94% of subjects 36 hours after injection.

Comment. Ease of administration characterized the injections of procaine penicillin in aqueous suspension. Clogging of needles occurred occasionally due to aggregation of procaine penicillin crystals. The frequency of this may be minimized by thorough shaking of the preparation prior to withdrawal into the syringe, immediate injection, and use of 18 or 19 gauge needles for administration. Pain, inflammatory reaction or subcutaneous nodules were not observed at the site of injections. No systemic reactions occurred.

The serum penicillin concentrations following similar doses of vegetable oil suspension and aqueous suspension of procaine penicillin G manifested the same general pattern differing from peanut oil-beeswax preparations by the absence of a high initial peak shortly after injection and the presence of a more uniform serum level during the 24 hours after administration.[1] However, it was noted that with 300,000 units a slightly higher percentage of patients maintained a level of 0.02 units per cc or more but a lower percentage maintained 0.08 units per cc with the aqueous suspension. In using 600,000 units the aqueous suspension group showed a lower percentage throughout of comparable serum levels.

Summary. 1. Administration of 300,000 units of crystalline procaine penicillin G in aqueous suspension produced demonstrable serum penicillin concentrations at 12 hours after administration in all subjects and at 24 hours after injection in 92% of subjects studied.

2. Administration of 600,000 units of this material produced demonstrable serum penicillin concentrations 24 hours after injection in all subjects.

3. Similar patterns of serum penicillin concentrations were obtained for both the oil and the aqueous suspensions of procaine penicillin G although slightly lower values were generally observed with the aqueous suspension.

4. Ease of administration and absence of untoward local or systemic reaction were uniformly observed with the administration of procaine penicillin G in aqueous suspension.

We are indebted to Miss Doris McCarthy for technical assistance.

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The Effect of Streptomycin on Well-Established Experimental Tuberculosis of Mice.*

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Previous studies have shown streptomycin to be effective for the suppression of experimental tuberculosis in mice when treatment was started at the time the animals were infected.[1,2,3] Although well-established tuberculosis of guinea pigs is known[4,5,6,7] to be favorably affected by streptomycin therapy, there is no information available as to the degree of effectiveness of this agent on well-established tuberculous infections of mice.

* This investigation was supported (in part) by a research grant from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service.

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TABLE I.
Amount of Pulmonary Tuberculosis in Mice Sacrificed at 7, 14, and 21 Days After Infection with *M. tuberculosis.*

No. of mice	Time sacrificed in days	Avg amt of gross pulmonary tuberculosis	Avg amt of microscopic pulmonary tuberculosis	Type of pulmonary lesions
15	7	0.8+	1.0+	P
15	14	2.8+	2.0+	P
15	21	3.8+	3.5+	NE and P

P—Proliferative lesions.
NE—Necrotic exudative lesions.
1+—0-10% of lung substance involved.
2+—11-25% of lung substance involved.
3+—26-50% of lung substance involved.
4+—Over 50% of lung substance involved.

Methods. Two separate experiments were conducted using white mice infected intravenously with 0.1 mg of the virulent H37Rv strain of *M. tuberculosis var. hominis.*[2] In the first experiment groups of mice were treated with 3000 μg of streptomycin given daily approximately 6 hours apart in 4 subcutaneous doses of 750 μg each. Treatment of the first group was started the day the mice were infected, while treatment of 3 other groups was started at 7, 14 and 21 days after infection. A fifth group received no streptomycin and served as a control on the other groups. A second similar experiment was performed, with the exception that the streptomycin was administered in 2 daily subcutaneous doses of 1500 μg approximately 8 hours apart. This alteration in technic was employed since other experiments in the meantime had shown that the degree of suppression of the tuberculous process, employing 3000.0 μg streptomycin daily, was essentially the same regardless of whether the streptomycin was given in 2 or 4 daily injections.

All animals, except the controls, were treated with streptomycin for 28 days, following which, therapy was discontinued and the mice observed until death supervened. At autopsy the viscera of all mice were fixed in 3.7% formaldehyde, and the amount of gross and microscopic tuberculosis determined and recorded as previously described.[8,9]

Three other groups of untreated mice were sacrificed at 7, 14 and 21 days respectively after infection with tubercle bacilli. The pathological findings in these animals served as an indication of the amount and type of pulmonary tuberculosis present at the time treatment of the other groups with streptomycin was started.

Results. Table I shows the amount of tuberculosis present in the animals sacrificed at 7, 14 and 21 days. The findings in these animals were similar to those previously reported,[2] and indicated the presence of well-established tuberculosis, even as early as 7 days after infection.

Table II indicates the results obtained in the first experiment with those mice in which treatment was started at 0, 7, 14, and 21 days after inoculation, and with the untreated controls.

Table III shows similar data for the mice used in the second experiment. The only animals included in the tables were those from which complete and suitable tissue specimens were available for pathological examination.

The numbers of animals in the groups in the first experiment (Table II) are rather small, but there is a significant difference between the average time of survival of the

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TABLE II.
Effect of Streptomycin on Well-Established Experimental Tuberculosis in Mice.

