SPECIAL ARTICLES

TOXIC EFFECTS OF DESOXYCORTICOSTERONE ESTERS IN DOGS[1]

IN the spring of 1939, a number of our patients with Addison's disease who were undergoing treatment with synthetic desoxycorticosterone acetate developed cardiac insufficiency. This varied considerably in degree but was severe in three instances. Furthermore, two patients complained of rapidly progressing weakness and died at home at a time when the blood pressure had been normal and no signs of adrenal insufficiency were recognized.

In view of the implication of these observations, it seemed imperative to study the effect of the protracted administration of synthetic desoxycorticosterone esters in animals. To this end, in July, 1939, two female dogs with carotid loops were selected for the experiment. One of these, S.E., was a normal animal, the other, N., one year before had had a unilateral nephrectomy, and the renal artery to the remaining kidney was slightly constricted by a Goldblatt clamp. The dogs were given the regular maintenance diet, containing some meat, and were injected subcutaneously with 20 mg of desoxycorticosterone acetate[2] in peanut oil daily for 70 days. In addition, two normal male dogs, maintained on a low potassium diet, have also been treated with daily injections of 25 mg of synthetic hormone. After 10 days of treatment in one instance and after 20 days of treatment in the other, both animals have developed changes similar to those shown by the other animals. The results may be summarized as follows:

(1) The changes in the blood constituents are shown in Table 1 and demonstrate a striking decrease in serum potassium, a slight increase in serum sodium, a slight decrease in serum protein and non-protein nitrogen and no definite change in blood sugar or serum calcium.

TABLE 1

Dog	Date	Na m.eq. per l.	K m.eq. per l.	Non-protein nitrogen mgm per 100 cc	Serum protein per cent.	Blood sugar mgm per 100 cc	Blood pressure mm Hg	Body weight Kgm
S.E.	Control 7/11	145.0	4.1	27	..	86	185	17.0
	Maximal effect	152.1	2.2	22	5.9	74	205	13.1
N.	Control 7/11	147.5	4.5	34	6.5	80	170	14.4
	Maximal effect	158.4	2.3	25	5.8	74	215	10.6
B.	Control 10/20	143.9	4.3	20	6.1	82	...	13.0
	Maximal effect	147.0	2.4	20	5.6	93	...	13.1
P.	Control 10/20	146.4	4.8	23	7.3	73	...	16.5
	Maximal effect	147.6	2.5	19	6.4	74	...	15.1

(2) All the dogs have shown curious periodic weakness with inability to stand or raise the head. The tendon reflexes have been active, and when the animals have been able to get on their feet they have held their limbs in marked hyperextension, but have usually been unable to raise their heads, apparently because of flaccidity of the neck muscles. These bouts of paralysis, except for persistence of reflexes, have closely resembled familial periodic paralysis in man, which is also associated with marked decrease in serum potassium. A number of the attacks of paralysis have remitted spontaneously in the course of one to three days, but others have been markedly alleviated in one half to eight hours apparently by the intravenous or oral administration of potassium chloride. (Intravenous dosage up to 1 gram in 2 per cent. solution and oral dosage up to 15 grams in one day). The administration of large doses of KCl appears to protect the animal from paralysis for one to three weeks.

(3) After several weeks of administration of synthetic hormone the ingestion and excretion of water have been definitely augmented. This "diabetes insipidus" has been most apparent at those times when varying degrees of paralysis have been present. The administration of KCl has further emphasized the disturbance of water regulation. The daily urine volume has frequently been in excess of 2.5 liters and has even exceeded 4,000 cc.

(4) X-ray studies of the heart before and at the conclusion of hormone administration showed no increase in cardiac contour. The dogs did not receive large amounts of salt in their diet in addition to hormone in contrast to the patients who developed cardiac dilatation.

(5) Electrocardiographic studies made by Dr. N. Williams showed irregular changes in the T waves, which were perhaps most apparent when the serum potassium content was strikingly decreased.

(6) In the dogs with carotid loops there was a maximal increase in arterial pressure of 45 mm and 20 mm Hg, respectively.

(7) Following the withdrawal of hormone all the abnormalities recorded disappeared in the course of

[1] From the Departments of Medicine and Neurology, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York.

[2] Supplied by Roche-Organon, Inc., Nutley, N. J.

three to four days and no manifestations of adrenal insufficiency appeared.

