transfers on roots of living cotton seedlings. When a fragment of infected root was placed against the root of a healthy seedling, it usually caused within a few hours a shrinking and discoloring of adjacent host tissues. This was followed by the formation of an encircling and penetrating hyphal weft, which produced within two or three weeks a thoroughly soft decay of the cortex along the entire root system. The immediately destructive action observed in such cases, occurring before penetration had been accomplished, suggested that a transfer of tissue-destroying substances from rotted to healthy roots had taken place, and led the writers to test the action of hyphal exudates apart from the fungus. It was found that small pieces of decayed roots, when squeezed with forceps, generally yield one or two drops of clear, amber-colored liquid. In one series of tests this fluid was applied directly, in the form of drops, to the surfaces of normal cotton seedling roots; in a parallel series healthy roots were treated with drops of liquid expressed from decayed roots which had been subjected to the temperature of boiling water for one hour. Similar tests were made for the action of liquid pressed from germinating sclerotia which had been formed on nutrient agar without access to host tissue. Special care was taken to avoid mechanical injury to the healthy roots and to maintain aseptic conditions throughout all experiments.

Liquid from unheated, decayed roots was usually absorbed into healthy roots in 4 or 5 hours, and frequently imparted a moist, water-soaked appearance to the tissue at the points of application. After 24 hours the tissue thus affected had begun to shrink and turn yellow or light-brown, forming sunken necrotic areas which resembled the lesions caused by the fungus on roots of young cotton seedlings. In most cases the shrinkage progressed around the root and extended somewhat longitudinally until the root appeared to be girdled or ringed almost to the central cylinder. After three or more days a number of small lateral roots began to break through the necrotic cortex, which suggests that the tissue destruction did not extend beyond the endodermis. Stained sections of roots fixed in different stages of cortical necrosis show a gradual disorganization of protoplasts, followed by swelling and distortion of cell walls from the epidermis inward. Finally the epidermis and cortex collapse into a deeply staining, disorganized mass, which shrinks toward the endodermis. Simultaneously, abundant cell division in the pericycle initiates the formation of lateral roots.

Cotton roots treated with liquid expressed from heated decayed roots reacted in a different manner; the liquid was not absorbed as readily, and usually only a slightly discolored spot resulted. No considerable shrinkage or disruption of tissue continuity was observed, although sections of the treated roots show that toxic effects had been exerted on protoplasts near the places of application. The experiments with unheated and heated liquid from germinating sclerotia gave results closely parallel to those described above.

Attempts to recover the fungus by placing on nutrient agar various samples of roots to which unheated fluid had been applied failed, and sections of such roots show no mycelium. Both of these circumstances tend to indicate that viable hyphae are rarely, if ever, transferred with the expressed drops of liquid, and that the induced lesions resulted from the activity of fungous secretions. The consideration that the lesions might have been brought about by possible toxic products of the chemical breakdown of host tissues is minimized by the fact that similar lesions resulted from the application of unheated liquid from germinating sclerotia. The demonstration of chemical action as an important factor in the pathogenic mechanism of *P. omnivorum* is of interest in connection with biochemical studies of the basis of resistance in certain plants to the root rot caused by this fungus.[4,5]

G. M. WATKINS
MATILDE OTERO WATKINS
U. S. DEPARTMENT OF AGRICULTURE

## URINE CHLORIDE CONCENTRATION IN PATIENTS WITH CUSHING'S SYNDROME

McQUARRIE, JOHNSON and Ziegler[1] and Anderson, Haymaker and Joseph[2, 3] recently reported changes in the serum electrolyte pattern and urinary excretion of sodium and potassium in patients with Cushing's syndrome that were in most respects diametrically opposite to those in patients with Addison's disease. These findings, together with the observation[2] that extracts of the blood of such patients prolonged the lives of adrenalectomized rats, suggested that Cushing's syndrome may be dependent upon or associated with a state of hypercorticoadrenalism.[1, 2]

Cutler, Power and Wilder[4] have demonstrated that under standardized conditions of low sodium and chloride and high potassium intake the presence of supernormal concentrations of sodium or chloride in the urine is suggestive of a state of adrenal cortical insufficiency. Theoretically, the opposite condition should obtain in hypercorticoadrenal states under conditions of high sodium and chloride intake. The fol-

[4] G. A. Greathouse, *Phytopath.*, 28: 592–593, 1938; *Amer. Jour. Bot.*, 25: 743–748, 1938.

[5] The senior writer's share of this work was done during his tenure of a National Research Fellowship in Botany, 1938–39.

[1] I. McQuarrie, R. M. Johnson and M. R. Ziegler, *Endocrinol.*, 21: 762, 1937.

[2] E. Anderson, W. Haymaker and M. Joseph, *Endocrinol.*, 23: 398, 1938.

