*Brit. J. Pharmacol.* (1950), **5**, 549.

# THE TOXIC AND ANTITUBERCULOUS EFFECTS OF TWO THIOSEMICARBAZONES AND STREPTOMYCIN IN DOGS, MONKEYS, AND GUINEA-PIGS

BY

J. FRANCIS AND A. SPINKS

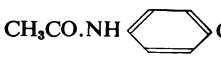
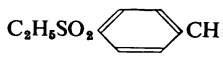
*From the Research and Biological Laboratories, Imperial Chemical Industries Limited, Hexagon House, Blackley, Manchester, 9*

AND G. T. STEWART

*From the Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School, London, W.2*

(Received August 24, 1950)

The earlier reports from Germany on the antituberculous action of the thiosemicarbazones have been reviewed by Domagk (1950) and Mertens and Bunge (1950). Experiments made in Britain by Hoggarth, Martin, Storey, and Young (1949) showed that activity in mice was maximal with the two thiosemicarbazones shown below:

| | *I.C.(P) reference* | *German reference* |
|---|---|---|
| *p*-acetylaminobenzaldehyde thiosemicarbazone<br>  | 9311<br>berculon A* | TbI/698<br>conteben |
| *p*-ethylsulphonylbenzaldehyde thiosemicarbazone<br>  | 8388<br>berculon B* | TbIII.Be 1374 |

This paper deals chiefly with 8388 because at the time the work was carried out we were more interested in that compound. Martin and Stewart (1950) showed that, in mice, 8388 was more active than *p*-aminosalicylic acid, but less active than streptomycin. In the early stages of murine tuberculosis, however, 8388 appeared in some respects superior to streptomycin. Toxicological tests with 8388 showed that the compound exhibited differences, not only in the doses tolerated, but also in the organs affected, in various animal species. It seemed possible that there might be major differences in the metabolism of 8388 in different animals, so experiments were performed on a wider range of experimental animals.

## EXPERIMENTAL

### *Administration of* 8388 *to normal monkeys*

The blood-concentrations produced by single doses of 8388 are shown in Table I; estimations were made by the method of Spinks (1949). This compound had been well tolerated by mice, rats, and guinea-pigs in doses of 500 mg./kg. a day for several weeks and post-mortem examination had revealed no significant changes.

* Trade marks of Imperial Chemical (Pharmaceuticals) Ltd.

TABLE I

BLOOD-CONCENTRATIONS PRODUCED BY 8388 IN MONKEYS AFTER SINGLE, ORAL DOSES

| Monkey No. | Weight kg. | Dose* mg./kg. | mg./100 ml. at various times after dosing | | |
|---|---|---|---|---|---|
| | | | 4 hr. | 7 hr. | 24 hr. |
| 10 | 5.9 | 50 | 0.91 | 0.99 | 0.67 |
| | | 100 | 0.98 | 1.0 | 0.68 |
| | | 400 | 1.4 | 1.7 | 1.4 |

* The interval between doses was four days.

TABLE II

BLOOD-CONCENTRATIONS PRODUCED BY REPEATED DOSES OF 8388 IN MONKEYS

| No. of days after 1st dose | 24 hr. after dosing | 6 hr. after dosing | | No. of days after 1st dose | 24 hr. after dosing* | 6 hr. after dosing | |
|---|---|---|---|---|---|---|---|
| | 8388 mg./100 ml. | 8388 mg./100 ml. | Blood urea mg./100 ml. | | 8388 mg./100 ml. | 8388 mg./100 ml. | Blood urea mg./100 ml. |
| Monkey 9. 500 mg./kg. once daily | | | | Monkey 10. 500 mg./kg. twice daily | | | |
| 44 | | 1.3 | | 13 | | 2.2 | |
| 50 | | 1.7 | 35 | 19 | | 2.8 | 48 |
| 58 | 0.83 | 1.6 | | 27 | 2.6 | 2.9 | |
| 65 | 1.1 | 2.7 | 50 | 34 | 3.9 | 4.2 | 52 |
| 72 | 1.1 | 1.9 | | 41 | 2.6 | 2.2 | 54 |
| | | | | 56 | 2.0 | 2.4 | |
| † Monkey 20. 500 mg./kg. once daily | | | | † Monkey 21. 500 mg./kg. once daily | | | |
| 9 | | 1.5 | | 9 | | 1.1 | |
| | | | | 14 | | 1.7 | |
| 42 | | 2.8 | | | | | |

* For animals dosed twice daily the interval was 18 hr. after the evening dose.
† Infection with tuberculosis: see Table VII.

TABLE III

CONCENTRATIONS OF 8388 OBTAINED AT POST-MORTEM EXAMINATION OF MONKEYS

| | Monkey 9 500 mg. 8388/kg. once daily. Killed 78 days. mg. 8388/100 ml. or 100 g. | Monkey 10 500 mg. 8388/kg. twice daily. Killed 78 days. mg. 8388/100 ml. or 100 g. |
|---|---|---|
| Blood | 1.2* | 2.5† |
| C.S.F. | 0.35 | — |
| Liver | 5.1 | 6.5 |
| Kidney | 3.4 | 4.2 |
| Thyroid | 1.1 | 11.0 |
| Lung | 4.1 | 2.5 |
| Stomach wall | 7.6 | — |
| Heart muscle | 1.6 | — |
| Abdominal muscle | 1.4 | — |
| Fat | 5.4 | 4.6 |

* Blood urea = 52 mg./100 ml. † Bilirubin = 0.1 mg./100 ml.

Monkey 9 was given this dose once daily and monkey 10 twice daily for 78 days (Table II). In the former animal residual blood-concentrations of about 1 mg./100 ml. were obtained before dosing, and levels of about 1.8 mg./100 ml. six hours after dosing. In monkey 10, dosed twice daily, concentrations about twice as high were obtained. The blood picture showed no significant change in either monkey. *Post mortem*, the organs and tissues contained concentrations of drug two to six times higher than in the blood, but the concentration in the cerebrospinal fluid was only about one-quarter of that in the blood (Table III). The liver of monkey 10, that had received the drug twice daily, had a " nutmeg " appearance and showed fatty degeneration on histological examination.

*Administration of* 8388 *and* 9311 *to normal dogs*

The blood-concentrations produced by single doses of 8388 and 9311 given to dogs are shown in Table IV. As in other animals, lower concentrations were produced by 9311 than 8388. Dog 63 was given 500 mg. 8388 per kg. twice daily.

