ship of MSH and ACTH was discussed. The adrenotropic tumor-bearing animal should be advantageous in the study of the action of MSH in the mammal.

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## Effect of Magnesium Ion on Glycogen Fraction Synthesis in Rat Tissues.* (22489)

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Magnesium is intimately associated with carbohydrate metabolism through its function as an activator in enzymatic processes involving phosphatase(1). However, opposing views exist regarding the promotion of glycogen synthesis by magnesium. Injection of Mg salts was claimed by Franke(2) to promote hepatic glycogenesis, but according to Peters and Van Slyke(3) the data have not been confirmed and are not convincing. Reid(4) on the other hand demonstrated a 6-fold increase in liver glycogen of cats after injecting Mg as chloride or gluconate. No reference to the effect of Mg on the synthesis of the glycogen fractions of Bloom(5) has been found by the writers.

This report presents the results of glycogen fraction studies in heart, liver and skeletal muscle of the rat in an attempt to elucidate the relationship of the TCA soluble and TCA insoluble glycogen fractions after Mg salt injections.

*Methods.* White rats weighing between 200 and 400 g were fasted 18 hours prior to injection I.P. with 0.2M Mg sulfate. Each rat received approximately 1.6 mg of Mg ion per 100 g of body weight. The sexes were equally mixed. Control rats were injected with equivalent molar concentrations of sodium chloride. The animals were then sacrificed by decapitation at one or 4 hours. The entire heart and samples of liver and gastrocnemius muscle were taken for analysis for TCA soluble and TCA insoluble glycogen fractions using the procedure outlined by Bloom, *et al.*(5). After hydrolysis of the glycogen in sulfuric acid, the isolated glycogen samples were measured quantitatively by the anthrone procedure of Seifter, Dayton, Novic and Muntwyler(6). A separate series of 12 fasted rats was injected with Mg sulfate at the same dose level as the rats in the glycogen series for the serum Mg data. These were sacrificed after one hour. Another 15 rats similarly treated were sacrificed after 4 hours. An additional 12 untreated rats were used as controls. Blood was withdrawn by direct heart stab and analyzed for serum Mg using the method of Orange and Rhein(7) as modified by Platner and Hosko(8).

The results of the glycogen and serum Mg determinations are presented in Table I.

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TABLE I. Glycogen Content (mg % ± S.E.) of Rat Tissues as Affected by Magnesium Ion.

| | Control | Magnesium inj. 1 hr | 4 hr |
|---|---|---|---|
| *Heart* | | | |
| TCA insoluble glycogen | 110± 9.7 (19)* | 131± 7.3 (15) | 155±11.6† (15) |
| TCA soluble glycogen | 187±25.5 (19) | 250± 28.3 (15) | 219±21.9 (15) |
| Total glycogen | 297±31.8 (19) | 381± 29.6 (15) | 374±22.3 (15) |
| *Muscle* | | | |
| TCA insoluble glycogen | 107±10.1 (19) | 137± 14.9 (15) | 184±13.3† (15) |
| TCA soluble glycogen | 167±16.7 (19) | 167± 12.7 (13) | 174±24.4 (15) |
| Total glycogen | 274±18.7 (19) | 312± 22.9 (13) | 358±27.4† (15) |
| *Liver* | | | |
| TCA insoluble glycogen | 164±17.5 (19) | 224± 13.7† (14) | 196±20.7 (30) |
| TCA soluble glycogen | 382±70.7 (17) | 796±144.8† (15) | 402±58.9 (28) |
| Total glycogen | 536±75.0 (18) | 958±150.9† (14) | 620±75.4 (27) |
| Serum magnesium | 1.73±.08 (12) | 5.21±.25 (12) | 1.45±.09 (15) |

* No. in parenthesis refers to No. of animals used.

† Significant to 98% level.

*Discussion.* One hour after Mg sulfate injection there was a sharp rise in total liver glycogen. Most of this rise is due to the TCA soluble fraction, although the increase in TCA insoluble fraction is also significant, ($P = 0.02$). Four hours following the Mg injection there was a significant increase in the TCA insoluble fraction in heart tissue. Skeletal muscle also showed at this time a significant increase in both the TCA soluble and TCA insoluble fractions. The data suggest that the liver reflects changes in blood Mg levels much more readily than does skeletal muscle or heart tissue. The serum Mg levels support this finding in that the highest serum Mg levels coincide in time with the greatest rise in liver glycogen values.

