Hanna

13, August 1993

TO: **Dr. Matthew Meselson**
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TOTAL PAGES 17

Dear Dr. Meselson:

As per our recent conversation, enclosed please find:

-Terri Koehler's address. She was a student of Thorne's and a recent post-doc with the Collier group. She is now a new professor studying gene expression of BA and has many, if not most, of Thorne's strains.

-Several abstracts from articles that may interest you. Please also see **Microbial Toxins III** Ed. by Montie, Kadis and Ajl. 1970. Academic Press NY. Chapter9 by Lincoln and Fish is a nice review. Also enclosed is an interesting article from 1964 New Yorker magazine.

-I can't put my finger on references concerning Sheep and inhalation anthrax or effect of peroxides on spore viability. I will continue to search.

-A good introduction to macrophages is **IMMUNOLOGY** by Roitt, Brostoff and Male. 2nd edition.J.B. Lippincott Company, Philadelphia1989, 1990.

Best travels to you and your crew and I look forward to our next meeting.

Sincerely,

Phil

ing strength of the bones of the birds between the two percheries. These observations suggest that there may be subtle differences between the design of the two percheries which allow the Gleadthorpe perchery birds to experience more accidents during flying and on landing. In this respect the differences between the overall heights of the perchery frames and in the distances between the perches and landing stages alongside the nest boxes may be important. The shorter the flight distance the less likely there is to be an accident on landing.

The experiments produced two other unexpected findings. First, in spite of their longer period of confined activity, end of lay battery hens (68 weeks old) which had been cage-reared as pullets for the first 18 weeks had stronger wing bones than battery hens which had been reared on deep litter for the first 18 weeks. In particular, the strength of the humerus at end of lay was 24 per cent greater in the cage-reared birds, and this difference was sufficient to cause a substantial difference in the extent to which the humerus was broken during depopulation. In one of the flocks the tibia was also significantly stronger in the cage-reared birds.

The second unexpected finding was that during moulting bone strength declined slightly but then improved during the second laying period; in the case of the tibia there was a 16 per cent increase in strength during this period.

The rearing of pullets on deep litter and the housing of hens in a perchery system are often thought to have advantages in terms of the welfare of laying hens. In contrast, forced moulting is sometimes considered to impose a stress on the birds. The results of these experiments suggest, first, that the presumed welfare advantages of the first two husbandry practices are offset to some extent by their effects on bone strength and the incidence of broken bones either during or at the end of lay and, secondly, that the effects of forced moulting are not wholly negative. It is clear that comprehensive information is essential when assessing the overall welfare benefits of particular methods of keeping livestock.

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Anthrax in wildlife in the Luangwa Valley, Zambia

P. C. B. Turnbull, R. H. V. Bell, K. Saigawa, F. E. C. Munyenyembe, C. K. Mulenga, L. H. C. Makala

Veterinary Record (1991) **128**, 399-403

An abnormally high mortality among hippos (*Hippopotamus amphibius*) in the Luangwa River valley between June and November 1987 and estimated to number more than 4000 deaths was attributed to anthrax. Several other species, particularly Cape buffalo (*Syncerus caffer*) and elephant (*Loxodonta africana*), appear to have been affected. A smaller outbreak of anthrax in hippos occurred between August and September 1988, approximately 100 km up-river. A field study was arranged in August 1989 to assess the extent of environmental contamination by *Bacillus anthracis* and the risks to people in the area, to study possible methods of control and to equip local laboratory staff for continued monitoring of the disease. The study confirmed the enzootic status of the region. The characteristics of the outbreaks of anthrax in 1987 and 1988, and the results of the field study are described.

ANTHRAX, once one of the major scourges of man and his livestock throughout the world, has been so well controlled over the past half-century by the vaccination of livestock and improvements in animal husbandry, factory hygiene and public health measures that it has become an almost forgotten disease in the western world. However, in African wildlife, which cannot easily be vaccinated and in which the other aspects of control are not relevant, the disease remains a major cause of uncontrolled mortality in herbivores (Turnbull 1990).

The North and South Luangwa National Parks in Zambia are situated along the Luangwa River and comprise areas of 4636 and 9050 km^2, respectively, 700 to 800 km north east of Lusaka. They have their origins in a game reserve proclaimed in 1904, predominantly to protect giraffe. They were gazetted under their existing names as game reserves in 1938 and made into national parks in 1972.