TABLE IV

BLOOD-CONCENTRATIONS PRODUCED BY SINGLE DOSES OF 9311 OR 8388 IN DOGS

| Drug and dose | Dog No. | mg. drug/100 ml. blood at various times | | | | |
|---|---|---|---|---|---|---|
| | | 1 hr. | 2 hr. | 4 hr. | 7 hr. | 24 hr. |
| 8388 .. .. .. | 70 | 0.44 | 0.80 | 1.40 | 1.50 | 0.82 |
| 500 mg./kg. .. .. | 67 | 0.58 | 0.67 | 0.82 | 1.10 | 0.27 |
| 9311 .. .. .. | 78 | 0.20 | 0.39 | 0.10 | 0.06 | 0.07 |
| 500 mg./kg. .. .. | 67 | 0.15 | 0.34 | 0.65 | 0.33 | 0.03 |

The blood-concentrations obtained are shown in Table V. The dog died after 13 days. The liver was slightly yellow, and on histological examination showed necrosis and fatty degeneration of the polygonal cells. The glomeruli of the kidney were congested and the tubular epithelium was desquamated. The lungs showed oedema and congestion.

Dog 64 was dosed with 500 mg. 8388 per kg. once daily. In this and the other dogs dosed with 8388 or 9311 the haemoglobin concentration fell slightly, the numbers of lymphocytes fell, and neutrophils increased. These changes are similar to those noted by Mertens and Bunge (1950) in man. The blood-concentrations obtained are shown in Table V. The dog was killed on the 29th day. *Post mortem*, the liver was found to be slightly yellow and on histological examination it showed many areas of necrosis, excess fat, and deficient glycogen. In the kidney there was desquamation of the epithelium of the convoluted tubules. The lungs were congested. No other changes were seen.

Dog 67, which six days previously had been given a single dose of 500 mg. 8388 per kg., was given 100 mg./kg. twice daily. Eleven days later it was very jaundiced, the blood-concentration of 8388 at six hours after dosing was 1.5 mg./100 ml., and, 24 hours after, 1.76 mg./100 ml. (Table V). The blood-urea concentration was 56 mg./100 ml. The dog was killed after 13 days' dosing, when the bilirubin-

TABLE V

BLOOD-CONCENTRATIONS PRODUCED BY REPEATED DAILY DOSES OF 8388 AND 9311 IN DOGS

| 8388 | | | | | | 9311 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dog No. | No. of days after 1st dose | 24 hr. after dosing* | | 6 hr. after dosing | | Dog No. | No. of days after dose | 24 hr. after dosing* | | 6 hr. after dosing | |
| | | mg./100 ml. | Blood urea | mg./100 ml. | Blood urea | | | mg./100 ml. | Blood urea | mg./100 ml. | Blood urea |
| | 500 mg./kg. twice daily | | | | | | 250 mg./kg. once daily | | | | |
| 63 | 2 | 2.4 | 60 | 2.7 | | 65 | 3 | 0.25 | | 0.82 | 66 |
| | 9 | 1.5 | | 1.9 | 70 | | 4 | 500 mg./kg. once daily | | | |
| | 500 mg./kg. once daily | | | | | | 7 | 0.04 | 61 | 0.53 | |
| 64 | 2 | 1.9 | 52 | 2.0 | | | 12 | 0.32 | | 0.64 | 52 |
| | 9 | 1.1 | | 3.3 | 89 | | 27 | 0.04 | | 0 | 34 |
| | 11 | 2.1 | | 2.8 | 57 | | | | | | |
| | 23 | 1.8 | | 2.7 | 54 | | | | | | |
| | 100 mg./kg. twice daily | | | | | | 250 mg./kg. twice daily | | | | |
| 67 | 11 | 1.5 | 58 | 1.8 | | 66 | 3 | 0.72 | | 1.4 | 60 |
| | | | | | | | 4 | 500 mg./kg. twice daily | | | |
| | | | | | | | 7 | 0.55 | 42 | 0.81 | |
| | | | | | | | 8 | 500 mg./kg. once daily | | | |
| | | | | | | | 12 | 1.3 | | 1.8 | 27 |

* For animals dosed twice daily the interval was 18 hr. after the evening dose.

TABLE VI

CONCENTRATIONS OF 8388 AND 9311 AT POST-MORTEM EXAMINATION OF DOGS

| | 8388. mg./100 ml. or 100 g. | | | 9311. mg./100 ml. or 100 g. | |
|---|---|---|---|---|---|
| | Dog 63 500 mg./kg. twice daily Died 13 days | Dog 64 500 mg./kg. once daily Killed 29 days | Dog 67 100 mg./kg. twice daily Killed 13 days | Dog 65 500 mg./kg. once daily Killed 52 days | Dog 66 500 mg./kg. once daily Killed 53 days |
| Blood | 2.2 | 2.8 | 1.6 | 0.77 | 0.73 |
| C.S.F. | | 0.35 | | | |
| Liver | 4.1 | 6.7 | 2.2 | 1.9 | 1.9 |
| Kidney | 3.5 | 4.6 | 2.3 | 1.7 | 1.9 |
| Thyroid | | 6.5 | 3.1 | 50 (?) | 1.7 |
| Lung | 4.7 | 21.0 | 2.4 | 0.74 | 2.1 |
| Stomach wall | | 6.5 | | | |
| Heart muscle | | 4.2 | | | |
| Abdominal muscle | | 4.6 | | | |
| Fat | | 1.2 | | | |
| Blood urea | | 66.0 | 86.0 | 61.0 | |
| Bilirubin | | | 17.2 | 0.12 | 0.8 |

concentration was 17.2 mg./100 ml. and the blood urea 86 mg./100 ml. The various blood-concentrations of 8388 are shown in Table V and tissue-concentrations in Table VI. The general nutritional condition of the dog was good, but the skin and fatty tissues were yellow and the liver again showed changes of acute yellow atrophy. There was some desquamation of the tubular epithelium in the kidney.

Dog 65 was dosed once daily and dog 66 twice daily with 250 mg. 9311 per kg. for three days, when the doses were increased to 500 mg./kg. Five days later dog 66 was unwell and it was dosed only once daily. The dogs were killed after 53 days' dosing. During most of this period they had each received 500 mg./kg. once daily. The blood-concentrations obtained are shown in Table V and the tissue-concentrations obtained at post-mortem examination in Table VI. No macroscopic changes were observed in the organs of dogs 65 and 66, but on histological examination the liver of dog 66 showed fairly marked fatty degeneration and absence of glycogen. There was desquamation of the tubular epithelium of the kidney. Dog 65 showed similar but slightly less marked changes.

