normal adrenal cortical function in these patients with scurvy. The observation, however, is amenable to other interpretations. First, ascorbic acid may not be necessary for those functions of the adrenal cortex studied. This interpretation is supported by the report of Long(9) that the injection of ACTH to scorbutic guinea pigs with but 4% of normal adrenal ascorbic acid remaining resulted in the usual fall in adrenal cholesterol and the development of lymphopenia. Secondly, although scorbutic, the patients studied may have had residual ascorbic acid in their adrenals.

It is impossible to decide between these alternatives at present. It is known, however, that scorbutic guinea pigs retain small but significant quantities of ascorbic acid in their adrenals(10). The same may be true in human scurvy. Moreover, patients dying of scurvy sometimes present a syndrome of hypotension and shock resembling an Addisonian crisis(11). Perhaps only terminally do the adrenals become completely deficient in ascorbic acid.

*Summary.* 1. Test doses of ACTH were administered to 5 patients with classical scurvy to assess their adrenal cortical activity. The test was repeated in 4 of these patients after therapy with ascorbic acid. 2. The blood eosinophil response to ACTH was that expected of individuals with normal adrenal function and did not change following treatment with ascorbic acid. 3. The urinary excretion of uric acid after ACTH was uninterpretable because of low urine volumes. 4. The blood glutathione, and the serum sodium concentrations were normal or low in these patients, but were unaltered by ascorbic acid therapy. Serum potassium concentrations were normal before and after ascorbic acid therapy. 5. To explain these evidences of normal adrenal cortical activity in scurvy, it may be postulated that either ascorbic acid is not necessary for these adrenal functions, or there was residual ascorbic acid in the adrenals of these patients.

*Addenda.* Dr. Frank H. Gardner, Peter Bent Brigham Hospital, made a similar study of one patient, a 76-year-old bachelor, with scurvy. A 48-hour ACTH test‖ was done, giving 40 mg of ACTH daily, divided into 4 equal doses. A normal eosinophil response and increase in 17-ketosteroid excretion was observed.

| | Control | 48 hr |
|---|---|---|
| Eosinophil count per cu mm | 27 | 0 |
| 17-ketosteroid excretion mg/24 hr | 2.3 | 13.6 |

The patient presented in addition to clinical evidence of scurvy, the defect in tyrosine metabolism reported in the disease.**

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## Failure of Aureomycin and Terramycin to Increase Resistance to Toxic Action of Influenza Virus.* (18251)

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Acute toxic damage is induced by the influenza virus in tissues which will not support its multiplication(1). When injected intravenously, intra-abdominally or intra-

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cerebrally, the virus may cause death even in animals which are not ordinarily susceptible to influenza infection(1). The toxic action of the influenza virus has caused hepatic, splenic, cerebral and probably myocardial damage(1,2), and pathologic changes have been demonstrated in organs outside of the respiratory tract in fatal cases of influenza A infection(3). It is possible, therefore, that certain of the clinical manifestations of influenza are due to the direct toxic action of the virus. The administration of aureomycin or terramycin to patients with severe cases of proved influenza is usually followed by prompt defervescence and relief from myalgia, fever and other clinical evidences of toxicity(4,5). However, influenza has been a relatively mild disease in recent years and equally rapid terminations of the disease have been observed in the absence of specific therapy(5,6). Aureomycin(7) and terramycin(5,8,9) do not inhibit the growth of the influenza virus in experimental animals although terramycin, under certain conditions, may affect growth of the virus in chick embryos to a slight extent(5,8,9). It has been suggested that these antibiotics may diminish the toxicity of the virus to the host and that this may explain their possible clinical effectiveness in the treatment of influenza. Acute toxicity induced by the intravenous injection of rickettsiae into mice is not prevented or reduced by therapeutic doses of terramycin and aureomycin(10). Likewise, aureomycin and penicillin do not affect the toxicity of viruses of the psittacosis-lymphogranuloma group for mice(11).

In the present study, terramycin and aureomycin were administered to rats and mice, and concentrated suspensions of influenza virus were then injected, to determine if the toxicity of the virus was diminished by the antibiotics.