The possibility that anthrax was a cause of mortality among the wildlife in the Luangwa Valley national parks does not seem to have been acknowledged before 1987, when a major epizootic in hippos was attributed to anthrax. Records from pre-independence (1964) days of cases of anthrax in the wildlife of that area would probably be difficult to obtain but discussions with local residents have provided strong anecdotal evidence that it had been recognised in those days.

This paper summarises the events which occurred during the 1987 epizootic, the situation in 1988 and the results of a field study and related work in 1989 to 1990.

The 1987 outbreak

Abnormal mortality in *Hippopotamus amphibius* began in mid-June 1987. A census of the hippo population in a selected 23 km section of the river, begun at the end of June, indicated that the epizootic reached its peak in early August and abated after the onset of the November rains. A census of the hippo populations in eight sections (14 to 31 km lengths) of the river totalling 167 km in June, July and November and a comparison of these with 1986 census figures indicated an average population loss of 21 per cent (5·7 to 55·5 per cent), or approximately 1420 hippos. Extrapolating this rate of loss over the total pop-

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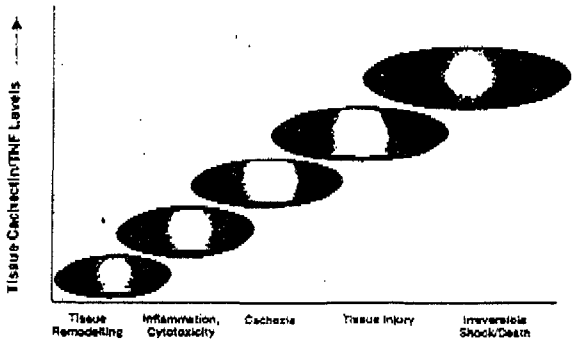
Review of TNF action & shock



Net biological effects of cachectin/TNF according to tissue levels.

At the lowest tissue levels its beneficial effects coordinate tissue remodelling and inflammation; chronic exposure to cachectin/TNF causes cachexia; and the release of large quantities, as occurs in overwhelming infection, triggers potentially catastrophic tissue injury and lethal shock.

possibly indicating ancestral tandem duplication. The biological function of cachectin/TNF also overlaps with that of another 17 kD cytokine, IL-1, but the molecules are structurally different and do not compete for a common receptor. The mRNA transcripts of these and several other inflammatory mediators contain a 33 nucleotide 3'-untranslated sequence composed of repeated and overlapping copies of the consensus octamer UUAUUUAU.[3] This sequence apparently shortens mRNA half-life, thereby reducing the chances for incidental or inappropriate production of large quantities of these potent polypeptides.

Synthesis

Cachectin/TNF is synthesised by various activated phagocytic and non-phagocytic cells, including macrophages/monocytes, lymphocytes, natural killer cells, astrocytes and microglial cells of the brain, and Kupffer cells of the liver. A pivotal role in inflammation is suggested by the wide variety of infectious or inflammatory stimulae capable of triggering cachectin/TNF biosynthesis—eg, bacterial endotoxin/lipopolysaccharide (LPS), enterotoxin, toxic shock syndrome toxin-1, mycobacterial cord factor, viruses, C5a, fungal or parasitic antigens, IL-1, and, in an autocrine manner, cachectin/TNF itself. In response to LPS, both transcription and translation of the cachectin/TNF precursor are increased and large amounts of mature protein are released within minutes. Dexamethasone inhibits cachectin/TNF biosynthesis, but this effect is not observed if the steroid is given after the cells have been exposed to LPS. By contrast, IFN-γ exerts a permissive influence that enhances cachectin/TNF biosynthesis. The up or down regulation of cachectin/TNF biosynthesis by IFN-γ or dexamethasone, respectively, probably contributes to the pro-inflammatory or anti-inflammatory effects of these mediators.[5]

In addition to the active secreted form of cachectin/TNF that appears in the circulation during endotoxaemia, some newly synthesised cachectin/TNF remains cell-associated as a transmembrane form.[6] The 26 kD transmembrane molecule is a precursor of the 17 kD secretory component, but may also have inherent bioactivity as a paracrine mediator.[7] Cachectin/TNF interacts with high-affinity membrane receptors in normal tissues, including liver, muscle, lung, bowel, and kidney. The receptor-ligand complex is internalised and degraded, but details of the receptor and post-receptor responses remain unclear.