*Administration of* 9311 *and* 8388 *to tuberculous dogs*

The observations of Innes (1940) and of Lovell and White (1940) indicate that healed lesions of tuberculosis do not occur in the dog, and in the twenty-seven cases described by Lovell and White lesions were present in both the abdominal and thoracic cavity of twenty-one. In three, lesions were confined to the abdominal, and in three to the thoracic cavity.

Experimental infection induced by the intratracheal injection of tubercle bacilli is quite different from the natural disease. We have found that when 10 mg. (moist weight) of human tubercle bacilli (Weybridge C) are injected intratracheally, under anaesthesia, localized lesions, which become fibrocaseous, develop in the lungs. When established these lesions are essentially chronic, and show a striking resemblance to the phthisical lesions which may develop soon after a primary tuberculous infection in young human adults (Francis, Pagel, and Stewart, 1950; see also Gunn and Sheehy, 1950). Up to the first week after infection the lesion consists of a lobar pneumonia. The lymph-spaces are distended and the alveoli packed with cells which are chiefly of the large mononuclear type. The alveolar walls are swollen but remain intact, and if treatment with streptomycin is begun at this stage almost complete resolution may occur. In the absence of streptomycin therapy necrosis, caseation, and even early cavitation develop within two to three weeks. Treatment with streptomycin at this stage causes resolution of the peripheral parts of the lesions where necrosis has not occurred, but cavities form in the central parts. It would seem that no drug could prevent this cavity formation; nevertheless the walls of the cavities in treated dogs undergo organization and may become sterile as judged both by cultural and biological tests. Details of the experimental technique, and a description of the course of the disease, together with the results of the two experiments outlined below will be published shortly.

Fifteen dogs were injected intratracheally with tubercle bacilli. After three weeks the dogs were arranged in four groups on the basis of *x*-ray changes, erythrocyte sedimentation rates, and general clinical appearance. One group served as a control, one group (three dogs) was treated with streptomycin, and the other two groups were given 8388 or 9311 (25 mg./kg. once daily). All the dogs receiving 8388

attempted to vomit after the second dose and dyspnoea developed soon afterwards. One dog died six hours after the second dose and another was killed at the same time. The two other dogs that had shown slight symptoms during the day died during the night. Post-mortem examination showed that death was due to pulmonary oedema. All the dogs dosed with 9311 were alive on the third morning, and dogs 80 and 82, which appeared to be in the best condition, received a third dose. Dog 82 died at 5 p.m. after showing similar symptoms to the other dogs. The remaining dogs that had received 9311 survived.

These results were quite unexpected, as larger doses had been tolerated by non-tuberculous dogs, and the experiment, which was designed to compare the action of 8388 and 9311 with that of streptomycin, had to be altered. All dogs except the controls were given 40 mg. streptomycin per kg. once daily. In addition, two were given 5 mg. 9311 per kg. once daily, and two 300 mg. *p*-aminosalicylic acid per kg. each day divided into two doses. These treatments were continued for 150 days (176 days after infection). At post-mortem examination arbitrary scores, which have been given to the pathological lesions, were slightly greater in the dogs treated with streptomycin plus *p*-aminosalicylic acid or with streptomycin and 9311 than with streptomycin alone. The dose of 9311 used (5 mg./kg.) produces maximum blood-concentrations of about 0.05 mg./100 ml., which is probably rather lower than the concentrations produced in man by doses of 3 mg./kg., but estimations at these low concentrations are inaccurate.

In a second experiment the following treatments were given once daily, beginning fifteen days after infection:

| | |
|---|---|
| Streptomycin, 20 mg./kg. | |
| ,, 10 mg./kg. | |
| ,, ,, | plus 4: 4'diaminodiphenylsulphone, 20 mg./kg. |
| ,, ,, | ,, *p*-aminosalicylic acid, 300 mg./kg. |
| ,, ,, | ,, 9311, 5 mg./kg. |

In this experiment 9311 caused no toxic signs of any sort. There was no evidence that the action of streptomycin at 10 mg./kg. was enhanced by any of the other drugs, and none of the treatments produced such good results as 20 mg./kg. of streptomycin alone. It will thus be seen that, although the first therapeutic experiment on dogs cannot be regarded as conclusive because of the early toxic symptoms, similar results were obtained in both experiments.

*Administration of* 8388 *to tuberculous monkeys*

Natural tuberculosis in monkeys has been studied by us during the past three years. A primary complex and subsequent disease closely resembling that observed in natural tuberculosis can be produced in monkeys by the intranasal inoculation of small doses of human tubercle bacilli (Francis, 1950). Although the monkey is more susceptible to tuberculosis than most people now living in urban communities are, it may be said that tuberculosis in the monkey more closely resembles human tuberculosis than tuberculosis in any other animal that we have studied. This conclusion is based on histological observations of the lesions and the fact that " secondary " or " reinfection " lesions, as in man, produce very little change in the regional lymph-nodes.

The experiment about to be described was carried out at an early stage in this work and a larger infecting dose was used than would now be employed. Each of nine monkeys was tested for tuberculin sensitivity by injecting 0.01 mg. of tuberculin purified protein derivative intrapalpebrally. None of the monkeys reacted. A 14-day culture of human tubercle bacilli (Weybridge C) was suspended in 5 per cent serum-saline to give 0.01 mg. in 0.4 ml. The monkeys were narcotized with nembutal and anaesthetized with ether until the swallowing reflex was abolished. A volume of the bacillary suspension to give a dose of 0.003 mg. bacteria per kg. body weight was then instilled intranasally into the right nostril with the monkey lying on its left side and, two minutes later, the same volume was instilled into the left nostril with the monkey on its right side (masks and goggles are worn during these manipulations); that is, each monkey received about 0.01 mg. of tubercle bacilli into each nostril.

The monkeys recovered from the anaesthesia and were all well the following day. They remained well for 13 days, when four showed slight malaise and anorexia. All the monkeys were *x*-rayed (without anaesthesia). The following day all monkeys showed varying degrees of malaise and anorexia, and four were coughing. The monkeys were then grouped on the basis of weight, general condition, and *x*-ray photographs. Dosing began 14 days after infection. Group 1 was untreated, and the monkeys died 9, 14, and 20 days respectively after treatment of the other monkeys had begun. Group 2 received 40 mg. streptomycin per kg. once daily by injection, and Group 3 received 500 mg. 8388 per kg. once daily by mouth. Dosing continued until the 11th week after inoculation. Within four days of commencing treatment the three monkeys treated with streptomycin showed some improvement in general condition. Treatment with 8388 kept the other three monkeys alive but weak and slightly jaundiced. Treatment with both drugs was stopped 11 weeks after inoculation. Two weeks later the monkeys were killed; during this period the monkeys that had been dosed with 8388 improved considerably in condition and lost their jaundiced appearance. It was evident that a single dose of 500 mg. 8388 per kg. once daily was more toxic to the three tuberculous monkeys than it had been to the normal monkey (No. 9), and probably more toxic than twice this dose given to normal monkey No. 10.

