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Colorimetric Detection of Barbital and its Applications.

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Barbital and other barbituric acid derivatives are usually detected by recrystallization (Bachem[1]), and only few chemical tests for their identity have been described. Most of these tests are obviously crude and unsatisfactory, or at least not specific. One cannot test for the presence of barbital with Millon's reagent, Denigès' reagent, phenylhydrazine and sodium nitroprusside, or sulphuric acid and naphthol (Parri[2]). The murexide test of Handorf[3] is likewise not sufficiently specific. Parri,[2] Zwikker,[4] and Bodendorf[5] used cobaltous salts for the detection of barbiturates, but proved neither the specificity of their tests, nor did they attempt to make them sensitive beyond 1 part in 2000.

We devised the following test for the determination of barbiturates. We acidulate the unknown solution with hydrochloric acid, and shake with 10 volumes of chloroform in a separatory funnel. Six cc. of the chloroform extract are divided into 3 equal parts, A, B, and C. To A we add 0.05 cc. of a 1% solution of cobalt acetate in absolute methyl alcohol; to B, 0.1 cc.; and to C, 0.2 cc. of the same. This gives no color beyond a dilution of the original color of the cobalt acetate solution. Then to A we add 0.1 cc. of 1% barium hydroxide solution in absolute methyl alcohol; to B we add 0.2 cc., and to C 0.4 cc. of the same. If malonyl urea derivatives are present in the extract, a blue color always appears in A, may appear in B, or even in C, according to the amount of barbiturates contained therein. This step gives us an approximate idea of the concentration of barbiturates in the solution, for we have found that small amounts of barbital react only to small amounts of the reagents. If only A gives a blue color, the solution contains less than 0.25 mg. per cc.; if B also yields a blue color, the extract contains 0.5 mg. per cc. or less; if A, B, and C are blue, the extract contains 0.5 mg. per cc. or more.

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[1] Bachem, *Arch. Exp. Path. u. Pharm.*, 1910, **63**, 228.

[2] Parri, *Boll. Chim. Farm.*, 1924, **63**, 401.

[3] Handorf, *Z. f. ges. Exp. Med.*, 1922, **28**, 56.

[4] Zwikker, *Pharm. Weekblad*, 1931, **68**, 975.

[5] Bodendorf, *Arch. d. Pharm.*, 1932, **270**, 291.

The next step consists of matching the color produced by the unknown solution against the color produced by chloroform solutions of barbital of known strength in appropriate ranges. For example, if only A yields a blue color, we prepare 0.025%, 0.01%, and 0.005% solutions of barbital in chloroform and treat these samples in the same manner as sample A was tested, and match the color. This colorimetric test allows us a very accurate estimate of the barbital present in the extract. If we test in the ranges of samples B or C, that is, if B or both are positive, the procedure is the same, except that to the known strengths of barbital solution the same amounts of reagents are added as used in samples B or C respectively. For instance, if sample C also yields a blue color, we must match its color against that given by solutions of barbital in chloroform in concentrations of 0.5%, 0.2%, 0.1%, etc., again adding the same amounts of reagents as in C.

By means of this test we can detect barbital in chloroform extracts in concentrations of at least 1 in 20,000. This means that by concentrating the chloroform extracts we can actually discover the presence of barbital in body fluids in such small amounts as 0.05 mg. per cc. Care must be taken to exclude water in all procedures.

This test yields a blue color, *i. e.*, positive, only with barbital and other barbituric acid derivatives, but gives a negative, *i. e.*, pinkish, dirty yellow, or a green color, with every other compound. We have shown that both malonic acid and urea give negative tests, as do sulfonals, urethanes, uric acid, xanthine, adenine, cysteine, leucine, and allontoin. The sulphocyanates, and some other compounds, give a blue color with cobalt acetate which turns negative upon the addition of barium hydroxide. In short, this test is positive only with barbiturates.

Using this test, we attacked certain problems pertaining to the pharmacology of barbital. We found that barbital sodium following injection of doses of 300 mg. per kg. intravenously disappears from the blood stream in 6 minutes or less. After 3 minutes, only traces can be detected in the blood. We have also investigated the rate of excretion of barbital in the urine of normal dogs, and in dogs treated with diuretics.

Into each of 6 dogs we injected 375 mg. per kg. of sodium barbital intravenously. Three received no additional treatment. One of these dogs died in 12 hours, excreting 50 cc. of urine, which contained 500 mg. of barbital, or one-sixth of the total dose. The 2 remaining dogs recovered, excreting in 24 hours 1,230 mg., or 37%, and 2,100 mg., or 52%, respectively. In 48 hours they excreted

1,940 mg. and 3,060 mg., respectively, representing 58% and 76% of the total doses injected.

The other 3 dogs received, in addition to 375 mg. per kg. of sodium barbital by vein, a total dose of 2 cc. novasurol intramuscularly and 150 mg. per kg. of ammonium chloride subcutaneously. These injections produced profound diuresis in 2 dogs. The third died an hour after the administration; no diuresis had occurred, and 0.15% of the total injected dose was detected in the 23 cc. of urine produced. One dog lived 18 hours, in which time 7% of the total amount of barbital sodium injected was excreted. The last excreted 26% in 40 hours. In these experiments, diuretic measures contributed neither to the recovery of the animals nor to the excretion rate of barbital.

The study of the excretion of other malonyl ureas in the urine by this test and the application of the same in a clinical diagnosis of acute and chronic poisonings and in legal medicine will be reserved for the detailed communication.

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Preparation of a Purified and Highly Potent Extract of Growth Hormone of Anterior Pituitary Lobe.

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Since the demonstration by Long and Evans[1] of the growth hormone of the anterior pituitary, various workers have confirmed the existence in alkaline extracts of the anterior lobes of animals of a growth-promoting substance. Van Dyke and Wallen-Lawrence[2] have suggested the use of totally hypophysectomized animals as a test object for determining the potency of extracts. The latter also made definite progress in the preparation of potent extracts which were less crude than those previously used.

We have been successful in preparing highly potent extracts of the anterior lobe of oxen. These extracts have been tested for

[1] Long and Evans, *Anat. Rec.*, 1921, **21**, 62.

[2] Van Dyke and Wallen-Lawrence, *J. Pharmacol.*, 1930, **40**, 413.