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.

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[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).

primary amino groups in the physiological activity of lactogenic hormone.

CHOH HAO LI
WILLIAM R. LYONS
MIRIAM E. SIMPSON
HERBERT M. EVANS
INSTITUTE OF EXPERIMENTAL BIOLOGY AND DIVISION OF ANATOMY, UNIVERSITY OF CALIFORNIA, BERKELEY

## MULTIPLE NATURE OF THE RAT "FILTRATE FACTOR"—A COMPONENT OF VITAMIN $B_2$[1]

IN our attempt to purify the "filtrate factor,"[2] a dietary component of vitamin $B_2$ essential for rat growth, we have obtained evidence that this factor must consist of at least two entities, one of which is extractable from acid solution by diethyl ether; the second factor remains in the residue.

The methyl alcohol soluble fraction from cane molasses was adsorbed on fuller's earth. The filtrate was acidified and extracted for 72 hours with ether in a continuous extractor (Preparation I). The residue was subjected to a second 72-hour extraction (Preparation II). These extracts, as well as the residue (Preparation III), were fed to female rats maintained on a vitamin B complex-deficient diet supplemented with thiamin, riboflavin and a source of $B_6$ in the form of a wheat germ eluate. Each preparation was fed for 56 days at the equivalent of 3 gm daily of the original molasses. The gain in weight (above that of the controls) was: Preparation I, 60 gm; Preparation II, no gain; Preparation III, 58 gm.

At this point it may be noted that black, gray and hooded rats receiving Preparation I exhibited no change in pelage coloring, while those receiving Preparation III showed a marked graying of black hair and a lightening of gray hair, although the nutritive state and growth were essentially the same in groups I and III. This experiment was repeated with new preparations and again the graying was observed in the rats receiving the residue and the coat was normal in those receiving the ether extractable fraction, although again growth was comparable in both groups. The graying of fur in "filtrate factor"-deficient rats was first noted by Morgan, Cook and Davison[3] and by Lunde and Kringstad.[4] The present work would seem to indicate that the "anti-graying" activity goes with the ether extractable component of the "filtrate factor."

The evidence for a relationship between the "chick anti-dermatitis factor" and the "rat filtrate factor" is conflicting. Woolley *et al.*[5,6] and Jukes[7,8] have demonstrated that pantothenic acid (Williams) is the "chick anti-dermatitis factor."

Hoffer and Reichstein[9] and Subbarow and Hitchings[10,11] have shown that the fraction extracted with ether is in all probability pantothenic acid and is a component of the rat "filtrate factor"; however, El Sadr and co-workers[12] found that β-alanine did not replace the liver or yeast "filtrate factor." Woolley *et al.*[5] have reported that the "chick anti-dermatitis factor" is readily destroyed by alkali. We have prepared an iso-amyl alcohol extract from a rice bran preparation. Its activity was not destroyed by heating in 1 N NaOH solution at 100° C. for 1 hour. It would, therefore, appear that the factor extractable with iso-amyl alcohol is not identical with the "chick anti-dermatitis factor."

ALI MOHAMMAD
OLIVER H. EMERSON
GLADYS A. EMERSON
HERBERT M. EVANS
INSTITUTE OF EXPERIMENTAL BIOLOGY, UNIVERSITY OF CALIFORNIA, BERKELEY

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# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## AN ELECTRIC STERILIZER FOR THE CULTURE ROOM

To avoid the use of an open flame in culture room or transfer case a small electric sterilizer has been used for a number of years and found to be highly satisfactory. The constant heating of the culture space resulting from the use of a gas jet or an alcohol lamp; the resulting convection currents of air carrying con-