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).

two cases, 30 days in one, 20 days in two and 14 days in another. Apparently life could have been maintained indefinitely with continued treatment. Dosages used varied from 0.5 to 5 mgm per day. The former was ineffective and the minimal life-maintaining dose seemed to be approximately 2 mgm per day. Marked weight gains occurred during treatment in 5 of 6 cases. The salt supplement usually added to the meat diet was not essential for the observed effects as determined by its omission in one case. All animals died of adrenal insufficiency when therapy was discontinued, showing that adrenalectomy was complete.

In two cases a commercial corpus luteum extract from hog ovaries proved toxic and reduced the life-span, perhaps because of estrin contamination.

Testosterone propionate, a substance chemically and in some ways biologically similar to progesterone, was slightly, if at all, effective in four animals, in doses of 5 or 10 mgm per day. The animals lived for 13, 12, 8 and 11 days. Similar results have been obtained in rats by others. Estrone given to two animals in doses of 0.1 and 0.2 mgm daily was toxic, as in the rat, and as was to be inferred from the effects of spontaneous estrus.

CONCLUSIONS

Crystalline progesterone will maintain adrenalectomized ferrets in excellent health and will relieve an established adrenal insufficiency. The phenomenon of life-maintenance in pseudopregnant adrenalectomized animals is therefore probably explained on the basis of progesterone secretion alone. Testosterone is non-toxic and non-beneficial to adrenalectomized ferrets, while estrone is toxic.

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NICOTINIC ACID AND THE GROWTH OF ISOLATED PEA ROOTS[1]

IN earlier publications[2,3] it has been shown that vitamin B_1 is an essential accessory factor for the growth of isolated pea roots. Pea roots were maintained for 14 weekly transfers in a medium containing only vitamin B_1 in addition to sucrose and mineral nutrients. It was also shown, however, that the growth rate of isolated pea roots decreases in successive transfers if vitamin B_1 is the only accessory growth factor. Such roots also become progressively thinner as the weekly transfers are continued. Vitamin B_1 alone is not then the only growth factor essential for the sustained growth of the isolated pea root. Other investigators have suggested that specific minor elements[4,5] or specific "essential" amino acids[6] constitute this second essential growth factor in the case of the isolated tomato root. The present authors also found that a mixture of 16 amino acids possessed activity as the second growth factor for the isolated pea roots.[7] The mixture of amino acids used in these earlier experiments was, however, a purely arbitrary one. In work which will be described in detail elsewhere, each individual amino acid was tested both alone and in combination with other amino acids (always in the presence of added vitamin B_1) for its ability to increase the growth of the isolated pea root above the level of growth rate of similar roots supplied with only vitamin B_1 as the accessory factor. In this way a mixture of amino acids having the composition shown in Table 1 and optimal for support of the growth rate of the isolated pea root was obtained. This mixture of amino acids was found capable of increasing the growth rate of isolated pea roots over the growth rate obtained with vitamin B_1 alone, but to be incapable of supporting growth at an undiminished rate for more than three weekly transfers. It is clear then that the second factor limiting the growth of isolated pea roots is not merely a combination of amino acids. It has also been found that the factor in question is not a specific minor element.

TABLE 1

AMINO ACID MIXTURE OPTIMAL FOR THE GROWTH OF PEA ROOTS

Amino acid	Concentration in medium in mg. per liter
Asparagine	0.1
Glycine	0.05
Glutamic Acid	0.5
Isoleucine	0.015
Leucine	0.5
Tryptophane	0.5
Valine	0.05

Nicotinic acid, in combination with vitamin B_1, exerts a very striking effect on the growth of the isolated pea root, as is demonstrated by the following experiment. Approximately 700 pea roots, which had been in culture on vitamin B_1 free medium for one week, were divided into 7 series. These separate series received as accessory growth factors either vitamin B_1, the amino acid mixture, nicotinic acid or combinations of these. The series receiving only vitamin B_1 will be considered the control. It may be seen in Table 2 that in all series in which nicotinic acid and vitamin B_1 were not both present there was a steady decrease of

[1] Report of work carried out with the assistance of the Works Progress Administration, Official Project No. 665-07-3-83, Work Project W-9809.

[2] J. Bonner, SCIENCE, 85: 183–184, 1937.

[3] J. Bonner and F. T. Addicott, *Bot. Gaz.*, 99: 144–170, 1937.

[4] W. J. Robbins and M. Bartley, SCIENCE, 85: 246–247, 1937; *Bot. Gaz.*, 99: 671–728, 1938.

[5] P. R. White, *Plant Physiol.*, 13: 391–398, 1938.

[6] *Ibid.*, 12: 793–802, 1937.

[7] J. Bonner and F. T. Addicott, *loc. cit.*