The heart tissue shows the next greatest rise and skeletal muscle appears to be least affected after one hour, but both tissues show significant increases after 4 hours. The delayed response of the heart and especially skeletal muscle may be due simply to the slow rate of entrance of the Mg ion into muscular tissue. Smith, *et al.*(9) showed that Mg distributes itself in the body more like Na than K and that complete distribution requires more than 4 hours to enter spaces other than extracellular. Delayed entrance of the Mg ion into muscular tissue may also be due to dilution by body fluids. After 4 hours, as the serum Mg falls to control levels, the total glycogen also declines in heart and liver but continues to rise slightly in skeletal muscle.

Although both glycogen fractions follow a similar pattern, the TCA soluble fraction appears to be synthesized at a slightly greater rate than the TCA insoluble fraction. The data imply that the Mg ion *in vivo* promotes synthesis of both glycogen fractions in the 3 tissues studied. This finding is quite significant since very few substances have been reported which increase the synthesis of the TCA insoluble fraction. The sharp increase of the soluble fraction in liver one hour following Mg injection implies a preferential effect of this ion on the synthesis of this fraction in liver pointing up the difference in metabolic pattern between this tissue and skeletal muscle.

*Summary.* Magnesium sulfate injected I.P. into rats promotes synthesis of both TCA soluble and TCA insoluble glycogen fractions in the liver after one hour. Heart and skeletal muscle show a significant rise in TCA insoluble glycogen fraction after 4 hours. Skeletal muscle also shows a significant rise in total glycogen after 4 hours. Magnesium appears to be one of the few substances which promote synthesis of the TCA insoluble glycogen fraction.

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## Potentiated Inhibition of *Escherichia coli* by Certain Combinations of Agents. (22490)

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Much useful information has been obtained on the modes of action of chemotherapeutic agents, including tumor-inhibiting agents, by inhibition analysis in bacterial systems. In a number of cases the mode of action appears to be the same in bacterial and mammalian systems. Therefore, it is not unreasonable to test agents or combinations of agents against bacteria to serve as a guide for tests in mammals. In this laboratory, candidate anticancer agents are tested singly and in combinations as growth inhibitors for bacteria prior to testing against neoplasms in animals. This report presents some of the data that have been obtained for combinations of agents in tests with bacteria.

*Methods.* To screen for potentiating growth-inhibitory activity, a simple test method was devised using *Escherichia coli* (ATCC No. 9637) as the test organism. The use of this organism grown in a simple, chemically defined glucose-salts medium is ideal for biochemical studies of this kind because the observed action of specific inhibitors or antimetabolites is not obscured by the presence in the medium of the chemically complex metabolites required in similar studies on more fastidious organisms. Duplicate paper discs saturated with known concentrations of a candidate compound were placed on the surfaces of minimal agar cultures of *E. coli* in a control plate and in a test plate. The control plate contained no test compound, whereas the test plate contained a subinhibitory concentration of the second member of the pair of candidate compounds being tested for potentiation. If the diameter of the zone of inhibition around the paper disc on the test plate was greater than 2 times that on the control plate, then the combination was considered worthy of further study. Of approximately one thousand pairs of compounds tested, approximately one hundred pairs gave evidence of significant additive or potentiated inhibition. Although the agar-plate method described above was quite useful for rapidly screening combinations for additive activities, it was considered desirable to subject the combinations that showed the more promising activities to another test, which would permit a quantitative evaluation of the combinations. For this purpose, the method of Elion, Singer, and Hitchings(1) was used, and *E. coli* (ATCC No. 9637) was used instead of *Lactobacillus casei* or *Streptococcus faecalis*. The minimal medium of Davis and Mingioli(2) was used with the omission of the agar. A 24-hour culture of *E. coli* was adjusted by addition of sterile water to have a transmission of 55 $\pm$ 2% (Bausch and Lomb Monochromatic Colorimeter with a 660 m$\mu$ interference filter), and then diluted 1:10 with sterile water. Each 10-ml tube of medium was inoculated with 0.1 ml of this suspension. The extent of growth was determined turbidimetrically after incubation at 37° for 16-18 hours. The effects of the 2 compounds being tested upon the growth of the bacteria were determined by testing various levels of the compounds alone and in combination. In each case, the quantity of the compound required to cause half-maximal inhibition was determined graphically as shown in Fig. 1 and 2. The fractional inhibitory concentra-