No. of mice	Days after infection treatment started	Streptomycin daily (μg)	Avg survival time (days)	Standard deviation	Pulmonary tuberculosis: Gross	Pulmonary tuberculosis: Microscopic	Pulmonary tuberculosis: Type lesion
7	0	3000.0	108.1	±36.1	2.4+	3.0+	P
11	7	3000.0	118.5	±27.2	3.9+	3.7+	P
8	14	3000.0	134.6	±30.6	3.6+	3.5+	P
				P = 0.2			
11	21	3000.0	109.0	±44.3	3.1+	3.5+	P
				P = <0.01			
13		0	38.0	±27.4	3.7+	4.0+	NE

TABLE III.
Effect of Streptomycin on Well-Established Experimental Tuberculosis in Mice.

No. of mice	Days after infection treatment started	Streptomycin daily (μg)	Avg survival time (days)	Standard deviation	Pulmonary tuberculosis: Gross	Pulmonary tuberculosis: Microscopic	Pulmonary tuberculosis: Type lesion
16	0	3000.0	115.3	±31.6	3.8+	4.0+	P
18	7	3000.0	105.1	±29.6	3.5+	3.1+	P
				P = 0.2			
17	14	3000.0	112.6	±15.3	3.6+	4.0+	P
15	21	3000.0	94.6	±58.3	3.8+	4.0+	P
				P = <0.01			
29		0	20.6	± 2.8	3.8+	4.0+	NE

treated animals as compared with the controls.

The numbers of animals employed in the second experiment were larger, and the results were essentially similar to those of the first experiment, even though, as indicated by the average survival time of the controls, the disease was somewhat more severe in the second experiment. Statistical analysis of the data in Table III indicates a significant difference between the average survival time of the control and streptomycin treated animals, but not between the groups of treated animals. The somewhat lower average survival time of the groups of mice in which treatment was delayed 21 days reflects the loss of several animals within 2 days following initiation of therapy. These animals were apparently moribund and derived no benefit from therapy —had they been excluded from the data the average survival time would have been even more similar to those of the 7 and 14 day groups.

The physical appearance of the mice confirmed the statistical data. Most of the mice in the 21-day group had lost from 3 to 6 g in weight, had ruffled fur and looked emaciated and listless. With the exception of the mice that died in 2 days, all the others began to improve in appearance and to gain weight 3 to 5 days following the institution of streptomycin therapy. By the end of 28 days' treatment normal weight had been restored.

All the mice eventually died and the extent of the pulmonary disease did not differ significantly from the control animals. All the treated subjects however exhibited proliferative lesions, whereas the lesions of the

controls were predominantly necrotic exudative. The lesions in the treated animals were extensive, occupied almost all alveoli, but the numbers of tubercle bacilli were considerably reduced as compared with the controls. The livers and spleens of such animals were extremely congested and this, in combination with the great reduction in space for gas exchange in the lungs, suggests that death may have resulted primarily from anoxia and right heart failure rather than from the toxic effects of the tuberculous infection.

These findings indicate that mice may be benefited even late in the course of tuberculosis induced by intravenous inoculation. All groups, whether treated from the day of inoculation or from the 7th, 14th, or 21st days had the same amount of disease on death, and since the average survival times were not significantly different, it would seem that the subjects with most severe disease at the time treatment was started must have obtained relatively greater benefit from streptomycin. The explanation for this phenomenon, however, is not readily apparent.

Conclusion. Streptomycin to the amount of 3000 μg per day had a favorable effect upon the course of experimental tuberculosis in mice when treatment was initiated 7, 14 and 21 days following infection.

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A New Chromosome Stain and Its Relationship to Atypical Cell Proliferation.

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The metabolic transformations of paraphenylenediamine and certain azo-dyes into quinone diimine (Mayer[1]) are accompanied by staining effects on epidermal and other cells. This would indicate that quinone diimine possesses a strong affinity for certain nuclear material to which it becomes attached. Since it seemed probable that the cell proliferations with precancerous and cancerous degeneration produced by aromatic amines are caused by the affinity of their oxidation products for certain vital components of the cell nuclei, we have investigated that part of the nucleus with which quinone diimine combines. We have therefore attempted to stain chromosomes with the oxidation products of paraphenylenediamine. and several of its derivatives.

Certain substitution products of paraphenylenediamine, such as dimethyl- and tetramethyl paraphenylenediamine and also metaphenylenediamine, have been used as microscopic or vital stains by Wurster,[2,3] Unna[4] and Joseph and Wurster.[5] In combination with β-naphthol, dimethyl paraphenylenediamine forms blue indophenol in the presence of oxidative cell enzymes (Ehrlich[6]), a reaction known as the "Nadi reaction". No reference in the literature has been found on similar uses of paraphenylenediamine itself.

Methods. Source of chromosomes. Salivary glands of *Drosophila melanogaster* and *Drosophila robusta* larvae* maintained on molasses agar were prepared in the usual

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