SEPTIC SHOCK

After intravenous administration of endotoxin/LPS in man, cachectin/TNF levels peak within two hours coinciding with the onset of fever, rigors, myalgia, headache and nausea.[8] Larger quantities of LPS stimulate much higher serum cachectin/TNF concentrations that trigger lethal shock and tissue injury. Administration of human recombinant cachectin/TNF that is virtually endotoxin-free produces a constellation of metabolic derangements and lethal tissue injury that is almost identical to fatal endotoxin or septic shock syndrome.[9,10] Cachectin/TNF-induced tissue injury is partly mediated by enhanced endothelial procoagulant activity that promotes intravascular coagulation and capillary thrombosis.[11] Increased expression of endothelial-leucocyte adhesion molecules (ELAMs) and intercellular-leucocyte adhesion molecules (ICAMs) also incites leucostasis. Adherent cells are simultaneously stimulated by cachectin/TNF to increase biosynthesis and release of reactive superoxide intermediates and arachidonic acid metabolites.[12] The magnitude of the biological response induced by the recombinant human preparation in different animal species may vary because of the species-specific nature of the receptor-ligand interaction.[13]

The deleterious effects of cachectin/TNF are largely caused by the induction of mediators, including other peptide regulatory factors and eicosanoids, but the importance of cachectin/TNF in lethal endotoxic and septi

TABLE I—SOME BIOLOGICAL PROPERTIES OF CACHECTIN/TNF IN VARIOUS DISORDERS

Physiological condition	Biological effects
Acute infection	
Septic shock; toxic shock syndrome; meningococcal infection; cerebral malaria	Shock; fever; respiratory arrest; capillary leak syndrome; haemorrhagic necrosis; lactic acidosis; stress hormone release; hyperglycaemia then hypoglycaemia; hyperaminoacidaemia; induces reactive oxygen intermediate platelet activating factor, and eicosanoids
Cachexia	
Chronic infection; AIDS?; malignancy?	Anorexia; weight loss; anaemia; fever; increased energy expenditure; hypertriglyceridaemia; increased whole-body lipolysis; net protein loss; acute phase protein biosynthesis; suppression of LPL
Inflammation	
Abscess formation; systemic lupus erythematosus; rheumatoid arthritis; autoimmunity; transplant rejection; graft-versus-host disease	Chemotactic for leucocytes; promotes leucostasis; enhances nonspecific host resistance; leucocyte and endothelial activation; induces expression of MHC antigens; neutrophil degranulation; phagocytosis against pathogens; fibroblast and thymocyte growth factor; induces reactive oxygen intermediates, collagenase, and eicosanoids
Tissue remodelling	
Wound healing	Fibroblast growth factor; angiogenesis factor; stimulates TGFs, GM-CSF, and PDGF; induces collagenase and eicosanoids; cell cytotoxicity

LPL = lipoprotein lipase; MHC = major histocompatibility complex; TGF = transforming growth factor; GM-CSF = granulocyte-macrophage colony stimulating factor; PDGF = platelet-derived growth factor.

Review

Anthrax vaccines: past, present and future

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Peter C.B. Turnbull

Most livestock vaccines in use throughout the world today for immunization against anthrax are derivatives of the live spore vaccine formulated by Sterne in 1937 and still use descendants of his strain 34F$_2$. Credit belongs to this formulation for effective control in many countries with considerable reduction, sometimes complete elimination, of the disease in animals and, since man generally acquires it from livestock, in man also. However, there are some contraindications of its use and situations in which it cannot be easily administered, and room for development of a successor is discussed. The human vaccines, formulated for at-risk occupations and situations, date from the 1950s (UK vaccine) and 1960s (US vaccine). The rather greater need for improvement of these as compared with the veterinary vaccine stimulated valuable research during the 1980s which has led to a number of promising candidate alternatives for the future.

