drugs maintained body weight, but those which received either drug alone lost weight.

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## Effect of Feeding Several Sulfonamides and Related Compounds on Growth of Chicks.* (20274)

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Stimulation of animal growth has been observed when certain non-absorbable sulfonamides, such as sulfasuxidine or sulfathalidine are fed continuously in the diet in substantial concentrations(1-6). However, the feeding of similar concentrations of absorbable sulfonamides has been found to have a deleterious or growth depressing effect(7). It was of interest, therefore, to ascertain the growth effect brought about by feeding high dietary concentrations of Gantrisin®,† (sulfisoxazole), a soluble, readily absorbable sulfonamide of low toxicity(8-10) and the sparingly soluble, non-absorbable γ-keto sulfones of the intestinal antiseptic type, Ro 2-0201‡ and Ro 2-0404 (11).§ Sulfamethazine, sulfadiazine, sulfaguanidine, sulfanilamide and sulfaquinoxaline were included for comparison. Because of its sensitivity to the neuropathological effects of sulfonamides(12) and its suitability for growth studies, the chick was chosen as the test animal.

*Procedure.* Day-old straight run sexed single comb White Leghorn chicks‖ were placed on raised wire floors in electrically heated brooders and were maintained on the basal ration for 3 days. The basal ration used was an antibiotic-free¶ commercial chick starting mash. After 3 days acclimatization on the basic diet, all runts and giants were discarded, the birds were wing banded and grouped into equally weighted groups of 20, containing equal numbers of males and females. The groups of birds were then placed on the experimental rations and maintained so in the brooders to the age of 4 weeks, after which time they were transferred to growing cages where they remained until the termination of the experiment. All birds were weighed weekly. Water and feed were provided *ad libitum*. The lights were controlled to keep the room well illuminated between the hours of 5 a.m.

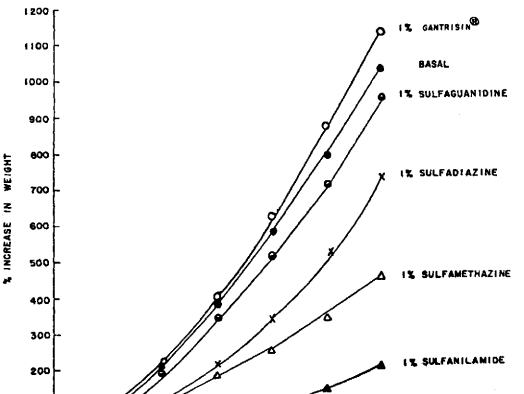


FIG. 1. Rate of increase in growth of chicks fed diets containing 1% sulfonamide.

* Roche Publication No. 344.

† 3,4-Dimethyl-5-sulfanilamido isoxazole.

‡ 4-Phenyl-4-sulfanilyl-2-butanone

§ β-Phenyl-β-sulfanilyl-propiophenone.

‖ Obtained from Kerr Chickeries, Frenchtown, N. J.

¶ "Start and Grow" chick mash, manufactured by GLF Feed Co-Op Mills.

TABLE I. Relative Weight Responses of White Leghorn Chicks at 6 Weeks when Fed Sulfonamides Continuously in the Diet.

