rial, equipment and technical assistance in the study of the molecular organization of cell surfaces in relation to biological activity and specificity 1,500

Detlev W. Bronk, for material, equipment and assistance in the study of the mechanism of chemical excitation of nerve cells 1,800

Detlev W. Bronk, for material, equipment and assistance in making a general investigation of the nervous control of body organs in terms of the properties of nerve cells and fibers which make up the sympathetic and para-sympathetic nervous systems; with special reference to the physico-chemical processes which occur within these systems 2,050

H. K. Hartline, for material and equipment in connection with the study of the photo-chemical and neural processes of vision 1,500

SPECIAL ARTICLES

IDENTIFICATION OF THE FILTRABLE, TRANSMISSIBLE NEUROLYTIC AGENT ISOLATED FROM TOXOPLASMA-INFECTED TISSUE AS A NEW PLEUROPNEUMONIA-LIKE MICROBE

THE isolation from Toxoplasma-infected tissues of a filtrable, transmissible agent capable of producing a characteristic nervous disease in mice was reported recently.[1] The nature and origin of this agent was perplexing because (a) it was not demonstrated in thousands of normal mice, (b) it did not appear to possess the properties necessary for natural host-to-host transmission either by itself or by means of an arthropod vector, and (c) it appeared to be intimately associated with certain strains of Toxoplasma. The morphological appearance of the minute structures observed intracellularly and extracellularly in some of the infectious material suggested a similarity (a) to "chromatin" structures within the Toxoplasma and (b) to stages described for the causative agents of pleuropneumonia and agalactia. The relationship to "chromatin" structures of Toxoplasma, admittedly a fantastic concept, was rendered even more so by the finding that they were Feulgen-positive, while the structures in the "neurolytic agent" material were Feulgen-negative. The relationship to the causative agents of pleuropneumonia and agalactia appeared unlikely at first because their minimal size of 125–150 mμ and their capacity to grow in a cell-free medium containing serum differentiated them from the neurolytic agent material which appeared to have a minimal size of 314 to 360 mμ and failed to grow in serum-containing media.

By a modification of technique, however, it has proved possible to grow the "neurolytic agent" in a cell-free medium, consisting of ordinary nutrient broth, 10 per cent. Seitz-filtered, sterile, bovine serum and 0.5 per cent. glucose. No growth was perceptible in the initial cultures, but one or two transfers of the apparently negative cultures to fresh medium invariably showed a faint opalescence which, upon dark-field examination, revealed structures not unlike those seen in "neurolytic-agent" infectious material. Successful cultures were thus obtained from each of six infectious brains—some of which had been kept in the dried state for months and others fresh—while simultaneous tests with the same medium to which no glucose was added failed in a number of instances; in the absence of serum, all attempts were negative. Subculture of the serum-glucose-broth cultures on serum-agar yielded colonies 20 to 100 μ in size, which on direct microscopic observation and study of Giemsa-stained impression films were quite similar to those described by Ledingham[2] for pleuropneumonia and agalactia. The evidence that the cultivated microorganisms are identical with the "neurolytic agent" is as follows:

(1) More than 18 subcultures have now been obtained, and the cultures continue to show in mice the same pathogenic effects (both clinical and pathological manifestations) as the animal-passage material.

(2) The active agent is filterable, although with culture material a smaller size was obtained for the minimal infectious particle (determined by animal inoculation as well as by culture), *i.e.*, 250 to 292 mμ instead of 310 to 360 mμ.[3]

(3) Its pathogenicity is inactivated at the same temperature (about 45° C. for 15 minutes).

(4) The cultivated and animal-passage materials give rise to complete immunity against each other.

Elaboration of exotoxin in vivo and in cultures: In the first report[4] it was indicated that the brain lesions produced after intraperitoneal or intrathoracic inoculation were not caused by the transmissible agent itself but rather by some non-transmissible substance formed when growth occurred in certain sites. Filtration of cultures through Seitz filters, which hold back the infective particles, yielded a material which upon intravenous injection (but not intracerebral or intraperitoneal) of 0.5 cc amounts in 3-weeks-old mice (old mice react irregularly or not at all), reproduced the typical nervous signs within 1 to 2 hours. Most of these mice died within a few hours, but the surviving ones continued with the nervous signs and exhibited

[1] A. B. Sabin, SCIENCE, 88: 189, 1938.

[2] J. C. G. Ledingham, *Jour. Path. Bact.*, 37: 393, 1933.

[3] Gradocol membranes and filters were kindly supplied by Dr. J. H. Bauer.

[4] A. B. Sabin, *loc. cit.*

the same acute necrotic lesion in the posterior pole of the cerebellum, with acidophilic necrosis of Purkinje cells, so often observed in virus affections, which has already been described.[5] Neither the brain nor the viscera of these mice contained a transmissible agent.

