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## Influence of Fasting on Retention and Conjugation of Sulfanilamide in Rabbits.

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It has been shown[1] that rats maintained on a low protein diet exhibited higher drug blood levels when treated with sulfanilamide than rats treated similarly but receiving adequate protein in their diet. In like manner therapeutic tests with sulfanilamide in mice with streptococcus infection showed the drug to be more effective in animals maintained on a low protein diet, and the degree of effectiveness appeared to be related to the blood concentration of the drug.[2] Since the food intake is likely to be much curtailed in animals restricted to low protein diets, it seemed desirable to ascertain the fate of sulfanilamide under fasting conditions.

Rabbits were selected for these experiments for the following reasons: (1) It was desirable to have an animal large enough to be suitable for frequent bleedings; (2) the rabbit is suitable for frequent catheterization so that the excretion curve could be followed; and (3) normally, sulfanilamide is acetylated in the rabbit to a very high extent and with considerable uniformity.

*Procedure.* The rabbits were catheterized and 0.5 g per kilo sulfanilamide suspended in about 25 cc 5% gum acacia was administered by stomach tube. The drug was washed in with about 100 cc water to insure moderate diuresis. Small blood samples were taken from the ear vein for sulfanilamide determinations at intervals of 1, 2, 4, 6, and 24 hours or longer if necessary. Urine was collected at 24-hour intervals up to 72 hours when necessary to determine the excretion rate of the drug. The method of Bratton and Marshall[3] was used for the colorimetric determination of free and total sulfanilamide.* The animals were on a diet of oats and cabbage for at least a week prior to the fasting period, during which time water alone was permitted.

*Results.* In the normal non-fasting rabbit the peak blood con-

[1] Smith, M. I., Lillie, R. D., and Stohlman, E. F., *Public Health Rep.*, 1941, **56**, 24.

[2] Rosenthal, S. M., *Public Health Rep.*, 1941, **56**, 188.

[3] Bratton, A. C., and Marshall, E. K., *J. Biol. Chem.*, 1939, **128**, 537.

* In some of the experiments the method of A. S. Giordano and M. C. Prestrud (*Am. J. Clin. Path.*, 1940, **10**, 88) was used.

TABLE I.
Absorption, Conjugation, and Excretion of Sulfanilamide in Rabbits Under Diverse Experimental Conditions. Dose—0.5 g per kg orally.

| Experimental condition | No. | Maximum blood level | | Urinary excretion in 24 hours | | |
|---|---|---|---|---|---|---|
| | | Mg% | Conjugation % | % of dose | Conjugation % | Urine, cc/hr |
| Normal | 2 | 41 | 79 | 75 | 90 | 9.8 |
| " | 3 | 45 | 88 | 89 | 91 | 8.5 |
| " | 25 | 50 | 59 | 90 | 83 | 11.8 |
| " | 55 | 38 | 79 | 70 | 85 | 10.4 |
| Avg | | 44 | 76 | 81 | 87 | 10.1 |
| Fasting, 3 days | 16 | 54 | 60 | 75 | 70 | 8.2 |
| " 7 " | 17 | 92 | 54 | 70 | 44 | 13.0 |
| " 9 " | 18 | 45 | 27 | 65 | 53 | 8.7 |
| " 14 " | 19 | 55 | 33 | 50 | 45 | 8.0 |
| Avg | | 61 | 43 | 65 | 53 | 9.5 |
| Carbon tetrachloride | 42 | 32 | 51 | 96 | 81 | 13.0 |
| " " | 43 | 41 | 46 | 71 | 75 | 6.3 |
| " " | 44 | 40 | 63 | 95 | 73 | 11.1 |
| Avg | | 37 | 53 | 87 | 76 | 10.1 |
| *Acidosis* | | | | | | |
| Oats diet, 9 days | 23 | 41 | 57 | 72 | 52 | 5.3 |
| " " 26 " | 26 | 31 | 69 | 84 | 79 | 10.9 |
| " " 28 " | 27 | 30 | 57 | 46 | 49 | 7.2 |
| $NH_4Cl$, 1 g per kg orally | 33 | 23 | 82 | 60 | 89 | 9.6 |
| " 0.5 " " " " | 34 | 29 | 68 | 70 | 88 | 12.6 |
| $NaH_2PO_4$, 1 " " " " | 64 | 30 | 30 | 51 | 81 | 8.3 |
| " 1 " " " " | 65 | 25 | 28 | 70 | 80 | 7.7 |
| " 1 " " " i.v. | 66 | 20 | 51 | 79 | 78 | 18.5 |
| " 1 " " " " | 68 | 27 | 55 | 61 | 81 | 11.2 |
| Avg | | 28 | 55 | 66 | 75 | 10.1 |

centration of sulfanilamide under these conditions was reached in 2 to 4 hours, with an average of 76% acetylation. The elimination of sulfanilamide in the urine during the first 24 hours was on an average 81% of the dose administered, and 87% of this was acetylated. The urine output, which varied in these experiments from 8.5 to 11.8 cc per hour, seemed to bear no relationship either to the excretion or the acetylation of the drug. There was usually little or no sulfanilamide in the blood at the end of 24 hours. This is shown in the first part of Table I.

