be handled with moderate care and no sand grains are knocked off in the handling. Likewise very fissile shale specimens have been preserved *en masse* for laboratory hand specimens.

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A SHORT METHOD FOR THE PREPARATION OF ANIMAL TISSUES FOR STAINING

CUT the tissue in pieces from two to four millimeters in thickness. Drop these in some ten per cent. formalin solution and heat to boiling. Transfer them to acetone for dehydration and leave them in this liquid for one hour, during which time the acetone should be poured off and renewed with fresh material two or three times. The dish should be covered and put in the paraffine oven, as diffusion takes place more rapidly at the higher temperature. Remove the tissue from the acetone and drop it into a container of melted paraffine. Leave this in the oven for one hour.

Embed, mount on block and cut in the usual manner. Smear the slides lightly with Mayer's albumen, float the sections over this on a drop of water, warm gently to expand them and drain off the excess.

Lay the slides out flat in the paraffine oven. At the end of one hour remove them. After they have cooled, put them in xylol to dissolve the paraffine and proceed in the usual way to stain and finish them.

Excellent slides have been made by this method. If quicker results are desired, cut the tissue in smaller pieces. If larger pieces must be used, allow more time for the infiltration of acetone and paraffine.

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SPECIAL ARTICLES

FURTHER EXPERIMENTS ON CORTICO-ADRENAL EXTRACT: ITS EFFICACY BY MOUTH

A RAPIDLY accumulating mass of evidence indicates that extracts of the adrenal cortex which have recently been developed are markedly potent in both experimental and clinical cases of adrenal insufficiency. The methods of Hartman and his associates[1] and Swingle and Pfiffner[2] have been widely used. In experiments reported from this laboratory full support has been given to the observations of the Princeton workers. We have employed a slightly modified Swingle-Pfiffner technique as described.[3]

Cortico-adrenal extract maintains completely adrenalectomized animals in good health for indefinitely long periods. It readily abolishes the severe symptoms of adrenal insufficiency when allowed to develop, and concurrently restores the blood sugar and non-protein nitrogen levels to normal.

Augmentation of the blood sugar to the normal value occurs in adrenalectomized cats showing symptoms within one to three hours after the extract is given. The increase is approximately proportional to the amount of the material injected; it is produced consistently and repeatedly when potent extracts are used. We have observed the effect at least five times within about ten days in the same adrenalectomized animal.

Cats with severe symptoms of adrenal insufficiency invariably show low blood sugar values. In over thirty cases in our experience, cortico-adrenal extract has raised the sugar percentage to within normal limits or higher. Notable increments in blood sugar also occur in unoperated cats, particularly if young animals are used. Biological assay of the extract based on the observed blood sugar effects is suggested from these results.[4]

The decreases in blood non-protein nitrogen, following extract injection, have not been so characteristic in our experiments. They usually occur more slowly than the glycemic changes, and sometimes are long delayed. These differences are possibly referable to the renal condition (damage?) in the different animals, and also to the fluid content of the tissues. In some cases there is considerable urination following extract administration.

Intraperitoneal injections of pituitrin, ephedrine and ergotamine solutions, known to influence the blood sugar in insulin hyperglycemia and other conditions, do not affect significantly the low blood sugar levels or the symptoms of adrenal insufficiency.

The administration of cortico-adrenal extract has been tested by mouth in a series of animals. The large doses which were found to be necessary, and the relatively small supplies of the extract available, as well as the considerable expense of the material, has somewhat curtailed our observations. Cats have been kept alive by oral treatment, however, for two weeks *after* severe symptoms (convulsions) of adrenal insufficiency became evident. Death rapidly fol-

[1] F. A. Hartman, K. A. Brownell and W. E. Hartman, *Amer. J. Physiol.*, 95: 670, 1930.

[2] W. W. Swingle and J. J. Pfiffner, *Amer. J. Physiol.*, 96: 153, 1931.

[3] S. W. Britton and H. Silvette, SCIENCE, 73: p. 322, March 20, 1931; *Ibid.*, p. 373, April 3, 1931; *Amer. J. Physiol.*, 97.

