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TEST FOR AMINO-BENZENE COMPOUNDS IN BODY FLUIDS

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It is the object of my invention to provide a simple, rapid, and convenient test for the presence in blood or in urine of amino-benzene chemotherapeutic compounds which contain the sulfanilyl group, which has a free amino group attached to a benzene ring; and one that can readily be made by a physician in a sick room without elaborate equipment, without heat, and without the need of other liquid than the blood or the urine itself and conveniently some water, which may be ordinary tap water.

These amino-benzene chemotherapeutic compounds are sulfanilamide and sulfapyridine, both of which contain the sulphanilyl group, and the many derivatives thereof which have been produced and which have varying degrees of effectiveness to combat infections. Both sulfanilamide and sulfapyridine contain a free amino group attached to a benzene ring, as that is inherent in the sulfanilyl group, and so do many if not all of their therapeutically effective derivatives.

These amino-benzene chemotherapeutic compounds, such as sulfanilamide and sulfapyridine, are recognized as tremendously important therapeutic agents. But it is essential for their effectiveness that their concentration in the blood of the patient be within certain limits. If their concentration in the blood is too low, they are without appreciable effect in combating the infection; while if such concentration is too high, they are also less effective in combating the disease, and in addition they then produce certain dangerous effects, both immediately and subsequently, such as acidosis, cyanosis, sulfhemoglobinemia, methemoglobinemia, fever, agranulocytosis, and anemia. Thus these amino-benzene chemotherapeutic agents have both a threshold blood-concentration and what may be called a safety-ceiling blood-concentration, and it is extremely important that the concentration of the amino-benzene chemotherapeutic agent in the blood be kept between those limits.

In addition, the determination of the concentration of these amino-benzene chemotherapeutic agents in the blood must be made promptly if it is to be of any value; for that concentration varies with considerable rapidity in a patient. Thus if the test were one which had to be sent away to a laboratory, for a report in a day or two, it would be practically useless. It is thus essential that the test be one which can be made by the physican himself in the sick room, or in the physician's office, in order that he may determine at once his course of action.

With sulfanilamide, for instance, it is the present consensus that the threshold dose is about 7 mg. per 100 cc. of blood, and that the safety-ceiling dose is about 12 mg. per 100 cc. of blood. If the concentration in the blood is less than about 7 mg. %, there is ordinarily no observable benefit; and if that concentration is above about 12 mg. %, the effect is markedly diminished and dangerous symptoms appear; while if the concentration in the blood is permitted to rise as high as 18 or 20 mg. %, there is almost always cyanosis, and even grave danger of death.

With sulfapyridine there is as yet considerable difference of opinion as to what the optimum range is; but there is absolute agreement among physicians that a concentration much in excess of the safety-ceiling concentration is dangerous. Probably the optimum concentration range for sulfapyridine has its threshold value somewhere between 4 and 10 mg. %, and its safety-ceiling value about 12 to 15 mg. %; with danger resulting if the concentration reaches 20 mg. %.

The situation is further complicated by the fact that the kidneys of the patient have some tendency and some ability to eliminate these amino-benzene chemotherapeutic agents; but this tendency and this ability vary markedly in different patients, and in addition many of the diseases being treated tend to impair the action of the kidneys to a greater or less extent. In addition, the rate of absorption of these amino-benzene chemotherapeutic agents from the intestinal tract varies considerably with different patients, so that it is not always (if ever) possible to predetermine accurately the concentration of the amino-benzene chemotherapeutic agent in the blood by the size of the dose given.

These are some of the reasons why it is highly desirable that there be a simple, rapid, and convenient test for these amino-benzene chemotherapeutic agents in the blood, and to a lesser extent for determining their presence in the urine.

My test can be applied to either blood or urine; and can be made readily by unskilled persons, with only a few drops of blood such as may be readily obtained from the patient's finger or ear lobe incidentally to other blood tests, as for red-corpuscle count or for hemoglobin determination, or with only a few drops of urine. In either case any kind of water may be used for dilution that may be available, such as tap water. Because the test is ordinarily more important as a blood test, rather than as a urine test, I shall describe it in the testing of blood.

In carrying out my invention, I proceed generally as follows:

I dilute approximately two drops of the patient's blood about 30 or 40 times with any available water, such as tap water. I precipitate the proteins present in this diluted blood; and, desirably but not necessarily in the same operation, diazotize the amino-benzene compound (such as sulfanilamide or sulfapyridine) present in the blood. For both the precipitation of the proteins and the diazotization, the diluted blood is acidulated to a pH at least as low as pH 4.5.

