The average value in subjects with preceding streptococcus infections (500 units) was definitely higher.

The wide range of titres, from 20 to 1000 units, observed in both the control and rheumatic series indicates the importance of knowing the antistreptolysin level of each individual for a control period before attaching any significance to a single determination. High titres, 333 to 1000 units, were maintained over periods of from 5 to 9 months in normal and in rheumatic subjects who were apparently well. It is also of interest that higher values were obtained during the winter and spring months.

A significant rise in the antistreptolysin titre was frequently noted in rheumatic subjects, unaccompanied by rheumatic activity. Only 20% of the subjects who developed rheumatic fever during the period of observation exhibited a rise in titre above their level during the control period. The rise in titre was not directly related to type, severity or duration of the rheumatic activity.

The evidence presented in this study does not indicate that the antistreptolysin content of the serum of children with rheumatic fever is of specific etiologic significance.

7413 C

Quantitative Colorimetric Estimation of Morphine in Biological Fluids by the Iodoxybenzoate Method.*

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Development of a satisfactorily accurate quantitative application of the usual color reactions for morphine has been retarded because of their general lack of (a) proper selectivity for this single opium alkaloid, (b) permanence of hue produced or (c) sufficient sensitivity. In addition such reactions are in most instances dependent to an undesirable degree on the temperature and pH of the test solution and the presence of commonly occurring contaminants. This situa-

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tion is not surprising since most of these reactions are supposedly due to the formation of complex unstable chromogenic substances. A more ideal quantitative test should be the stoichiometric conversion of morphine to a simple colored form.

Leake[1] suggested that such an effect could be obtained for many substances by reacting their free phenolic groups with an aqueous solution of ammonium iodoxybenzoate, the oxidative powers of which had been studied in another regard by Loevenhart[2] and others. Morphine rapidly develops the same color when treated with this agent as is produced by slow spontaneous oxidation of morphine solutions in air, and it was recommended[1] that this reaction be used not only as a qualitative test to differentiate morphine from codeine, heroin, dionin and apomorphine, but also as the basis of a quantitative colorimetric estimation. We have found that when 25 to 50 mg. of powdered ammonium iodoxybenzoate are added to 5 to 10 cc. of 0.005 M to 0.02 M aqueous solutions of morphine salts sufficient agreement with Beer's Law holds so that colorimeter readings yield results accurate to $\pm 3\%$ if the comparison with a standard is made after 30 minutes are allowed for full development of the color. Spectroscopic examination of color absorption by the treated solutions reveals that in concentrations higher than 0.03 M extinction extends further toward the yellow band than with the lower concentrations, which is represented grossly by a change in shade from orange to garnet and considerable deviation in colorimetric measurements unless a suitable filter is used. In practice it is simpler to adjust the concentration of the unknown solution after one approximation, which also serves the purpose of a qualitative test, to about 0.01 M or 0.005 M and then compare it with a standard accurately made to 0.01 M or 0.005 M. The reaction is sufficiently sensitive to yield fair results down to 0.0015 M morphine solutions, but the error increases somewhat at the lower limits.

Estimations in serum and urine are not as satisfactory, the error amounting to $\pm 7\%$ and $\pm 5\%$ respectively. Trichloracetic acid filtrates may be used or extraction of ammoniacal solutions of serum or urine containing morphine salts with $CHCl_3$ and subsequent conversion of the extractive to a water-soluble salt may be carried out to permit a more accurate determination in distilled water. Sucrose and lactose do not interfere with the quantitative estimation, thus allowing direct determinations on syrups and solid preparations

[1] Leake, C. D., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 148.

[2] Loevenhart, A. S., and Grove, W. E., *J. Pharmacol. and Exp. Therap.*, 1911, **3**, 101.

contaminated with milk sugar. Semi-quantitative estimations may be carried out in oil solutions as well. The nature of the anion of the morphine salt has no influence on the color produced. Heavy metals precipitate iodoxybenzoate and so interfere with the test. The amount of iodoxybenzoate used has no effect on the intensity of the color produced, but maximum intensity is reached in a shorter time if larger amounts are used; excessive amounts cause a troublesome precipitate, however. Standards once prepared remain constant as to intensity of color for long periods. Attempts to concentrate the color in test solutions of concentrations less than 0.001 M morphine by precipitating it with $Al(OH)_3$, $Zn(OH)_2$ or kaolin have not been successful, and since the method is sensitive to less than 0.1 mg. of morphine per cc. no further attempts to increase the sensitivity have been made.

Since most of the illicit morphine confiscated in California is in the form of morphine hydrochloride, a rapid check accurate to $\pm 0.5\%$ can be made on the customary extraction-titration methods used routinely for such evidence by following the colorimetric morphine determination with a microchloride determination. If the chloride determination falls within the calculated value obtained to $\pm 3\%$ colorimetrically, it is safe to assume the corresponding amount of morphine is present to within $\pm 0.5\%$ because of the rarity of diluents having a similar Cl content which at the same time themselves react with iodoxybenzoate. The economy of time and amount of substance used in the test is a notable advantage over the older methods.

Summary. The qualitative iodoxybenzoate test of Leake has been found suitable for quantitative adaptation to the estimation of morphine. Methods for determining morphine in biological fluids and a rapid method of accurately checking the morphine content of morphine hydrochloride are suggested.