*Assessment of results*

At post-mortem examination the lungs were inflated and fixed by introducing formol-saline into the trachea. Sagittal sections were cut and the lesions in the lungs and in the bronchial lymph-nodes assessed as shown below:

*Lungs (right or left):*

| Extent of change | Score of area showing morbid change | Score of caseation | Score of cavitation |
|---|---|---|---|
| Less than $\frac{1}{3}$ of cut surface .. .. | 1–2 | 1 | 1–2 |
| $\frac{1}{3}$–$\frac{2}{3}$ of cut surface .. .. | 3–4 | 2 | 3–4 |
| More than $\frac{2}{3}$ of cut surface .. | 5–6 | 3 | 5–6 |

## TABLE VII

THE EFFECT OF STREPTOMYCIN AND *p*-ETHYLSULPHONYLBENZALDEHYDE THIOSEMICARBAZONE (8388) ON TUBERCULOSIS IN THE MONKEY

Monkeys inoculated intranasally into both lungs with 0.01 mg. human tubercle bacilli (Weybridge C) under ether-anaesthesia

| Treatment | Exp. No. | Time to death in days (1) | Macroscopic pathological assessment | | | | | | | | | | | Weights | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Lungs | | | | | Bronchial lymph-nodes | | | | Total score: lungs + bron. lymph nodes | Other organs (3) | Total scores | 0 days | 14 days | End of exp. | Difference |
| | | | (2) | Area affected | Degree of caseation | Extent of cavitation | Total score | Degree of enlargement | Degree of caseation | Extent of cavitation | Total score | | | | | | | |
| Streptomycin—40 mg./kg. once daily | 14 | K. 85 | L | 3 | 1 | 3 | 12 | 1 | 0 | 0 | 2 | 14 | 0 | 14 | 3.6 | 3.4 | 3.4 | −0.2 |
| | | | R | 2 | 0 | 3 | | 1 | 0 | 0 | | | | | | | | |
| | 15 | K. 87 | L | 3 | 1 | 2 | 13 | 1 | 1 | 0 | 4 | 17 | 0 | 17 | 3.6 | 3.4 | 3.18 | −0.32 |
| | | | R | 3 | 1 | 3 | | 1 | 1 | 0 | | | | | | | | |
| | 16 | K. 87 | L | 3 | 1 | 2 | 11 | 2 | 1 | 0 | 5 | 16 | 0 | 16 | 3.2 | 2.9 | 2.98 | −0.22 |
| | | | R | 4 | 1 | 0 | | 1 | 1 | 0 | | | | | | | | |
| | Aver. scores | | | 3.0 | 0.8 | 2.2 | 12 | 1.2 | 0.7 | 0 | 3.7 | 15.7 | 0 | 15.7 | 3.5 | 3.2 | 3.2 | −0.3 |
| 8388—500 mg./kg. once daily | 20 | K. 87 | L | 5 | 0 | 3 | 16 | 1 | 1 | 0 | 5 | 21 | 0 | 21 | 3.4 | 3.0 | 2.98 | −0.42 |
| | | | R | 4 | 1 | 3 | | 1 | 2 | 0 | | | | | | | | |
| | 21 | K. 83 | L | 5 | 1 | 4 | 21 | 1 | 1 | 0 | 4 | 25 | 1 | 26 | 3.1 | 3.6 | 3.07 | −0.03 |
| | | | R | 5 | 1 | 5 | | 1 | 1 | 0 | | | | | | | | |
| | 22 | K. 83 | L | 6 | 0 | 6 | 22 | 3 | 2 | 2 | 13 | 35 | 0 | 35 | 3.2 | 2.9 | 2.5 | −0.7 |
| | | | R | 5 | 0 | 5 | | 2 | 2 | 2 | | | | | | | | |
| | Aver. scores | | | 5 | 0.5 | 4.3 | 19.7 | 1.5 | 1.5 | 0.7 | 7.3 | 27 | 0.3 | 27.3 | 3.23 | 3.2 | 2.9 | −0.4 |
| Controls | 11 | D. 28 | L | 4 | 2 | 2 | 16 | 2 | 2 | 2 | 10 | 26 | 0 | 26 | 5.1 | 4.66 | Dead 27 days | |
| | | | R | 4 | 2 | 2 | | 2 | 2 | 0 | | | | | | | | |
| | 12 | D. 23 | L | 5 | 2 | 0 | 11 | 3 | 1 | 0 | 7 | 18 | 0 | 18 | 3.0 | 2.84 | Dead 22 days | |
| | | | R | 3 | 1 | 0 | | 1 | 2 | 0 | | | | | | | | |
| | 13 | D. 34 | L | 5 | 1 | 2 | 14 | 2 | 1 | 0 | 7 | 21 | 3 | 24 | 3.0 | 2.7 | Dead 33 days | |
| | | | R | 5 | 1 | 0 | | 2 | 2 | 0 | | | | | | | | |
| | Aver. scores | | | 4.3 | 1.5 | 1.0 | 13.7 | 2.0 | 1.7 | 0.3 | 8.0 | 21.7 | 1.0 | 22.7 | 3.7 | 3.4 | | |

(1) K = killed, D = died. (2) L = left, R = right. (3) The following lesions can each be given a total score of 5 if severe: pleurisy or pleural fluid, peritonitis or ascites, lesions in liver, lesions in spleen, lesions in kidney—possible total 25.