The acidulating agent is most conveniently glutamic acid hydrochloride, or (less desirably) oxalic acid, because they will give the desired acidity and may be carried in solid form; but, save for that convenience of availability in solid form, any sufficiently strong acid can be used, such as hydrochloric acid or sulfuric acid or acetic acid. The protein-precipitating agent is most conveniently "sodium hexametaphosphate," which is available on the market under the trademark "Calgon," because it is readily available and may be used in dry form; but in cases where that dry form is not deemed necessary, other protein-precipitating agents may be used, such for instance as trichloracetic acid, or sulfosalicylic acid, or a mixture of zinc sulfate and sodium hydroxide.

The diazotizing agent is most conveniently sodium nitrite; but other nitrites, such as potassium nitrite or ammonium nitrite, or even nitrous acid, may be used. To facilitate the precipitation of the proteins I deem it desirable to have also present a coagulating agent, such as talc.

All of this is done most conveniently by using the agents which are mentioned above as preferred, and putting them in a single capsule; which may be called Reagent A, and which contains the following:

	Mg.
Sodium nitrite	2
Sodium hexametaphosphate	50
Glutamic acid hydrochloride	30
Talc	120

With such a capsule (Reagent A), the desired protein precipitation and the diazotization may be obtained quickly, usually in about one minute, by simply dumping the contents of one capsule into the diluted blood above described.

The precipitated proteins are removed by filtration; and the clear filtrate containing the diazotized amino-benzene compound is then further treated, as follows:

The amount of sodium nitrite, or other diazotizing agent used, is an excess amount, so that there will certainly be enough of it present to diazotize all of the amino-benzene compound which the blood may contain. In consequence, there is some excess nitrous acid in the filtrate. This nitrous acid is removed by the addition of a sufficient quantity of ammonium sulfamate; of which the amount is not critical, as an excess does no harm. I find it convenient to add the ammonium sulfamate, which may be called Reagent B, in the form of a tablet containing 120 mg. of it. The ammonium sulfamate breaks down the nitrous acid into nitrogen and water, and the nitrogen escapes.

Then I add a definite amount of 1-amino-8-naphthol-3,6-disulfonic acid—which is the so-called "H acid" of the dye industry. A very small quantity of this is all that is required, and I actually use 1.5 mg. Because this is such a small amount, I preferably put it in a capsule with some diluting solids with which it does not react, and which need serve no other purpose although I prefer to include among them some which do play a part in facilitating the observation demanded by the test. This capsule may be called Reagent C, and contains the following:

	Mg.
1-amino-8-naphthol-3,6-disulfonic acid	1.5
Sodium sulfate	200
Milk sugar	20
Talc	30

It is simply necessary to dump the contents of this capsule into the clear filtrate after the addition of the ammonium sulfamate tablet.

On this dumping, with the filtrate acid in character, very little noticeable change occurs, although there may be a slight pink coloration. This pink coloration is not the test coloration.

The sodium sulfate in the capsule of Reagent C is a mere diluent; but the talc is to furnish a background for the color that is to be produced at the next step in the test, so that that color will be observable wholly by reflected light and not by transmitted light; and the milk sugar serves as a protective colloid to hold the talc in suspension.

Up to this point, the filtrate has been acid in character. At this point I make it alkaline, to a pH of between 8.5 and 9.5, by adding some suitable alkalinizing agent; which is preferably sodium (or potassium) carbonate monohydrate, because it is stable in solid form and is non-corrosive and non-deliquescent. To get the desired pH, with the proportions given above, I find that a tablet containing 180 mg. of sodium carbonate monohydrate is suitable. I may put nothing else in that tablet, but I prefer also to have a little milk sugar co-present, to make the tablet rapidly soluble; so that I preferably include in each of these tablets 60 mg. of milk sugar in addition to the 180 mg. of sodium carbonate monohydrate. This tablet may be called Reagent D.

Upon the addition of the tablet containing sodium carbonate, I shake the tube and contents vigorously. There develops at once, as the result of the alkalinization, the color which is dependent upon the amount of the amino-benzene compound that is present in the original blood.