[3] E. Anderson and W. Haymaker, *Proc. Soc. Exper. Biol. and Med.*, 38: 610, 1938.

lowing procedure was adopted: The basic diet was identical with that employed in the procedure outlined by Cutler *et al.*,[4] containing 0.949 gm Cl, 0.592 gm Na and 4.062 gm K daily. On the first day of the study period the fluid intake was fixed at 20 cc per kilogram of body weight, and 10 gm of NaCl (in capsules) was given with the morning and again with the evening meal. The same régime was followed on the second day. On the third day the bladder was emptied at 8 A.M. and urine was collected for the subsequent four-hour period. On this day 5 cc of fluid per kilogram was given before 11 A.M. Under these conditions, the intake of Na and Cl on each of the first two days was approximately 8.6 and 12.95 gm, respectively.

Of the several chemical studies performed during the test period, I wish to mention here only the chloride concentration in the four-hour urine specimen obtained on the morning of the third day. Sixteen subjects were studied. Three of these were patients presenting the characteristic clinical picture of Cushing's syndrome, with normal urinary findings and normal renal function (urea clearance); one was a patient with excessive hirsutism; the remainder were patients with miscellaneous diseases, including rheumatic fever, convalescent, afebrile (2 cases), mild essential hypertension with normal renal function (2 cases), hypertrophic arthritis (2 cases), bronchial asthma (2 cases), maxillary sinusitis (1 case) and inguinal hernia (2 cases).

In the three subjects with Cushing's syndrome the Cl concentrations were 0.193, 0.243 and 0.357 per cent., respectively, with urine volumes of 475, 450 and 500 cc. The corresponding values in the patient with excessive hirsutism were 0.179 per cent. and 535 cc. In the twelve subjects with miscellaneous disorders with no evidence of endocrine dysfunction the Cl concentration ranged from 0.462 to 1,265 per cent. (mean 0.642) and the urine volume from 680 to 1,120 cc. It appears, therefore, that under the conditions of the experiment the subjects with Cushing's syndrome and with hirsutism (suspected hyperadrenalism) were unable to eliminate chloride in the urine in as high concentration as subjects with various disorders not apparently associated with endocrine dysfunction. This test procedure may prove to be of value in detecting states of hypercorticoadrenalism. It must be kept in mind, however, as demonstrated by Thorn and Harrop,[5] that various sex hormones may exert an effect upon the urinary excretion of sodium, chloride and potassium similar to that exerted by the adrenal cortical hormone.

ABRAHAM CANTAROW
JEFFERSON MEDICAL COLLEGE, PHILADELPHIA

[4] H. H. Cutler, M. H. Power and R. M. Wilder, *J. A. M. A.*, 111: 117, 1938.

[5] G. W. Thorn and G. A. Harrop, SCIENCE, 86: 40, 1937.

## ESSENTIALITY OF PRIMARY AMINO GROUPS FOR SPECIFIC ACTIVITY OF THE LACTOGENIC HORMONE[1]

THE primary amino groups are essential for the action of the protein hormone of pituitary origin which stimulates the formation of the pigeon crop gland. We have recently demonstrated this by treating the lactogenic hormone with ketene.[2] Philpot and Small[3] have shown that nitrous acid resembles ketene in first attacking the primary amino groups in the protein molecule. They found that on the treatment of pepsin with nitrous acid, nitrogen liberation was complete within one half hour and that the secondary reaction—diazo compound formation—was scarcely started by this time.[4]

TABLE 1

EFFECT OF NITROUS ACID ON LACTOGENIC HORMONE*

| Conditions of treatment | Total dose/squab (intramuscular) mg | Number of 30-day-old pigeons | Crop gland reaction |
|---|---|---|---|
| Untreated ..... | 1.0 | 3 | Pronounced |
| 22–23° C, 30 minutes. | 1.0 | 3 | Negative |
| | 2.0 | 3 | Negative |
| 0° C, 30 minutes .... | 1.0 | 3 | Negative |
| | 2.0 | 3 | Negative |
| | 1.0 | 3 | Negative |

* L 250: potency, 10 systemic units per mg.

It therefore seemed desirable to treat lactogenic hormone with nitrous acid in an effort to confirm the findings with ketene. A 1.5 per cent. solution of a highly purified lactogenic preparation was dissolved in 0.5 M acetate buffer (pH 4) and was treated for 30 minutes with an equal volume of 2 M $NaNO_2$ at 22° C. and 0° C. The mixture was then adjusted to about pH 5, was centrifuged, and the precipitate was redissolved and reprecipitated isoelectrically. As can be seen in the table, the crop stimulating activity of the preparation was completely destroyed by nitrous acid in this period. The results therefore confirm those obtained with ketene, indicating the essentiality of the

[1] Aided by grants from the Research Board of the University of California, from the Rockefeller Foundation of New York and from Parke, Davis Company of Detroit. Assistance was rendered by the Federal Works Progress Administration, Project OP 665-08-3-30, Unit A-5.

[2] C. H. Li, M. E. Simpson and H. M. Evans, SCIENCE, 90: 140, 1939.

[3] J. St.L. Philpot and P. A. Small, *Biochem. Jour.*, 32: 542, 1938.

[4] This specific action of nitrous acid has recently been confirmed by us in a study of the gonadotropic hormones. Pituitary follicle stimulating hormone, interstitial cell stimulating hormone and pregnant mare serum are inactivated quickly (one half hour) by nitrous acid, whereas human chorionic gonadotrophin is only inactivated *very slowly*. The same results were secured with ketene. (To be published).