

ABSTRACTS

(On melanosis and hypermelanosis; cytoplasm structure in *M. leprae*; elongation of *M. lepraemurium* in a cell-free medium; initiation of a trial of BCG vaccine for leprosy; inoculation of golden hamsters producing histiocytic granulomatous leprosy-like lesions; preliminary report of the effect of DDS on malaria in N. Nigeria)

Melanosis, a Peculiar and Rare Dermatosiis seen in the African of Mashonaland. M. GELFAND. The Central African J. of Med., 5, 11; Nov. 1959, pp. 595–598: 5 illustrations.

Dr. Gelfand records an important observation of his of the occurrence of black, oval, or circular macules of hyperpigmentation or melanosis in adult Shona of both sexes. He describes 3 cases, with clear photographs. The macule has an average diameter of 2 cm. and a few or many appear widely on the body, the trunk being the most typical site. The only difference between macule and healthy skin is its deeper black colour, and skin biopsy shows no apparent abnormality except for the excess of melanin pigment in the skin. The patches seem to be permanent, though the patients say that they fade away and are followed by others. In addition to the apparently more permanent lesions, the patient may describe a much more transitory eruption consisting of reddish papules which fade after several days only to be followed later by the black lesions at a fresh site. Most cases also have attacks of abdominal pains of a few days duration which may be so severe as to end up in laparotomy. This pain may be felt in any part of the abdomen and end up in vomiting. The prognosis as to life appears to be excellent. Gelfand has occasionally seen cases with temporary non-recurring black circular macules as described by M. Rose (1958) in Med. Proceedings, Baragwanath Hosp., Johannesburg 4, 314, in which the skin is lustreless and sooty in appearance and after 3 to 4 days the pigmented area begins to shrink and peel, leaving a paler skin underneath.

In the illustrative cases described by Dr. Gelfand, some further interesting points emerge:—(a) In the first patient, a male, there were crops of melanotic macules all over the body. When they were young, there was *itching* in them. Before the macules appeared there would invariably be *severe pains in the abdomen and chest*, immediately followed by pink papules, which itched, could appear anywhere, and disappear after about 3 weeks. The pain could be independent of the papules. There was no fever at any time, and he was fit and well-nourished. Laboratory investigations were negative. Skin biopsy showed only a *diffuse fibrosis in the corium*. No urobilins or porphyrins in the urine. A second skin biopsy showed *slight hyperkeratosis*, and *perivascular accumulations of pigment* in the cutis, but no significant lesion. (b) The second patient was an adult African Shona female, aet. 26 years. Since 1951 she complained of lower abdominal and vague upper abdominal pain for which no cause could be found. There was occasional vomiting. In 1955 black circular hypermelanotic

macules first appeared on the lower abdomen and continued to appear there until June, 1958, when they also turned up on her legs. The early macules were preceded by localised itching, and the early macules were first of reddish colour, but very soon became black. Latterly pain instead of an itch has preceded the appearance of a macule, and the lower abdominal pains have become more severe and intermittent, with no fixed time relationship to anything. All examinations were negative, and the patient continues to look fit and well, except for the black circular patches on the skin, which remain unaltered. (c) The third patient was an African male aged about 50 years. In 1958 he first began to complain of painful ulcers on his tongue and gums, and at the same time dark circular patches appeared on his arms, chest, and right leg. There was an occasional rise of temperature to 99°F. (37.3 C.), and he took 1 aspirin tablet at the beginning. The temperature soon returned to normal and remained so. His B.P. was 170/100. After several days the black patches began to crack and the dead skin peeled off in tiny fragments, leaving a hypopigmented but otherwise healthy-looking skin underneath. Dr. M. Rose has recorded a similar case to this, in which the rash peels after a while, and attributes it to the taking of phenolphthalein previously as a purgative. The rash in the first 2 cases is entirely different and requires explanation. (In connection with Dr. Gelfand's paper, see letters by B. NICHOLSON and S. G. BROWNE in *Leprosy Review*, 30, 4, and 31, 1, of Oct. 1959 and Jan. 1960 respectively; also the two Abstracts which follow here immediately. EDITOR).

A Hypermelanotic Rash Complicating Sulphone Therapy: S. G. BROWNE. *Trans. Roy. Soc. of Trop. Med. & Hyg.*, 53, 6; Nov. 1959, pp. 495-505.