[4] S. W. Britton and H. Silvette, *Amer. J. Physiol.*, in press.

lowed withdrawal of the extract. Complete recovery from the convulsions which usually immediately preceded death was readily brought about, in several cases in the same animal.

The low blood sugar, high non-protein nitrogen and increased blood cell volume are restored to within normal limits when the extract is given by mouth. All the effects which were produced by oral administration were in keeping, indeed, with those which resulted from intraperitoneal injection of the material. Approximately three to five times the intraperitoneal dosage was necessary to produce comparable results. It is definitely clear from the experiments, however, that the effective hormone of the adrenal cortex is not wholly if at all destroyed in its passage through the gastro-intestinal mucous membrane.

Numerous attempts to maintain the lives of adrenalectomized cats by feeding whole and demedullated adrenal glands have not to date been successful. The animals do not readily eat the glandular tissue; if they are forcibly fed, vomiting often follows.

Adrenalin given by mouth in similar concentration to that present in the cortical extract which was used had no noteworthy influence on adrenal insufficiency. Glucose solutions given orally were also without significant effect.

The development of a method by which potent extracts of the adrenal cortex can be consistently produced suggested that tests of extracts of other tissues be made. Knowledge that specific chemical agents or hormones may be separated from widely different body tissues, and also that certain characteristics of the adrenal cortex find a resemblance in other extra-adrenal tissues (*e.g.*, the brain and testes), gave some hope of success in these experiments. Extracts were made of the following tissues: testes, brain, liver, spleen, and heart muscle. The method of preparation of these extracts was in all details similar to that used concurrently in making potent preparations of the adrenal cortex. When the tissue extracts were tested on adrenalectomized cats showing different degrees of adrenal insufficiency, however, the results were in all cases wholly negative. No effects were observed on the muscular weakness or other symptoms, or on the length of survival after operation.

In a recent report[5] the striking influence of cortico-adrenal extract, in bringing about precocious sexual maturation in rats, has been pointed out. Maintenance of the lives of adrenalectomized animals and the effects on sexual maturity—two apparently widely-separated functions—which are brought about by cortico-adrenal extract indicate the presence in the material of two distinct chemical agents or hormones.

It is suggested that the primary action of the life-preserving hormone of the adrenal cortex, which is effective in conditions of adrenal insufficiency, is concerned with preservation of the normal carbohydrate balance in the body.

Observation that animals from which the adrenal glands have been completely removed may be kept alive with extracts of the cortex indicate the dispensability but not the inutility of adrenal medullary secretion.

SUMMARY

Increase of the blood sugar which invariably follows administration of cortico-adrenal extract to adrenalectomized cats with symptoms of insufficiency is a reliable index of potency and affords a means of bio-assay of the material.

Cortico-adrenal extract is effective when given by mouth. The lives of adrenalectomized animals are prolonged and all the signs and symptoms of severe insufficiency are rapidly abolished by oral administration of large doses of the extract.

Extracts which have been prepared similarly to cortico-adrenal extract from many different (extra-adrenal) body tissues have no influence on the symptoms of adrenal insufficiency.

The existence of two hormones in cortico-adrenal extract is postulated.

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VARVED CLAY IN HOLMES COUNTY, OHIO[1]

IN the course of field study of the glacial geology of Holmes County, Ohio, several deposits of layered glacial clay and silt have been discovered.

Holmes County is in northeast central Ohio. The region is one of maturely dissected upper Mississippian (Waverley) and Lower Pennsylvania (Pottsville and Allegheny) horizontal sediments. The usual relief is about 300 feet. The Late Wisconsin drift border crosses the county in a general east-west direction, except in the eastern portion, where the glacial boundary trends northeast-southwest. The farthest extent of the Late Wisconsin ice sheet is not well marked by a terminal moraine, except in some of the valleys which have their courses across the glacial boundary.

[5] E. L. Corey and S. W. Britton, SCIENCE, 74: 101–102, July 24, 1931.

[6] W. T. Porter Fellow in Physiology.

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[1] Published by permission of the Director, Geological Survey of Ohio.