*Bronchial lymph-nodes (right or left):*

| Degree of enlargement | Score | Caseation | Score | Cavitation | Score |
|---|---|---|---|---|---|
| Slight .. .. | 1 | Less than $\frac{1}{3}$ of cut surface | 1 | Less than $\frac{1}{3}$ of cut surface | 1–2 |
| Moderate .. | 2 | $\frac{1}{3}$–$\frac{2}{3}$ of cut surface | 2 | $\frac{1}{3}$–$\frac{2}{3}$ of cut surface | 3–4 |
| Gross .. | 3 | More than $\frac{2}{3}$ of cut surface | 3 | More than $\frac{2}{3}$ of cut surface | 5–6 |

The number of tubercle bacilli in microscopic sections was assessed as follows:

| *Code* | *Number of bacilli in tissues* |
|---|---|
| A.F.B., 1 | Scanty, difficult to find. |
| „ 2 | Isolated bacilli found easily. |
| „ 3 | Small clumps found easily, or fairly numerous isolated bacilli. |
| „ 4 | Numerous small clumps and isolated bacilli. |
| „ 5 | Large clumps in all diseased parts. |
| „ 6 | Masses, as seen in leprosy or infection with the vole acid-fast bacillus. |

Sagittal sections of the lungs from a representative monkey in each group are shown in Figs. 1–3, and the pathological scores in Table VII.

In all of the untreated monkeys, which died early in the experiment, there was an acute tuberculous bronchopneumonia with caseous enlargement of the bronchial lymph-nodes (Fig. 1). Macroscopic tuberculous lesions were present in the liver and spleen of monkey 13 that lived the longest. The area affected in the streptomycin monkeys was less than that in the controls, caseation was slight, but there was a moderate amount of cavitation; lesions in the lymph-nodes were minimal. In the monkeys treated with 8388 the area affected was larger, and the amount of cavitation was extraordinary, the lungs of monkey 22 consisting of little more than two sacs of watery fluid containing yellow flecks of caseous pus. It appeared at first that all the changes might not be due to tuberculosis, but the fluid and cavity walls contained acid-fast bacteria, and no tapeworm scolices or other parasites were found. Post-mortem examination of the other monkeys demonstrated a continuous series of changes leading up to those in monkey 22, so there could be no doubt about the nature of the lesions.

*Histological examination*

*Untreated monkeys.*—The lungs showed widespread consolidation. The centres of the consolidated areas were mainly caseous; in some parts, the alveolar walls could be distinguished, but in other parts softening and necrosis were evident. The caseous areas were surrounded by broad zones of cellular consolidation, where the alveoli were packed with polymorph and mononuclear phagocytes. Peripherally, the alveoli contained an albuminous exudate and scanty cells, and this zone merged outwardly into normal lung tissue or adjacent lesions. Acid-fast bacilli were numerous in the cellular zones and especially at the edges of the caseous zones, where they could be seen with the 2/3rds objective. Strands of fibrous tissue were seen in lesions, but there was no encapsulation. Deposition of calcium had occurred in one monkey. Representative lesions are shown in Figs. 1 and 4.

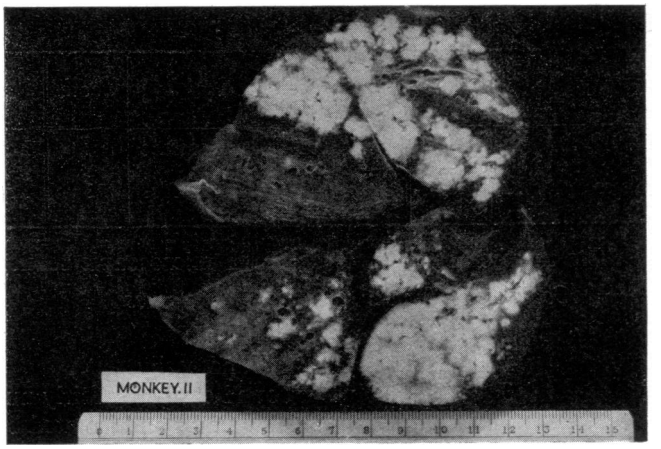



FIG. 1.—Lungs of monkey 11; control. Solid white areas containing caseous and necrotic foci and inflammatory cells.

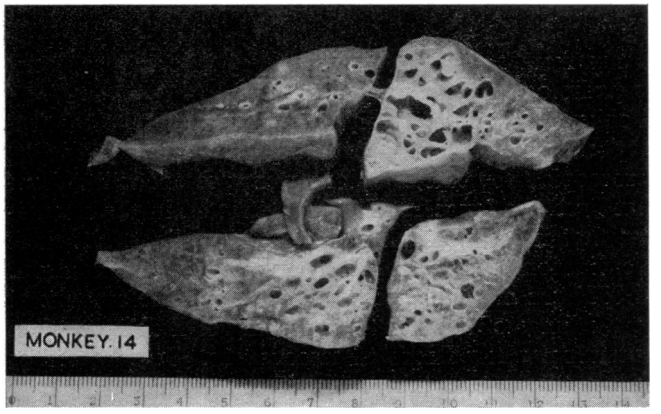



FIG. 2.—Lungs of monkey 14; streptomycin. Cavities surrounded by a small amount of firm white tissue.

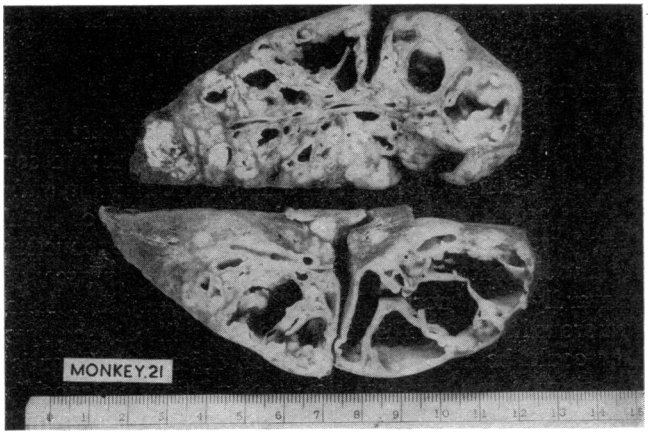



FIG. 3.—Lungs of monkey 21. Very large cavities and smaller caseous areas.

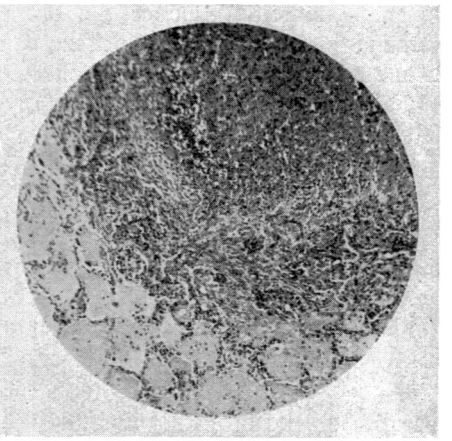


FIG. 4.—Lung of monkey 13; control. Central necrotic focus surrounded by a zone of epithelioid and macrophage cells. Surrounding alveoli filled with exudate. H.E. × 60.

