

LETTERS TO THE EDITORS

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An Antituberculous Substance in Tuberculous Lymph Nodes

THIS preliminary communication deals with the isolation from bovine tuberculous lymph-nodes of a substance which has an inhibitory effect on bovine and human tubercle bacilli.

Tuberculous lymph-nodes were reduced to pulp, and diluted in a proportion of 10 ml. of Sauton's medium to every 100 gm. of pulp. After seven to eight days at a temperature of $+4^{\circ}\text{C}$., the suspension was squeezed through gauze. The resultant fluid, which was viscous and opaque, was centrifuged until the supernatant was free from coarse particles and relatively clear. Seitz filtration was not used, because preliminary experiments suggested that part or all of the antituberculous property was lost by adsorption on the pad.

Bottles each containing 2 ml. of the extract were inoculated with 1 mgm. of tubercle bacilli, bovine type. The strain was of fairly high virulence, since 0.0001 mgm. regularly produced tuberculosis in guinea pigs in a six-week period. Some bottles were left at room temperature for periods of 48 hr., four days and seven days respectively. Others were incubated at 37°C . for 24 hr. and for 48 hr. Control suspensions prepared from tubercle bacilli suspended in saline solution were also exposed at room temperature and at 37°C . for similar periods of time. After incubation, all samples were then inoculated, each into two guinea pigs, and these were killed after six weeks. The extent of the tuberculous changes, where present, was estimated and an examination made for the presence of tubercle bacilli.

It was found that all the guinea pigs receiving the suspension in the extract kept either at room temperature for four or seven days or for 48 hr. at 37°C . were free from visible signs of disease, whereas controls inoculated with the saline suspension showed extensive generalized tuberculosis. Contact at 37°C . for 24 hr. or at room temperature for 48 hr. was not sufficient to inactivate tubercle bacilli. The extract of tuberculous lymph-nodes was equally active against a human strain (*H37Rv*).

In order to exclude the possibility that the tubercle bacilli were still living but were prevented from exerting a pathogenic effect through a protective coating of products of the lymph-node suspension, bacilli exposed to extract for six days at room temperature were washed three times with saline. Guinea pigs inoculated with the washed bacilli did not show any sign of tuberculosis when killed four weeks later; but those killed eight weeks later showed tuberculous lesions in the regional lymphatic nodes. Controls showed extensive lesions after four weeks and progressive tuberculosis after six weeks.

Tubercle bacilli suspended in extract and left at room temperature for seven days or at 37°C . for two days were examined microscopically. The organisms were aggregated into large clumps suggesting that they had been agglutinated. The staining properties, however, were not obviously altered.

Most of the observations on the activity of this tuberculous extract have been made on tubercle bacilli from cultures; but inactivation also occurred

when the extract was allowed to act on bacilli present in the spleen and liver of the tuberculous guinea pig. A suspension in saline of spleen and liver from a tuberculous guinea pig showing large numbers of tubercle bacilli in each microscopical field was added to double the quantity of the extract and left at room temperature for seven days. A control suspension of the spleen pulp, in saline without extract, was treated in a similar manner. Each sample was inoculated into two guinea pigs, which were killed six weeks later. At post-mortem examination only the controls showed tuberculosis.

Preliminary experiments on the effect of the antituberculous substance on the growth of *H37Rv* on fluid media showed complete inhibition at a dilution of 1:50, and partial inhibition at 1:100. As the crude extract was not sterile, it was filtered four times through Kieselguhr and then through 'Gradocol' collodion membranes ($0.68\ \mu$), a process which might have considerably reduced the antituberculous activity.

Attempts to separate the antituberculous substance from the lymph-nodes of non-tuberculous cattle and from the spleen of a guinea pig failed to produce any substance which would inactivate a suspension of tubercle bacilli.

Sufficient experiments have not been undertaken to determine whether the agent present in lymph-nodes of the ox is a form of immune body, or whether it is similar to 'spermine' recently described by Hirsch and Dubos¹ as having an inhibitory action on tubercle bacilli. The value of extract-treated bacilli as an immunizing agent and the possible therapeutic value of the extract will be reported in a subsequent communication.

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May 5.

¹ Hirsch, J. G., and Dubos, R. J., *J. Exp. Med.*, **95**, 191 (1952).

Action of some Water-soluble Poly- α -Amino-Acids on Bacteria

SOME of the antibiotics formed by fungi and bacteria have been found to consist of α -amino-acids linked by peptide bonds¹. The recent work of Bloom, Watson, Cromartie and their associates² showed that even the cells of higher animals may, in certain circumstances, for example, in infection with anthrax, excrete antibacterial substances of peptide nature. These facts encouraged us to study the biological properties of some synthetic water-soluble poly- α -amino-acids, which were synthesized in this laboratory³.

The water-soluble poly-amino-acids investigated may be classified, according to their electrochemical properties, into neutral, acidic and basic poly-amino-acids. In the present study, poly-DL-alanine was chosen as representative of the neutral poly-amino-acids (it is the only known water-soluble representative of this group); the acidic poly-amino-acids were represented by synthetic poly-L-aspartic acid and the natural poly-D-glutamic acid, and the basic ones by poly-L-lysine, poly-DL-lysine, poly-DL-ornithine and poly-DL-arginine.

It was found that the basic poly-amino-acids are able to inhibit the growth of Gram-positive and