| Compound | % conc. in diet | Avg wt ± S.E.* | % deviation from control† | Exp. No. |
|---|---|---|---|---|
| Sulfisoxazole‡ | 1 | 460 ± 9 | +12 | 8 |
| | .3 | 437 ± 6 | + 6 | 8 |
| | .1 | 477 ± 10 | +12 | 7 |
| | .1 | 448 ± 6 | + 9 | 8 |
| | .1 | 543 ± 9 | + 6 | 9 |
| | .03 | 447 ± 5 | + 5 | 7 |
| | .03 | 551 ± 5 | + 7 | 9 |
| | .01 | 465 ± 12 | + 9 | 7 |
| | .01 | 520 ± 11 | + 1 | 9 |
| Sulfaquinoxaline | .3 | 206 ± 11 | —60 | 9 |
| | .1 | 421 ± 8 | —18 | 9 |
| | .05 | 387 ± 8 | — 6 | 8 |
| | .025 | 391 ± 10 | — 5 | 8 |
| | .0125 | 493 ± 8 | — 4 | 9 |
| Sulfanilamide | 1 | 108 ± 10 | —79 | 9 |
| | .3 | 350 ± 12 | —32 | 9 |
| | .1 | 423 | — 1 | 7 |
| | .03 | 427 | 0 | 7 |
| | .01 | 435 | + 2 | 7 |
| Sulfamethazine | 1 | 181 ± 16 | —56 | 8 |
| | .03 | 325 ± 9 | —21 | 8 |
| | .1 | 493 ± 9 | — 4 | 9 |
| | .03 | 473 ± 8 | — 8 | 9 |
| | .0125 | 509 ± 10 | — 1 | 9 |
| Sulfadiazine | 1 | 350 ± 9 | —32 | 9 |
| | .3 | 463 ± 14 | —10 | 9 |
| | .1 | 524 | + 2 | 7 |
| | .03 | 499 | — 3 | 7 |
| Sulfaguanidine | 1 | 371 ± 11 | —10 | 8 |
| | .3 | 404 ± 9 | — 2 | 8 |

* Stand. error of mean: $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$.

† Avg wt of control groups

| | g | S.E.* |
|---|---|---|
| Exp. 7 | 427 | ±10 |
| 8 | 412 | ± 9 |
| 9 | 514 | ±12 |

‡ Gantrisin ®

and 9 p.m. Humidity and temperature varied with the weather and the temperature ranged from 22 to 28°C. The experimental diets were made by mixing thoroughly the desired amount of a compound into the basal diet. All compounds were employed as the commercially available free form of the drug.

*Results.* The rates of increase in weight of birds fed diets containing 1% of the sulfonamide are shown in Fig. 1. It is interesting to observe from these data that Gantrisin® effects a slight enhancement in growth over the control whereas sulfanilamide, sulfamethazine, sulfadiazine and sulfaguanidine depress growth. After 6 weeks of continuous feeding of 1% of the sulfonamide in the diet, the birds getting Gantrisin® gained 12% in weight over the drug free control, whereas birds getting sulfanilamide showed a growth repression of 79% and those getting sulfamethazine showed a growth repression of 56%. Birds fed 1% sulfadiazine and 1% sulfaguanidine showed growth repressions of 32% and 10% respectively. With diets containing 0.3% of drug, Gantrisin® still exerts a small growth enhancing effect. The growth depressing effects of sulfaguanidine and sulfadiazine are considerably reduced whereas sulfanilamide and sulfamethazine are still markedly inhibitory to growth. Of the compounds examined in this series, sulfaquinoxaline was the most inhibitory to growth.

TABLE II. Growth Response Obtained on Adding Ro 2-0201* or Ro 2-0404† to Basal Ration.

| Compound | % conc. in diet | Total wt at 5 wk ± S.E. | % gain over control |
|---|---|---|---|
| | Basal | 436 ± 9 | — |
| Ro 2-0404 | .03 | 471 ± 8 | 8 |
| | .1 | 500 ± 7 | 15 |
| Ro 2-0201 | .03 | 498 ± 7 | 14 |
| | .1 | 488 ± 8 | 12 |
| | .3 | 489 ± 8 | 12 |

* 4-phenyl-4-sulfanilyl-2-butanone.
† β-phenyl-β-sulfanilyl-propiophenone.

It was of interest to ascertain if any of the compounds tested might be stimulatory to growth at markedly reduced dietary concentrations. It may be seen from the data in Table I that a stimulation of growth was not observed when sulfamethazine, sulfadiazine or sulfaquinoxaline were fed at dietary levels as low as .01% and sulfadiazine as low as .03%.

Statistically significant growth stimulation was obtained when small amounts of Ro 2-0404 and Ro 2-0201 were added to the basal ration. These results may be seen in Table II. It is of interest to note that Ro 2-0201 exerts a maximum stimulatory effect at a diet level of .03% and maintains this effect at levels of up to 10 times this concentration. Diet concentrations lower than .03% were not tested.