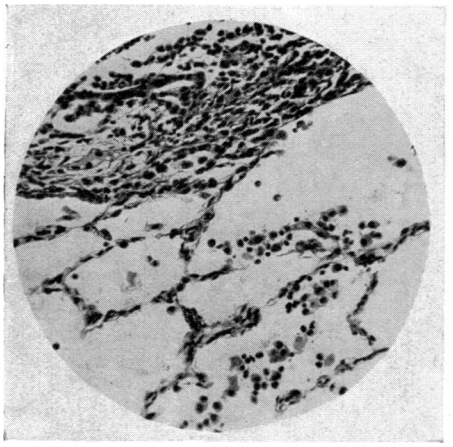


FIG. 5.—Lung of monkey 14; streptomycin. Well-defined edge to lesion, cells acquiring a fibroblastic appearance. Surrounding alveoli free of exudate, but they do contain "alveolar phagocytes." H.E. × 175.

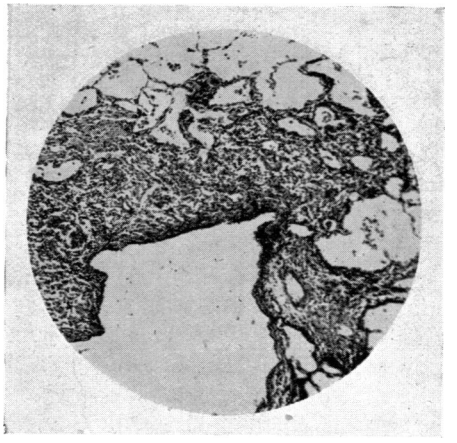


FIG. 6.—Lung of monkey 14; streptomycin. Cavity partly lined by bronchial epithelium and surrounded by a zone of lymphocytes and macrophages. H.E. × 60.

The lymphoid tissue of the bronchial lymph-nodes was largely replaced by epithelioid cells and necrotic tissue containing fairly numerous acid-fast bacilli (2). In one monkey there were several foci of epithelioid cells in the liver, another had a focus in the spleen, and in monkey 13 there were numerous epithelioid foci in both the liver and spleen (acid-fast bacilli, 2).

*Monkeys treated with streptomycin.*—The picture was quite different from that in the untreated monkeys. In monkey 14 the cavities in the lung were lined by fibroblastic cells, although some areas still contained neutrophils and mononuclear phagocytes. Representative lesions are shown in Figs. 5 and 6. The bronchial lymph-nodes were normal, except for a few foci of epithelioid cells. Two resolving epithelioid foci were seen in the liver but no lesions in any other organs. No acid-fast bacilli were observed.

The changes in the other two monkeys were rather more severe, with some caseation in the bronchial lymph-nodes. In addition to cavities in the lungs there were fairly extensive areas of necrotic tissue, but only one acid-fast bacillus was seen in a cavity wall. The tuberculous lesions were fairly sharply demarcated from normal lung by a fibroblast layer with only occasional extensions of interstitial pneumonia. The cavity walls consisted of unorganized epithelioid and neutrophil cells, only small portions being lined by epithelium.

*Monkeys treated with* 8388.—The changes were rather similar to those in the streptomycin monkeys, except that acid-fast bacteria could be found fairly easily (1–2) and the lesions were less well separated from normal lung tissue. In monkey 22 there was very little normal lung tissue left. The bronchial lymph-nodes in all monkeys showed more severe changes than in the monkeys treated with streptomycin, there being fairly extensive caseous zones containing a few acid-fast bacteria. Small epithelioid foci were observed in the liver. Apart from these tuberculous changes monkeys 20 and 22 showed marked adenomatous enlargement in the thyroids: the acini were dilated and irregular, the glandular epithelium grossly distorted by hyperplasia and metaplasia, the colloid deficient or absent, and some areas were haemorrhagic. The thyroids of monkey 21 were not examined, but similar changes were not observed in the thyroids of monkeys in the other group or in other monkeys. Fatty degenerative changes in the liver, similar to those observed in the uninfected monkeys but relatively more extensive, were also present. Minor changes of this type were noted in the liver of one of the streptomycin-treated monkeys and also in the controls.

*Administration of* 8388 *to tuberculous guinea-pigs*

Fifty-two guinea-pigs were inoculated intramuscularly with 0.005 mg. of human tubercle bacilli (Weybridge C). Thirty-eight days later the guinea-pigs were weighed and six representative animals killed. They had fairly advanced lesions of tuberculosis, some having large necrotic areas in the spleen. The average score of the lesions was 2.6. The remaining guinea-pigs were divided into the three groups shown in Table VIII. It will be seen from this Table that 8388 slightly increased the survival time of the guinea-pigs, and streptomycin had a greater effect. An assessment of lesions at death also placed the groups in the same order. It will

## TABLE VIII

THE EFFECT OF STREPTOMYCIN AND *p*-ETHYLSULPHONYLBENZALDEHYDE THIOSEMICARBAZONE (8388) ON TUBERCULOSIS IN THE GUINEA-PIG

The guinea-pigs were injected intramuscularly with 0.005 mg. human tubercle bacilli (Weybridge C) and treated with streptomycin and *p*-ethylsulphonylbenzaldehyde thiosemicarbazone (8388) 40 days later

