mycobacteria. using agar diffusion technic. 2. The reaction has occurred in sera of 28 of 32 patients, 3 of which changed from negative to positive as lesions improved. 3. The data permit no conclusion as to specificity of this reaction, but suggest its value as an additional diagnostic method in sarcoidosis.

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Chemical Interaction of S^{35}-6-Mercaptopurine and Ribonucleic Acids.* (26615)

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The question as to whether S^{35}-labelled 6-mercaptopurine. (S^{35}-6 MP) is incorporated into nucleic acids has not been settled. Elion *et al.*, injected mice with S^{35}-6 MP and within 4 hours found the label associated with RNA and DNA isolated from visceral organs(1). Brockman has recently questioned this observation as indicating incorporation of 6-mercaptopurine and states that one would not expect 6-MP to be incorporated into nucleic acid because it is an analog of hypoxanthine and not an analog of adenine or guanine(2). 6-MP, however, is converted to thioxanthine and thiouric acid by mouse tissue and by bacteria(2). It is possible that small amounts of a given dose of 6-MP administered to rats might be converted to thioguanine. Lepage has reported that thioguanine is incorporated into nucleic acid(3). In view of these considerations it was thought worthwhile to examine the nature of incorporated S^{35}-6 MP in rat liver RNA.

In vivo incorporation of S^{35}-6 MP into rat liver RNA was accomplished as follows: Three groups (3 rats per group) of adult white male rats were injected intraperitoneally with 2 mg S^{35}-6 MP. After 4 hours the rats were killed; the livers were removed and frozen in dry ice. They were homogenized in a Waring blender and RNA was isolated by the Kirby phenol extraction method(4). The liver RNA from each of the rats in the first group was assayed for radioactivity in an open window gas flow well counter. Fifty mg liver RNA from each of the rats in the second group were dissolved in 5 ml H_2O and treated with an equal volume of 10% trichloracetic acid at 5°C. The precipitate was collected by centrifugation, washed with 95% EtOH and the radioactivity per mg RNA was measured. Fifty mg liver RNA from each of the rats in the third group were dissolved in 5 ml of water containing 100 μg unlabelled 6-MP and aerated with H_2S gas for one hour at room temperature. The

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RNA was precipitated with 2 volumes 95% EtOH, dialysed against saline for 24 hours and radioactivity per mg RNA was measured. Average amount of mμg S^{35} per mg liver RNA was 4.3, 4.1 and 2.1 respectively for RNA from the livers of rats in Groups 1, 2 and 3. These data indicate that at least half of the S^{35}associated with the RNA fraction could be removed by H_2S treatment at room temperature but was not split off by precipitation with trichloracetic acid at 5°C.

An attempt was made to increase the amount of S^{35} associated with purified extracted rat liver RNA. Each of 3 groups of 3 rats was given 5 mg S^{35}-6 MP intraperitoneally every day for 3 days. Rat liver RNA was isolated as described for Rat Group 3 above. The livers of these 3 groups showed an average of 3.0, 2.3, and 1.1 mμg S^{35} per mg RNA. There was no significant increase in associated S^{35}. These data confirm the experimental results of Elion, Bieber and Hitchings in that S^{35} is associated with purified isolated liver RNA after injection of S^{35}-6 MP into rats. However, the findings with H_2S suggest that part of the "incorporated" S^{35} may be bound as a mercaptide.

Two types of experiments demonstrated that 6-MP combines with RNA to form a stable complex. It was found that S^{35}-6 MP combines with RNA of homogenized rat liver and that it combines with pure yeast RNA to form a stable complex.

1. Combination of S^{35}-6 MP with rat liver RNA. Ten grams of rat liver from each of 3 rats were separately homogenized in a Waring blender for one minute with 4 volumes of water containing 5 mg S^{35}-6 MP. RNA was isolated by the Kirby method and aerated with H_2S for one hour at room temperature. The RNA was precipitated with 2 volumes of 95% EtOh, dialysed against saline for 24 hours and radioactivity per mg RNA was measured. 2.1, 1.1 and 0.2 mμg S^{35} were associated with the liver RNA fractions. Two or 3 times as much S^{35} per mg RNA was present prior to H_2S treatment. A small but measurable fraction appears to resist separation by H_2S.