*Discussion.* In our experiments, no evidence of drug toxicity was observed when Gantrisin® (sulfisoxazole) was fed continuously for 6 weeks at high diet concentration to young chicks. In fact, Gantrisin® was observed to produce a stimulation of growth when added to the diet in concentrations ranging from 0.03% to 1%. These findings stand in marked contrast to the growth depressing effects observed when sulfamethazine, sulfadiazine, sulfanilamide, sulfaguanidine, or sulfaquinoxaline were fed to chicks at comparable or even markedly reduced levels, and no growth stimulation was observed to occur with any of the above compounds at any level tested.

The toxicity of sulfamethazine for the chick has also been shown by Asplin *et al.*(13), Levine and Barber(14), and Waletzky and Hughes(15). The toxic effects resulting from the continuous administration of sulfadiazine, sulfanilamide or sulfaguanidine are well known(7,16,17). Our findings are in agreement with the observations(18) that sulfaquinoxaline is relatively non-toxic when fed continuously in the diet at a concentration of 0.03% but higher concentrations may repress growth.

The growth-stimulating activity of low diet concentrations of Ro 2-0201 and Ro 2-0404 is of considerable interest since these compounds are quite insoluble and are only very slightly, if at all, absorbed from the intestinal tract. It has been shown(19) that in spite of their low solubility, these compounds exert a definite anti-coli effect on the intestinal microbial population of the mouse. From the data obtained in our experiments it would seem that the presence of these compounds in the diet has favored the development of an intestinal flora highly favorable to the chick.

*Summary.* 1. Young chicks fed diets containing Gantrisin® (sulfisoxazole) at levels of up to 1% of the diet showed no physical abnormalities, were more uniform and thrifty in weight and appearance than the controls and weighed heavier than the controls at 6 weeks of age. 2. Young chicks fed diets containing .03% of Ro 2-0404 or from .03 to 0.3% of Ro2-0201 were also thriftier in appearance and heavier than the controls and showed no physical abnormalities. Young chicks fed diets containing 1% or 0.3% of sulfamethazine, sulfadiazine, sulfanilamide, sulfaguanidine or sulfaquinoxaline were markedly retarded in their growth.

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## Non-Protein Nitrogen Changes in Serum and Plasma of Rats Following Thermal Injury.* (20275)

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Disturbances in nitrogen metabolism are commonly observed after severe burns. Azotemia is one of the principal features of the metabolic changes. In general, the distribution of the elevated blood non-protein nitrogen is normal, but, occasionally, in patients(1,2) and rats(3,4) with very extensive deep burns, a large part (as much as 80%) of the azotemia is due to abnormal amounts of "residual" nitrogen whose nature has not been determined. Duval *et al.*(5) and Lambret *et al.*(6) postulated that severe burns result in a polypeptidemia. They based their conclusions on an observed increase of that amino nitrogen which is soluble in trichloracetic acid, but precipitated by phosphotungstic acid. Christensen(7) has presented evidence which casts doubt on the reliability of such determinations and interpretations.

In the present investigation, ultrafiltrates of plasma and serum from normal and thermally injured rats were utilized in a study of the non-protein nitrogen changes. In addition to the routine chemical measurements, absorption spectra in the ultraviolet were obtained, and changes of individual amino compounds were followed by ion exchange chromatography.

*Methods.* A. *Thermal technic.* Male albino rats of the Wistar strain, weighing between 225 and 275 g were anesthetized with ether and thermally injured deeply over about 30% of the body surface by dipping their backs in hot water (90°C) for 35 seconds(8). Rosenthal and McCarthy(4) have found that the increase in plasma residual nitrogen in similarly injured rats is at a maximum 10 to 12 hours after trauma. Accordingly, in the present study 11 to 12 hours after injury, the surviving rats (80% of those injured) were sacrificed, and their blood collected by heart punctures. Control rats, anesthetized but not burned, were studied simultaneously. Purina chow and water were offered *ad libitum* to all rats. B. *Chemical and spectrophotometric measurements.* Bloods from 16 injured rats

* The experimental work was done at the Medical College of Virginia.