DANIEL KUHLMANN
ROCKEFELLER FOUNDATION FELLOW FROM UNIVERSITY OF STRASBOURG, FRANCE
CHARLES RAGAN
JOSEPH W. FERREBEE
DANA W. ATCHLEY
ROBERT F. LOEB

THE ENCEPHALITOGENIC PROPERTY OF HERPES VIRUS[1]

MODIFICATIONS have recently been reported in the virulence of the viruses of human influenza,[2] vaccinia,[3,4] infectious bronchitis of fowls[5] and pseudorabies,[6,7] following cultivation on the chorio-allantois of chick embryos. This communication is to describe an increase in virulence for chick embryos and a reduction in virulence for rabbits which have occurred in a neurotropic strain of herpes simplex (HF, Rockefeller Institute) during 70 serial passages on the chorio-allantois of chick embryos. The technique of establishing and maintaining membranal strains of viruses has been described elsewhere.[8]

The original HF virus on membranes metastasized rarely to the heart and brain. After the twentieth passage it regularly induced metastatic lesions in the heart, liver, lungs, spleen and kidneys of embryos. Lesions in the heart, liver and spleen are grossly visible areas of necrosis. By subinoculation of membranes, embryonic blood was shown to contain virus at 24, 48, 72 and 96 hours. There was no localization of virus in the brain from the blood stream. The lethal effect of infection has increased so that at the 75th passage embryos rarely survived 96 hours, whereas at the tenth, most embryos survived infection 6 or 7 days.

As virulence for chick embryos has increased, it has diminished for the rabbit. After the 25th passage the membranal strain no longer induced a typical keratitis in rabbits. Fifteen rabbits scarified and inoculated on the cornea at intervals between the 25th and 65th membranal generations have shown only slight lacrimation, mild inflammation of conjunctiva without suppuration, no elevation of temperature and no symptoms of encephalitis. This is in contrast to an invariably fatal encephalitis which follows a suppurative keratitis induced by the original strain.

To determine whether or not this modified virus traveled along the fifth nerve from the cornea to the brain without causing encephalitis, two rabbits were inoculated on their right corneas: one with the 50th membranal generation, the other with HF. Corneal reactions typical of each strain developed. The rabbits were killed at 96 hours. The right Gasserian ganglion and brain at the entrance of the fifth nerve were removed from each animal and inoculated onto membranes. Herpes virus transmissible in series was recovered from both the Gasserian ganglion and the brain of the HF control rabbit. Virus could not be demonstrated grossly or microscopically on membranes inoculated with material from the rabbit with the modified virus. This experiment was repeated with the same result using rabbits inoculated with the 53rd generation virus and sacrificed at 96 hours and at 7 days. Material from the medulla of one of these rabbits inoculated intracerebrally into another rabbit induced no symptom.

These experiments led to the conclusion that the HF strain of herpes virus had lost its ability to invade the central nervous system following corneal inoculation in rabbits. A second series of passages through 30 generations over a period of 6 months has not induced a similar modification. Modification of the first strain was observed at the 25th generation 11 months after initiation of the chick strain. Whether the already established modification is a repeatable *Dauermodification* induced by prolonged passage of the virus on chick embryos or a suddenly appearing mutation remains to be determined.

To test the virulence of the modified strain following intracerebral inoculation, suspensions of HF virus (1st membranal generation) and the 62nd membranal generation in dilutions of 10^{-3}, 10^{-4} and 10^{-5} were inoculated intracerebrally into rabbits. All 3 rabbits receiving HF virus died within 5 days of herpetic encephalitis. Each rabbit receiving the modified virus had a remarkably delayed elevation of temperature. One (10^{-4}) is alive 60 days after inoculation; one (10^{-3}) died on the 20th day, and the other (10^{-5}) died on the 26th day.

Preliminary experiments indicate that corneal inoculation with the modified strain induces a stable degree of immunity to HF inoculated on the cornea.

KATHERINE ANDERSON
VANDERBILT UNIVERSITY MEDICAL SCHOOL

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[2] F. M. Burnet, *Brit. Jour. Exp. Path.*, 17: 283, 1936.
[3] C. Levaditi, *et al.*, *Revue d'Immunologie*, 4: 481, 1938.
[4] L. Molina, *Zbl. f. Bakt., I. Orig.*, 139: 493, 1937.
[5] F. R. Beaudette (personal communication).
[6] F. M. Burnet, Dora Lush and A. V. Jackson, *Aust. Jour. Exp. Biol. and Med. Sc.*, 17: 35, 1939.
[7] R. E. Glover, *Brit. Jour. Exp. Path. and Med.*, 20: 150, 1939.
[8] E. W. Goodpasture and G. J. Buddingh, *Am. Jour. Hyg.*, 21: 319, 1935.

THE CHEMICAL COMPOSITION OF SUCKLEYA SUCKLEYANA

FOLLOWING the discovery of hydrocyanic acid in *Suckleya suckleyana* during 1937, together with the experimental work which proved its toxicity to cattle, sheep, rabbits and guinea pigs, the etiology of many of the heretofore unexplained losses in cattle and sheep in eastern Colorado was explained.[1]

[1] Frank Thorp, Jr., A. W. Deem, H. D. Harrington and J. W. Tobiska, *Colo. Exp. Sta. Tech. Bul.*, 22: 1–19, 1937.