2. Combining of yeast RNA with S^{35}-6 MP.† Commercial yeast RNA was obtained from Nutritional Biochemical Corp. Fifty aliquots were dissolved in 5 ml of 2% KAc solution. One tube served as control, while to the others were added copper and iron salt solutions in the amounts of 1 μm/50 mg RNA. Two volumes of alcohol were added and the resulting precipitates of RNA were collected by centrifugation. The precipitates were dissolved in 5 ml of 2% KAc and alcohol precipitation was repeated. The precipitates were re-dissolved in 5 ml of 2% KAc solution containing 100 μg of S^{35}-6 MP per 5 ml salt solution. After 15 minutes RNA was precipitated with alcohol, the precipitate collected by centrifugation and re-dissolved in 5 ml of 2% KAc. This step was repeated 4 times to remove free S^{35}-6 MP. The RNA was assayed for S^{35} per mg RNA. The results given in Table I represent averages of 4 such experiments.

TABLE I.* Effects of Metals on Combining of S^{35}-6 MP by Yeast RNA.

Metal added	mμg S^{35}/mg RNA
None	13
Cu^{+}	133
Cu^{++}	95
Fe^{++}	35
Fe^{+++}	13

* Avg of 4 experiments.

Discussion. When S^{35}-6 MP is injected into rats, small amounts of radioactivity are associated with liver RNA isolated by the Kirby procedure involving phenol extraction, alcohol precipitation and dialysis. Since at least half of the radioactivity can be removed by treatment with H_2S at room temperature, it would appear that RNA may be combined with 6-MP by a metal bridge bond as shown below:

RNA - Metal - S-6 MP

The same reaction appears to take place immediately when rat liver is homogenized in presence of S^{35}-6 MP—a speed of reaction which would seem to preclude "incorporation". Commercial yeast RNA also com-

† S^{35}-6 MP was synthesized in this laboratory by methods and materials kindly supplied by Dr. George Hitchings, Burroughs Wellcome Lab., Tuckahoe, N. Y.

bines with small quantities of S^{35}-6 MP and addition of metals (Table I) enhances the combination. Various metals have been reported to be combined with nucleic acids(5, 6). The interaction of 6-MP with metals in nucleic acid may interfere with cellular metabolism.

Summary. These data indicate that 6-mercaptopurine can combine with ribonucleic acid both *in vitro* and *in vivo*. Binding is enhanced by added metals and the combination is stable to ribonucleic acid isolation procedures.

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Studies on Murine Hepatitis Virus (MHV3) *in vitro*.* (26616)

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The results of several investigations from various approaches(1,2) have focused attention upon murine hepatitis virus and have indicated the possible use of studies of these viruses *in vitro*.

Until recently, knowledge of the murine hepatitis viruses has been based mainly on experiments *in vivo*, since in spite of the observations that these viruses multiply in tissue culture(3-5), no cytopathic effect has been demonstrated. However, Bang(6) has recently shown that MHV2 (Nelson virus) destroys macrophages spreading out from liver explants obtained from a strain of Princeton mice susceptible to hepatitis virus. Parallel observations were made by Bang (7) in macrophage cultures from Princeton mice which were lysed by the virus; macrophages from resistant C3H mice showed no cytopathic effect. This report concerns the behavior of another strain of murine hepatitis (MHV3) in tissue culture, since this strain has been used in experiments designed to show the protective action of antihistamines against liver injury(8,9).

Methods. 1. *Virus.* The MHV3 virus, obtained from Dr. Gledhill, was injected intraperitoneally into adult C57Bl/6 mice, and the infected livers were harvested from moribund mice. The livers were pale and under histological examination appeared to be completely necrotic. For experiments *in vitro* viral pool suspensions were prepared from 10% homogenates of these livers. The homogenate was centrifuged at 1500 rpm for 15 minutes and the supernatant diluted 10-fold with culture medium.

2. *Tissue culture.* Liver explants from 16 to 20-day old fetuses of C57Bl/6 mice were cultivated on reconstituted rat-tail collagen as described by Hillis *et al.*(10). Collagen was prepared and stored according to Ehrmann *et al.*(11). Fetal livers pooled from the same litter were minced with scissors, and 0.5 mm^3 pieces were placed on coverslips on collagen, which were placed in roller tubes. Rotation speed was adjusted to 8 rph. Culture medium consisted of 30% active horse serum, 5% chick embryo extract, and 65% Eagle's basal medium in Earle's balanced salt solution. Tubes were gassed with 5% CO_2 before being stoppered.

In addition, dispersed spleen cell cultures were prepared according to the method of Manaker *et al.*(12).

3. *Passage of virus in tissue culture.* Virus was passed in tissue culture either by using 10-fold diluted infected culture medium or by transferring homogenates from infected explants. This was accomplished by breaking

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