| Drug and dose | Survival time* | Macroscopic pathological score | | | | | | Drug and dose | Survival time* | Macroscopic pathological score | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Spleen | Liver | Lungs | Bronch. lymph node | Local lesions | Aver. | | | Spleen | Liver | Lungs | Bronch. lymph node | Local lesions | Aver. |
| Animals killed 38 days after infection, i.e., before dosing commenced | | 4 | 4 | 2 | 2 | 3 | 3.0 | 8388—0.25% in food | 18 | 2 | 0 | 2 | 3 | 3 | 2.0 |
| | | 3 | 2 | 2 | 2 | 3 | 2.4 | | 21 | 4 | 1 | 1 | 2 | 3 | 2.2 |
| | | 2 | 3 | 2 | 2 | 3 | 2.4 | | 21 | 1 | 1 | 3 | 2 | 3 | 2.0 |
| | | 3 | 1 | 1 | 1 | 2 | 1.6 | | 19 | 2 | 1 | 0 | 1 | 2 | 1.2 |
| | | 5 | 5 | 2 | 3 | 3 | 3.6 | | 22 | 3 | 2 | 1 | 2 | 3 | 2.2 |
| | | 3 | 1 | 1 | 4 | 4 | 2.6 | | 27 | 2 | 1 | 1 | 3 | 3 | 2.0 |
| | | | | | | | | | 20 | 2 | 1 | 2 | 2 | 3 | 2.0 |
| | | | | | | | | | 25 | 1 | 0 | 0 | 1 | 3 | 1.0 |
| | | | | | | | | | 19 | 2 | 2 | 0 | 2 | 4 | 2.0 |
| | | | | | | | | | 18 | 2 | 2 | 2 | 2 | 3 | 2.2 |
| | | | | | | | | | 21 | 3 | 0 | 1 | 2 | 3 | 1.8 |
| | | | | | | | | | 21 | 3 | 1 | 1 | 3 | 4 | 2.4 |
| | | | | | | | | | 21 | 3 | 1 | 1 | 3 | 3 | 2.2 |
| | | | | | | | | | 24 | 5 | 3 | 1 | 2 | 3 | 2.8 |
| Aver. | | 3.33 | 2.66 | 1.66 | 2.33 | 3.0 | 2.6 | Aver. | 21.2 | 2.5 | 1.14 | 1.14 | 2.14 | 3.07 | 2.0 |
| Controls | 19 | 3 | 0 | 1 | 5 | 4 | 2.6 | Streptomycin—20 mg./kg. subcutaneously twice daily | 22 | 4 | 2 | 1 | 1 | 3 | 2.2 |
| | 18 | 5 | 5 | 4 | 5 | 5 | 4.8 | | 22 | 1 | 0 | 0 | 1 | 3 | 1.0 |
| | 20 | 4 | 2 | 2 | 4 | 5 | 3.4 | | 20 | 1 | 0 | 0 | 1 | 3 | 1.0 |
| | 18 | 5 | 5 | 5 | 5 | 5 | 5.0 | | 28 | 2 | 3 | 3 | 2 | 3 | 2.6 |
| | 15 | 3 | 1 | 1 | 3 | 4 | 2.4 | | 19 | 1 | 0 | 0 | 2 | 2 | 1.0 |
| | 20 | 5 | 5 | 5 | 5 | 5 | 5.0 | | 39 | 2 | 0 | 1 | 1 | 3 | 1.4 |
| | 18 | 5 | 5 | 3 | 3 | 5 | 4.2 | | 18 | 1 | 1 | 0 | 1 | 4 | 1.4 |
| | 18 | 3 | 0 | 1 | 3 | 3 | 2.0 | | 39 | 1 | 0 | 0 | 0 | 3 | 0.8 |
| | 12 | 2 | 4 | 1 | 3 | 2 | 2.4 | | 18 | 3 | 2 | 1 | 3 | 3 | 2.4 |
| | 18 | 5 | 5 | 5 | 5 | 5 | 5.0 | | 26 | 2 | 2 | 1 | 2 | 3 | 2.0 |
| | 17 | 3 | 1 | 2 | 3 | 4 | 2.6 | | 27 | 3 | 1 | 1 | 2 | 2 | 1.8 |
| | 20 | 5 | 5 | 2 | 4 | 4 | 4.0 | | 28 | 2 | 2 | 1 | 2 | 3 | 2.0 |
| | 20 | 5 | 5 | 4 | 5 | 5 | 4.8 | | 15 | 3 | 0 | 1 | 2 | 2 | 1.6 |
| | 18 | 2 | 3 | 3 | 4 | 4 | 3.2 | | | | | | | | |
| Aver. | 17.9 | 3.93 | 3.29 | 2.8 | 4.07 | 4.28 | 3.67 | Aver. | 24.6 | 2.0 | 1.0 | 0.77 | 1.53 | 2.84 | 1.64 |

* Days after commencement of treatment.

be seen that there was no progression of the lesions in any of the treated groups, and it is a little difficult to understand why the streptomycin guinea-pigs died, because there was obviously a regression of the lesions.

## Discussion

The published experimental work on conteben (9311) (Domagk, 1950 and earlier papers) is scanty and not very convincing: Domagk states that " on an egg culture medium, containing *p*-aminobenzoic acid, *p*-aminosalicylic acid shows an inhibition value which lies at 1: 5,000 or even lower, streptomycin shows an inhibition of the growth of tubercle bacilli at from 1: 50,000 to 1: 100,000, while Tb I (9311) in solution still shows it at 1: 300,000. Here we already see the same gradations which are obtained on administration in humans in order to obtain a therapeutic effect in tuberculous infections." It must be pointed out, however, that what happens in the presence of *p*-aminobenzoic acid is largely irrelevant: the sulphonamides are inactive in the presence of *p*-aminobenzoic acid, whereas the acridines are fully active, but this does not prove that the acridines are better therapeutic agents. We found that in egg medium *p*-methoxybenzaldehyde thiosemicarbazone (6057) had about the same activity as *p*-aminosalicylic acid, and 8388 was considerably less active. *p*-Aminosalicylic acid inhibited virulent tubercle bacilli at a dilution of about 1/20,000,000 (it was much less active against avirulent strains). Domagk found an inhibitory dilution of 1/100,000.

From Domagk's Table I it would appear that conteben (9311) is more active weight for weight in tuberculous guinea-pigs than streptomycin. In his experiments (one animal per group) the effect of streptomycin fell off when the dose was reduced below about 300 mg./kg. This is entirely contrary to the findings of Karlson and Feldman (1948), who found that there was no difference in the effect of streptomycin whether one gave 20, 6, or 4 mg. of streptomycin per kg. daily, but below 4 mg. the effect was less. It may be argued that these doses are of an entirely different order from those used by Domagk, but they are of the same order as those used by us and they produced better effects than large doses of the thiosemicarbazones. In addition, Hoggarth and Martin (1950) have shown that the optimum effect that can be obtained with 8388 or 9311 in mice is considerably less than the optimum effect obtained with streptomycin. They also found that the maximum anti-tuberculous effect produced in mice by these compounds is similar, although 9311 has to be given in larger doses than 8388 to achieve its maximum effect. It would seem, therefore, that although many of the experiments in this paper were carried out with 8388, there would have been little essential difference if 9311 had been used. Large doses of 8388 were fairly well tolerated by monkeys, but although blood-concentrations of 1–2 mg./100 ml. were obtained they produced only about half the effect of streptomycin on established tuberculous infection in these animals. A concentration of 0.25 per cent in the food of guinea-pigs (about 200 mg./kg. daily) also produced less effect on established tuberculosis, which is similar to the result obtained by Karlson, Gainer, and Feldman (1950).

