filtered through a Berkefeld (W) candle to remove bacterial bodies, and then injected intravenously into the dog. The results in Table I show that the acacia after bacterial growth becomes "reactive".

Conclusions. 1. Acacia *per se* is not "reactive" or pyrogenic. 2. Commercial acacia prepared for intravenous use may be divided into 2 categories, reactive and non-reactive. 3. The growth in acacia of *B. subtilis* and 2 water organisms, all pyrogen-producers, changes non-reactive acacia into reactive acacia. 4. The febrile agent in reactive acacia is probably pyrogen.

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Formation of Peroxide and a Reversible Oxidation-Reduction in Solutions of Sulfanilamide.

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In accordance with a previous concept[1] of the mechanism of sulfhemoglobin formation I investigated whether sulfanilamide which often causes sulfhemoglobinemia forms H_2O_2 in presence of molecular oxygen. Aqueous solutions of sulfanilamide were prepared in different concentrations. The phenolphthalin reagent and, as a catalyst, copper sulfate, both as described by Schales,[2] were added. Oxygen was bubbled through the solutions. Parallel controls contained distilled water instead of sulfanilamide solution. The development of a red color resulting from the oxidation of phenolphthalin into phenolphthalein under the given conditions is specific for the presence, which implies the formation of hydrogen peroxide or labile peroxides. Concentrations of H_2O_2 as low as $1:10^8$ are detectable by means of this method.[2] In solutions containing sulfanilamide in a concentration of 250 mg per 100 cc and more the formation of H_2O_2 was regularly found. In lower concentrations, *e. g.*, 100 mg per 100 cc, no convincing specific effect was detectable.

From the failure to find H_2O_2 in the less concentrated solutions one could conclude that possibly not the sulfanilamide itself but rather

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[1] Barkan, G., and Schales, O., *Hoppe-Seyler's Z. f. physiol. Chemie*, 1938, **254**, 241; **253**, 83; 1937, **248**, 96; Barkan, G., "*Kongressbericht II*" *des XVI Internat. Physiol. Kongresses*, Zürich (Schweiz), 1938, 250.

[2] Schales, O., *Berichte Dtsch. Chem. Gesellsch.*, 1938, **71**, 447.

some other substance accompanying the drug as an impurity might be responsible for the H_2O_2 formation as described above. Two facts make such a presumption improbable but do not entirely exclude it. (1) There could not be found any characteristic differences in the behavior of different preparations of sulfanilamide. (2) Neither could there be found any shift in the limit after purifying the drug by recrystallization.

Thus the formation of H_2O_2, although under the given experimental conditions in a rather slight amount, seems to be due to the sulfanilamide itself. That makes it likely that *in vivo* by means of any catalyst present, H_2O_2 can be formed from an oxidation of sulfanilamide. An anticatalase activity of sulfanilamide or one of its oxidation products was claimed[3] to be responsible for an accumulation of H_2O_2 formed by streptococci and pneumococci. Hydrogen peroxide formation quite independently of the presence of bacteria may explain the increase of the oxidation-reduction potential which was noticed not only in streptococcic cultures[4, 5] but also in sterile broth[5] in presence of sulfanilamide. H_2O_2 formation from the drug itself may take part also in the mechanism of both the chemotherapeutic and toxicologic actions of sulfanilamide.

Aside from the H_2O_2 formation there was found another rather striking phenomenon. In all solutions containing sulfanilamide, phenolphthalin reagent and copper, the reddish pink color of the alkaline phenolphthalein as developed by H_2O_2 changed more and more into violet-blue. At first within ½ to 1 hour there is only a slight violet shade. The violet becomes more intense and after some hours, certainly on standing over night, usually a nearly pure blue color is formed. In no one of the tests containing sulfanilamide was the slow development of the violet-blue color in the previously red solutions missing. No corresponding change in the color of phenolphthalein was noticed if sulfanilamide was absent.

After cautiously adding small amounts of $Na_2S_2O_4$ the blue color disappears and a pure reddish-pink is formed again. Larger amounts of the reducing substance destroy the color throughout leaving a colorless or slightly yellow-brown solution.

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[3] Locke, A., Main, E. R., and Mellon, R. R., *Science*, 1938, **88**, 621; *Immunol.*, 1939, **36**, 183; Main, E. R., Shinn, L. E., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 272; Shinn, L. E., Main, E. R., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 591; 1939, **40**, 640.

[4] Fox, C. L., Jr., German, B., and Janeway, C. A., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 184.

[5] Warren, J., Street, J. A., and Stokinger, H. E., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 208.

By shaking with air, the reddish color is transformed within a few minutes into the previous violet-blue one. By adding again a small amount of $Na_2S_2O_4$ one can repeat the color change several times, always getting at first the red by reduction and then the blue by oxidation. After repeated transformations in both directions, however, mixed colors occur instead of the pure red and blue.

Using ascorbic acid as a reducing substance the same phenomena were observed; other reductants were less suitable. The reoxidation by shaking the reduced solutions with air seems to be a true autoöxidation. However, one must consider that both $Na_2S_2O_4$ and ascorbic acid belong to those reducing substances which may react with molecular oxygen forming H_2O_2.[2] It is not yet proved, but not excluded that the slow formation of the blue-colored substance on standing as mentioned above, may also be due to the influence of nascent H_2O_2. There must be supposed some interaction of phenolphthalein, since sulfanilamide solutions even in presence of copper, under the same conditions do not change their color. On the other hand it is at least probable that the violet-blue colored substance is a similar if not the identical derivative as formed from sulfanilamide by ultraviolet irradiation in presence of oxygen.[6]

Even in the deep blue solutions the red phenolphthalein ions are still present. One can see spectroscopically the characteristic absorption band of phenolphthalein with the middle at about 554.7 mμ. No other distinct absorption band is noticeable in the visible part of the spectrum, but a general absorption can be observed disappearing at once when after reduction the pure reddish color has come back.

On acidifying the reduced solutions after the red has reappeared the phenolphthalein as usual becomes colorless. By adding Na_2CO_3 solution cautiously while shaking slightly the blue color reappears almost instantaneously. The influence of pH upon the "autooxidation" and the mechanism of this process together with the peroxide formation is under further investigation.

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[6] Ottenberg, R., and Fox, C. L., Jr., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 479.