The toxin is produced early during growth, is present in very small amounts, and disappears from the culture within 2 days of its appearance. It is thermolabile, being inactivated at 50° C. but not at 45° C. for one-half hour. It is apparently antigenic and the same as that produced *in vivo* during infection, since recovered mice are immune to it.

Natural habitat and transmission: Previous attempts to isolate a similar agent from thousands of normal mice by animal inoculation and subinoculation failed. A new attempt was made by the technique of cultivation and subcultivation just described. The viscera and brains of ten 1-month-old mice from the normal stock were cultured separately with negative results in the initial culture tubes, but on transfer to fresh medium, a pure culture of a pleuropneumonia-like microbe was obtained from the brain but not from the viscera (liver, spleen, lung) of one of the mice. This strain has been repeatedly subcultured since, and still requires serum although not the added glucose for its growth. It is not, however, pathogenic for mice, several passages in mice have not yet made it so, and no toxin has been found in its cultures. Since the cultivated as well as the animal-passage material does not appear to be capable of multiplication in the peripheral sites which permit of direct host-to-host transmission by means of an arthropod vector, it is necessary to consider the possibility of a new type of vector for these microorganisms, namely, bacterial or perhaps even protozoal. Klieneberger[6] has already shown the existence of a pleuropneumonia-like microorganism in natural symbiosis with a streptobacillus and has also experimentally induced a symbiosis with *B. tetani* and *B. tetanomorphus*. The intimate association of the present pleuropneumonia-like microbe with Toxoplasma may perhaps be another form of symbiosis resulting from a chance encounter in normal mice, maintained by repeated passage with a consequent development of the special pathogenic properties in what might originally have been a non-pathogenic pleuropneumonia-like microorganism. I have been informed by Dr. G. M. Findlay that he has encountered a similar disease among mice in London in the course of routine passage of the yellow fever and lymphocytic choriomeningitis viruses and that Dr. E. Klieneberger has isolated a pleuropneumonia-like microorganism from the material. He has compared the infective agent from English mice with that of mice which I sent him and found that the two were essentially similar and immunologically identical.[7]

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[5] *Ibid.*

[6] E. Klieneberger, *Jour. Path. Bact.*, 40: 93, 1935; *Jour. Hyg.*, 38: 458, 1938.

[7] To be reported in a forthcoming issue of *Lancet*.

THE LIFE-MAINTAINING EFFECT OF CRYSTALLINE PROGESTERONE IN ADRENALECTOMIZED FERRETS[1]

IN the dog, ferret and cat the condition of pseudopregnancy prevents the appearance of adrenal insufficiency following adrenalectomy. Estrus, contrariwise, increases the need for cortical hormone therapy. Somewhat similar but less striking results occur in the rat. Thorn and Harrop found resemblances between cortical extracts and several sex hormones on electrolyte excretion of intact dogs. The chemical similarity between cortical substances and progesterone suggests that the latter might be the life-maintaining agent during pseudopregnancy, but direct tests in several laboratories have failed to demonstrate it. Furthermore, the estrogens which have an effect like progesterone and cortical hormone in intact dogs are toxic in adrenalectomized rats.[2]

With the idea that dosage or species variables may have been the cause of previous failures to maintain life, we have investigated the effect of progesterone and other sex hormones in adrenalectomized ferrets.[3]

Anestrus adrenalectomized ferrets fed a diet of fresh milk and fresh, ground, lean meat, to which is added a 2 per cent. Na-salt supplement, demand, with a good uniformity, one cc of cortical extract per day (equivalent of 30 gms fresh adrenal tissue) for maintenance. Without therapy adrenal insufficiency (loss of weight, anorexia and asthenia) occurs on the average (of 15 cases) at 6 days with a range of 3 to 10 days. During estrus the cortical hormone requirement is at least doubled.

The effect of prolonged progesterone treatment on adrenalectomized ferrets has been observed in six instances on five animals. Ovariectomized and non-ovariectomized females and one castrate male were used. With adequate dosage all signs of adrenal insufficiency were prevented for the duration of treatment, or, as occurred in three cases, when dosage was reduced to inadequate levels so that adrenal insufficiency appeared, revival to excellent health was effected by giving 5 mgm progesterone daily without any cortical hormone. Treatment was continued for 32 days in

[1] This work was aided by a grant (to R. G.) from the Penrose Fund of the American Philosophical Society.

[2] Most references to above work are cited by R. Gaunt, *Cold Spring Harbor Symp. Quant. Biol.*, 5: 395, 1937.

[3] The authors are indebted to Dr. W. W. Swingle, of Princeton University, for the cortical extract used here and for other assistance; and to Dr. Erwin Schwenk, of the Schering Corporation, who furnished the progesterone (Proluton) and testosterone propionate (Oreton).