In a recent experiment (Francis, 1950) guinea-pigs were infected with $10^{-5}$ mg. of tubercle bacilli into each nostril and killed five weeks later. Under these conditions the average scores of the macroscopic lesions were: controls, 9.9; strepto-

mycin, 4.04; 8388, 1.32. The finding that streptomycin has relatively less effect on the early stages of tuberculosis in the guinea-pig than on established tuberculosis is in accord with the results of Smith, Emmart, and McClosky (1948). It is an interesting fact, but does not alter the main conclusions based on the experiments reported in this paper.

Both 8388 and 9311 were more toxic to dogs than monkeys. When we observed pulmonary oedema and rapid death in tuberculous dogs receiving doses of 25 mg. 9311 or 8388 per kg., we thought that this might be a specific reaction of tuberculous dogs, but in a recent toxicity test a single dose of 100 and even 10 mg. 8388 per kg. produced pulmonary oedema and death in normal dogs. In the same test 100 mg./kg. of 9311 daily produced no symptoms of pulmonary oedema. It will be recalled that α-naphthyl thiourea produces pulmonary oedema and death in rats and other animals (Dubois, Holm, and Doyle, 1946).

Martin (1950) has shown that 9311 or 8388 markedly increase the antituberculous effect of suboptimal doses of streptomycin in mice. No evidence was obtained that doses of 5 mg./kg. of 9311 increased the action of suboptimal doses of streptomycin in the dog. This dose of 9311 probably produces maximum blood-concentrations of 0.05 mg./100 ml. in the dog. The dose of 9311 recommended for man is 3 mg./kg., and in one person who received this dose in our laboratories maximum concentrations of about 0.1 mg./100 ml. were produced.

Published work does not indicate that any thorough studies were made in Germany on the toxicity of the thiosemicarbazones in experimental animals, but Heepe (1949) has reported various toxic symptoms following the use of conteben (9311) in man. These include vague malaise, various dyspeptic manifestations, palpitations, and arrhythmias, dizziness, pain in the eyes and photophobia, exanthematous rashes, a tendency to bleeding, and, in severe cases, loss of weight, granulocytopenia, enlargement of the liver, which may be very great and very rapid, jaundice, convulsions, and coma. Three deaths have occurred, all in children, in all of whom necropsy revealed marked fatty degenerative changes. Individual susceptibility varies greatly and some children may not tolerate even small amounts of the drug. Readministration of the drug after recovery from toxic symptoms may cause no ill effects.

It will be seen that the individual variation and the major symptoms mentioned above are not dissimilar from the various toxic manifestations we have observed in dogs, and the work described in this paper indicates that neither 9311 nor 8388 will be a very safe or potent antituberculous agent in man. It does, however, illustrate the necessity for caution when first using a new drug in man. Monkeys tolerated 500 mg. of 8388 per kg. once daily, and yet the recommended dose of 9311 for human tuberculosis is only 3 mg./kg. and this dose may produce toxic effects. It is generally assumed that the metabolism of the monkey is more closely related to that of man than is the metabolism of other animals, but in this instance it was the dog only that gave an indication of the dose that should be used in man. On the other hand, there are doubtless substances that would be better tolerated by man than by experimental animals.

## SUMMARY

1. Published experimental work indicated that the thiosemicarbazones might be more potent antituberculous agents than streptomycin.

2. Monkeys tolerated doses of 500 mg. *p*-ethylsulphonylbenzaldehyde thiosemicarbazone (8388) per kg. daily, which is more than one hundred times greater than the dose of *p*-acetylaminobenzaldehyde thiosemicarbazone (9311) used in man. This large dose of 8388 maintained levels of 1–2 mg./100 ml. in the monkey, but it had only about half the effect produced by a dose of 40 mg. streptomycin per kg. once daily on experimental tuberculosis in the monkey.

3. A daily intake of about 200 mg. 8388 per kg. had less effect on established tuberculosis in the guinea-pig than 20 mg. streptomycin per kg. given twice daily.

4. Both 9311 and 8388 were more toxic to dogs than monkeys. Two or three doses of 25 mg./kg. once daily caused pulmonary oedema and death of tuberculous dogs. It was first thought that these drugs were more toxic to tuberculous than to non-tuberculous dogs, but a toxicity test showed that 8388 might produce the same symptoms and death in normal dogs. It is evident that there is great individual variation in susceptibility to the thiosemicarbazones in some species.

5. No evidence was obtained that doses of 5 mg. 9311 per kg. daily, which were well tolerated by tuberculous dogs, increased the therapeutic effect of suboptimal doses of streptomycin. Similar negative results were obtained with *p*-aminosalicylic acid and 4:4′diaminodiphenylsulphone.

We are indebted to W. Russell for his assistance with this work, and to A. B. Hallam and E. Young for the photographs.

## References

Domagk, G. (1950). *Amer. Rev. Tuberc.*, **61**, 8.
Dubois, K. P., Holm, L. W., and Doyle, W. L. (1946). *Proc. Soc. exp. Biol., N.Y.*, **61**, 102.
Francis, J. (1950). To be published.
Francis, J., Pagel, W., and Stewart, G. T. (1950). Demonstration to Pathological Society.
Gunn, F. D., and Sheehy, J. J. (1950). *Amer. Rev. Tuberc.*, **61**, 77.
Heepe, F. (1949). *Mon. f. Kinderheilkunde*, **97**, 349.
Hoggarth, E., and Martin, A. R. (1950). *Brit. J. Pharmacol.*, **5**, 188.
Hoggarth, E., Martin, A. R., Storey, N. E., and Young, E. H. P. (1949). *Brit. J. Pharmacol.*, **4**, 248.
Innes, J. R. M. (1940). *Vet. J.*, **96**, 42, 96, 391.
Karlson, A. G., and Feldman, W. H. (1948). *Amer. Rev. Tuberc.*, **58**, 129.
Karlson, A. G., Gainer, J. H., and Feldman, W. H. (1950). *Proc. Mayo Clin.*, **25**, 160.
Lovell, R., and White, E. G. (1940). *Brit. J. Tuberc.*, **34**, 117.
Martin, A. R. (1950). To be published.
Martin, A. R., and Stewart, G. T. (1950). *Brit. J. exp. Path.*, **31**, 189.
Mertens, A., and Bunge, R. (1950). *Amer. Rev. Tuberc.*, **61**, 20.
Smith, M. I., Emmart, E. W., and McClosky, W. T. (1948). *Amer. Rev. Tuberc.*, **58**, 112.
Spinks, A. (1949). *Brit. J. Pharmacol.*, **4**, 254.