tations are within the spinal canal and involve both anterior and posterior nerve roots.

The gross manifestations are: (1) extensive and striking herniations of the nerve roots into the intervertebral foramina distorting and, to some degree, displacing laterally the spinal ganglia; (2) herniations into the bodies of the vertebrae (ventral wall of spinal canal) which become symmetrically deeply pitted and filled with irregularly coiled nerve roots. The localization of these pits is probably due to the vascular cushion ventral to the nerve roots which enter the spinal ganglia. We believe that the veins in this location yield to the crowding and thus determine the regions of bone resorption; (3) increase in size of the contents of the cranium as evidenced thus far by—(*a*) the presence of herniations of the cerebrum, cerebellum, and posterior colliculus into the transverse venous sinus. These herniations presumably occur at the sites of arachnoidal villi. The most notable herniations are those of the cerebellum which enter the transverse sinus where the superior cerebellar veins open into it; (*b*) distortion of the brain, made conspicuous by moulding of the mid-brain and cerebellum and partial obliteration of the cisterna magna; (*c*) changes in the contours of the fossae of the floor of the skull, due to bone resorption.

Thus far no striking gross changes have been noted in the peripheral nerves. In some instances the optic nerves close to the eyeball have shown a symmetrical globoid enlargement.

As it is well known that growth of bone ceases with the establishment of vitamin A deficiency, the obvious explanation of the unequal growth is that the growth of the central nervous system continues after the growth of the skeleton has ceased. We have endeavored to test this by two procedures—(1) the study of rats whose growth has been retarded at an equal rate and degree through inadequate diet with full vitamin complement; (2) a study of rats retarded by riboflavin deficiency. In spite of the facts that these control rats paralleled in rate of weight increase and skeletal growth the A-deficient rats, no neurological lesions were produced and dissection showed normal relations of the nervous system to spinal canal and cranium. This was also true in rats where the stunting was severe and prolonged.

Experiments indicate that the unequal growth of bone and nerves occurs between the fortieth and sixtieth days of age. In a few experiments we have found that if vitamin A in the form of carotene is added to the diet at 42 days of age and the diet restricted in amount so that the growth parallels littermates in continued A deficiency, no manifestations of nervous lesions occur. The rats retained on the deficiency became paralyzed on about the fiftieth day. On postmortem examination at 63 days of age, those given carotene showed either no changes in the central nervous system or only very slight evidences of beginning herniation of nerve roots, in contrast to the striking lesions found in the litter-mates maintained on the deficiency for the entire period.

The unequal growth is most strikingly exhibited in the lumbar and sacral regions. It is manifest, to some degree however, throughout the entire length of the spinal cord. Rough estimates would indicate that nerve trunks taking exit in the lower lumbar and sacral foramina may be 4–6 mms longer than the distance from their origins to the foramina of exit.

It is possible that the compression of the cerebrum and cerebellum may be responsible for degeneration of descending fibers in the cord. At present the evidence points to a marked predominance of ascending tract lesions. The consequence of the herniations is obviously essentially that of more or less complete transection of the nerve roots and the few microscopic studies made support this view. Evidences of regeneration of nerve fibers proximal to the cell of origin have been found to take place during the deficiency. Observations in gross indicate that small excrescences comparable to amputation neuromata are present. Extensive formations of this sort have been seen after a period of extended repair on diets containing carotene.

Elucidation of the significance of the above observations is being attempted through a series of experiments designed to give information concerning the normal growth relations of the central nervous system and bony enclosure. As no readily discernible changes in nerve cells of the central nervous system or spinal ganglia are to be found other than those explainable through mechanical factors, an obvious conclusion is that the growth and physiology of the nervous system is independent of vitamin A, although the possibility that vitamin A deficiency accelerates the growth of the central nervous system can not be definitely eliminated without further work.

