the antigens prior to heating. The amount and dilution of the antigens exposed to heating, as well as titer and protein content of the different samples of the same antigen, were not kept strictly constant when repeating the same experiments.

The results reported refer to single experiments, but very similar, if not identical, results were obtained repeatedly with the same kinds of antigen, under the same conditions. The only exception was experienced when testing mumps antigens, with which no reproducible results were obtained under the conditions used.

Although glycerine was found to have a protective effect against flocculation of Q Fever antigen at higher temperatures, glycerinated preparations do not seem to present any advantage because of the remarkable heat stability of this antigen even in the absence of glycerine.

Glycerine alone or in combination with any of the antigens tested, as well as glycerine mixed with positive or negative sera, consistently failed to give either pro- or anti-complementary effects.

After autoclaving for 30 minutes at 110°C or 120°C, both saline and glycerinated preparations of some antigens (*e.g.*, Nine Mile Q Fever antigen) became somewhat anti-complementary. However, the anti-complementary level never reached that of the specific antigen titer and since it appeared only after exposure to temperatures higher than 100°C, it seems evident that it does not add to the problem at hand.

The results obtained seem to justify therefore the addition of 50% glycerine as preservative to the complement-fixing antigens tested so far in our laboratory.

Summary. The stabilizing action of glycerine on some complement-fixing antigens of viral and rickettsial origin was investigated. Its ability to prevent rapid deterioration of antigen-titers and visible flocculation following long storage or exposure to unduly high temperatures has been reported.

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Binding of Histamine by Animal Proteins. (20594)

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Recent reports(1,2) have given evidence for binding of histamine by components of normal human blood serum, especially γ-globulin. The availability of C^{14}-histamine* made it possible to check these results by the dialysis-equilibrium technic(3).

Guinea pig lung has been reported(4) to contain relatively high concentrations of histamine. However, it was demonstrated(5) in experiments with isotopically labeled histamine and *L*-histidine that injected histamine is rapidly metabolized and excreted in the urine by guinea pigs and does not appear to any appreciable extent in the internal organs, whereas injected *L*-histidine is converted to histamine which is present in the organs for long periods. It was therefore of interest to determine whether added C^{14}-histamine would be bound by guinea pig lung homogenates or equilibrate with histamine already present.

Experimental. Human plasma proteins†

* We are grateful to Dr. R. W. Schayer of this Institute for supplying the labeled histamine.

† These human plasma fractions were generously furnished by Dr. E. J. Cohn of Harvard University Medical School.

were dissolved in Tyrode solution, dialyzed against Tyrode overnight in a cold room, and finally diluted to a concentration of 0.5% of the protein as weighed. Normal human blood serum was dialyzed against Tyrode solution and diluted with Tyrode to 3.3 times the original volume of serum. Guinea pig lung was homogenized with Tyrode solution in a Potter-Elvehjem homogenizer, dialyzed, and diluted to 1% by weight of wet tissue. Aliquots (10 ml) of each solution or suspension were placed in cellulose bags in test tubes containing solutions of 1 mg, 10.4 γ, and 0.4 γ of C^{14}-histamine in 11 ml of Tyrode solution, each having the same total radioactivity. Identical tubes were set up simultaneously containing only Tyrode solution inside the cellulose bags. Each protein solution or suspension was equilibrated against each concentration of histamine by shaking the stoppered tubes for 18-20 hours in a cold room (2-5°C), all experiments being run in duplicate. Portions (10 ml) of the solutions outside the bags were then added to 66.4 mg of histamine dihydrochloride carrier, acidified with HCl, evaporated to dryness, and converted to picrates. The picrates were dissolved in aqueous alcohol, treated with norit, recrystallized, plated in stainless steel cups, and counted to 5% accuracy at infinite thickness in a flow counter. Under these conditions the C^{14}-histamine in each tube had a total activity of 200 c.p.m. above background. If the histamine became distributed uniformly, each 10 ml portion of solution should have had an activity of 95 c.p.m. above background under the conditions used. Within experimental error this was the radioactivity found not only in the tubes containing only Tyrode solution inside the bags, but also in the tubes containing 0.5% solutions of human γ-globulin, β_1-globulin, and plasma albumin as well as normal human serum and the 1% guinea pig lung homogenate. No evidence was obtained for binding of histamine at equilibrium levels of 47.6, 0.5, and 0.02 γ per ml by these proteins.

