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SURVIVAL AFTER ISONIAZID TREATMENT OF EXPERIMENTAL TUBERCULOSIS IN GUINEA PIGS AND RESISTANCE TO REINFECTION

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INTRODUCTION

In a report to the Illinois State Academy of Science (Sher and Kloeck, 1953) it was indicated that sulfones prolonged the lives of tuberculous guinea pigs. These animals lived three and four times as long as the infected but untreated controls, despite an early progressive tuberculous process which manifested itself even before the prescribed two months of therapy were ended. This prolonged survival was attributed to an immunity which protected the animal against endogenous spread of the disease. However, no attempt was made to determine whether these animals were resistant to an external source of reinfection with virulent tubercle bacilli.

It is known that primary infections in human beings are for the most part spontaneously healed and that the individuals are thereafter more resistant to a subsequent infection than if they had no previous history of tuberculosis (Pinner, 1945). Yet little attention has been given to immunity which might develop when the disease is suppressed by drugs, although this has become increasingly important with the extensive use of antituberculosis agents. It would be interesting to compare immunity developed by spontaneous healing with that produced by chemotherapeutic suppression; but it is difficult if not impossible to formulate experiments for this purpose because spontaneous healing in animals is exceedingly rare, and it is impractical to produce this experimentally. Consequently, our experiments are limited, first, to the determination of the increase in survival time due to administration of the drug, and second, to the determination of resistance to reinfection after therapy is discontinued.

METHODS

The same groups of experimental animals may be used to obtain the essential data for both of the above objectives. Increased survival time is found by calculating the average survival of each group of animals from the date of the primary infection. This survival time was compared with that of the control Group 1B, of Table 1, which was infected simultaneously with the primary infection given to the treated groups. The resulting data were used to calculate the "Index of Protection" as indicated in Table 1.

TABLE 1.—Survival Time Following Primary Tuberculous Infection in Guinea Pigs.

Group	Primary infection	Number animals	Isoniazid therapy	Re-infected	Ave. survival (days)	Observed diff. (days)[1]	Index protection[2]
1A[3]	none	12	none	no	631	520	...
1B[4]	H37RV	22	none	no	111	...	...
2A	H37RV	10	early	no	348	237	3.1
2B	H37RV	12	early	yes	295	184	2.6
3A	H37RV	20	delayed	no	246	135	2.2
3B	H37RV	21	delayed	yes	218	107	2.0

[1] Difference between survival of controls (1B) and the treated animals following primary infection.
[2] Average survival of treated group divided by that of control group (1B).
[3] 1A animals were controls for 1B animals.
[4] The 1B primary infected animals were controls for groups 2A, 2B, 3A and 3B.

The degree of resistance to reinfection, our second objective, was obtained by calculating the average survival time of each group from the date of reinfection which took place 131 days after the primary infection. The observed difference between the survival time of each of these groups and that of Group 1B, Table 2, gave a measure of resistance to reinfection. Group 1B was the control group for the reinfected animals and was given primary infection 131 days later than the control Group 1B of Table 1, to coincide with the reinfection date of Groups 2B, 3B and 4B of Table 2.

Isoniazid was selected for these studies because it is more effective than any drugs in our previous work (Sweany, Sher, and Kloeck, 1946; and Sher and Kloeck, 1946). Earlier observation had shown that no tuberculosis is evident for several

TABLE 2.—Survival Time of Guinea Pigs Following Reinfection With Tuberculosis.

Group	Number animals[1]	Primary infection	Isoniazid therapy	Re-infection	Ave. survival after re-infection (days)[2]	Observed diff. in survival[4]	Index reliability[5]
1A	12	none	none	none	500	374	5
1B	11	none	none	H37RV[3]	126	...	...
2A	8	H37RV	early	none	309	183	3.1
2B	10	H37RV	early	H37RV	193	67	1.9
3A	13	H37RV	late	none	226	100	2.7
3B	9	H37RV	late	H37RV	223	98	2.5
4A	6	BCG	none	none	292	166	2.0
4B	18	BCG	none	H37RV	206	80	2.8

[1] Only animals living two weeks after reinfection included.
[2] Calculated from date of reinfection.
[3] Late primary infection inoculated simultaneously with reinfection.
[4] Difference between survival controls (1B) and treated animals after reinfection.
[5] Observed difference divided by standard error of that difference gives a measure of reliability of observed value.

months after the drug is discontinued. Since it is not known to what degree tuberculosis must be established before immunity develops, it is possible that this failure of tuberculous development might result in no protection at all. Therefore, the rate of development was altered by varying the beginning and the duration of treatment. Therapy was started at intervals varying from 7 to 40 days after the inoculation; the duration varied from 30 to 62 days. On the basis of our observation and for expedience these experiments were regrouped into early (7th to 12th day) and delayed (19th to 40th day) treatment groups. Non-infected normals, non-treated tuberculous controls, and BCG vaccinated animals were included as indicated in Tables 1 and 2. Each treated animal received 20 mg. of isoniazid daily in 1 cc. of 10% sugar solution mixed with fresh green food. The inoculating dose for infection and reinfection was 1/10 mg. wet weight of H37RV, a virulent human strain of tubercle bacilli, injected subcutaneously in the lower left inguinal region. One-tenth mg. of BCG, obtained from the Tice Laboratories, was used to immunize the BCG groups.