EARLY LIVESTOCK VACCINE: PASTEUR, CARBOZOO AND STERNE

Some 80 years after Jenner's celebrated vaccination and publication of *An Inquiry Into the Cause and Effects of Variolae Vaccinae* (Sampson Low, London, 1798), microbiology's founding fathers had begun the first systematic studies on protection afforded by vaccination against a number of the most troublesome animal diseases of the day. Most noted of these were Pasteur's demonstrations of protective immunizations against fowl cholera in 1880[1], anthrax in 1881[2] (*Figure 1*) and rabies in 1885[3]. In fact, in the case of anthrax, close examination of the records[4] has revealed that credit for the first recorded demonstration of protection induced by attenuated strains of *Bacillus anthracis* really belonged to W.S. Greenfield at the Brown Animal Sanatory Institution in London[5,6].

It was, however, Pasteur's vaccine schedule that became adopted for use. This involved two inoculations 2 weeks apart. The first dose consisted of *B. anthracis* cells from cultures which had been incubated at 42–43°C for 15–20 days (Pasteur I vaccine) and which was pathogenic only for mice and young guinea-pigs. The second dose consisted of cells from cultures incubated at 42–43°C for only 10–12 days and which were rather less attenuated (Pasteur II vaccine).

The Pasteur duplex vaccine became widely used for cattle and sheep in Europe and South America over the next 50 years. In the 1920s and 1930s, the procedure was modified[7]. First, in the 1920s suspension of spores in 50–60% glycerine was found to increase longevity and improve the immunizing efficiency of the spores and the double Pasteurian vaccine was replaced by single vaccines consisting of spores suspended in 50% glycerol. The strains were attenuated to such an extent as to be non-virulent for rabbits but virulent for guinea-pigs and the intricate manipulations needed to meet this requirement rendered these vaccines impractical in the long run. In the 1930s, the practice of adding saponin (1–10%) to the Pasteur II or other virulent or slightly attenuated strains was introduced. Saponin at these concentrations provoked a violent inflammatory response at the inoculation site which limited generalization of the anthrax infection.

As reviewed by Sterne *et al.* (1939)[8], there was an epidemic of reports between 1929 and 1937 on the application and merits of vaccines consisting of spores from isolates designated as having various levels of virulence suspended in 4–10% saponin which was reported to neutralize the virulence. Particularly popular for a while, it seems, was 'Carbozoo', initially produced at the Istituto Sieroterapico Milanese and later in the

Figure 1 From an original monograph on anthrax vaccination entitled 'Vaccinations Preventives contre le Charbon du Betail' (Compagnie de Vulgarisation du Vaccin Charbonneux Pasteur) 1886. The operator is probably Louis Pasteur himself. (Photograph kindly supplied by Dr J. Ezzell.)

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In: Pharmacology of Bacterial Toxins
F. Dorner and J. Drews, Eds.
Pergamon Press, Oxford 1986 p 381-397

CHAPTER 19

ANTHRAX TOXIN

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1. INTRODUCTION

This topic was actively researched from the early 1950s to the late 1960s in the United States of America and Britain at which point the work came to a halt. During the 1970s a trickle of publications from the Soviet Union continued to appear on the production of toxin (Lesnyak and Saltykov, 1974; Vylchev *et al.*, 1976; Derbin *et al.*, 1977), immunizing antigen preparations (Fedotova, 1974; Kuzmich *et al.*, 1976) biological testing for toxicity (Lesnyak, 1975) and the effect of anthrax toxin on cultured cells (Fedotova, 1970). Very recently, new studies have appeared from American workers on the genetics of anthrax toxin (Mikesell *et al.*, 1983) and on the mode of action of one of its three components in chinese hamster ovary (CHO) and other cells (Leppla, 1982). The bulk of this review will concentrate on the earlier work. The fact that the author was not personally involved in research carried out during the halcyon period from the early 1950s to the late 1960s might enhance rather than detract from this article which is mainly one of retrospective appraisal, since this era closed on a note of as yet unresolved controversy. No attempt will be made to exude a pseudo-scholarship by remarshalling the many primary papers which emanated mainly from the American group at Fort Detrick, the British group at the Microbiological Research Establishment (Porton) and others, since these have been collated and reviewed on several occasions (Smith, 1958; Lincoln *et al.*, 1964; Nungester, 1967; Lincoln and Fish, 1970). My aims are to present, to a later generation, a concise outline of the discovery of this complex toxin, describe its nature and role in the pathogenesis of disease, discuss the controversy that developed as to the pathophysiological changes induced by anthrax toxin and finally to comment on the recent developments which if pursued could, and probably will, lead to a resolution of the extant problems.