The author describes and discusses a little-recognised complication of sulphone therapy in leprosy, namely hypermelanotic macules in the skin and mucosae of leprosy patients, who were all deeply-pigmented Bantu of the Belgian Congo. Apart from drug therapy, no cases were observed. Apart from sulphones, only 2 cases were observed, 1 with sulphaguanidine and 1 with sulphathiazole. Bantu are known to respond excessively to stimuli causing hyperpigmentation, much as they do to factors causing a fibrotic and keloid response. In all, 160 cases of the hypermelanotic macules were observed in a population of 43,035 persons, of whom 5,349 were under treatment for leprosy. Rounded, black, or purplish-black macules appeared suddenly in the skin or mucosae, typically 1 cm. in diameter, but varying from 0.5 cm. to a less well-defined diffuse larger type. Crops of macules may appear over months or years. After sulphone therapy ceases, the macules tend to disappear in 9 to 18 months. Scratching may modify the course and even introduce

areas of light blue colouration. There are a few cases of acute severe onset, with irritation, vesicles, bullae, and oedema. Sometimes there is desquamation. There has been no family incidence. The sulphone therapy was either Dapsone with iron by mouth, or 50% Solapsone by injection, or Dapsone in 25% suspension in hydrocarpus oil. The incidence of the rash was a bit lower with the oral than the injection treatment, but all three methods could cause it. The time before the rash appeared under sulphones varied considerably, average 18 months to 2 years. Distribution of macules on the body was apparently haphazard, even in relation to the form of leprosy and to the existence of a lesion or not in the site of appearance of the macules, nor is there any relation to the occurrence or speed of the clinical arrest of the leprosy. The fading out of the hypermelanotic macules seems to be independent of the form of sulphone given. Of the 160 patients, 48 had symptoms which could be attributed to the macules, mostly moderate or severe skin irritation. A few had pain which interfered with sleep, or a generalized pruritis, or a local burning sensation with objective heat. Histologically in the macules there is remarkably little change. There is an increase in melanophores and pigment, and the suggestion is of a disturbance in the functional activity of the melanoblasts, perhaps a direct toxic action by DDS on areas of sensitive melanoblasts, with consequent alteration in the mechanism of formation or storage or transport of melanin.

Hypermelanotic Rash Associated with Sulphonamide Therapy:

S. G. BROWNE. Brit. Med. J., Feb. 27, 1960, p. 621.

He describes an eruption identical to that observed by him in sulphone therapy, in 2 cases in the Belgian Congo where sulphaguanidine had been taken, in the one for diarrhoea and in the other for pustular dermatitis. There was widespread skin irritation, small bullae, macules coming out in crops which were hypermelanotic and faded slowly.

Cytoplasm Structure in Mycobacterium leprae. E. M. BRIEGER, AUDREY M. GLAUERT, and JENNIFER M. ALLEN. Experimental Cell Research, **18**, 1959, pp. 418-421. 1 plate.

The authors earlier had noted laminated structures in electron micrographs of *Mycobacterium avium* but could not identify them nor assign a function to them. These round or oval bodies lay in the cytoplasm and resembled the unidentified bodies seen in *B. subtilis* by Ryter and colleagues. Later Shinohara and colleagues described laminated structures in avian tubercle bacilli and thought that they might be related to the nuclear apparatus and to mitochondria. Later Glauert and Hopwood found a remarkable system of intracytoplasmic membranes in study of thin sections of hyphae of *Streptomyces coelicolor*, which seemed to be continuous with the plasma

membrane. The present authors have now seen systems of membranes in the cytoplasm of human leprosy bacilli obtained from Africa (fixed in a standard buffered osmium tetroxide solution in Africa and then stored in 70% alcohol until they were embedded in n-butyl methacrylate in Cambridge many weeks later. Good sections were obtained by a thin-sectioning microtome designed by A. F. Huxley). These membranes are sometimes seen in parallel arrangement, and sometimes form stacks at the point of cell division of the mycobacterium. The membranes are often connected with the plasma membrane, which appears as a "double" structure made up of 2 dense layers, each about 3 m μ thick, separated by a less dense layer about 4 m μ thick. The membranes in the cytoplasm are about the same thickness, and sometimes they form structures which resemble those noted earlier in avian tubercle bacilli. These peculiar cytoplasmic membranes seem common to *Streptomyces* and *Mycobacterium*, but it is not known whether they are peculiar to them, nor whether they appear only at certain stages of development. It may be that their presence in leprosy bacilli is an indication of growth. There are morphological similarities between them and the endoplasmic reticulum of mammalian cells and of the plastids of plant cells. A complete identification awaits a knowledge of the function of this cytoplasmic system in the bacterial cell.