*Materials and methods.* The PR8 strain of influenza virus was used throughout, and was propagated in the allantoic fluids of 10- or 11-day-old chick embryos by the usual methods(12). The virus was concentrated 10- to 20-fold in the original allantoic fluid by adsorption on and elution from embryonic erythrocytes. The $ID_{50}$ of the resulting viral concentrates was between $10^{-7.6}$ and $10^{-8.0}$ when titrated in 10-day-old chick embryos. Aureomycin hydrochloride buffered with sodium glycinate‡ and terramycin hydrochloride¶ were used throughout but the dosages are expressed in terms of their respective free bases. Fresh solutions were prepared in saline for subcutaneous injection and in water for oral administration. The subcutaneous dose was 2.5 mg in 0.5 ml given twice daily to mice, and twice this amount, given every 4 hours to rats. The oral dose was 10 mg in 0.2 ml administered twice daily directly into the stomachs of the mice.

White Swiss male mice of the Tumblebrook Farm strain, weighing 18-22 g were used. In a few observations, female mice were found to be as susceptible as males to the toxic action of the influenza virus. The mice were divided into groups of 10 and were given either terramycin, aureomycin, or saline twice daily until the time of their death. Immediately after the second ad-

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‡ Supplied by Dr. Stanton M. Hardy of Lederle Laboratories.

¶ Furnished by Dr. Gladys L. Hobby of Chas. Pfizer & Co.

ministration of antibiotic, 1.5 ml of concentrated virus per 100 g body weight were injected intravenously. In one series, the virus was not injected until after the fourth injection of antibiotic. Control animals were given antibiotics but no virus. The amount of virus used resulted in the death of 50-70% of mice within 24 hours. Only mice succumbing during the first 24 hours after injection of the virus were considered to have died of toxemia.

The rats were males of the Sprague-Dawley strain, weighing 100-150 g. Groups of 5 rats were injected subcutaneously with one of the antibiotics or with saline every 4 hours until death occurred or the experiment was terminated. The virus was injected intravenously after the second dose of antibiotic, using the same dose as in mice. In the rats, this amount of virus regularly produced prostration in 6-8 hours and death in 9-14 hours.

Other groups of control mice and rats were immunized by intravenous injection of sublethal doses of virus 2 weeks before they were challenged.

*Results.* The immunized mice and rats were completely protected against otherwise lethal doses of the influenza virus. The control animals which received antibiotic but no virus showed no adverse effects save for slight hyperirritability and ruffled fur. There was no significant prolongation of life in rats (Table I) or mice (Table II) treated with aureomycin or terramycin, as compared with animals receiving saline alone. Neither subcutaneous nor oral administration of the antibiotics was effective.

*Discussion.* The failure of aureomycin and terramycin to alter the toxic action of the influenza virus, the lack of any effect of either of these antibiotics on influenza viral infections in mice(5,7-9), and the failure at least of aureomycin to affect multiplication of the virus in chick embryos(5,7) all would seem to indicate that any clinical benefits derived from the use of these antibiotics in the treatment of influenza are probably not the result of an effect on the virus or on the capacity of the host to resist its toxic action.

TABLE I. Effect of Aureomycin and Terramycin on Toxicity of Influenza A Virus to Rats.

| Substance inj. | Result | | | | |
|---|---|---|---|---|---|
| Saline | 9, | 9, | 10, | 11, | 12* |
| Aureomycin | 8, | 9, | 10, | 10, | 14 |
| Terramycin | 9, | 9, | 9, | 11, | 12 |
| Immune | S, | S, | S, | S, | S |

Test animals each received 1.5 ml of virus concentrate per 100 g of body wt intravenously.

Control rats receiving antibiotic and no virus all survived.

* Hours after virus was inj. when death occurred.

S = Survived.