Deaths and Complications

Some of the animals included in Table 1 died before the date set for reinfection. This was expected because under our laboratory environment normal animals had a death rate of 15% within this period of time. Since we know of no stresses that would cause early death when treatment was started early enough or when BCG vaccine was given, it was expected that the death rate would be similar for these groups. However, when treatment was delayed, the tuberculous process developed rapidly enough in some animals for death to occur before treatment was started; in others, the disease was too far advanced for effective therapy, and consequently, the death rate was higher, 40%. The death rate of the tuberculous controls was 70%, for this group received no protection whatsoever.

All of the animals receiving the primary infection are included in Table 1. All animals dying within 145 days after the primary infection, or within 2 weeks after the reinfection, were not included in Table 2 because these deaths were considered to be due to the primary infection rather than the reinfection.

Discussion

It is evident, from the observed difference and the indices of protection in Table 1, that isoniazid is an effective antituberculous drug. These figures are high enough to eliminate any need for detailed statistical analysis; it is apparent by inspection that the beneficial effects of the drug, rather than chance variation were responsible for these values. Thus, in Group 3B in which the more severe handicap of delayed treatment and a subsequent reinfection were imposed, the life span was increased two-fold over that of the controls, and the remaining groups showed correspondingly greater observed differences and higher index values. Under the optimum conditions of Group 2A, early treatment with no reinfection, the index of protection was 3.1. On the

other hand, the difference between groups 2A and 1A shows that the treated animals did not live as long as the noninfected normals. Therefore it is evident that chemotherapy, while it is highly effective, did not eradicate the disease; it merely suppressed it.

A comparison of the survival times after reinfection (Table 2) shows a moderate degree of protection against reinfection. The observed difference between the average survival time of any experimental group and that of the controls, 1B, gives a measure of immunity or resistance to reinfection.

The early treated group (2B) demonstrated a moderate degree of protection against reinfection with an observed difference, or increase in survival, of 67 days over that of the control group (1B). This value is statistically significant, and compares favorably with the survival of the challenged BCG vaccinated group in which an observed difference of 80 days was obtained. This latter figure is a measure of the protection obtained by immunization with BCG vaccine. This comparison is all the more favorable in view of the fact that the BCG animals had no virulent tubercle bacilli prior to reinfection.

The value of 98 days obtained with the delayed treated group (3B) *prima facie*, appears to show a greater degree of protection against reinfection. This greater value instead resulted from the fact that Group 3B became a more resistant one because the more susceptible animals were selectively culled out by the 40% mortality prior to reinfection. This factor, if it prevails in the early treated or BCG vaccine groups, does so to a lesser degree because the pre-reinfection death rate was 15%, and these deaths were not due to tuberculosis.

The average survival of 225 days was obtained for the unchallenged delayed group (3A) and 223 days for the challenged group (3B). It may be assumed that these almost identical values arise from the fact that the animals had extensive tuberculosis at the time of reinfection by virtue of the delayed treatment. Therefore, the effect of the reinfection was masked and no measurable difference was obtained.

These were preliminary experiments in which orientation was necessary; therefore, only small numbers of animals were available for each group. Appropriate precautions were taken in each instance in the analysis of the data by estimating the statistical reliability on the basis of the small numbers used.

Conclusions

It may be postulated that resistance to reinfection is immunological in nature. With BCG vaccination this may be due to an immunity induced by a non-virulent organism, whereas, under chemotherapy the tuberculous process was highly suppressed for several months and during this interim the rate of bacterial development was retarded. The animal body was thereby enabled to develop a moderate degree of resistance against an endogenous spread as well as against an externally ad-

ministered infection. The data recorded in Tables 1 and 2 show that benefits are derived from isoniazid therapy because the tuberculous process is suppressed and there is protection against reinfection, subsequent to chemotherapy.

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LITERATURE CITED

PINNER, MAX. 1945. Pulmonary tuberculosis in the adult. Chap. 3 of *Immunological principles*. Charles C. Thomas, Springfield, Ill., 121-123.

SHER, BEN C., and JOHN M. KLOECK. 1946. The combined action of P,P'-diaminodiphenylsulfone and immunization in experimental tuberculosis. Amer. Rev. Tuberc., 53 (3): 250-253.

SHER, BEN C., and JOHN M. KLOECK. 1953. Survival following treatment with sulfones in experimental tuberculosis in guinea pigs. Trans. Ill. Acad. Sci., 46: 63-67.

SWEANY, HENRY C., BEN C. SHER, and JOHN M. KLOECK. 1946. Derivatives of P,P'-diaminodiphenylsulfone and sulfanilamide in experimental tuberculosis. Amer. Rev. Tuberc., 53 (3): 254-263.