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EFFECTS OF STEROID GLYCOSIDES AND CORTIN ON INSULIN CONVULSIONS AND BLOOD SUGAR

PREVIOUS work from this laboratory has shown that extracts of beef adrenal cortex can affect both the blood sugar level and the electrolyte pattern of normal and adrenalectomized animals. Because our preparations were not pure substances, it could not be determined whether the effects were produced by one or several hormones. Nevertheless, our work presented evidence that the adrenal cortex was intimately con-

cerned with carbohydrate metabolism. The first step was the proof that the low blood sugars following adrenalectomy were due to the removal of the cortex and not of the medulla.[1] Subsequent work[2] showed that this fall in blood sugar of adrenalectomized cats could be prevented by our extract[3] and that the blood sugar of normal animals could be elevated by the use of this extract. Removal of the adrenal cortex delays the recovery of normal blood sugar values after insulin to a greater extent than does the removal of the adrenal medulla.[4]

Recent experiments having shown the cortin-like effects of steroid glycosides on blood potassium,[5] we next tried the effects of these substances in protecting against insulin convulsions and on the blood sugar level. Unpublished preliminary studies by Zwemer and Hrubetz on the prevention of blood sugar depression and insulin convulsions in rabbits by the use of adrenal cortex extract are in accord with recent work on rats which shows that an anti-insulin effect in regard to blood sugar[6] and convulsions[7] is obtained by previous treatment with adrenal cortex extract.

Experiments: We used a Rockland-Swiss strain of white mice weighing 16 to 24 grams in the particular groups tabulated. The animals were divided into small groups to facilitate accurate observations, individually marked and fasted for 18 hours previous to insulin injection. Strophanthin in oil was injected at the start of the fasting period; cortin or the aqueous solutions of glycosides were administered in 3 doses, at 6, 3 and 1 hours preceding the insulin injection. In studies on anaphylactic shock, we[8] found that it was best to give the aqueous cortical extract protective dose from 2 to 6 hours before antigen. This timing is also important in protection against insulin. Controls were treated in an identical manner with the exception that no strophanthin or cortical extract was given. Following intraperitoneal administration of a lethal dose of insulin, the animals were kept at a constant environmental temperature of 36°–38° C. for a period of 2 hours (Hemmingsen test).

One series of control mice are listed in Table 1–A. A significant number of animals was protected by previous treatment with adrenal cortex extract (Table 1–B). The third group of animals were treated with U. S. P. Reference Ouabain, U. S. P. Strophanthin Merck or Digitalin NNR (Pure Merck German). The protective injection was given in split doses, beginning 6 hours before insulin. Each of these offered protection against insulin similar to that given by adrenal cortex (Table 1–C). Protection which persisted for 18 or more hours was obtained by injection of strophanthin in oil (Table 1–D). In calculating the protective effects, the incidence of death and that of convulsions is given.

TABLE 1

ALL MICE RECEIVED A LETHAL CONVULSIVE DOSE OF INSULIN (2 OR 2.5 UNITS PER KG)

Number of mice	Protection	Per cent. convulsed	Per cent. died
(A) Controls			
34	None	97	91
(B) Adrenal Cortex Extract (Upjohn)			
14	2.5 dog units per gram split in 3 doses given 6, 3 and 1 hours before insulin	28.5	21.5
(C) Glucosides in water			
5	0.0015 mg Oubain (U. S. Ref.) per gram*	20	0
8	0.0025 mg strophanthin per gram body weight*	12.5	0
5	0.075 mg digitalin per gram*	0	0
(D) Strophanthin in oil			
5	0.0025 mg per gram body weight†	60	40
5	0.0020 mg per gram body weight†	40	40
15	0.0015 mg per gram body weight†	20	6.7

* The doses were divided so as to give ½, ¼ and ¼ at 6, 3 and 1 hours before insulin.
† The protective dose was given in olive oil 18 hours or more before insulin.

Equally good results were obtained with aqueous and oily solutions of strophanthin against other amounts of insulin. Details of the various experiments on more than 150 mice will be given in the completed paper. Similar experiments on 40 rats gave a total of 62.5 per cent. deaths after insulin alone, but only 14.6 per cent. deaths in animals protected with strophanthin.

Since in earlier work, we had found that blood sugar could be elevated by adrenal cortex extracts, we next followed the blood sugar in cats following injection of strophanthin. In all six animals there was a uniform elevation of the blood sugar well outside the limits of experimental error and running concomitantly with a decrease in plasma potassium and plasma proteins as previously reported. The data from the six animals is briefly summarized in Table 2.

We take this opportunity to thank the Upjohn Company for a grant in aid of our adrenal research, and to thank Miss Ruth Rawson for the determinations of blood sugar.

