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TEST FOR SULPHANILYL COMPOUNDS

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My present invention relates to a process for testing for sulphanilyl compounds in a body fluid, such as blood or urine. Sulphanilamide, sulphapyridine, sulphathiazol and sulphadiazine are prominent among a group of sulphanilyl derivatives which are widely used in medicine. It is well known that these drugs should be administered in quantities just sufficient to produce a predetermined optimum sulphanilyl concentration in the blood. Below the range of optimum concentration the drugs are ineffective, and above that range they may be very injurious.

Since the amount of the sulphanilyl compound absorbed by the blood of different individuals varies greatly, and also varies in the same individual with change of health, it is necessary to make frequent tests in order to maintain the desired optimum sulphanilyl concentration. It is therefore a main object of my present invention to provide a test of the type indicated which may be made by relatively unskilled persons, in a minimum of time, and without the aid of laboratory facilities.

A further object of my invention is the provision of reagents, for use in the aforementioned test, which may be provided in a compact dry form, in which form they retain full effectiveness for a relatively indefinite length of time.

Hitherto-known tests for sulphanilyl compounds have usually required the services of skilled technicians with well equipped laboratories at their disposal, partly on account of the instability of some of the reagents employed which have to be periodically freshly prepared.

Other proposed tests, using more stable reagents, are limited in accuracy by the requirement that the sulphanilyl determination be made by the comparison of a colored substance, formed in the tests, with a color chart, i. e., by reflection, in distinction to transmission, of light. Such comparison is necessary in those tests because of a cloudiness formed by certain of the reagents employed, which cloudiness precludes colorimetric comparison. Since the range of optimum sulphanilyl concentration is very narrow (for example, between 8 mg. and 12 mg. of sulphanilamide per 100 cc. of blood, according to some authorities) it is essential that the determination be made as accurately as possible. It is therefore another object of my invention to provide a test of the character described wherein the determination is made by colorimetric comparison of a transparent liquid, produced in the test, with a standard color solution.

Still further objects and advantages of my

invention will be found in the following description and the appended claims.

The general known procedure in conducting a test for a sulphanilyl compound in the blood (or in urine, in a slightly modified manner) is as follows: The proteins are precipitated from a diluted sample, the filtrate is treated with sodium nitrite to diazotize the sulphanilyl compound, the excess nitrous acid is removed by the aid of ammonium sulphamate, and the diazo compound is transformed into a colored compound (for comparison with a color standard) by the addition of a "coupling" reagent consisting of one of the following:

1. Dimethyl-a-naphthylamine,
2. 1-amino - 8 - naphthol-3,6-disulphonic acid ("H" acid),
3. N-(1-naphthyl)-ethylene-diamine dihydrochloride.

Known protein-precipitating reagents include: trichloroacetic acid, sodium hexametaphosphate, and a combination of molybdic and oxalic acids. I have discovered a very effective protein-precipitating agent that does not effect fading of the color produced by the sulphathiazol compound, which color is probably the least stable of the colors formed by the group. It is a combination of sulphosalicylic acid and another dry acid, preferably tartaric; talc being added for bulk to form a tablet, having the following composition, which may be called:

Reagent A (protein-precipitating)

	Mg.
Sulphosalicylic acid.....	50
Tartaric acid.....	50
Talc.....	100

As a diazotizing agent, I preferably employ sodium nitrite. From this I form a tablet, adding sodium chloride for bulk in the following proportions, which may be called:

Reagent B (diazotizing)

	Mg.
Sodium nitrite.....	0.3
Sodium chloride.....	30

For removing the excess nitrous acid formed from the sodium nitrite, it has been customary to employ ammonium sulphamate which breaks down the nitrous acid into nitrogen and water. However, in its place I preferably employ sulphamic acid in combination with another dry acid, since the reaction is then much more rapid; it being completed in a few seconds as compared with about three minutes with ammonium sulph-

amate. The tablet which I prepare consists of the following, and may be called:

Reagent C (nitrous-acid-removing)

Sulphamic acid.....	Mg. 5
Tartaric acid.....	0.5
	30

Coupling agents, for transforming the diazo compound into a colored compound, have been listed hereinabove. The first of these, dimethyl-a-naphthylamine, can be used only in liquid form and deteriorates very rapidly. The second, 1-amino-8-naphthol-3,6-disulphonic acid, works only in an alkaline medium and hence it is necessary to change the pH of the solution, which at this stage is normally acid. The third, and most effective coupling agent, N-(1-naphthyl)-ethylene-diamine dihydrochloride, has heretofore been employed in the laboratory with excellent results, fresh quantities of the agent having to be prepared every few weeks. Since a very small quantity of the coupling agent is required for each test, one could employ tablets containing a small amount of this agent together with other chemicals for bulk. But small quantities of this agent do not keep in tablet form and produce a cloudiness in the test, unless the tablet also contains a dry acid. I have discovered that the agent will retain its original properties relatively indefinitely (for over two years, in actual practice) if it is mixed with a dry acid, such as tartaric acid; no cloudiness then resulting from its use even when it is quite old. The tablet which I form, together with sodium chloride for bulk, consists of the following, and may be called:

Reagent D (coupling)

N-(1-naphthyl)-ethylene-diamine dihydrochloride.....	Mg. 40
Tartaric acid.....	0.3
Sodium chloride.....	1.5
	29

Test

Employing the reagents specified above, a test is made as follows: To 2 cc. of water in a test tube is added 0.1 cc. of blood. One tablet of

Reagent A is then added and the test tube shaken. This reagent, dissolving almost immediately, precipitates the blood proteins and produces the minimum pH necessary for subsequent reactions. After about 30 seconds the proteins (along with the talc) are filtered off, and to 1 cc. of the filtrate are added, in succession, one tablet of each of Reagents B, C and D, the test tube being shaken well after the addition of each tablet. The clear colored liquid resulting is then compared in a colorimeter with a standard color solution to determine the sulphanilyl content.

The term "dry acid," as employed in the description and claims in connection with the protein-precipitating and the coupling reagents, is intended to include any of the common dry acids which are stable in dry form, readily soluble in water, and colorless in solution, such as tartaric acid, citric acid, or oxalic acid.

I claim as my invention:

1. The process for testing for a sulphanilyl compound in a body fluid, which consists in: diluting a fixed quantity of said fluid, adding a protein-precipitating reagent, removing the precipitated proteins, adding to the fluid a diazotizing reagent, adding a nitrous-acid-removing reagent, transforming the resultant diazo compound into a colored compound by the addition of a dry mixture of N-(1-naphthyl)-ethylene-diamine dihydrochloride and a dry acid chosen from the group consisting of tartaric acid, citric acid and oxalic acid, and colorimetrically determining the color produced.

2. A stable coupling reagent for a determination of sulphanilyl compounds and effective in an acid solution, which consists of a dry mixture of N-(1-naphthyl)-ethylene-diamine dihydrochloride and a dry acid chosen from the group consisting of tartaric acid, citric acid and oxalic acid.

3. A stable coupling reagent for the determination of sulphanilyl compounds and effective in an acid solution, which consists of a tablet of N-(1-naphthyl)-ethylene-diamine dihydrochloride and tartaric acid.

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