Elongation of a Leprosy Bacillus (Mycobacterium lepraemurium) in a Cell-free Medium. P. D'ARCY HART and R. C. VALENTINE. *Nature*, **185**, No. 4705, Jan. 1960, pp. 58–60.

Though *M. leprae* was one of the first bacteria to be identified as the causative organism of a disease, full success in its culture lags far behind, and this has led to much study of the only other closely related organism, which is *M. lepraemurium*, which in rats and mice causes a disease with some of the features of human leprosy. Like *M. leprae*, it is unusually slow-growing in the body, with 10 days generation time. In 1958 there was an important advance when Rees and colleagues, Garbutt and colleagues, Wallace and colleagues observed limited multiplication of *M. lepraemurium* in tissue culture, but so far in a cell-free medium it remains uncultivated, and in its respiratory metabolism it shows an almost complete lack of response to many substances (Gray, 1952). A completely degenerate-form of *M. lepraemurium* has been distinguished by electronmicroscopy by McFadzean and Valentine (1959, 1960). This form is non-viable and is unable to produce disease. In conventional culture media it appears after incubation of a few weeks at 37°C. In one experiment where the medium was a liquid nutrient with 20% added sucrose, electron-microscopy showed at 2 months that among the degenerated bacilli there were some which looked unusually long, as if some limited growth had occurred before death of the bacilli. Therefore

the authors investigated the frequency distribution of lengths after varying times of incubation of *M. lepraemurium* in different media. The bacillary suspensions were added to 6 different media and incubated at 37°C. The lengths of 100 or more bacilli from each subsequent sample were measured at $\times 10,000$ under the electron microscope, and the proportions also estimated of completely degenerated bacilli. In 3 non-nutrient media there was no elongation of bacilli, and degeneration was rapid. In the ordinary nutrient culture media a small amount of lengthening occurred. But in the same medium with 10% sucrose or 8% glucose the mean length nearly doubled and for bacilli longer than 2.5μ the proportion rose from 6 to 67% with the added sucrose and 51% with the added glucose, the greater part of the increase being in the first 2 weeks. There was also greatly slowed degeneration. INH was incorporated in one of the media and had the effect of preventing the lengthening of bacilli, which means that the lengthening is not due to a passive stretching. The long bacilli showed no change in electron density: there was a slight increase in width. This points to a real increase in bacterial protoplasm in the cultures. Thus *M. lepraemurium* in culture medium has not a complete inability to metabolize and grow. Multiplication fails because it fails to divide. If means could be found to encourage division their culture in cell-free media might at last become possible.

The basal nutrient medium used contained:

- 2.5 g. of Difco "Casamino" Acids
- 0.3 g. of asparagine
- 2.5 g. of Anhydrous disodium hydrogen phosphate
- 1.0 g. of potassium dihydrogen phosphate
- 1.5 g. of sodium citrate
- 0.6 g. of crystalline magnesium sulphate
- 25.0 ml. of glycerol
- 1000.0 ml. of aqua dest.
- added to 0.25% of bovine plasma albumin fraction (V)
- added 1 to 20% of sucrose
- or added 0.5 to 8% of glucose

The Initiation of a Trial of BCG Vaccine for Leprosy: J. A. MCFADZEAN and R. BHAGWAN SINGH., Trans. Roy. Soc. Trop. Med. & Hygiene, **54**, 1; Jan. 1960, p. 8.

These authors in a Laboratory Meeting described this trial going on in a static population of 7,500 Chinese on an island off the coast of Malaya. The population from birth to 25 years of age has been examined for clinical signs of leprosy and tuberculin-tested. Those reacting with 9 mm. or less to the tuberculin, which was 5 T.U. of R.T.22, have been grouped at random. One group was given freeze-dried BCG and the other left as a control. The population is to be

followed for 10 years and examined at intervals for leprosy. It was found that the intake of new born children would be too low, and this part of the trial has been abandoned, but the present population should be enough to demonstrate a level of protection by the BCG of 50%.