TABLE II. Effect of Aureomycin and Terramycin on Toxicity of Influenza A Virus to Mice.

| Substance inj. | Route | No. dead/No. inj. |
|---|---|---|
| Saline | Subcut. | 8/10 |
| Aureomycin | ,, | 9/10 |
| Terramycin | ,, | 8/10 |
| Saline | Oral | 6/10 |
| Aureomycin | ,, | 7/10 |
| ,, * | ,, | 8/10 |
| Terramycin | ,, | 6/10 |
| ,, * | ,, | 8/10 |
| Immune | | 0/10 |

Test animals each received 1.5 ml of virus concentrate per 100 g of body wt intrav.

Control mice given aureomycin and terramycin alone did not succumb.

* Virus inj. after the 4th dose of antibiotic. In the other groups, virus was inj. after the second dose of antibiotic.

The clinical aspects, therefore, require further investigation.

It has been suggested that some of the toxic effects of the influenza virus may be due to incomplete and atypical replication of viral particles in tissues in which the virus is not ordinarily considered to have multiplied(13). After intracerebral injection of a non-neurotropic strain of influenza virus, complement-fixing and hemagglutinating substances in the cerebrum of the mouse increased, but infectivity titers did not increase. If this observation should prove to be generally applicable to all tissues in which the titer of infectivity of the influenza virus does not increase, the findings presented here may simply reflect the failure of aureomycin and terramycin to interfere with viral multiplication in the experimental animal. It has not been demonstrated, however, that

13. Schlesinger, R. W., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 541.

atypical replication of the influenza virus will occur in tissues other than the brain of the mouse, or in any of the tissues of the rat, which is not susceptible to influenza infection.

*Summary.* Aureomycin and terramycin, administered subcutaneously and orally, did not increase the resistance of mice or rats to the acute toxic action of influenza A virus.

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## Mustard Inactivated Rabies Vaccine. (18252)

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Our preliminary paper(1) on the production of vaccines by treating virus suspensions with mustard was based in part on studies in this laboratory on the effect of mustard on proteins. These studies(2), which were published subsequently to our paper, showed among other things that relatively small amounts of mustard inactivated enzymes and that mustard reacted with the carboxyl groups of protein molecules but not with the free amino groups. After our preliminary paper, one of us(3) studied the action of mustard on animal, plant, and bacterial viruses, as well as on bacteria, yeasts, and a pneumococcus-transforming agent, and found that viruses and cells were inactivated faster than enzymes and that viruses containing desoxyribose nucleic acid were inactivated faster than those containing ribosenucleic acid. Mustard gas seems an ideal inactivating agent because its action is complete within a relatively short time and in our experiments the end products were not toxic. Mustard is bactericidal; and if the contamination of the starting material is not too great, sterile vaccines are secured. It is in fact much more like ultraviolet light in its action than like most other chemical agents. When tested for antigenicity it is found that inactivated rabies and equine encephalomyelitis viruses immunize when diluted $10^{-3}$ or higher, hog cholera and Newcastle viruses immunize when undiluted, while very preliminary experiments with pseudorabies and Baker's cat viruses have failed to show antigenicity.

In the present paper some experiments made with inactivated rabies virus are reported, since the vaccine promises to be of practical importance and since the findings may be useful as a guide for the preparation of other vaccines.

*Materials and methods.* The fixed viruses used were a strain obtained from the Bureau of Laboratories of the Department of Health of the City of New York, through the kindness of Dr. Jules Freund, the Pitman-Moore strain 980 from Dr. R. B. Gochenour, and a strain adapted to mice from Dr. Harald Johnson. The street virus used for challenging the guinea pigs also came from Dr. Johnson. Rabbits, guinea pigs, and mice were inoculated intracerebrally and the brains removed when the animals were completely paralyzed. Small amounts of brain material were suspended in water, using a glass grinder; larger amounts were suspended in water in a Waring blender. Water was used rather than saline as the reaction is retarded by halid ions.

*Test of immunity.* The least amount of antigen injected into the peritoneal cavity that would immunize guinea pigs weighing 250-300 g against street virus was determined. This test has been described in some detail(1).

*Mustards.* Sulfur mustard (*bis* (β-chloroethyl) sulfide) was prepared in this laboratory

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3. Herriott, R. M., *J. Gen. Physiol.*, 1948, v32, 321.