in both the supernatant liquid and the insoluble residue of insulin treated by interfacial adsorption, is shown in Table I, where the prolonged action of the treated insulin may be observed. The insulin in each case was injected subcutaneously.

TABLE I.

Hours	0	1	2	3	4	5	6	7	8	10	12	13	18	22	25	30
A	89	31	30	33	52	82	93	93								
B	82	63	56	59		65		74			78					
C	83	42	36	41	42		51		55			56	80	78	80	83
D	87	78	71	77	77	74	75	68		80	78					

A represents the standard dose of crystalline insulin required to bring the blood sugar of a rabbit to the convulsive level. B is a dose of supernatant fluid representing 3 times the original volume of untreated insulin normally used as in A. C represents the insoluble fraction of 30 units of crystalline insulin after denaturation. D represents 5 times a normal dose of insulin contained in a mixture of the supernatant liquid and insoluble residue of another sample of treated insulin.

Variations in the methods of procedure are being tried to note whether the apparent decrease in activity per unit of insulin can be prevented while retaining its prolonged activity after injection.

As will be shown elsewhere, this method has also been used to bring about the attenuation of bacterial toxin.

9296 P

A Colorimetric Assay for Male Sex Hormones in Urine.

RALPH B. OESTING.* (Introduced by Bruce Webster.)

From the Barbara Henry Research Laboratory, New York Hospital, and the Department of Medicine, Cornell University Medical College, New York City.

Zimmerman[1] described a color reaction for the R-CH_2-CO-R group of the sex hormones using meta dinitrobenzene as his reagent. Although the reaction is not specific the author after some preliminary trials has been able to adapt it so that it can be used as an index to the male sex hormone content of urine and as a guide for capon assays of urinary extracts where more complete characterization of the extract is desired.

Capon assays were performed using the alcoholic inunction technique described by Fussgänger[2] and elaborated by Dessau.[3] The

* General Education Board Fellow.

1 Zimmerman, W., *Z. f. Physiol. Chem.*, 1935, **233**, 257.

2 Fussgänger, R., *Medicine in its Chemical Aspects*, 1934, **2**, 185.

3 Dessau, F., *Acta Brev. Neer.*, 1935, **5**, 139.

growth of the comb was determined by measuring with a planimeter the area of a shadow photograph of the comb taken under standard conditions. The author's standard deviation, calculated from 5 measurements each on a group of 10 capons, according to the statistical methods of Fisher,[4] was ±3.9%. Growth of combs is expressed in percent increase in area using the size of the starting comb as the baseline. Since the relation between comb growth response and the color assay on urine extracts is the important feature of this study no effort is made to describe the value of the color unit in gammas of androsterone. It is generally agreed that the inunction assay for androsterone is about 50 times as sensitive as the intramuscular injection technique.

A 24-hour specimen of urine is brought to a pH below 1.0 with concentrated sulfuric acid and autoclaved for 15 minutes at 15 pounds pressure. After cooling, the urine is extracted with benzene in a continuous extractor until extraction is complete. The benzene is distilled off and the residue is dissolved in about 100 cc. of ethyl ether. This is transferred to a separatory funnel where the ether solution is extracted 5 times with 25 cc. portions of 10% aqueous solution of sodium hydroxide. The ether solution is then washed 3 times with 30 cc. portions of distilled water and transferred to a beaker, where it is stirred with 0.5 gm. of Norit (decolorizing charcoal) and filtered into a 125 cc. distilling flask. The charcoal is washed once with fresh ether. The ether solution at this point should be practically free from color. The ether is then distilled off and 20 cc. of 60% ethyl alcohol solution (60 cc. of 95% alcohol diluted to 95 cc.) is carefully measured into the flask to dissolve the hormone residue.

Five cubic centimeters of this alcohol solution, or less of it diluted to 5.0 cc. with 60% alcohol, is placed in one of the tubes of the colorimeter and 1.0 cc. of a 2% alcoholic solution of meta dinitro-benzene (2.0 gm. per 100 cc. of 95% alcohol) is added. One cubic centimeter of a 15% aqueous solution of KOH (15.0 gm. per 100 cc. of distilled water) is added, the tubes are shaken and set aside for an hour and a half in a dark corner of the room. A blank tube containing 5 cc. of 60% alcohol plus the reagents is prepared along with the unknowns. The color is measured in a simple colorimeter prepared for this purpose under the direction of the author by the Hellige Manufacturing Company. Color is expressed directly in color units read from the color disc of the colorimeter and the number of color units per 24-hour specimen is calculated.

[4] Fisher, R. A., 1930, Statistical Methods for Research Workers, 283 pages.

The alkali treatment of the ether solution removes the female sex hormones and the use of charcoal in small amounts does not remove male hormone activity to any significant extent. This point has been tested several times by capon assays.

Table I summarizes the data of a series of assays comparing the colorimetric assay with the comb growth assay. The latter is expressed in percent increase in comb area after 5 daily applications of 0.3 cc. of the alcoholic hormone solution to the combs of at least 2 capons. Each comparison in the table is a specimen of urine from a different individual.

TABLE I.

Comparison of the Colorimetric and the Inunction Assays of Urine Extracts for Male Sex Hormone.

The comb growth response is expressed as the average percent increase in area after 5 daily applications of 0.3 cc. of the alcoholic hormone solution to the combs of at least 2 capons.

Color Units per 24 Hour Specimen	Comb Growth Response
Units	%
Doubtful	4.5
3.2	5.0
11.2	17.4
24.0	18.0
27.0	28.0
40.0	42.0
56.0	36.0
64.0	25.0
80.0	33.0
96.0	38.0
104.0	63.0
112.0	58.0
112.0	68.0
112.0	84.0
120.0	45.0
120.0	68.0
144.0	120.0
152.0	55.0
168.0	77.0
200.0	87.0

These data show a satisfactory correlation between the two methods of assay. In examining the data it should be remembered that the color developed is not specific for male sex hormones. The author believes, however, that the colorimetric assay can be used in clinical studies with as much confidence as is now placed in the comb growth assay using intramuscular injections. The colorimetric assay is decidedly more sensitive to changes in the hormone content of urine and requires a less complicated set-up.

Summary. A colorimetric method is described for male sex hormone assays on urine. The color assays are compared with comb growth assays on capons.