The data given in Table II show generally higher blood levels, decreased rate of conjugation and an increasing rate of retention of the drug with progressive fasting. Thus after a fast of 18 hours the free sulfanilamide was only 12% of the total in the blood 6 hours

TABLE II.
Effect of Fasting on Blood Concentration and Conjugation of Sulfanilamide in Rabbits. Dose 0.5 g per kg orally.

| Hrs after administration | Blood sulfanilamide concentration T = total, mg%; F = free, % of total | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 18 hrs | | 3 days | | 7 days | | 9 days | | 14 days | |
| | T | F | T | F | T | F | T | F | T | F |
| 1 | 19 | 80 | 17 | 68 | 50 | 67 | 30 | 73 | 50 | 67 |
| 2 | 44 | 49 | 36 | 40 | 60 | 74 | 50 | 64 | 55 | 79 |
| 4 | 45 | 21 | 42 | 55 | 92 | 46 | 43 | 81 | 44 | 77 |
| 6 | 43 | 12 | 54 | 40 | 80 | 72 | 45 | 73 | 43 | 80 |
| 24 | 4 | 0 | 22 | 1 | 45 | 74 | 27 | 74 | 33 | 76 |
| 48 | 0 | 0 | 7 | 0 | 17 | 59 | 10 | 69 | 18 | 72 |
| 72 | 0 | 0 | 0 | 0 | 5 | 58 | 10 | 45 | 12 | 63 |

after administration of 0.5 gm per kilo; while after a 14-day fast, it was 80% of the total. After a 14-day fast, the blood level 24 hours after the administration of the drug was 33 mg %, 76% of which was free; while after an 18-hour fast, the blood level at this time was only 4 mg %, and all of this was conjugated. In an animal fasting several days there may still be considerable sulfanilamide in the blood as long as 72 hours after its administration. In like manner the excretion of the drug is nearly complete in 24 hours in the normal animal but may not be more than 50% of the dose administered in the fasting animal at this time (Part 2, Table I).

The present experiments confirm and extend the earlier observations.[1] A satisfactory explanation for these findings is not at hand, though several possibilities suggest themselves. The possibility of impaired hepatic or renal function in prolonged fasting cannot be excluded, though this does not seem probable. A direct approach to this was made by administering sulfanilamide in the usual manner to rabbits that had been previously treated for a week with daily doses of 1 cc per kilo of carbon tetrachloride. The fate of sulfanilamide in such animals was not materially different from normal, as may be seen in Part 3 of Table I, except possibly for some slight reduction in the rate of conjugation. This is consistent with the reported site of sulfanilamide conjugation in the liver of the rabbit.[4, 5] The urine output in our fasting animals during sulfanilamide treatment was within normal limits.

The possibility of a state of acidosis developing in the course of fasting was also considered. With this in view the fate of sulfanilamide was examined in a series of rabbits on an exclusive oats diet, which is acid-producing, and also in animals receiving ammonium

[4] Stewart, J. D., Rourke, G. M., and Allen, J. G., *Surgery*, 1939, **5**, 232.
[5] Van Winkle, W., and Cutting, W. C., *J. Pharm. and Exp. Ther.*, 1940, **69**, 40.

chloride or sodium acid phosphate simultaneously with the sulfanilamide. These experiments indeed showed a reduced rate of conjugation in the blood and a somewhat decreased rate of elimination of the drug as compared with controls, as shown in Part 4 of Table I. The effects, however, were not as pronounced as in fasting. The lower peak in the blood under these conditions as compared with controls suggests a reduction in the rate of absorption of the drug. Obviously more work is needed to elucidate the effects of fasting or low protein diet on the fate of sulfanilamide in the body.

*Summary.* Prolonged fasting in the rabbit favors the absorption and retention of sulfanilamide, producing higher blood levels of the drug and over a longer period of time as compared to normally fed animals. This also favors reduction of acetylation of the drug. An acid producing diet or the administration of drugs favoring a state of acidosis appears to have an effect on the fate of sulfanilamide similar to that of fasting.

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## Action of Phlorizin on Acid Phosphatase Activity and on Glucose Phosphorylation of Kidney Cortex Extracts.*

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1. *Phosphatase Experiments.* Several authors[1, 2, 3] have reported that phlorizin does not appreciably affect the phosphatase activity of kidney extracts. In all cases their findings are apparently concerned with the alkaline phosphatase, since the determinations were made within the pH range of 7.6 to 9.2. It was thought of interest to determine the effects of phlorizin on the acid phosphatase activities of kidney cortex and intestinal mucosa extracts.

The filtered extracts were prepared as described by Kay,[4] brought

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1 Lambrechts, A., *Arch. intern. physiol.*, 1937, **44** Suppl., 136.

2 Walker, A. M., and Hudson, C. L., *Am. J. Physiol.*, 1937, **118**, 130.

3 Kritzler, R. A., and Gutman, A. B., *Am. J. Physiol.*, 1941, **134**, 94.

4 Kay, H. D., *Biochem. J.*, 1928, **22**, 855.