Histiocytic Granulomatous Mycobacterial Lesions Produced in the Golden Hamster (Cricetus Auratus) Inoculated with Human Leprosy. Negative Results in Experiments using Other Animals.

CHAPMAN H. BINFORD, "Laboratory Investigation", Washington, 8, 5: Sept.—Oct., 1959, pp. 901—923.

Dr. Binford reports on his comprehensive project in animal inoculation with human leprosy, begun in 1956 with the ultimate aim of producing lesions due to *M. leprae* which could be reproduced with regularity in an animal. The first step was to produce local progressive lesions at the site of inoculation. On about 1,500 small animals 35 inoculation experiments were undertaken, and also on 31 monkeys, and several methods for reducing host resistance were used, especially irradiation of the whole body and administration of cortisone. The factor of temperature of body sites was taken into consideration, for it is known that a temperature relatively lower than that of the internal organs is a common factor in all body sites preferred by *M. leprae*. Therefore the cooler parts of animals were selected for inoculation, such as the external ear, tail, foot, testis, scrotum, and skin, and the hair kept clipped on hairy sites of inoculation. Feldman with *M. ulcerans* found this influence of low temperature of skin was important. Binford studied the average temperatures of body surfaces in golden hamsters and found, for example, that at room temperature of 61°F. (15.1°C.) the ear temperature was 72.4°F. (20.5°C.). In monkeys the ulnar and femoral nerves were inoculated, because *M. leprae* has a predilection for peripheral nerves. In other animals the inoculations were made by multiple punctures, scarification, and intracutaneous routes, in the hope that bacilli might gain access to the terminal parts of tiny skin nerves. Human material for inoculating the animals was derived from 3 sources, Philippines, Carville, and Washington. The specimens were homogenized and the concentrations of the bacilli in the Oil-Immersion field varied from 10 to 100 bacilli. Heat-treated inoculum was used in controls. Histological studies of the inoculation sites were made regularly. Histiocytic granulomatous lesions appeared about 18 months after the inoculation in the testes and ears of the golden hamster. These lesions resembled human lepromatous leprosy in their histological picture, in the number of intracellular acid-fast bacilli, and in the presence of bacilli within nerves. Even with skin specimens that had been frozen with solid carbon dioxide and stored for 10 months, a heavy growth was produced in the ears

of hamsters when inoculated. Total body irradiation produced no evidence of influence on the infection. The animals treated with cortisone died too early to permit of an assessment of its influence. Transfer of the infection to other hamsters seems indicated by 5 months of preliminary work on this aspect. Further studies of the mycobacteria and further direct inoculation trials will be needed before final conclusions can be arrived at.

A Preliminary Report on the Effect of Diamino-Diphenyl Sulphone on Malaria in Northern Nigeria. H. M. ARCHIBALD and C. M. ROSS, Journ. of Trop. Med. & Hyg. 63, 2: Feb., 1960, pp. 25—27.

For the past 7 years DDS has been extensively used for the treatment of leprosy in Northern Nigeria, reaching 195,000 cases at the present time. There has been widespread and rapid subjective clinical improvement and solid and steady amelioration of the disease itself. The authors wondered if there were an effect on malaria which might explain the early clinical improvement. They conducted a preliminary investigation to determine the comparative prevalence of malaria in leprosy patients under treatment and in the healthy subjects at 4 centres in Zaria Province. It was found that *P. malariae* parasitaemia had disappeared from those taking DDS and that *P. falciparum* trophozoites occur about one tenth as often compared to the corresponding untreated. There are also lesser parasite densities in those of the DDS group who show infections, and less pronounced splenic enlargement. The suppression in the DDS group is incomplete. Activity of DDS was compared with chloroquine phosphate, and it was found to be positive and considerable, but slower than chloroquine (200 mgm. of DDS was compared with 300 mgm. of chloroquine base). DDS achieves trophozoite clearance. There are very few toxic effects from 800 mgm. weekly of DDS as used in leprosy and it is cheap, and could be used as an active prophylactic or a valuable synergist with other antimalarial drugs. If, as is probable, DDS acts on the metabolic processes of the parasites at a point different from proguanil or pyrimethamine it may be able to combat the survival of strains resistant to a single drug.