CONCLUSION

We present evidence that insulin convulsions and death of mice and rats may be prevented by the previ-

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TABLE 2

EFFECT OF STROPHANTHIN ON SOME BLOOD CONSTITUENTS OF CATS

Cat	Sugar mg per cent.			Potassium mg per cent.			Protein gm per cent.		
	Initial	Max. change	Time*	Initial	Min. value	Time*	Initial	Min. value	Time*
30 gamma per kilo									
A ...	111	+17	45	22.0	20.0	15	7.58	6.02	75
B ...	100	+45	15	21.0	19.9	15	7.21	6.02	90
C ...	71	+25	10	27.6	20.8	10	7.41	7.08	30
D ...	50	+37	45	20.5	18.6	15	8.50	6.85	90
50 gamma per kilo									
E ...	61	+35	45	25.1	19.9	5	7.06	6.50	105
F ...	58	+34	60	27.3	21.3	5	7.06	6.64	75

* Times given are in minutes from injection to greatest change.

ous administration of cardiac glycosides, and that the blood sugar level of normal cats can be significantly elevated by injection of these glycosides.

Both effects are similar to those obtained with adrenal cortex extract.

In view of the cortin-like effect of steroid glycosides on potassium, previously reported by us,[9] it is important to know that the same crystalline substances will also affect carbohydrate distribution and counteract the convulsive action of insulin. The fact that in some experiments the protection was effective when given 18 hours or more before insulin is particularly noteworthy.

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A PHYSIOLOGICALLY ACTIVE PRINCIPLE FROM CANNABIS SATIVA (MARIHUANA)

WHILE it has long been known that the physiological activity of *Cannabis sativa* (marihuana or hashish) is associated with its contained resins, no physiologically active crystalline material has heretofore been isolated. We report in this note the isolation of such a substance.

The hydrocarbon nonacosane and an oily product termed *cannabinol* were first isolated by Wood, Spivey and Easterfield.[1] In 1938 Bergel, Todd and Work[2] reported the preparation of a crystalline p-nitro benzoate of cannabinol which could be used to separate the cannabinol from the oil by chromatographic adsorption methods. Recently an oily product which was named *cannabidiol* was isolated by Adams, Hunt and Clark.[3] None of these well-defined products has exhibited the characteristic physiological activities that are shown by the crude drug though *cannabinol* was found to be quite toxic. Reviews of the earlier work on the chemistry of *Cannabis* have been published by Walton[4] and by Blatt.[5]

Work on the separation of physiologically active fractions from alcoholic extracts of *Cannabis sativa* has been in progress for the past year in our laboratories. The extracts of Minnesota wild hemp used for the work were generously supplied by the Narcotics Laboratory, United States Treasury Department, and we are indebted to Messrs. H. J. Anslinger and H. J. Wollner for their collaboration which made this work possible.

The alcohol extract of the crude drug was diluted with water to yield a seventy per cent. alcohol solution, and this was partitioned into petroleum ether. Salt-forming compounds were extracted and then colored substances were largely removed by adsorption on zinc carbonate. The resultant resinous material was fractionally precipitated from methanol with water and there was obtained a physiologically active fraction of about one twentieth the weight of the crude resin material. This purified product was fractionally distilled under 0.005 mm pressure, with the most active fraction distilling at 128°–135° C. This fraction is a red-colored oil which shows typical activity in dogs following an oral dose of 1.0 mg per kg. By cooling a solution of this oil in a methanol-acetic acid mixture, some crystalline material was obtained. This was then recrystallized several times from methanol to yield colorless needles melting at 128°–129° C.

When crystalline material thus isolated was administered orally to a dog in a dose of 0.1 mg per kg, incoordination of movements followed after two hours and persisted for about four hours. A dose of 0.38 mg per kg was administered to the same dog three days later. Within two hours incoordination of movements was apparent and this effect persisted notably for six hours. A transitory catatonic depression was also observed, during which the animal gazed fixedly at particular objects for long periods of time; breathing was deep and dyspneic, and cardiac arrhythmia was noted. Fibrillary tremors of the left leg were observed as were, also, periods in which the dog engaged in vigorous though seemingly useless scratching. The dog used for these studies had been standardized for response to the crude drug material from which the crystalline material was prepared and had shown similar effects to those above noted following a dosage of 20 mg per

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[1] Wood, Spivey and Easterfield, *Jour. Chem. Soc.*, 69: 539, 1896; 75: 20–36, 1899.

[2] Bergel, Todd and Work, *Chem. and Ind.*, 57: 86, 1938.

[3] Adams, Hunt and Clark, *Jour. Am. Chem. Soc.*, 62: 196, 1940.

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