The above experiments were performed under different conditions than those used by the previous workers(1,2). For γ-globulin more comparable experiments were carried out. The bags contained 4 γ of histamine (2000 c.p.m. above background when assayed for radioactivity as above) and 5 mg of γ-globulin in 10.2 ml of Tyrode solution and were placed in 5.1 ml of Tyrode containing 2 γ of histamine (1000 c.p.m.). As above, controls were run in which γ-globulin was absent. The tubes were shaken 3 hours at room temperature (26-8°C), and 1.0 ml aliquots of the solutions outside the cellulose bags were assayed for radioactivity. The histamine was uniformly distributed in the tubes equilibrated with γ-globulin, as well as the controls, all samples assaying 195 c.p.m. above background per ml (0.39 γ/ml) within the counting error of 5%. There was therefore no evidence for binding of histamine by γ-globulin under these conditions. The solutions both inside and outside the cellulose bags were checked for their histamine content by pharmacological assay on guinea pig intestine.‡ Within experimental error the solutions outside the bags contained the equilibrium concentration of histamine (0.45 γ/ml), but the solutions within the bags containing γ-globulin gave low assays (0.25 γ/ml). The latter levels were increased by acid hydrolysis or trichloroacetic acid precipitation of the protein, but the experimental uncertainty was greater than any uptake of histamine which might have occurred.

An attempt was also made to demonstrate binding of histamine at room temperature by a much higher level of γ-globulin. The solutions within the cellulose bags contained a 5% solution of the protein and the concentration of labeled histamine was 0.33 γ/ml (163 c.p.m. per ml by the usual assay procedure). Both the solution equilibrated with protein and that equilibrated with Tyrode assayed as expected for uniform distribution of histamine on both sides of the cellulose membrane.

Discussion and summary. The results with labeled histamine show that it is not bound reversibly by blood proteins. As found by Parrot *et al.*(1,2), γ-globulin causes a diminution of the pharmacological activity of hista-

‡ Dr. G. Ungar of this Institute kindly carried out these assays.

mine solutions, but on the basis of the dialysis-equilibrium experiments this effect cannot be attributed to binding of histamine by the protein. Schayer(5) has pointed out the non-equilibration of guinea pig organs with exogenous histamine *in vivo*. The present results show that *in vitro* guinea pig lung is incapable of binding added histamine or of equilibrating added with bound histamine. Apparently, binding of histamine requires formation at the binding site.

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Anti-mycobacterial Properties of a New Derivative of Isoniazid. (20595)

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Since the first description of the marked anti-tuberculous properties of isoniazid(1,2,3, 4) and iproniazid, it has been shown that derivatives of isoniazid in which one or both of the terminal hydrogens have been replaced by substitutions might possess similar high activity both *in vitro* and *in vivo*(5,6,7,8). Of the numerous compounds of this type synthesized by Dr. H. H. Fox in the Roche Chemical Research Laboratories, which were studied for their activity against *Mycobacterium tuberculosis* and were found to exert the expected effect, one substance appeared to have favorable properties of both toxicity and chemotherapeutic activity. This substance, 1,1'-methylenebis(2-isonicotinylhydrazine) from now on designated as Ro 2-4969, which contains 2 isoniazid molecules linked by the methylene group is characterized by the lack of solubility in water, alcohol and most other organic solvents. Despite this physical property which distinguishes it sharply from other active members of the hydrazine series, it exhibited an effect comparable to the parent substance. The results of the experimental studies carried out with Ro 2-4969 are reported in the present note.

Materials and methods. The H37Rv strain of *M. tuberculosis* obtained through the courtesy of Mr. W. Steenken, Jr., of Trudeau Sanatorium on December 20, 1947 was used in all experiments. Adult albino mice of 18-20 g from our own colony were used throughout the investigation. The technics of the experiments in mice used in the evaluation of Ro 2-4969 are identical with those described in earlier publications(2,9) and are based on the effect of a 21 day treatment with medicated diet and examination of the lungs both immediately after discontinuation of treatment as well as 21 days after termination of therapy. The lack of solubility of Ro 2-4969 prompted also experiments in which small pellets were inserted in the subcutaneous tissue. This was followed by intravenous infection with *M. tuberculosis* H37Rv at different intervals after the implantation. Further details of the procedure are given in the text.

Toxicity for mice. Acute toxicity tests carried out by administering a single dose of the compound by gavage gave an LD_{50} of 3900.0 mg/kg.*

In vivo experiments with M. tuberculosis. 1. *Effect of oral drug administration.* If medicated diet with Ro 2-4969 was started immediately after the intravenous infection with strain H37Rv and continued for 21 days at which time the mice were sacrificed and examined, the majority of animals were found to be protected at a dose of 25.0 mg/kg/day

* Experiments in different species of animals including chronic toxicity tests have been carried out by Dr. Benson and associates in the Roche Pharmacology Laboratory and will be reported elsewhere.