Immediately upon the production of that color, it is compared with the colors on a standard color chart which is graduated in terms of milligrams of the amino-benzene compound (as for instance of sulfanilamide or sulfapyridine) present in 100 cc. of blood. The color steps on this color chart conveniently correspond to 2.5 mg. % units, starting with 0 and going at least as high as 15 mg. %. With such a color chart, the best results of medication with sulfanilamide are probably obtained when the color produced in the filtrate obtained from the blood sample is between a rather light pink which corresponds to 7.5 mg. % and a medium dark purple which corresponds to 12.5 mg. %. The colors of the color chart will vary for different amino-benzene compounds; for while all of those amino-benzene compounds produce much the same general range of colors they vary in the amount of the amino-benzene compound required to produce any given color, and they also may vary in the amount which gives the best results of medication. In addition, different physicians will have somewhat different ideas of optimum values for any given compound.

In the foregoing example I have given the procedure for testing the amount of an amino-benzene compound in a blood sample. The same procedure is used for testing the amount of such

an amino-benzene compound in a urine sample. The reading will show the amount of the unacetylated amino-benzene compound in the urine sample. The total amount of the amino-benzene compound in the urine sample, acetylated and unacetylated, may if desired be determined, although it usually takes more time than is allowable. To do that, the urine sample is hydrolyzed before the test procedure starts, as by making the diluted urine barely acid to litmus and then boiling it for about half an hour, to convert any acetylated amino-benzene compound to the unacetylated form; and then the test is performed as outlined above.

A quantitative example of making my test on blood is as follows:

Example

0.15 cc. (approximately two drops) of the patient's blood, obtained from his finger, is diluted to 6 cc., with tap water; conveniently in a test tube marked at 6 cc. and 4 cc. levels. Then the contents of one capsule of Reagent A are emptied into the test tube, and the whole is stirred and then allowed to stand for one minute; whereupon the contents of the test tube are filtered to remove the protein which has been precipitated, and the filtrate is further treated. For that further treatment, I use 4 cc. of the filtrate; and to it add one tablet of Reagent B, which is ammonium sulfamate. Then, after about a minute, I add the contents of one capsule of Reagent C and one tablet of Reagent D, adding Reagents C and D in either order or simultaneously if desired. I at once shake the tube vigorously, and immediately compare the color produced with the colors of the standard color chart.

This test can usually be made in less than 5 minutes; and gives the physician immediate information as to the content of the amino-benzene compound in the blood so that he may shape his further treatment in accordance with the indication which the color gives.

I claim as my invention:

1. The process for testing for a sulfanilyl compound in a body fluid of the class consisting of blood and urine, which consists in diluting a fixed quantity of the body fluid, adding to the diluted body fluid a protein-precipitating reagent and a diazotizing reagent and removing the protein which is precipitated, neutralizing any excess nitrous acid in the filtrate by adding ammonium sulfamate, then adding to the filtrate enough of an alkalinizing agent to produce a pH between 8.5 and 9.5 and a predetermined amount of 1-amino-8-naphthol-3,6-disulfonic acid; to produce a color which by comparison with a color chart indicates the quantity of the sulfanilyl compound in the body fluid.

2. The process for testing for a sulfanilyl compound in a body fluid as set forth in claim 1, in which the diazotizing agent is sodium nitrite.

3. The process for testing for a sulfanilyl compound in a body fluid as set forth in claim 1, in which the protein-precipitating reagent is sodium hexametaphosphate and an acidifying agent.

4. The process for testing for a sulfanilyl compound in a body fluid as set forth in claim 1, in which the protein-precipitating reagent is sodium hexametaphosphate and glutamic acid hydrochloride.

5. The process for testing for a sulfanilyl compound in a body fluid as set forth in claim 1, in which in addition to the 1-amino-8-naphthol-3,6-disulfonic acid milk sugar and talc are added to the filtrate.

6. The process for testing for a sulfanilyl compound in a body fluid as set forth in claim 1, in which the alkalinizing agent is sodium carbonate monohydrate.

7. A sulfanilyl-coupler-reaction unit, effective in alkaline solution, which reaction unit contains 1-amino-8-naphthol - 3,6 - disulfonic acid, milk sugar, and talc.

8. A combined diazotant and protein-precipitant reaction unit, which reaction unit contains a nitrite, sodium hexametaphosphate, and a solid-form acid sufficient to produce an acidity as great as pH 4.5.

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