The remainder of this introductory section sets out the context in which the work was conceived and developed.

The disease anthrax occurs in two forms. Localized cutaneous infections occur in man, swine, rabbits and horses (Lincoln *et al.*, 1964; Lamb, 1973) in which the most characteristic superficial feature is the black eschar which gives its name to the disease and the causative organisms (Gr. anthrakos = coal). The incidence in humans is low, occurring mainly among veterinarians, meat workers and workers in woollen mills, hence the name wool sorter's disease. This form is readily treatable by antibiotics. The septicaemic form may develop from untreated cutaneous infections, or by primary infection via the respiratory or gastrointestinal routes or by infection of wounds and it is nearly always fatal. In animals, a peracute form of the disease occurs in which the first sign of the disease is often death itself; a less acute form occurs in which signs may be evident over a period of 2–10 days before the, nearly always, fatal outcome (Lincoln *et al.*, 1964). Herbivores are the most usual victims: cattle, sheep, horses and goats, in that order, being most susceptible. The organism is highly invasive, spreading throughout the body from the initial portal of entry and producing a characteristic massive terminal bacteraemia. In certain parts of the world it is of sufficient economic importance to warrant vaccination of animals at risk with attenuated vaccines (Sterne, 1967).

The study of anthrax and its causative organism, *B. anthracis*, has attracted the attention of microbiologists from Davaine, Pasteur and Koch onward. In the immediate post-war

The New Yorker
April 24, 1964

ANNALS OF MEDICINE
A Man Named Hoffman

Around ten o'clock on the morning of Wednesday, March 4, 1964, a man named Donald Hoffman presented himself for treatment at the Student Health Clinic of Miami University, in Oxford, Ohio, some thirty miles northwest of Cincinnati. Hoffman was thirty-six years old, married, and a resident of Cincinnati, but, as he explained to the receptionist, he was currently employed as an installer of insulation on a remodelling job at McCullough-Hyde Memorial Hospital, in Oxford, and his company had an arrangement with the clinic. He was here, he added, because his foreman had sent him. That was the only reason. His trouble was nothing - an itchy sore on the side of his neck. He had probably picked up a sliver of glass-wool fibre. It had happened several times before. It was a common complaint in his trade.

The doctor who saw him was inclined to agree. There was no good reason not to. Hoffman worked with fibre glass, and his lesion had the look of a fibre-glass lesion. The history of the lesion, the doctor found, was equally suggestive. It had first appeared on Monday evening as a tiny red swelling that might have been caused by a chafing shirt collar. It was larger on Tuesday, and somewhat sensitive. This morning it was larger still, and it alternately itched and burned. The doctor slipped a thermometer under Hoffman's tongue, and picked up a scalpel and nicked the edge of the lesion. There was no discharge. He removed and read the thermometer. Hoffman had a temperature of 99.2 degrees. The doctor noted the reading on his record of the case and added, "Has erythematous swollen area at base of neck anteriorly on left, extending over chest. A firm furuncle is present in the center of this area. Impression: fibre-glass dermatitis with secondary infection." The doctor then turned his attention to treatment. He covered the lesion with a bacitracin dressing and got out a hypodermic needle. In view of the threat of infection, he said, a course of penicillin was indicated. He proposed to begin with an intramuscular injection of three hundred thousand units. Hoffman stood up. That wouldn't be necessary, he said. He had had all the treatment he wanted. He didn't believe in taking penicillin every time he had a little scratch. He put on his jacket and left.

Hoffman drove back to the job and resumed his work. He worked until noon, and then knocked off and sat down to lunch with one of his friends. He thought he was hungry, but after a couple of bites he changed his mind. His appetite had vanished. He only wanted to sit and rest. Nevertheless, when the lunch hour was over he went back to work and finished out the day. When he got home, a little before six, he was exhausted. He stretched out on the living-room sofa for a rest before dinner and instantly fell asleep. His wife let him sleep, and he slept two hours. He awoke feeling worse than ever. His head ached, his bones ached, and it hurt him to move his neck. He looked so sick that his wife was frightened. She insisted that he see a doctor at once. The Hoffmans had no regular doctor, but Mrs. Hoffman knew the name of a general practitioner in the neighborhood who kept evening office hours, and she looked up his address.